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Studies on Antimicrobial Sesquiterpenes in the Leaves of Rugosa
Rose (*Rosa rugosa* Thunb.)

ハマナス (*Rosa rugosa* Thunb.) 葉中の抗菌性セスキテルペンに関する研究

Volume II

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3-6 Rugosic Acid A Metabolites in *Rosa rugosa* Leaves

3-6-1 Introduction

As described in the previous section, rugosal A (1) is probably synthesized from carota-1,4-dienaldehyde (3) and further oxidized to rugosic acid A (2) in the leaf tissues of *Rosa rugosa*. Those carotane peroxides tend to be accumulated in the tissues. However, the author noticed that old and slightly senescent leaves of *rosa rugosa* (for example, sample VIII) contained quite small amount of those compounds. Albeit the dramatic decrease of 1 and 2, the leaves were still rich in oily substance. The author therefore speculated that those peroxides were metabolized in the senescent leaf tissues. Accordingly, the presumable metabolites were surveyed in the constituents of senescent *Rosa rugosa* leaves, especially in the acidic extractives. In this section, the author describes isolation and identification of those metabolites of rugosic acid A (2), and discusses on the biogenesis of carotanoids originated in *Rosa rugosa*, focussing on rugosal A (1) as a key compound.

3-6-2 Rugosic Acid A Methyl Ester

Rugosic acid A methyl ester (5), a neutral metabolite of rugosic acid A (2) was found during a survey of peroxides in *Rosa rugosa* leaf-constituents, using a peroxide test with *N,N*-dimethyl-*p*-phenylenediamine sulfite reagent. Firstly, 9.6 kg of leaves (sample V) were mechanically damaged and then soaked in 76 liters of tap water for 24 hr, at which point the water layer was collected and the exudates were partitionated with an equal volume of EtOAc. After being concentrated to ca 5 liters, the EtOAc

extracts were once washed with 10 liters of 5 % NaHCO_3 to ca 17 g of neutral substances in the organic layer. The neutral constituents coated on 70 ml of silica gel were chromatographed on silica gel column settled in benzene (gel volume 1500 ml) to obtain 9 fractions as shown in Table 3-90 and Fig. 3-235. Since an unknown spot clearly positive to the peroxide reagent was detected in Fr-V-1, the compound denoted as RL-PERO-3 was isolated by PTLC successively in *n*-hexane-EtOAc 3:1, successive benzene-EtOAc 5:1, *n*-hexane-EtOAc 4:1 and CHCl_3 -MeOH 50:2; *Rf* 0.66, 0.58, 0.36 and 0.81, respectively) to give colorless needles (6.5 mg, from ca 1/2 of Fr-3) (Fig. 3-236).

Table 3-90 Fractionation of the extractives from Sample V by silica gel column chromatography

No	Solvent	Volume
Fr-V-1	20 % EA/B	500 ml
Fr-V-2	20 % EA/B	500 ml
Fr-V-3	50 % EA/B	500 ml
Fr-V-4	50 % EA/B	500 ml
Fr-V-5	50 % EA/B	500 ml
Fr-V-6	50 % EA/B	500 ml
Fr-V-7	100 % EtOAc	500 ml
Fr-V-8	100 % EtOAc	500 ml
Fr-V-9	100 % EtOAc	500 ml

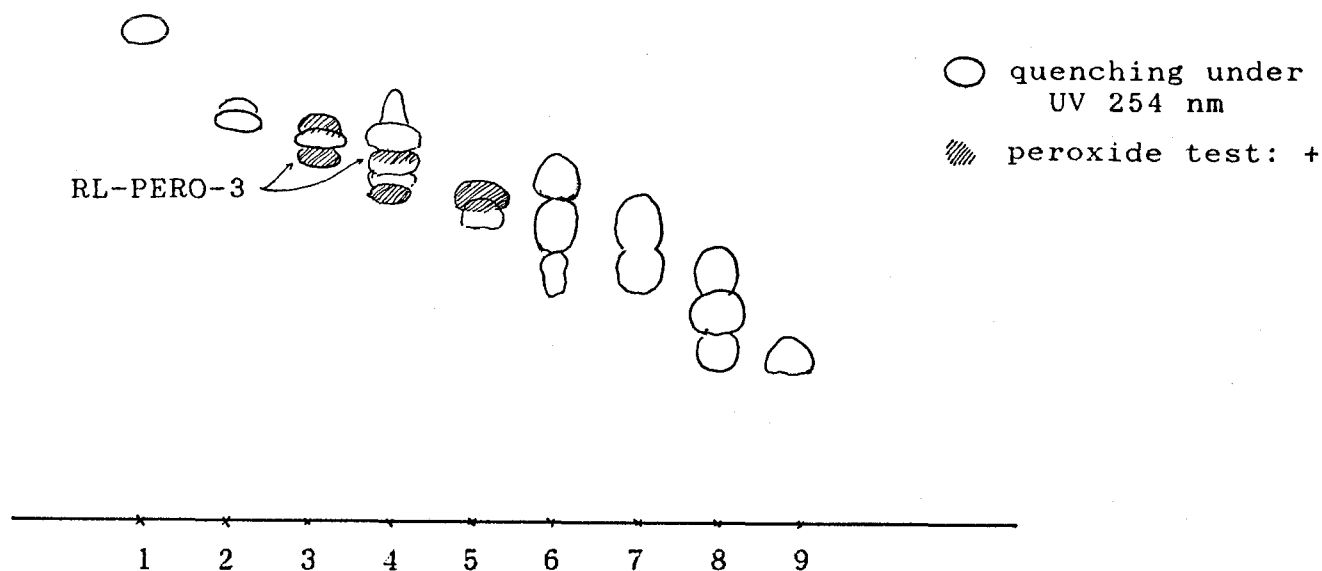


Fig. 3-235 TL Chromatogram of Column Eluants of Neutral Constituents in the Exduates from Damaged *Rosa rugosa* Leaves

The isolate showing the molecular ion at m/z 296 (0.8 %) was agreeable with that of RSA-CN or RDA-ME 1k in the EI-MS (Fig. 3-237). In the ^1H -NMR spectrum, RL-PERO-3 was also indistinguishable from them (Fig. 3-238). RL-PERO-3 was thus identified as 5. The fifth naturally occurring carotanoid from *Rosa rugosa* named rugosic acid A methyl ester was thus confirmed. As the optical rotation of the isolate $[\alpha]_D + 150^\circ$ indicates an absolute configuration identical with that of rugosic acid A (2), 5 is presumed to be derived from 2 in the plant. The possibility that 5 is an artifact was reasonably rejected, because the leaf material was extracted not with MeOH but with water.

Rugosic acid A methyl ester (5) was also isolated from sample VI (ca 4.5 mg/kg). The content of 5 in the overmatured leaves (collected in late August) was about 3.5 times higher than that of leaves during the flowering season (June-July). Therefore, this methyl ester was regarded as an ordinary metabolite of rugosic acid A (2).

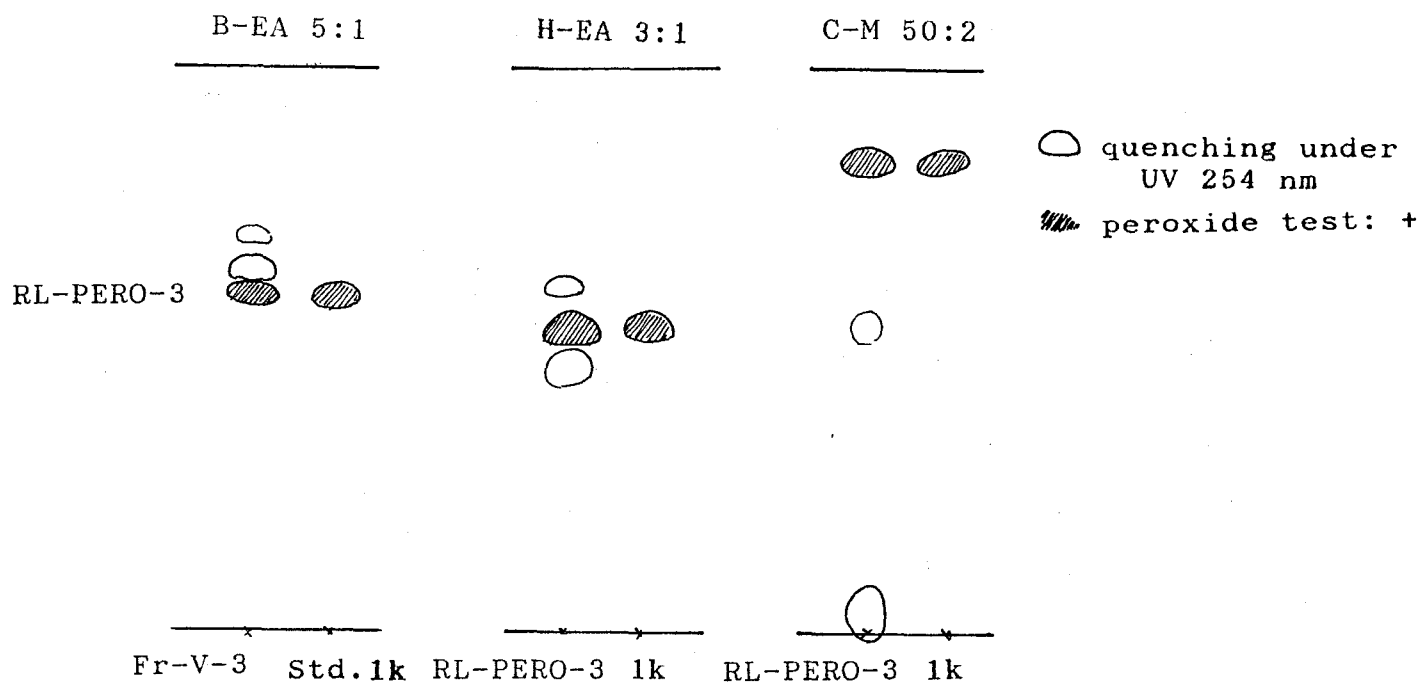


Fig. 3-236 TL Chromatograms of Rugosic Acid A Methyl Ester (RL-PERO-3)

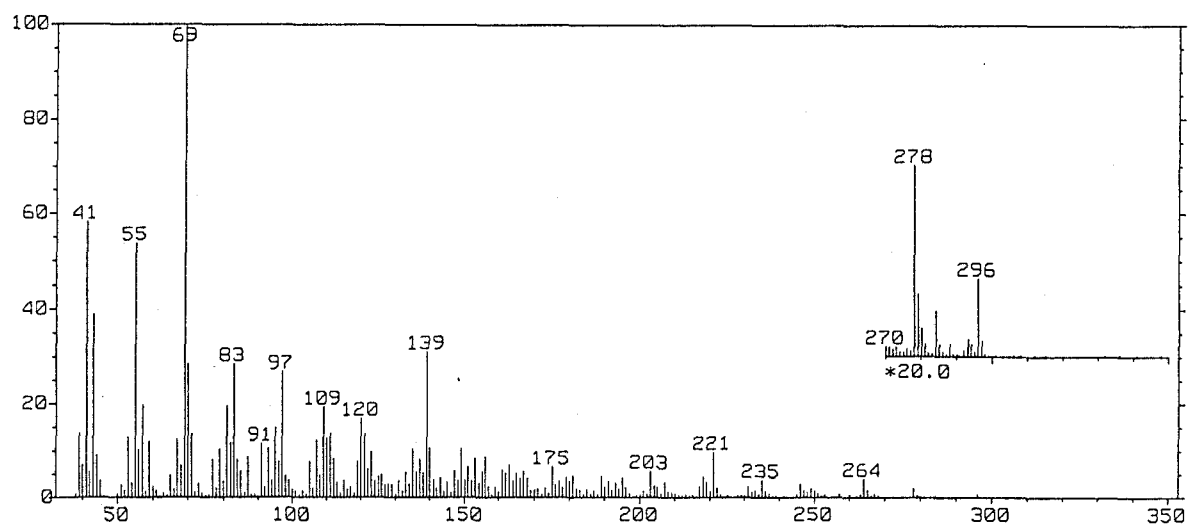


Fig. 3-237 EI-Mass Spectrum of Rugosic Acid A Methyl Ester (RL-PERO-3)

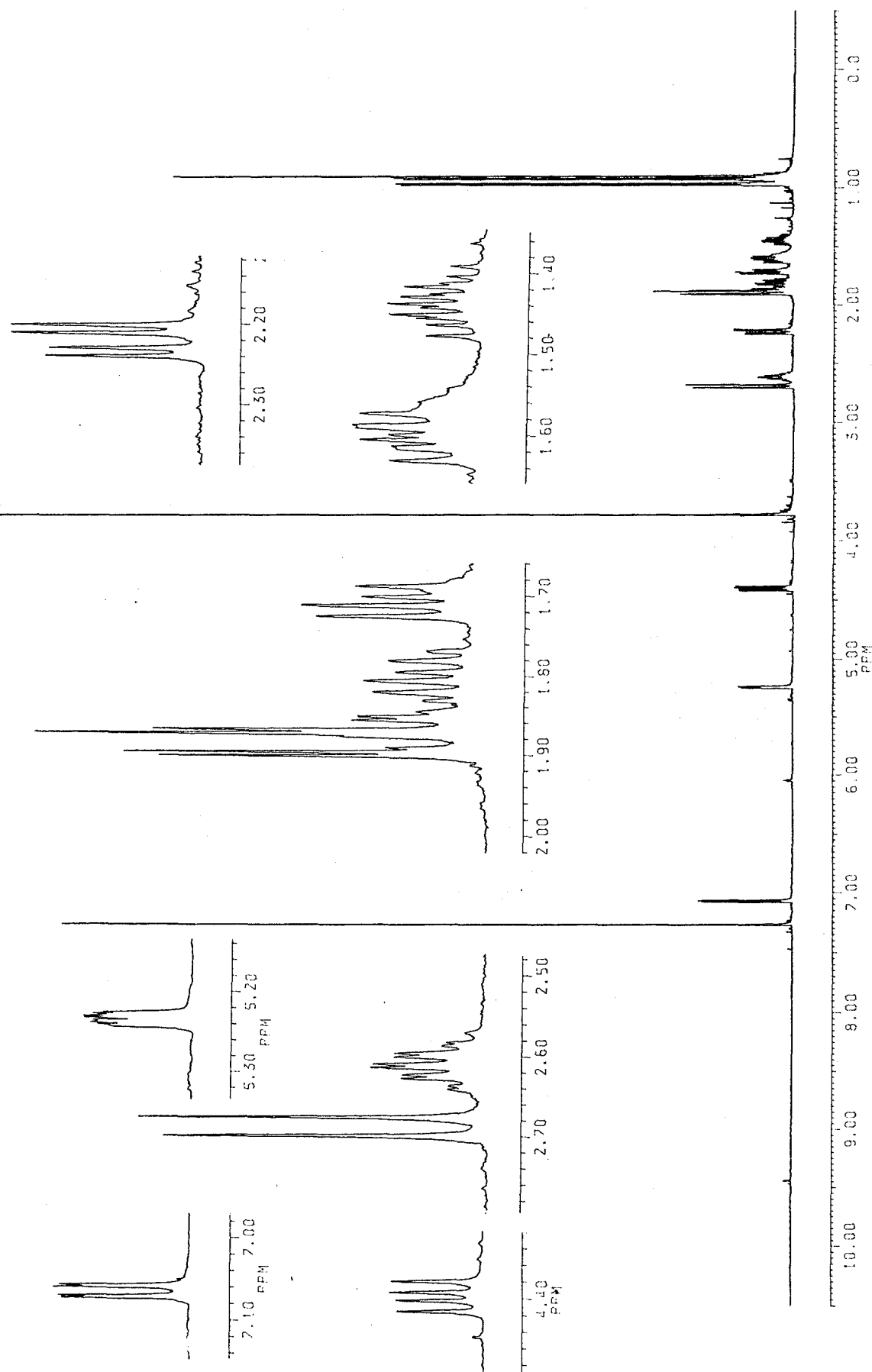
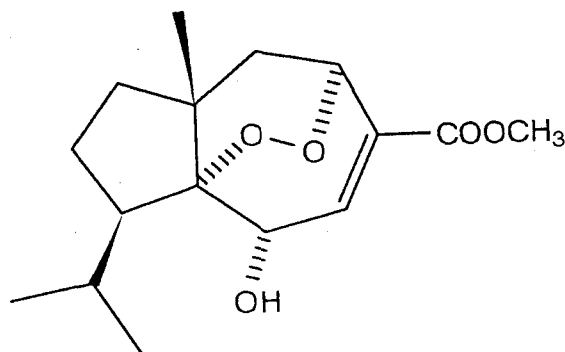


Fig. 3-238 ^1H -NMR Spectrum of Rugosic Acid A Methyl Ester (RL-PERO-3) (500 MHz, in C_6D_6)

Table 3-91 Physicochemical properties of methyl rugosate A (5=
RDA-ME)



5= 1k

Colorless needles, mp 144-147 °C

Rf: 0.66 (H-EA 3:1), 0.56 (B-EA 5:1), 0.34 (H-EA 4:1), 0.81
(C-M 50:2)

Vanillin-H₂SO₄ color: pinkish grey

N,N-dimethyl-p-phenylenediamine sulfate test: positive (pink)

[α]_D : + 150 ° (c 0.02 in MeOH)

FI-MS m/z (%): 296 (M⁺, 100)

EI-MS m/z (%): 296 (M⁺, 0.8), 278 (M⁺-H₂O, 2.0), 264 (4.0), 246
(2.9), 235 (3.8), 221 (9.6), 203 (5.7), 175 (6.7), 139
(31), 120 (17), 109 (19), 97 (27), 83 (29), 81 (20), 70
(29), 69 (100), 57 (20), 55 (54), 43 (39), 41 (59).

¹H-NMR δ_{TMS}^{CDCl₃} (500 MHz): 4.397 (1H, dd, J= 11.5 and 6.5 Hz, C-2-H),
2.687 (1H, d, J= 11.5 Hz, C-2-OH), 7.064 (1H, dd, J= 6.4
and 1.2 Hz, C-3-H), 5.234 (1H, ddd, J= 5.1, 2.4 and 1.2 Hz,
C-5-H), 2.220 (1H, dd, J= 14.2 and 5.1 Hz, C-6-Ha), 1.884
(1H, dd, J= 14.2 and 2.4 Hz, C-6-Hb), 1.813 (1H, ddd, J=
12.8, 12.8 and 7.1 Hz, C-8-Ha), 1.706 (1H, dd, J= 12.1 and
6.5 Hz, C-8-Hb), 1.603 (1H, ddd, J= C-9-Ha), 1.434 (1H,
dddd, J= 13.0, 13.0, 10.8 and 6.6 Hz, C-9-Hb), 1.873 (1H,
ddd, J= 10.3, 8.5 and 2.2 Hz, C-10-H), 2.611 (1H, double
sept., J= 6.8 and 2.2 Hz, C-11-H), 0.966 (3H, d, J= 6.8 Hz,
C-12-H₃), 0.922 (3H, d, J= 6.8 Hz, C-13-H₃), 0.899 (3H, s,
C-15-H₃), 3.776 (3H, s, C-7'-H₃). These signals showed a
good accordance to those of RSA-CN (1k).

3-6-2 Rugosic Acid B

As the metabolic fate of carotanooids, it is expectable that rugosic acid A (2) is converted into some polar compounds through some rearrangement at the endoperoxide bridge, since 2 is the largest pool of carotanooids in *Rosa rugosa* tissues. The leaf materials (sample VII and VIII) were both extracted with MeOH, and the concentrated extract was dissolved in EtOAc. The organic layer of V (ca 1 liter) was once washed with 700 ml of 5 % NaHCO₃. The washing was acidified to pH 3.0 with 5 N HCl, and then extracted with 500 ml of EtOAc. The acidic substances (ca 8 g) were successively chromatographed on a silica gel column. As shown in Table 3-92, the acid constituents coated on 90 ml silica gel was put onto 250 ml of Wako gel C-200 settled in *n*-hexane, and then eluted with a EtOAc/hexane mixture containing 0.33 % of formic acid. The eluates were immediately concentrated to dryness to remove the formic acid, and then the residue was dissolved in small volume of EtOAc. The TLC pattern of the each fraction was shown in Fig. 3-239.

Table 3-92 Silica gel column chromatography of acidic constituents from sample VII

No	Solvent	Volume	Weight (g)
Fr-VIIA-1	CH ₂ Cl ₂ -HCOOH 300:1	100 ml	0
Fr-VIIA-2	CH ₂ Cl ₂ -HCOOH 300:1	200 ml	) 0.9
Fr-VIIA-3	CH ₂ Cl ₂ -MeOH-HCOOH 400:10:1.5	200 ml	
Fr-VIIA-4	CH ₂ Cl ₂ -MeOH-HCOOH 400:10:1.5	200 ml	) 0.9
Fr-VIIA-5	CH ₂ Cl ₂ -MeOH-HCOOH 400:20:1.5	200 ml	
Fr-VIIA-6	CH ₂ Cl ₂ -MeOH-HCOOH 400:20:1.5	200 ml	5.4
Fr-VIIA-7	CH ₂ Cl ₂ -MeOH-HCOOH 400:60:1.5	200 ml	
Fr-VIIA-8	CH ₂ Cl ₂ -MeOH-HCOOH 400:60:1.5	200 ml	
Fr-VIIA-9	CH ₂ Cl ₂ -MeOH-HCOOH 400:60:1.5	200 ml	
Fr-VIIA-10	acetone	300 ml	

In Fr-VIIA-8 and -9, some spots showing lower R_f than that of 2 were detected on TLC, and one of them indicated a yellow coloration by the vanillin- H_2SO_4 test. As RDA-KOH (2e) was also colorized yellow with vanillin- H_2SO_4 reagent, the acidic fraction was again developed in H-EA-F 25:25:1 with authentic 2e. As the result, the focused compound and 2e were agreeable in their R_f values (0.27) and responses to vanillin- H_2SO_4 reagent. Accordingly, the focused compound was isolated by PTLC in H-EA-F 30:30:1 and C-M-F 30:2:1 (R_f 0.21, in Fig. 3-240), to give 10.4 mg of a colorless syrup (ca 14 mg/kg of sample VII). EI-MS and ^1H -NMR spectra of the isolate were indistinguishable from those of 2e (Fig. 3-241 and 242, cf. ^{13}C -NMR spectrum in Fig. 3-243), and accordingly the isolate named rugosic acid B (6= 2e) was revealed to be identical to RDA-KOH and present in the fresh leaves of *Rosa rugosa*.

Although rugosic acid B (6) is convertible from rugosic acid A (2) by an alkali-reaction, the isolate (6) from the exudates seems not to be an artifact but a naturally occurring carotane acid, since the MeOH extract was ordinarily acidic (> pH 5.5).

H-EA-F 30:10:1

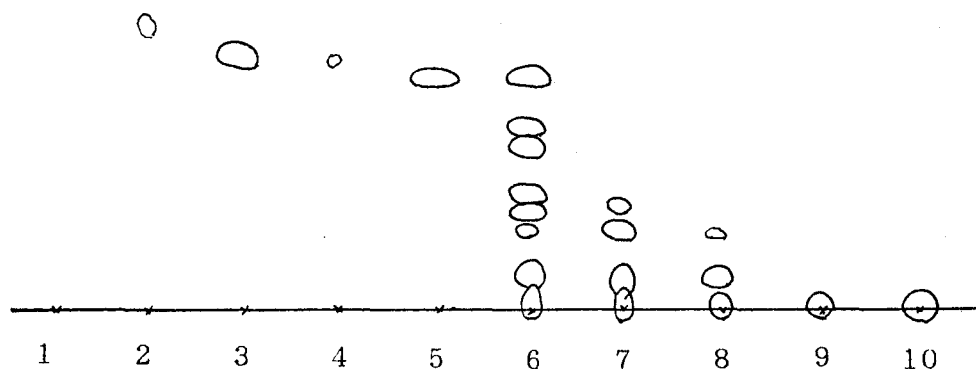


Fig. 3-239 TL Chromatogram of Column Eluants of Acidic Constituents in the Methanol Extracts of *Rosa rugosa* Leaves (Sample V)

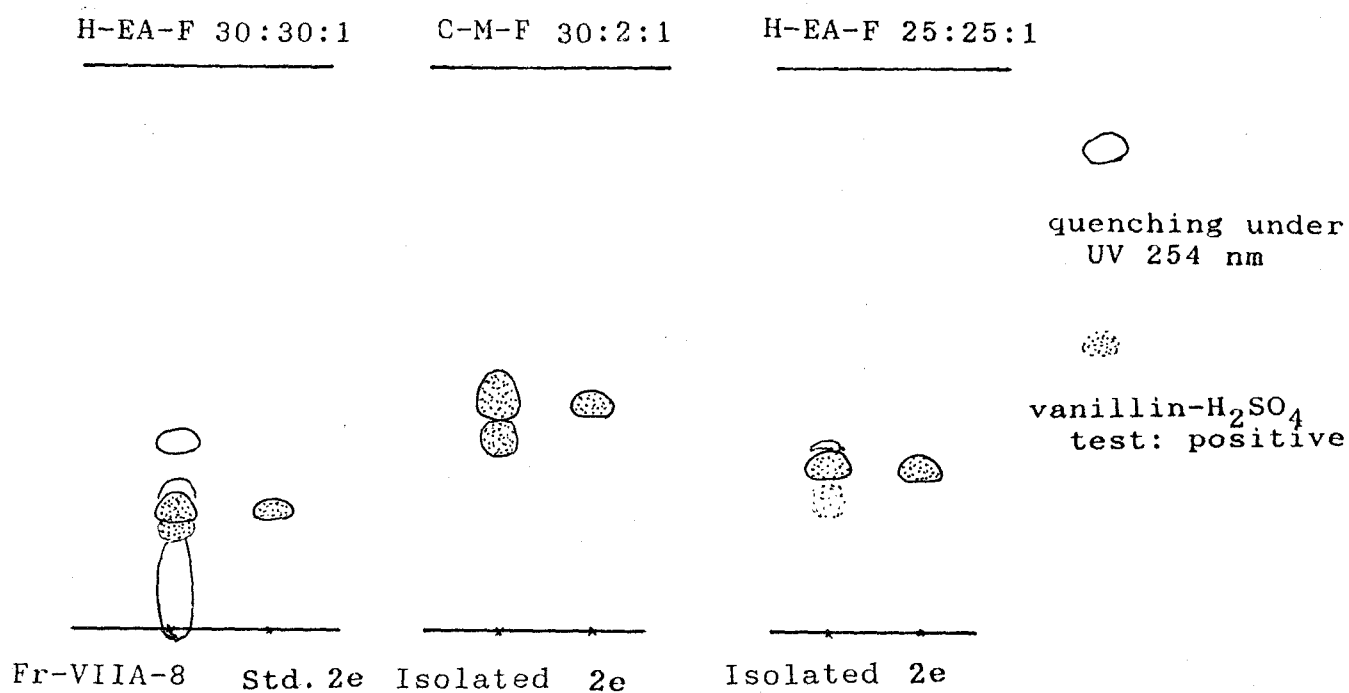


Fig. 3-240 TL Chromatograms of Rugosic acid B and Authentic Compound 2e

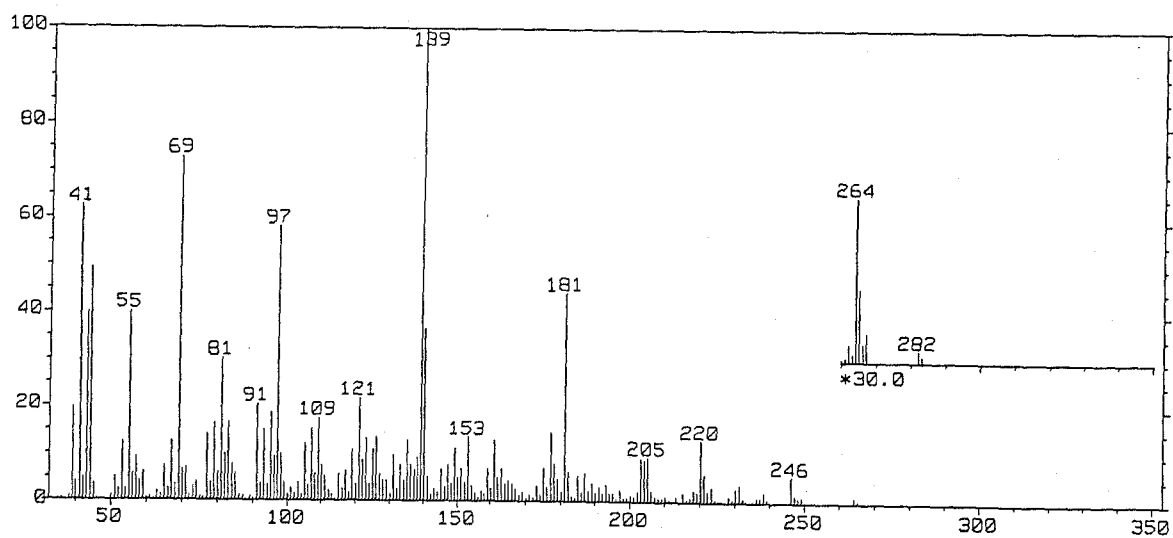


Fig. 3-241 EI-Mass Spectrum of Rugosic Acid B

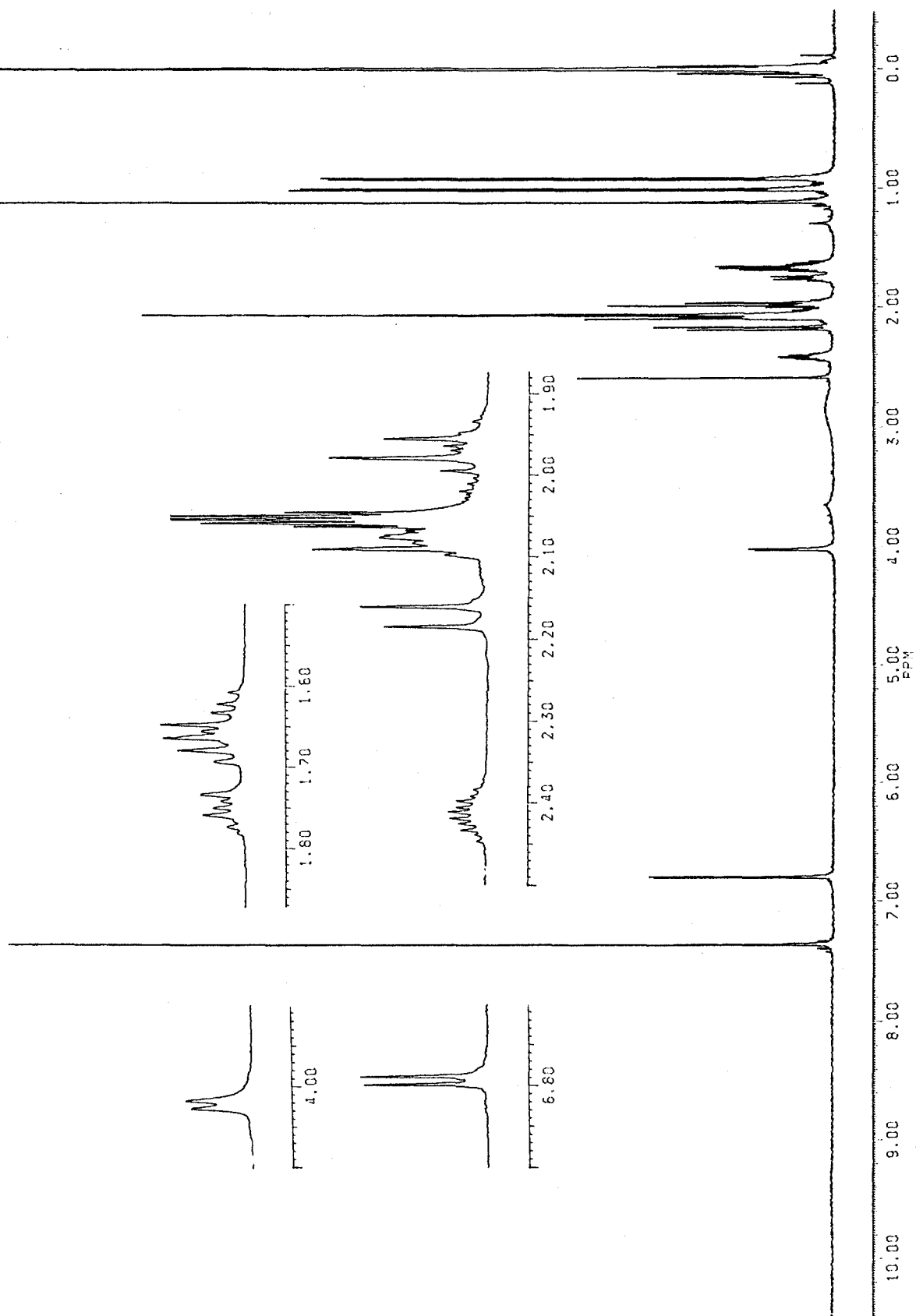


Fig. 3-242 ^1H -NMR Spectrum of Rugosic Acid B (500 MHz, in acetone- d_6)

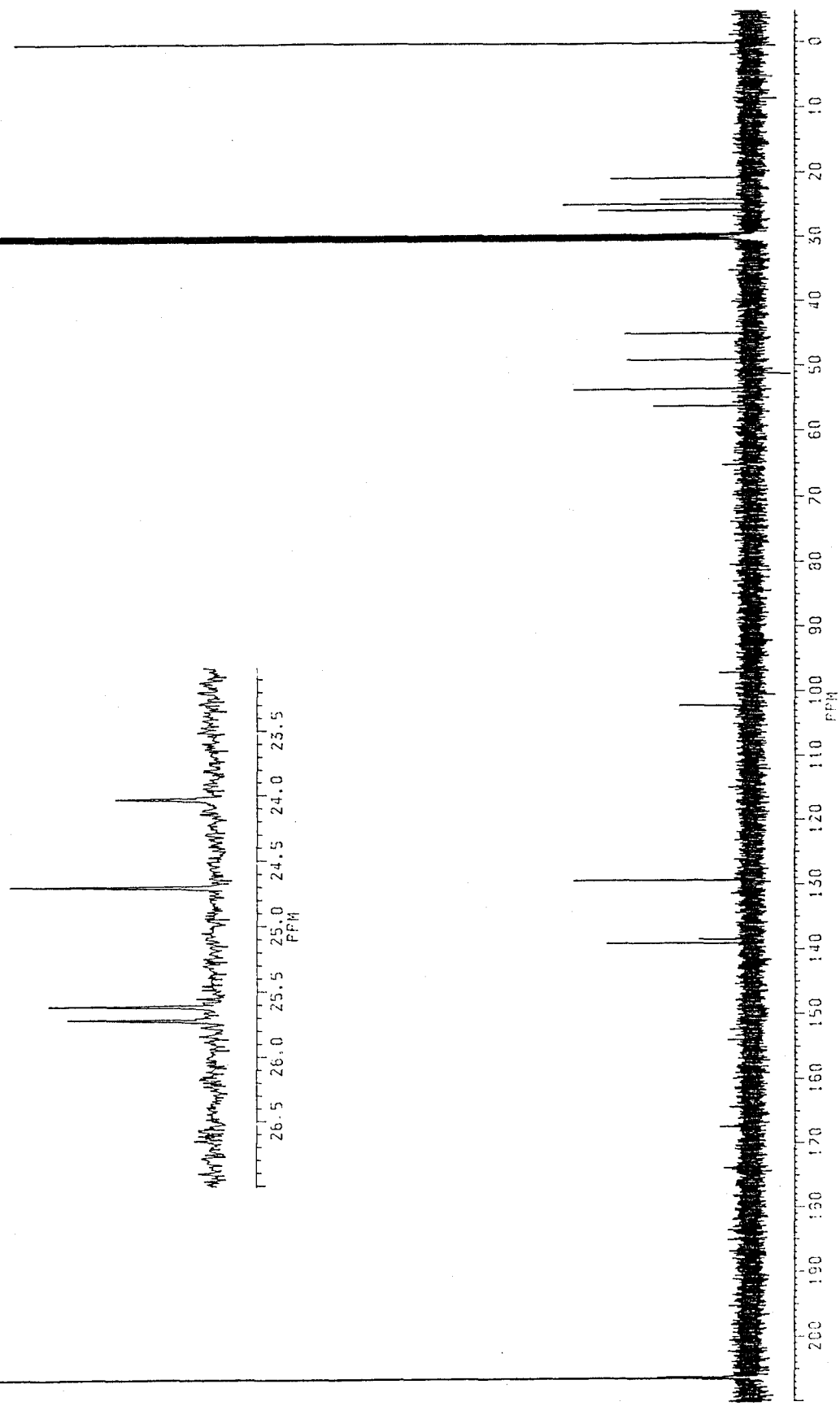
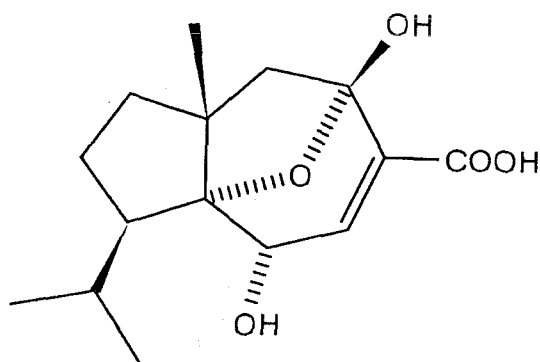


Fig. 3-243 ^{13}C -NMR Spectrum of Rugosic Acid B (125 MHz, in acetone- d_6)

In this extractives (Sample VIII), rugosic acid B (6) appeared only as an ambiguous spot detected by Vanillin-H₂SO₄ test, while rugosic acid C (7) was undetected. On the contrary, 8 was also detectable in the acidic extractives of the younger leaves (sample VII). These facts allowed a speculation that rugosic acid A (2) is further transformed to various minor metabolites including rugosic acids B~D (6, 7 and 8), and that only 8 among them can be accumulated in the old leaves. Probably, 8 is one of the most stable and physiologically inactive carotane acids in *Rosa rugosa* tissues.

Table 3-93 Physicochemical properties of rugosic acid B (6= RDA-KOH)



6= 2e

Colorless columns, mp

Vanillin-H₂SO₄ color: clear yellow

EI-MS m/z (%): 282 (M^+ , 0.1), 264 ($M^+ - H_2O$, 1.2), 246 (5.6), 231 (3.7), 220 (13), 205 (9.5), 204 (8.6), 203 (9.0), 181 (44), 140 (37), 139 (100), 121 (22), 109 (17), 97 (58), 95 (19), 91 (20), 83 (17), 81 (30), 79 (16), 69 (73), 55 (40), 44 (49), 43 (40), 41 (63)

¹H-NMR $\delta_{TMS}^{COMe_2}$ (500 MHz): 6.791 (1H, d, J = 4.9 Hz, C-3-H), 4.019 (J = 4.9 Hz, C-2-H), 2.414 (1H, d sept., J = 6.9 and 3.9 Hz, C-11-H), 2.172 (1H, d, J = 11.9 Hz, C-6-Ha), ca 2.09 (1H, overlapped, C-10-H), 1.966 (1H, d, J = 11.9 Hz, C-6-Hb), 1.966 (1H, m, C-9-Ha), 1.753 (1H, m, C-8-Ha), 1.662 (1H, ddd, J = 15.8 and 7.2 Hz, C-8-Hb), 1.638 (1H, ddd, J = 12.5, 11.4 and 7.0 Hz, C-9-Hb), 1.112 (3H, s, C-15-H₃), 1.002 (3H, d, J = 6.9 Hz, C-12-H₃), 0.911 (3H, d, J = 6.9 Hz, C-12-H₃).

¹³C-NMR $\delta_{TMS}^{COMe_2}$ (125 MHz): 167.2 (C-14), 138.9 (C-4), 129.2 (C-3), 102.0 (C-5), 97.0 (C-1), 65.1 (C-2), 56.0 (C-10), 53.4 (C-6), 48.9 (C-7), 44.8 (C-8), 25.7 (C-12), 25.6 (C-11), 24.7 (C-15), 24.0 (C-9), 20.7 (C-13).

3-6-4 Rugosic Acid C

From the column fraction (Fr-VIIA-8 and -9) containing rugosic acid B, a substance with *Rf* 0.23 in *n*-hexane-EtOAc-HCOOH 25:25:1 identical to that of authentic RSA-TU (2a) was also found. After PTLC in the solvent, the substance was further purified by PTLC in CHCl₃-MeOH-HCOOH 30:2:1 (*Rf* 0.30) and finally isolated by PTLC in the first solvent system to give 1.9 mg of a colorless syrup (Fig. 3-244). The isolate was elucidated as 7, by direct comparison with 2a in EI-mass and ¹H-NMR spectra, and was named rugosic acid C (Fig. 3-245 and 246). Rugosic acid C (7) was involved in a comparatively small amount in the leaf tissues (*ca* 5 mg/kg). This compound was considered as one of the metabolites of rugosic acid A (2), as with rugosic acid B (6). Since these acidic metabolites were hardly found in senescent leaves collected in late September (Sample VIII), it was expected that these compound were further metabolized or modified in the tissues while the leaves were functioning.

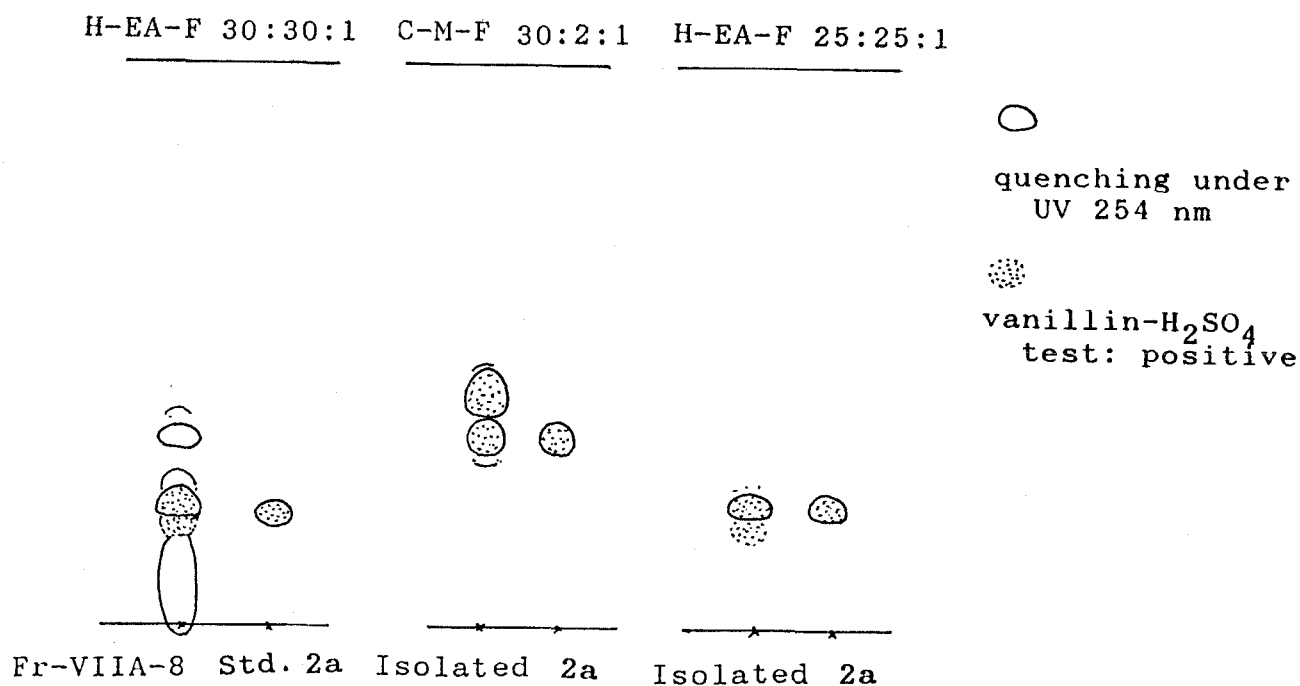


Fig. 3-244 TL Chromatogram of Rugosic Acid C and Authentic Compound 2a

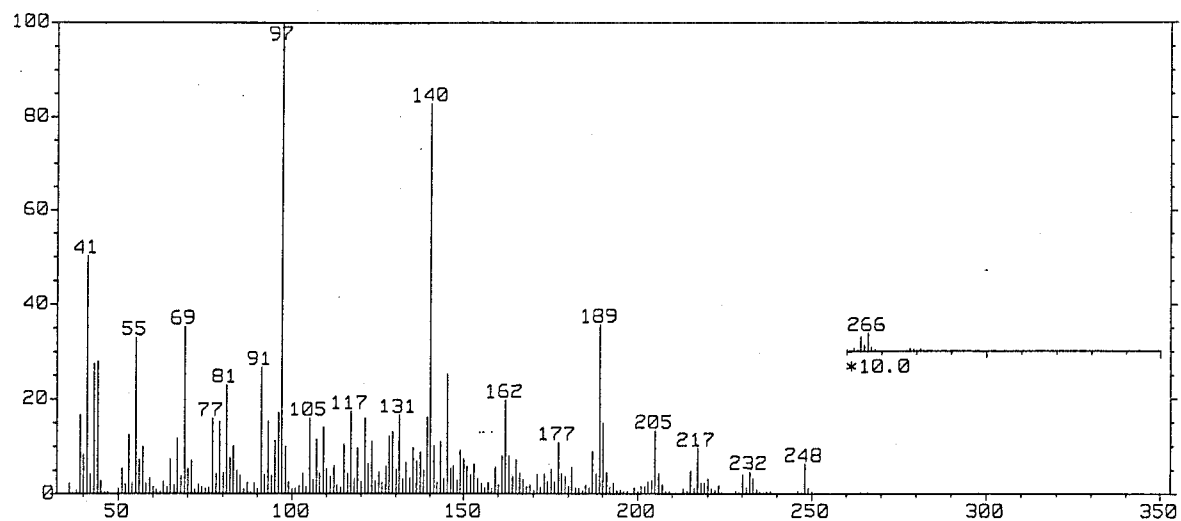


Fig. 3-245 EI-Mass Spectrum of Rugosic Acid C

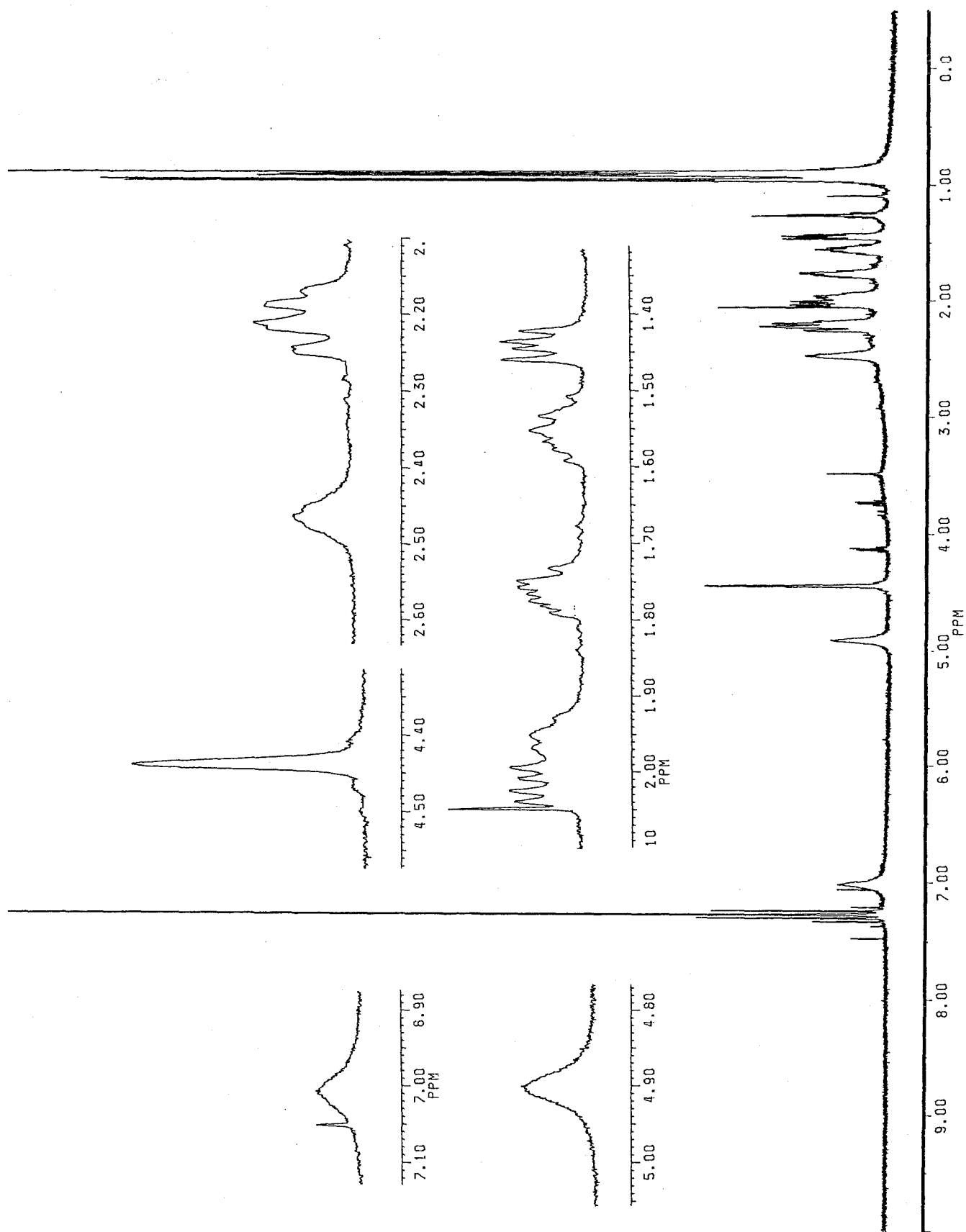
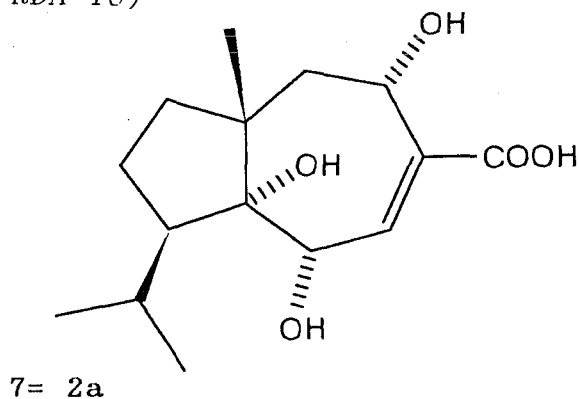


Fig. 3-246 ^1H -NMR Spectrum of Rugosic Acid C (500 MHz, in CDCl_3)

Table 3-94 Physicochemical properties of rugosic acid C (7=
RDA-TU)



A colorless syrup

Vanillin-H₂SO₄ color: grayish yellow → grayish purple

EI-MS *m/z* (%): 266 (M⁺-H₂O, 0.4), 248 (M⁺-2H₂O, 6.4), 232 (4.7), 230 (4.1), 217 (9.7), 205 (13), 190 (15), 189 (36), 162 (20), 145 (25), 140 (83), 131 (17), 117 (17), 97 (100), 96 (17), 91 (27), 81 (23), 69 (35), 55 (33), 44 (28), 43 (28), 41 (50).

¹H-NMR δ_{TMS}^{CDCl₃} (500 MHz): 7.005 (1H, br., C-3-H), 4.900 (1H, br., C-5-H), 4.436 (1H, br., C-2-H), 2.461 (1H, br., C-11-H), *ca* 2.21 (1H, m, C-6-H_a), 2.188 (1H, m, C-10-H), 2.016 (1H, dd, *J* = 15.4 and 7.1 Hz, C-6-H_b), 1.959 (1H, m, C-9-H_a), 1.769 (1H, m, C-9-H_b), 1.551 (1H, m, C-8-H_a), 1.438 (1H, dd, *J* = 11.6 and 7.0 Hz, C-8-H_b), 0.947 (3H, d, *J* = 6.8 Hz, C-12-H₃), 0.901 (3H, d, *J* = 6.8 Hz, C-13-H₃), 0.878 (3H, s, C-15-H₃).

Rugosic acid D (8), a naturally occurring carotanoic acid equal to RDA-TSA (2c) was isolated from the exudates of old *Rosa rugosa* leaves. Sample VIII (550 g) collected in late September was once extracted for 3 weeks with ca 4 liters of MeOH. The extractives were defatted twice by shaking with a 1/3 volume of *n*-hexane, and the resulting lower layer was concentrated to ca 500 ml of a water suspension. The water layer was saturated with NaCl and extracted with 600 ml of EtOAc (x 2). The combined EtOAc extracts were repeatedly washed with 700 ml of 5 % NaHCO₃ solution. The washings were acidified with 5 N HCl to pH 3.5 and re-extracted with 500 ml of EtOAc. The EtOAc layer containing the acidic constituents was then dried over Na₂SO₄, and concentrated *in vacuo* to give ca 3 g of acidic extracts.

The acidic extractives were chromatographed (PTLC) in *n*-hexane-EtOAc-HCOOH 25:25:1 (Fig. 3-247). As a constituent corresponding to RSD-TSA (2c) was detected as the major one, the compound was focused to isolate. After the PTLC in the H-EA-F system, the focused compound (*R_f* 0.51) was further purified by PTLC in CHCl₃-MeOH-HCOOH 50:2:1 (*R_f* 0.34). By re-PTLC in the first solvent system, the compound was eventually isolated as 11.2 mg of a colorless syrup (calculated ca 41 mg/kg). In EI-mass and ¹H-NMR spectra, the isolate and RDA-TSA was completely identical (Fig. 3-248 and 249). The fourth naturally occurring carotanoic acid named rugosic acid D was thus formulated as 8 (= 2c).

In this extractives (Sample VIII), rugosic acid B (6) appeared only as an ambiguous spot detected by Vanillin-H₂SO₄ test, while rugosic acid C (7) was undetected. On the contrary, 8 was also detectable in the acidic extractives of the younger leaves (sample V). These facts allowed a speculation that rugosic acid A (2) is further transformed to various minor metabolites including rugosic

acids B~D (6, 7 and 8), and that only 8 among them can be accumulated in the old leaves. Probably, 8 is one of the most stable and physiologically inactive carotane acids in *Rosa rugosa* tissues.

H-EA-F 25:25:1

C-M-F 50:2:1

○ quenching under
UV 254 nm

● vanillin-H₂SO₄
test: positive

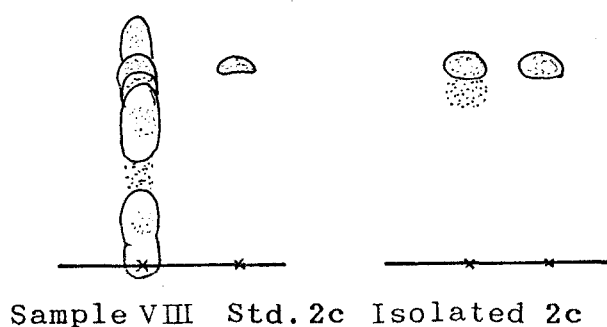


Fig. 3-247 TL Chromatographic Detection of Rugosic Acid D in Acidic Extractions (Sample VIII)

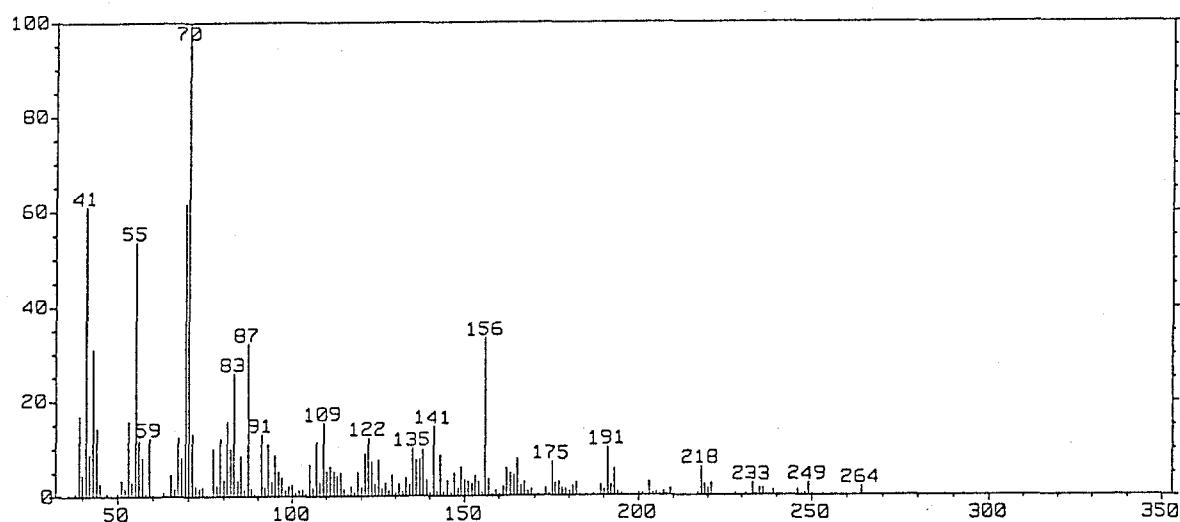


Fig. 3-248 EI-Mass Spectrum of Rugosic Acid D

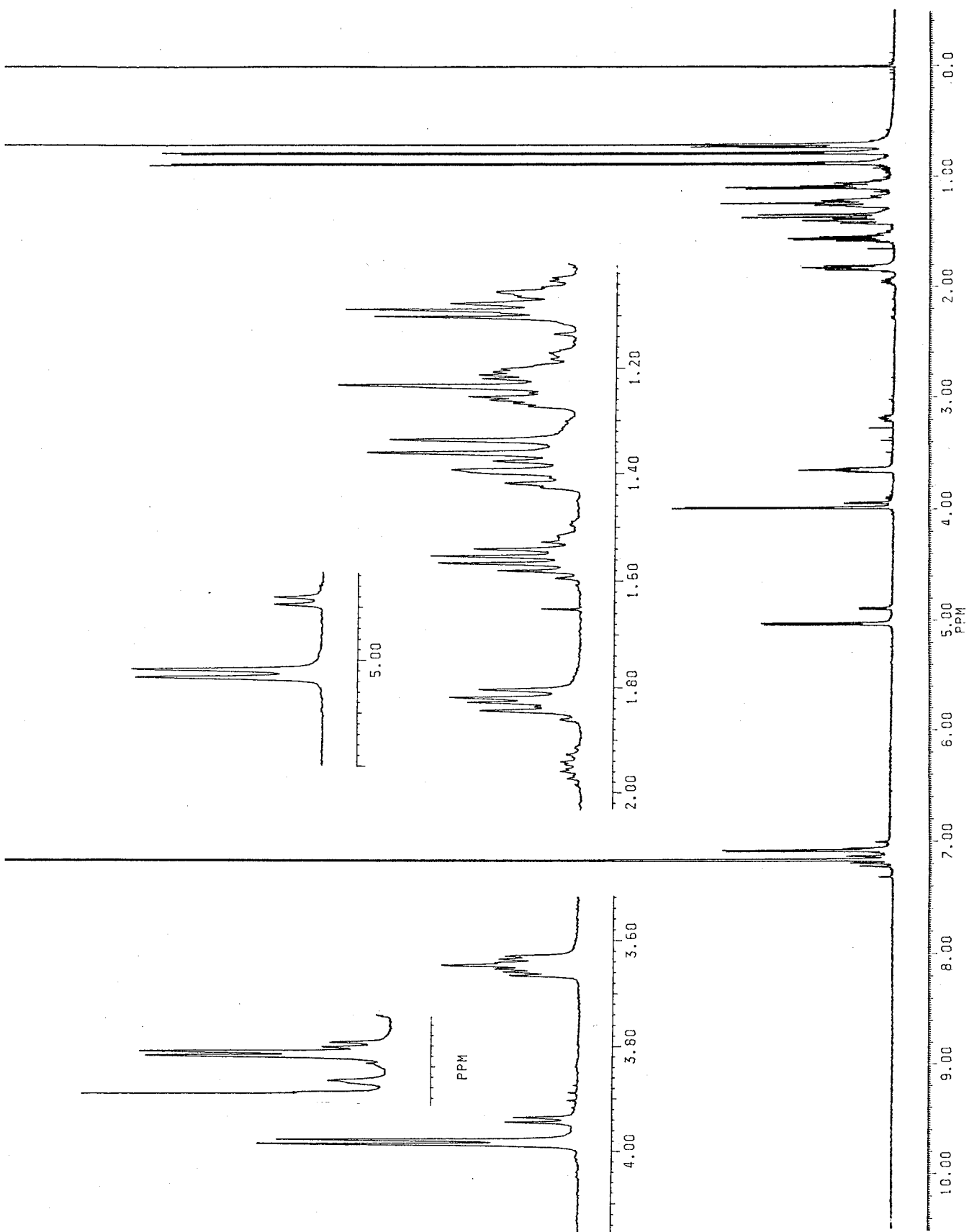
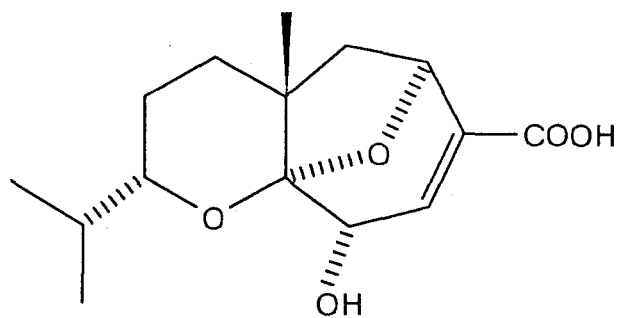


Fig. 3-249 ^1H -NMR Spectrum of Rugosic Acid D (500 MHz, in C_6D_6)

Table 3-95 Physicochemical properties of rugosic acid D (8= RDA-TSA)



2c

A colorless syrup

Rf: 0.51 (H-EA-F 25:25:1), 0.34 (C-M 100:5:2)

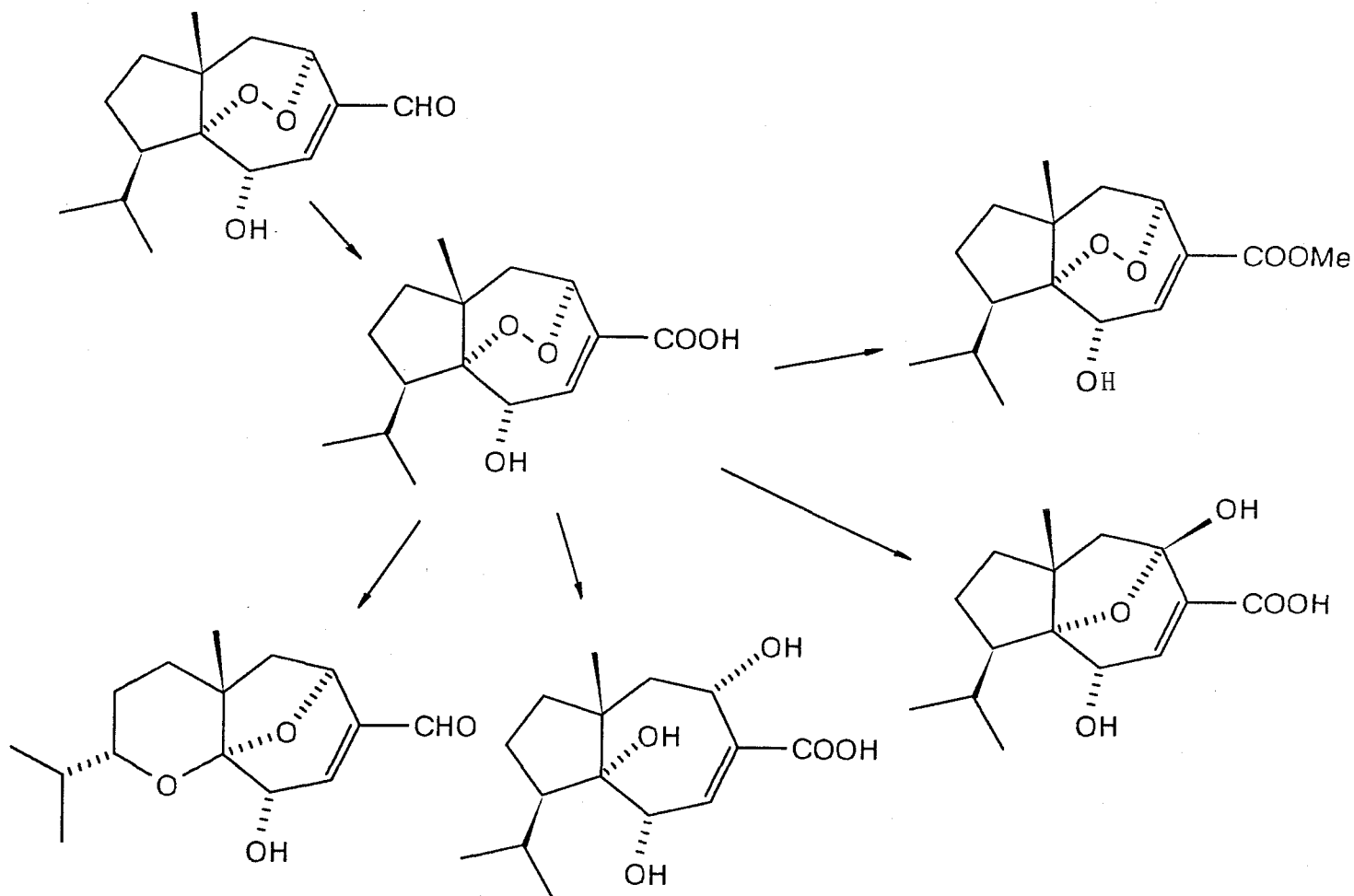
Vanillin-H₂SO₄ color: grayish brown

EI-MS m/z (%): 264 ($M^+ - H_2O$, 2.1 Hz), 249 ($M^+ - CH_3 - H_2O$, 2.8), 233 (2.8), 218 (6.3), 203 (3.0), 191 (10), 175 (7.2), 165 (7.9), 156 (33), 141 (15), 138 (10), 135 (10), 122 (12), 109 (15), 91 (13), 87 (32), 83 (26), 70 (100), 69 (62), 55 (54), 53 (16), 43 (31), 41 (61).

¹H-NMR $\delta_{TMS}^{C_6D_6}$ (500 MHz): 7.076 (1H, d, $J = 4.1$ Hz, C-3-H), 5.025 (1H, br. d, $J = 7.7$ Hz, C-5-H), 3.940 (1H, d, $J = 4.1$ Hz, C-2-H), 3.647 (1H, ddd, $J = 8.5, 6.5$ and 3.0 Hz, C-10-H), 1.824 (1H, dd, $J = 12.2$ and 7.7 Hz, C-6-Ha), 1.560 (1H, d sept., $J = 6.8$ and 6.5 Hz, C-11-H), 1.394 (1H, m, C-8-Ha), 1.350 (1H, br. d, $J = 12.2$ Hz, C-6-Hb), ca 1.26 (1H, m, C-8-Hb), ca 1.21 (1H, m, $J =$ C-9-Ha), 1.070 (1H, m, C-9-Hb), 0.873 (3H, d, $J = 6.8$ Hz, C-12-H₃), 0.775 (3H, d, $J = 6.8$ Hz, C-13-H₃), 0.698 (3H, s, C-15-H₃).

3-6-6 Conclusion

Rugosic acid A (2) as a large pool of carotanoids in *Rosa rugosa* was found to be further metabolized into more polar and stable compounds. As the result of these surveys, at least four metabolic pathways for 2 were presumed (Scheme 3-32). Those metabolic reaction, involving methylation at the carboxyl group and peroxide rearrangements, were presumed as only a part of the complicated carotanoid metabolism in *Rosa rugosa*, since more and more constituents were detectable on TLC. However, those metabolites are considered to occupy an initial part in the metabolism of 2. Significance of those metabolites are not known. As *Rosa rugosa* can control the pathogenic fungus, those sesquiterpenes related to 2 may be immune from pathogens.



Scheme 3-46 Metabolic pathways from rugosic acid A (2)

3-7 Minor Carotenoids in *Rosa rugosa*

3-7-1 Introduction

According to the survey of terpenoids in *Rosa rugosa* leaves, it was revealed that the pathway from carota-1,4-dienaldehyde (3) to rugosic acids A-D and rugosic acid A methyl ester (2 and 5-8) is the main flow in carotenoid metabolism. However, *Rosa rugosa* leaves also contain various kinds of minor carotenoids, which are considered to be originated in some isomers of 3 found in the RL fraction. Here, minor carotenoids isolated and identified by spectroscopic and/or chemical methods are described.

3-7-2 Daucenaldehyde and Its Related Compounds

1) Daucenaldehyde

As described in Section 5 of this chapter, some less polar constituents detected in GC-MS analysis of RL fraction indicated a sesquiterpene nature isomeric to carota-1,4-dienaldehyde (3). Following by the HPLC for isolation of carota-1,4-dienaldehyde (3), these minor constituents were also purified. A compound appearing as the second peak in HPLC (Unisil Q 100-5, 2.5 % EtOAc/*n*-hexane) was isolated as a colorless oil (Fig. 3-250) identical with RL-A in MS (the former EI-MS and the latter GC-MS, Fig. 3-251). RL-A was considered as an isomer of carota-1,4-dienaldehyde (3) because of the same molecular weight (M^+ 218, $C_{15}H_{22}O$) as that of 3 and similar MS fragmentation to that of 3. Furthermore, RL-A showed $\lambda_{\max}^{\text{MeOH}}$ 229 nm, which was indicative of the presence of an α,β -unsaturated aldehyde group in the molecule.

In ^1H -NMR spectrum, an isopropyl group (δ_{H} 0.897 and 0.869 each 3H, d, J = 6.9 Hz, and 2.515, 1H, sept., J = 6.9 Hz), an

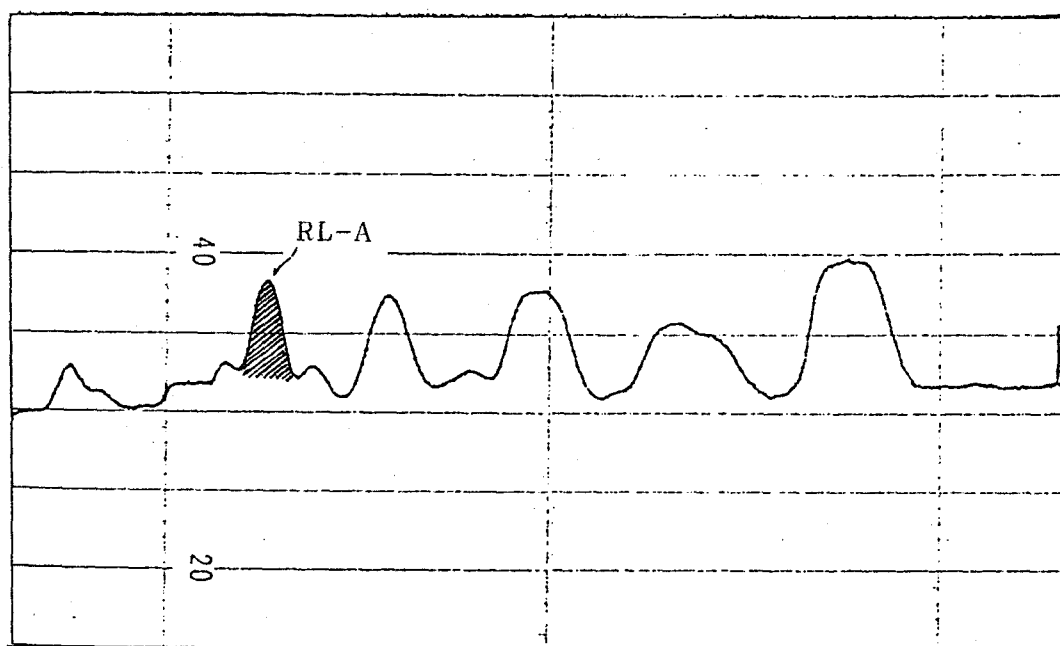


Fig. 3-250 HPL-Chromatogram of RL Fraction (at UV 230 nm): The second peak isolated by HPLC revealed the compound was identical to RL-A depicted in Fig. 3-162.

aldehyde group (δ_H 9.263, 1H, s) and bridgehead methyl group (δ_H 0.811, 3H, s) all characteristic of a carotane aldehyde were observed (Fig. 3-252 and Table 3-95). As a clear difference of RL-A from 3 showing two olefinic protons in the ^1H -NMR spectrum, only one olefinic proton was detected at δ_H 6.101 (1H, ddd, J = 8.2, 5.2 and 2.0 Hz). In addition, the methine proton of the isopropyl group was observed as a clear septet signal (δ_H 2.515, J = 6.9 Hz), which suggested that the isopropyl group was attached to a non-hydrogen-bearing carbon. Therefore, a carotane structure for RL-A which possesses a C,C-double bond on C(10)-C(1) was tentatively proposed. To confirm the estimation, decoupling experiments were carried out.

As the results, a part of proton sequence was newly deduced as shown in Fig. 3-253. The olefinic proton was vicinally coupled with a pair of methylene protons at δ_H 2.053 (1H, br. dd, J = 14.3 and 5.2 Hz) and 1.935 (1H, dd, J = 14.3 and 8.2 Hz), and further coupled with one of allylic methylene protons at δ_H 1.763 (1H, br. dd, J = 14.9 and 14.5 Hz, geminally coupled with δ_H 3.049) by J = 2.0 Hz of an allyl coupling. This methylene protons being relayed to another pair of methylene protons with further vicinal couplings, the partial structure $-CH_2-CH=C-CH_2-CH_2-$ was eventually revealed. Because of the deshielding effects on the olefinic proton and the allylic methylene protons (δ_H 3.049 and 1.763), the α,β -unsaturated aldehyde group was allocated to the disubstituted non-hydrogen bearing olefinic carbon.

Furthermore, another proton sequence was also revealed. Analysis of the proton coupling patterns revealed part structure $\blacksquare-CH_2-CH_2-\blacksquare$, and equivalent methylene protons at δ_H 2.125 showed a homoallyl coupling with a proton at δ_H 1.616. This result was suggestive that the tetrasubstituted olefinic bonds locate between two carbons bearing these protons (See Fig. 3-253).

On the other hand, ^{13}C -NMR spectra (COM, DEPT and CH-COSY) were taken to assign all the proton bearing carbons (Fig. 3-254,

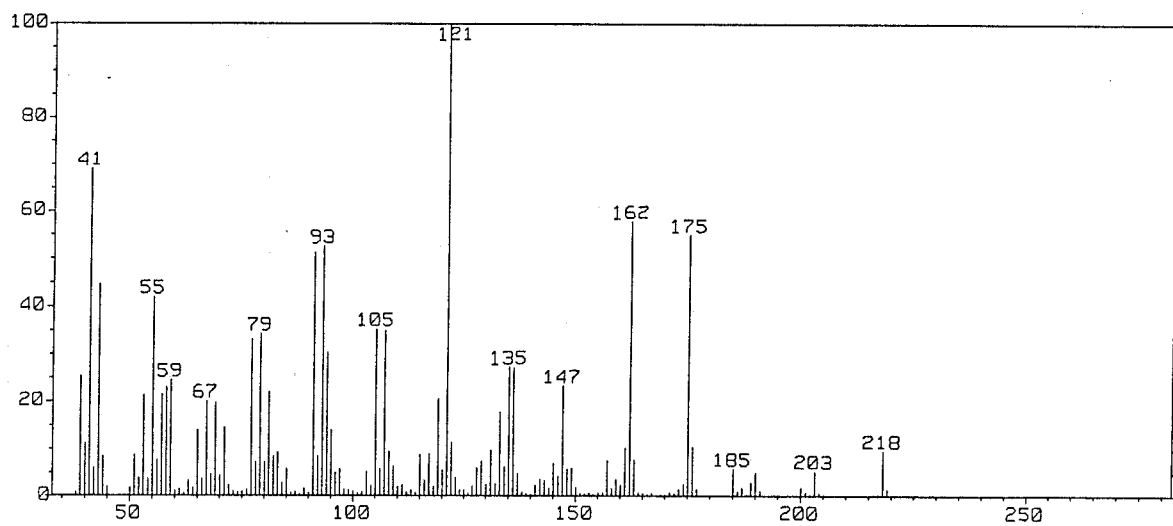
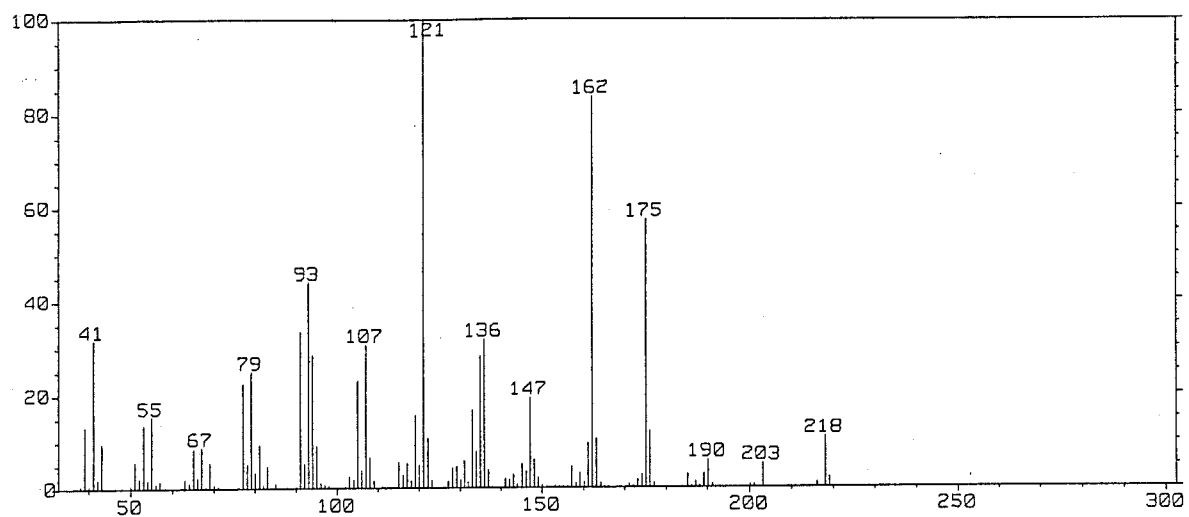


Fig. 3-251 GC-Mass Spectrum of RL-A (top) and EI-Mass Spectrum of the Isolated Compound (bottom)

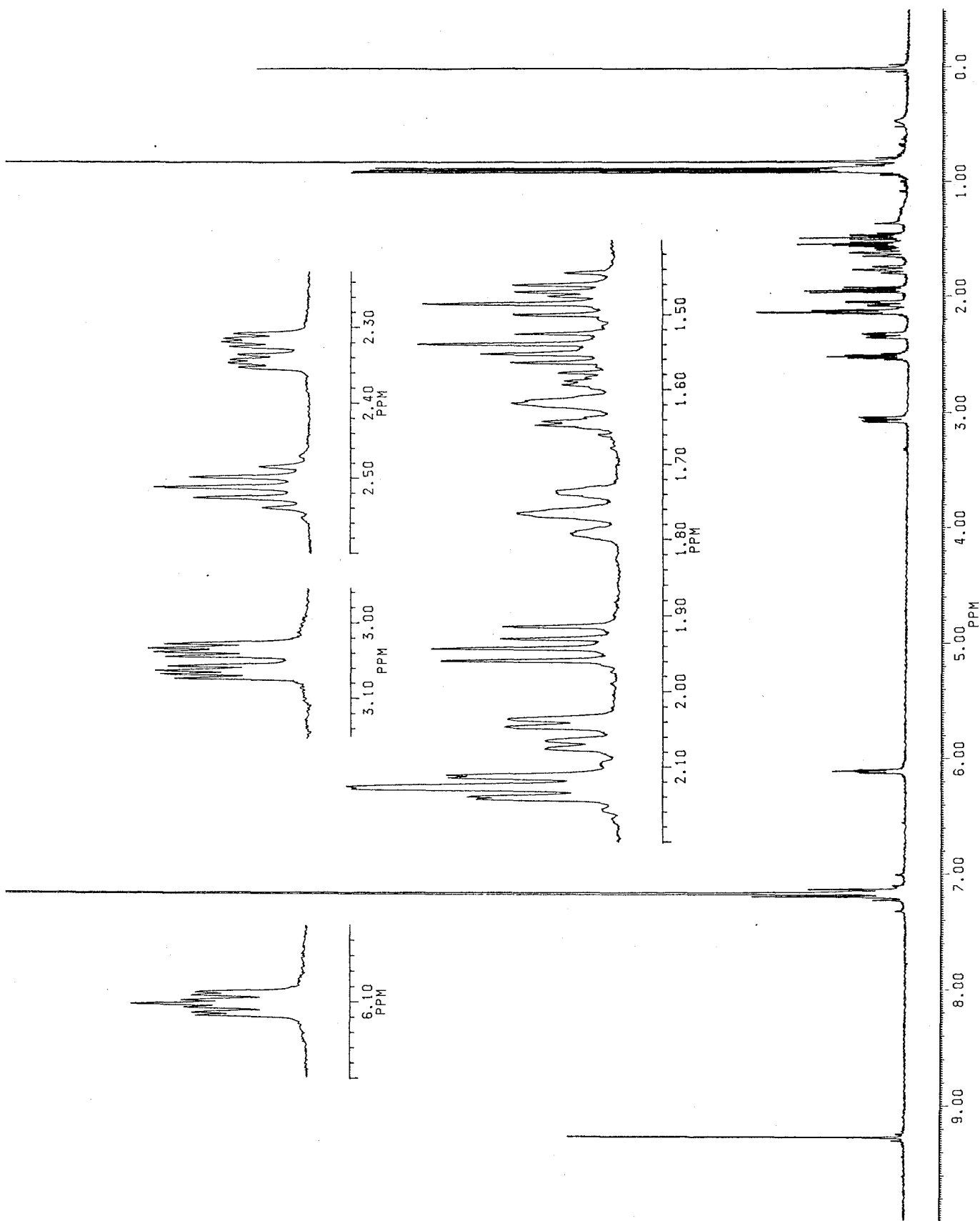


Fig. 3-252 ^1H -NMR Spectrum of RL-A (500 MHz, in C_6D_6)

Table 3-96 Proton chemical shift values of RL-A

(500 MHz, in C₆D₆, TMS as an int. std.)

δ_H	Coupling	Assignment
9.263	s	C-14-H
6.101	ddd $J= 8.2, 5.2, 2.0$ Hz	C-5-H
3.049	ddd $J= 14.9, 5.4, 2.9$ Hz	C-3-Ha
2.512	sept $J= 6.9$ Hz	C-11-H
2.330	ddd $J= 14.0, 5.4, 3.0$ Hz	C-2-Ha
2.125	br ddd $J= 7.7, 7.4, 2.9$ Hz	C-9-H ₂
2.053	br dd $J= 14.3, 5.2$ Hz	C-6-Ha
1.935	dd $J= 14.3, 8.2$ Hz	C-6-Hb
1.763	br.dd $J= 14.9, ca 14.5, 3.0, 2.0$ Hz	C-3-Hb
1.616	br.dd $J= ca 14.5, 14.0, 2.9$ Hz	C-2-Hb
1.550	dd $J= 12.4, 7.4$ Hz	C-8-Ha
1.471	dd $J= 12.4, 7.7$ Hz	C-8-Hb
0.897	d $J= 6.9$ Hz	C-12-H ₃
0.869	d $J= 6.9$ Hz	C-13-H ₃
0.811	s	C-15-H ₃

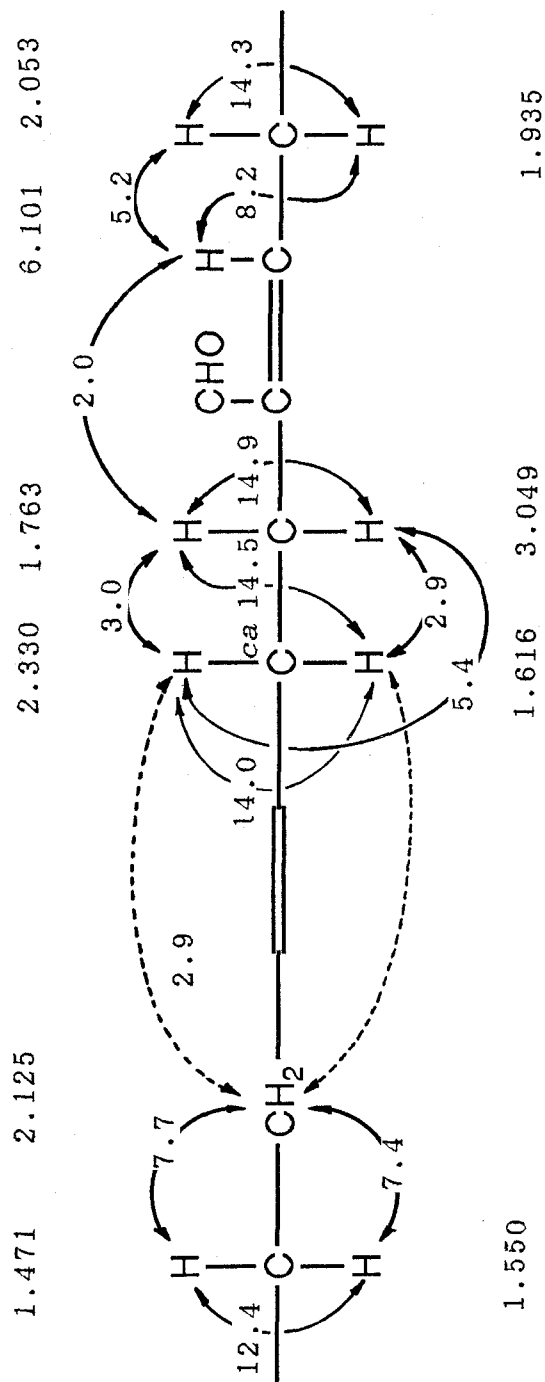


Fig. 3-253 Proton-Proton Coupling Sequences Further Elucidated
by Decoupling Experiments

←→ : possible to read its coupling constant

←.....→ : coupling constant unreadable

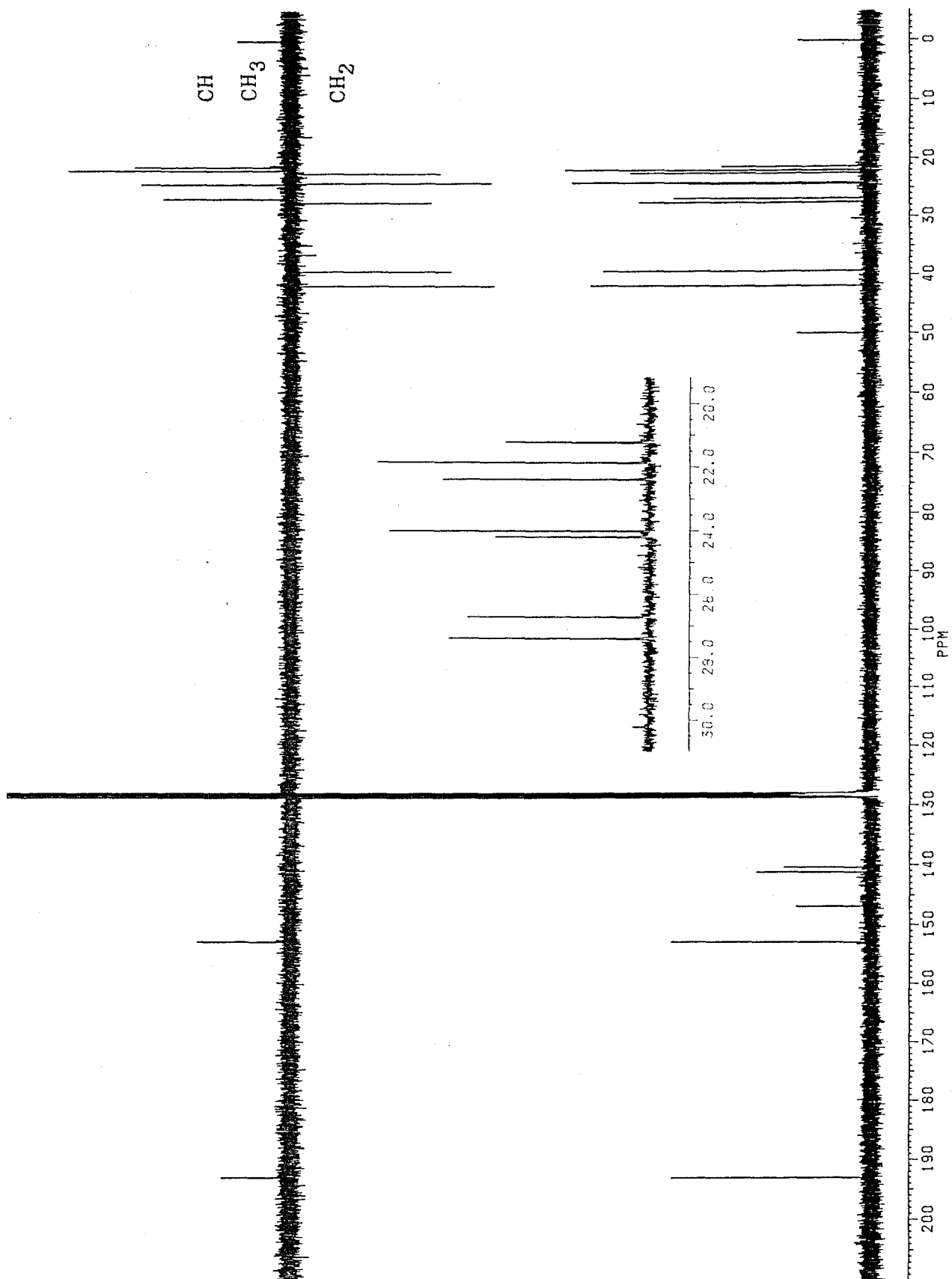


Fig. 3-254 ^{13}C -NMR Spectra of RL-A (500 MHz, in C_6D_6 , COM and DEPT)

Table 3-97 ^{13}C -NMR chemical shift values of RL-A

(COM and DEPT, 125 MHz, in C_6D_6 , TMS as an int. std.)

δ_{C}	Properties	Assignment
193.0	-CHO	C-14
152.8	=CH-	C-5
146.7	=C	C-1 (or C-4, C-10)
141.0	=C	C-10 (or C-1, C-4)
140.2	=C	C-4 (or C-1, C-10)
49.8	-C-	C-7
41.7	CH_2	C-8 (or C-6)
39.2	CH_2	C-6 (or C-8)
27.4	CH_2	C-9 (or C-2, C-3)
26.7	CH	C-11
24.2	CH_3	C-15
24.0	CH_2	C-2 (or C-3, C-9)
22.4	CH_2	C-3 (or C-2, C-9)
21.7	CH_3	C-12 (or C-13)
21.2	CH_3	C-13 (or C-12)

255 and Table 3-97, 98). Three (one quarternary carbon at δ_C 49.8 and two of disubstituted olefinic carbons) non-hydrogen bearing carbons out of four were contributed to the connection of these partial structures. Accordingly, the partial structures including the isopropyl group and the bridgehead methyl group were combined with those non-hydrogen bearing carbons. Since the bridgehead methyl group must be allocated to the quarternary carbon and the homoallyl coupling must be present over two partial structures, only two possible forms were depicted - one has a carotane skeleton (A) and the other a 4,8-bicyclic skeleton (B) (Fig. 3-256).

To elucidate the correct structure, a COLOC experiment on RL-A was carried out. The COLOC spectrum of RL-A, indicative of a H-C-C and H-C-C-C sequence together with H-C, showed some long range cross peaks that made the non-hydrogen-bearing carbons assignable as shown in Fig. 3-257. Accordingly, it was revealed that the structure A only fulfilled a cross peak between δ_H 3.049 proton and δ_C 141.0 (Table 3-99 and Fig. 3-258). Furthermore, a cross peak between δ_H 2.330 and δ_C 49.8 was also explicable only with the structure A. Thus, the planar structure of RL-A was established as 9. Since RL-A was a novel carotane aldehyde having C-14-oxo-daucene structure, to this compound was given a trivial name, daucenaldehyde. All the carbon signals were finally assignable as shown in Table 3-97.

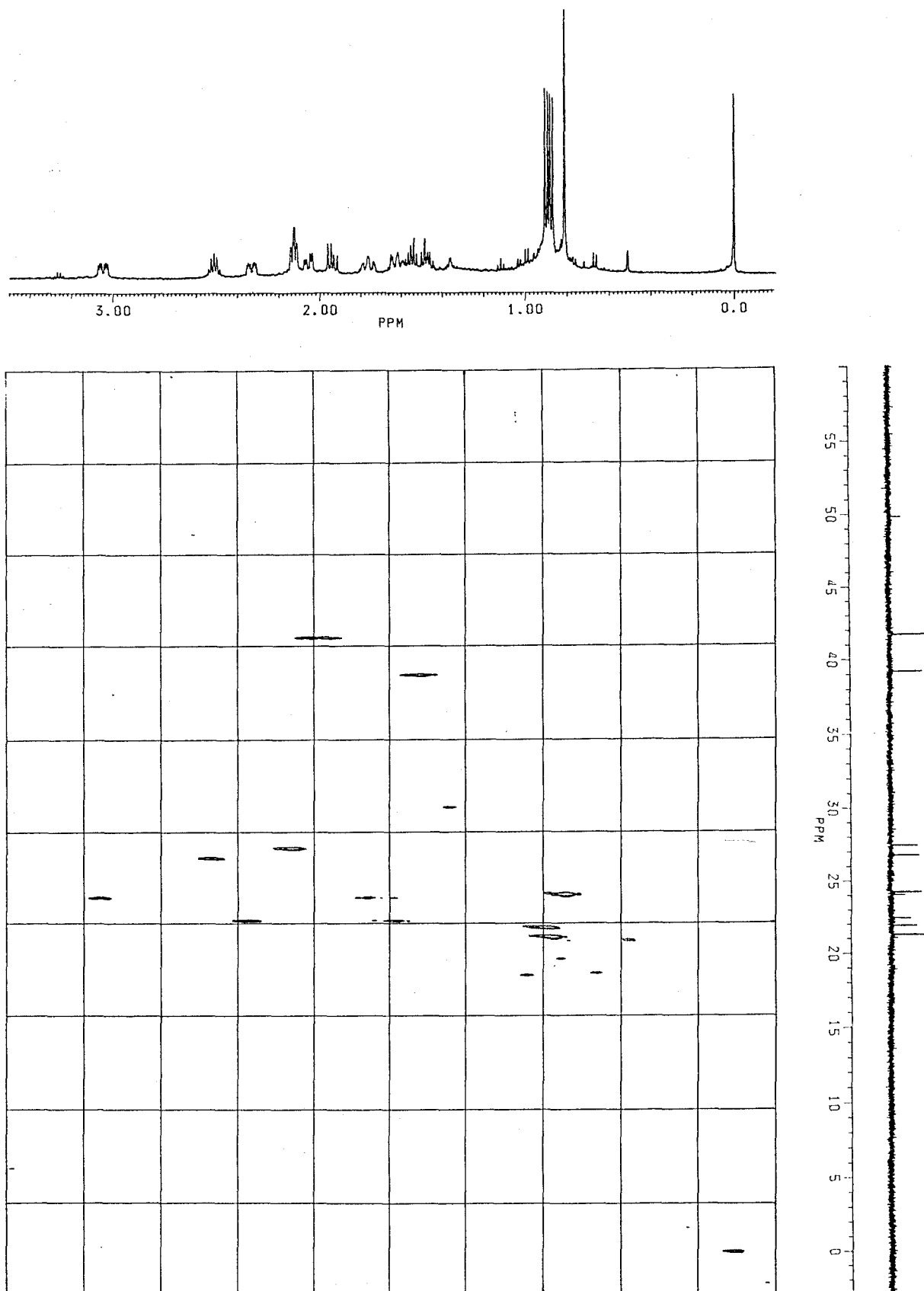
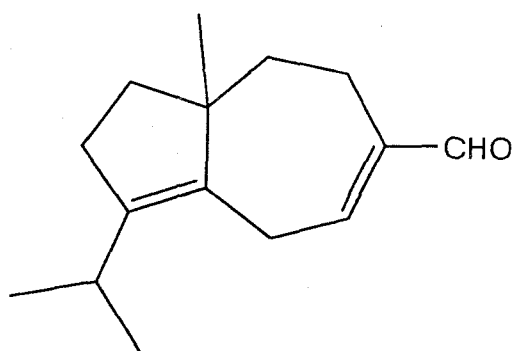


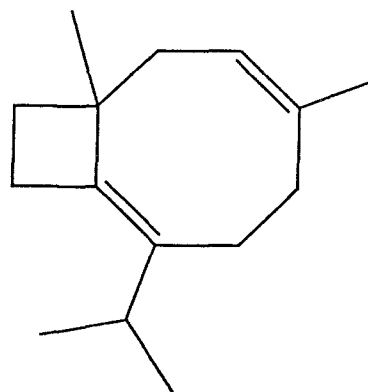
Fig. 3-255 CH-COSY Spectrum of RL-A (500 and 125 MHz, in C_6D_6)

Table 3-98 *Corelations between carbons and protons in CH-COSY of
RL-A*

Carbon	Proton
193.0	9.263
152.8	6.101
41.7	2.053, 1.935
39.2	1.550, 1.471
27.4	2.125
26.7	2.512
24.2	0.811
24.0	3.049, 1.763
22.4	2.330, 1.616
21.7	0.897
21.2	0.869



structure A



structure B

Fig. 3-256 Possible Structure of RL-A: Structure A affords
a carotadiene skeleton

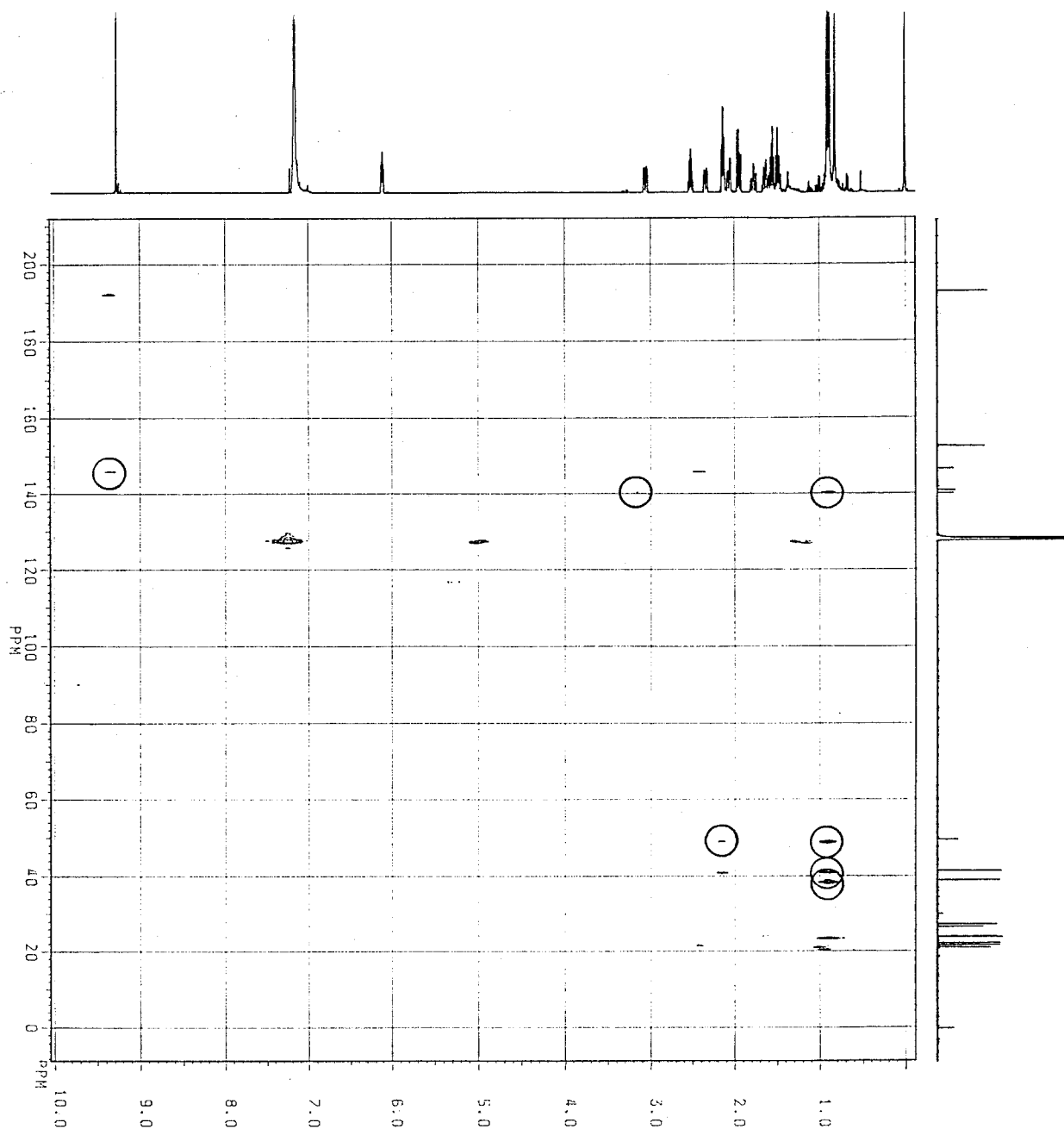


Fig. 3-257 COLOC Spectrum of RL-A (500 and 125 MHz, in C_6D_6):
The long range cross peaks (C-C-H, or C-C-CH) were marked with
a circle.

Table 3-99 Proton-carbon peaks in COLOC experiment of RL-A

Proton (δ_H)	Carbon (δ_C)
3.049 ---->	141.0
0.811 ---->	141.0
0.811 ---->	49.8
0.811 ---->	41.7
0.811 ---->	39.2
2.330 ---->	49.8
9.263 ---->	146.7

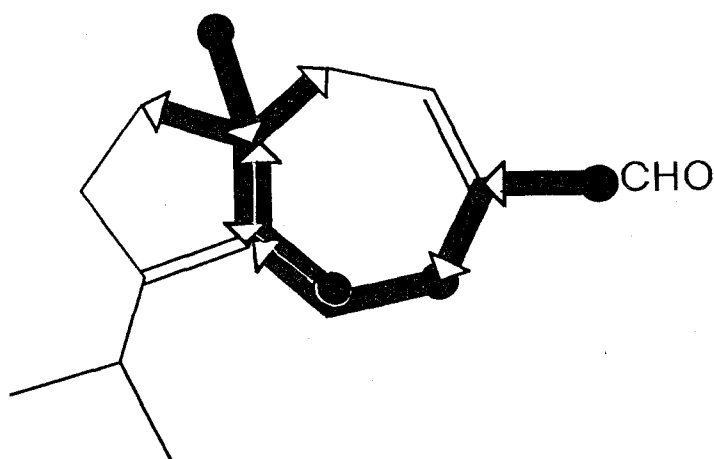
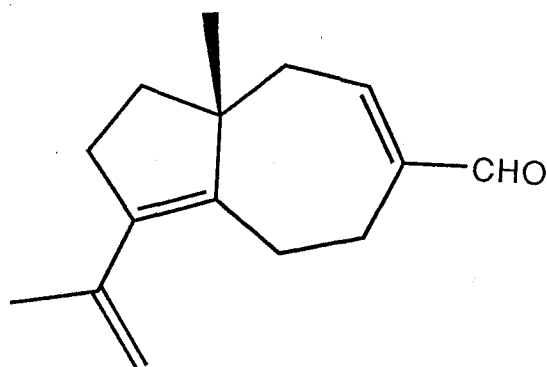


Fig. 3-258 Correlation between Non-Hydrogen-Bearing Carbons and Protons in RL-A Revealed by CH-COSY and COLOC Experiment: $\bullet \longrightarrow$ indicates a long range correlation (Proton to carbon).

Table 3-100 Physicochemical properties of daucenaldehyde

(RL-A, 9)



9

A colorless oil

Vanillin- H_2SO_4 color: grayish purple

GC-MS m/z (%): 218 (M^+ , 11), 203 ($\text{M}^+ - \text{CH}_3$, 5.2), 190 (6.0), 176 (12), 175 ($\text{M}^+ - \text{C}_3\text{H}_7$, 58), 162 (84), 147 (19), 136 (32), 135 (28), 133 (17), 122 (11), 121 (100), 107 (31), 105 (23), 94 (29), 93 (44), 91 (33), 79 (25), 77 (23), 55 (15), 53 (14), 41 (32).

EI-MS m/z (%): 218 (M^+ , 9.8), 203 ($\text{M}^+ - \text{CH}_3$, 5.4), 190 (4.9), 185 (5.9), 176 (11), 175 ($\text{M}^+ - \text{C}_3\text{H}_7$, 55), 162 (58), 147 (23), 136 (27), 135 (27), 133 (18), 122 (11), 121 (100), 107 (35), 105 (35), 94 (30), 93 (53), 91 (51), 79 (34), 77 (33), 55 (42), 53 (21), 43 (45), 41 (69).

^1H - and ^{13}C -NMR data are shown in Tables 3-97 and 3-98, respectively.

2) Epoxydaucenaldehydes

While constituents of the rugosa rose leaves were surveyed, a quenching spot was detected on TLC around R_f 0.55 in H-EA 4:1 (RL-109), and the compound corresponding to the spot was accordingly isolated from Fr-B-5 (Sample VII, See Section 3-5, pp. 324) and Fr-I-5 (Sample I, See Section 3-8, pp. 529). The isolated compound (ca 30 mg) afforded the parent ion at m/z 234 in FI-MS. RL-109 was however found to be a mixture of two compounds in ^1H -NMR analysis. For separation of two constituents from each other, some solvent systems for TLC were examined to find *n*-hexane-dichloromethane-acetone 25:25:1 (H-DCM-AT) as an effective solvent (Fig. 3-259). Accordingly, the mixture was separated to RL-109A (8.2 mg) and RL-109B (18.2 mg) by HPLC (Unisil Q 100-5, *n*-hexane-*iso*-PrOH 100:0.5 and H-DCM-AT 100:100:10) equipped with a UV detector at 230 nm (Fig. 3-260).

The major constituent RL-109B, providing M^+ 234 in FI- and EI-MS (Fig. 3-261, 262) showed some signals characteristic of carotane aldehydes (*cf.* carota-1,4-dienaldehyde, 3) in ^1H -NMR and HH-COSY spectra as shown in Fig. 3-263, 264 and Table 3-101. Furthermore, some methylene protons were quite similar to those of daucenaldehyde (9) in the signal pattern. The presence of clear septet signal assignable to C-11-H (δ_{H} 1.468) and isolated $-\text{CH}_2-\text{CH}=\text{C}-\text{CH}_2-\text{CH}_2-$ coupling sequence allowed to presume that C-1 and C-10 were replaced by non-hydrogen-bearing sp^3 carbons. Since two non-hydrogen-bearing and oxygenated carbons were detected at δ_{C} 76.8 and 75.4 in place of the two sp^2 carbons in the ^{13}C -NMR spectrum (Fig. 3-265 and Table 3-101), those were attributable to C-1 and C-10. According to the ^{13}C -NMR (INEPT), RL-109B was deduced to have the molecular formula $\text{C}_{15}\text{H}_{22}\text{O}_2$ ($\text{CH} \times 3$, $\text{CH}_2 \times 5$, $\text{CH}_3 \times 3$ and $\text{C} \times 4$), which was only compatible with a structure having an epoxy group on C(1)-C(10). Thus, structure 10 was proposed for RL-109B.

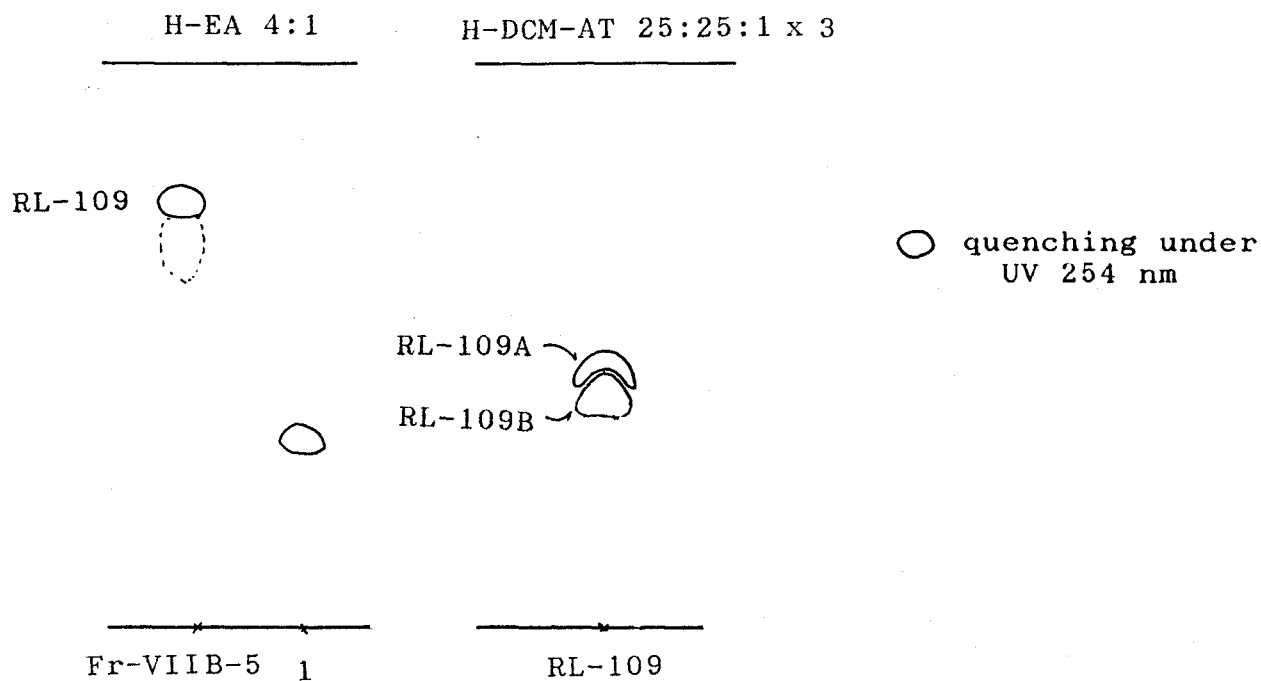


Fig. 3-259 TL Chromatograms of RL-109A and RL-109B

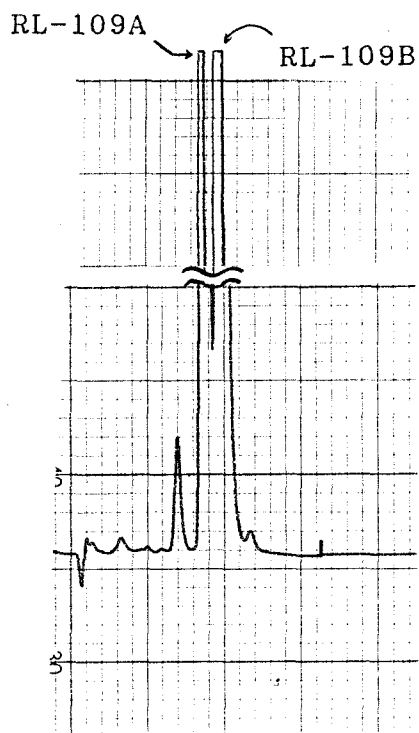


Fig. 3-260 HPL-Chromatogram of RL-109 Mixture (Unisil Q 100-5,
H-DCM-AT 25:25:1, UV 230 nm)

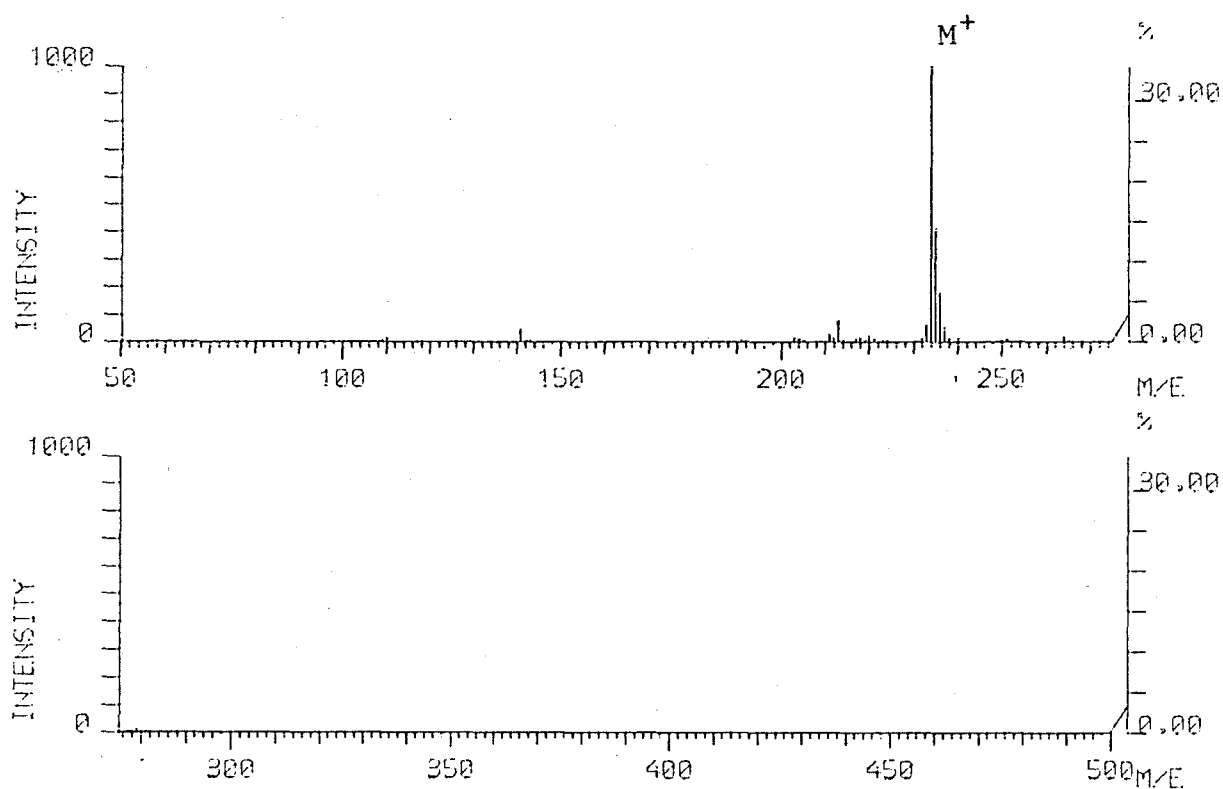


Fig. 3-261 FI-Mass Spectrum of RL-109B

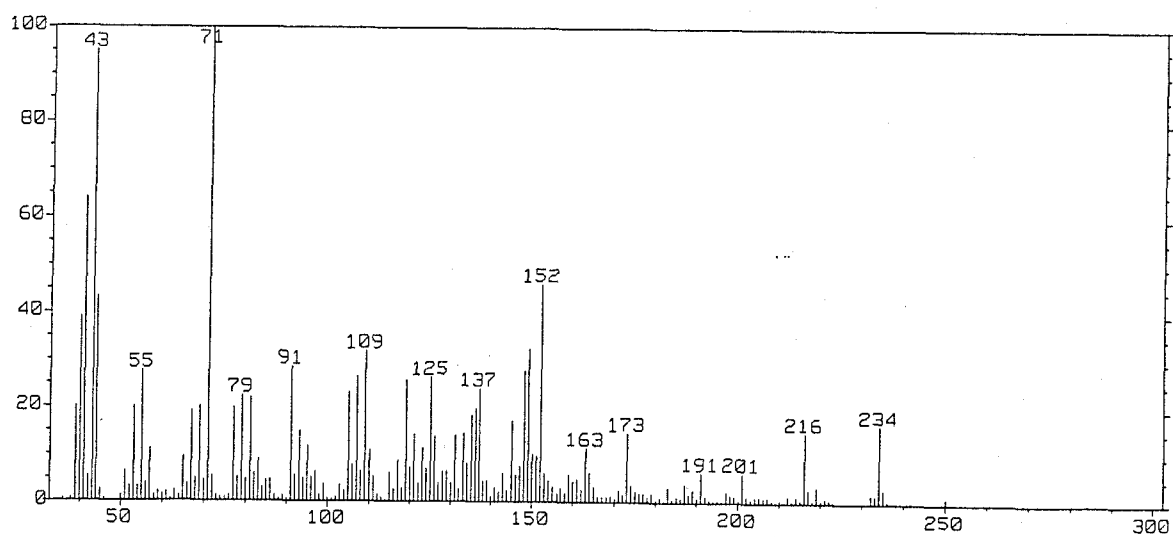


Fig. 3-262 EI-Mass Spectrum of RL-109B

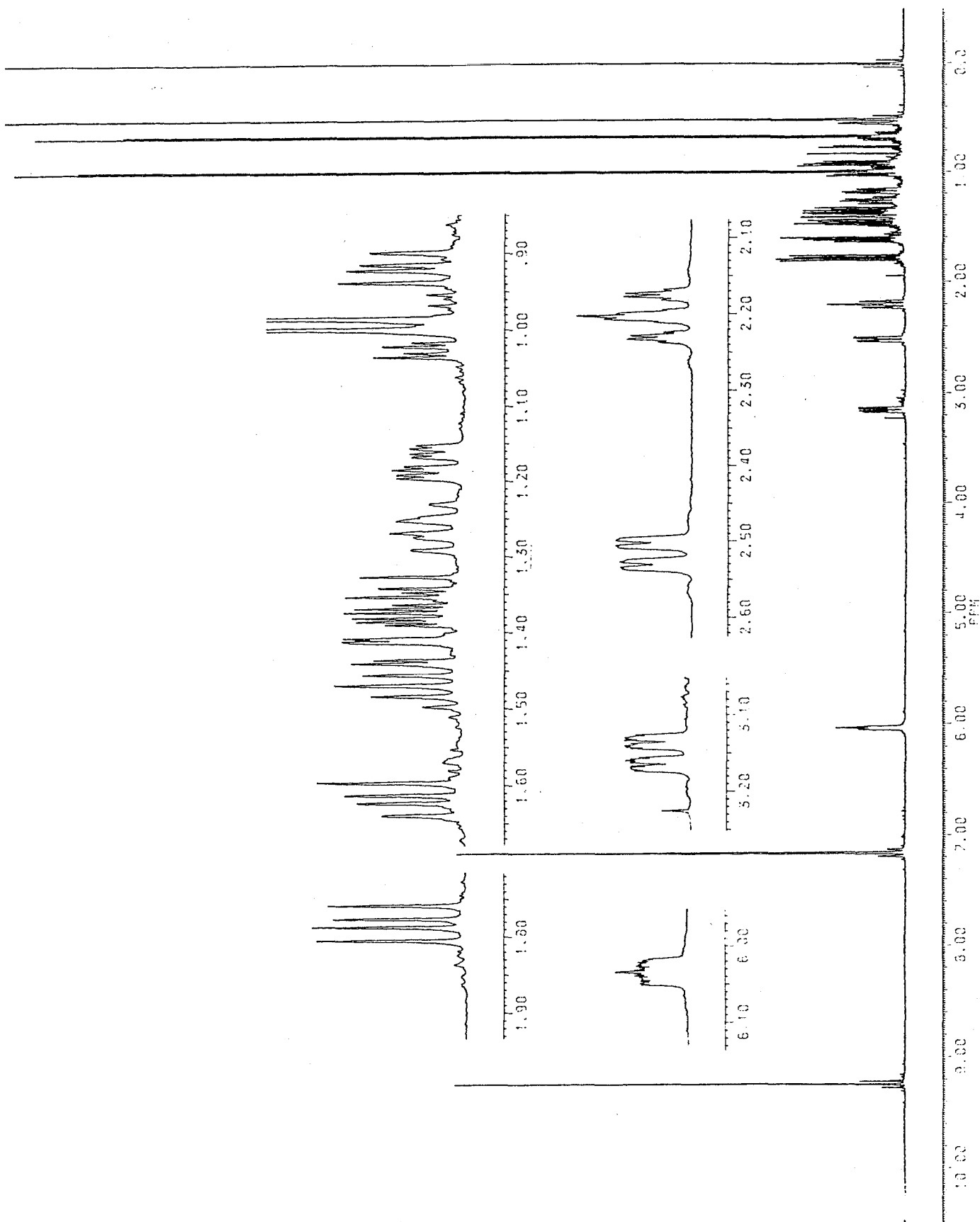


Fig. 3-263 ^1H -NMR Spectrum of RL-109B (500 MHz, in C_6D_6)

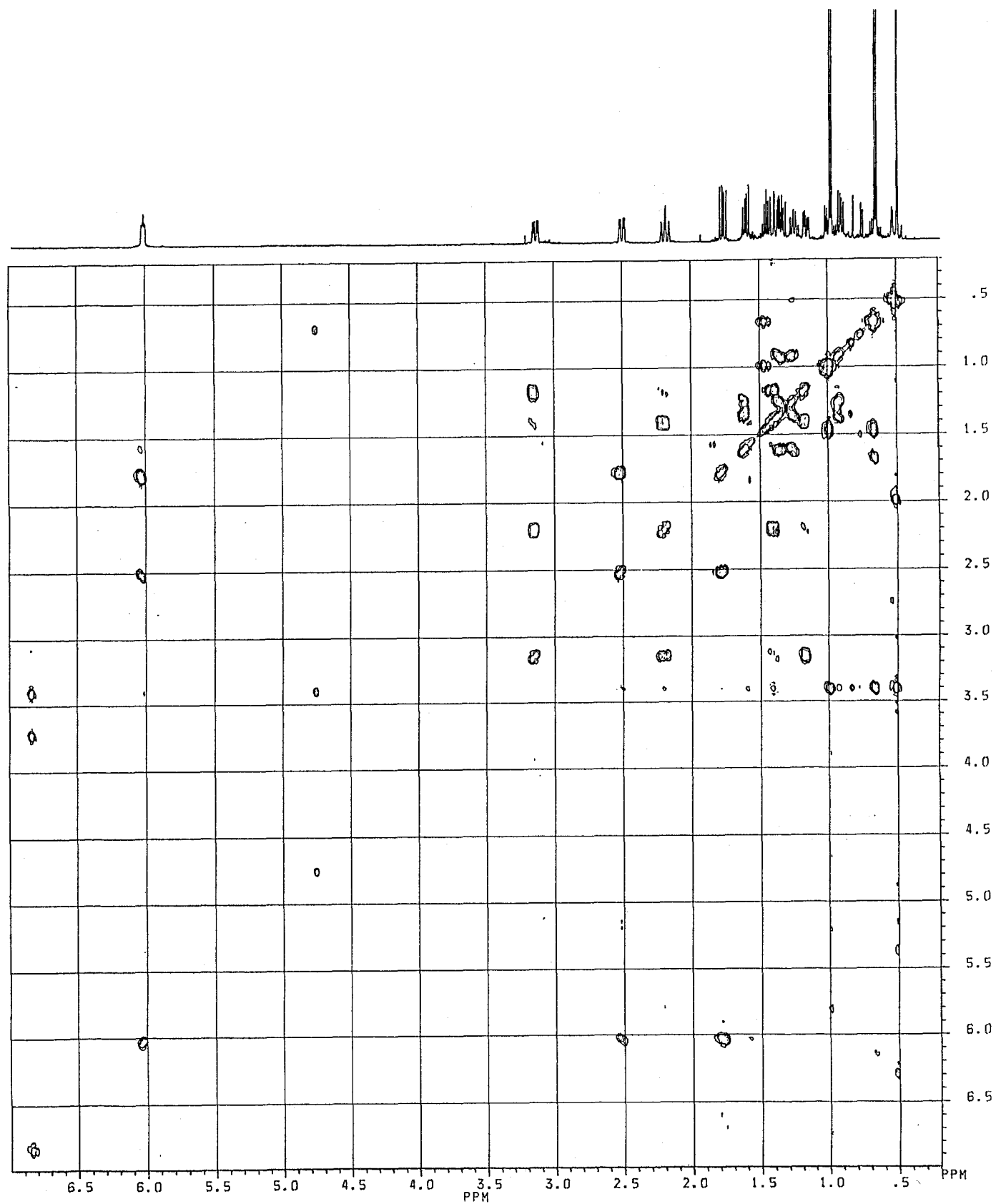


Fig. 3-264a HH-COSY Spectrum of RL-109B (500 MHz, in C_6D_6)

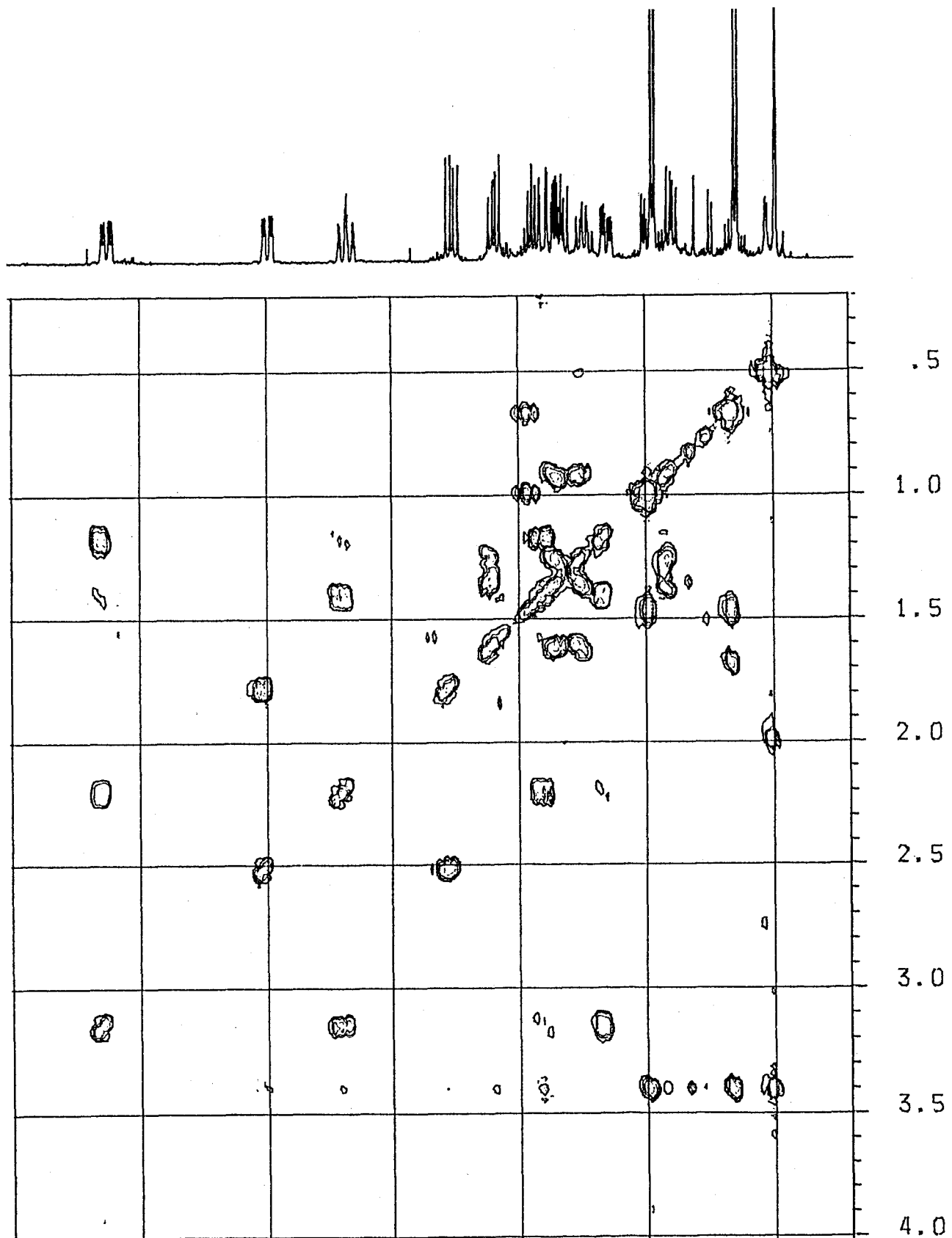


Fig. 3-264b Continued (Magnified in High Magnetic Field)

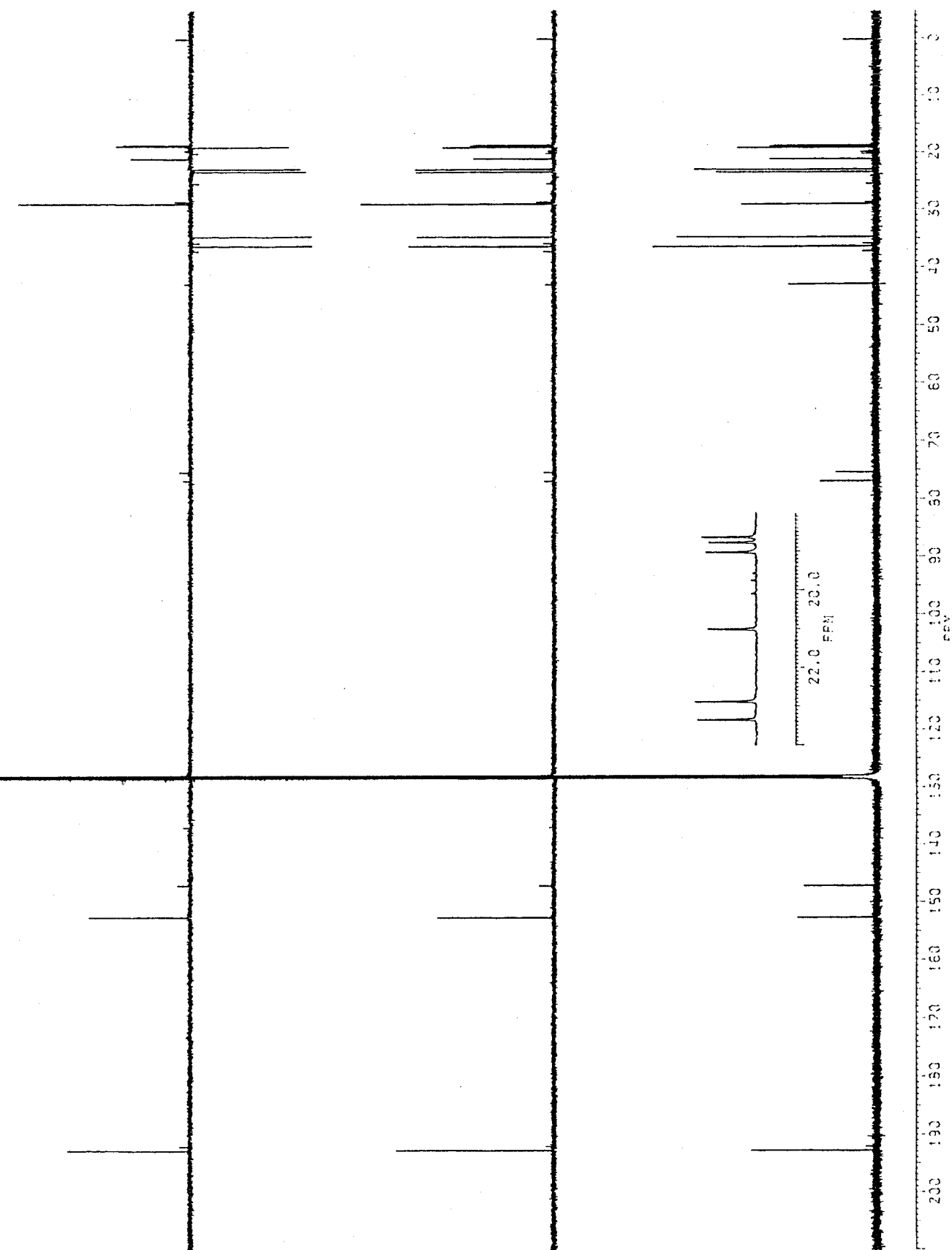
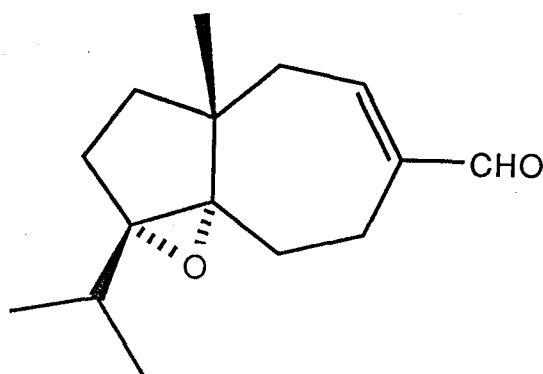


Fig. 3-144 ^{13}C -NMR Spectrum of RL-109B (125 MHz, in C_6D_6 , COM and DEPT)

Table 3-101 Physicochemical properties of epoxydaucenaldehyde A
(= RL-109B, 10)



10

A colorless syrup

EI-MS m/z (%): 234 (M^+ , 17), 216 (15), 201 (6.4), 191 (6.4), 173 (15), 163 (11), 152 (46), 149 (33), 148 (28), 137 (24), 125 (27), 119 (26), 109 (32), 107 (27), 105 (23), 91 (29), 81 (22), 79 (22), 71 (100), 55 (28), 44 (43), 43 (95), 41 (64).

$^1\text{H-NMR}$ $\delta_{\text{TMS}}^{\text{C}_6\text{D}_6}$ (500 MHz): 1.407 (1H, ddd, J = 14.1, 13.3 and 2.2 Hz, C-2-Ha), 1.173 (1H, br. ddd, J = 14.1, 5.5 and 2.2 Hz, C-2-Hb), 3.149 (1H, m-dvid. dd, J = 14.8 and 5.4 Hz, C-3-Ha), 2.202 (1H, m-dvid. dd, J = 14.8 and 13.2 Hz, C-3-Hb), 6.033 (1H, br. m, C-5-H), 2.516 (1H, br. dd, J = 14.4 and 4.7 Hz, C-6-Ha), 1.781 (1H, dd, J = 14.4 and 8.8 Hz, C-6-Hb), 1.260 (1H, br. ddd, J = 11.7, 10.5 and 8.0 Hz, C-8-Ha), 0.918 (1H, dd, J = 11.7 and 7.8 Hz, C-8-Hb), 1.617 (1H, dd, J = 13.4 and 8.0 Hz, C-9-Ha), 1.357 (1H, ddd, J = 13.4, 10.5 and 7.8 Hz, C-9-Hb), 1.469 (1H, sept., J = 6.9 Hz, C-11-H), 0.991 (3H, d, J = 6.9 Hz, C-12- H_3), 0.666 (3H, d, J = 6.9 Hz, C-13- H_3), 9.240 (1H, s, C-14-H), 0.511 (3H, s, C-15- H_3).

$^{13}\text{C-NMR}$ $\delta_{\text{TMS}}^{\text{C}_6\text{D}_6}$ (125 MHz): 76.8 (C, C-1), 23.3 (CH_2 , C-2), 19.0 (CH_2 , C-3), 147.0 (C, C-4), 152.5 (CH, C-5), 36.2 (CH_2 , C-6), 42.8 (C, C-7), 34.6 (CH_2 , C-8), 22.9 (CH_2 , C-9), 75.3 (C, C-10), 28.8 (CH, C-11), 18.8 (CH_3 , C-12), 18.6 (CH_3 , C-13), 192.6 (CH, C-14), 21.0 (CH_3 , C-15).

While RL-109B was elucidated as **10**, the other isolate, RL-109A was also analyzed spectroscopically. RL-109A whose FI-MS showed the parent ion at m/z 234 (100 %) (Fig. 3-266) resembled to **10** in the EI-MS fragmentation (Fig. 3-267). Although the ^1H -NMR spectrum of RL-109A (in C_6D_6) was quite different from that of RL-109B (Fig. 3-268), in CDCl_3 the minor isolate showed a similar ^1H -NMR signal pattern (Fig. 3-269). HH-COSY of RL-A was therefore measured in CDCl_3 (Fig. 3-270) to prove two $\blacksquare\text{-CH}_2\text{-CH}_2\text{-}\blacksquare$ coupling systems on RL-109A. ^{13}C -NMR data of RL-109A were indicative of its diastereoisomeric nature to **10** at the C1/C10-epoxy ring, showing a good accordance of carbon chemical shift values to those of **10** (Fig. 3-271). Accordingly, structure **11** was proposed for RL-109A.

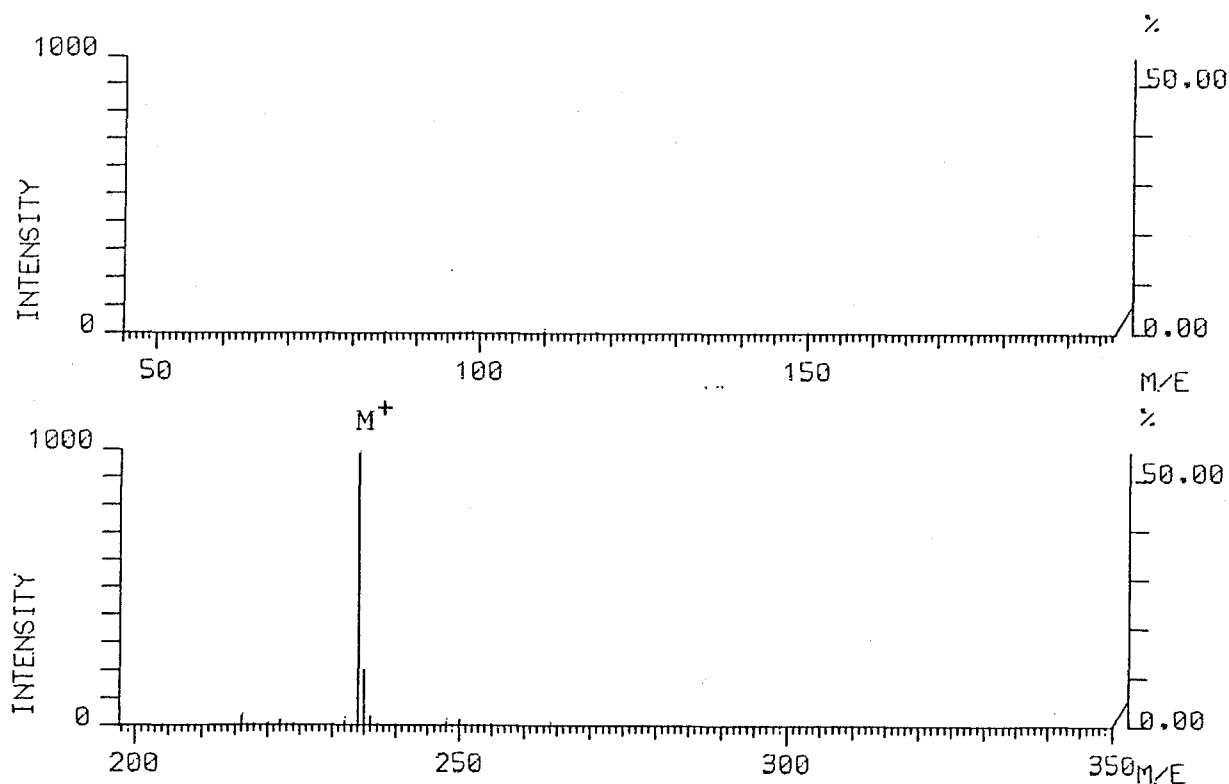


Fig. 3-266 FI-Mass Spectrum of RL-109A

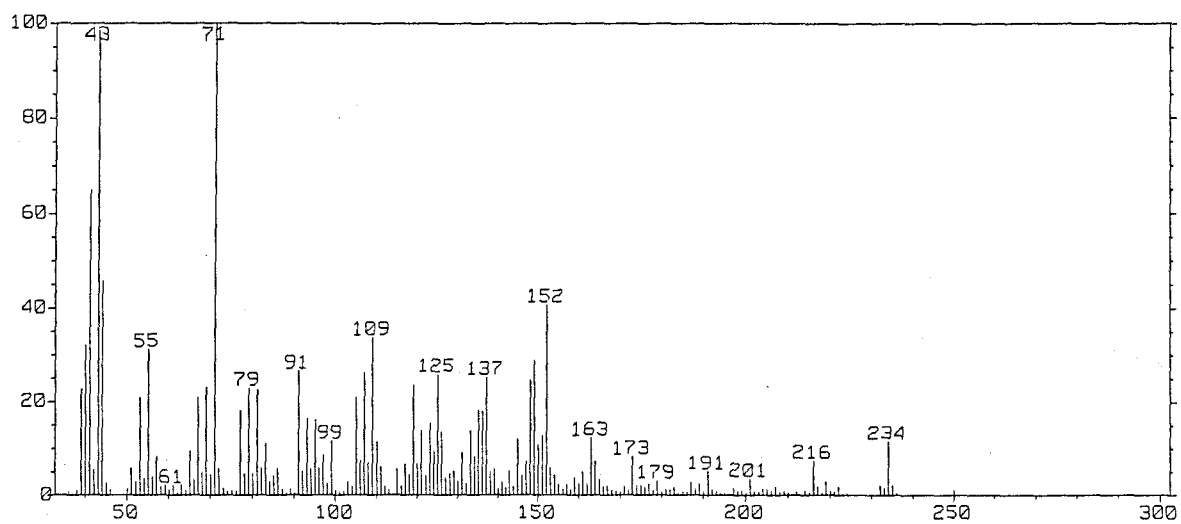


Fig. 3-267 EI-Mass Spectrum of RL-109A

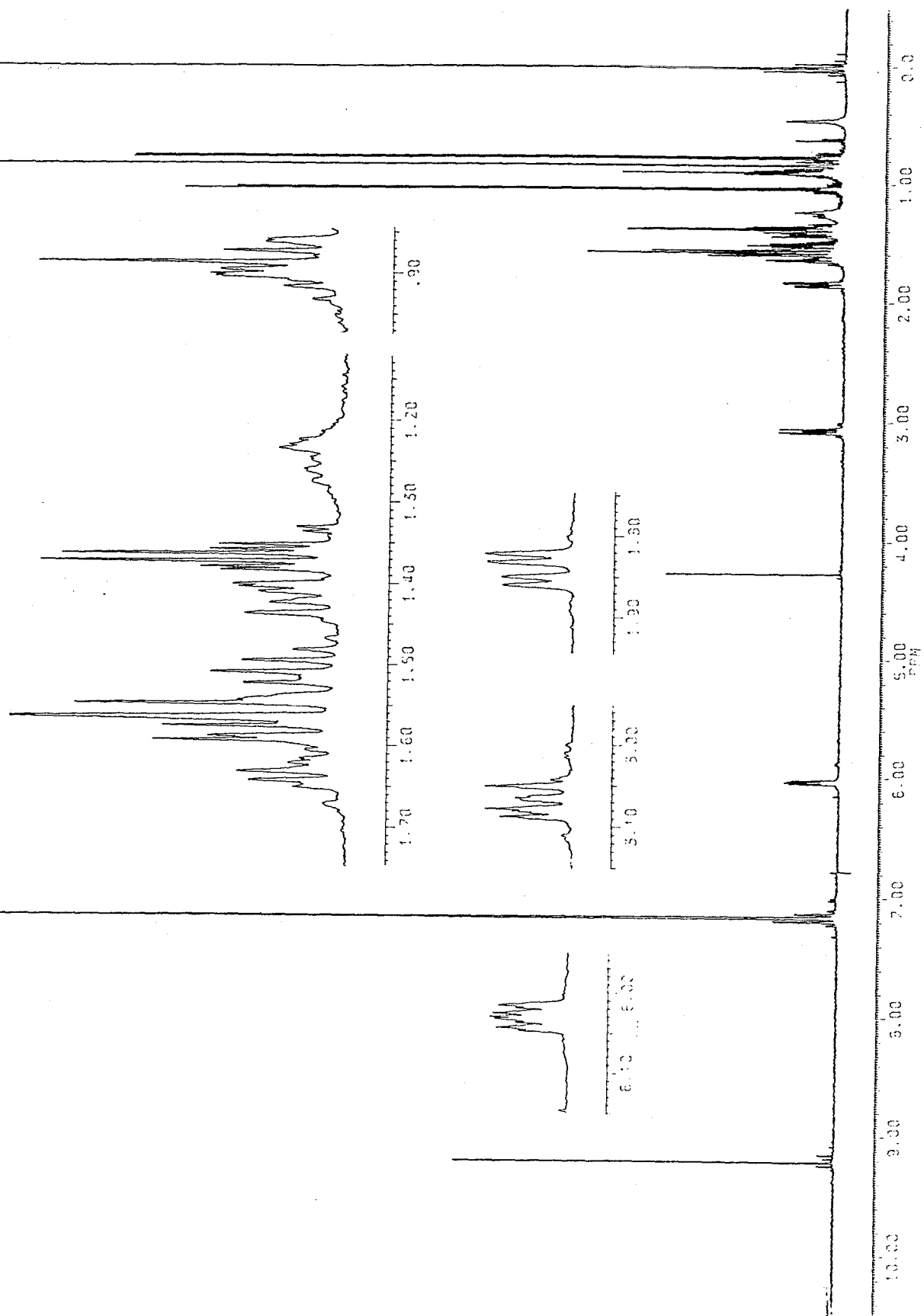


Fig. 3-268 ^1H -NMR Spectrum of RL-109A (500 MHz, in C_6D_6)

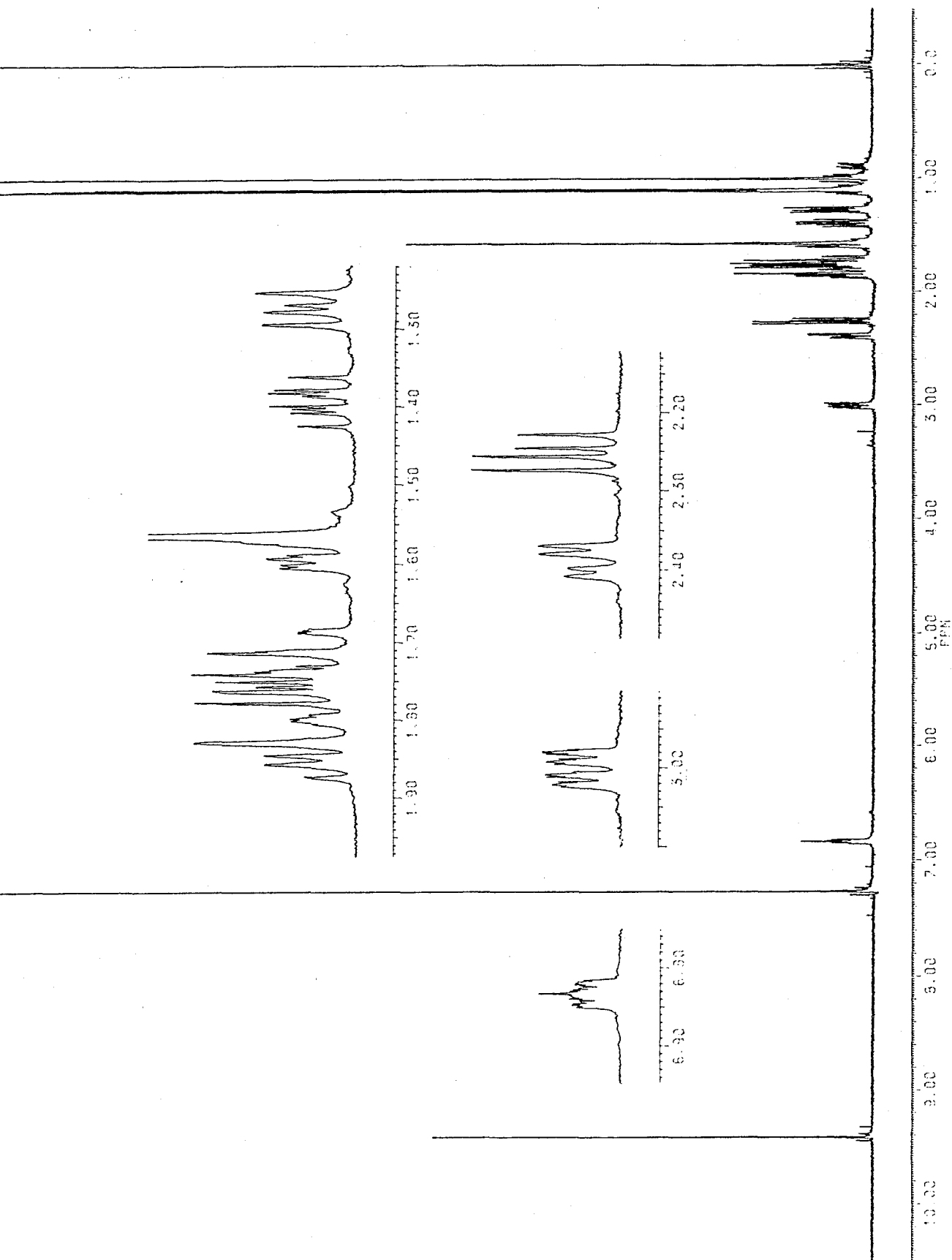


Fig. 3-269; ^1H -NMR Spectrum of RL-109A (500 MHz, in CDCl_3)

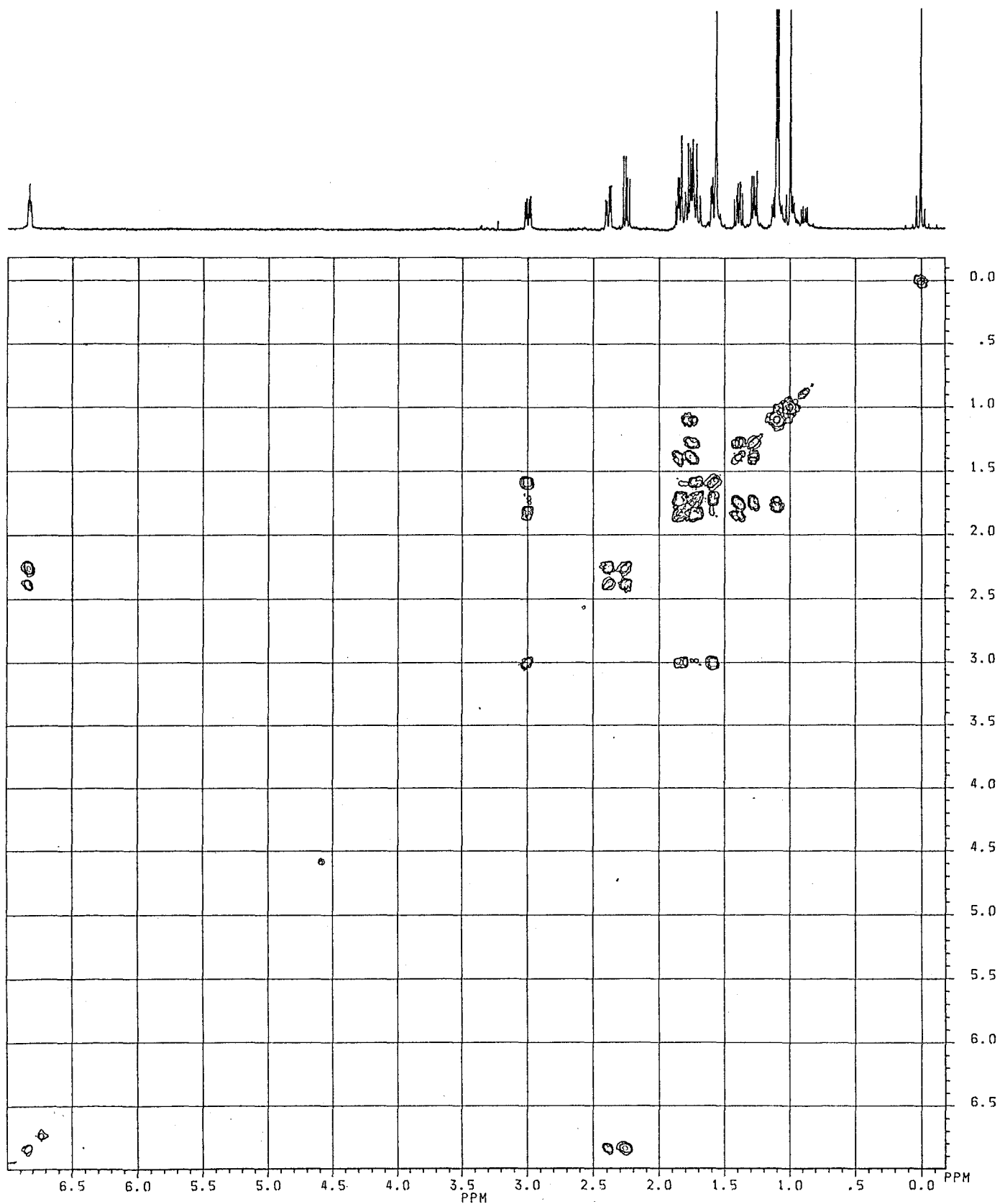


Fig. 3-270a HH-COSY Spectrum of RL-109A (500 MHz, in CDCl₃)

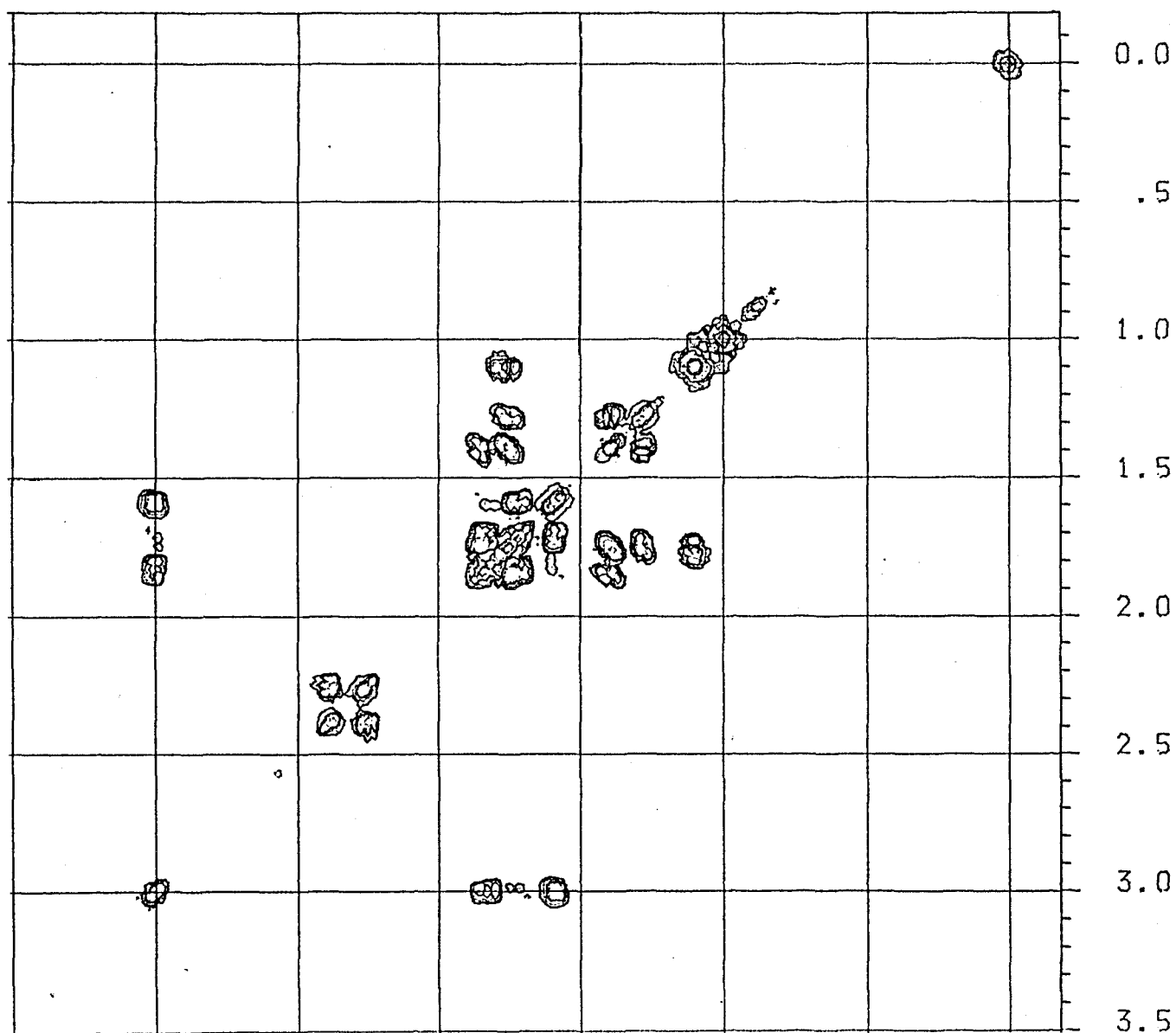
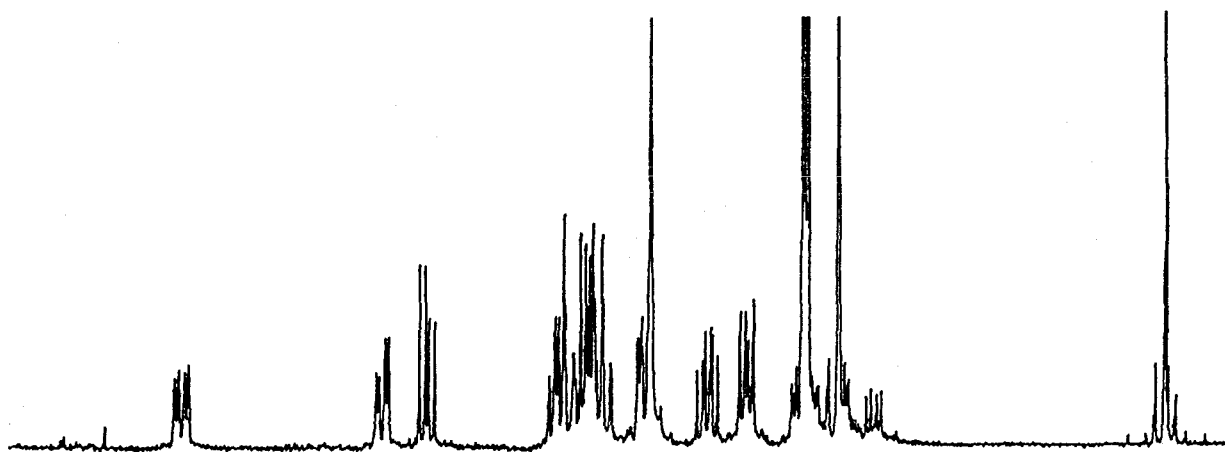


Fig. 3-270b Continued (Magnified in High Magnetic Field)

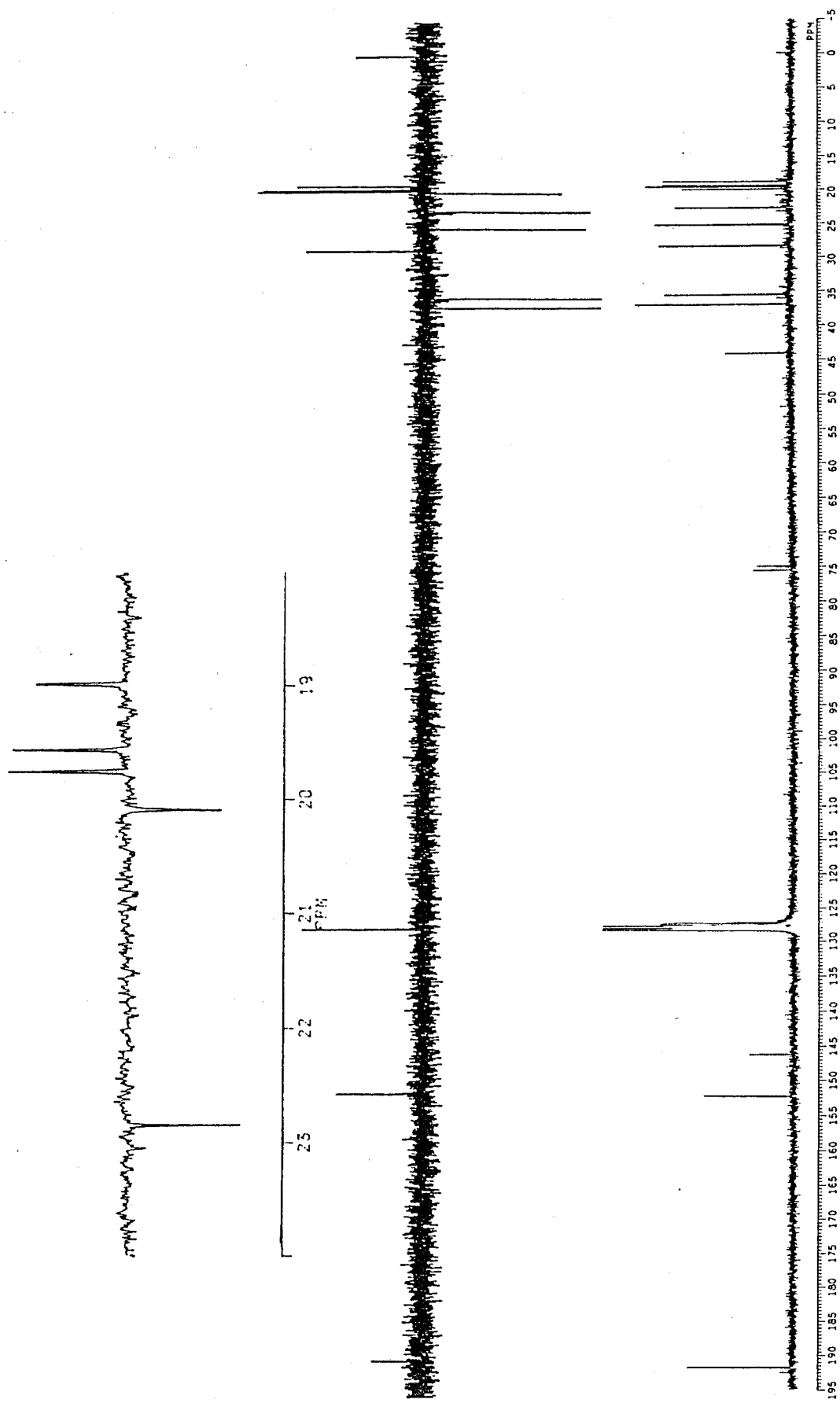
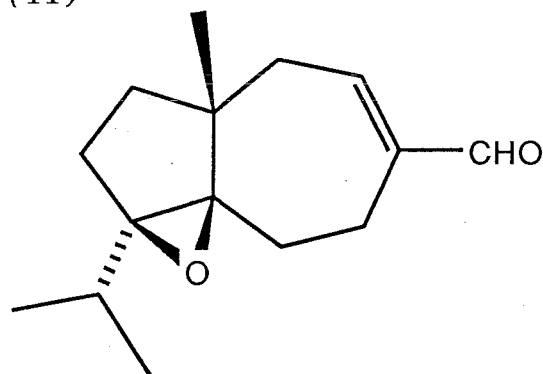


Fig. 3-271 ^{13}C -NMR Spectrum of RL-109A (68 MHz, in C_6D_6 , COM and DEPT)
DEPT spectrum was measured at 125 MHz)

(11)



11

A colorless syrup

EI-MS m/z (%): 234 (M^+ , 12), 216 (7.5), 201 (3.6), 191 (5.3), 173 (8.4), 163 (13), 152 (41), 149 (29), 148 (25), 137 (25), 125 (26), 119 (24), 109 (34), 107 (26), 91 (27), 79 (23), 71 (100), 55 (31), 44 (46), 43 (99), 41 (65).

$^1\text{H-NMR}$ $\delta_{\text{TMS}}^{\text{C}_6\text{D}_6}$ (500 MHz): 9.211 (1H, s, C-14-H), 6.028 (1H, m, C-5-H), 3.068 (1H, m, C-3-Ha), 1.840 (1H, dd, $J = 14.1$ and 5.0 Hz, C-6-Ha), 1.522 (1H, sept., $J = 6.9$ Hz, C-11-H), 1.028 (3H, d, $J = 6.9$ Hz, C-12- H_3), 0.828 (3H, s, C-15- H_3), 0.763 (3H, d, $J = 6.9$ Hz, C-13- H_3). Other signals were mostly overlapped in C_6D_6 .

$^1\text{H-NMR}$ $\delta_{\text{TMS}}^{\text{CDCl}_3}$ (500 MHz): 1.712 (1H, br. dd, $J = 13.4$ and 12.9 Hz, C-2-Ha), ca 1.58 (1H, overlapped with H_2O peak, C-2-Hb), 3.000 (1H, m-dvid dd, $J = 14.8$ and 6.3 Hz, C-3-Ha), 1.829 (1H, t-like m, C-3-Hb), 6.832 (1H, m, C-5-H), 2.388 (1H, dd, $J = 14.1$ and 5.2 Hz, C-6-Ha), 2.250 (1H, dd, $J = 14.1$ and 8.8 Hz, C-6-Hb), 1.393 (1H, ddd, $J = 12.5$, 10.5 and 8.3 Hz, C-8-Ha), 1.273 (1H, br. dd, $J = 12.5$ and 7.9 Hz, C-8-Hb), 1.851 (1H, br. dd, $J = 13.8$ and 8.3 Hz, C-9-Ha), 1.746 (1H, m, C-9-Hb), 1.751 (1H, sept., $J = 6.9$ Hz, C-11-H), 1.097 (3H, d, $J = 6.9$ Hz, C-12- H_3), 1.092 (3H, d, $J = 6.9$ Hz, C-13- H_3), 9.409 (1H, s, C-14-H), 0.994 (3H, s, C-15- H_3).

$^{13}\text{C-NMR}$ $\delta_{\text{TMS}}^{\text{CDCl}_3}$ (125 MHz): 75.7 (C, C-1), 22.8 (CH_2 , C-2), 20.1 (CH_2 , C-3), 146.3 (C, C-4), 152.3 (CH, C-5), 37.1 (CH_2 , C-6), 44.2 (C, C-7), 35.7 (CH_2 , C-8), 25.4 (CH_2 , C-9), 28.5 (CH, C-11), 19.5 (CH_3 , C-12), 18.9 (CH_3 , C-13), 191.8 (CH, C-14), 19.7 (CH_3 , C-15).

The proposed structures 10 for RL-109B and 11 for RL-109A was confirmed by direct comparison with the epoxidation products of 9 with *m*-CPBA as shown in Scheme 3-33 (TLC in Fig. 3-272).

RL-A (3.2 mg)
|
dissolved in 1.5 ml of CHCl₃
|
cooled to 0 °C
|
added 2.4 mg of *m*-CPBA
|
stirred 30 min.
|
concentrated and dissolved in ca 0.2 ml of EtOAc
|
subjected to PTLC (*n*-hexane-EtOAc 20:1)
|
Product (RL-A-CPBA, *R*_f 0.06, 2.1 mg, 61 % yield)

Scheme 3-33 Process of RL-A epoxidation and isolation of the product

The product showing *m/z* 234 as the parent ion in EI-MS was, as expected, found to be a mixture of diastereomers by the ¹H-NMR analysis with a ratio of 3:1 (Fig. 3-273). The product was therefore separated by HPLC (H-DCM-AT 100:100:10, UV 230 nm, Fig. 3-274) to obtain RL-A-CPBA-1 (0.5 mg) and RL-A-CPBA-2 (1.5 mg). The EI-MS fragmentation and proton signals of the products were each coincident well with those of RL-109A and RL-109B, respectively, proving their proposed structures (11 and 10) (Fig. 3-275, 276, 277 and 278).

C-M 50:4

vanillin-H₂SO₄
test: +

peroxide test: +

Reaction Std.1c
mix.

Fig. 3-272 TL Chromatograms of Reaction Product

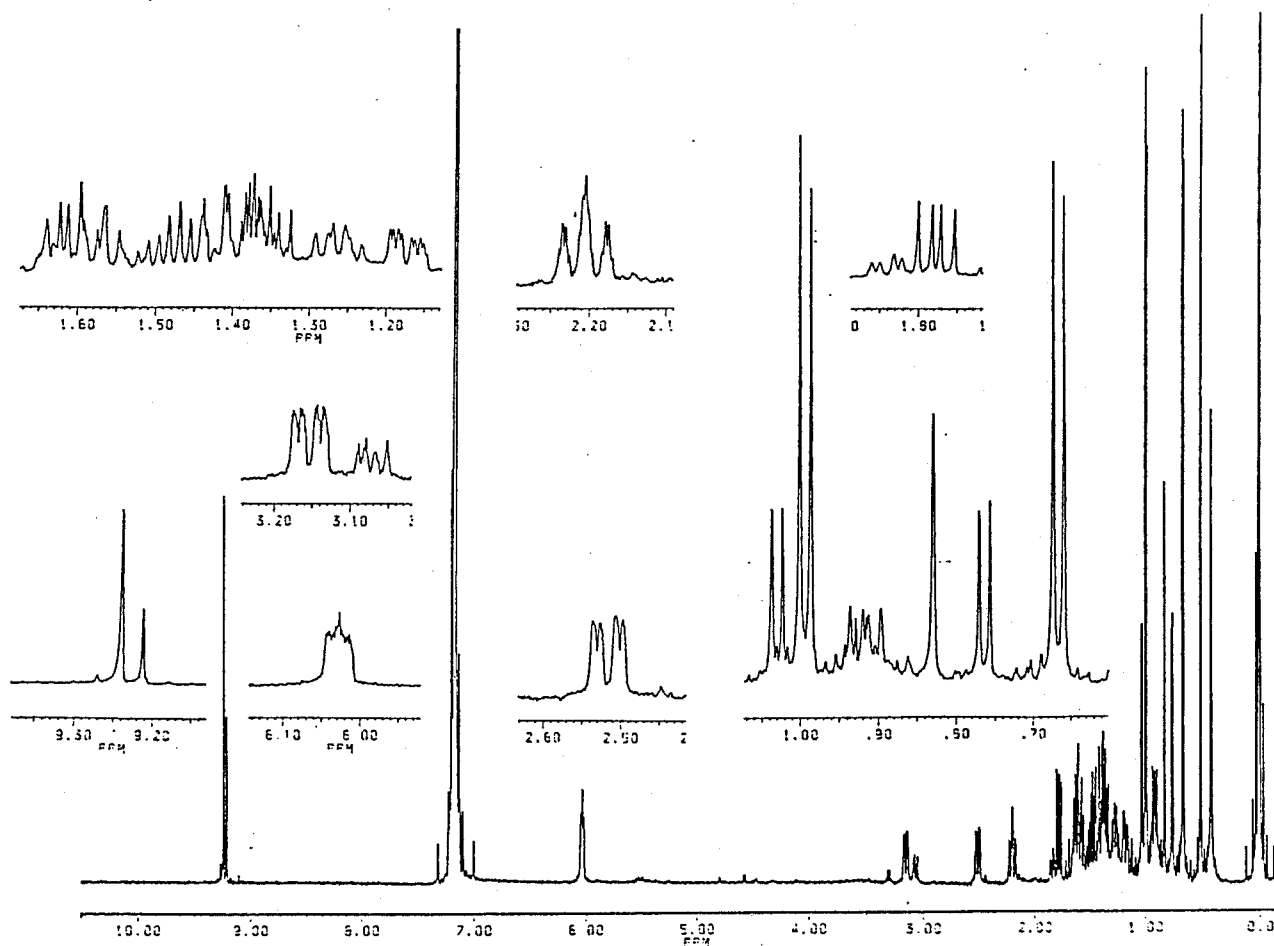


Fig. 3-273 ¹H-NMR Spectrum of RL-A-CPBA Mixture (500 MHz, in C₆D₆)

Thus, it was suggested that the epoxy carotanoids RL-109B (10) and RL-109A (11) were biogenetically derived from 9, and were named epoxydaucenaldehyde A (RL-109B) and epoxydaucenaldehyde B (RL-109A), respectively. Carbon chemical shift values of 10 and 11 were quite similar to those of a known epoxycarotane (46) found in *Ferula communis subsp. communis* by Miski *et al.* (Table 3-103) [46].

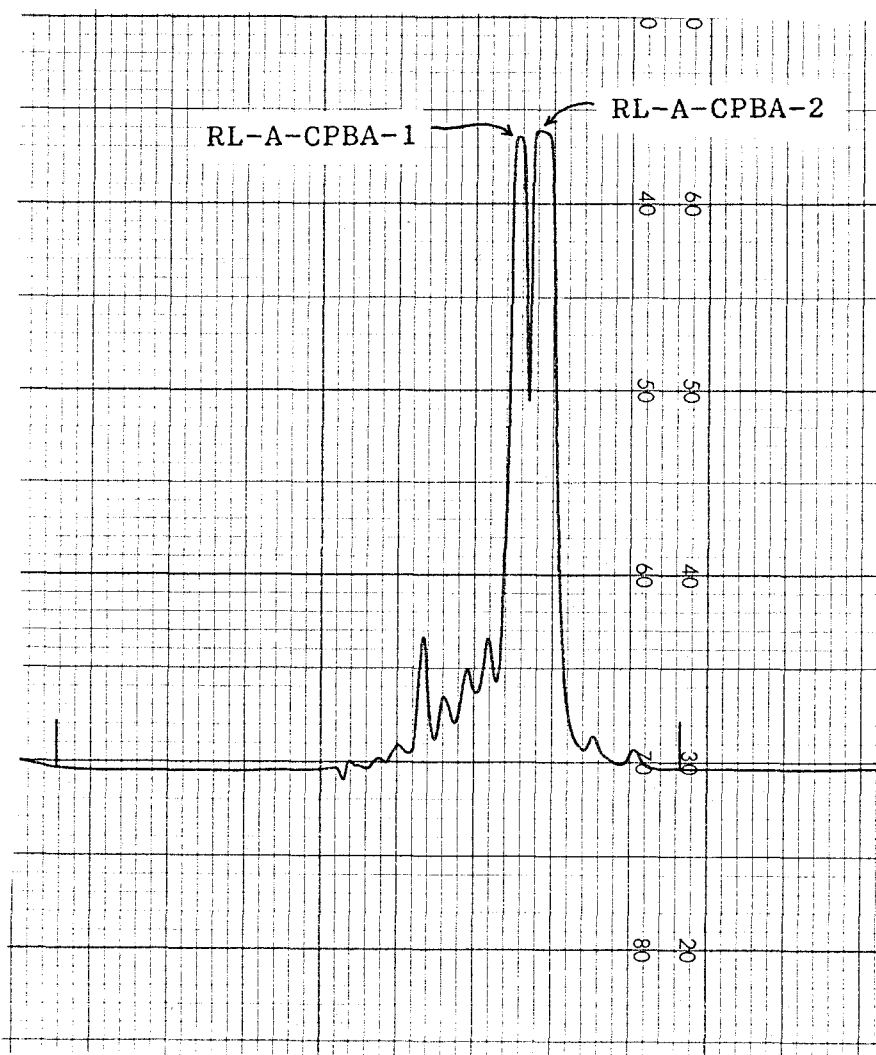


Fig. 3-274 HPL-Chromatogram of RL-A-CPBA Mixture (UV 230 nm)

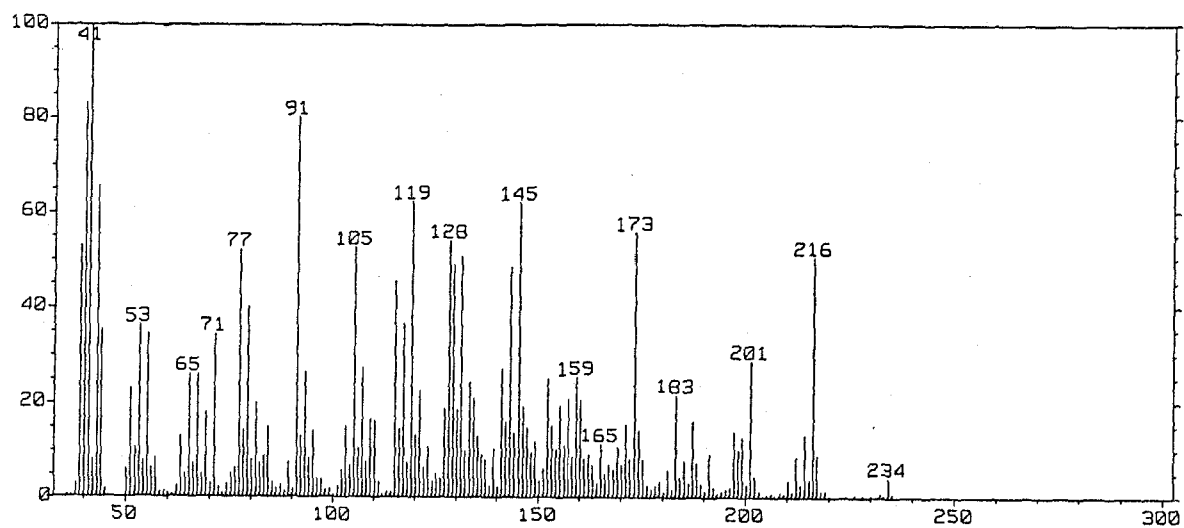


Fig. 3-275 EI-Mass Spectrum of RL-A-CPBA-2

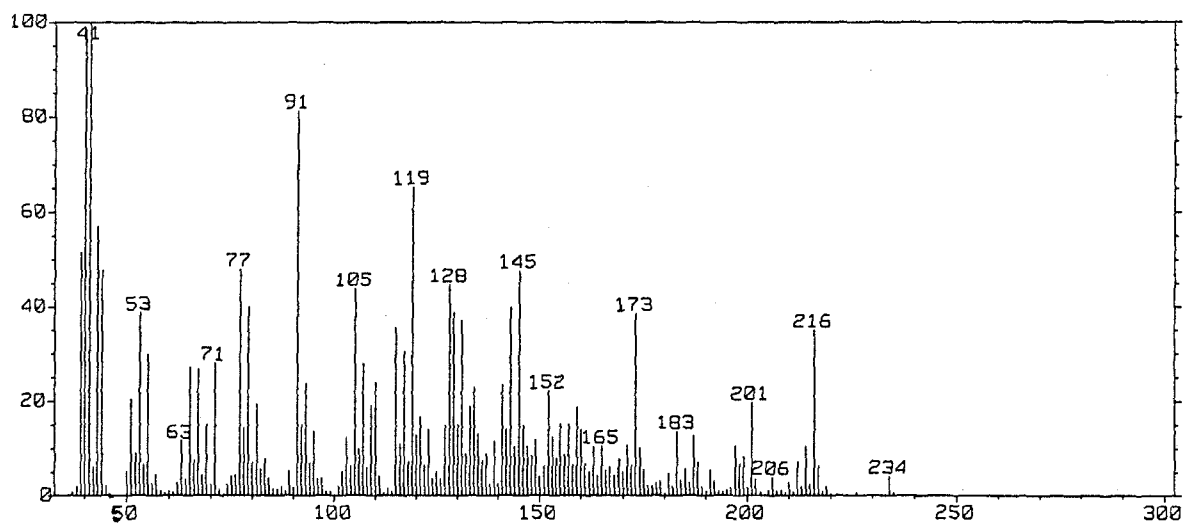


Fig. 3-276 EI-Mass Spectrum of RL-A-CPBA-1

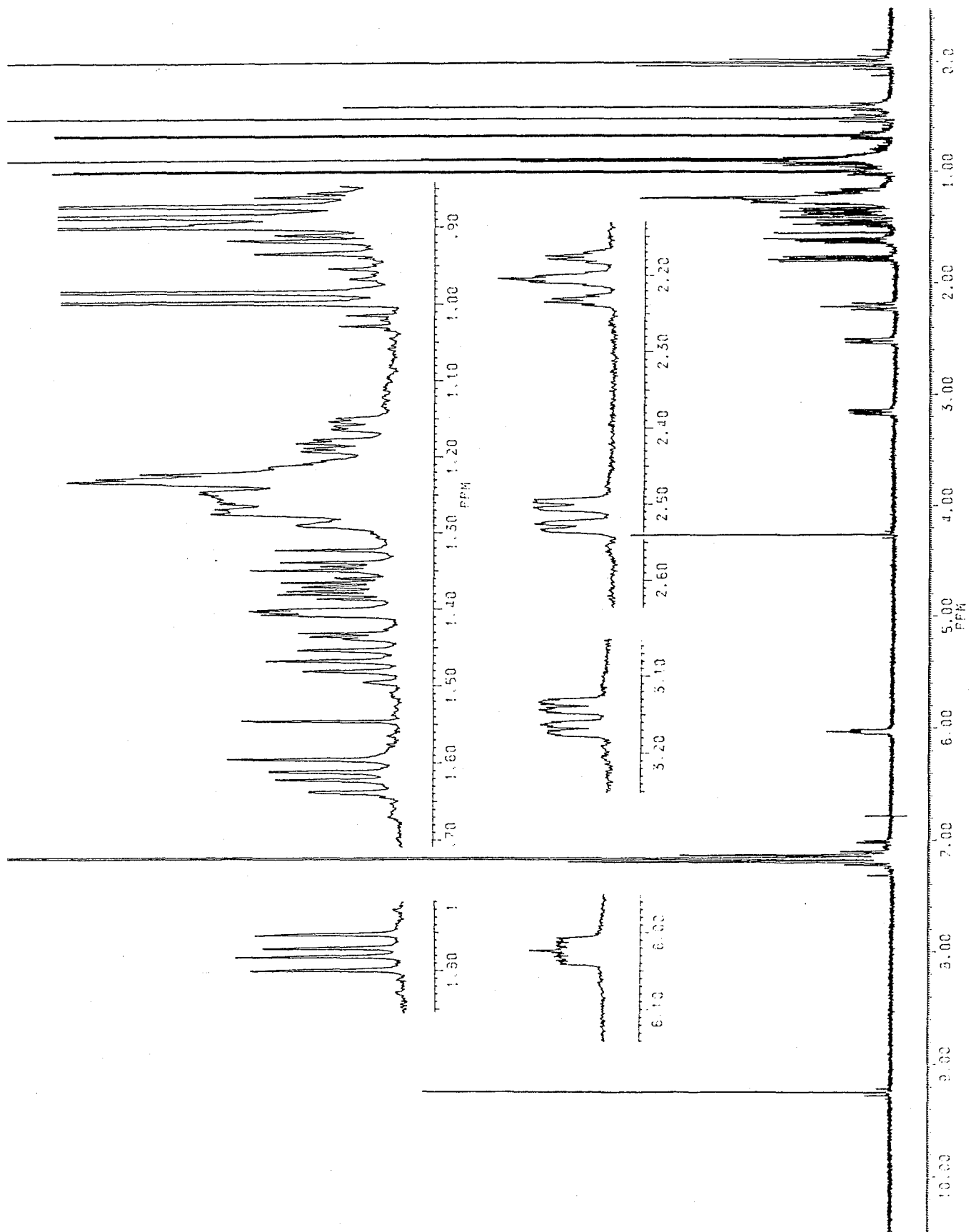
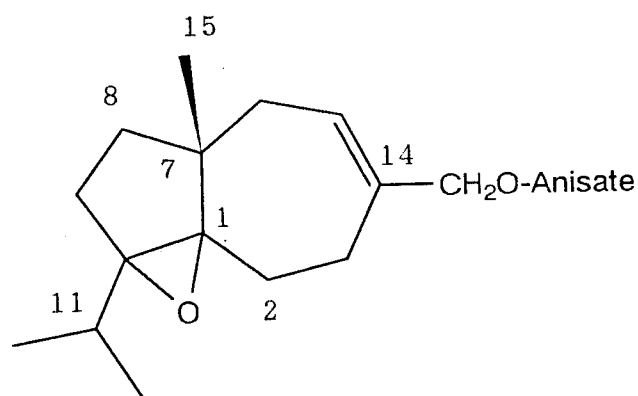


Fig. 3-277 ^1H -NMR Spectrum of RL-A-CPBA-2 (500 MHz, in C_6D_6)

Table 3-103 Carbon chemical shift of an epoxy carotanoid (46)
isolated from an *Umberifellae* plant



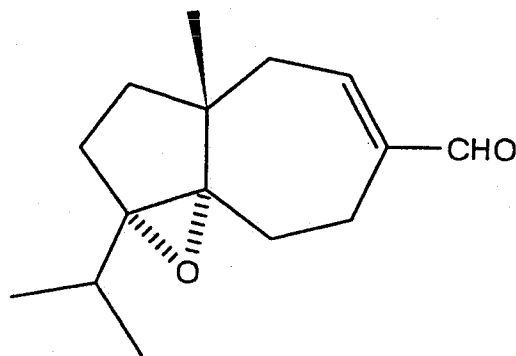
No	46*	10**	11***
1	76.0	76.8	75.7
2	24.7	23.3	22.8
3	22.2	19.0	20.1
4	138.1	147.0	146.3
5	127.2	152.5	152.3
6	33.3	36.2	37.1
7	41.9	42.8	44.2
8	23.4	34.6	35.7
9	28.0	22.9	25.4
10	76.8	75.3	75.0
11	33.9	28.8	28.5
12	18.2	18.8	19.5
13	17.2	18.6	18.9
14	69.3	192.6	191.8
15	20.3	21.0	19.7

* 50 MHz in CDCl_3

**125 MHz in C_6D_6 , assigned by CH-COSY

***68 MHz in C_6D_6

Table 3-104 Physicochemical properties of RL-A-CPBA-2 (10)



10

A colorless syrup

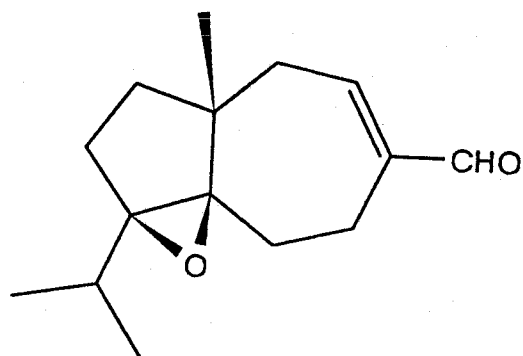
Rf: 0.35 (H-EA 4:1), 0.19 (H-DCM-AT 25:25:1)

Vanillin-H₂SO₄ color: brown

EI-MS *m/z* (%): 234 (M⁺, 4.3), 216 (51), 201 (29), 183 (22), 173 (56), 159 (25), 145 (62), 143 (49), 131 (51), 129 (49), 128 (54), 119 (62), 117 (37), 115 (46), 105 (53), 91 (80), 79 (40), 77 (52), 71 (34), 53 (36), 43 (66), 41 (100).

¹H-NMR δ_{TMS}^{C₆D₆} (500 MHz): 1.403 (1H, ddd, *J*= 14.2, 13.8 and 1.8 Hz, C-2-Ha), 1.171 (1H, br. dd, *J*= 14.1, 5.5 and 2.3 Hz, C-2-Hb), 3.155 (1H, m-divid. dd, *J*= 14.8 and 5.5 Hz, C-3-Ha), 2.204 (1H, m-divid. dd, *J*= 14.8 and 13.8 Hz, C-3-Hb), 6.023 (1H, br. m, C-5-H), 2.511 (1H, dd, *J*= 14.4 and 5.1 Hz, C-6-Ha), 1.775 (1H, dd, *J*= 14.4 and 8.7 Hz, C-6-Hb), *ca* 1.25 (1H, overlapped, C-8-Ha), 0.914 (1H, dd, *J*= 11.7 and 8.2 Hz, C-8-Hb), 1.617 (1H, dd, *J*= 13.2 and 8.2 Hz, C-9-Ha), 1.355 (1H, ddd, *J*= 13.2, 10.7 and 7.8 Hz, C-9-Hb), 1.403 (1H, sept., *J*= 6.9 Hz, C-11-H), 0.991 (3H, d, *J*= 6.9 Hz, C-12-H₃), 0.663 (3H, d, *J*= 6.9 Hz, C-13-H₃), 9.238 (1H, s, C-14-H), 0.507 (3H, s, C-15-H₃).

Table 3-105 Physicochemical properties of RL-A-CPBA-1 (11)



11

A colorless syrup

Rf: 0.35 (H-EA 4:1), 0.21 (H-DCM-AT 25:25:1)

Vanillin-H₂SO₄ color: brown

EI-MS m/z (%): 234 (M^+ , 4.1), 216 (35), 201 (20), 183 (14), 173 (39), 145 (48), 143 (40), 131 (37), 129 (39), 128 (44), 119 (65), 115 (36), 105 (44), 91 (81), 79 (40), 77 (48), 53 (38), 44 (48), 43 (57), 41 (100).

¹H-NMR $\delta_{TMS}^{C_6D_6}$ (500 MHz): 9.211 (1H, s, C-14-H), 6.020 (1H, m, C-5-H), 3.072 (1H, m, C-3-Ha), 1.837 (1H, br. dd, J = 13.7 and 4.5 Hz, C-6-Ha), 1.507 (1H, sept., J = 6.9 Hz, C-11-H), 1.028 (3H, d, J = 6.9 Hz, C-12-H₃), 0.827 (3H, s, C-15-H₃), 0.761 (3H, d, J = 6.9 Hz, C-13-H₃).

By the NOE experiments, the relative configuration were revealed as **rel.* [1(*S*^{*}), 7(*R*^{*}), 10(*S*^{*}) in 10, and 1(*R*^{*}), 7(*R*^{*}), 10(*R*^{*}) in 11, respectively (Fig. 3-279). Tendency to be formed more 10 in the epoxidation of 9 is explicable with a steric hindrance on C-15 bridgehead methyl group. Compared with highly restricted stereo-selectivity during epoxidation of carota-1,4-dienaldehyde (3), this stereo-selection is rather loose. This fact is suggested that the stereo-selectivity in the oxygenations of 3, including the epoxidation and peroxylation, was mainly due to the steric hindrance of the isopropyl group on C-10 (Fig. 3-280).

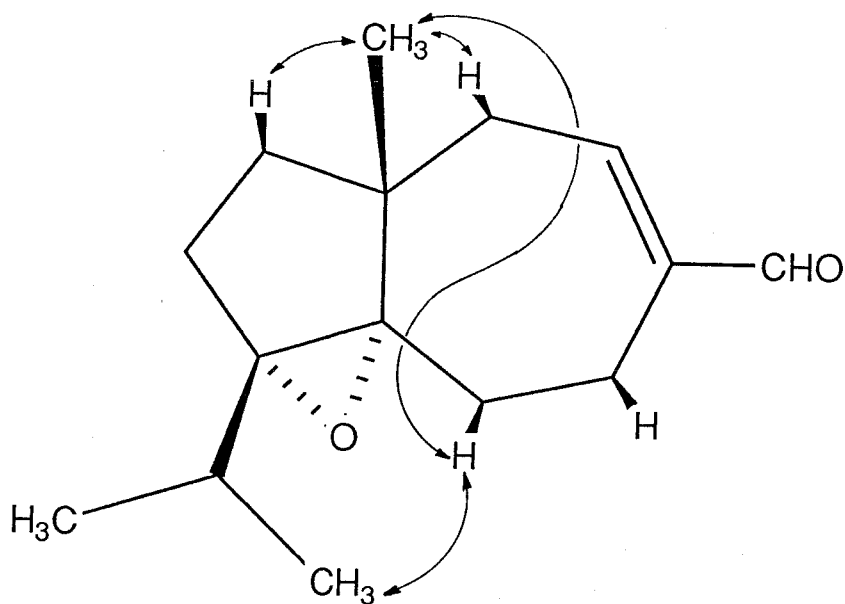
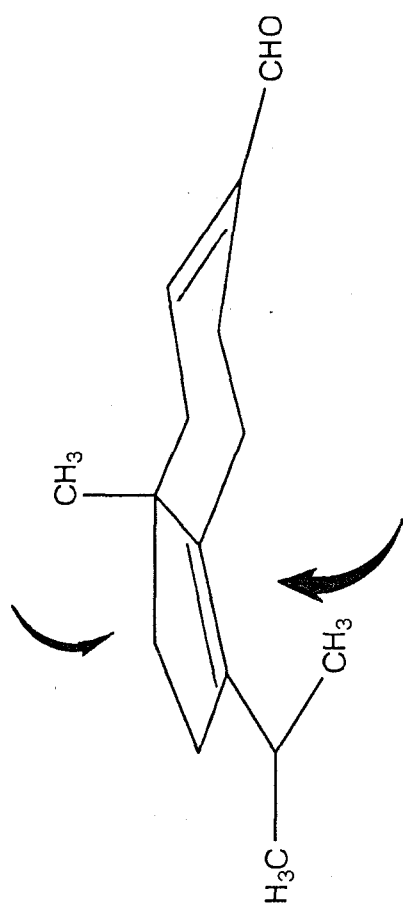
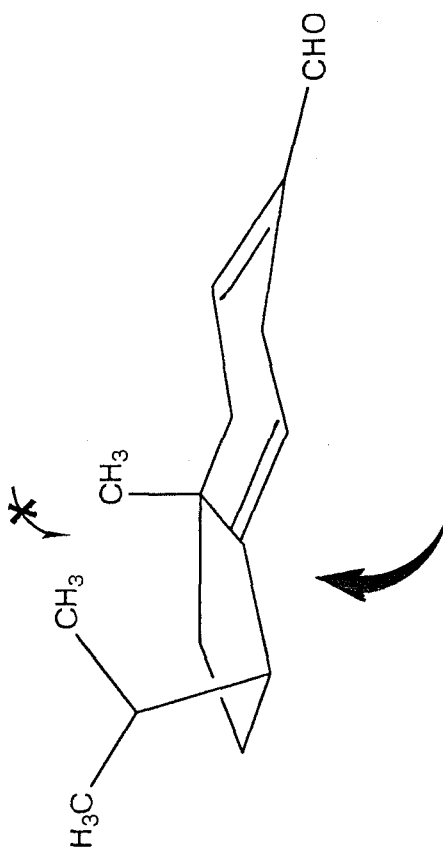


Fig. 3-279 Some NOEs Observed on RL-109B



RL-A (daucenaldehyde)



RL-C (carota-1,4-dienaldehyde)

Fig. 3-280 Steric Hindrance of the C-15 Methyl Group and the Isopropyl Group in Epoxidation of Daucenaldehyde (11) and Carota-1,4-dienaldehyde (3)

3-7-3 Isodaucenaldehyde and Its Related Compounds

1) Isodaucenaldehyde

Third peak of RL fraction (Fig. 3-281) isolated as colorless oil (ca 3 mg) was identical to RL-E in MS analysis (Fig. 3-282). This minor compound was revealed to possess an isopropenyl group instead of an isopropyl group (δ_{H} 4.811 and 4.720, each 1H, coupling with $J = 2.4$ Hz, assignable to exomethylene protons, and 1.690, 3H, br s, allylmethyl protons) in the ^1H -NMR spectrum. In addition, a methyl group on a quaternary carbon (δ_{H} 0.814, 3H, s) and a formyl proton at δ_{H} 9.348 both characteristic of *Rosa rugosa* carotane skeleton were detected (Fig. 3-283 and Table 3-106). Furthermore, its ^{13}C -NMR spectrum revealed the presence of two methine carbons at δ_{C} 56.2 and 50.3, in spite of the disappearance of the isopropyl methine carbon (Fig. 3-284 and Table 3-107).

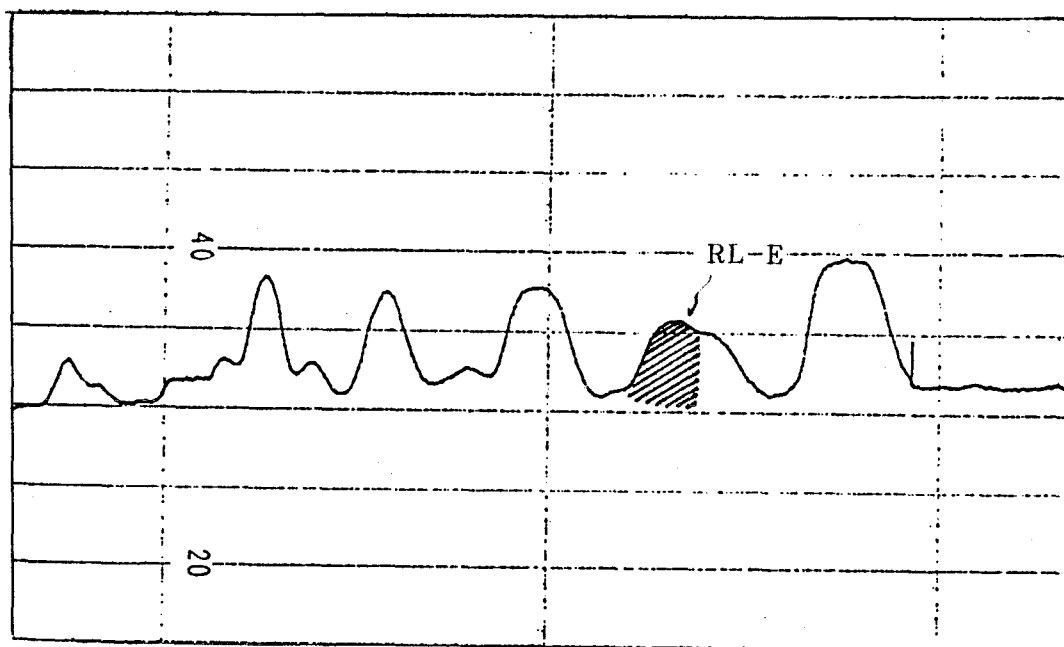


Fig. 3-281 HPL-Chromatogram of RL-E Detected at UV 230 nm

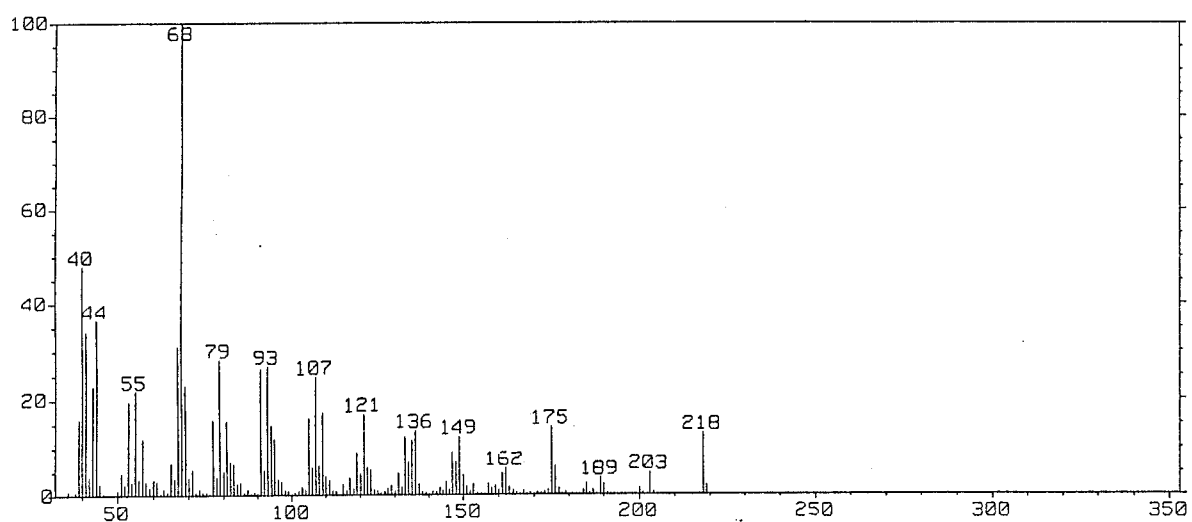
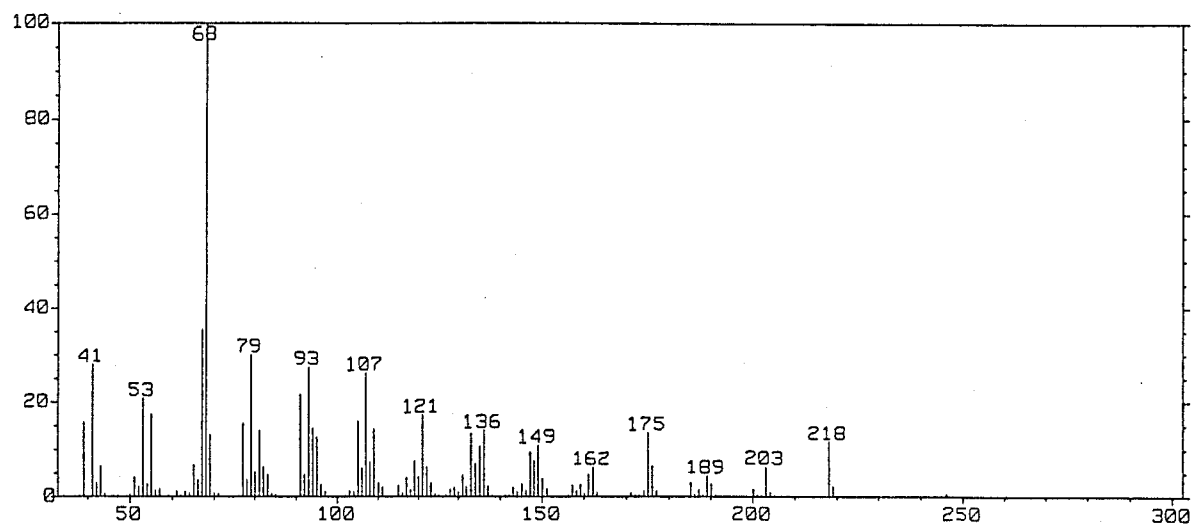


Fig. 3-282 GC-Mass (top) and EI-Mass (bottom) Spectra of RL-E

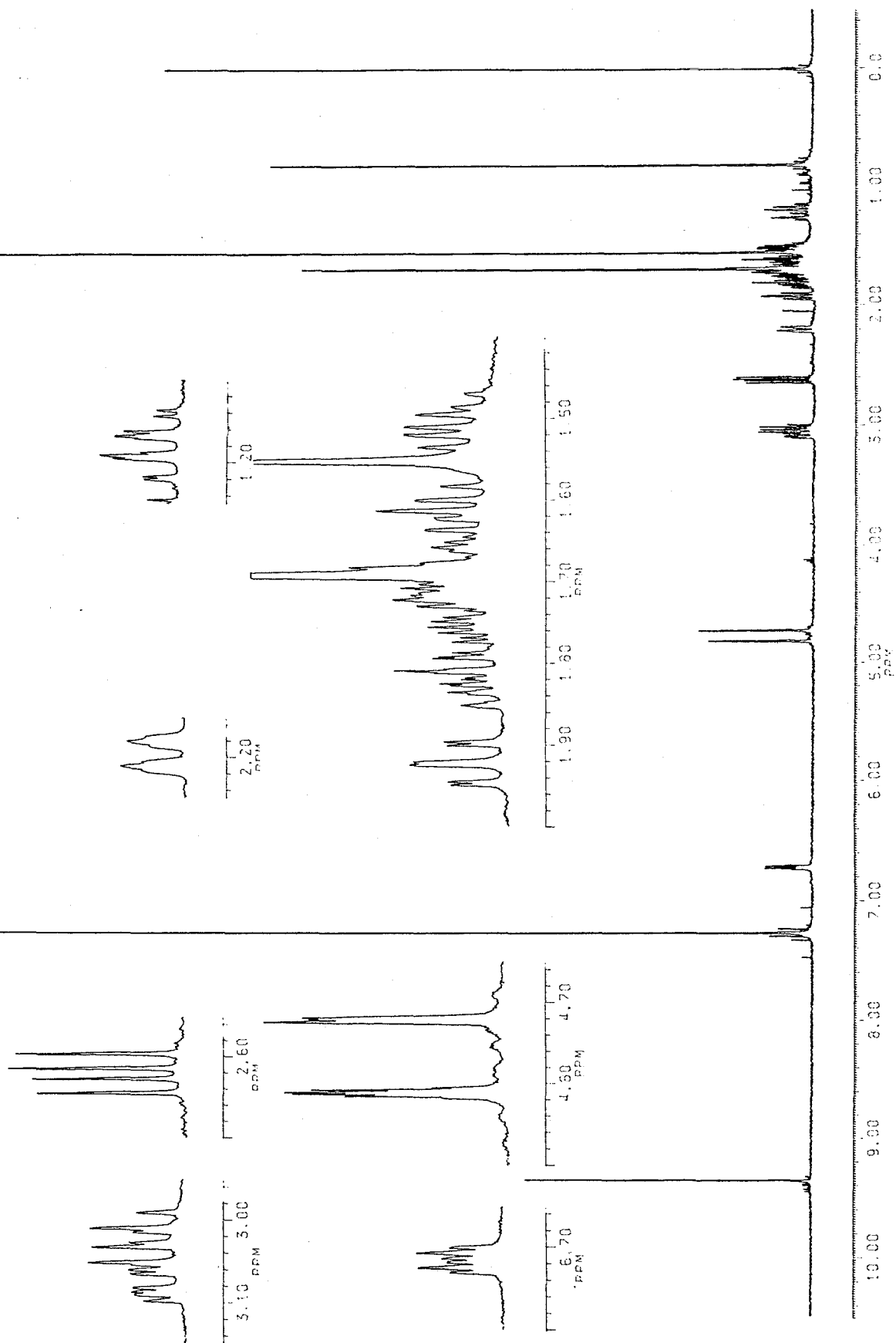


Fig. 3-283 ^1H -NMR Spectrum of RL-E (500 MHz, in CDCl_3)

Table 3-106 ^1H -NMR chemical shift values of RL-E

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}	Coupling			Assignment
1.921	1H	ddd	$J = 12.3, 11.6$ and 2.0 Hz	C-1-H
1.182	1H	dddd	$J = 13.6, 12.8, 12.3$ and 1.7 Hz	C-2-Ha
1.71 (approx.)	1H	m		C-2-Hb
3.073	1H	m dvid. d	$J = 16.5$ Hz	C-3-Ha
1.666	1H	m		C-3-Hb
6.751	1H	ddd	$J = 8.8, 3.3$ and 3.0 Hz	C-5-H
2.619	1H	dd	$J = 15.3$ and 8.8 Hz	C-6-Ha
2.195	1H	br. d	$J = 15.3$ Hz	C-6-Hb
1.617	1H	br. dd	$J = 11.7$ and 6.7 Hz	C-8-Ha
1.502	1H	ddd	$J = 12.4, 11.7$ and 8.2 Hz	C-8-Hb
1.757	1H	ddd	$J = 12.4, 9.2$ and 8.2 Hz	C-9-Ha
1.821	1H	dddd	$J = 12.4, 12.4, 6.7$ and 0.9 Hz	C-9-Hb
3.020	1H	ddd	$J = 11.6, 9.2$ and 9.1 Hz	C-10-H
4.811	1H	dd	$J = 2.4$ and 1.2 Hz	C-12-Ha
4.720	1H	d	$J = 2.4$ Hz	C-12-Hb
1.690	3H	d	$J = 1.2$ Hz	C-13- H_3
9.348	1H	s		C-14-H
0.814	3H	s		C-15- H_3

Since the single oxygen was attributable to the aldehyde group these methine carbons rather resonating in a lower magnetic field were deduced to be the C-10 tertiary methine and the C-1 bridgehead methine carbons, respectively. Spin-spin decoupling experiments and CH-COSY spectrum of RL-E (Fig. 3-285) clearly revealed a sequence of proton coupling to form a carotane skeleton as shown in Fig. 3-286. The methine carbons at δ_C 56.3 and δ_C 50.3 each showed a cross peak with proton signals of C-1-H (δ_H 1.921) and C-10-H (δ_H 3.020), respectively. Thus, RL-E was proved as an isomeric carotane dienaldehyde (12) and was named isodaucenaldehyde.

Stereostructure of 12 was determined by NOE experiments. By the irradiation on the C-15 methyl protons, an NOE's were observed on C-6-H_a, C-8-H_b, C-12-H_a and C-13-H₃, suggesting *rel** [7(*R**), 10(*R**) for 12 (Fig. 3-287). On the other hand, stereostructure at C-1 was determined by its proton coupling constant with vicinal C-10-H ($J = 11.6$ Hz). If C-1 are *R** (namely *cis*-fusion on C-1 and C-7), the dihedral angle between C10-C1-C1H and C1-C10-C10H provides

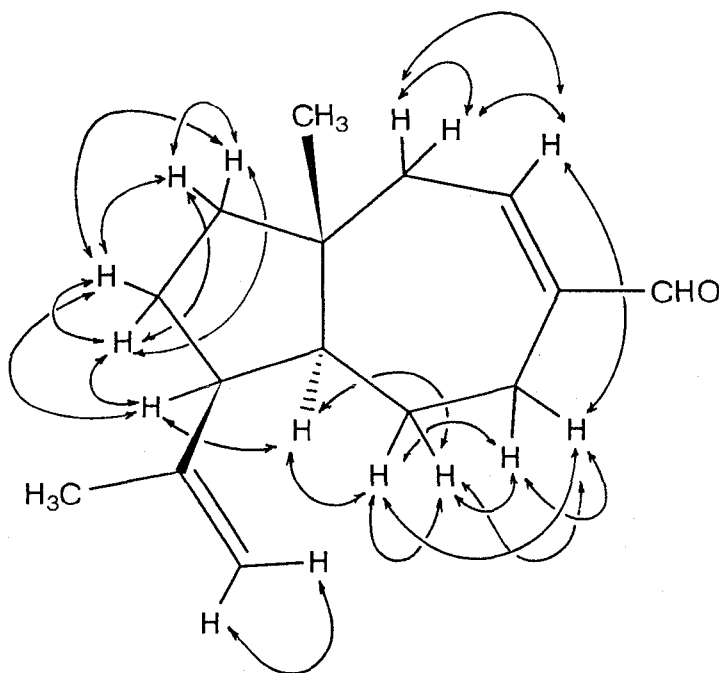


Fig. 3-286 Proton-Proton Coupling Sequence Elucidated by Decoupling Experiment on RL-E

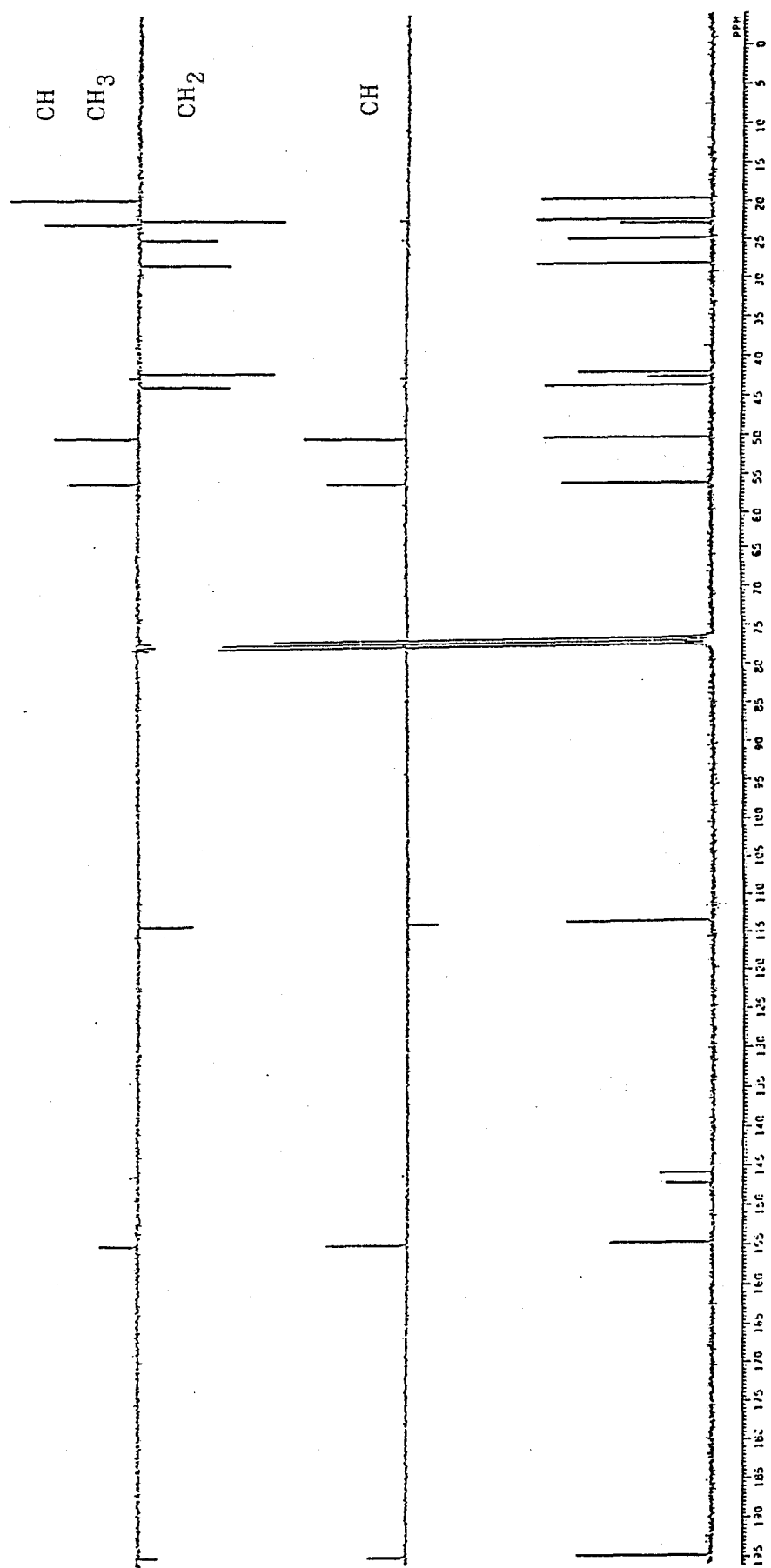


Fig. 3-284' ^{13}C -NMR Spectra of RL-E (68 MHz, in CDCl_3 , COM and INEPT)

Table 3-107 ^{13}C -NMR chemical shift values for RL-E

(78 MHz, COM and INEPT, in CDCl_3 , TMS as an int. std.)

δ_{C}	CHx	Assignment*
195.0	CH	C-14
154.7	CH	C-5
147.2	C	C-4
145.9	C	C-11
113.4	CH_2	C-12
56.2	CH	C-1
50.3	CH	C-10
43.7	CH_2	C-6
42.7	C	C-7
42.0	CH_2	C-8
28.2	CH_2	C-9
24.9	CH_2	C-3
23.0	CH_3	C-13
22.9	CH_2	C-2
19.7	CH_3	C-15

* The assignment was confirmed by the CH-COSY experiment on RL-E

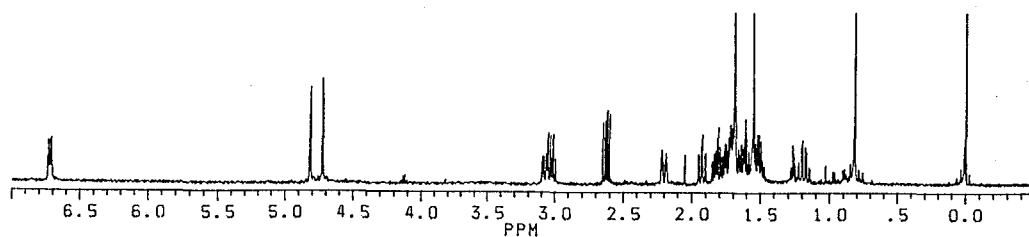
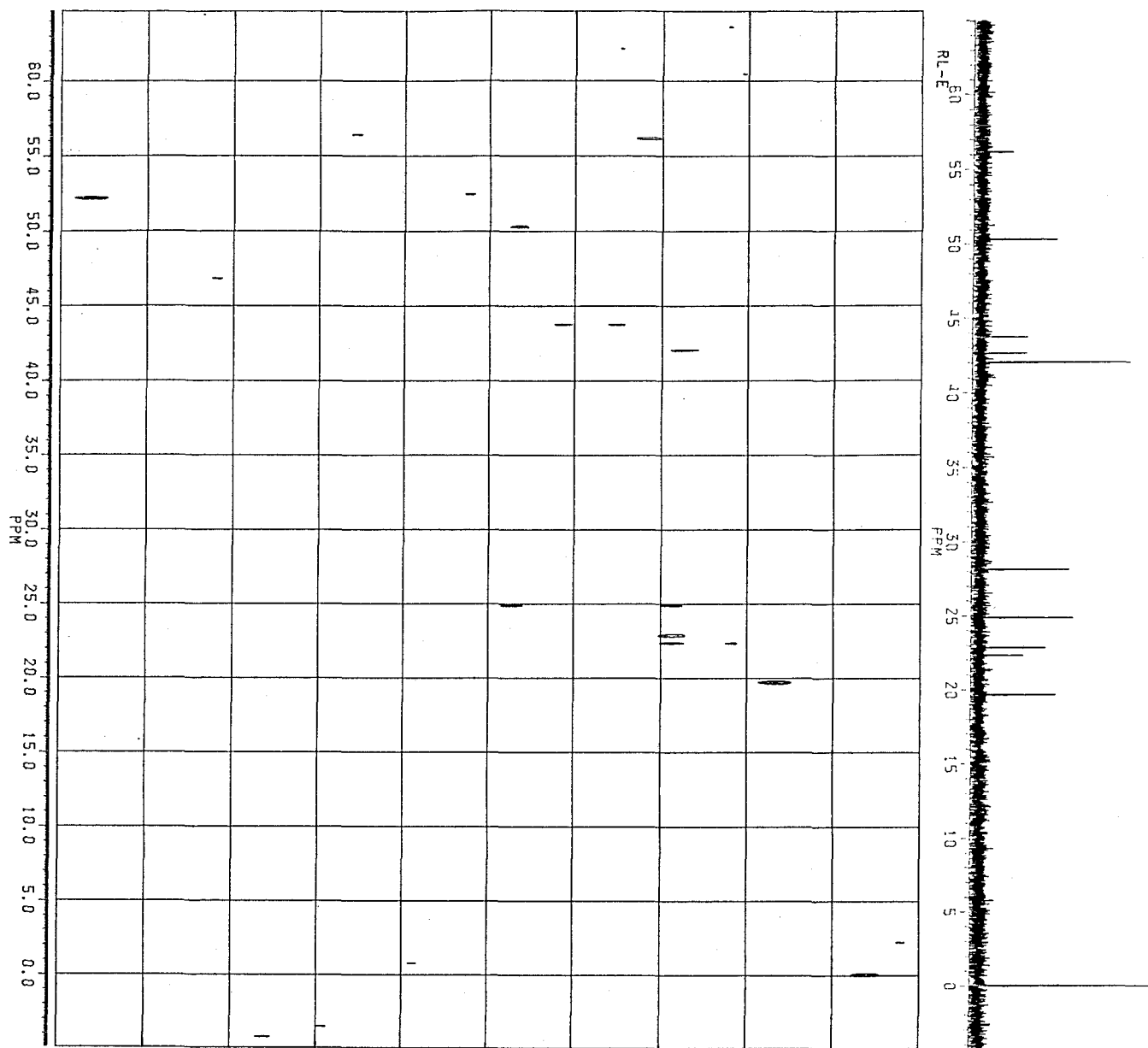


Fig. 3-285 CH-COSY Spectrum of RL-E (500 MHz, in C_6D_6): The methylene carbon at δ_C 28.2 unexpectedly showed no cross peak with proton signals; however, the carbon was eventually assigned to be C-9 whose protons were also not attributable by the spectrum.

nearly 90° in the molecular model, while a model possessing S^* configuration at C-1 (*trans*-fused 5,7-bicyclic) indicates a small angle (Fig. 3-288). In the light of Jackman-Sternhell function, the coupling constant $J = 11.6$ Hz between C-1-H and C-10-H is obviously compatible with C-10 S^* configuration.

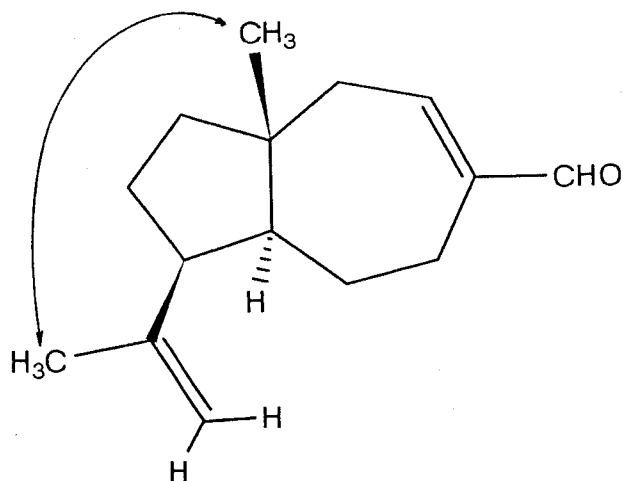


Fig. 3-287 NOE's Observed on RL-E: The mutual NOE between C-15-H₃ and C-13-H₃ indicated that those groups were on the same side of the five-membered ring.

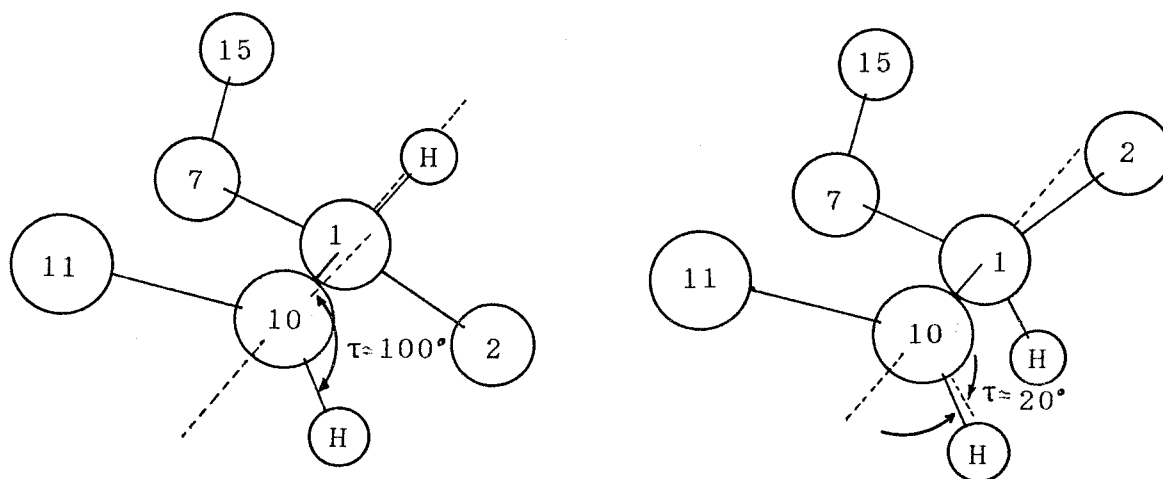
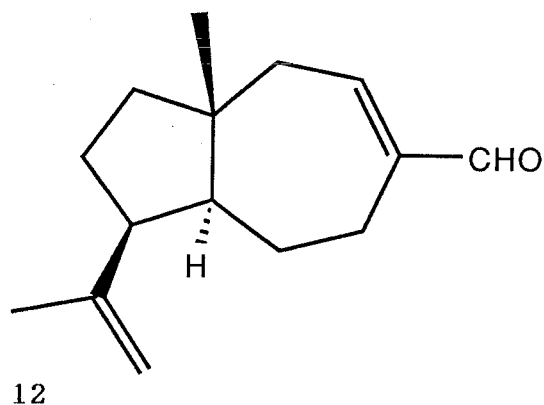


Fig. 3-288 Dihedral Angles between C10-C1-C1H and C1-C10-C10H in the Cases of C-1 R^* and C-1 S^*

Table 3-108 Physicochemical properties of isodaucenaldehyde (12)



A colorless oil

Vanillin- H_2SO_4 color: brown

GC-MS m/z (%): 218 (M^+ , 12), 203 (6.3), 189 (4.6), 176 (6.8), 175 (14), 162 (6.4), 149 (11), 147 (9.7), 136 (14), 135 (11), 133 (14), 121 (17), 109 (15), 107 (26), 105 (16), 95 (13), 94 (15), 93 (28), 91 (22), 81 (14), 79 (30), 77 (16), 68 (100), 67 (35), 55 (17), 53 (21), 41 (28).

EI-MS m/z (%): 218 (M^+ , 13), 203 (4.9), 189 (3.8), 176 (6.2), 175 (14), 162 (5.8), 149 (12), 147 (9.0), 136 (14), 135 (12), 133 (12), 121 (17), 109 (17), 107 (25), 105 (16), 95 (12), 94 (15), 93 (27), 91 (26), 81 (16), 79 (28), 77 (16), 68 (100), 67 (31), 55 (22), 53 (20), 44 (37), 41 (34), 40 (48)

^1H - and ^{13}C -NMR data are shown in Table 3-106 and 3-107, respectively.

2) Isodaucenoic Acid

As described in Section 5, the fraction containing carota-1,4-dienoic acid (4) further involved an unknown substance isomeric to 4 and indicative of some carotane nature. After 4 was autoxidized to yield RL-115MA-OX (4b) and rugosic acid A (2), the residue recovered by PTLC yielded fine crystallines during the concentration (Fig. 3-289). When the crystallines were carefully washed with cool *n*-hexane, ca 2.0 mg of colorless plates were obtained. This acidic substance (RL-115M-B) indicating M^+ 234 in EI-MS (Fig. 3-290) was revealed by ^1H - and ^{13}C -NMR analyses to be a carotane acid (structure 13) corresponding to isodaucenaldehyde (12) (Fig. 3-291 and 292). Most of the proton signals were similar to those of 12, except C-14 formyl proton. The acid was the sub-major constituent of the RL-115M fraction, unlike the carotane diene aldehyde mixture (RL faction) in which 12 was only a minor constituent.

From the mixture of RL-115-M methylation products (See pp. 382), the methyl ester of 13 (RL-115M-B-ME) was also isolated. Its spectroscopic data are shown in Fig. 3-293, 294.

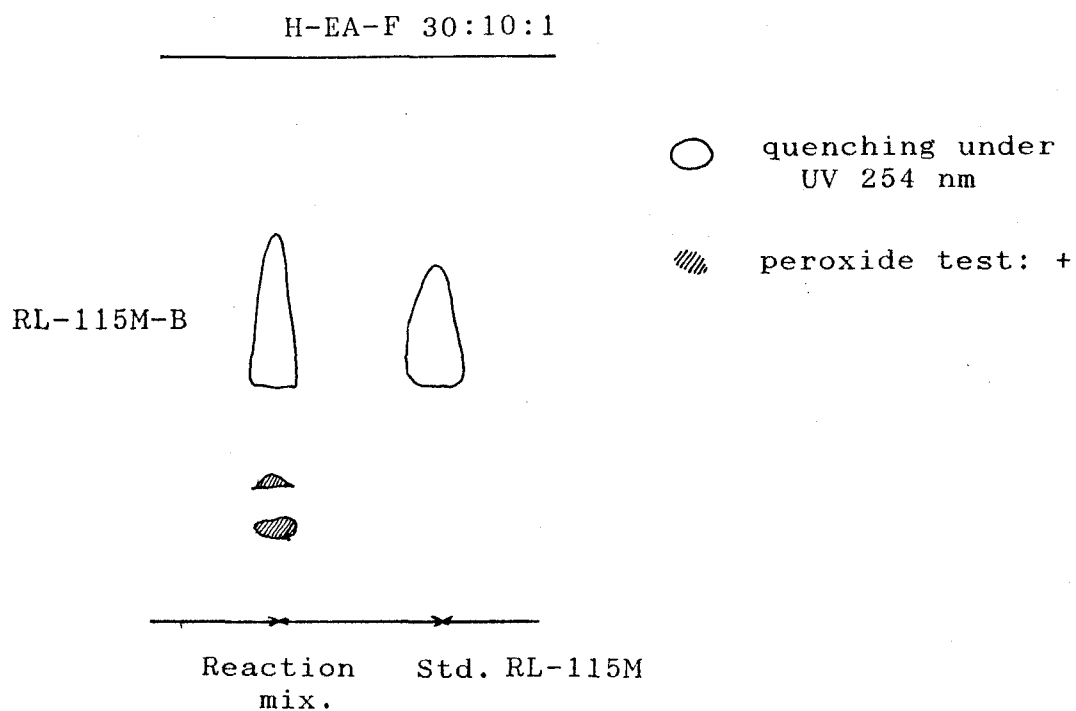


Fig. 3-289. TL Chromatograms of RL-115M Fraction Contained RL-115M- B

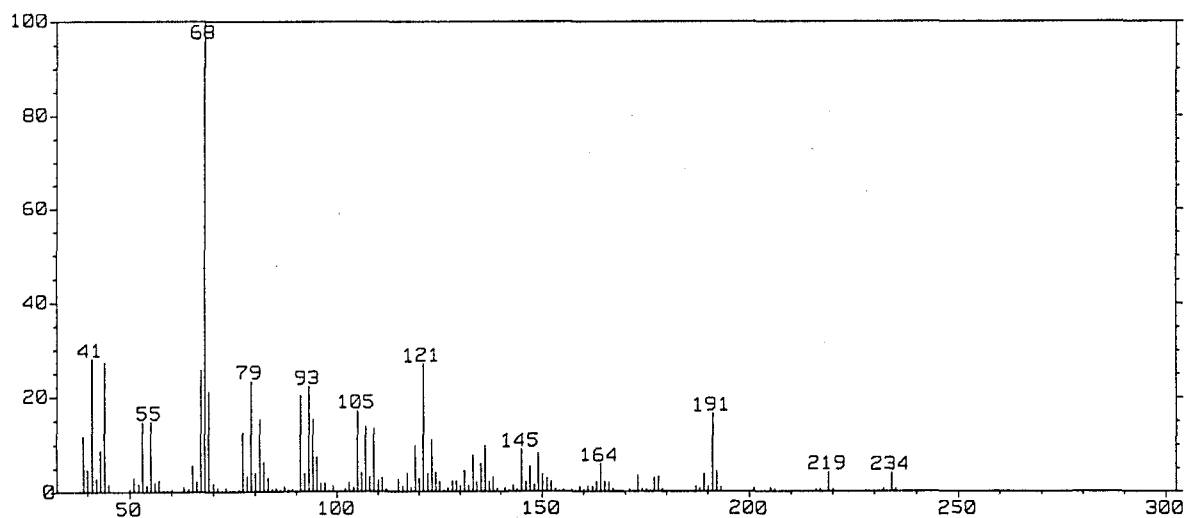


Fig. 3-290 EI-Mass Spectrum of RL-115M-B

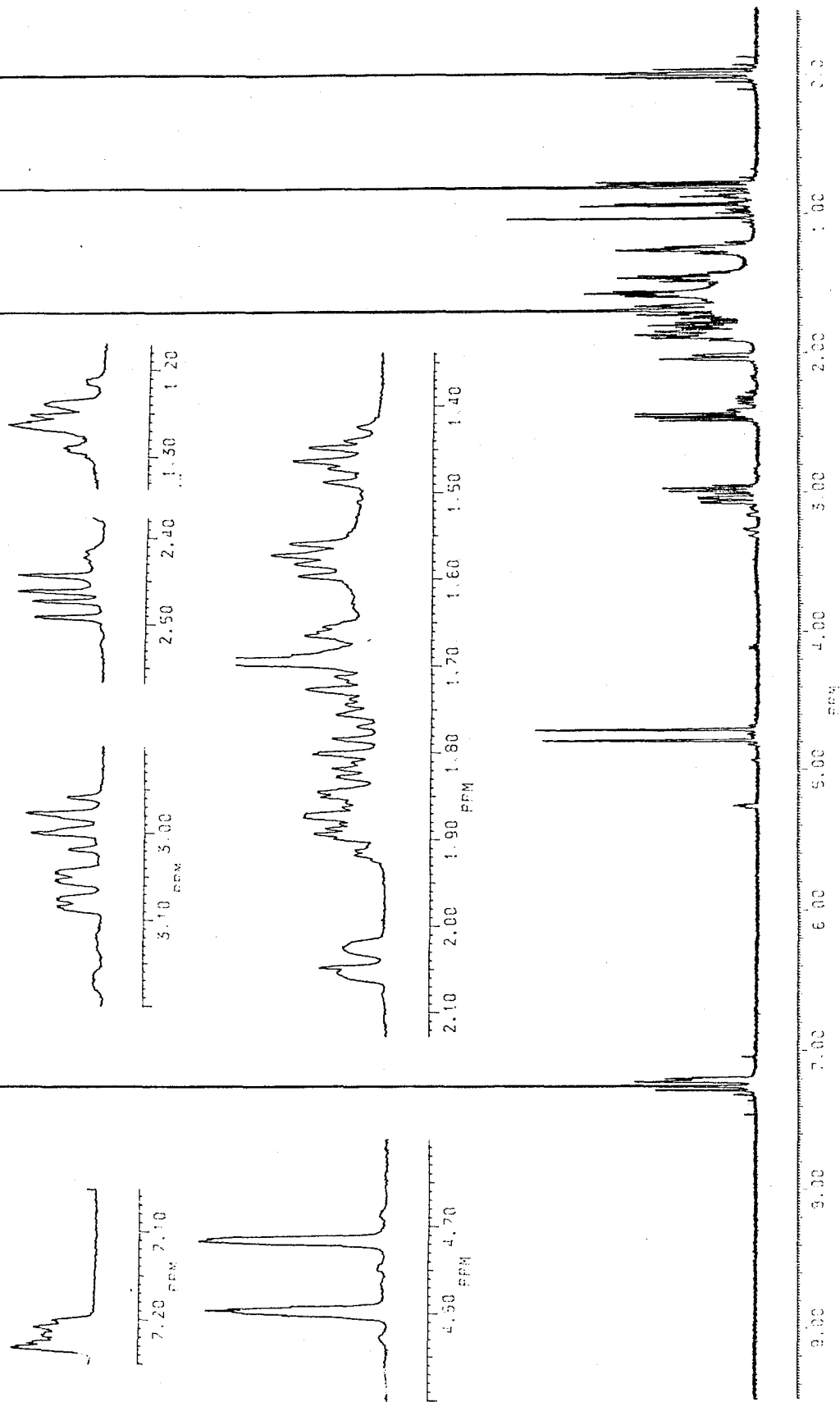


Fig. 3-291 ^1H -NMR Spectrum of RL-115M-B (500 MHz, in CDCl_3)

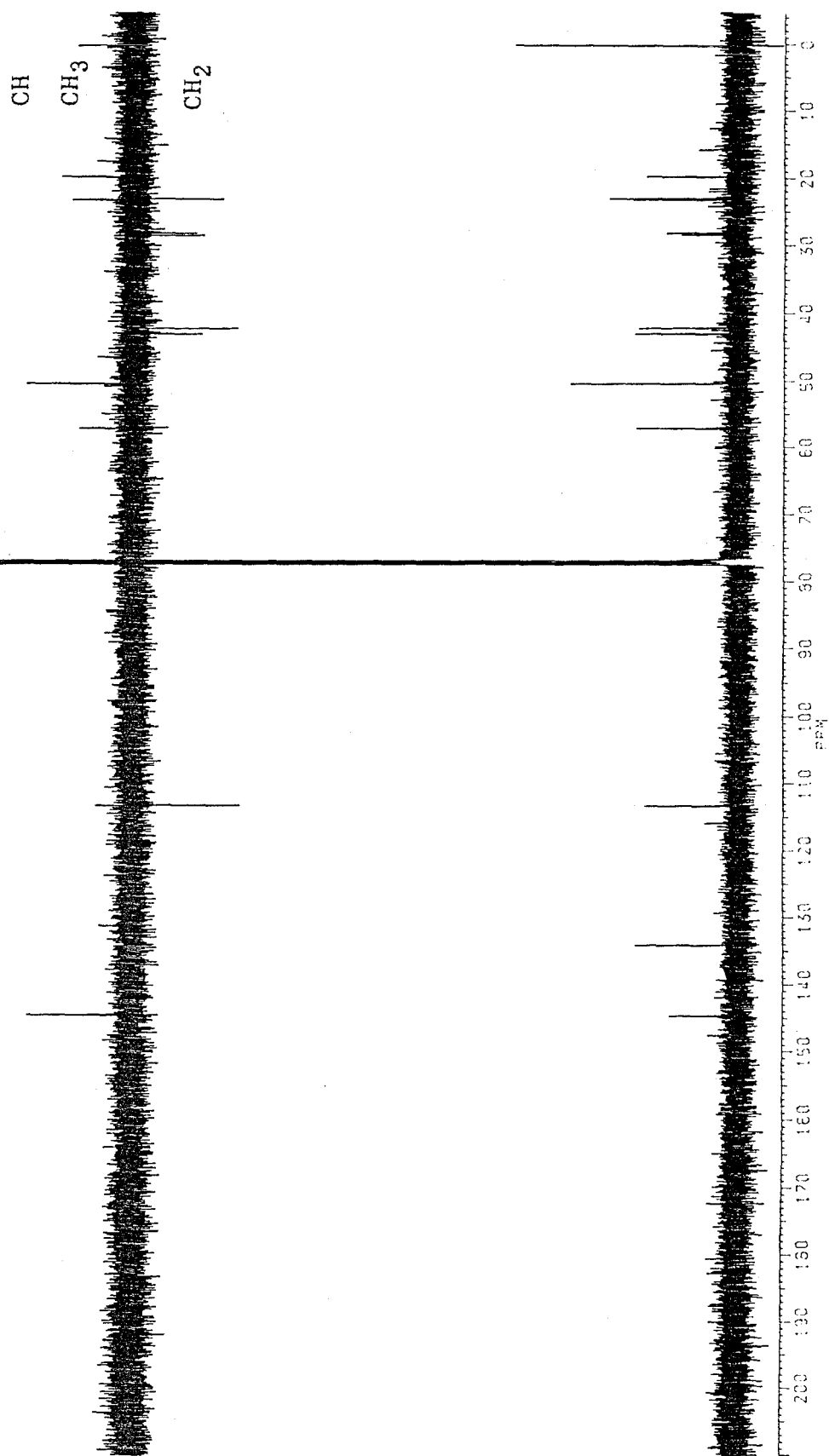


Fig. 3-292 ^{13}C -NMR Spectra of RL-115M-B (125 MHz, in CDCl_3 , COM and DEPT)

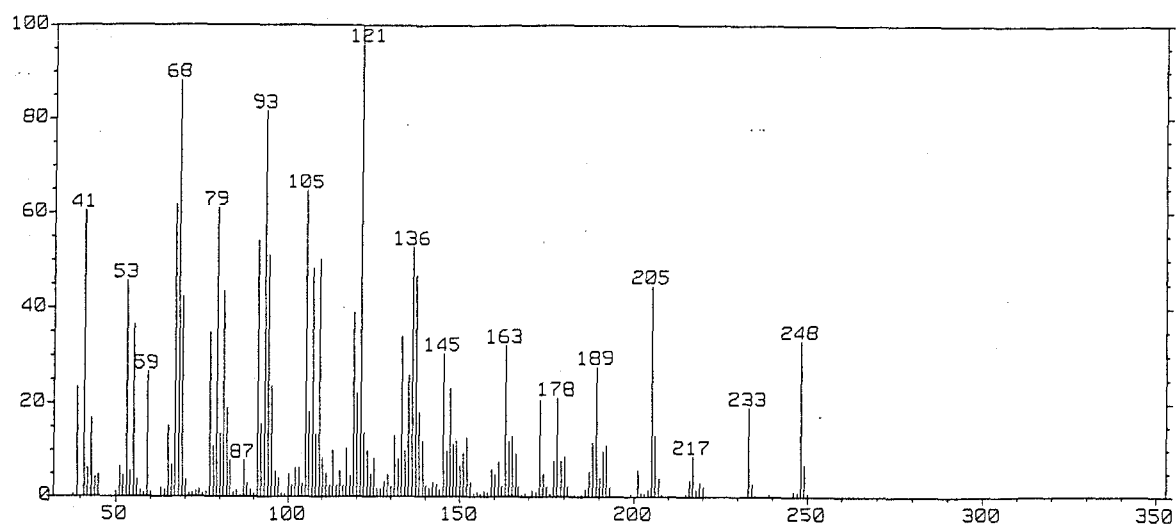


Fig. 3-293 EI-Mass Spectrum of RL-115M-B-ME

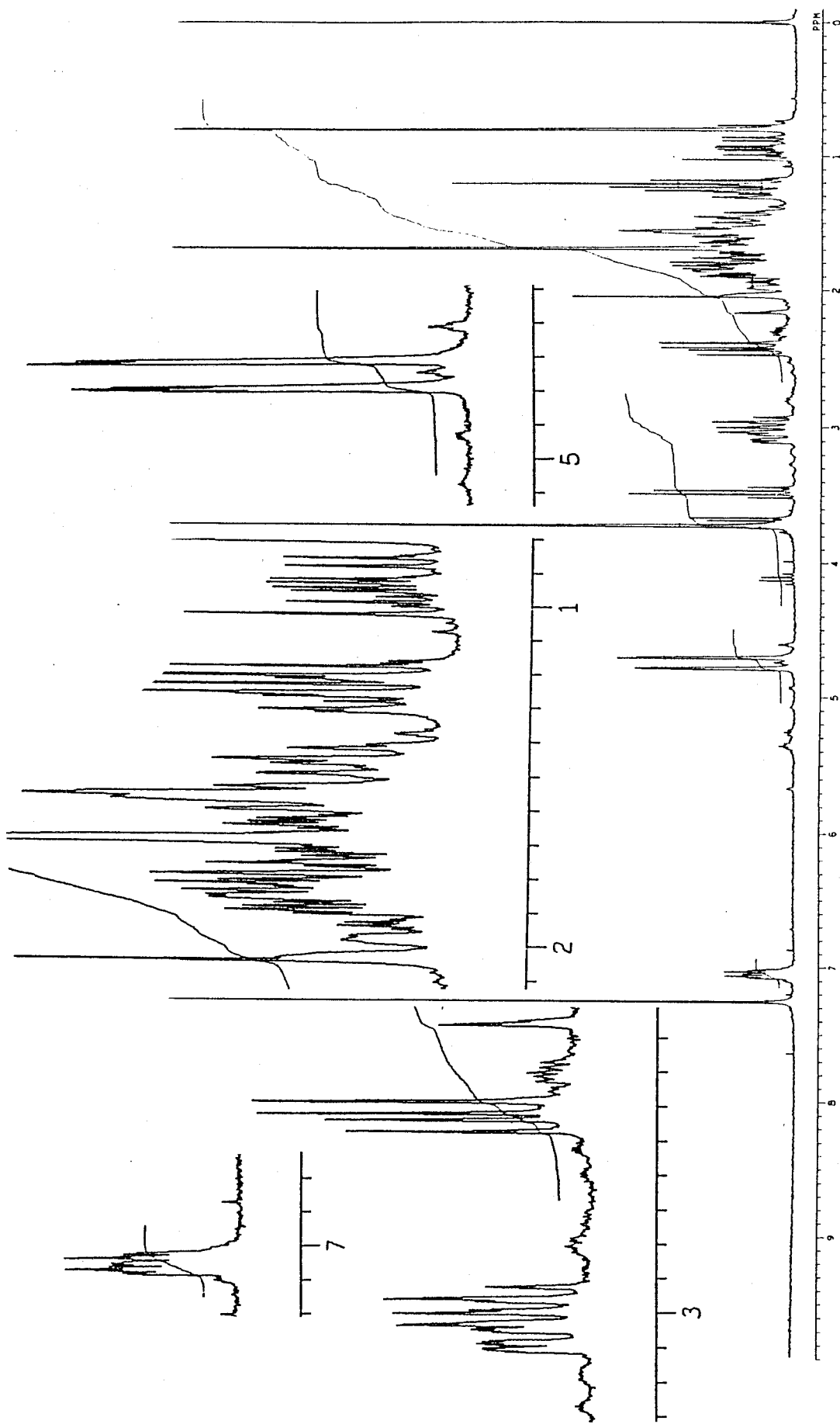
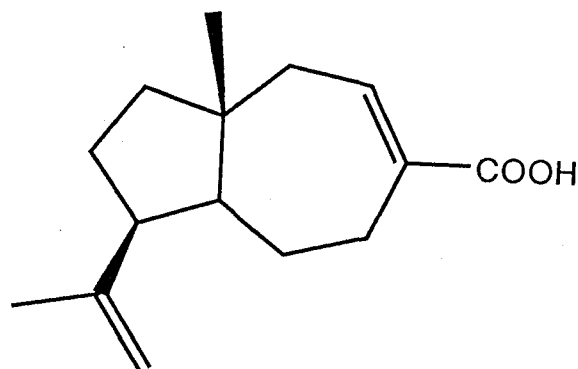


Fig. 3-294 ^1H -NMR Spectrum of RL-115M-B-ME (270 MHz, in CDCl_3)

Table 3-109 Physicochemical properties of isodaucenoic acid (RL-115M-B, 13)



13

Colorless powder

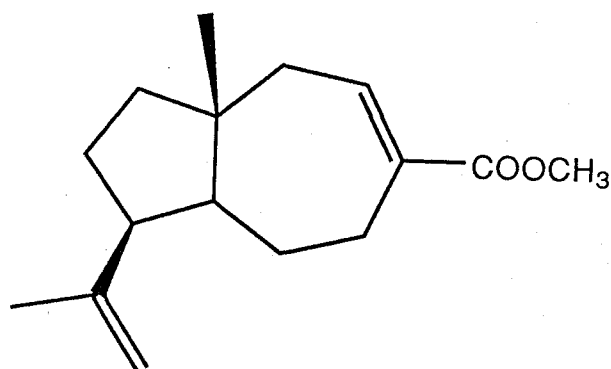
Vanillin-H₂SO₄ color: clear pink

EI-MS m/z (%): 234 (M^+ , 4.0), 219 (4.2), 191 (17), 164 (6.0), 149 (8.3), 145 (9.1), 136 (9.9), 123 (11), 121 (27), 109 (13), 107 (14), 105 (17), 94 (15), 93 (22), 91 (20), 81 (15), 79 (23), 77 (12), 69 (21), 68 (100), 67 (26), 55 (15), 53 (15), 53 (15), 44 (27), 41 (28).

¹H-NMR $\delta_{TMS}^{CDCl_3}$ (500 MHz): 1.847 (1H, overlapped, C-1-H), 1.261 (1H, m, C-2-Ha), 1.716 (1H, m, C-2-Hb), 3.050 (1H, m dvid. dd, J = 15.4 and 5.4 Hz, C-3-Ha), *ca* 1.68 (1H, m, C-3-Hb), *ca* 7.22 (1H, m, C-5-H), 2.469 (1H, dd, J = 14.8 and 9.3 Hz, C-6-Ha), 2.039 (1H, br. d, J = 14.8 Hz, C-6-Hb), 1.577 (1H, br. dd, J = 11.8 and 6.9 Hz, C-8-Ha), 1.455 (1H, ddd, J = 12.2, 11.9 and 8.0 Hz, C-8-Hb), 1.792 (1H, m, C-9-Ha), *ca* 1.88 (1H, m, C-9-Hb), 2.989 (1H, ddd, J = 11.4, 9.1 and 9.1 Hz, C-10-H), 4.797 (1H, dd, J = 2.3 and 1.3 Hz, C-12-Ha), 4.716 (1H, d, J = 1.3 Hz, C-12-Hb), 1.694 (3H, br. s, C-13-H₃), 0.814 (3H, s, C-15-H₃).

¹³C-NMR $\delta_{TMS}^{CDCl_3}$ (78 MHz): 56.9 (CH, C-1), 22.8 (CH₂, C-2), 27.9 (CH₂, C-3), 134.0 (C, C-4), 144.6 (CH, C-5), 42.7 (C-H₂, C-6), 42.3 (C, C-6), 41.9 (CH₂, C-7), 28.2 (CH₂, C-9), 50.1 (CH, C-10), 147.4 (C, C-11), 113.1 (CH₂, C-12), 23.0 (CH₃, C-13), 172.2 (C, C-14), 19.5 (CH₃, C-15).

Table 3-110 Physicochemical properties of RL-115MB-ME (13a)



13a

A colorless syrup

Vanillin-H₂SO₄ color: clear pink

EI-MS m/z (%): 248 (M^+ , 33), 233 (19), 217 (8.4), 205 (45), 189 (28), 178 (21), 173 (21), 163 (32), 145 (30), 137 (47), 136 (53), 133 (34), 121 (100), 119 (50), 107 (48), 105 (65), 94 (51), 93 (82), 91 (54), 81 (43), 79 (61), 77 (35), 69 (42), 68 (88), 67 (62), 55 (37), 53 (37), 53 (37), 41 (60).

¹H-NMR $\delta_{TMS}^{C_6D_6}$ (500 MHz): 3.069 (1H, dddd, J = 15.0, 5.5, 2.2 and 1.1 Hz, C-3-Ha), 7.053 (1H, m, C-5-H), 2.431 (1H, dd, J = 14.6 and 9.2 Hz, C-6-Ha), *ca* 2.00 (1H, br. d, J = 14.6 Hz, C-6-Hb), 2.982 (1H, ddd, J = 11.4, 9.2 and 9.2 Hz, C-10-H), 4.792 (1H, dd, J = 2.2 and 1.1 Hz, C-12-Ha), 4.712 (1H, d, J = 2.2 Hz, C-12-Hb), 1.692 (3H, d, J = 1.1 Hz, C-13-H₃), 3.724 (1H, s, C-14'-H₃), 0.806 (3H, s, C-15-H₃).

3) Isodaucenaldehyde Hydroxyl Derivative

Hydroxycarotaldehyde was found as a spot with *R_f* 0.26 and positive to DNPH reagent (orange) on silica gel plates charged Fr-V-8 (from Sample V, See pp. 589) and developed in H-EA 3:1 (Fig. 3-295). By column chromatography and PTLC of the fraction Sample V, ca 4 mg of the focused substance was isolated as a colorless syrup. The compound denoted as RL-117C was also expected to be a carotane aldehyde, which showed M^+ 236 (18 %) with base peak at m/z 59 in FI-MS (236.173 in FI-HR-MS, $C_{15}H_{22}O_2$, calcd. 236.178) (Fig. 3-296). On the other hand, a dehydration peak at m/z 214 was observed in EI-MS (Fig. 3-297). The presence of a hydroxyl group was suggested by its IR spectrum appearing as a broad absorption at 3430 cm^{-1} (Fig. 3-298).

In the ^1H -NMR and HH-COSY analyses (Fig. 3-299, 300 and Table 3-111), RL-117C showed a similar signal pattern to that of isodaucenaldehyde (12) except protons attributable to C-11, 12 and 13. The signals for the side-chain revealed the presence of $\blacksquare$ -C(OH)-(CH₃)₂. The ^{13}C -NMR of the compound indicated a non-hydrogen-bearing oxygenated carbon at δ_{C} 73.3 assignable to the C-11 (Fig. 3-301 and Table 3-112). Furthermore, two methine carbons resonating in a downfield at δ_{C} 56.5 and 53.1 resonating downfield were characteristic of a tertiary or a bridgehead carbon. As C-1 and C-10 methine carbons of 12 were detected at δ_{C} 56.2 and 50.3, respectively, the former methine carbons of RL-117C were similarly assigned to those positions. Consequently, structure of RL-117C was elucidated as 14, and this novel carotanoid was named hydroxycarotanaldehyde.

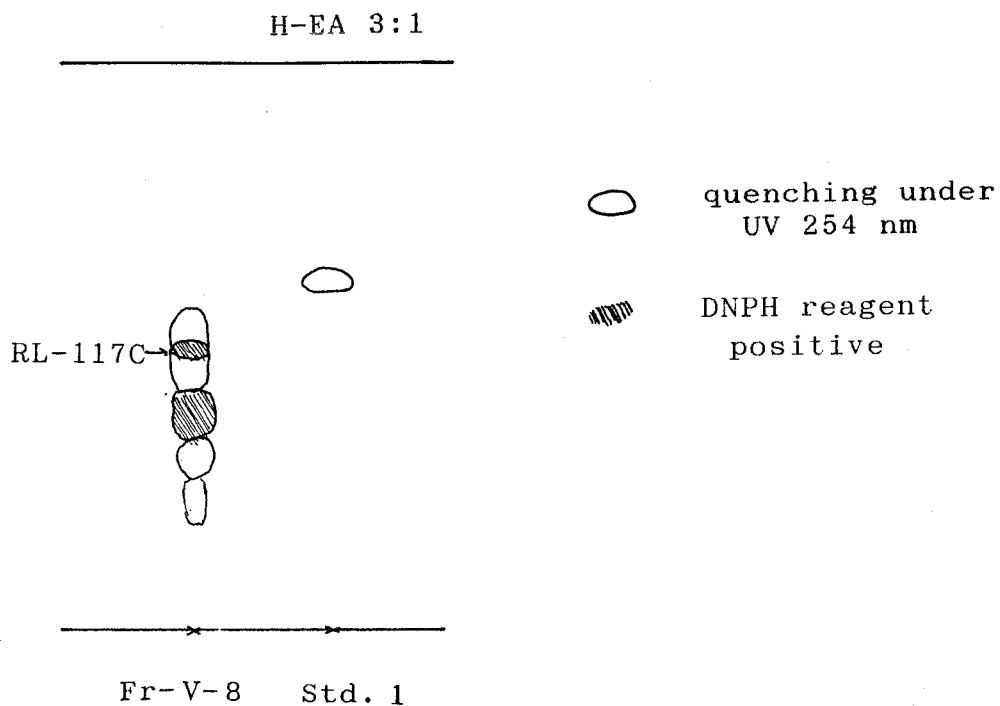


Fig. 3-295 TL Chromatogram of RL-117C

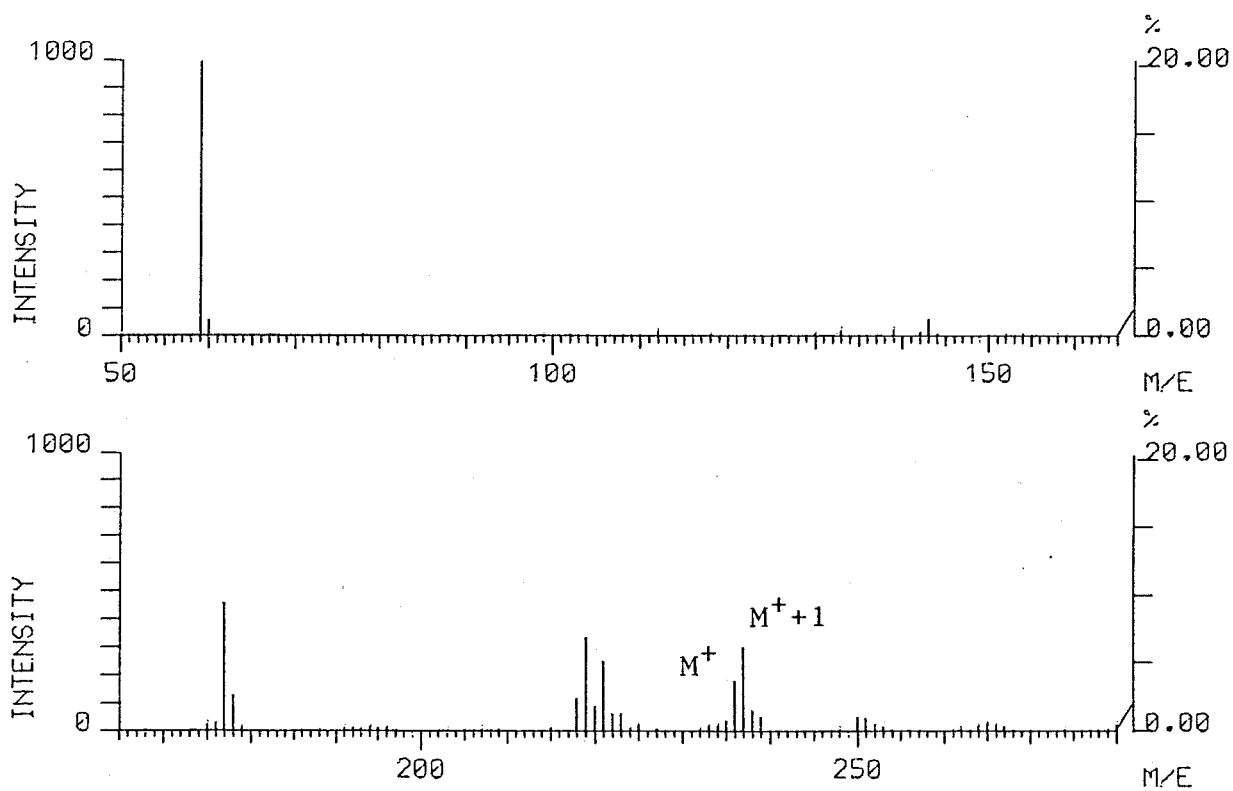


Fig. 3-296 FI-Mass Spectrum of RL-117C

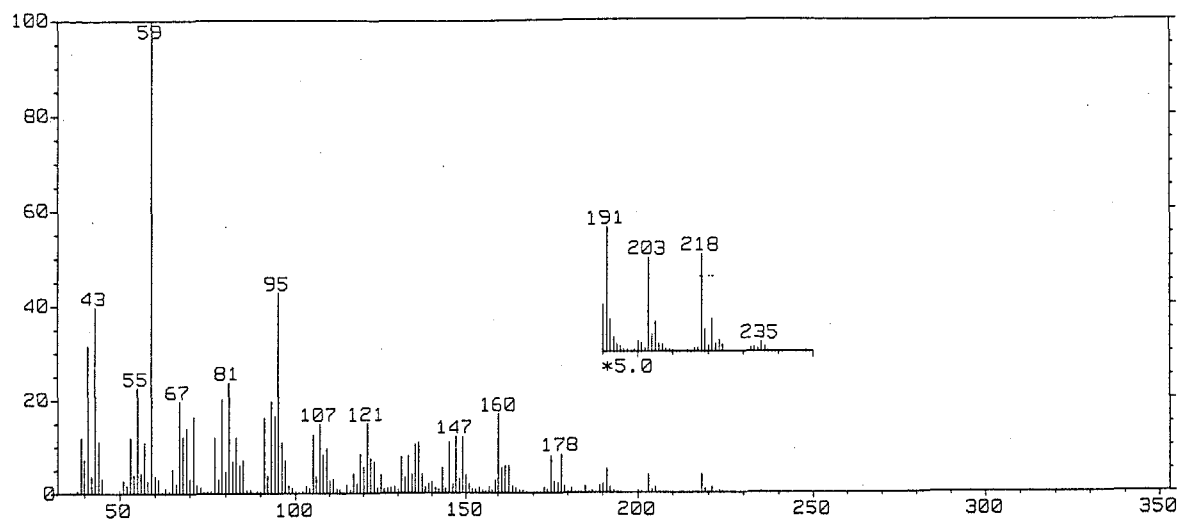


Fig. 3-297 EI-Mass Spectrum of RL-117C

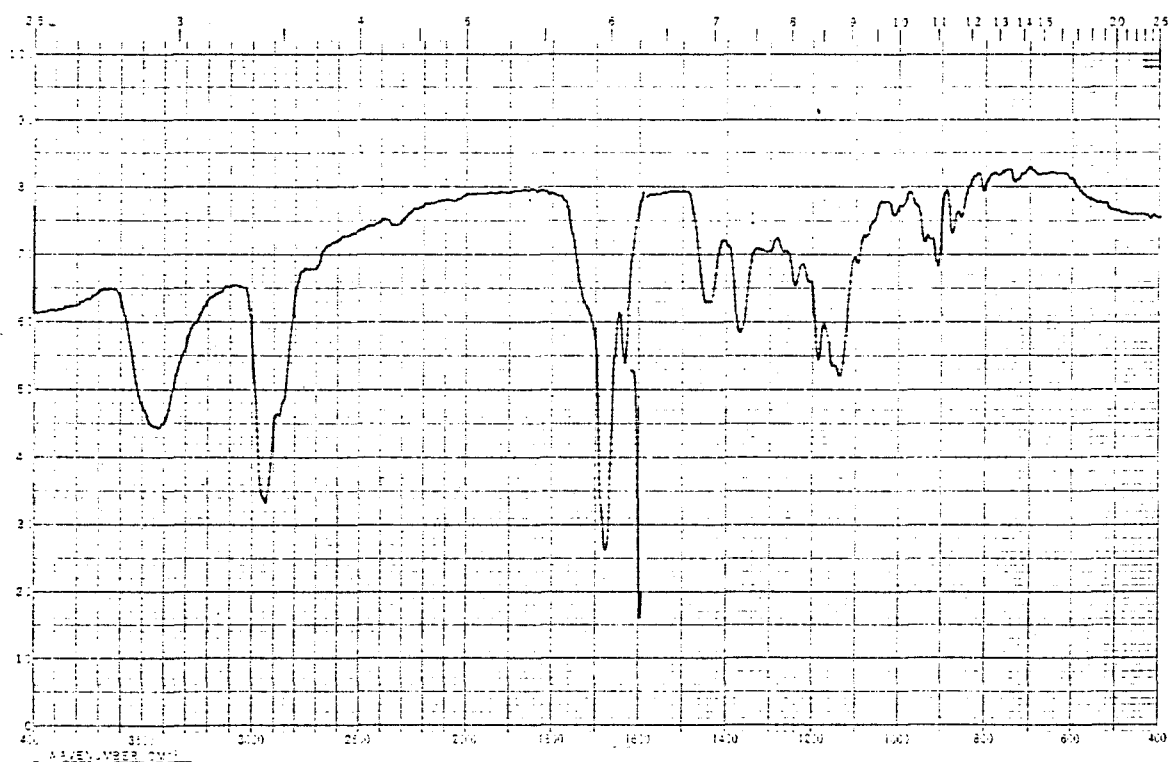


Fig. 3-298 IR Spectrum of RL-117C

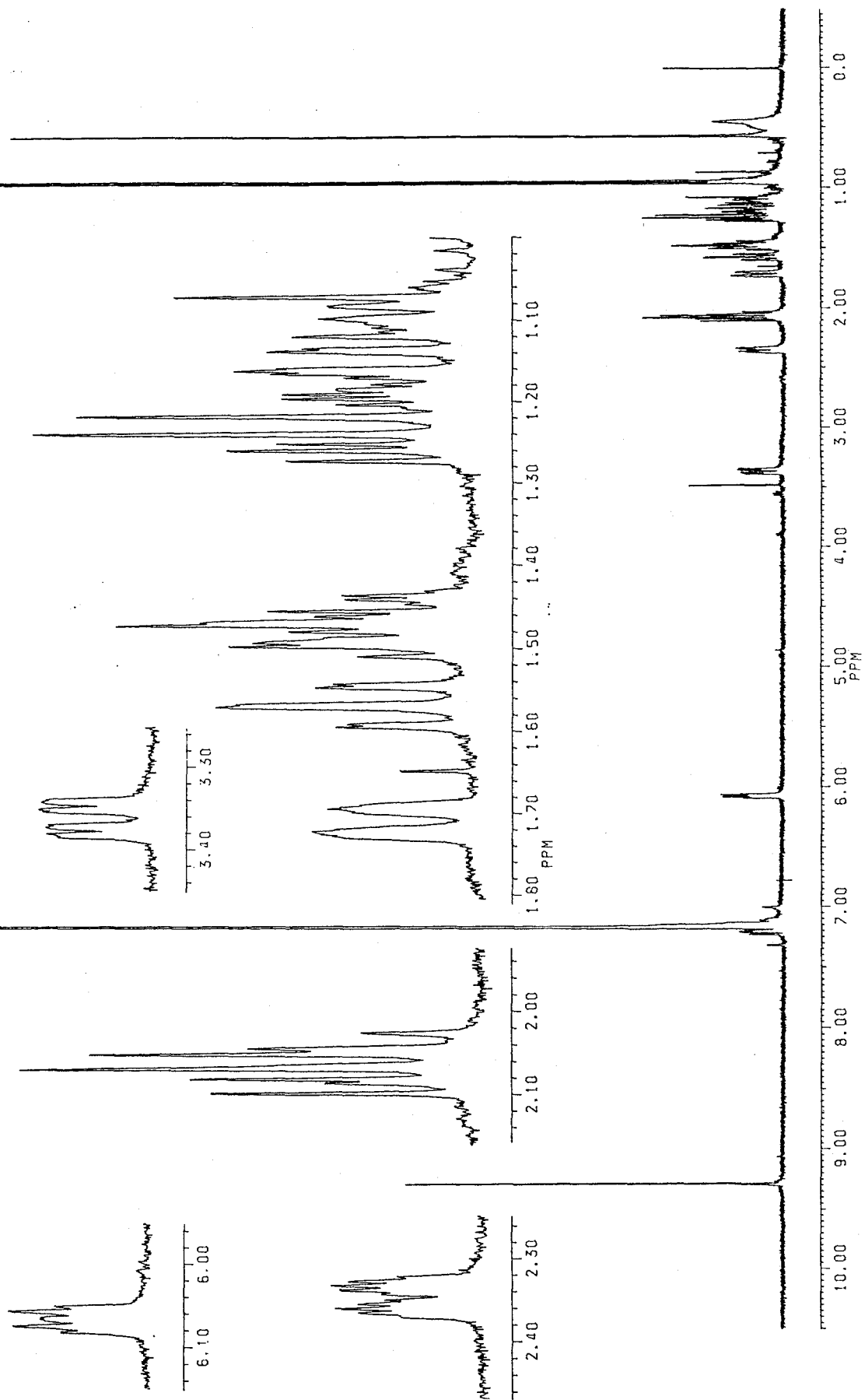


Fig. 3-299 ^1H -NMR Spectrum of RL-117C (500 MHz, in C_6D_6)

Table 3-111 ^1H -NMR chemical shift values of RL-117C

(500 MHz, in C_6D_6 , TMS as an int. std.)

δ_{H}	Coupling		Assignment
9.283	1H	s	C-14-H
6.067	1H	ddd $J= 8.8, 3.3, 3.2$ Hz	C-5-H
3.361	1H	dddd $J= 15.3, 5.1, 2.3, 0.8$ Hz	C-3-Ha
2.345	1H	m dvid. d $J= 13.9$ Hz	C-2-Ha
2.074	1H	dd $J= 15.1, 8.8$ Hz	C-6-Ha
2.056	1H	ddd $J= 12.2, 9.1, 9.0$ Hz	C-10-H
1.708	1H	br. d $J= 15.1$ Hz	C-6-Hb
1.569	1H	ddd $J= 12.2, 11.1, 1.7$ Hz	C-1-H
1.482	1H	m	C-9-Ha
1.466	1H	m	C-3-Hb
1.245	1H	m	C-8-Ha
1.20 (approx.)	1H	overlapped d-like	C-9-Hb
1.148	1H	dddd $J= 12.6, 12.6, 12.5, 2.2$ Hz	C-2-Hb
1.088	1H	ddd $J= 11.0, 10.5, 8.1$ Hz	C-8-Hb
0.951	3H	s	C-12- H_3
0.935	3H	s	C-13- H_3
0.564	3H	s	C-15- H_3

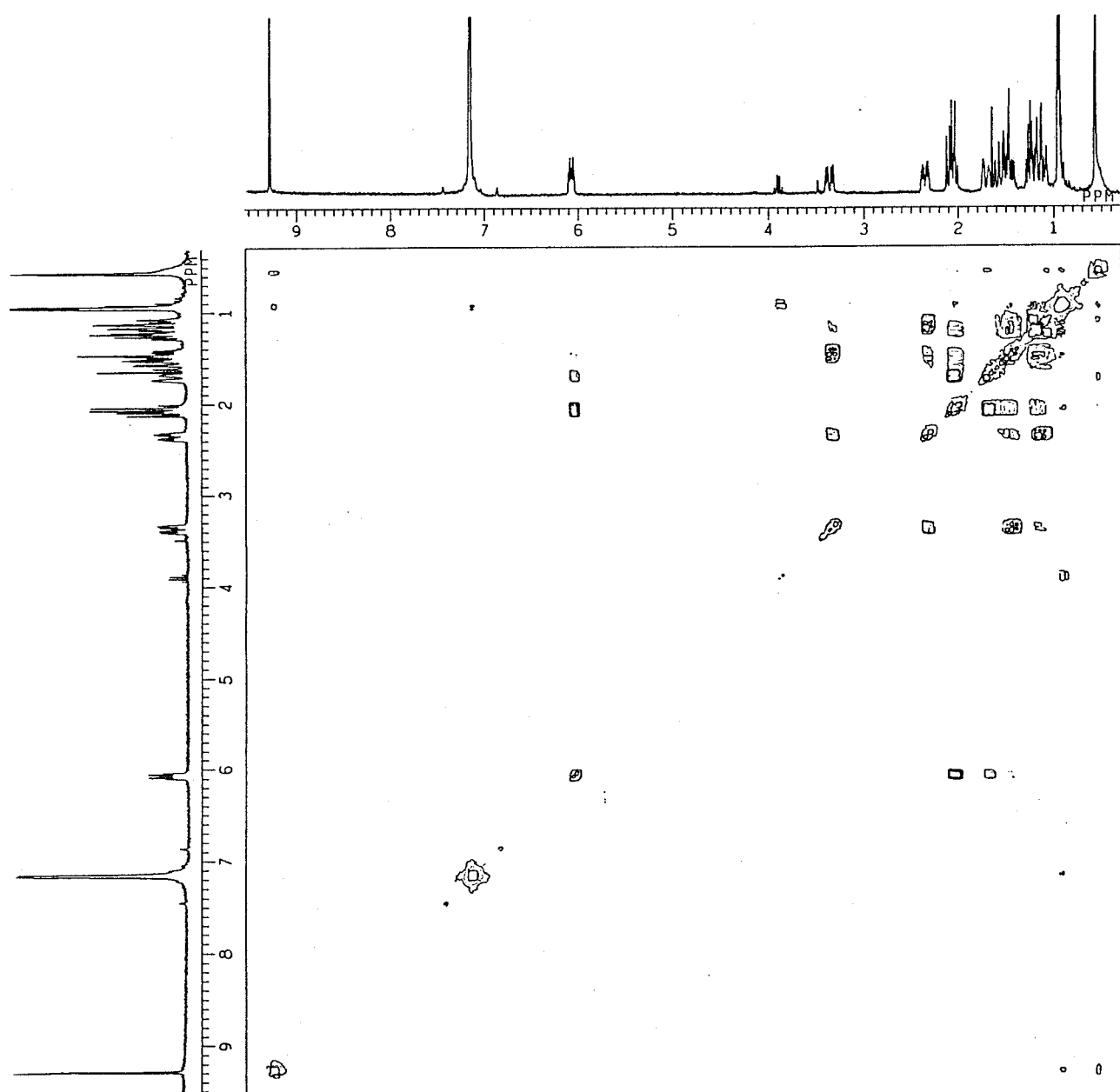


Fig. 3-300 COSY Spectrum of RL-117C (270 MHz, in C_6D_6)

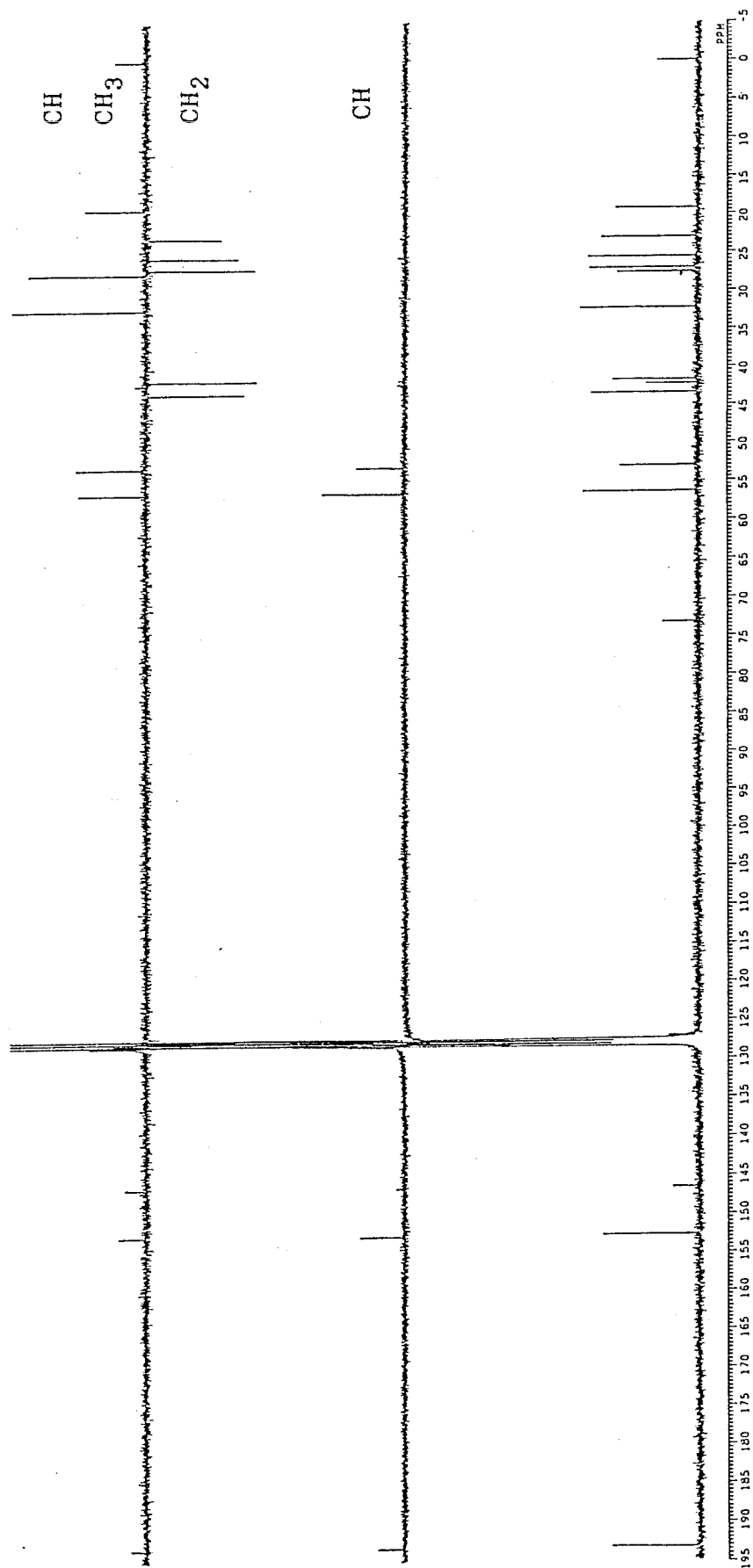


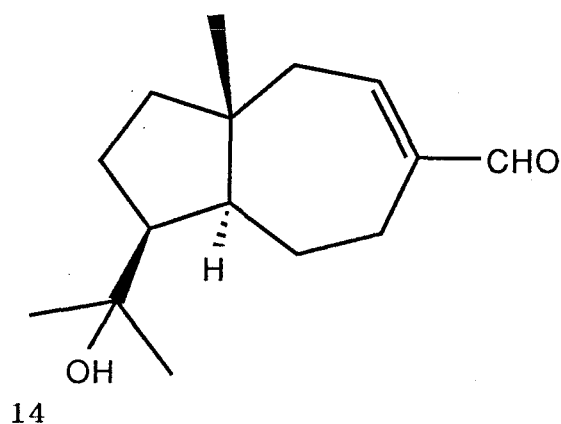
Fig. 3-301 ^{13}C -NMR Spectra of RL-117C (500 MHz, in C_6D_6 , COM and INEPT)

Table 3-112 ^{13}C -NMR chemical shift values of RL-117C

(78 MHz, COM and INEPT, in C_6D_6 , TMS as an int. std.)

δ_{C}	CHx	Assignment*
193.3	CH	C-14
152.8	CH	C-5
146.6	C	C-4
73.3	C	C-11
56.5	CH	C-1
53.1	CH	C-10
43.5	CH_2	C-6
42.3	C	C-7
41.8	CH_2	C-8
32.3	CH_3	C-12
27.7	CH_3	C-13
27.1	CH_2	C-9
25.6	CH_2	C-3
23.1	CH_2	C-2
19.3	CH_3	C-15

Table 3-113 Physicochemical properties of hydroxycarotanaldehyde
(14)



A colorless syrup

Vanillin- H_2SO_4 color: brown $\rightarrow$ pale pink

FI-MS m/z (%): 237 ($\text{M}^+ + 1$, 30), 236 (M^+ , 18), 221 (25), 219 ($\text{M}^+ - \text{H}_2\text{O} + 1$, 34), 177 (46), 59 (100).

EI-MS m/z (%): 236 (M^+ , 0.2), 235 (0.4), 221 (1.4), 218 ($\text{M}^+ - \text{H}_2\text{O}$, 4.2), 203 (4.0), 191 (5.3), 178 (8.4), 175 (8.2), 160 (17), 149 (12), 147 (12), 145 (11), 136 (11), 135 (11), 121 (15), 107 (15), 105 (13), 95 (43), 93 (20), 81 (24), 79 (20), 67 (20), 59 (100), 55 (23), 43 (40), 41 (32).

^1H - and ^{13}C -NMR data are shown in Tables 3-111 and 112, respectively.

3-7-4 Carota-trienaldehyde and Its Related Compounds

1) Dehydrodaucenaldehyde

RL-D showing M^+ 216 in GC-MS was a further dehydrogenated form of carota-1,4-dienaldehyde (3), daucenaldehyde (10) or isodaucenaldehyde (13). During the HPLC of the RL fraction, RL-D was detected as the third peak (Fig. 3-302) and was obtained as a colorless oil (1.8 mg). The EI-MS of the isolate was agreeable with GC-MS of RL-D (Fig. 3-303), and its ^1H - and ^{13}C -NMR spectra were indicative of the triene form (Fig. 3-304, 305 and Tables 3-114, 115). Decoupling experiments revealed two $-\text{CH}_2-\text{CH}_2-$ units relayed by a homoallyl coupling each other. In addition, $-\text{C}(\text{CHO})=\text{CH}-\text{CH}_2-$, $-\text{C}(\text{CH}_3)=\text{CH}_2$ and $-\text{C}-\text{CH}_3$ moieties were visible. These results formulated RL-D as 15, and the proposed structure was supported by detection of four non-hydrogen-bearing olefinic carbons at δ_{C} 146.7, 144.7, 141.9 and 137.0. Compound 15, a dehydrogenation product of 9 or 12 was given a trivial name, dehydrodaucenaldehyde.

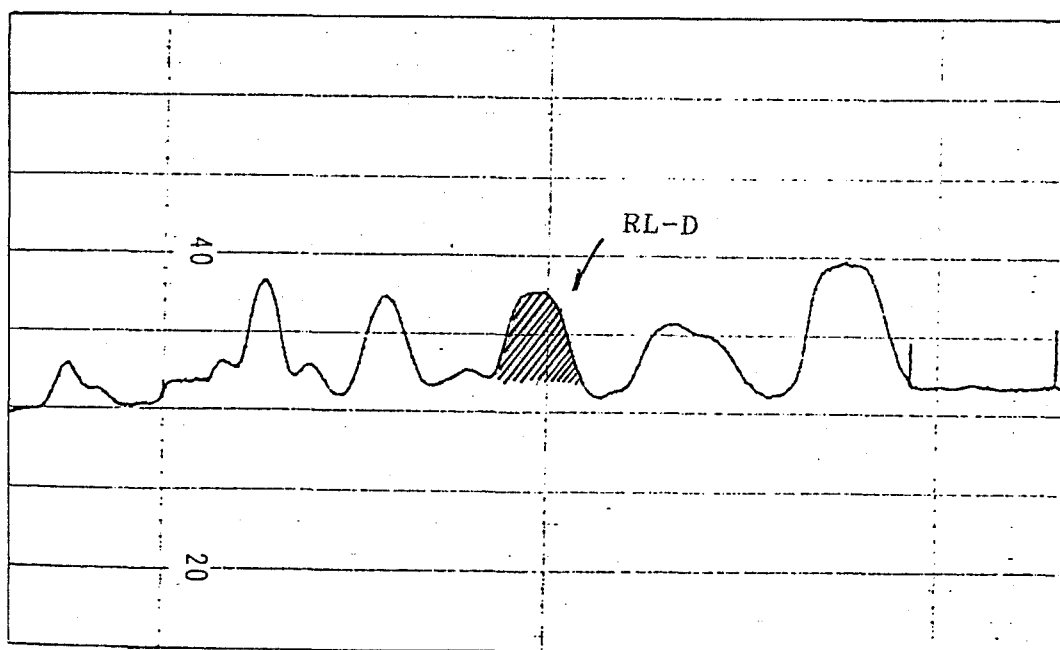


Fig. 3-302 HPL-Chromatogram of RL-D Detected at UV 230 nm

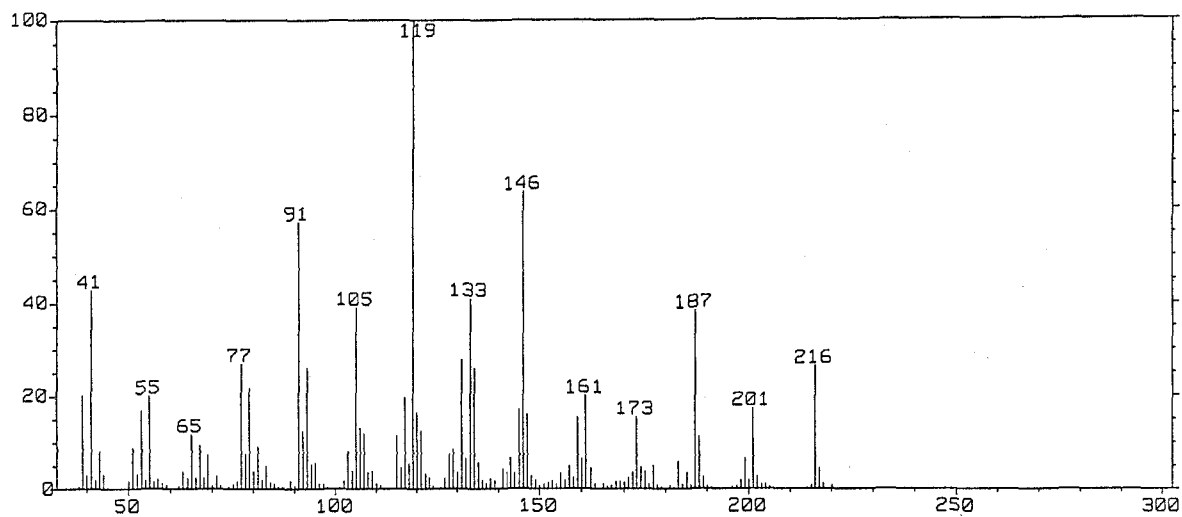
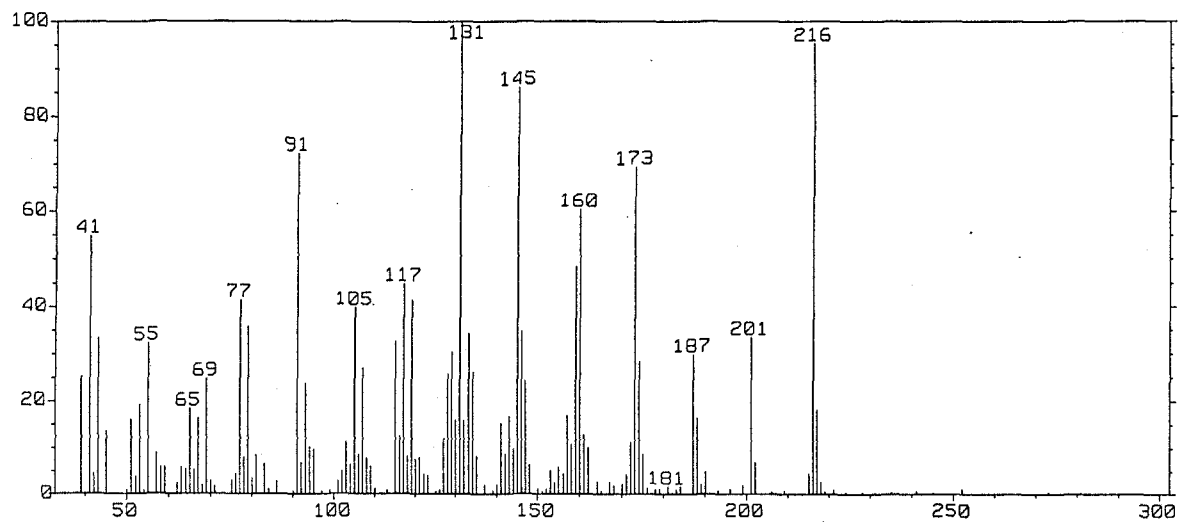


Fig. 3-303 GC-Mass (top) and EI-Mass (bottom) Spectra of RL-D

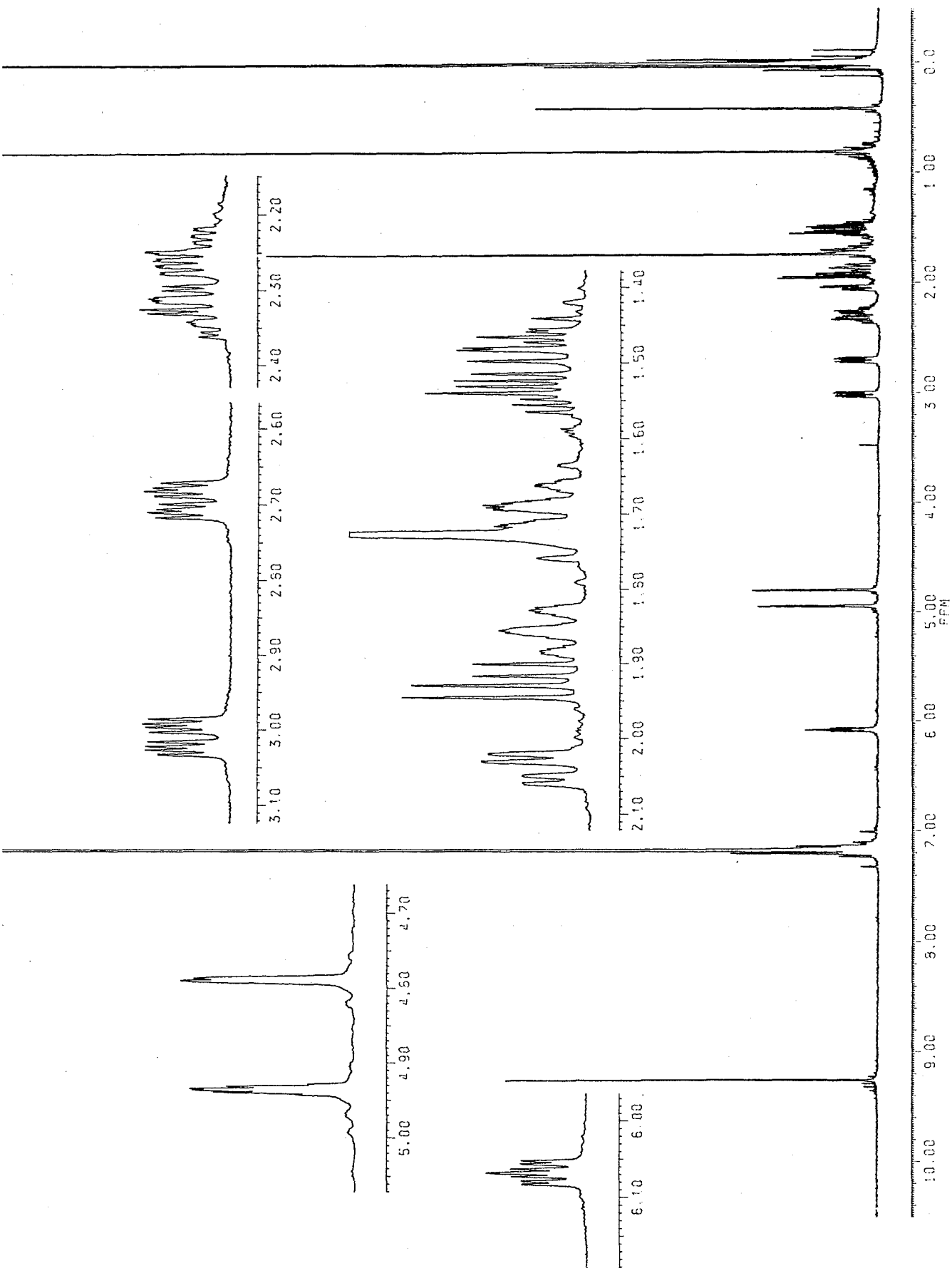


Fig. 3-304 ^1H -NMR Spectrum of RL-D (500 MHz, in C_6D_6)

Table 3-114 ^1H -NMR chemical shift values for RL-D

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}	Coupling		Assignment
9.247	1H	s	C-14-H
6.066	1H	ddd $J= 8.1, 5.3, 2.0$ Hz	C-5-H
4.935	1H	dd $J= 2.9, 1.5$ Hz	C-12-Ha
4.788	1H	d $J= 1.5$ Hz	C-12-Hb
3.008	1H	ddd $J= 15.1, 5.5, 3.1$ Hz	C-3-Ha
2.693	1H	ddd $J= 16.2, 5.5, 3.3$ Hz	C-2-Ha
2.327	1H	dddd $J= 15.8, 8.7, 8.4, 2.8$ Hz	C-9-Ha
2.248	1H	dddd $J= 15.8, 7.3, 4.6, 1.0$ Hz	C-9-Hb
2.039	1H	dd $J= 14.4, 5.3$ Hz	C-6-Ha
1.922	1H	dd $J= 14.4, 8.1$ Hz	C-6-Hb
1.854	1H	br. dd $J= 15.1, \text{ca } 14$ Hz	C-3-Hb
1.726	3H	br. s	C-13-H ₃
1.689	1H	br. dd $J= \text{ca } 16, \text{ca } 14$ Hz	C-2-Hb
1.539	1H	ddd $J= 12.4, 8.4, 4.6$ Hz	C-8-Ha
1.468	1H	ddd $J= 12.4, 8.7, 7.3$ Hz	C-8-Hb
0.800	3H	s	C-15-H ₃

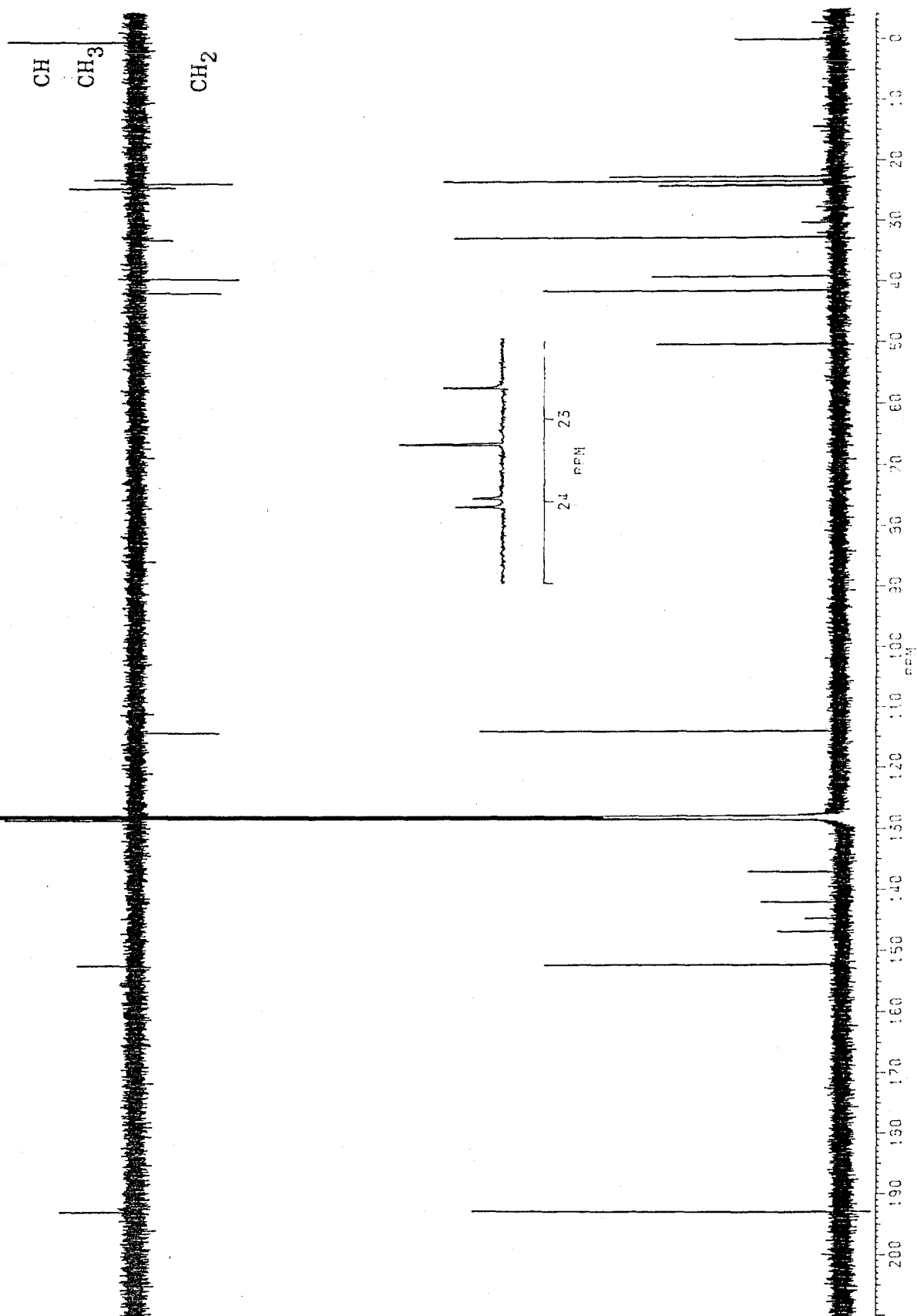


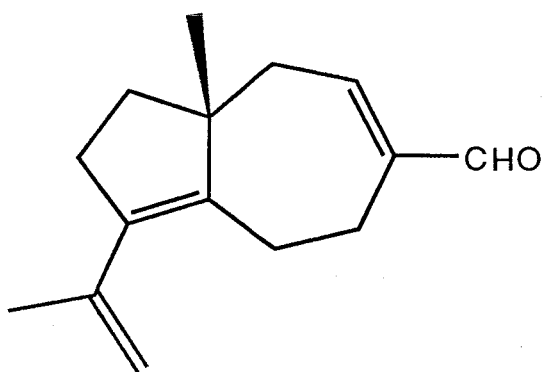
Fig. 3-305 ^{13}C -NMR Spectra of RL-D (125 MHz, in C_6D_6 , COM and DEPT)

Table 3-115. ^{13}C -NMR chemical shift values of RL-D

(125 MHz, COM and INEPT, in C_6D_6 , TMS as an int. std.)

δ_{C}	CHx	Assignment
192.8	CH	C-14
152.1	CH	C-5
146.7	C	C-1
144.7	C	C-4
141.9	C	C-11
137.0	C	C-10
113.9	CH_2	C-12
50.2	C	C-7
41.4	CH_2	C-6
39.0	CH_2	C-8
32.6	CH_2	C-9
24.1	CH_3	C-13
24.0	CH_2	C-3
23.3	CH_2	C-2
22.6	CH_3	C-15

Table 3-116 Physicochemical properties of dehydrodaucenaldehyde
(15)



15

A colorless oil

Vanillin- H_2SO_4 color: brown

GC-MS m/z (%): 218 (M^+ , 12), 203 (6.3), 189 (4.6), 176 (6.8), 175 (14), 162 (6.4), 149 (11), 147 (9.7), 136 (14), 135 (11), 133 (14), 121 (17), 109 (15), 107 (26), 105 (16), 95 (13), 94 (15), 93 (28), 91 (22), 81 (14), 79 (30), 77 (16), 68 (100), 67 (35), 55 (17), 53 (21), 41 (28).

EI-MS m/z (%): 216 (M^+ , 27), 201 ($\text{M}^+ - \text{CH}_3$, 17), 199 (6.2), 188 (11), 187 ($\text{M}^+ - \text{CHO}$, 38), 173 (16), 161 (20), 159 (16), 146 (64), 134 (26), 133 (41), 131 (28), 119 (100), 105 (39), 93 (26), 91 (57), 79 (22), 77 (27), 55 (20), 41 (43).

^1H - and ^{13}C -NMR data are shown in Table 3-114 and 3-115, respectively.

2) Dehydrodaucenoic Acid

In the Fr-VIIA-6, a quenching spot was detected (Fig. 3-306). From the fraction of 1/10 volume, the focused compound was successfully isolated by PTLC (H-EA-F 30:20:1) to give a colorless syrup (ca 3 mg). The isolate showing M^+ 232 in EI-MS (Fig. 3-307) was tentatively deduced as 16, since the compound exhibited a quite similar $^1\text{H-NMR}$ spectrum to that of dehydrodaucenaldehyde (15) (Fig. 3-308). As well as carota-1,4-dienoic acid (4) or isodaucenoic acid (13), this sesquiterpene acid whose corresponding aldehyde was also contained was also present in *Rosa rugosa* leaves as a minor constituents, and was named dehydrodaucenoic acid.

H-EA-F 30:10:1

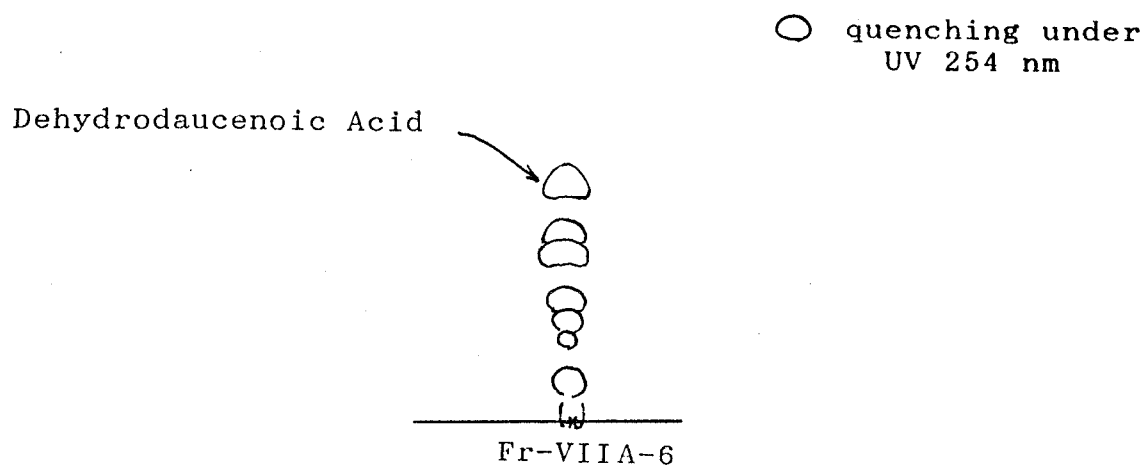


Fig. 3-306 TL Chromatogram of Fr-VIIA-6 Containing a Carotane Trienoic Acid

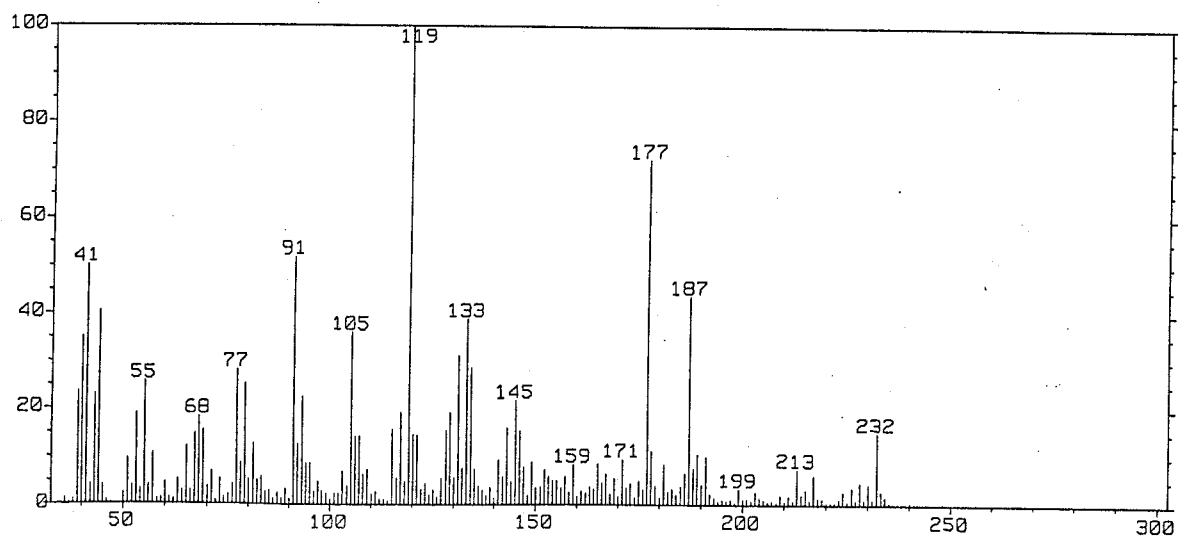


Fig. 3-307 EI-Mass Spectrum of Dehydrodaucenoic Acid

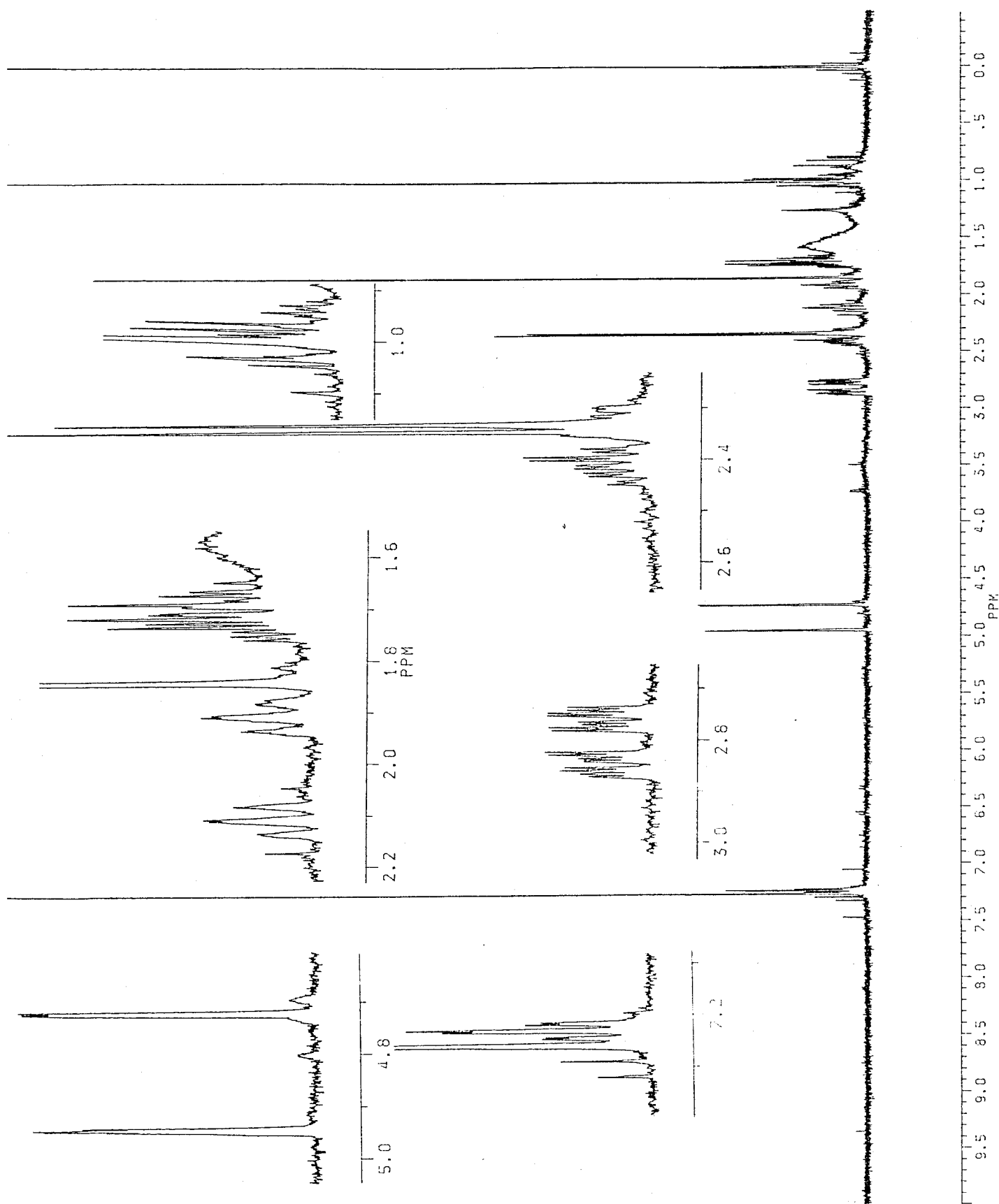
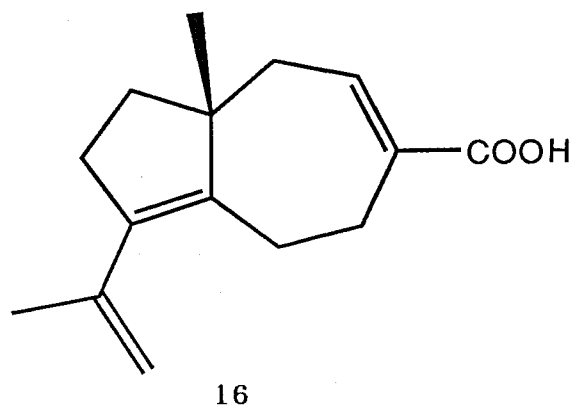


Fig. 3-308 ^1H -NMR Spectrum of Carotatriene Acid (500 MHz, in CDCl_3)

Table 3-117 Physicochemical properties of dehydrodaucenoic acid
(16)



Colorless syrup

Vanillin- H_2SO_4 color: dark pink

EI-MS m/z (%): 232 (M^+ , 15), 217 (6.3), 187 (44), 177 (72), 145 (22), 134 (29), 133 (39), 131 (31), 119 (100), 105 (36), 93 (23), 91 (52), 77 (28), 55 (26), 44 (41), 41 (50).

$^1\text{H-NMR}$ $\delta_{\text{TMS}}^{\text{CDCl}_3}$ (500 MHz): 2.763 (1H, ddd, $J=14.1, 6.0$ and 2.9 Hz, C-2-Ha), 1.909 (1H, br. dd, $J=14.1$ and 13.5 Hz, C-2-Hb), 2.852 (1H, ddd, $J=15.0, 6.0$ and 2.9 Hz, C-3-Ha), 2.107 (1H, br. dd, $J=15.0$ and 13.8 Hz, C-3-Hb), 7.233 (1H, ddd, $J=7.2, 7.2$ and 1.8 Hz, C-5-H), 2.335 (2H, d, $J=7.2$ Hz, C-6- H_2), 1.738 (1H, m, C-8-Ha), 1.681 (1H, m, C-8-Hb), 2.411 (1H, dddd, $J=16.1, 8.3, 8.2$ and 2.9 Hz, C-9-Ha), ca 2.30 (1H, overlapped, C-9-Hb), 4.941 (1H, dd, $J=2.3$ and 1.4 Hz, C-12-Ha), 4.719 (1H, d, $J=2.3$ Hz, C-12-Hb), 1.842 (3H, br. s, C-13- H_3), 1.005 (3H, s, C-15- H_3).

3) Dehydrodaucenaldehyde Hydroxyl Derivative

RL-119A was mainly isolated from Fr-VH-9 (See pp. 586) as a minor constituents. By PTLC, the compound was isolated as a colorless syrup (Fig. 3-309). In EI-MS, the parent ion (m/z 234, 11 %) and successive dehydration peak (m/z 216, 23 %) were observed, suggesting a hydroxylated compound for RL-119A (Fig. 3-310). In the ^1H -NMR spectrum, some signals characteristic of a carotane aldehyde were visible [e.g. an α,β -unsaturated aldehyde group at δ_{H} 9.345, a bridgehead methyl group at δ_{H} 0.822 and an isopropenyl group at δ_{H} 5.104, 4.988 (exomethylene) and 1.787 (allyl methyl)] (Fig. 3-311 and Table 3-118). As the signal pattern was similar to that of isodaucenaldehyde (13, See Fig. 3-275, pp. 486), the compound was expected to be a hydrate of 13.

By the ^{13}C -NMR analyses of RL-119A (COM and INEPT), a oxygenated sp^3 carbon was detected at δ_{C} 86.7 (Fig. 3-312 and Table 3-119). On the other hand, only one methine carbon was observed at δ_{C} 66.5, unlike 13. The presence of these two carbons suggested that either C-1 or C-10 was hydroxylated and gave a deshielding effect on the adjacent methine carbon. The position of hydroxylation was considered to be C-10 by comparison of the ^1H -NMR spectrum with that of 13. Namely, C-10 methine proton which may be deshielded characteristically around 3 ppm by the C12/C13 olefinic bond (e.g. δ_{H} 2.989 in 13) was not detectable in RL-119A. The methine proton of RL-119A resonated in an upfield (δ_{H} 1.946, dd, J = 11.8 and 1.5 Hz) was rather favorably assigned to C-1-H (cf. C-1-H of 13; δ_{H} 1.921, ddd, J = 12.3, 11.6 and 2.0 Hz). Thus, the compound was proposed to be 17 which is a relative of dehydrodaucenaldehyde (15), and was named hydroxyisodaucenaldehyde.

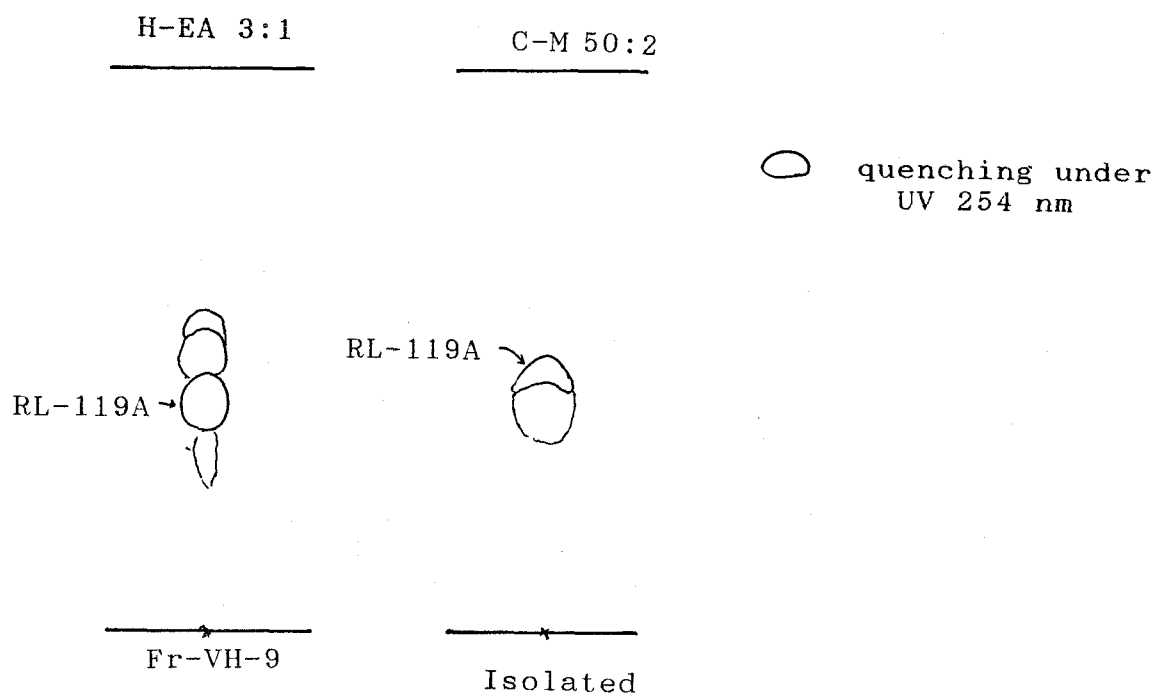


Fig. 3-309 TL Chromatogram of RL-119A

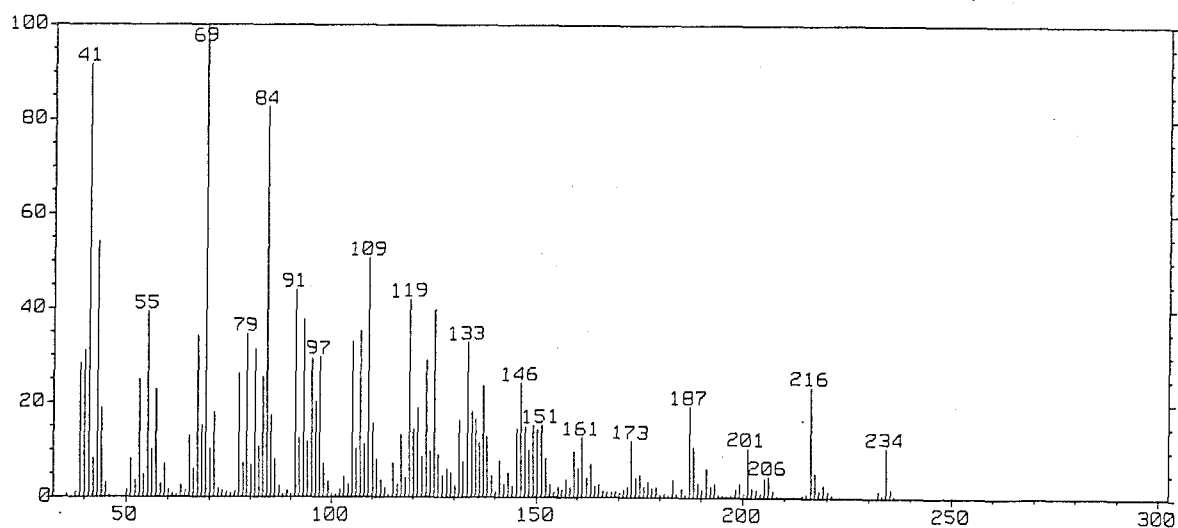


Fig. 3-310 EI-Mass Spectrum of RL-119A

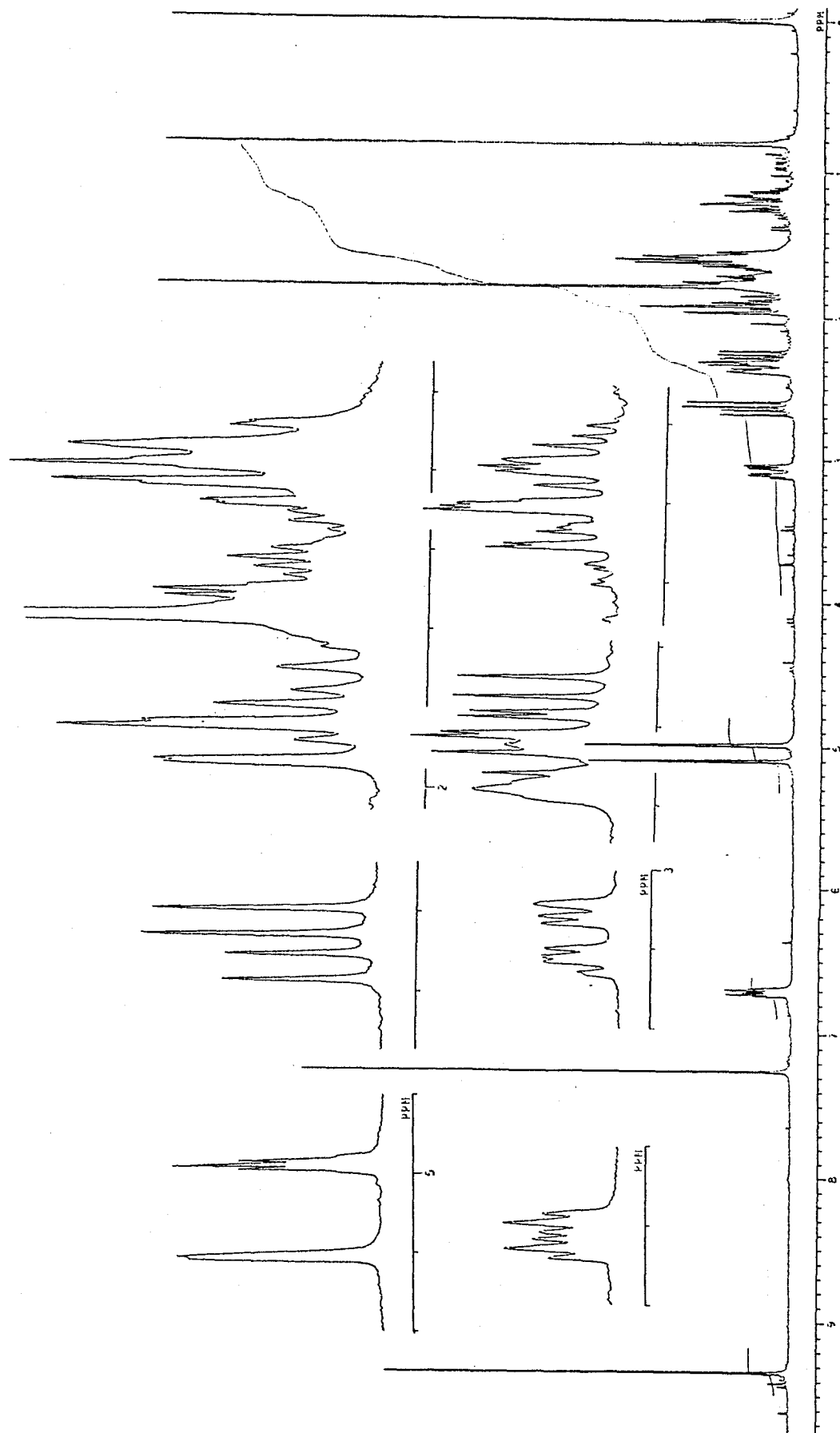


Fig. 3-311 ^1H -NMR Spectrum of RL-119A (270 MHz, in C_6D_6)

Table 3-118 ^1H -NMR chemical shift values of RL-119B

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}	Coupling		Assignment
9.345	1H	s	C-14-H
6.713	1H	ddd $J= 8.8, 3.3$ and 3.3 Hz	C-5-H
5.104	1H	br. s	C-12-Ha
4.988	1H	dd $J= 1.5$ and 0.7 Hz	C-12-Hb
3.083	1H	ddd $J= 13.6, 4.8$ and 2.9 Hz	C-3-Ha
2.643	1H	dd $J= 15.4$ and 8.8 Hz	C-6-Ha
2.354	1H	br d $J= 15.4$ Hz	C-6-Hb
2.300	1H	ddd $J= 13.6, 11.7$ and 6.6 Hz	C-3-Hb
1.946	1H	dd $J= 11.8$ and 1.5 Hz	C-1-H
1.912	1H	ddd $J= 12.5, 12.1$ and 7.7 Hz	C-9-Ha
1.787	3H	d $J= 0.7$ Hz	C-13-H ₃
1.77 (approx.)	1H	m	C-2-Ha
1.690	1H	ddd $J= 15.8, 6.2$ and 2.9 Hz	C-9-Hb
1.596	1H	dd $J= 12.5, 7.0$ and 5.5 Hz	C-8-Ha
1.58 (approx.)	1H	m	C-8-Hb
1.208	1H	ddd-like m	C-2-Hb
0.822	3H	s	C-15-H ₃

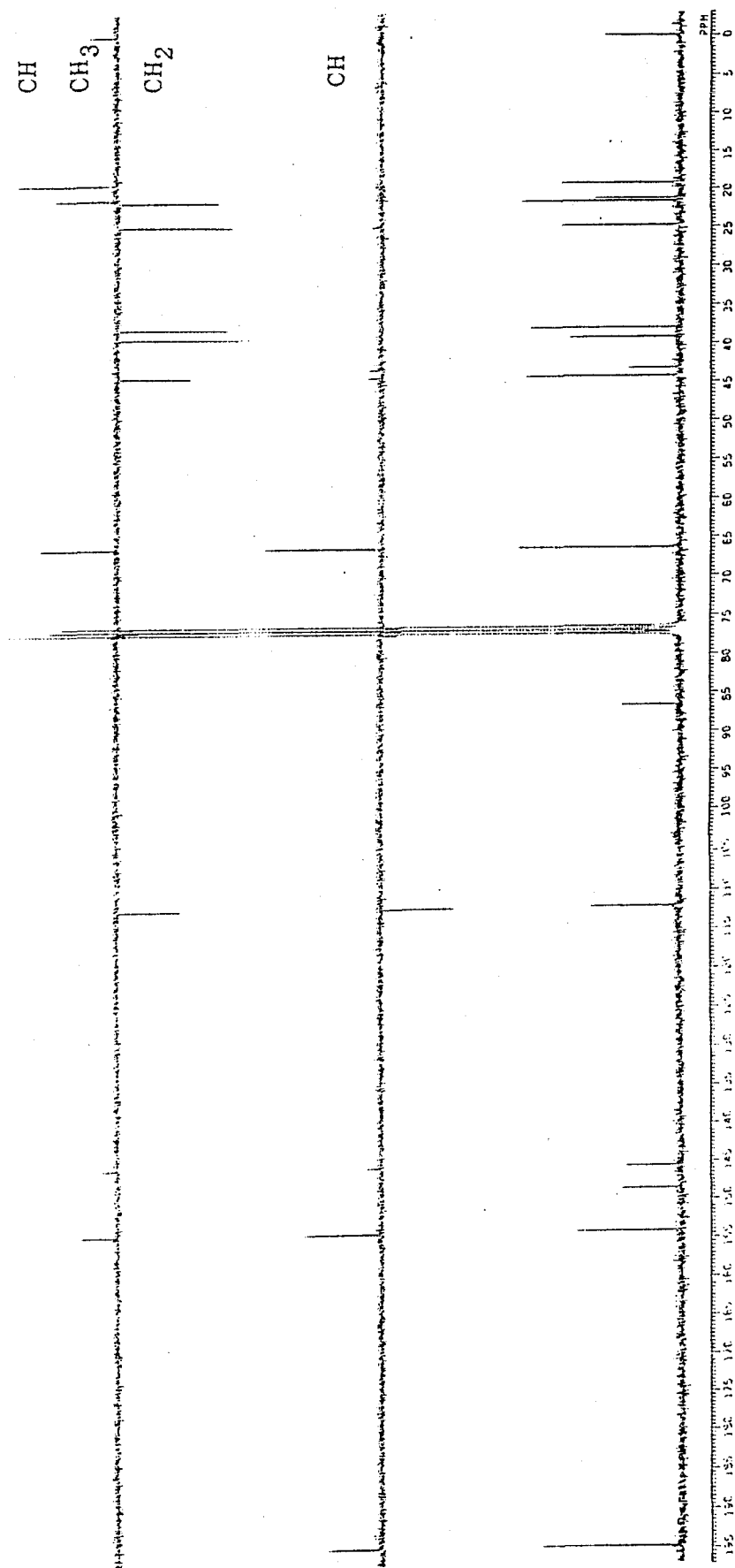


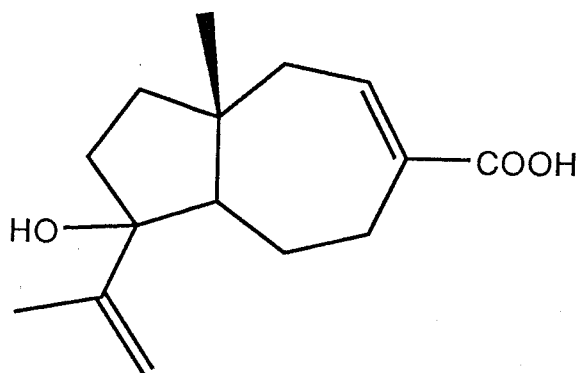
Fig. 3-312 ^{13}C -NMR Spectrum of RL-119A (68 MHz, in C_6D_6)

Table 3-119 ^{13}C -NMR chemical shift values of RL-119B

(78 MHz, COM and INEPT, in CDCl_3 , TMS as an int. std.)

δ_{C}	CHx	Assignment
194.9	CH	C-14
154.3	CH	C-5
148.7	C	C-4
145.7	C	C-11
112.2	CH_2	C-12
86.7	C	C-10
66.5	CH	C-1
44.4	CH_2	C-6
43.3	C	C-7
39.3	CH_2	C-8
38.1	CH_2	C-9
25.0	CH_2	C-3
21.8	CH_2	C-2
21.4	CH_3	C-13
19.4	CH_3	C-15

Table 3-120 Physicochemical properties of hydroxyisdaucenaldehyde
(17)



17

A colorless syrup

Vanillin- H_2SO_4 color: brown

EI-MS m/z (%): 234 (M^+ , 11), 216 ($\text{M}^+ - \text{H}_2\text{O}$, 23), 206 (4.6), 201 (11), 187 (19), 173 (12), 161 (13), 151 (15), 150 (14), 149 (15), 146 (24), 137 (24), 133 (33), 125 (40), 123 (29), 119 (42), 109 (51), 107 (35), 105 (33), 97 (30), 95 (29), 93 (38), 91 (44), 84 (83), 81 (31), 79 (35), 77 (26), 69 (100), 67 (34), 55 (39), 53 (25), 43 (54), 41 (92).

^1H - and ^{13}C -NMR data are shown in Tables 3-118 and 3-119, respectively.

3) Carotane Trienaldehyde Derivative

During the survey of rugosic acid A methyl ester (5), a compound showing a dull orange color on the peroxide test was detected in the fraction contained 5 (See pp. 402). This compound denoted RL-PERO-4 was also isolated by PTLC (Fig. 3-313). As the result, 12.8 mg of a colorless syrup denoted as RL-PERO-4 was obtained. The isolate showing M^+ 232 in FD- and EI-MS (Fig. 3-314 and 315), was expected to be a dioxygenated carotanoid. In the ^1H -NMR spectrum, it was revealed that RL-PERO-4 possessed an olefinic bond in the seven-membered ring and the olefinic protons were vicinally coupled with each other ($J = 12.0$ Hz of isolated *cis* coupling) (Fig. 3-316, 317 and Tables 3-121, 122).

Information for its oxygenation form was provided by ^{13}C -NMR analyses (COM and DEPT) (Fig. 3-318 and Table 3-123). Only a methine carbon δ_C 29.0 and two oxygenated carbons (δ_C 78.3 and 72.3) were clearly differentiated among 10 sp^3 carbons. Especially, the latter carbons were both non-hydrogen-bearing. These facts were suggestive of the presence of an epoxy ring on C-1/C-10. A proton coupling sequence of $-CH_2-CH_2-$ was assignable to C-8/C-9. However, there were two possible structures (D and E) for RL-PERO-4 as shown in Fig. 3-319. As a deshielding effect of C-14 aldehyde group was observed on an olefinic proton at δ_H ca 6.79 in $CDCl_3$ (cf. δ_H 5.904, br. dd, $J = 8.1$ and 2.3 Hz, in C_6D_6), it was suggestive of the presence of α, β -unsaturated aldehyde group. Since its UV_{max} was observed at 270 nm, that olefinic bond was expected to be further conjugated with this aldehyde group to form $\alpha, \beta; \alpha', \beta'$ -unsaturated one.

By the NOE experiments, NOE between one of the allylic methylene proton (δ_H 1.981 in C_6D_6) exhibited an NOE with C-15- H_3 and C-12- H_3 . These NOEs are only compatible when the methylene carbon allocates to C-2 (Fig. 3-320). Accordingly, the structure was presumed to be D. To confirm the proposed structure, chemical conversion of this compound was carried out.

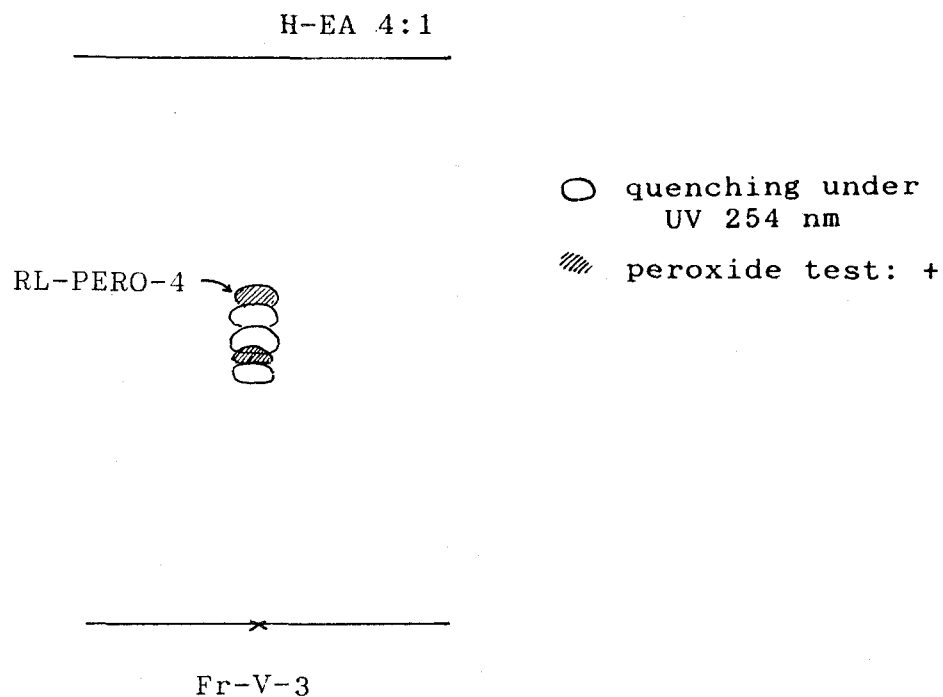


Fig. 3-313 TL Chromatogram of RL-PERO-4

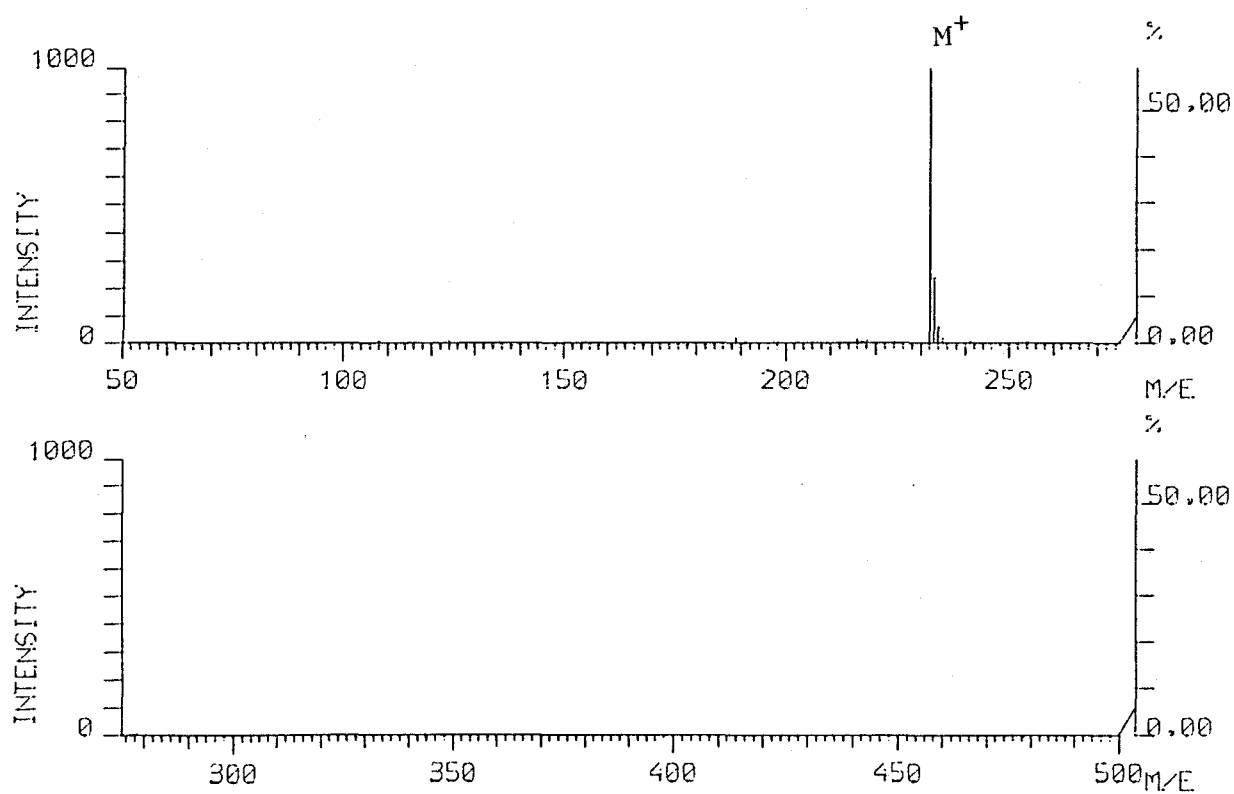


Fig. 3-314 FD-Mass Spectrum of RL-PERO-4

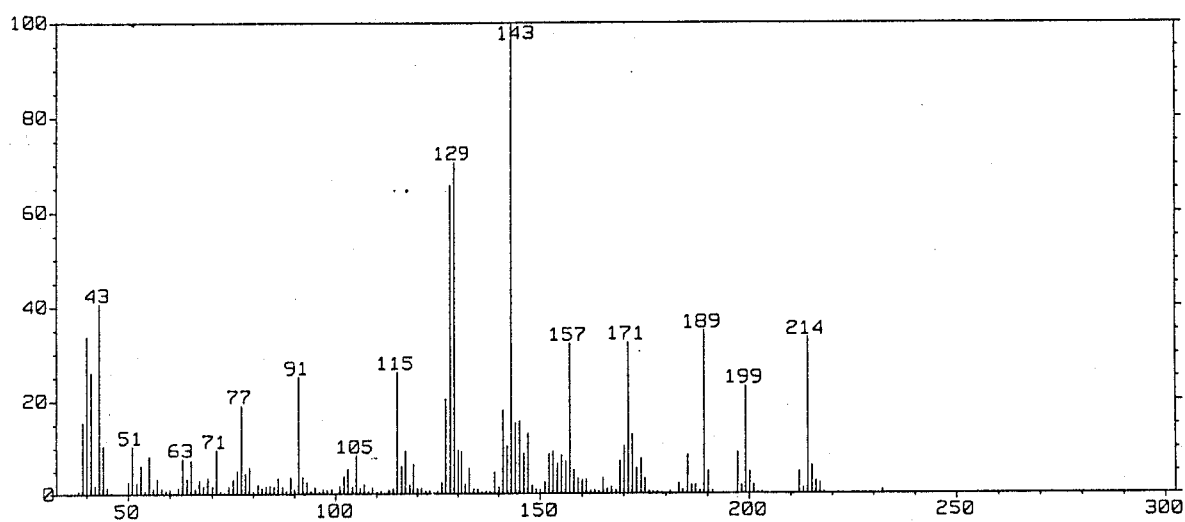


Fig. 3-315 EI-Mass Spectrum of RL-PERO-4

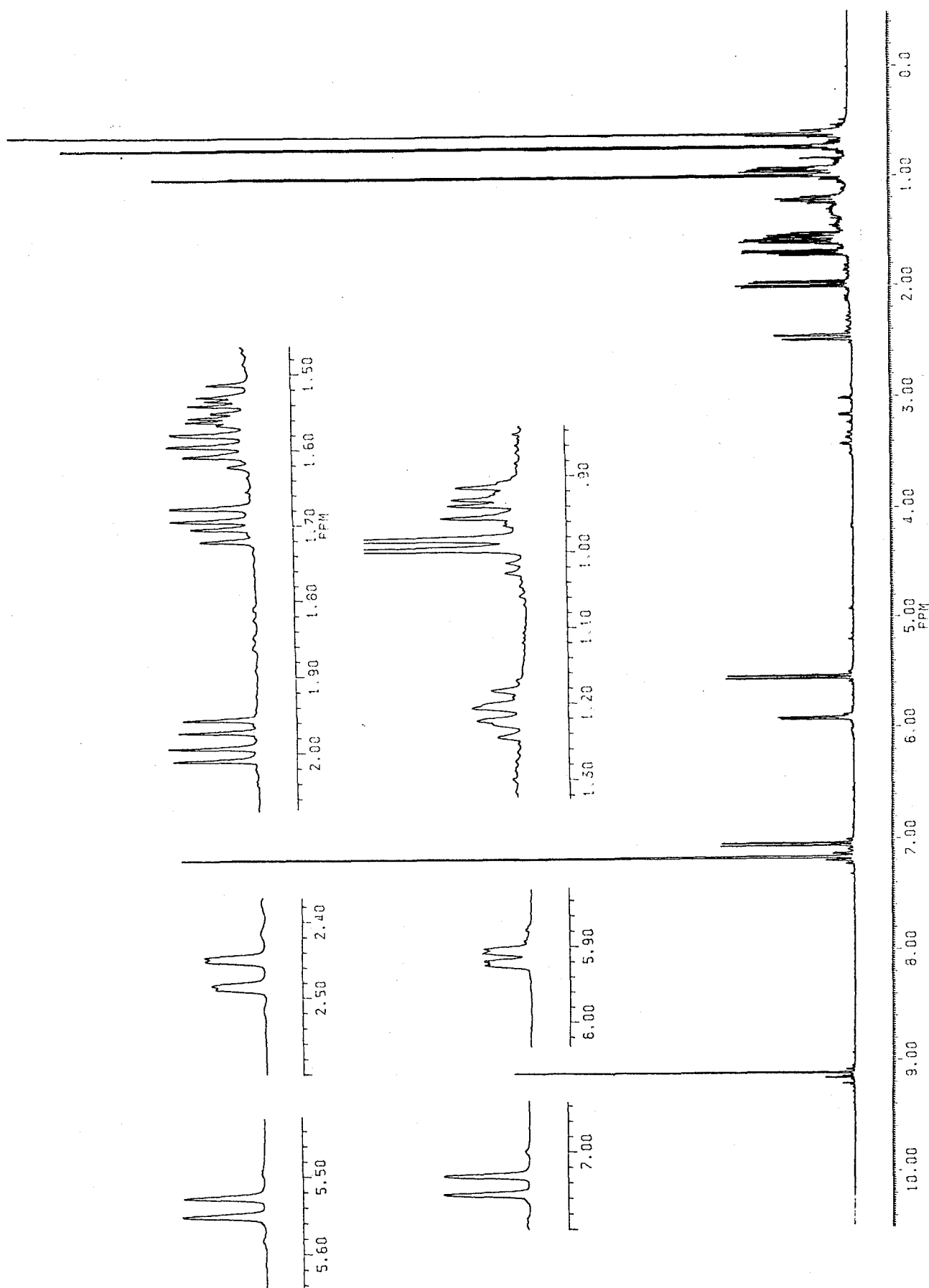


Fig. 3-316 ^1H -NMR Spectrum of RL-PERO-4 (500 MHz, in C_6D_6 .)

Table 3-121 ^1H -NMR chemical shift values for RL-PERO-4

(500 MHz, in C_6D_6 , TMS as an int. std.)

δ_{H}	Coupling			Assignment (for 18)
9.107	1H	s		C-14-H
7.042	1H	d	$J = 12.0$ Hz	C-5-H
5.904	1H	dd	$J = 8.1$ and 2.3 Hz	C-3-H
5.537	1H	d	$J = 12.0$ Hz	C-4-H
2.447	1H	dd	$J = 18.5$ and 2.3 Hz	C-2-Ha
1.981	1H	dd	$J = 18.5$ and 8.1 Hz	C-2-Hb
1.710	1H	dd	$J = 13.6$ and 8.2 Hz	C-9-Ha
1.592	1H	sept	$J = 6.9$ Hz	C-11-H
1.544	1H	ddd	$J = 13.6, 10.5$ and 8.0 Hz	C-9-Hb
1.212	1H	br. ddd	$J = 11.9, 10.5$ and 8.2 Hz	C-8-Ha
0.987	3H	d	$J = 6.9$ Hz	C-12-H ₃
0.923	1H	dd	$J = 11.9$ and 8.0 Hz	C-8-Hb
0.718	3H	d	$J = 6.9$ Hz	C-13-H ₃
0.603	3H	s		C-15-H ₃

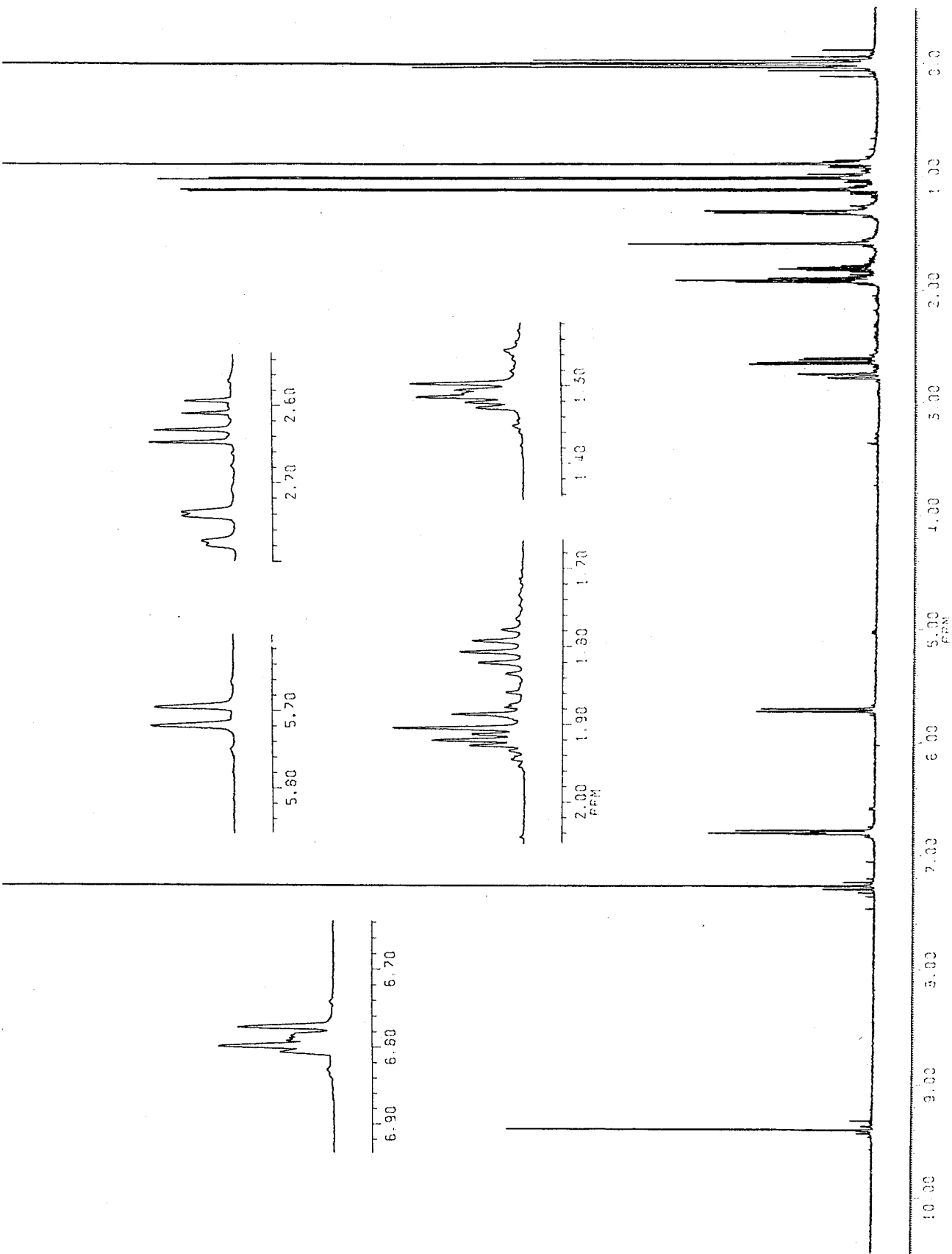


Fig. 3-317 ^1H -NMR Spectrum of RL-PERO-4 (500 MHz, in CDCl_3)

Table 3-122 ^1H -NMR chemical shift values for RL-PERO-4

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}	Coupling			Assignment (for 18)
9.397	1H	s		C-14-H
6.79 (approx.)	1H	m		C-5-H
6.784	1H	d	$J = 12.2$ Hz	C-3-H
5.705	1H	d	$J = 12.2$ Hz	C-4-H
2.757	1H	dd	$J = 18.6$ and 2.6 Hz	C-2-Ha
2.619	1H	dd	$J = 18.6$ and 8.0 Hz	C-2-Hb
1.916	1H	m		C-9-Ha
1.904	1H	m		C-9-Hb
1.806	1H	sept	$J = 6.9$ Hz	C-11-H
1.313	1H	m		C-8-Ha
1.308	1H	m		C-8-Hb
1.111	3H	d	$J = 6.9$ Hz	C-12-H ₃
1.009	3H	d	$J = 6.9$ Hz	C-13-H ₃
0.884	3H	s		C-15-H ₃

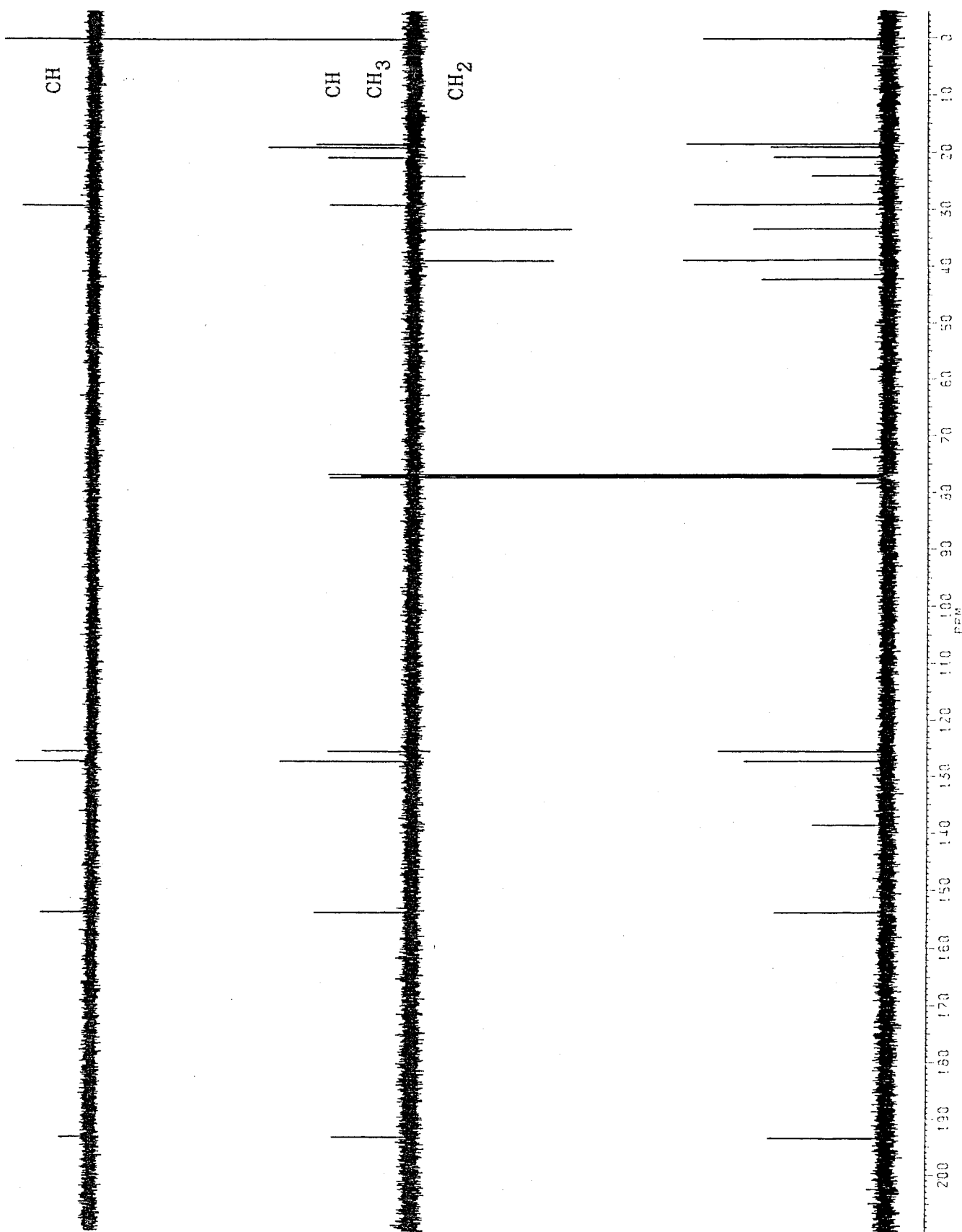


Fig. 3-318 ^{13}C -NMR Spectra of RL-PERO-4 (125 MHz, in CDCl_3 , COM and DEPT)

Table 3-123 ^{13}C -NMR chemical shift values of RL-PERO-4

(78 MHz, COM and INEPT, in CDCl_3 , TMS as an int. std.)

δ_{C}	CHx	Assignment*
193.5	CH	C-14
153.8	CH	C-3
138.6	C	C-4
127.2	CH	C-5
125.5	CH	C-12
78.3	C	C-1
72.3	C	C-10
42.1	C	C-7
38.8	CH_2	C-8
33.3	CH_2	C-9
29.0	CH	C-11
24.0	CH_2	C-2
20.7	CH_3	C-15
19.0	CH_3	C-12
18.4	CH_3	C-13

* The assignment for RL-PERO-4 is according to the proposed structure 18.

By treatment of RL-PERO-4 with thiourea/MeOH, a polar product possessing MW 232 was obtained in a 34 % yield (fine prisms, 2.3 mg from 6.8 mg, *Rf* 0.26 in C-M 50:2) (Fig. 3-321). As the spectroscopic data were indicative of 10-hydroxycarota-1,3,5-triene aldehyde, the product was introduced an olefinic bond through the epoxy ring cleavage (18a) (Fig. 3-322, 323 and Tables 3-124, 125). The olefinic bond formation should occur by electron transformation via β -hydrogen transference (C-2) to result in the epoxy cleavage (C-1-O) (Scheme 3-34). Accordingly, the structure of RL-PERO-4 was proved to be 18.

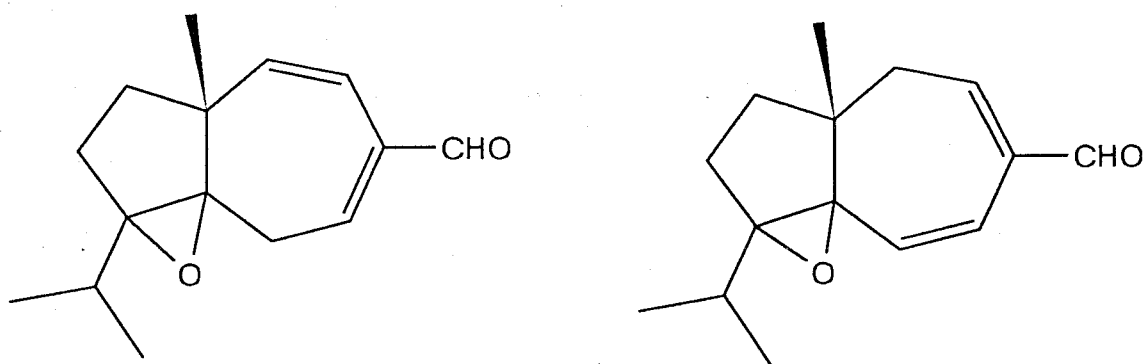


Fig. 3-319 Two Possible Structure for RL-PERO-4

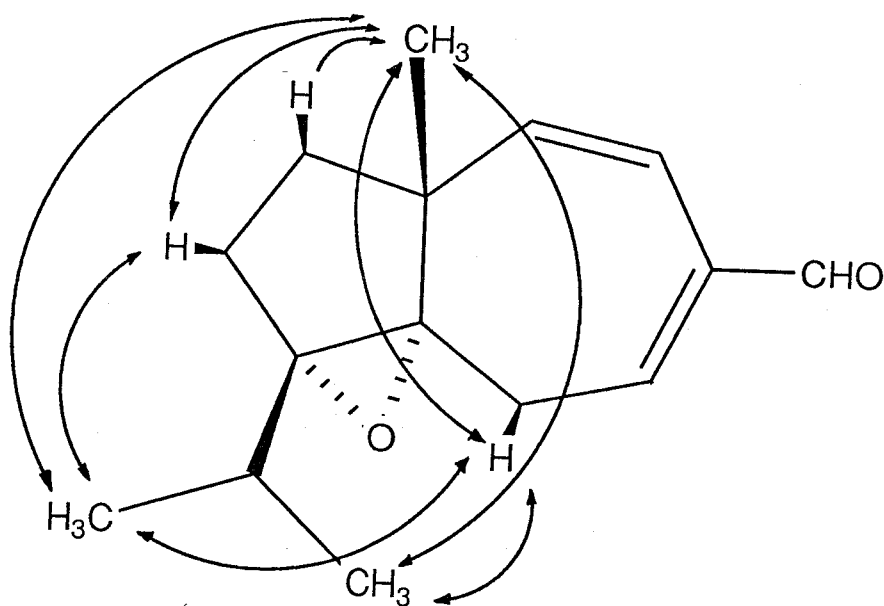


Fig. 3-320 NOEs Observed on RL-PERO-4

H-EA 3:1

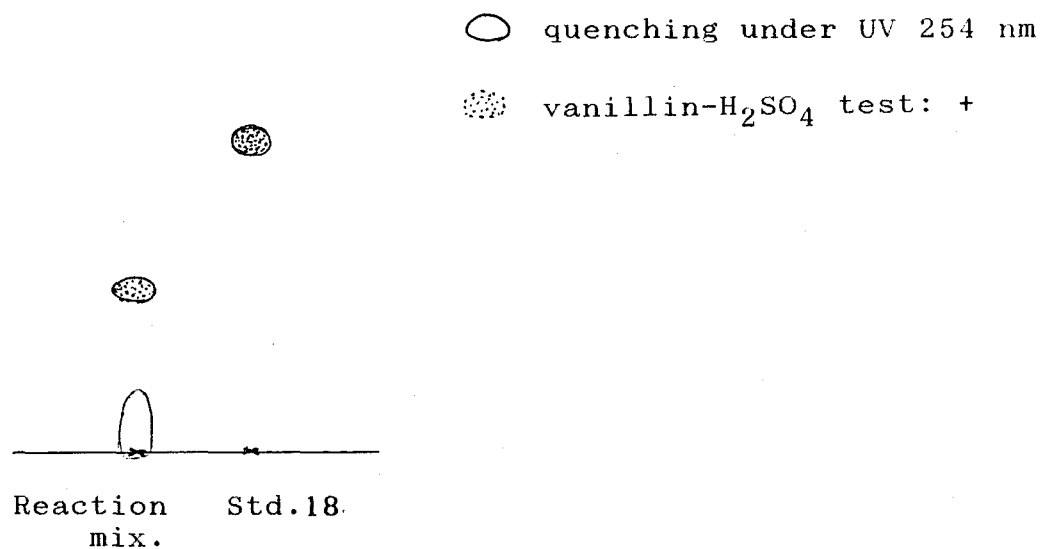
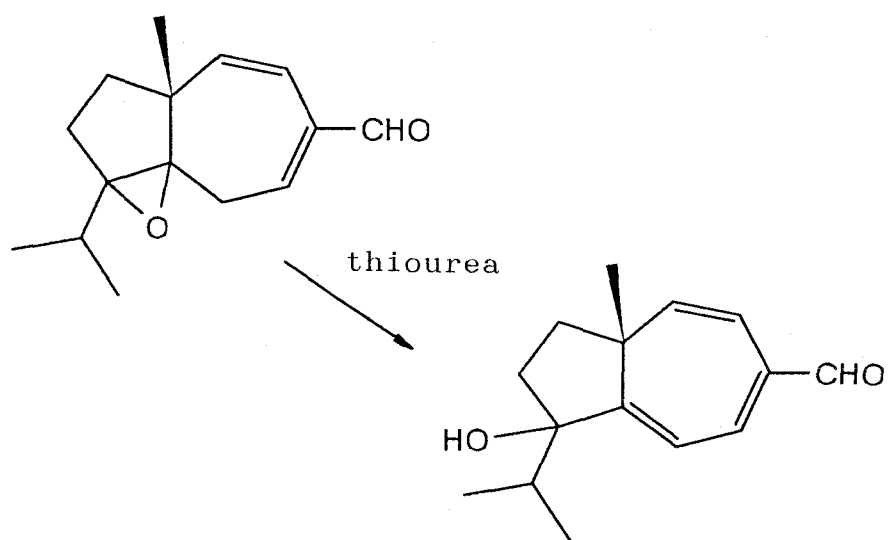


Fig. 3-321 TL Chromatogram of RL-PERO-4-TU Obtained by Treatment of RL-PERO-4 with Thiourea



Scheme 3-34 Conversion of RL-PERO-4 into a hydroxyl derivative

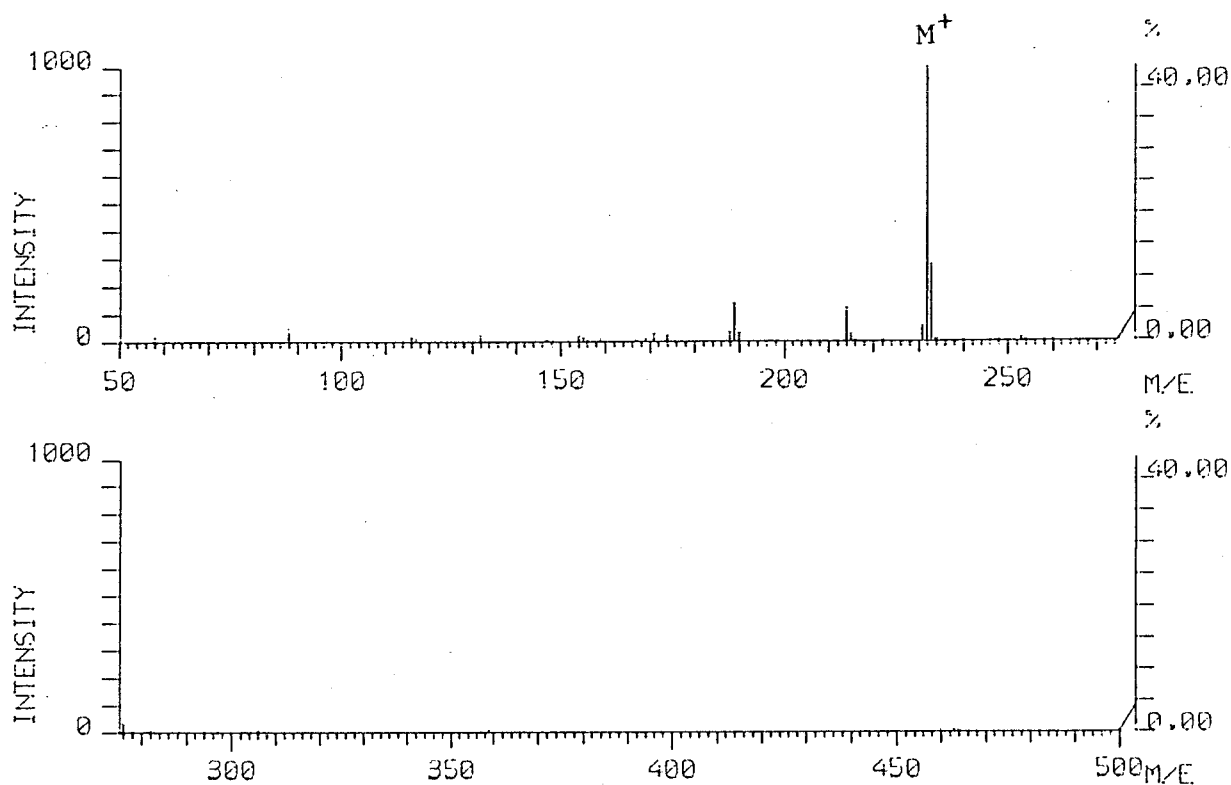


Fig. 3-322 FD-Mass Spectrum of RL-PERO-4-TU

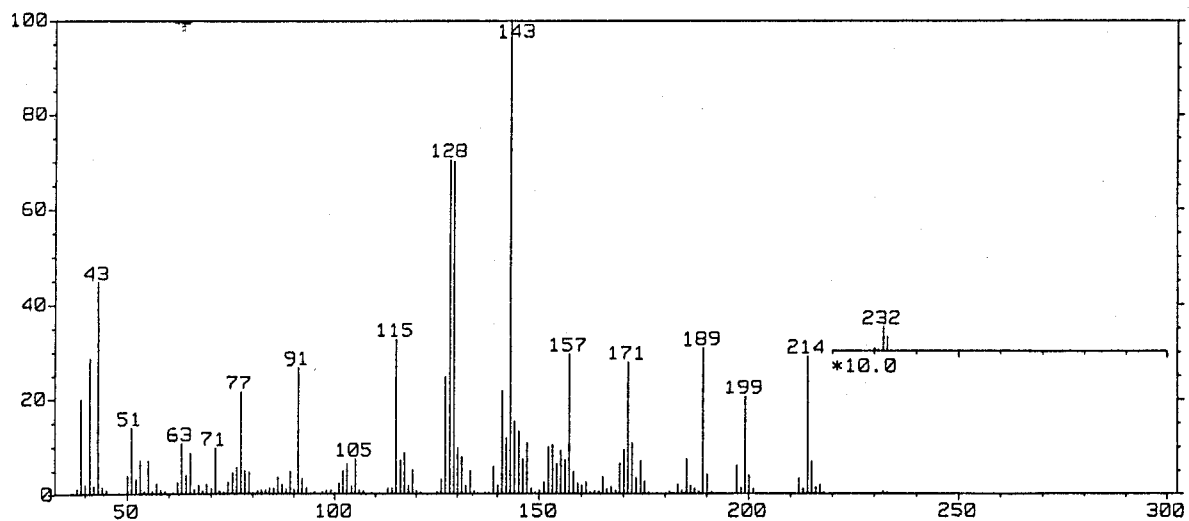


Fig. 3-323 EI-Mass Spectrum of RL-PERO-4-TU

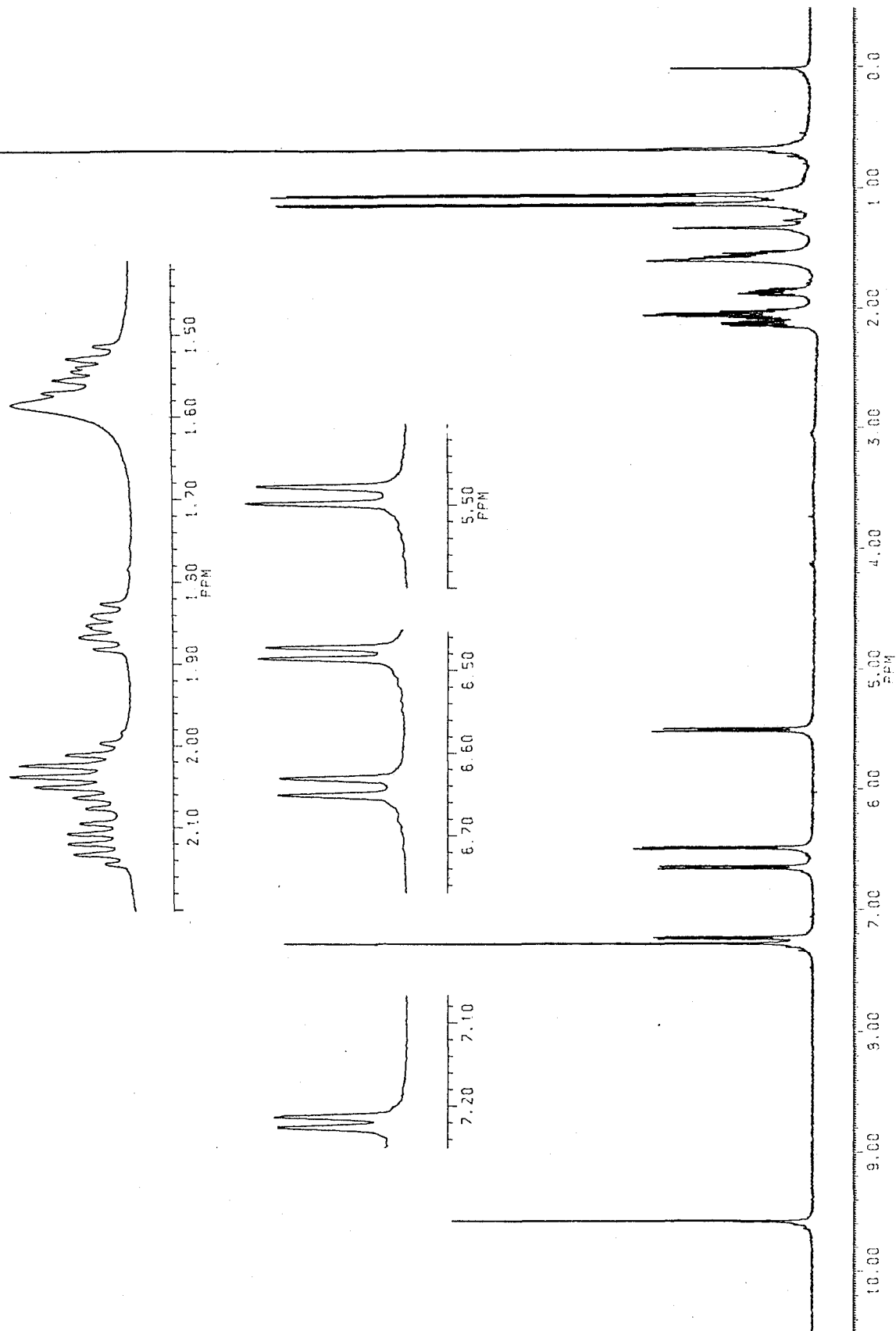


Fig. 3-324 ^1H -NMR Spectrum of RL-PERO-4-TU (500 MHz, in CDCl_3)

Table 3-124 ^1H -NMR chemical shift values for RL-PERO-4-TU

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}	Coupling			Assignment (for 18)
9.567	1H	s		C-14-H
7.219	1H	d	$J = 6.7$ Hz	C-5-H
6.640	1H	d	$J = 10.2$ Hz	C-3-H
6.478	1H	d	$J = 6.7$ Hz	C-6-H
5.487	1H	d	$J = 10.2$ Hz	C-2-H
2.118	1H	ddd	$J = 12.7, 6.4$ and 6.1 Hz	C-9-Ha
2.056	1H	dd	$J = 12.7, 6.5$ Hz	C-8-Ha
2.023	1H	sept.	$J = 6.8$ Hz	C-11-H
1.853	1H	ddd	$J = 12.9, 7.3$ and 7.2 Hz	C-8-Hb
1.541	1H	ddd	$J = 12.8, 7.8$ and 7.4 Hz	C-9-Hb
1.119	3H	d	$J = 6.8$ Hz	C-12- H_3
1.041	3H	d	$J = 6.8$ Hz	C-13- H_3
0.664	3H	s		C-15- H_3

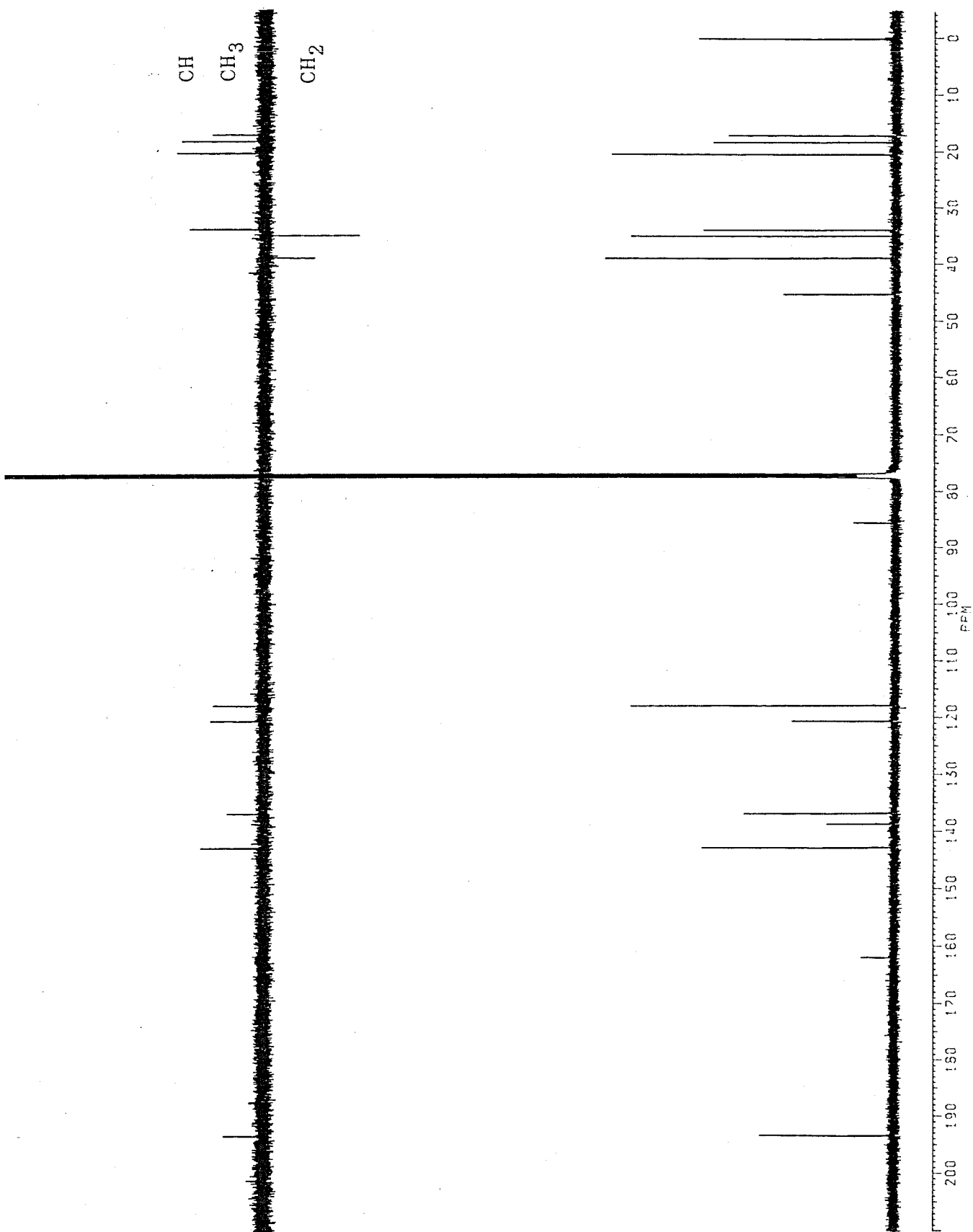


Fig. 3-325 ^{13}C -NMR Spectra of RL-PERO-4-TU (125 MHz, in CDCl_3 , COM and DEPT)

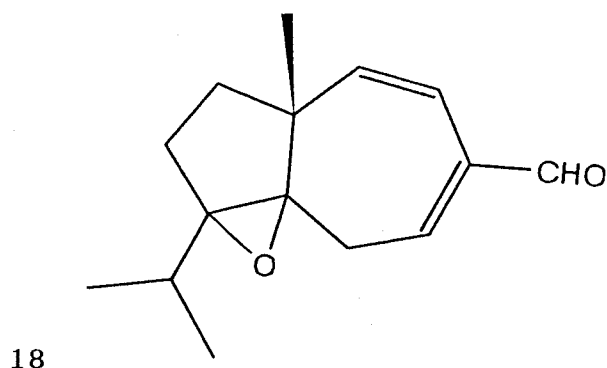
Table 3-125 ^{13}C -NMR chemical shift values of RL-PERO-4-TU

(78 MHz, COM and INEPT, in CDCl_3 , TMS as an int. std.)

δ_{C}	CHx	Assignment*
193.3	CH	C-14
161.6	C	C-1
142.7	CH	C-3
138.5	C	C-4
136.7	CH	C-5
120.5	CH	C-6
111.8	CH	C-2
85.5	C	C-10
45.1	C	C-7
38.7	CH_2	C-8
34.8	CH_2	C-9
33.7	CH	C-11
20.3	CH_3	C-15
18.2	CH_3	C-12
17.0	CH_3	C-13

* The assignment for RL-PERO-4 is according to the proposed structure 18.

Table 3-126 Physicochemical properties of isotrienecatoanal
epoxide (18)



A colorless oil

Vanillin-H₂SO₄ color: brown

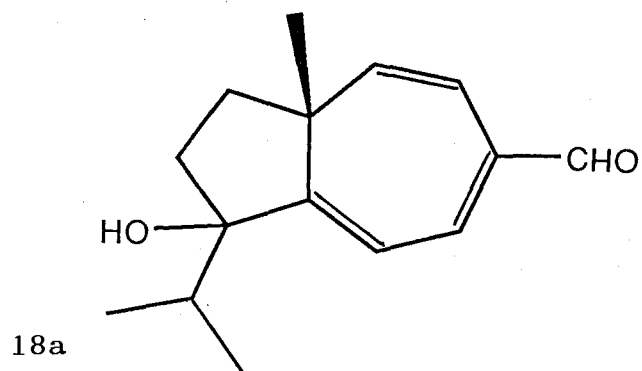
UVλ_{max}^{MeOH}: 270 nm

FI-MS *m/z* (%): 233 (M⁺+1, 24), 232 (M⁺, 100)

EI-MS *m/z* (%): 232 (M⁺, 13), 214 (4.9), 189 (3.8), 176 (6.2), 175 (14), 162 (5.8), 149 (12), 147 (9.0), 136 (14), 135 (12), 133 (12), 121 (17), 109 (17), 107 (25), 105 (16), 95 (12), 94 (15), 93 (27), 91 (26), 81 (16), 79 (28), 77 (16), 68 (100), 67 (31), 55 (22), 53 (20), 44 (37), 41 (34), 40 (48).

¹H- and ¹³C-NMR data are shown in Tables 3-124 and 3-125, respectively.

Table 3-127 Physicochemical properties of RL-PERO-4-TU (18a)



A colorless prism from n-hexane, mp. 145-146°C

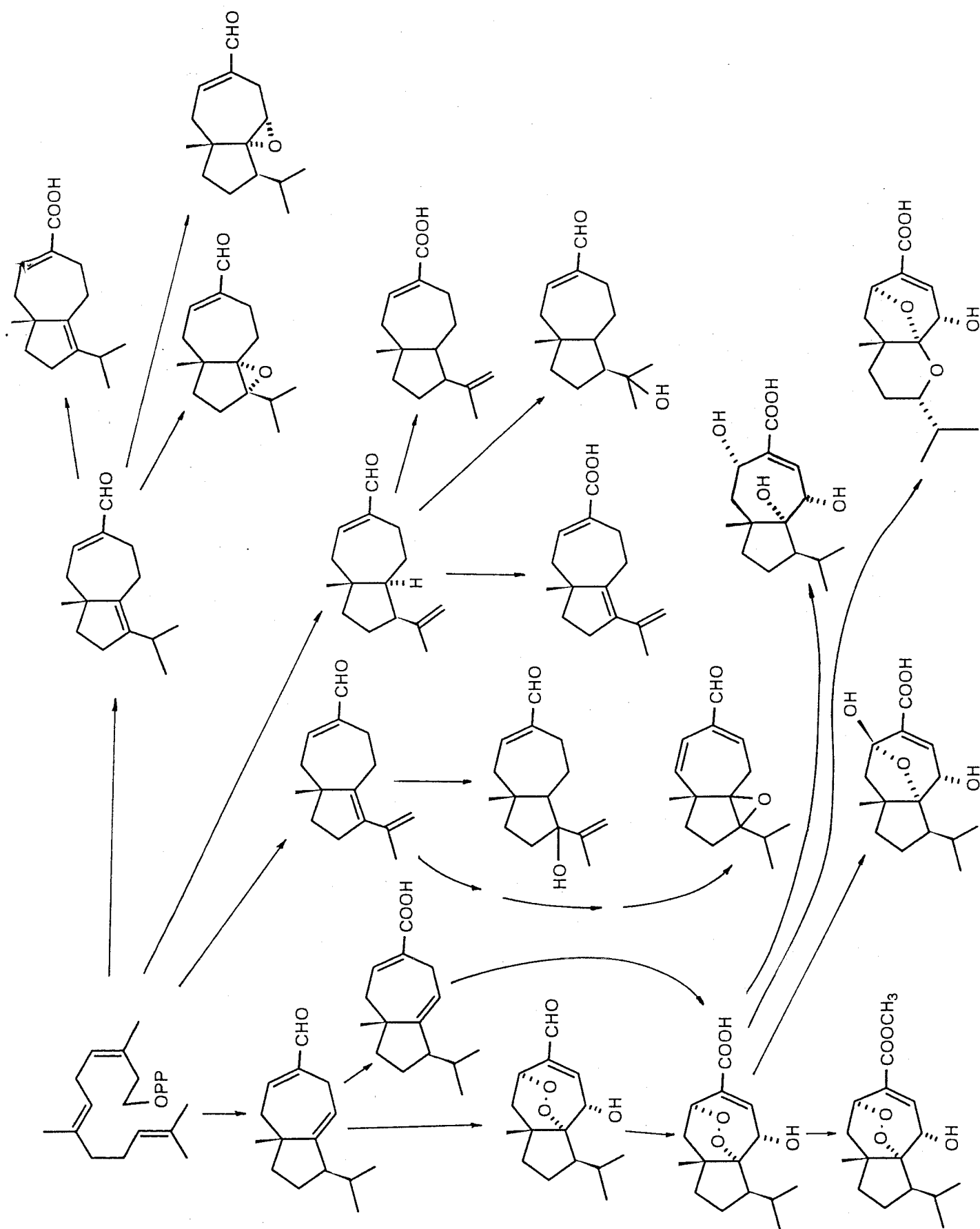
Vanillin-H₂SO₄ color: brown

FI-MS *m/z* (%): 232 (M⁺, 100)

EI-MS *m/z* (%): 232 (M⁺, 13), 203 (4.9), 189 (3.8), 176 (6.2), 175 (14), 162 (5.8), 149 (12), 147 (9.0), 136 (14), 135 (12), 133 (12), 121 (17), 109 (17), 107 (25), 105 (16), 95 (12), 94 (15), 93 (27), 91 (26), 81 (16), 79 (28), 77 (16), 68 (100), 67 (31), 55 (22), 53 (20), 44 (37), 41 (34), 40 (48)

¹H- and ¹³C-NMR data are shown in Tables 3-153, 3-154 and 3-155, respectively.

As the result of a survey of carotanoids in *Rosa rugosa* leaves, it was found that some carotane aldehydes and their oxygenated derivatives were also contained. In particular, simple carotane aldehydes, daucenaldehyde (10), isodaucenaldehyde (12) and 11,12-dehydrodaucenaldehyde (16) are in the position as precursors of further oxygenated minor carotanoids mentioned above. Those isomers and the triene aldehyde are speculated to be formed as a by-product in the biosynthesis of carota-1,4-dienaldehyde (3) pathway, since the contents of those compounds are markedly lower than that of 3. The author cannot discuss the significance of those minor carotanoids; however, they provided several information about the spectroscopic properties of carotanoids. The proposed biosynthetic relations among isolated compounds are shown in Scheme 3-35.



Scheme 3-35 Biogenesis of carotenoids in *Rosa rugosa* leaves

3-8 Bisabolanoids of *Rosa rugosa*

3-8-1 Introduction

Rugosa rose (*Rosa rugosa*) leaves were revealed to contain various kinds of carotane sesquiterpenoids, which were regio-specifically oxygenated at the C-14 carbon to yield an α,β -unsaturated aldehyde, an α,β -unsaturated carboxyl or an α,β -unsaturated methoxycarbonyl part structure. On the other hand, carotanoids of Umberifellae and Compositae are mostly not oxygenated at C-14, with only a exception [43]. Umberifellae and Compositae are also known as terpenoid-rich plants. Especially, both families contain some species as an abundant source of bisabolanoid [55,56] whose biosynthetic pathway is partly overlapped with that of carotanoid. The presence of bisabolanoids in Compositae and Umberifellae may be recognizable as an evidence for relationships of bisabolanoids to carotanoids in their biosynthesis. Indeed, for both sesquiterpenoids *cis-trans*-farnesyl-pyrophosphate pathway is proposed [57].

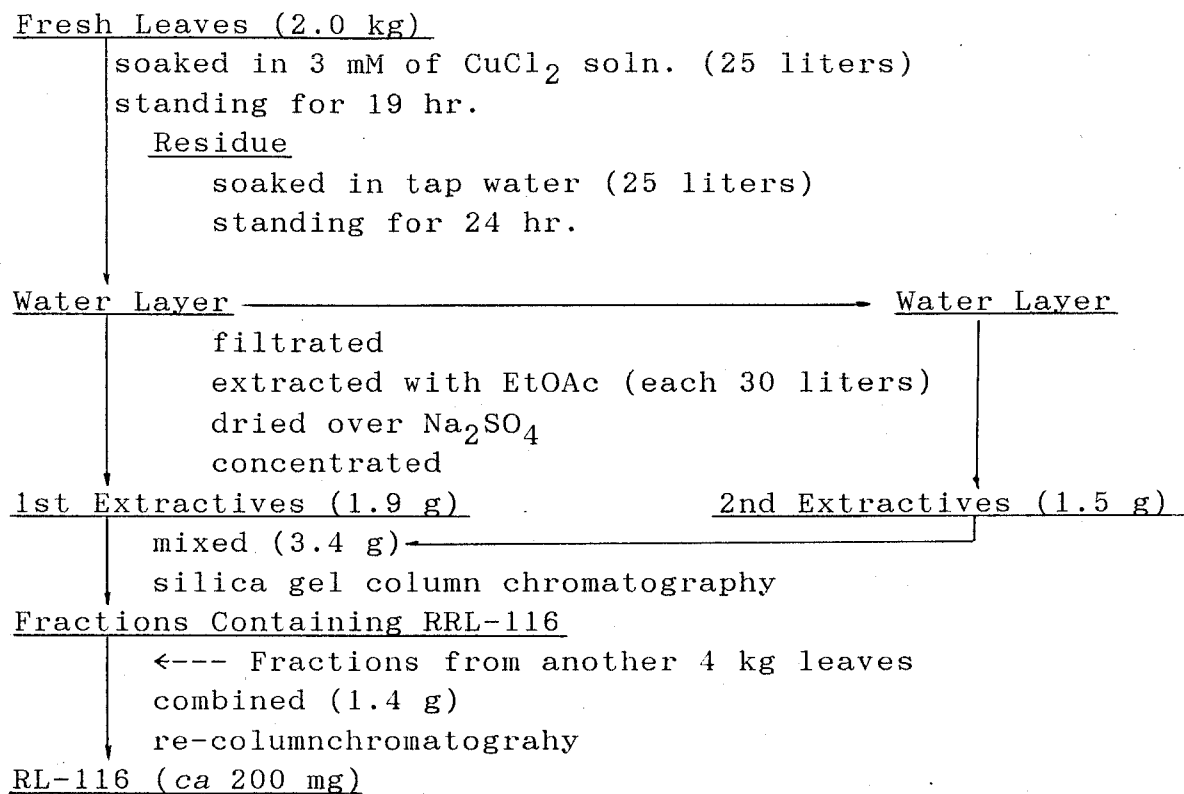
In *Rosa rugosa* leaves, some sesquiterpenes of non-carotane class are also found. During a survey of constituents in *rugosa rose* (*Rosa rugosa*) leaves, those non-carotane sesquiterpenes were obtained and elucidated their structures to be bisabolanoids. In this section, the author describes those *Rosa rugosa* bisabolanoids and discusses on the relationship between carotanoids and bisabolanoids in this plant.

1) Isolation

During the author's initial experiments, the fresh and non-damaged leaves of *Rosa rugosa* were soaked in aq. 3 mM CuCl_2 solution to survey some stress compounds, which were expected to be diffusable into the water layer [136]. Although no stress compound was detected in the preliminary experiment, a noticeable quenching spot showing a reddish purple color with vanillin- H_2SO_4 reagent was found as one of the major extractives. The focused compound denoted RL-116 was detectable both in the CuCl_2 solution and in the fresh water (control experiment). RL-116 was successively aimed to isolate and elucidate the structure. To obtain the compound, 2.0 kg of fresh leaves of *Rosa rugosa* were collected (Sample I) and soaked in 3 mM CuCl_2 solution (25 liters) for 19 hr and successively in the same volume of tap water for another 24 hr. The former and the latter layers water were respectively extracted with EtOAc (1000 ml water/600 ml x 2), and the organic layer was dried over Na_2SO_4 and concentrated. As the results, total 3.6 g of extractives (2.1 g and 1.5 g, respectively) were obtained (Scheme 3-36).

Since these extracts showed almost indistinguishable pattern in TLC, those were combined and coated on 40 ml of silica gel. The gel was put onto the Wako gel column settled in *n*-hexane (gel volume, 150 ml), and the constituents were eluted with Et_2O /hexane mixtures (Table 3-128 and Fig. 3-326).

Another 4.0 kg of leaves (sample I) was also soaked in 30 liters of 3 mM CuCl_2 solution and successively in 30 liters of tap water. The total 60 liters of water layers were extracted with EtOAc as described above to obtain 9.5 g of constituents which were also fractionated over silica gel column (volume 500 ml) with Et_2O /*n*-hexane mixtures as eluting solvent (Table 3-129 and Fig. 3-327).



Scheme 3-36 Fractions containing RL-116 obtained by column chromatography of the Sample I (TLC pattern is shown in Fig. 3-309)

Table 3-128 Fractions obtained by silica gel column chromatography of extractives from Sample I

Fraction	Eluting solvent	Volume
Fr-Ia-1	10 % Et_2O /hexane	100 ml
Fr-Ia-2	10 % Et_2O /hexane	200 ml
Fr-Ia-3	10 % Et_2O /hexane	200 ml
Fr-Ia-4	20 % Et_2O /hexane	200 ml
Fr-Ia-5	35 % Et_2O /hexane	150 ml
Fr-Ia-6	35 % Et_2O /hexane	150 ml
Fr-Ia-7	50 % Et_2O /hexane	150 ml
Fr-Ia-8	50 % Et_2O /hexane	150 ml
Fr-Ia-9	100 % Et_2O /hexane	150 ml

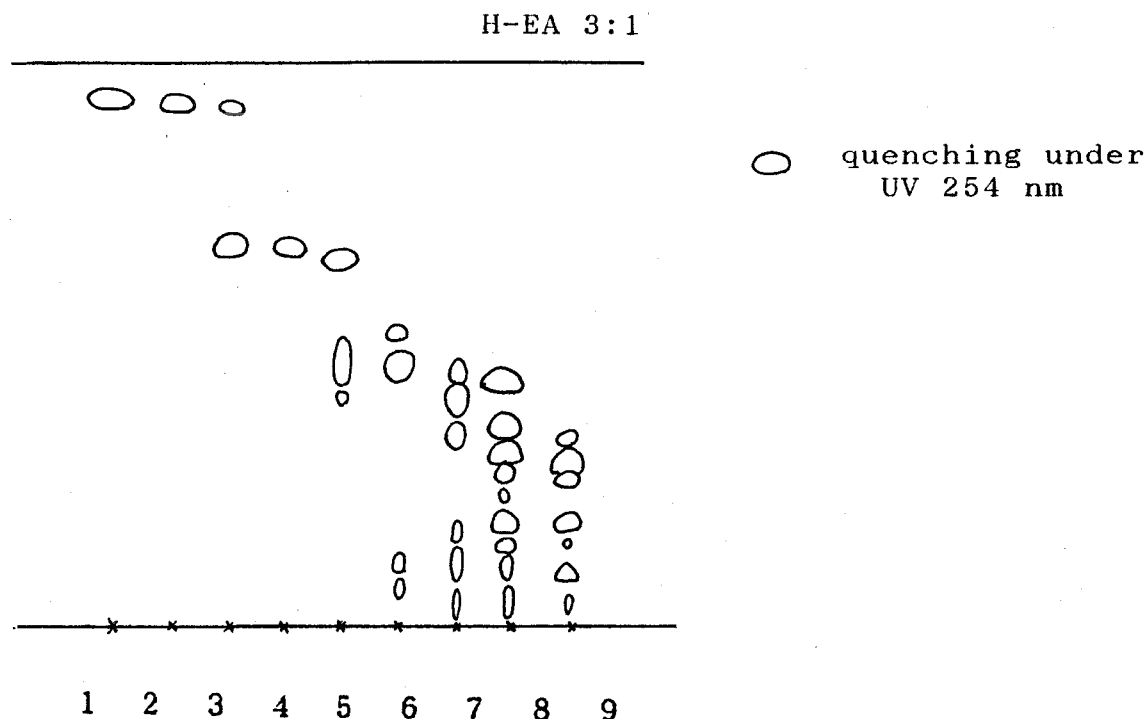


Fig. 3-326 TL Chromatogram of Fractions Obtained by Silica Gel Column Chromatography of Exudates from Sample I

A fraction containing RL-116 in the second extractives (Fr-Ia-8 and Fr-Ib-8) was combined with the former one (total 1.4 g) to rechromatograph over silica gel column (volume 150 ml). The constituents were successively eluted with EtOAc/*n*-hexane mixtures as follows: FFr-1; washings with 25 % EtOAc/hexane (100 ml), FFr-2; eluted with 25 % EtOAc/hexane (50 ml), FFr-3; eluted with 25 % EtOAc/hexane (50 ml). The focused substance appearing as a single spot on TL plate was contained in FFr-2. The pure colorless syrup (ca 200 mg of a colorless syrup) was finally obtained by PTLC in hexane-acetone 4:1 (*R_f* 0.28). For the spectroscopic analyses, RL-116 was further purified by PTLC in hexane-EtOAc 4:1. FFr-3 also contained marked amount of RL-116 (Fig. 3-328).

Table 3-129 Second fractions obtained by silica gel column chromatography of extractives from Sample I (4 kg)

Fraction	Eluting solvent	Volume
Fr-Ib-1	30 % Et ₂ O/hexane	500 ml
Fr-Ib-2	30 % Et ₂ O/hexane	200 ml
Fr-Ib-3	50 % Et ₂ O/hexane	200 ml
Fr-Ib-4	50 % Et ₂ O/hexane	200 ml
Fr-Ib-5	55 % Et ₂ O/hexane	200 ml
Fr-Ib-6	Et ₂ O	200 ml
Fr-Ib-7	Et ₂ O	200 ml
Fr-Ib-8	EtOAc	200 ml
Fr-Ib-9	EtOAc	200 ml

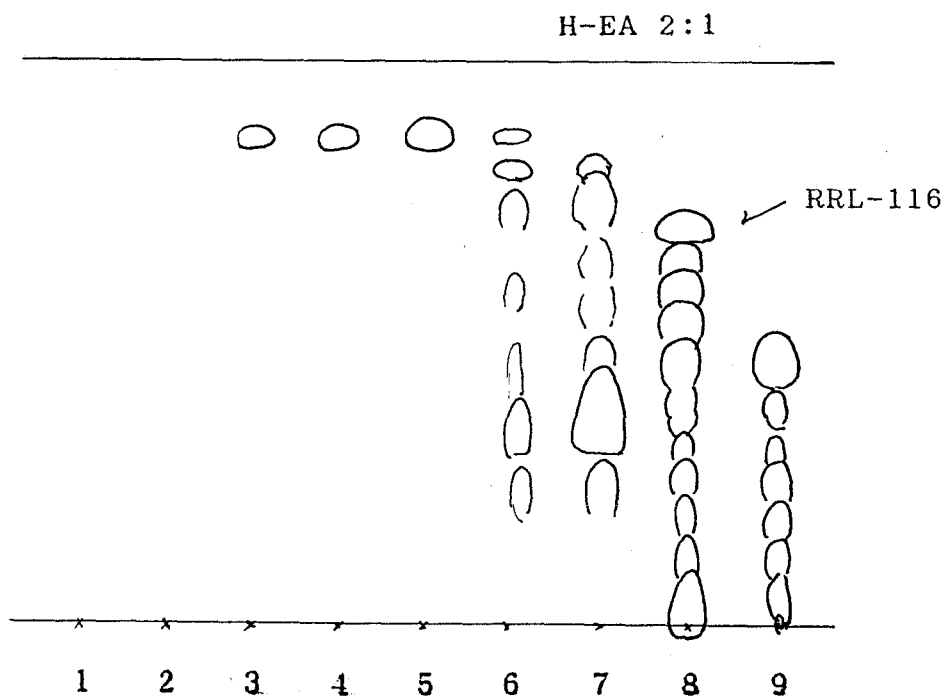


Fig. 3-327 TL Chromatogram of Fractions Obtained by Silica Gel Column Chromatography of Exudates from Sample I (4 kg)

H-EA 2:1

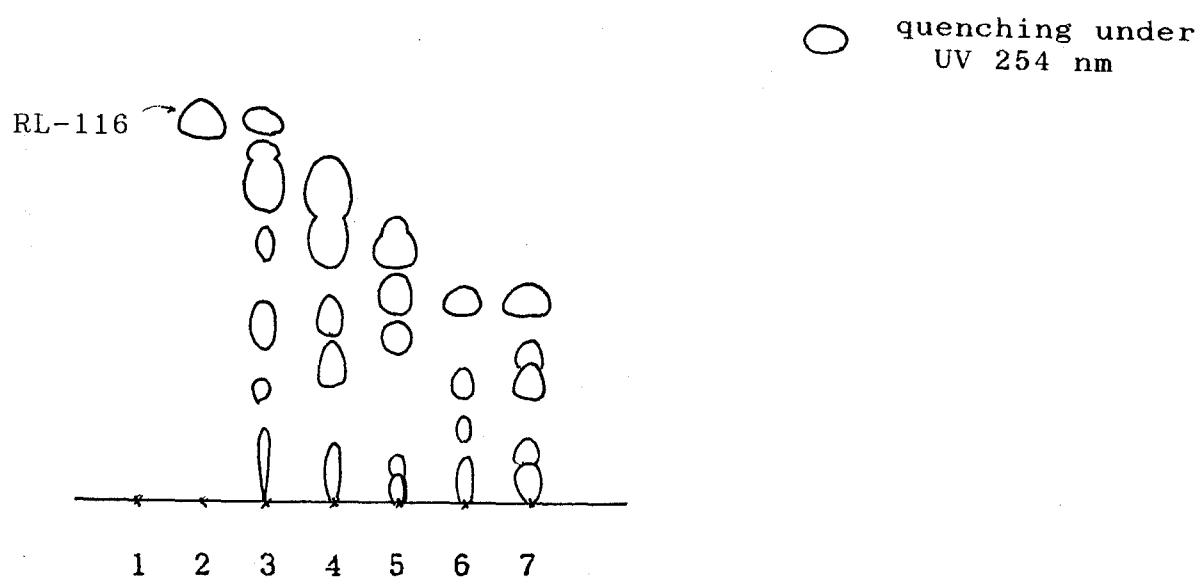


Fig. 3-328 TL Chromatogram of Fractions Obtained by Re-column Chromatography

2) Structure Elucidation by Spectroscopic Methods

RL-116 showed the parent ion M^+ at m/z 266 (100 %) in FI-MS (Fig. 3-329) and the EI-HR-MS revealed its molecular formula $C_{16}H_{26}O_3$ (M^+ 266.184, calcd. 266.188). The EI-MS fragments at m/z 266 (M^+ , 1.1 %), 248 ($M^+ - H_2O$, 23 %) and 217 ($M^+ - H_2O - OCH_3$, 5.2 %) indicated the presence of an -OH group (Fig. 3-330). The UV spectrum showed the absorption maximum at 218 nm due to an α, β -unsaturated hydroxycarbonyl group. Its infrared spectrum (KBr) was also indicative of the presence of hydroxyl (3500 cm^{-1} , broad), carbonyl (1720 cm^{-1} , sharp) and ether (1260 cm^{-1} , sharp) moieties (Fig. 3-331).

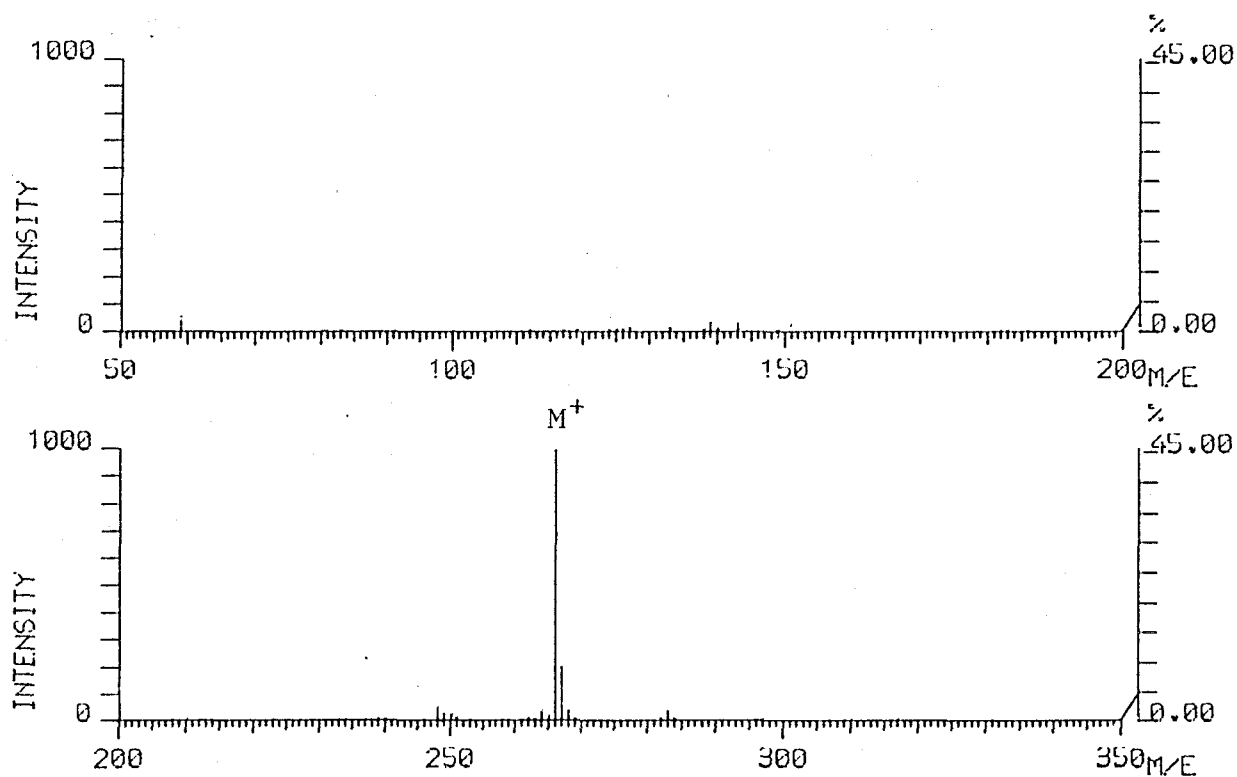


Fig. 3-329 FI-Mass Spectrum of RL-116.

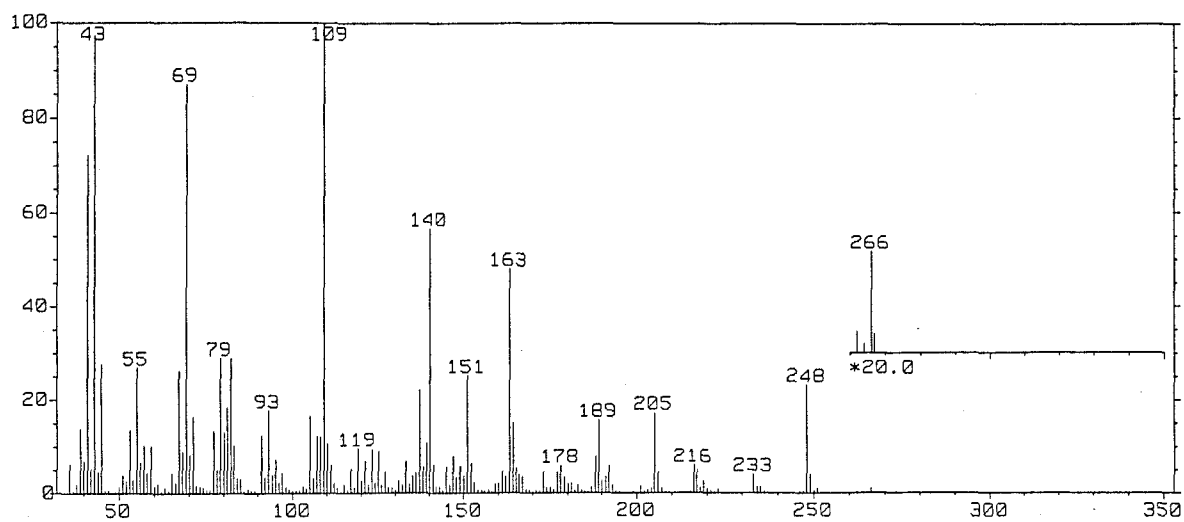


Fig. 3-330 EI-Mass Spectrum of RL-116

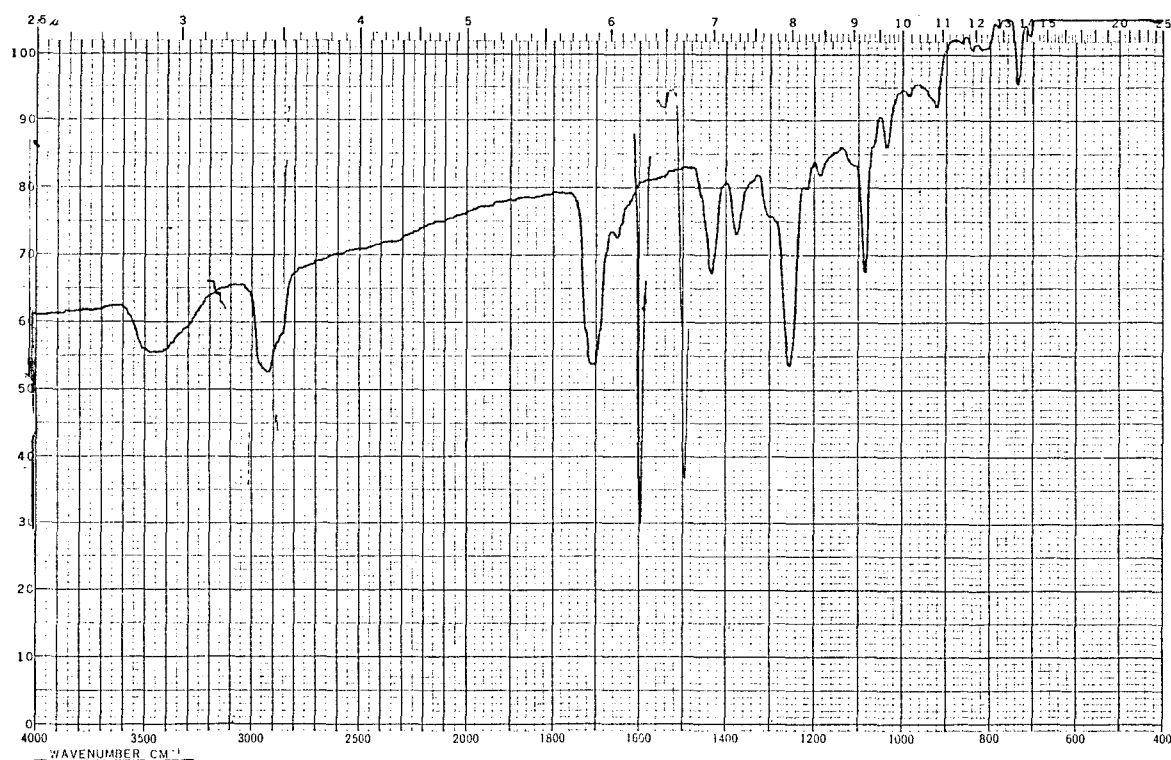


Fig. 3-331 IR Spectrum of RL-116

In ^1H -NMR spectrum (in CDCl_3 : 500 MHz), two isolated olefinic protons were detected at δ_{H} 7.005 and 5.125 (Fig. 3-332 and Table 3-130). The ^{13}C -NMR spectrum revealed four sp^2 carbons [δ_{C} 139.8 (C), 132.0 (CH), 129.9 (CH) and 124.3 (C)] attributable to two $\text{C}=\text{CH}-$ moieties (Fig. 3-333 and Table 3-131). Two allylmethyl groups (δ_{H} 1.696, br. s, and 1.665, br. d, $J=1.0$ Hz) and an olefinic proton (δ_{H} 5.125, 1H, multiply divided triplet, $J=7.2$ and 1.0 Hz) were all characteristic of protons of 3,3-dimethylallyl group. In COSY-NMR spectrum, the proton network system (substructure A) including this 3,3-dimethylallyl group was revealed (Fig. 3-334 and 335). The olefinic proton showed a cross peak with a pair of methylene protons (multiplet, 2H around δ_{H} 2.0), in addition to a cross peak with one of the allylmethyl signals due to an allyl coupling. Furthermore, the methylene proton (δ_{H} 2.0) indicated another coupling with a triplet signal at δ_{H} 1.536 (2H, $J=8.3$) attributable to a pair of equivalent methylene protons. The proton-proton coupling sequence was closed there to give a side chain $[(\text{CH}_3)_2\text{C}=\text{CHCH}_2\text{CH}_2-]$. Substructure A was also confirmed by the two point-homodecoupling experiment irradiated at 5.125 ppm and 1.536 ppm. As a result, the non-equivalent methylene protons (δ_{H} 2.090 and 2.029 geminally coupled with $J=14.6$ Hz) became feasible (Fig. 3-336).

On the other hand, another substructure involving the conjugation system was also raised by the ^1H -NMR spectrum. A methyl proton assignable to a methoxycarbonyl group was detected at δ_{H} 3.750 (3H, s). The presence of the methoxycarbonyl group was also proved by ^{13}C -NMR detection of two carbons at δ_{C} 51.5 (CH_3) and 167.9 (C) both attributable to the $-\text{COOMe}$ carbon. Since two sp^2 carbons out of four have had been attributed to substructure A, the remaining two had to be assigned to the olefinic bond to form a conjugation with the carbonyl group. The geometry of the conjugated C,C-double bond was deduced to be *E* (*cis* regarding the $-\text{COOMe}$ and an olefinic proton at δ_{H} 7.005) configuration since the

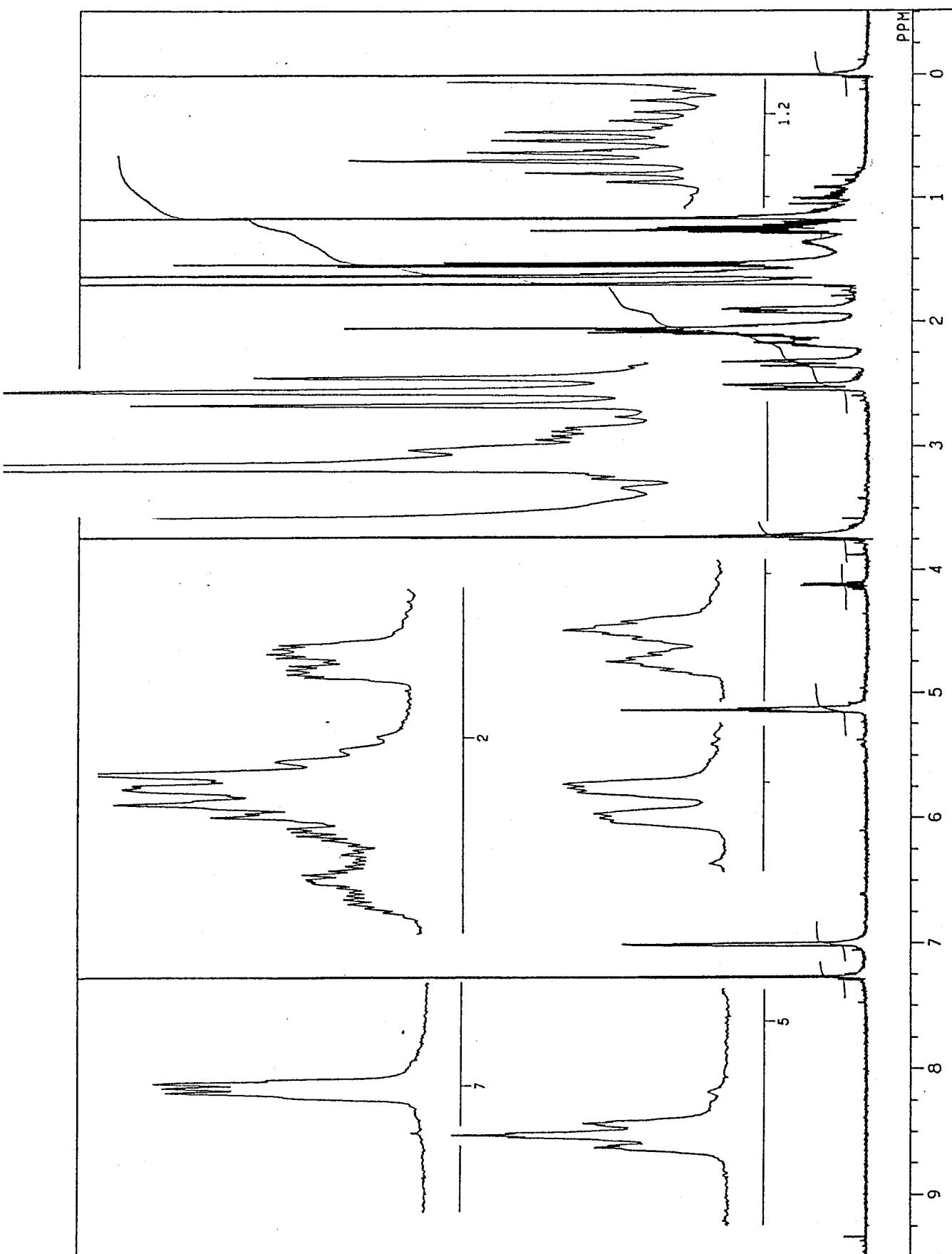


Fig. 3-332 ^1H -NMR Spectrum of RL-116 (500 MHz, in CDCl_3)

Table 3-130 ^1H -NMR chemical shift values of RL-116

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}		Coupling	Assignment
7.005	1H	br. m	C-2-H
2.337	1H	m-dvid. d* $J= 18.6$ Hz	C-3-Ha
2.087	1H	m-dvid. dd $J= 18.6$, ca 15 Hz	C-3-Hb
1.610	1H	m	C-4-H
1.907	1H	m	C-5-Ha
1.249	1H	dddd $J= 12.7, 12.2, 12.2, 5.4$ Hz	C-5-Hb
2.525	1H	m-dvid. d $J= 17.6$ Hz	C-6-Ha
2.168	1H	m-dvid. d $J= 17.6$ Hz	C-6-Hb
3.729	3H	s	C-7'-H ₃
1.536	2H	t $J= 8.3$ Hz	C-9-H ₂
1.160	3H	s	C-10-H ₃
2.090	1H	ddd $J= 14.6, 8.3, 7.3$ Hz	C-11-Ha
2.029	1H	ddd $J= 14.6, 8.3, 7.3$ Hz	C-11-Hb
5.135	1H	m-dvid. dt $J= 7.3, 1.0$ Hz	C-12-H
1.692	3H	d $J= 1.0$ Hz	C-14-H ₃
1.626	3H	br. s	C-15-H ₃

* multiply divided doublet

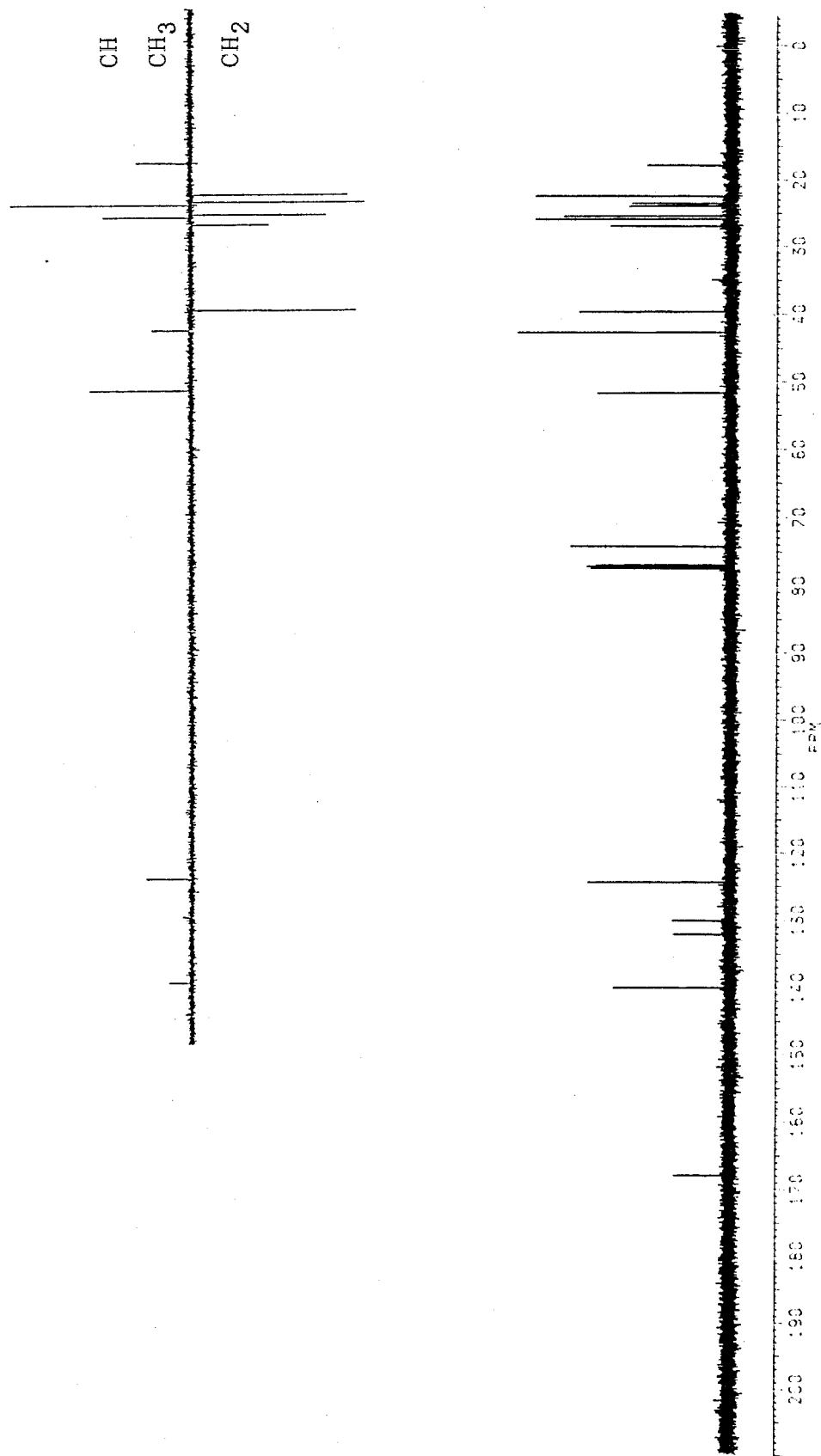


Fig. 3-333 ^{13}C -NMR Spectrum of RL-116 (125 MHz, in CDCl_3 , COM and INEPT)

INEPT was measures at 68 MHz.

Table 3-131 ^{13}C -NMR chemical shift values of RL-116 and comparisons of the shifts with those of α -bisabolol (94)

(125 MHz, in CDCl_3 , TMS as an int. std.)

δ_{C}	Property	Assignment	94 [137]
167.9	-COO-	C-7	23.15
139.8	=CH-	C-2	120.53
132.0	=C-	C-1 (or C-13)	134.00
129.9	=C-	C-13 (or C-1)	131.54
124.3	=CH-	C-12	124.58
74.1	-C-O	C-8	74.21
51.5	-O-CH ₃	C-7'	-
42.5	CH	C-4	42.94
39.5	CH ₂	C-9	40.08
26.8	CH ₂	C-3 (or C-5, C-6, C-11)	26.88
25.7	CH ₂	C-6 (or C-3, C-5, C-11)	31.00
25.2	CH ₃	C-14	25.65
23.8	CH ₃	C-10	23.28
23.3	CH ₂	C-5 (or C-3, C-6, C-11)	22.02
22.3	CH ₂	C-11 (or C-3, C-5, C-6)	23.25
17.7	CH ₃	C-15	17.59

* Hydrogenation degree are elucidated by INEPT experiment
(at 68 MHz)

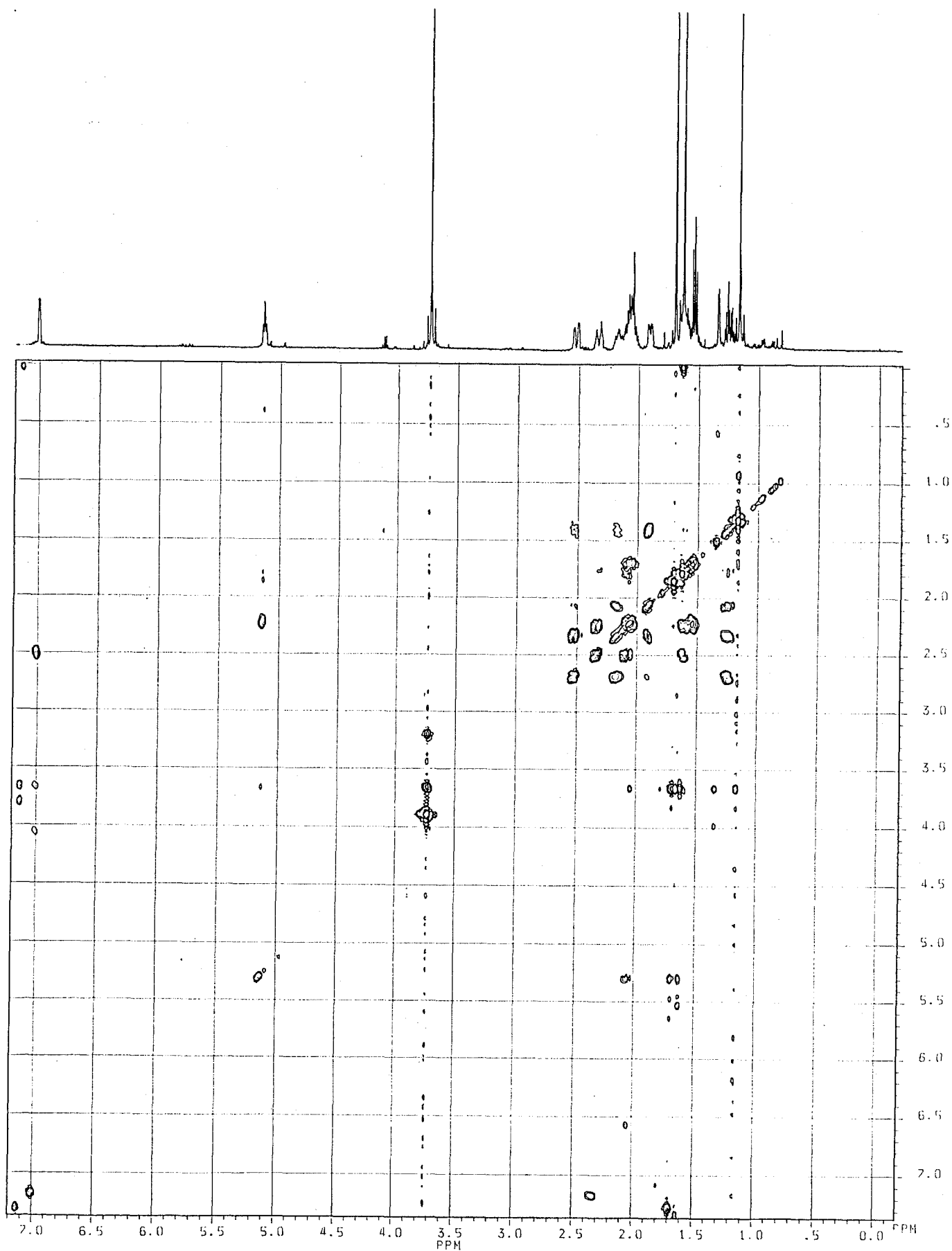


Fig. 3-334a HH-COSY Spectrum of RL-116 (500 MHz, in CDCl₃)

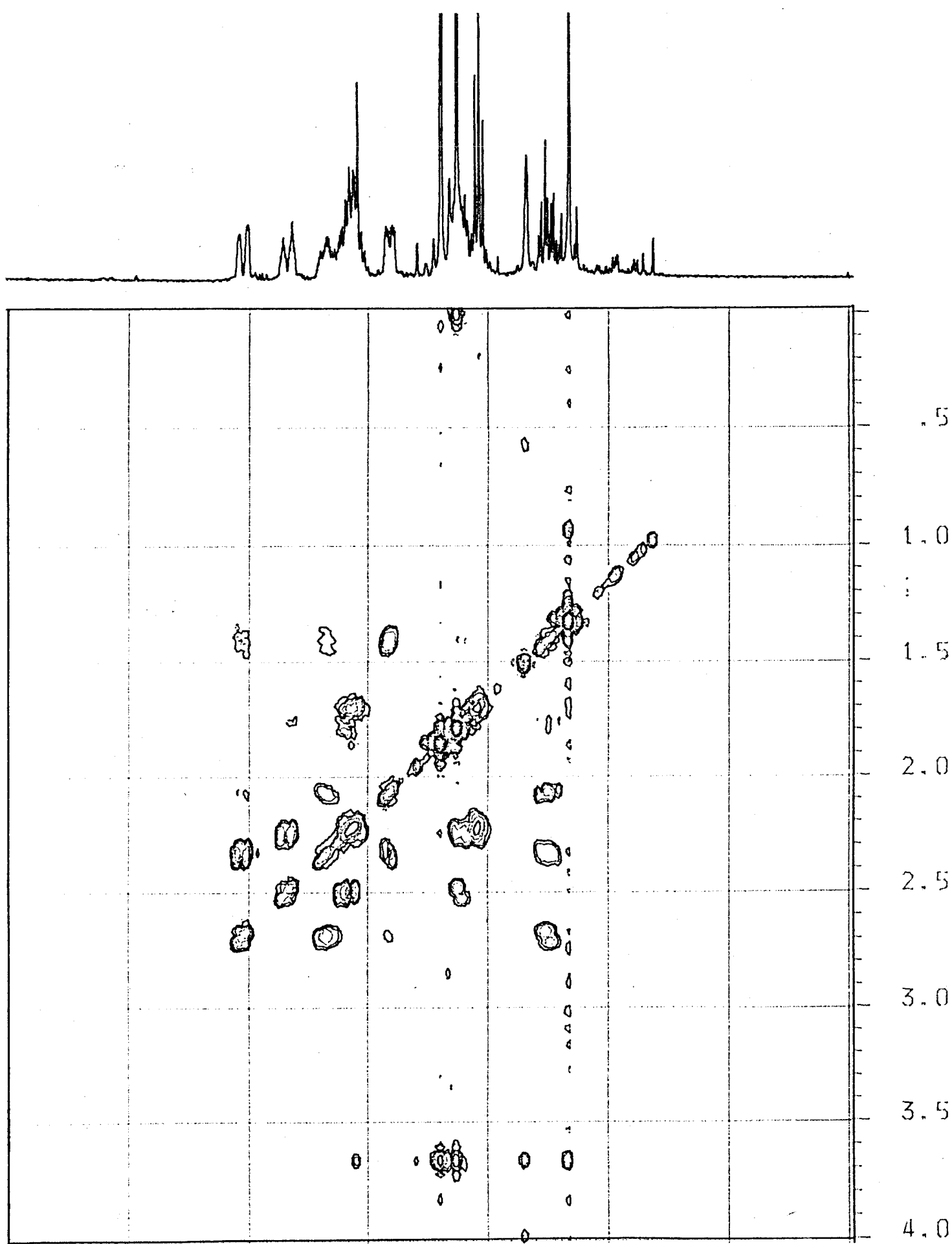
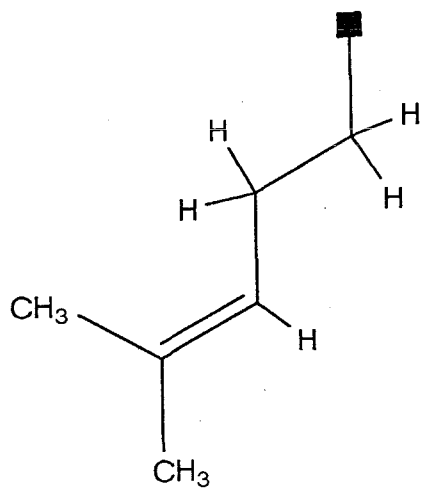
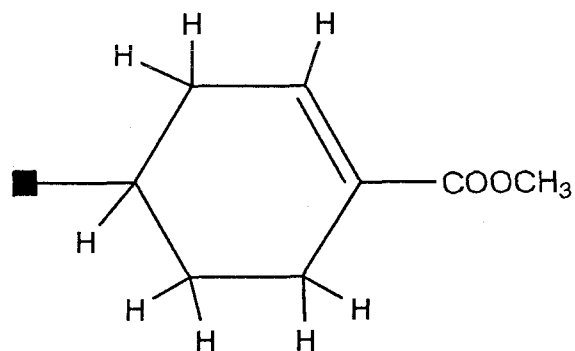


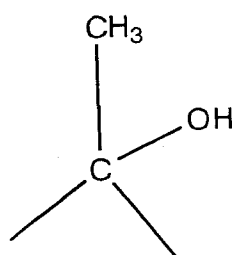
Fig. 3-334b Continued (Magnified in High Magnetic Field)



substructure A



substructure B



substructure C

Fig. 3-335 Substructures (A, B and C) of RL-116 Elucidated by Proton Coupling Sequence

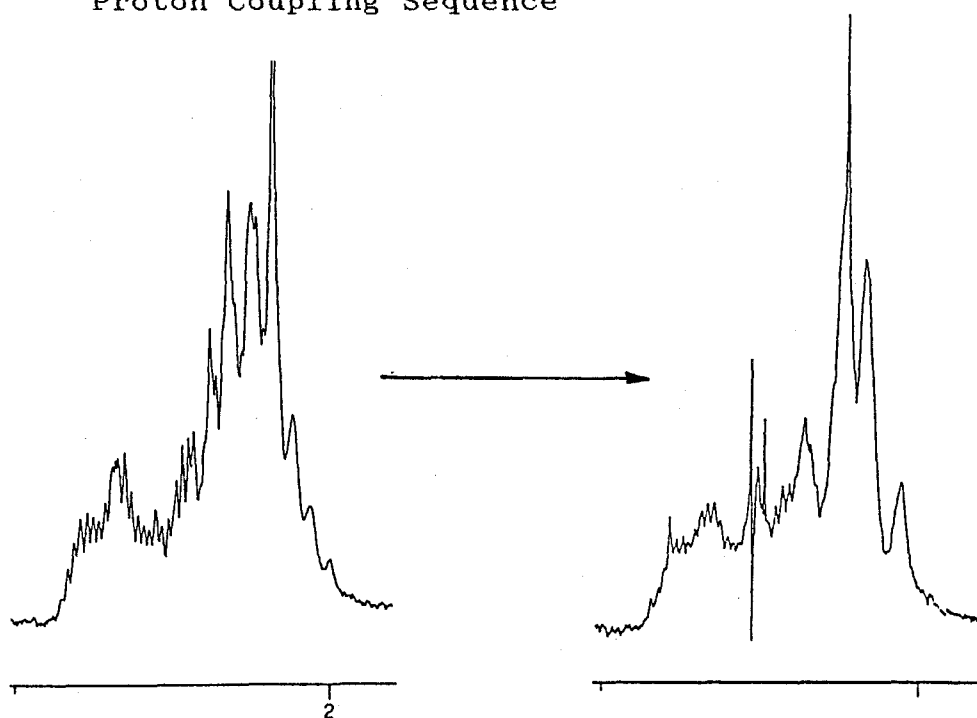


Fig. 3-336 Proton Signals Changed by Irradiation of δ_H 5.125 and 1.536

chemical shift value of the olefinic proton on the β -position was detected at a markedly lower field, due to a deshielding effect of the carbonyl group.

The HH-COSY, CH-COSY and spin-spin decoupling experiments also proved the presence of a proton coupling network for substructure B (Fig. 3-337 and Table 3-132). The olefinic proton resonated at δ_H 7.005 showed a vicinal coupling with a pair of methylene protons at δ_H 2.337 and 2.087 geminally coupled each other with $J = 18.6$ Hz (cross peak with C-3 methylene carbon at δ_C 26.8). In the HH-COSY, this coupling sequence showed a further extension to a proton signal resonating at δ_H 1.610 (1H, overlapped) that afforded a cross peak with C-4 methine carbon at δ_C 42.5 in the CH-COSY spectrum. When the olefinic proton was irradiated, together with those vicinal methylene protons (δ_H 2.337 and 2.087), another pair of methylene protons at δ_H 2.525 (br. d, $J = 17.6$ Hz) and 2.168 (m) both assignable to methylene carbon δ_C 25.7 (C-6) were changed their signal patterns. The latter fact was suggestive of the presence of an allyl coupling between them. These allylic methylene protons were remarkably deshielded to show their chemical shifts in a downfield by the carbonyl group, and further coupled with a pair of methylene protons at δ_H 1.907 (1H, br. d, $J = 12.7$ Hz) and 1.249 (1H, dddd, $J = 12.7, 12.2, 12.2$ and 5.4 Hz) revealing a cross peak with a methylene carbon at δ_C 23.3 (C-5). Coupling sequence of the $\text{CH}=\text{C}-\text{CH}_2-\text{CH}_2$ side was further extended to the methine proton at δ_H 1.610 that was coupled with the methylene protons at δ_H 2.337 and 2.087. Consequently, the substructure B was revealed to be a 1,4-disubstituted 1-cyclohexene ring (See Fig. 3-335). The only sp^3 methine carbon at δ_C 42.5 which showed a cross peak with the proton at δ_H 1.610 in CH-COSY was reasonably assigned to the C-4 methine carbon.

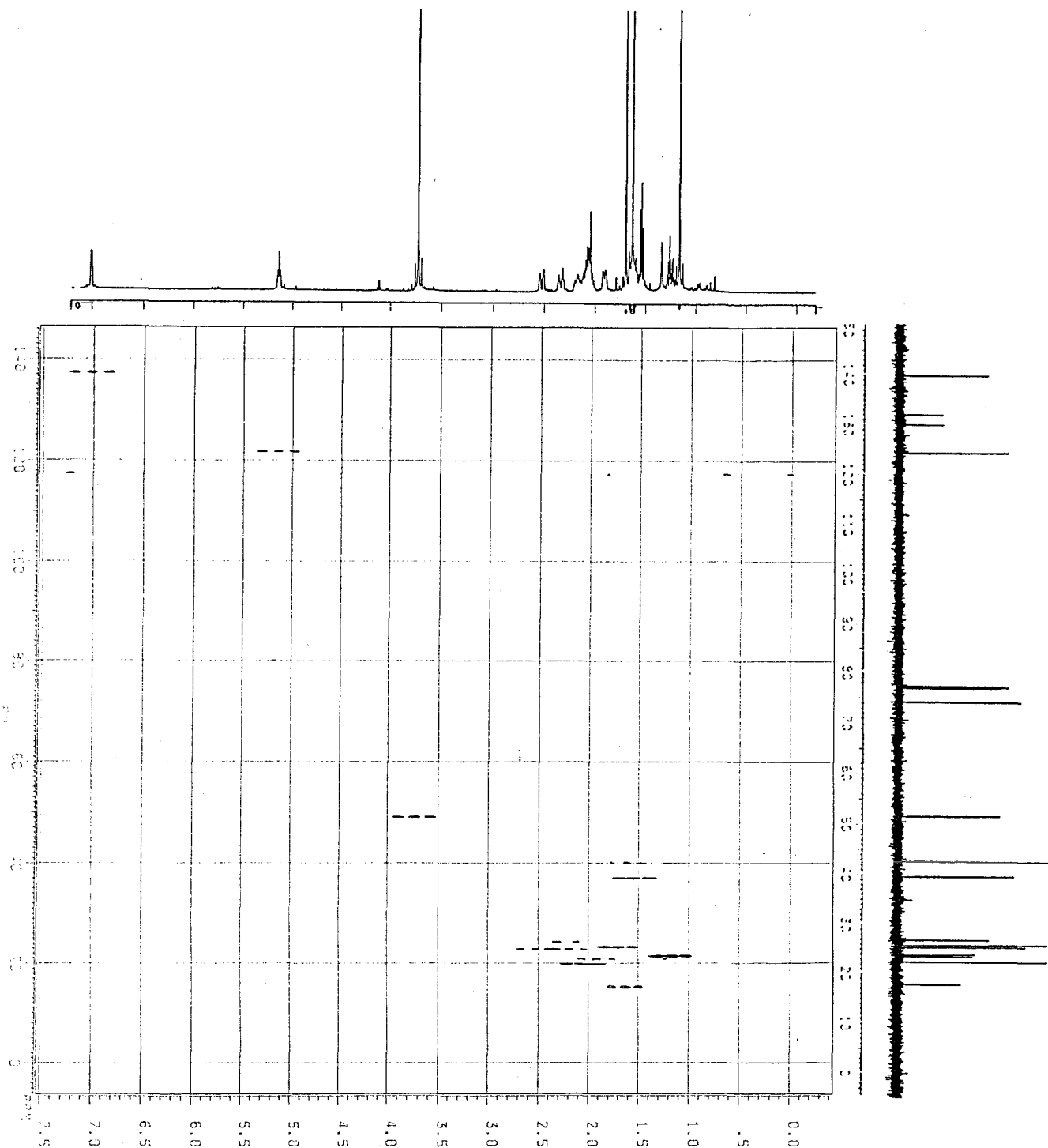


Fig. 3-337a CH-COSY Spectrum of RL-116 (500 and 270 MHz, in CDCl_3)

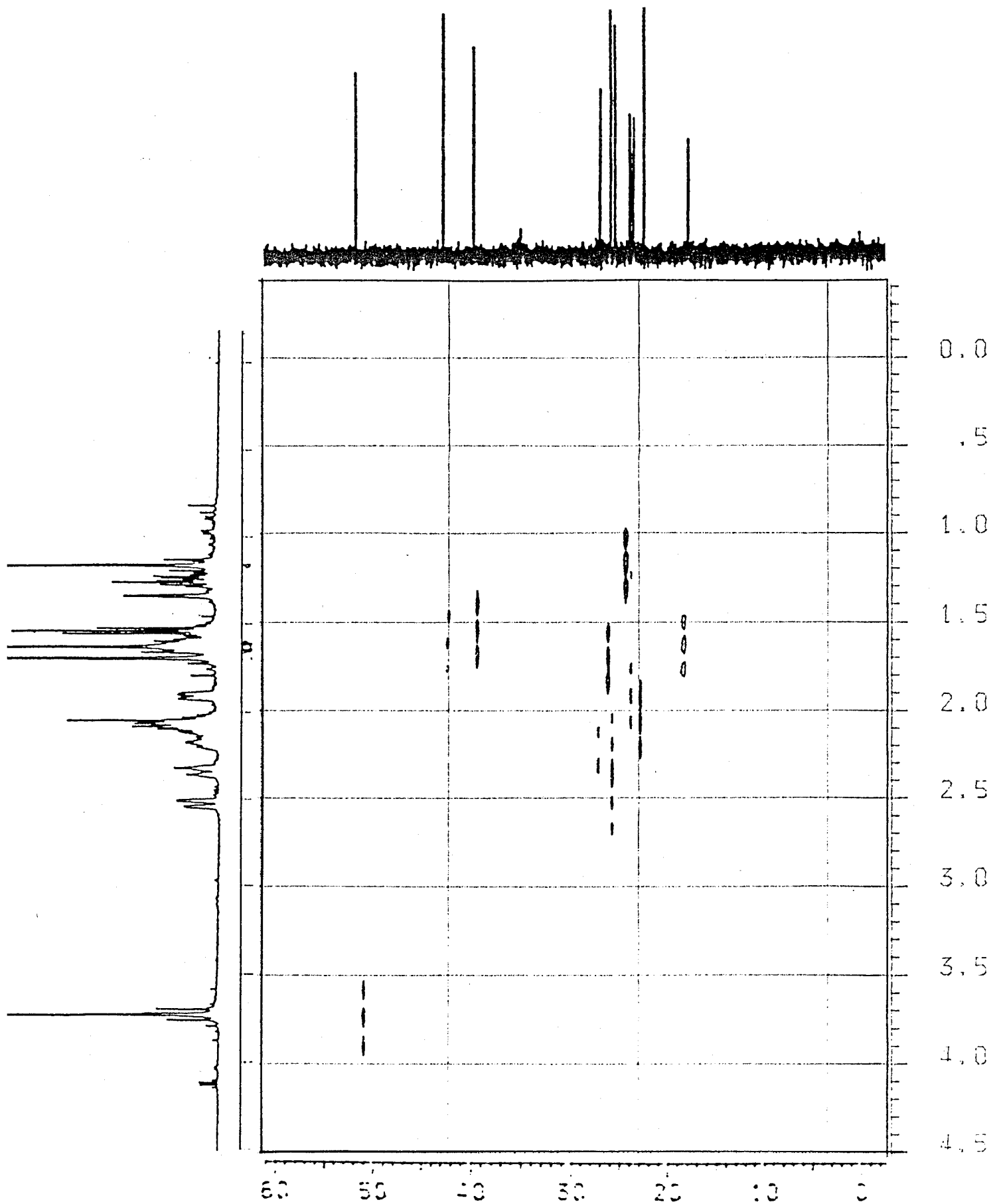


Fig. 3-337b Continued (Magnified in High Magnetic Field)

*Table 3-132 Relation between carbon and proton signals by CH-COSY
of RL-116*

(500 and 125 MHz, in CDCl_3 , TMS as an int. std.)

Carbon (δ_{C})	Proton (δ_{H})	Assignment
139.7	7.005	C-2
124.3	5.135	C-12
51.5	3.729	C-7'
42.5	1.610	C-4
39.5	2.090, 2.029	C-9
26.8	2.337, 2.087	C-3
25.7	1.692	C-14
25.3	2.525, 2.168	C-6
23.8	1.160	C-10
23.4	1.907, 1.249	C-5
23.1	1.536	C-11
17.7	1.626	C-15

The methyl group detected at δ_H 1.160 as an isolated signal in the ^1H -NMR was favorably allocated to a hydroxylated tertiary carbon (C-8) resonating at δ_C 74.1 (C) (See Fig. 3-335), and this methyl group on the tertiarily hydroxylated carbon was deduced to be the third substructure (C). Thus, all the carbons of total 16 were characterized.

To give a planar structure for the compound, substructures A and B should be combined through the tertiary hydroxylated carbon in the substructure C. Then, these three substructures form a bisabolane skeleton known as one of typical sesquiterpenoid skeleton. Both α -carbons to the hydroxylated one gave a reasonable chemical shift values (δ_C 39.5, in substructure A, and δ_C 42.5, in substructure B), which were compatible well with the proposed planar structure 19 (Fig. 3-338).

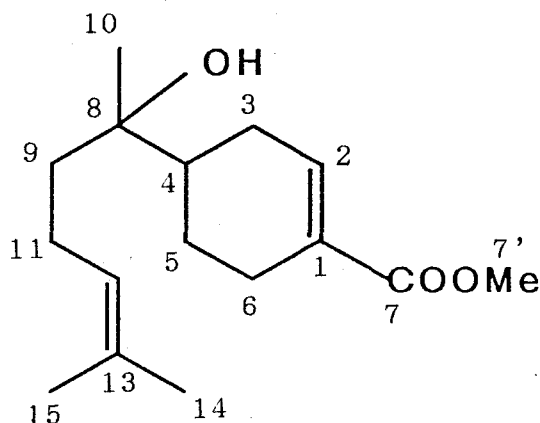
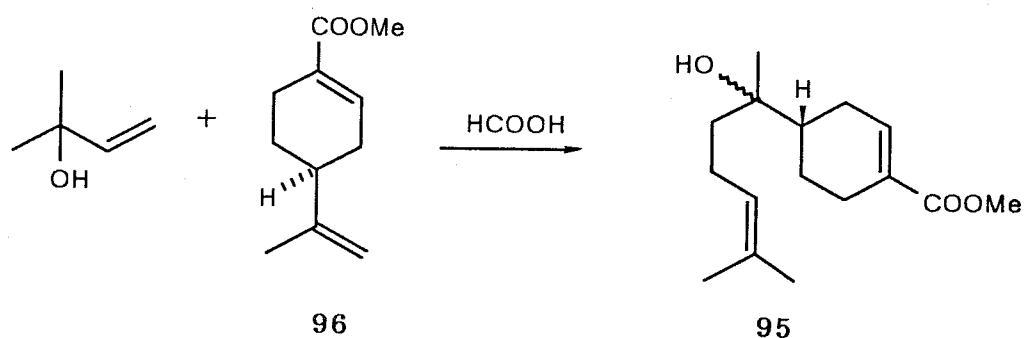


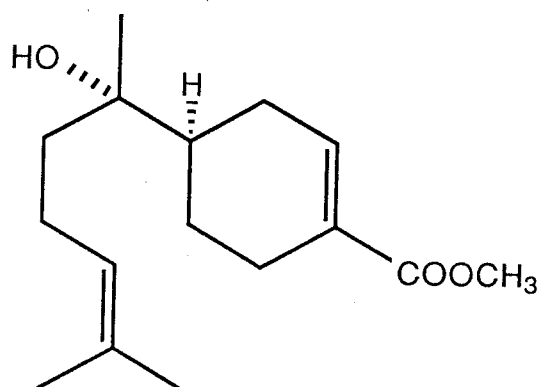
Fig. 3-338 Planar Structure of RL-116

According to the literature search, it was found that this compound had been reported by Harwood *et al.* as an epimeric by-product (**95a** + **95b**) during an alkyl cation reaction of (-)-methyl perillate (**96**) (Scheme 3-37) [138], and its EI-MS, IR and ^1H -NMR (100 MHz) spectra of **95a** + **95b** were approximately agreeable with those of **19**. However, isolation of this compounds as a natural product was the first example. Therefore, **19** was given a trivial name "bisaborosaol A". From Rosacea, bisabolane class sesquiterpene is the first report, so far as the author's investigation. Even though some C-7 oxygenated compounds are known among naturally occurring bisabolanoids, those are quite rare [139-145] (Fig. 3-339).



Scheme 3-37 Alkyl radical addition to yield a bisabolane sesquiterpene [138]

Table 3-133 Physicochemical properties of bisaborosaol A (19)



19

A colorless syrup

Vanillin- H_2SO_4 color: reddish purple

$[\alpha]_D$: + 78 ° (c 0.06 in acetone)

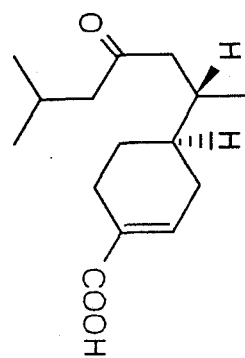
UV $\lambda_{\text{max}}^{\text{MeOH}}$: 218 nm (ϵ 17400)

FI-MS m/z (%): 266 (M^+ , 100)

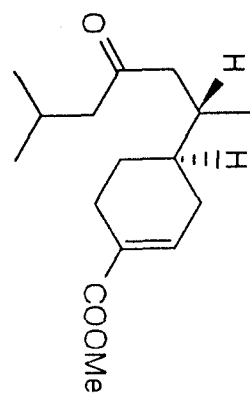
EI-HR-MS: 266.184 ($\text{C}_{16}\text{H}_{26}\text{O}_3$, calcd. 266.188)

EI-MS m/z (%): 266 (M^+ , 1.1), 248 ($\text{M}^+ - \text{H}_2\text{O}$, 23), 233 ($\text{M}^+ - \text{H}_2\text{O} - \text{CH}_3$, 4.4), 217 ($\text{M}^+ - \text{H}_2\text{O} - \text{CH}_3\text{O}$, 5.2), 216 ($\text{M}^+ - \text{H}_2\text{O} - \text{CH}_3\text{OH}$, 6.3), 205 (17), 192 (6.9), 189 ($\text{M}^+ - \text{H}_2\text{O} - \text{COOCH}_3$, 16), 188 (8.0), 178 (6.2), 164 (15), 163 (48), 151 (25), 140 (57), 139 (11), 137 (22), 110 (11), 109 (100), 108 (12), 107 (12), 105 (17), 93 (18), 91 (12), 83 (10), 82 (29), 81 (19), 80 (13), 79 (29), 77 (13), 71 (17), 69 (87), 67 (26), 59 (10), 57 (10), 55 (27), 53 (13), 45 (28), 43 (97), 41 (72).

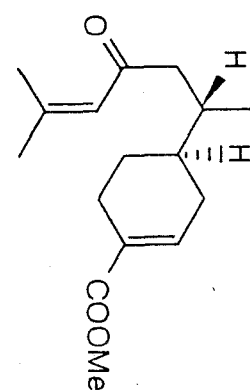
^1H -NMR and ^{13}C -NMR data are shown in Tables 3-131 and 3-132, respectively.



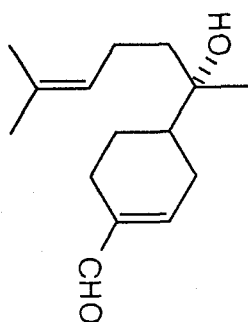
todomatsuiic acid (97)
[139]



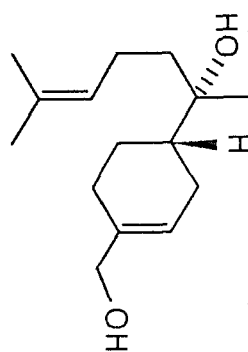
juvabione (98)
[140]



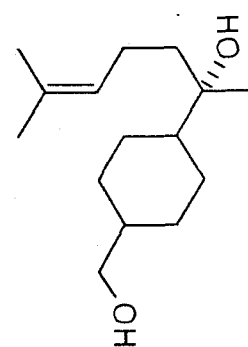
dehydrojuvabione (99)
[141]



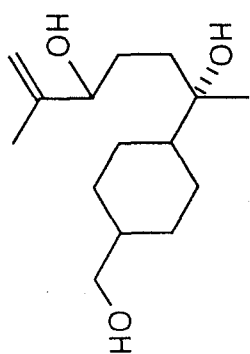
(100) [143]



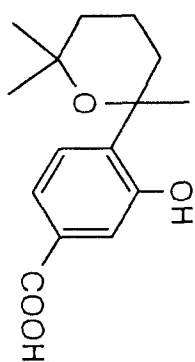
(101) [144]



(102) [145]



(103) [145]



sydowic acid (104) [142]

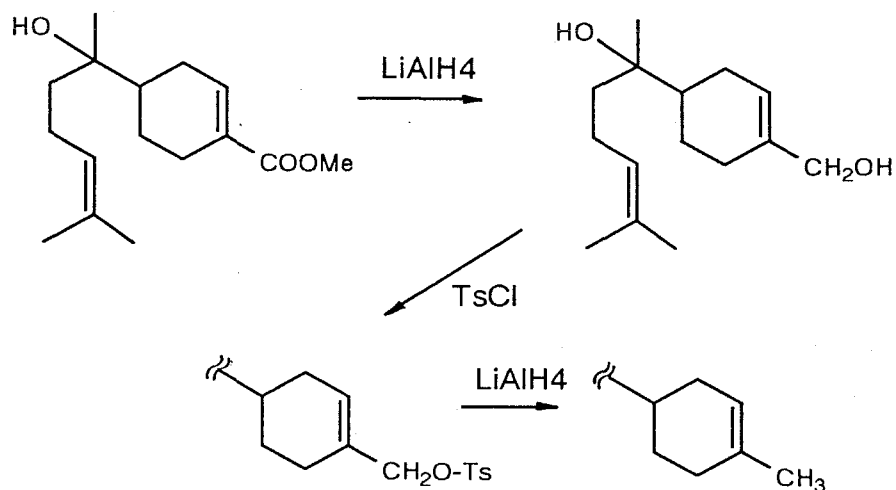
Fig. 3-339 Some Naturally Occurring Bisabolanoids Oxygenated at C-7

3) Stereochemistry

To elucidate the absolute stereostructure of bisaborosaol A (19), chemical modification of 19 at the C-7 methoxy carbonyl group into allylic methyl group was conducted. The expected product would be directly compared with authentic 4*S*,8*S*-(-)- α -bisabolol (94) in ^{13}C -NMR and $[\alpha]_{\text{D}}$. According to the conversion scheme (Scheme 3-38), the methoxycarbonyl group of 19 was reduced with LiAlH_4 as the first step.

To 24.4 mg of 19 dissolved in 1.5 ml of CHCl_3 was added an excess amount of LiAlH_4 (ca 50 mg), and the reaction mixture was stirred overnight at room temp. After that, 1 ml of EtOAc was added under cooling in an ice bath to stand for further 3 hr, and the mixture was then diluted with 15 ml of EtOAc. The resulting solution was washed with a saturated NaCl solution containing 0.2 N HCl. The organic layer was dried over Na_2SO_4 , concentrated, and then chromatographed (PTLC) in hexane-EtOAc 1:1. Together with unchanged material (6.4 mg, 29 %, *Rf* 0.71), a non-quenching product (RL-116-LAH, 19a) at *Rf* 0.32 showing pinkish blue with vanillin- H_2SO_4 reagent was isolated as a colorless syrup 13.1mg in a yield of 60 % (Fig. 3-340).

The product was confirmed its structure by FI- and EI-MS, and ^1H - and ^{13}C -NMR analyses (Fig. 3-341, 342, 343, 344 and Table 3-134). In addition to the molecular weight 238, the C-7 carbinol group was detected at δ_{H} 4.026 and 3.994 (each d, $J = 11.1$ Hz, $-\text{CH}_2\text{OH}$) in ^1H -NMR, and at δ_{C} 67.4 ($-\text{CH}_2\text{OH}$) in ^{13}C -NMR, respectively.



Scheme 3-38 Reduction of bisaborosaol A with LiAlH_4 and further scheme for conversion into α -bisabolol

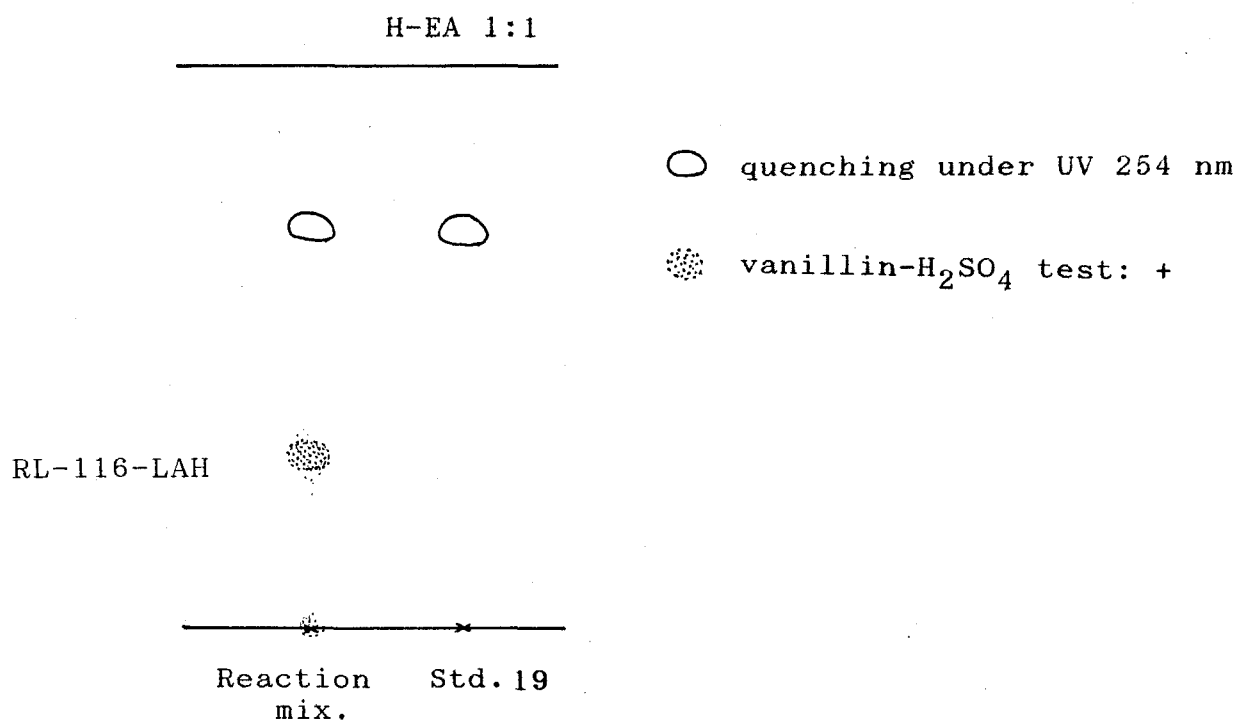


Fig. 3-340 TL Chromatogram of Reduction Product Obtained by Treatment of Bisaborosaol A

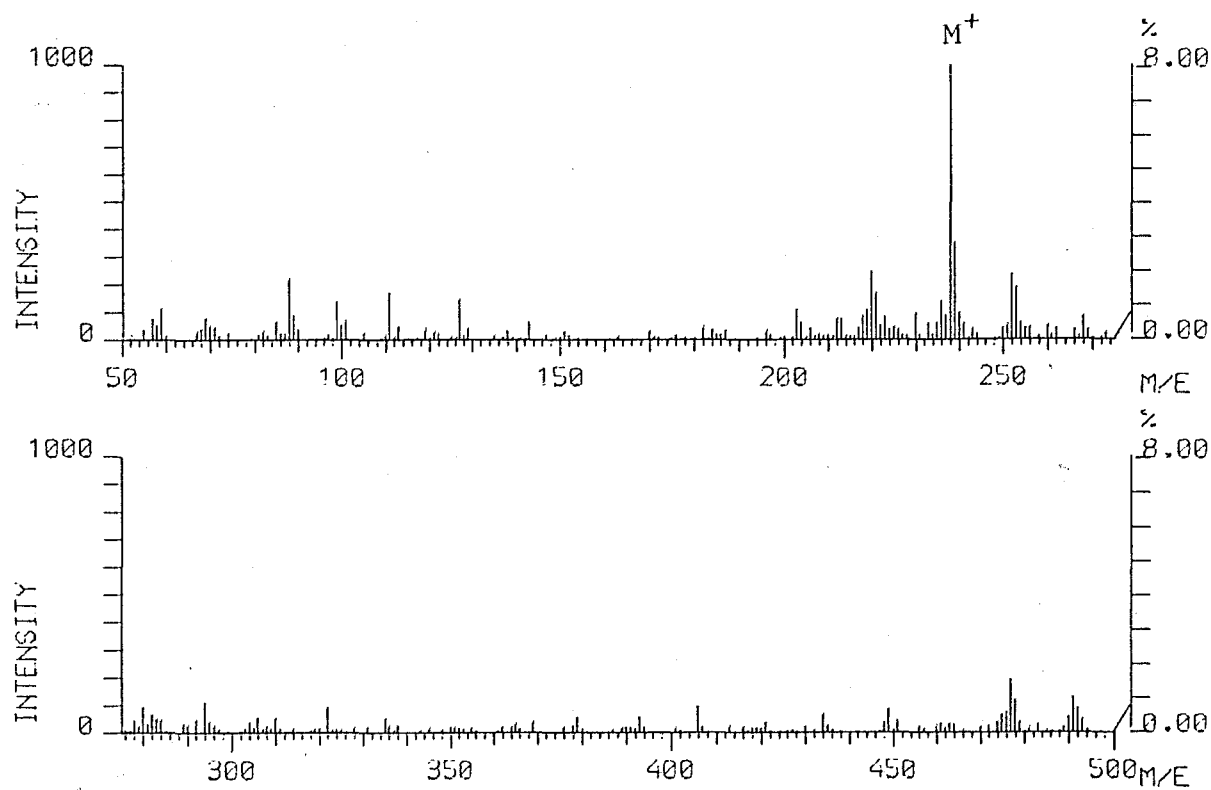


Fig. 3-341 FD-Mass Spectrum of RL-116-LAH

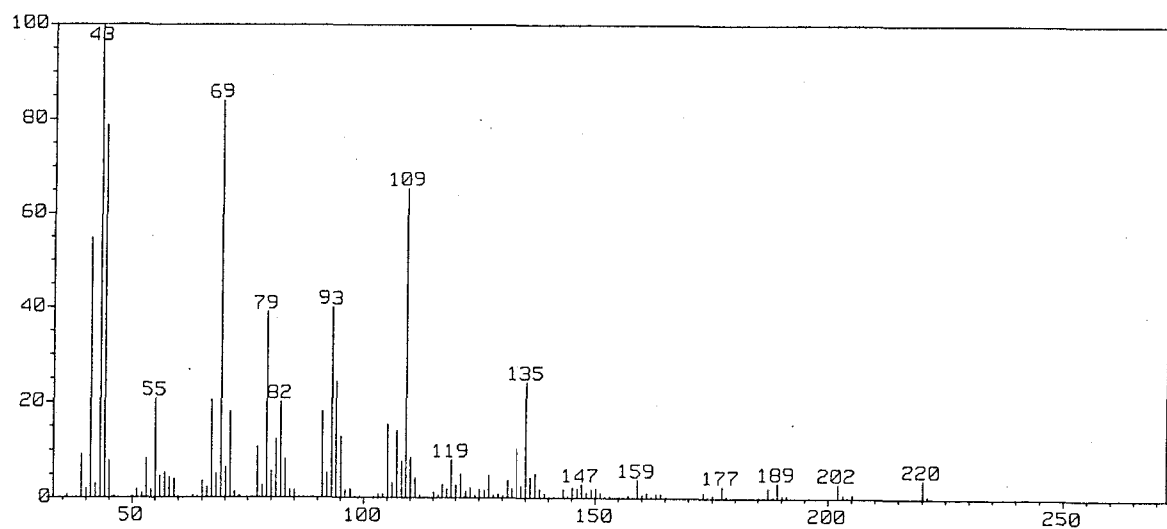


Fig. 3-342 EI-Mass Spectrum of RL-116-LAH

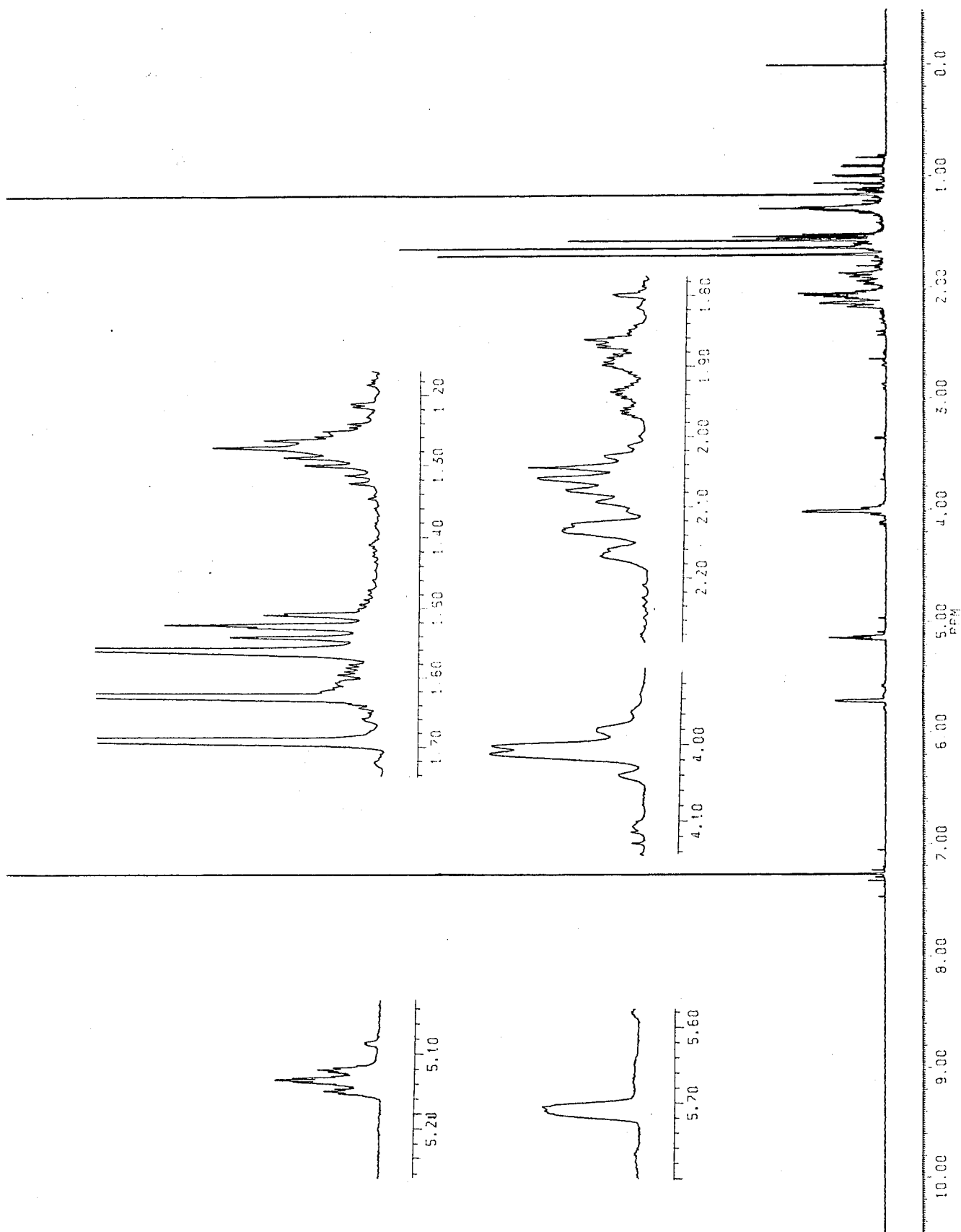


Fig. 3-343 ^1H -NMR Spectrum of RL-116-LAH (500 MHz, in CDCl_3)

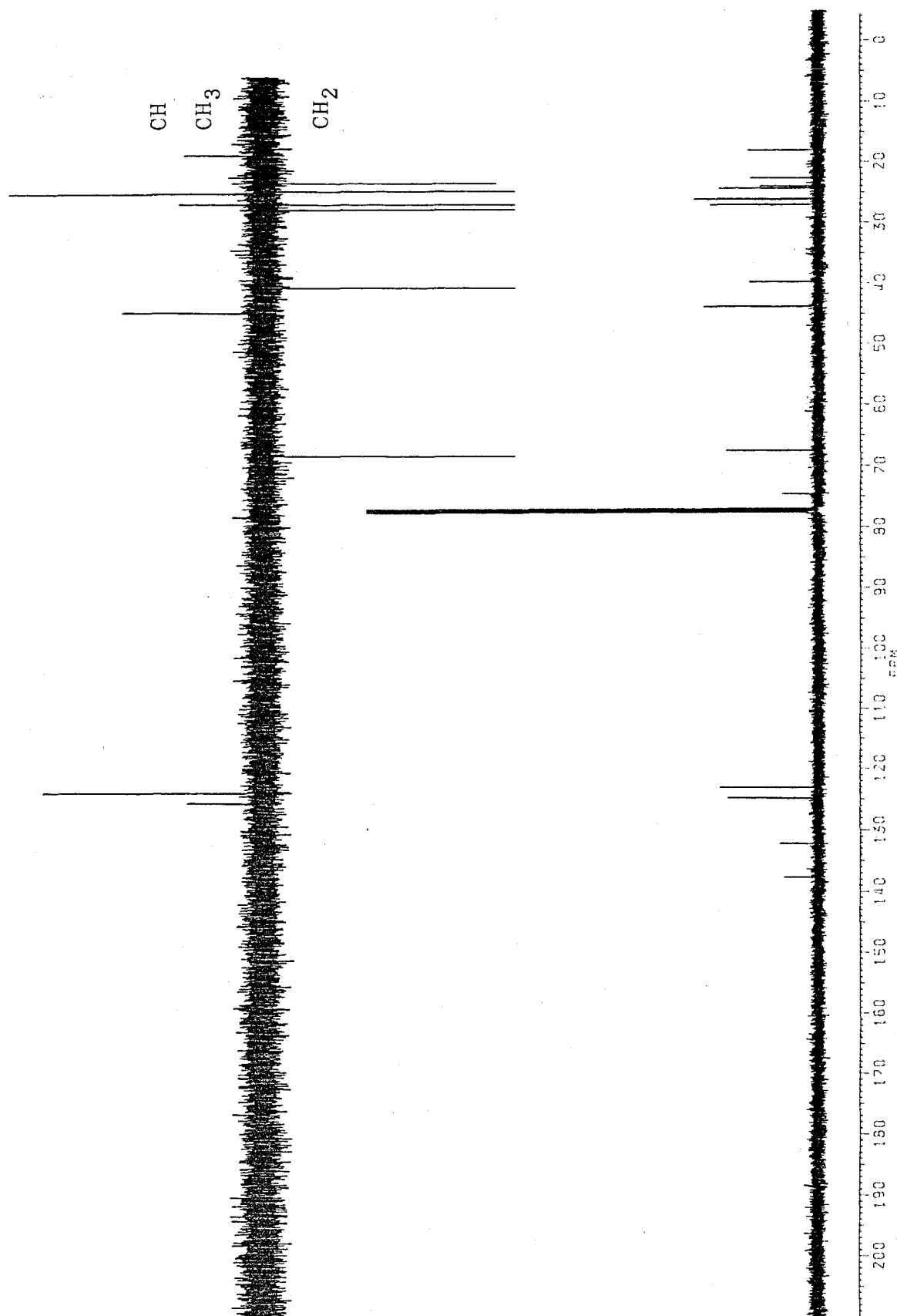


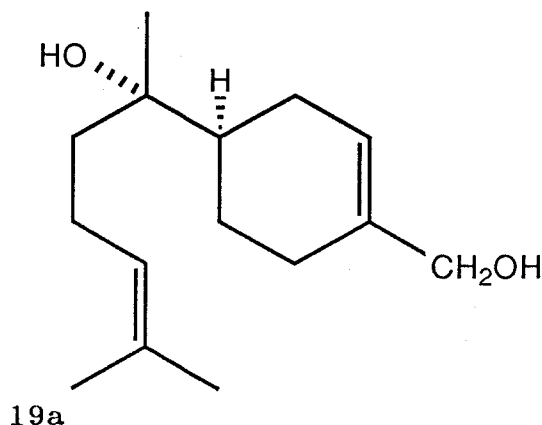
Fig. 3-344 ^{13}C -NMR Spectra of RL-116-LAH (125 MHz, in CDCl_3 , COM and DEPT)

Table 3-134 ^{13}C -NMR chemical shift value of RL-116-LAH (19a)

(125 MHz, in CDCl_3 , COM and DEPT, TMS as an int. std.)

δ_{C}	H	Assignment
137.5	C	C-1
132.1	C	C-13
124.6	CH	C-12
122.9	CH	C-2
74.5	C	C-8
67.4	CH_2	C-7
43.6	CH	C-4
39.6	CH_2	C-9
26.8	CH_2	C-3
26.0	CH_3	C-10
25.9	CH_2	C-6
24.2	CH_3	C-14
23.8	CH_2	C-5
22.5	CH_2	C-11
17.9	CH_3	C-15

Table 3-135 Physicochemical properties of RL-116-LAH (19a)



A colorless syrup

Rf: 0.32 (H-EA 1:1)

Vanillin-H₂SO₄ color: pinkish blue

UVλ_{max}^{MeOH}: featureless above 210 nm

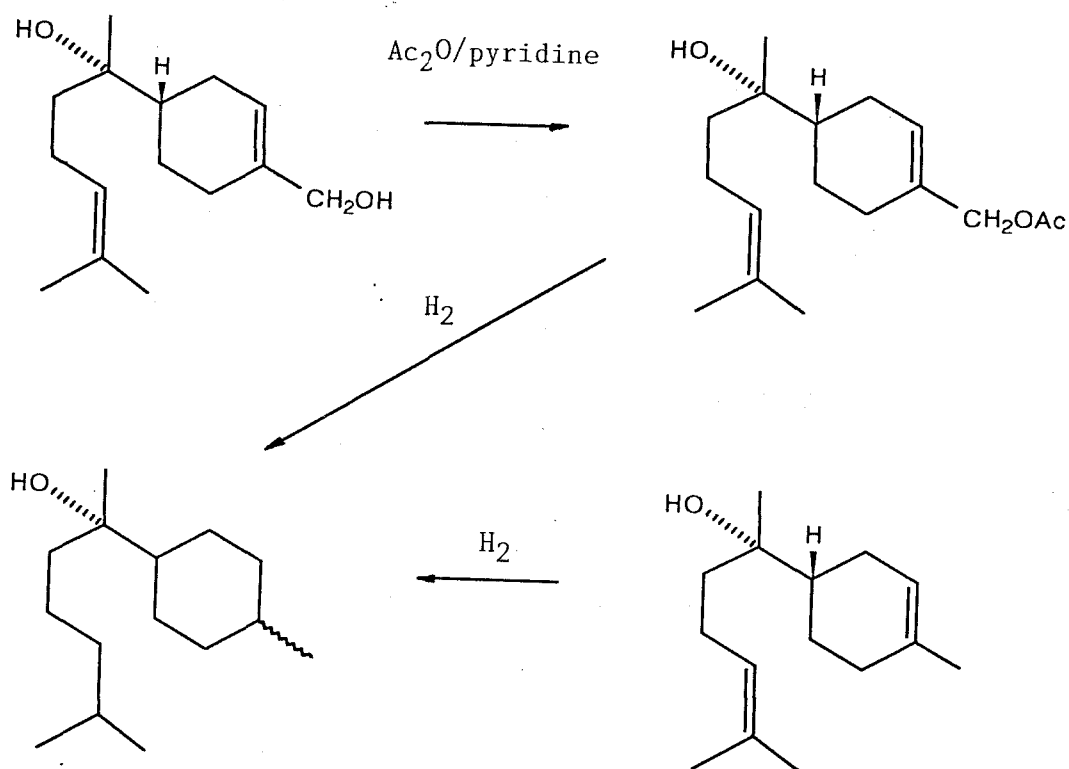
FI-MS *m/z* (%): 238 (100)

EI-MS *m/z* (%): 220 (M⁺-H₂O, 4.1), 202 (M⁺-2H₂O, 3.2), 189 (3.4), 187 (2.2), 177 (2.6), 159 (4.0), 147 (3.0), 135 (24), 133 (10), 119 (8.1), 109 (66), 107 (14), 105 (16), 95 (13), 94 (24), 93 (40), 91 (18), 82 (20), 81 (12), 79 (39), 71 (18), 69 (84), 67 (21), 55 (21), 44 (79), 43 (100), 41 (55).

¹H-NMR δ_{TMS}^{CDCl₃} (500 MHz): *ca* 5.71 (1H, br. C-2-H), 5.137 (1H, br. t, *J*=7.1 Hz, C-11-H), 4.026 (1H, d, *J*=11.1 Hz, C-7-Ha), 3.994 (1H, d, *J*=11.1 Hz, C-7-Hb), *ca* 2.15 (2H, d x 2, C-3-Ha and C-6-Ha), *ca* 2.07 (1H, overlapped, C-3-Hb), 2.062 (2H, m, C-10-H₂), 1.937 (1H, m, C-6-Hb), 1.881 (1H, m, C-3-Hb), 1.693 (3H, br d, *J*=0.7 Hz, C-14-H₃), 1.629 (3H, br s, C-13-H₃), 1.612 (1H, m, C-4-H), 1.527 (2H, t, *J*=8.1 Hz, C-9-H₂), 1.283 (1H, dddd, *J*=12.2, 12.2, 12.1 & 5.5 Hz, C-5-Hb), 1.154 (3H, s, C-15-H₃).

¹³C-NMR data are shown in Table 3-172.

On the other hand, 4*S*,8*S*-(-)-7-hydroxy- α -bisabolol (**101**) having the same planar structure with that of RL-116-LAH has been isolated as a naturally occurring sesquiterpene from *Vanillosmopsis arborea* (Compositae) by Matos *et al.* [144]. As the absolute stereostructure of **101** has also been determined by a chemical correlation method (Scheme 3-39), **19a** was compared its physicochemical properties with those of **101**. In their paper, however, $[\alpha]_D$ and ^{13}C -NMR chemical shift values were recorded only for the 7-acetyl derivative (**101a**). The diol **19a** (8.2 mg) was therefore acetylated in acetic anhydride/pyridine [1 ml (1:1, v/v), at 80 °C for 1.5 hr]. From the reaction mixture diluted with excess amount of toluene was removed all the solvent. The main product RL-116-LAH-AC (**16b**) was obtained by PTLC (hexane-EtOAc 3:1, *R_f* 0.43) as a colorless syrup (7.0 mg, yield 73 %) (Fig. 3-345). The structure was confirmed by EI-MS and ^1H -NMR (Fig. 3-346 and 347).



Scheme 3-38 Conversion scheme to elucidate the absolute configuration at C-8 of compound **80**

H-EA 3:1

vanillin-H₂SO₄
test: positive

RL-116-LAH-AC

Reaction Std. 19a
mix.

Fig. 3-345 TL Chromatogram of RL-116-LAH-AC

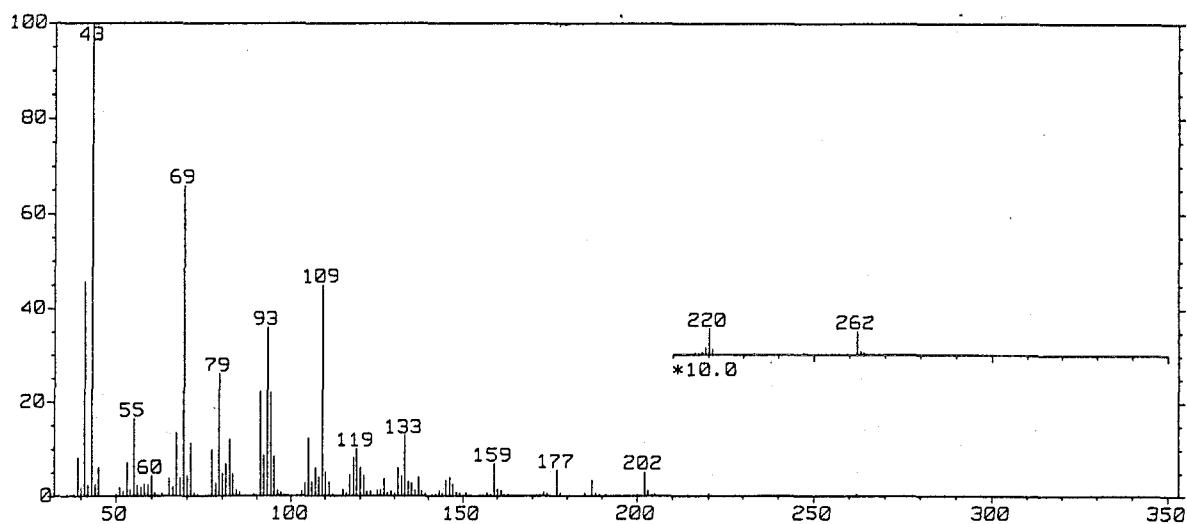


Fig. 3-346 EI-Mass Spectrum of RL-116-LAH-AC

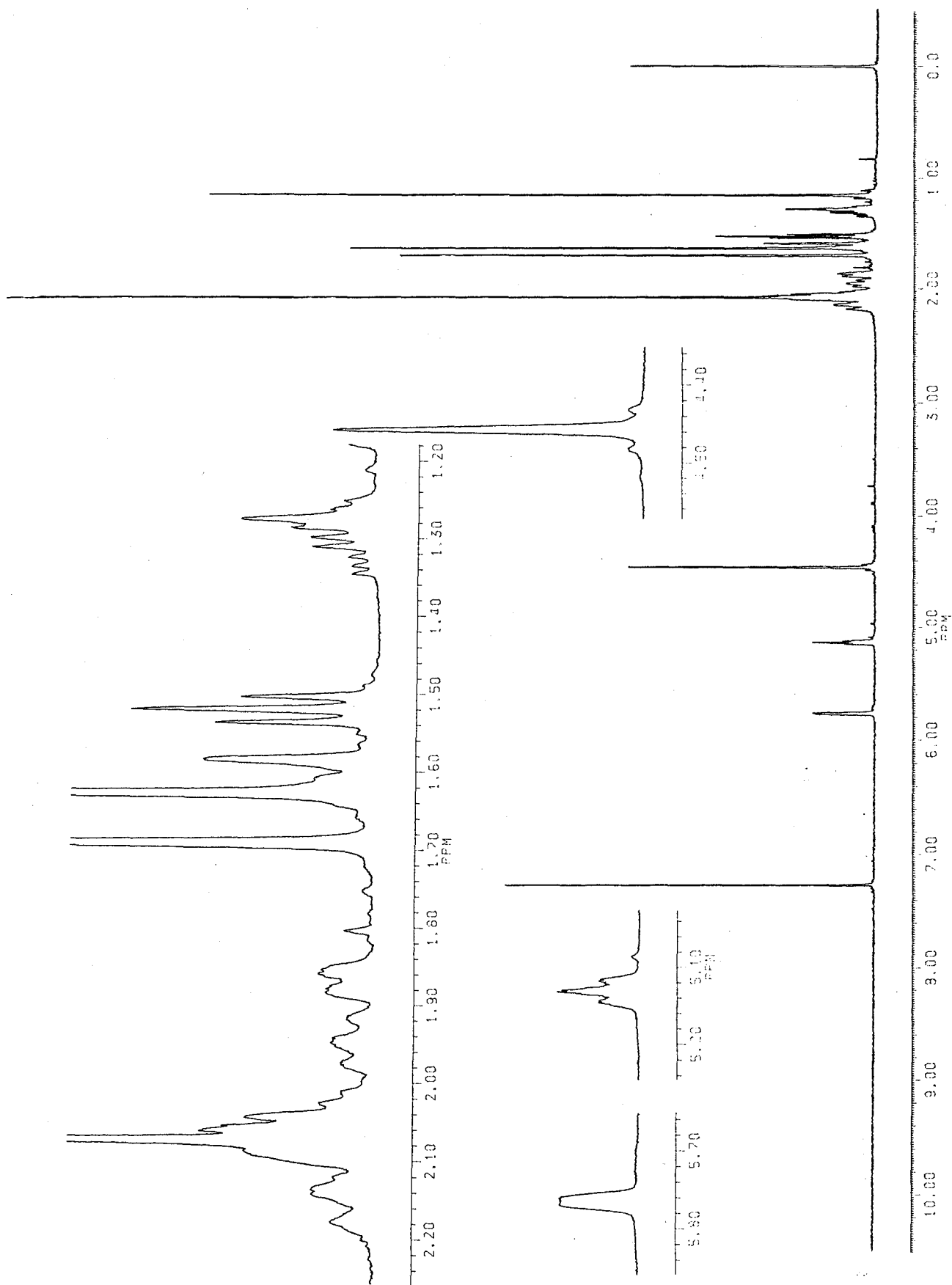
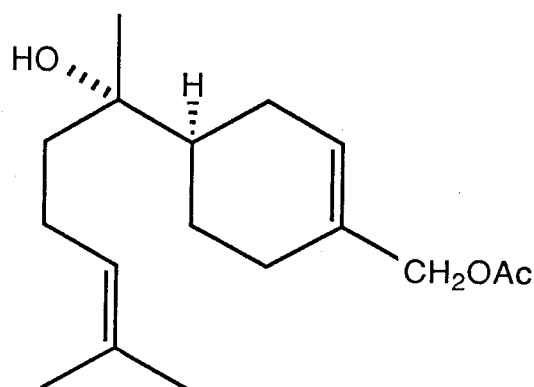


Fig. 3-347 ^1H -NMR Spectrum of RL-116-LAH-AC (500 MHz, in CDCl_3)

Table 3-136 Physicochemical properties of 116-LAH-AC (19b)



19b

A colorless syrup

Vanillin-H₂SO₄ color: pinkish blue

$[\alpha]_D$; + 33 ° (*c* = 0.1 in EtOH)

UVλ_{max}^{MeOH}: featureless above 210 nm

EI-MS *m/z* (%); 262 (M⁺, 0.5), 220 (M⁺-CH₂CO, 0.6), 202 (M⁺-CH₃COOH, 5.3), 187 (3.4), 177 (5.6), 159 (7.0), 133 (13), 119 (10), 109 (45), 105 (12), 94 (22), 93 (36), 91 (22), 79 (26), 69 (66), 55 (16), 43 (100), 41 (46).

¹H-NMR δ_{TMS}^{CDCl₃} (500 MHz): 5.765 (1H, br, C-2-H), 5.131 (1H, br t, *J* = 6.9 Hz, C-12-H), 4.456 (2H, s, C-7-H₂), 2.158 (1H, br d, *J* = 18.0 Hz, C-6-Ha), *ca* 2.15 (1H, d, *J* = 19.8 Hz, C-3-Ha), *ca* 2.08 (1H, overlapped, C-6-Hb), 2.073 (3H, s, C-7'-COCH₃), *ca* 2.06 (2H, overlapped, C-11-H₂), 1.948 (1H, br. t-like m, C-3-Hb), 1.868 (1H, br d, *J* = 12.4 Hz, C-5-Ha), 1.691 (3H, br s, C-14-H₃), 1.627 (3H, br s, C-15-H₃), *ca* 1.61 (1H, overlapped, C-4-H), 1.519 (2H, t, *J* = 8.3 Hz, C-9-H₂), 1.292 (1H, dddd, *J* = 12.5, 12.4, 12.4 & 5.7 Hz, C-5-Hb), 1.148 (3H, s, C-10-H₃).

¹³C-NMR data are shown in Table 3-135.

Compound **19b** was, in the ^{13}C -NMR, quite similar to (4*S*, 8*S*)-(-)-7-hydroxybisabolol 7-monoacetate (**101a**) prepared from **101** by Matos *et al* (Fig. 3-348 and Table 3-137) [143]. In ^{13}C -NMR, the difference of a chemical shift value between two corresponding carbons in **19b** and **101a** was approximately within ± 1.0 ppm, except the C-2 carbon. This result suggested that **19b** has at least the same planar structure with that of **101a**. However the optical properties of **19b** was opposite to that of **80a**. Contrary to **101a** showing laevorotatory ($[\alpha]_{\text{D}} - 48^\circ$, c 1.0 in EtOH), **19b** was dextrorotatory with $[\alpha]_{\text{D}} + 33^\circ$ (c 0.1, in EtOH).

On the other hand, the 4*S*,8-*epi*-7-keto-7-methoxy- α -bisabolol mixture (**95a** + **95b**) synthesized by Harwood *et al.* indicated laevorotately ($[\alpha]_{\text{D}} - 68.0^\circ$). Moreover, they prepared two stereoisomers, 4*S*,8*S*- and 4-*S*,8*R*-7-keto- α -bisabolols (aldehyde group at C-7) (**105a** and **105b**) which were successfully isolated by a chromatographic method. The latter isomers both showed laevorotatory with close values (**105a**: -68.2° , **105b**: -71.3°) [137]. This fact was suggestive that optical rotations of those α -bisabolol derivatives are mostly affected by the absolute configuration at C-4. Furthermore, it was expected that the C-8 chiral carbon provides an only small contribution to the optical rotatory values (Fig. 3-349). To prove this speculation, direct comparison with an authentic compound was requested. As it was impossible to purchase 7-hydroxy- α -bisabolol (**94**), the initial conversion reaction (Scheme 3-54) was applied to **19a**. However, this plan was failed since the tosylation of **19a** was unexpectedly unsuccessful. Therefore, another method was applied to the determination of absolute configuration.

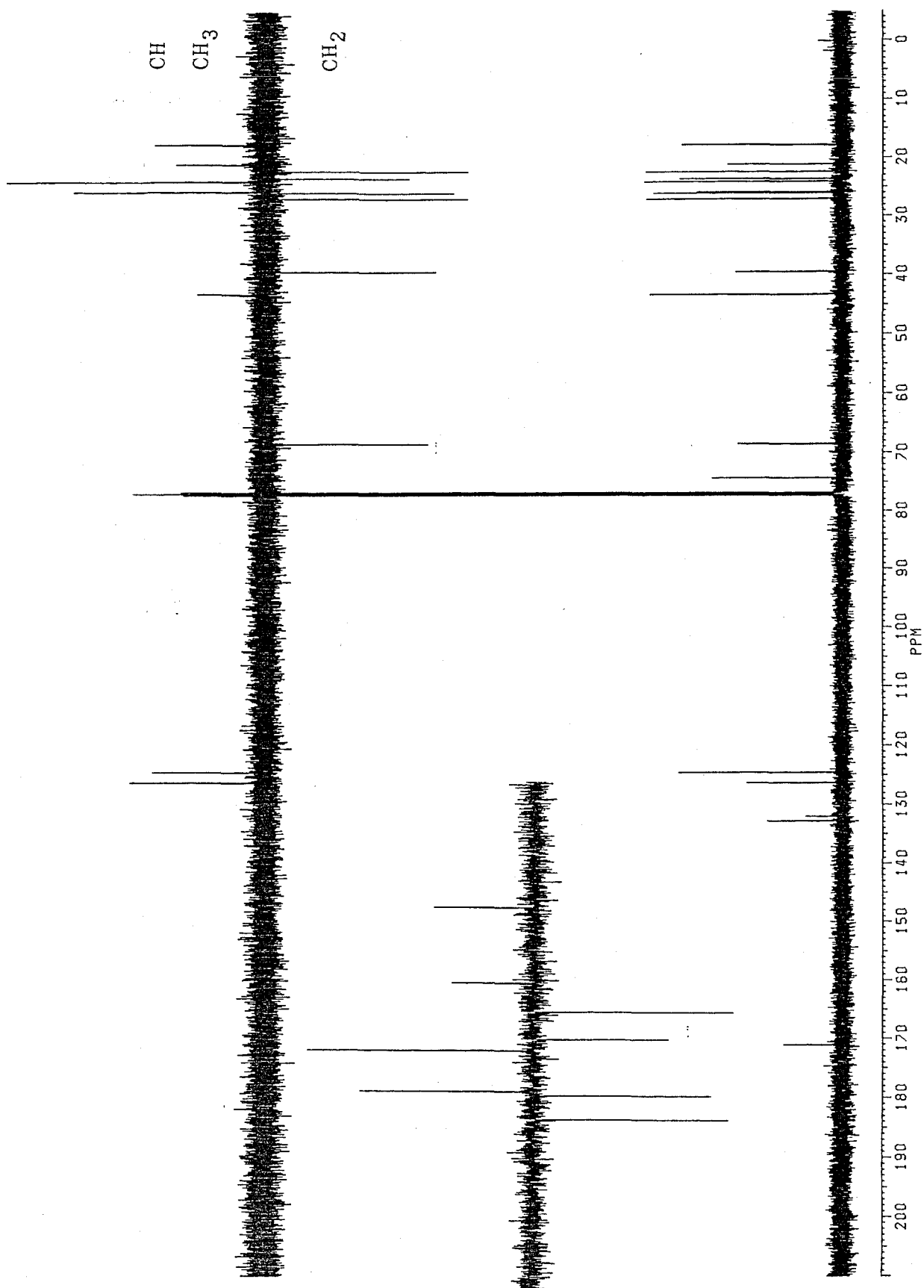


Fig. 3-348 ^{13}C -NMR Spectra of RL-116-LAH-AC (125 MHz, in CDCl_3 , COM and

Table 3-137 ^{13}C -NMR chemical shift values of RL-116-LAH-AC and comparisons of its chemical shifts with those of 7-acetoxyl bisabolol (101a), (-)- α -bisabolol (94) and bisaborosaol A (19)

C-No	RL-116-LAH-AC (19b)	101a [143]	94 [137]	19
1	132.7 (C)	132.75	134.00	132.0
2	126.2 (CH)	124.33	120.53	139.8
3	26.9 (CH ₂)	26.68	26.88	26.8
4	43.1 (CH)	42.59	42.94	42.5
5	23.5 (CH ₂)	22.61	22.02	23.3
6	25.9 (CH ₂)	26.50	25.65	25.2
7	68.4 (CH ₂)	68.27	23.15	167.9
8	74.2 (C)	73.91	74.21	74.1
9	39.4 (CH ₂)	39.89	40.08	39.5
10	24.0 (CH ₃)	23.00	23.28	23.8
11	22.3 (CH ₂)	21.89	23.25	22.3
12	124.4 (CH)	124.33	124.58	124.3
13	131.9 (C)	131.50	131.54	129.9
14	25.7 (CH ₃)	25.53	31.00	25.7
15	17.7 (CH ₃)	17.50	17.59	17.7
7'	171.0 (C)	170.91	-	51.5
7'	21.0 (CH ₃)	20.91		

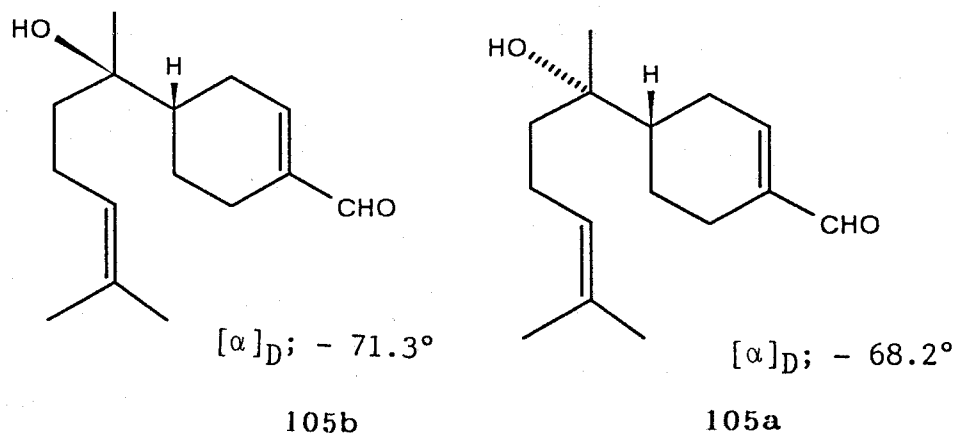
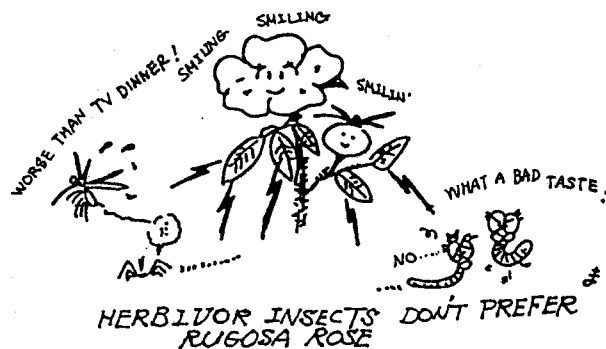
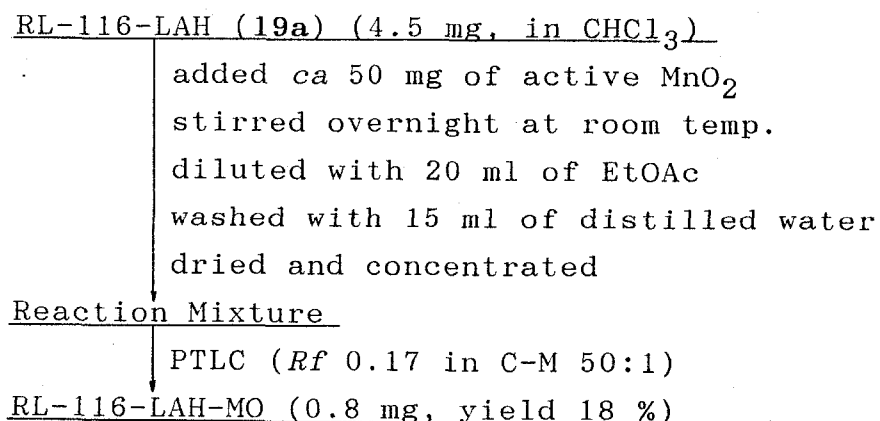


Fig. 3-549 Two Epimeric Isomers of a Bisabolane Aldehyde
 Synthesized by Harwood *et al.*: These derivatives were obtained by
 alkyl cation reaction of an isoprene + (-)-*S*-perillaldehyde in
 HCOOH. These products were isolated from each other by
 chromatographic method.



4) Determination of Absolute Configuration at C-4

Since two diastereomers 105a and 105b (both *S* at C-4) were reported their $[\alpha]_D$ values, bisaborosanol A (19) can be determine its stereostructure at C-4 after the compound is converted to the corresponding aldehyde derivative. The desired derivative was successively prepared from 19a by oxidation with active manganese dioxide as shown in Scheme 3-40. The product RL-116-LAH-MO detectable with UV 254 nm and DNPH reagent on TLC was obtained as a colorless syrup (Fig. 3-350).



Scheme 3-40 Preparation of RL-116-LAH-MO

Structure of RL-116-LAH-MO (19c) was confirmed by EI-MS, ^1H - and ^{13}C -NMR (Fig. 3-351, 352 and 353), and the optical rotation $[\alpha]_D + 77^\circ$ (c 0.1 in MeOH) clearly indicated the stereochemistry of C-4 in 19c to be *R*.

C-M 50:1

○ quenching under
UV 254 nm

● vanillin-H₂SO₄ test: +

DNPH test: +

RL-116-LAH-MO

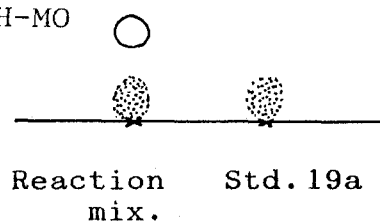


Fig. 3-350 TL Chromatogram of RL-116-LAH-MO

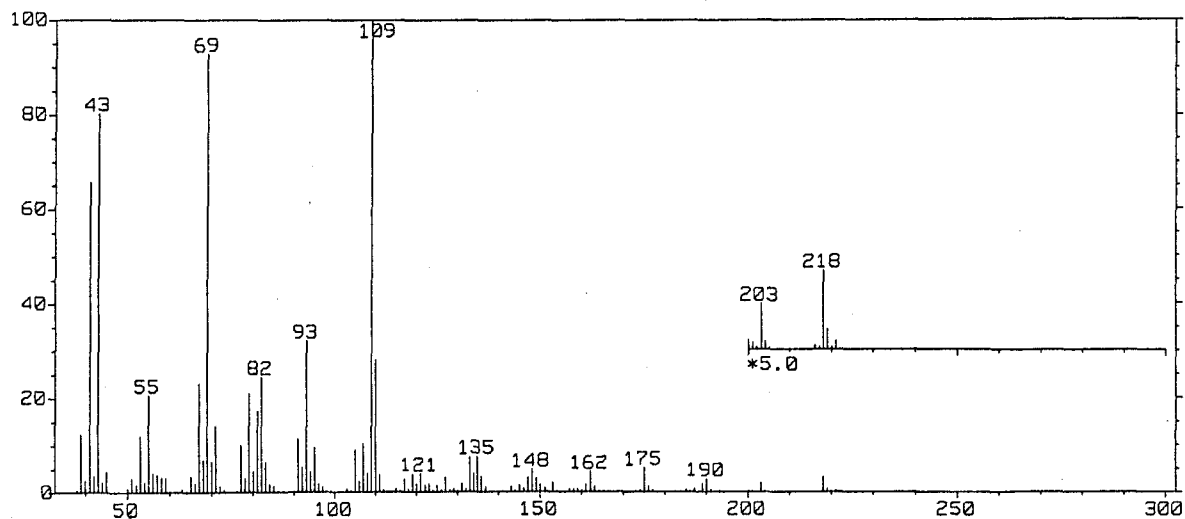


Fig. 3-351 EI-Mass Spectrum of RL-116-LAH-MO

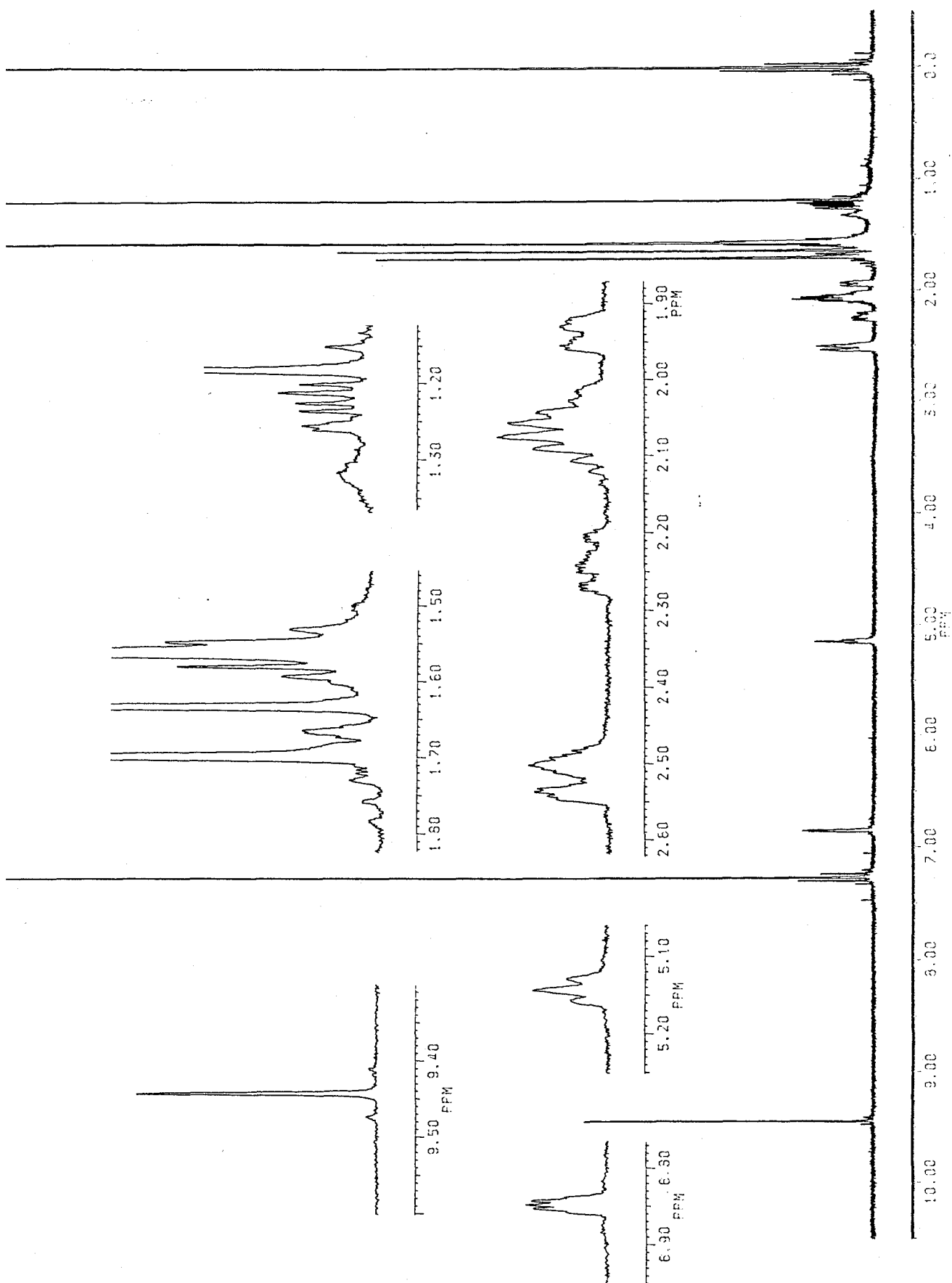


Fig. 3-352 ^1H -NMR Spectrum of RL-116-LAH-MO (500 MHz, in CDCl_3)

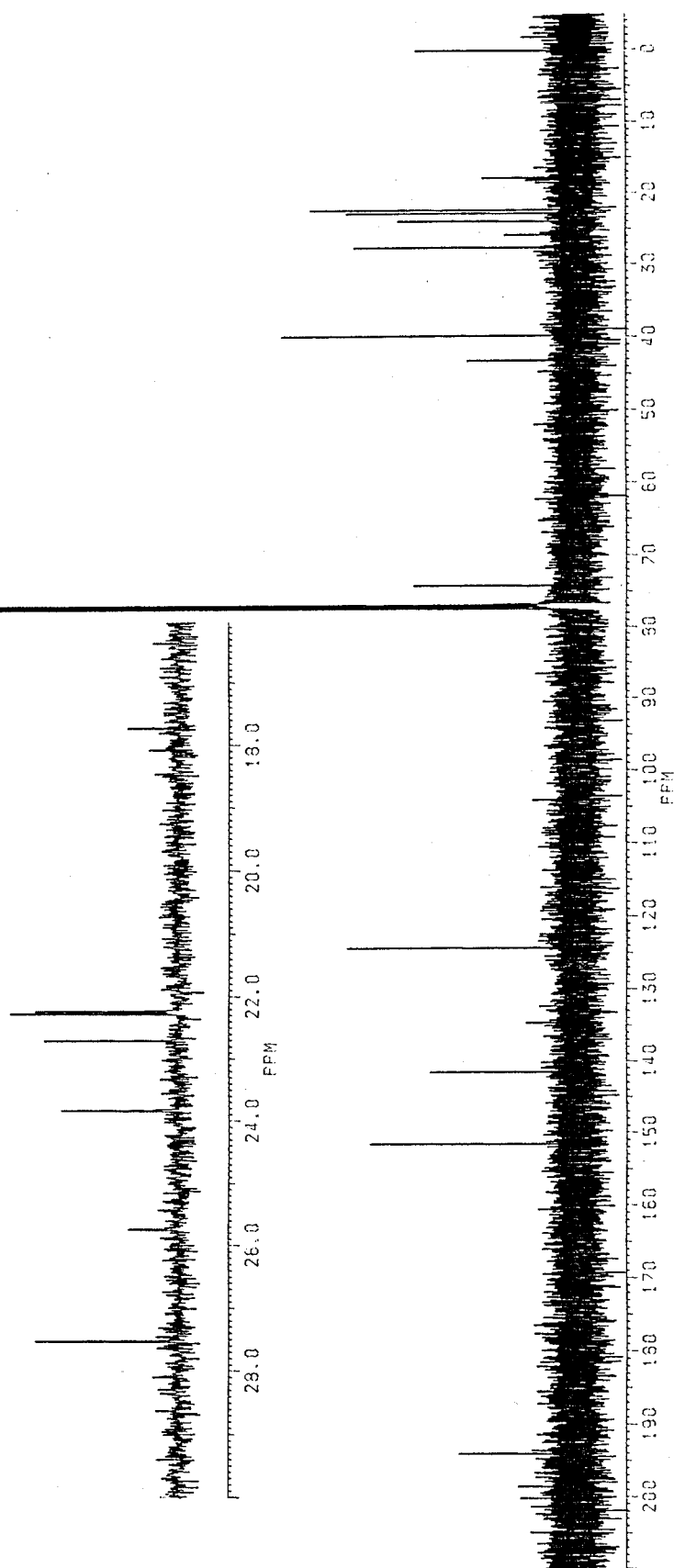
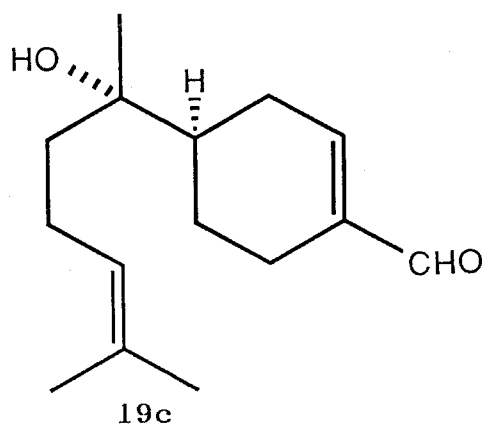


Fig. 3-353 ^{13}C -NMR Spectrum of RL-116-LAH-MO (125 MHz, in CDCl_3)

Table 3-138 Physicochemical properties of RL-116-LAH-MO (19c)



A colorless syrup

Vanillin- H_2SO_4 color: pinkish blue

$[\alpha]_D$; + 77 ° (c 0.1 in MeOH)

EI-MS m/z (%): 218 (M^+ , 3.4), 203 ($\text{M}^+ - \text{CH}_5\text{O}$, 2.0), 190 ($\text{M}^+ - \text{CO}$, 3.0), 175 (5.3), 162 (4.5), 148 (5.0), 135 (7.8), 133 (7.6), 110 (28), 109 (100), 93 (32), 82 (24), 79 (21), 69 (93), 67 (23), 55 (21), 43 (80), 41 (66).

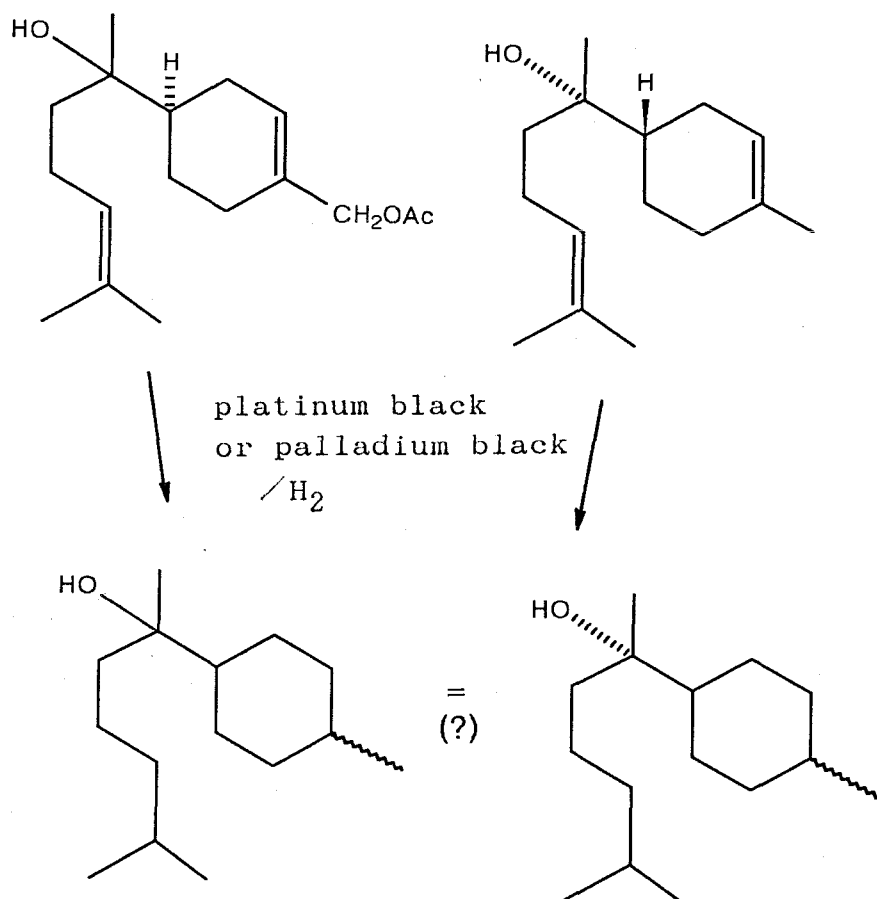
$^1\text{H-NMR}$ $\delta_{\text{TMS}}^{\text{CDCl}_3}$ (500 MHz): 9.442 (1H, s, C-14-H), 6.848 (1H, br. m, C-2-H), 5.143 (1H, br t, $J = 7.0$ Hz, C-12-H), ca 2.53 (1H, overlapped, C-6-Ha), 2.520 (1H, m-divid d, $J = 20.0$ Hz, C-3-Ha), 2.237 (1H, t-like m, C-6-Hb), 2.075 (2H, m, C-11- H_2), ca 2.04 (1H, m, C-3-Hb), 1.938 (1H, d-like m, C-5-Ha), 1.698 (3H, s, C-14- H_3), ca 1.68 (1H, overlapped, C-4-H), 1.633 (3H, s, C-15- H_3), 1.564 (2H, overlapped with H_2O , C-9- H_2), 1.218 (1H, dddd, $J = 12.4, 12.4, 12.1$ & 5.4 Hz, C-5-Hb), 1.182 (3H, s, C-10- H_3).

$^{13}\text{C-NMR}$ $\delta_{\text{TMS}}^{\text{CDCl}_3}$ (125 MHz): 193.9 (C-7), 151.3 (C-2), 141.3 (C-1), 134.5 (C-13), 124.2 (C-12), 74.1 (C-8), 43.1 (C-4), 39.6 (C-9), 27.5 (C-3), 25.7 (C-6), 23.8 (C-10), 22.7 (C-14), 22.3 (C-5), 22.2 (C-11), 17.7 (C-15).

5) Determination of Absolute Configuration at C-8

As Matos *et al.* has reported [144], C-7-monoacetate (101a and 19b) can be converted into a mixture of *cis*- and *trans*- 1-methyl-4(1'-hydroxy-1'-methyl-5'-methylhexa)-cyclohexanes by hydrogenation catalyzed with platinum black or palladium black. As (-)- α -bisabolol (94; 4*S*, 8*S*) [144] was also convertible to the same products, the planar structure of bisaborosaol A (19) can be confirmed in spectroscopic comparisons of those reduction products. If the yield rate of *cis* and *trans* was approximately constant, furthermore, not only the planar but also stereostructure at C-8 can be deduced on 19b (namely C-8 of 19) by the direct comparison of the optical data with those of 94-derived reduction product (Scheme 3-41).

Hydrogenation of 19b was accordingly performed to yield a major and a minor products (Scheme 3-42).



Scheme 3-41 Catalytic hydrogenation of RL-116-LAH-AC (19b) and (-)- α -bisabolol (82) to tetrahydrobisabolol

RL-116-LAH-AC (19.5 mg, in 2 ml of MeOH)

added 6.3 mg of platinum black
bubbled H₂ gas
stirred for 5 hr. at room temp.
added further 6 mg of platinum black
stirred for 5 hr.

Reaction Mixture

filtrated
concentrated
PTLC (H-EA 6:1)

Major Product RL-116-LAC-PLH1 (R_f 0.59, 8.9 mg, yield 57 %)

Minor Product RL-116-LAC-PLH2 (R_f 0.26, 3.1 mg, yield 16 %)

Scheme 3-42 Hydrogenation of RL-116-LAH-AC with H₂/platinum black

The minor product RL-116-LAC-PLH2 obtained by PTLC (Fig. 3-354) was, by FI-MS and ¹H-NMR analyses, identified to be a mixture of *cis*- and *trans*-1-acethylmethyl-4-(1'-hydroxy-1'-methyl-5'-methylhexa)-cyclohexanes (19d + 19e) (Fig. 3-355, 356 and Table 3-139).

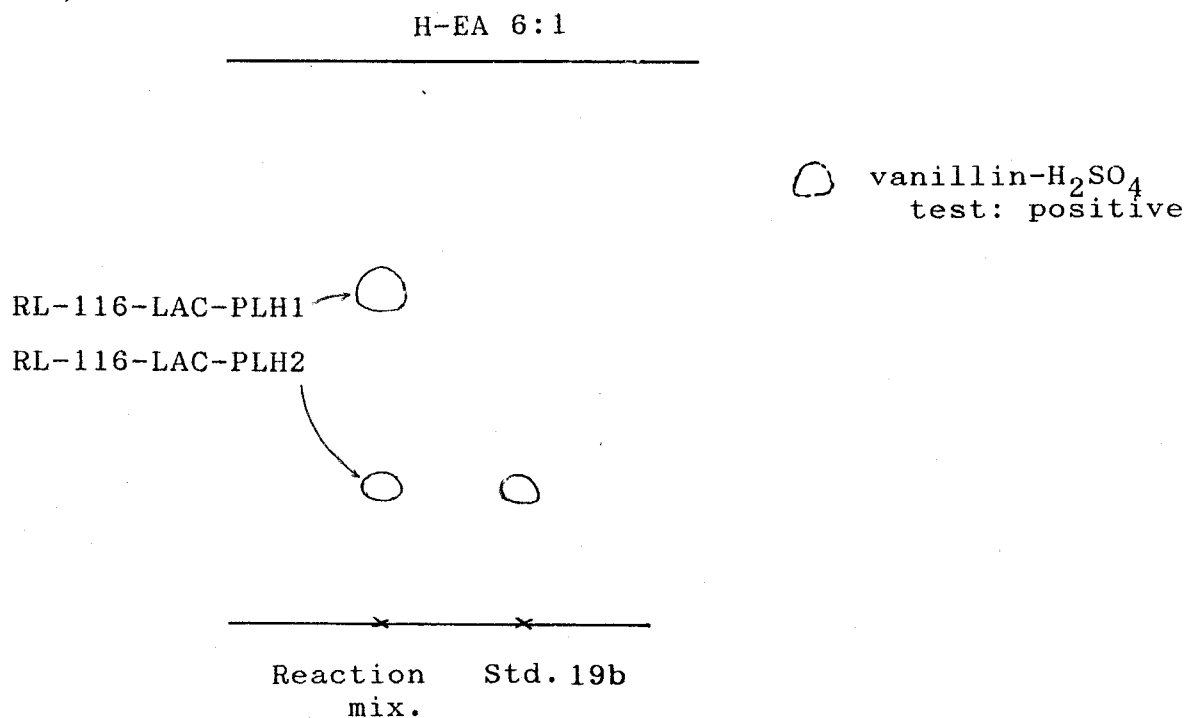


Fig. 3-354 TL Chromatogram of RL-116-LAH-AC Reduction Products

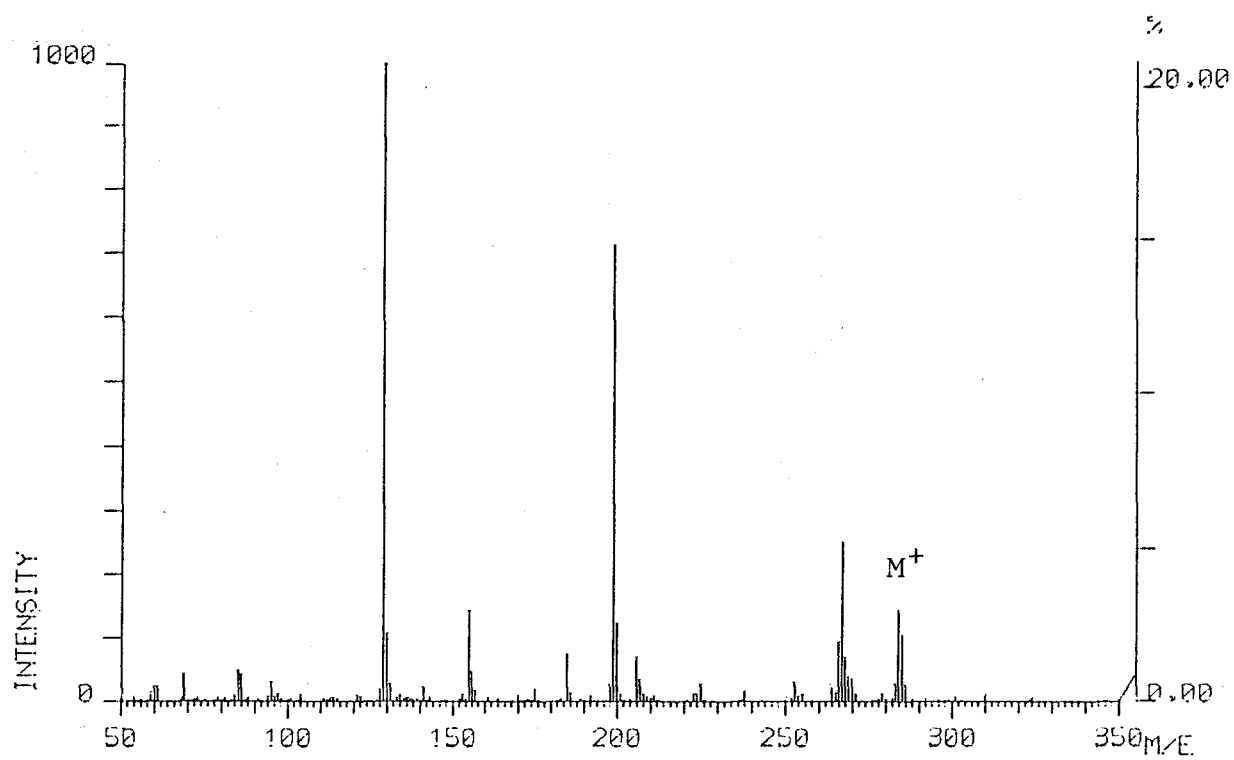


Fig. 3-355 FI-Mass Spectrum of RL-116-LAC-PLH2

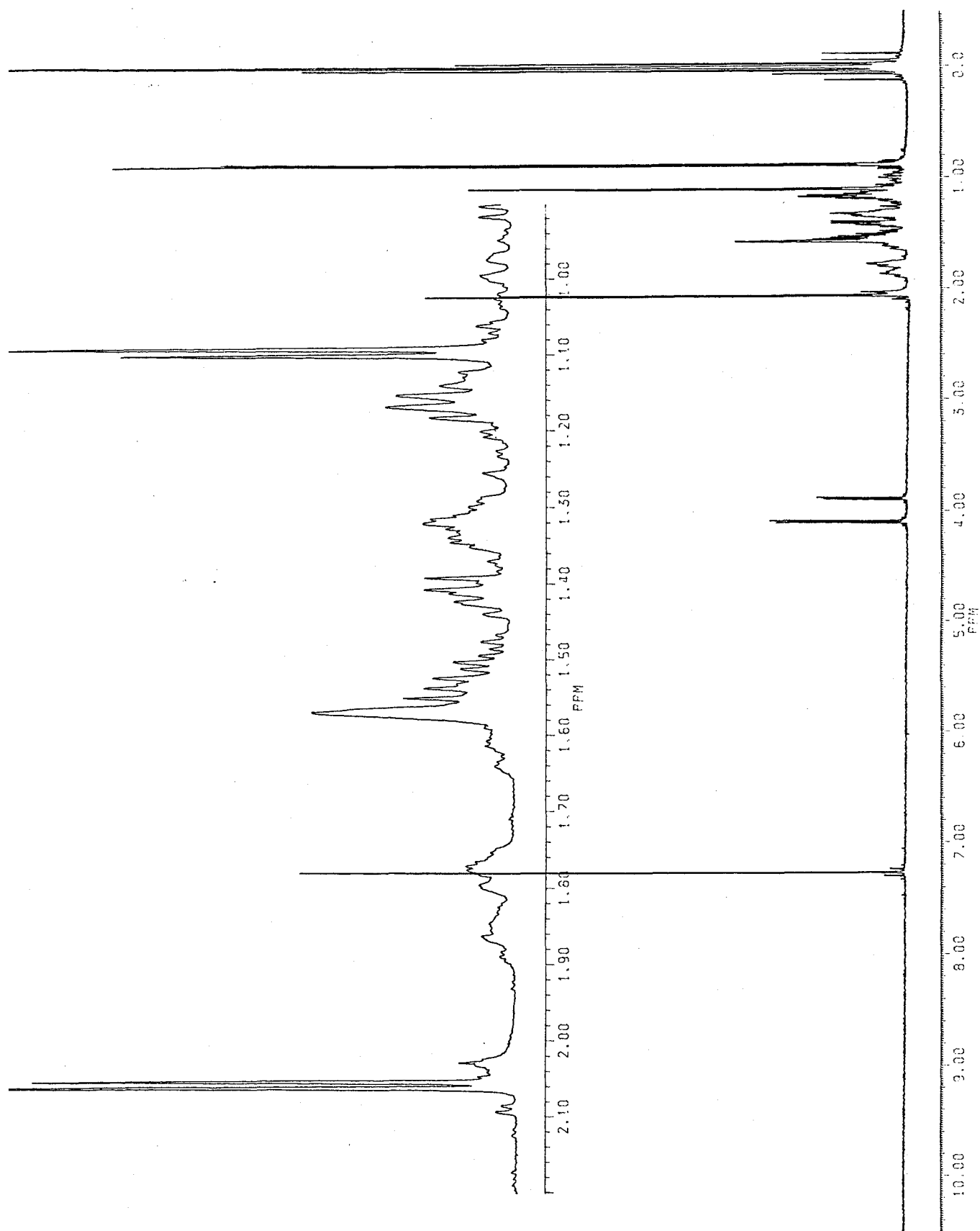
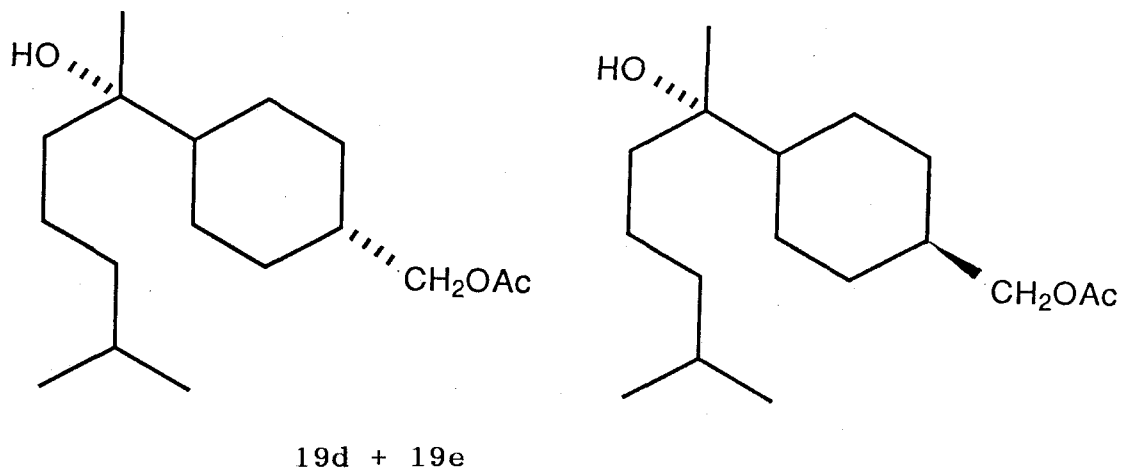


Fig. 3-356 ^1H -NMR Spectrum of RL-116-LAC-PLH2 (500 MHz, in CDCl_3)

Table 3-139 Physicochemical properties of RL-116-LAC-PLH2 (19d + 19e)



A colorless syrup

R_f: 0.26 in H-EA 6:1

Vanillin-H₂SO₄ color: pinkish blue

FI-MS *m/z* (%); 285 (M⁺+1, 11), 284 (M⁺, 15), 267 (25), 199 (71), 155 (15), 129 (100).

¹H-NMR δ_{TMS}^{CDCl₃} (500 MHz): 4.093 (d, *J*= 7.8 Hz), 3.885 (d, *J*= 7.8 Hz), 2.062 (s), 2.054 (s), 1.160 (d, *J*= 7.3 Hz), 1.100 (s), 1.091 (s).

On the other hand, RL-116-LAC-PLH1 was confirmed as mixture of the desired products (19f + 19g) by FI-MS, ^1H - and ^{13}C -NMR analyses (Fig. 3-357, 358, 359 and Table 3-140). In the ^1H -NMR spectrum, these geometric isomers 19f and 19g showed the C-7- H_3 protons as quite different signals in their chemical shift values each other. It seemed possible to calculate the relative ratio of *cis* to *trans* form.

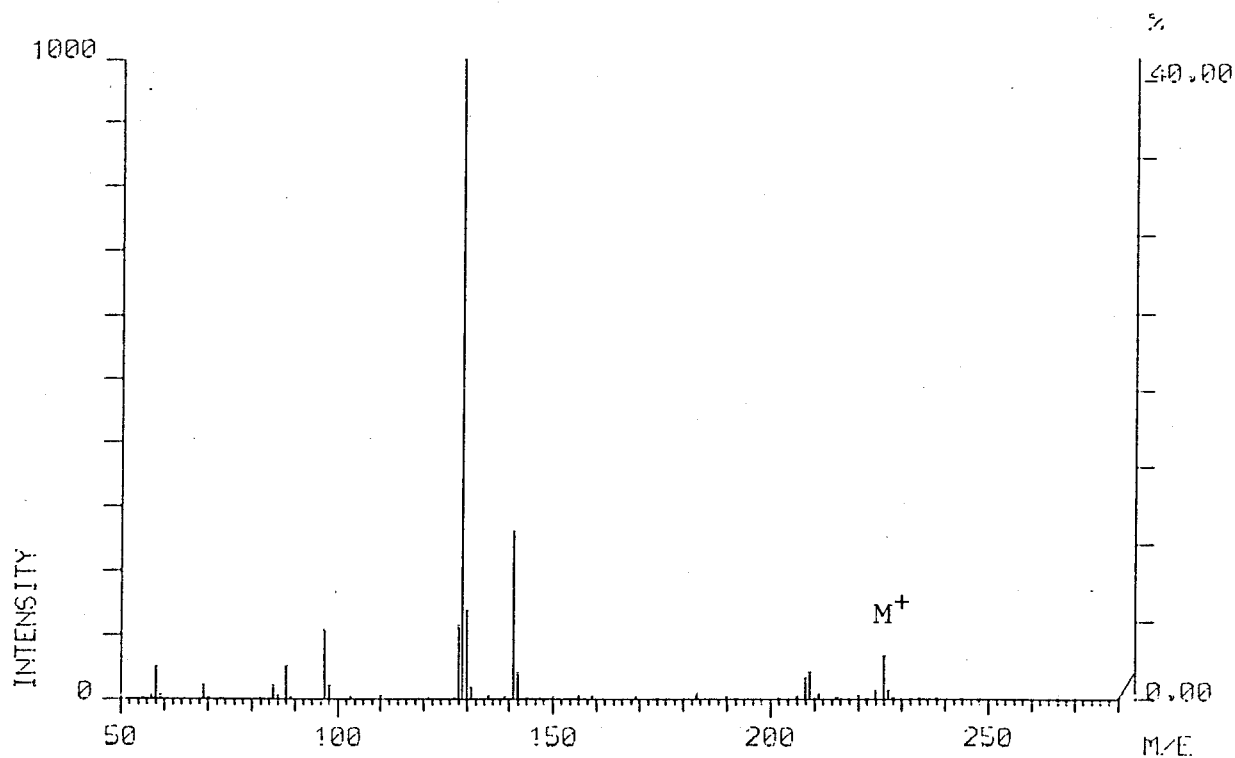


Fig. 3-357 FI-Mass Spectrum of RL-116-LAH-PLH2

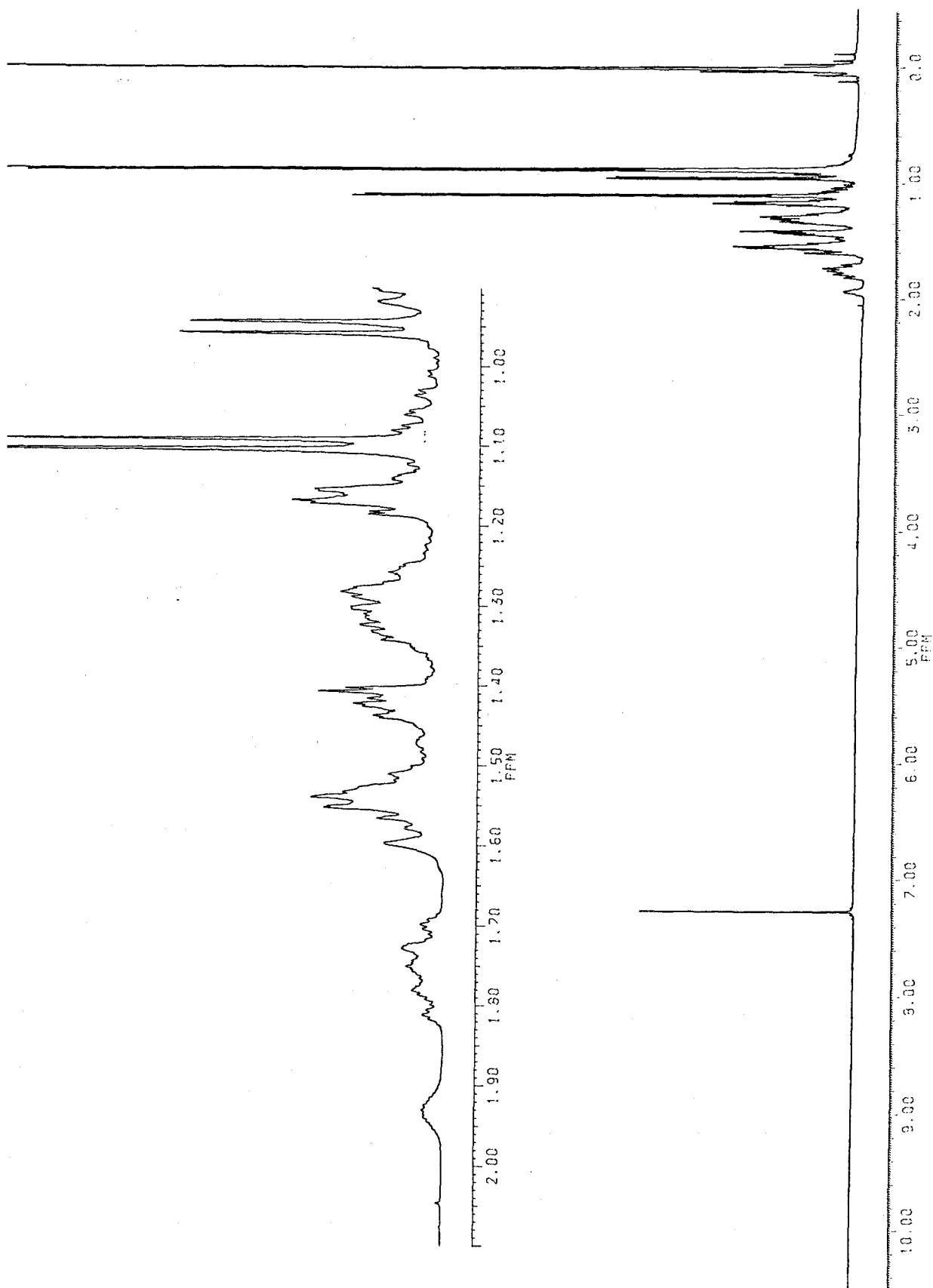


Fig. 3-358 ^1H -NMR Spectrum of RL-116-LAH-PLH2 (500 MHz, in CDCl_2)

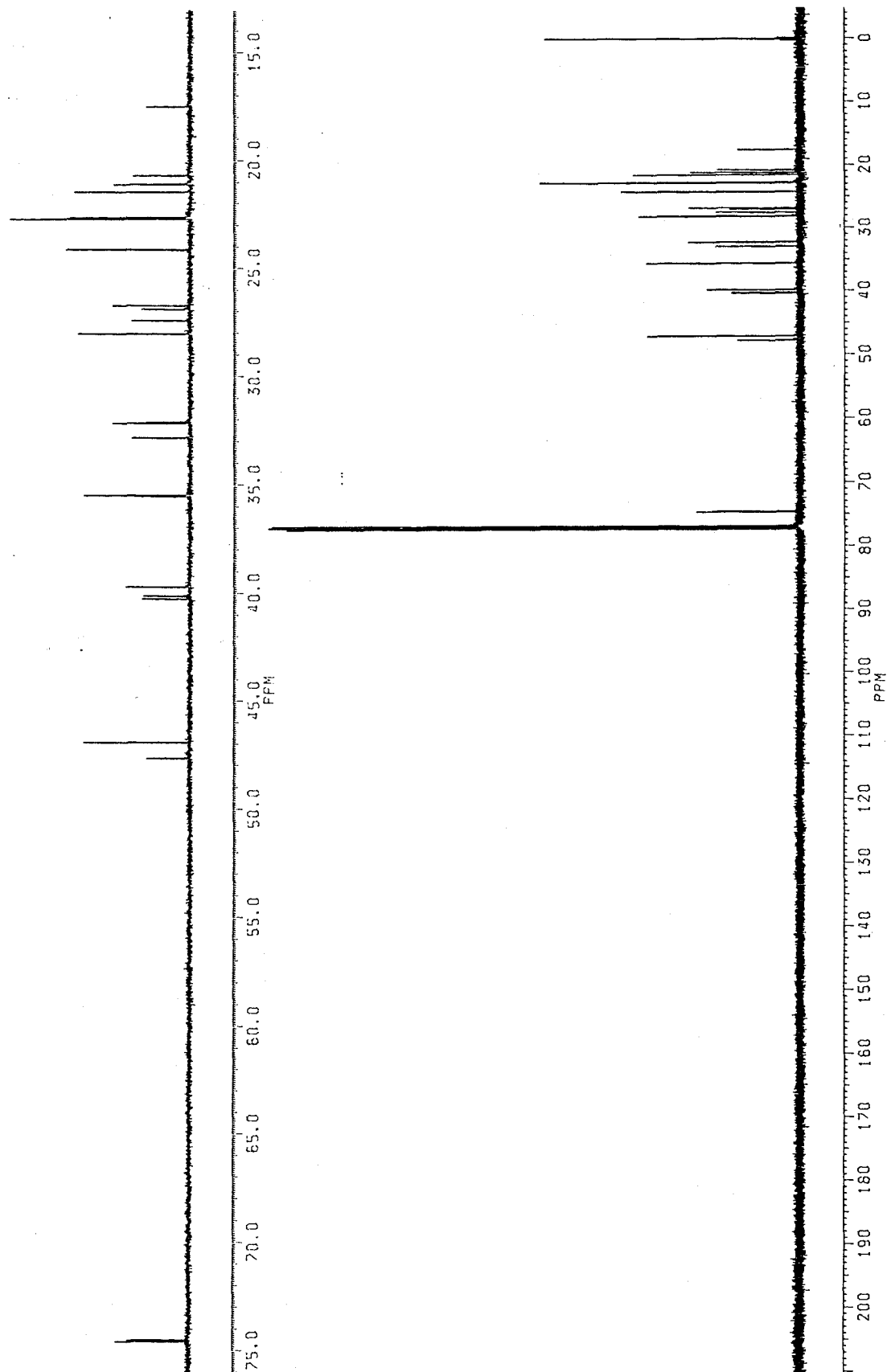
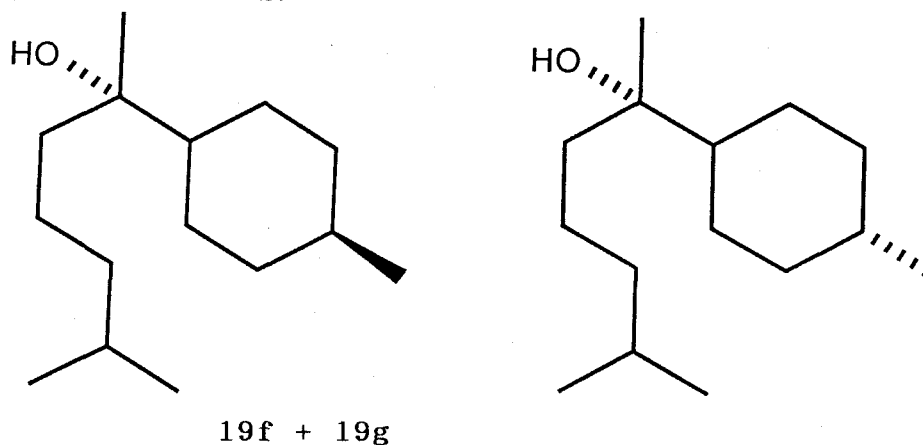


Fig. 3-359 ^{13}H -NMR Spectrum of RL-116-LAC-PLH2 (125 MHz, in CDCl_3)

Table 3-140 Physicochemical properties of RL-116-LAC-PLH1 (19f + 19g)



A colorless syrup

Vanillin-H₂SO₄ color: pinkish blue

EI-MS m/z (%); 226 (M^+ , 7.0), 209 (4.6), 208 ($M^+ - H_2O$, 3.6), 141 (27), 130 (14), 129 (100), 97 (11).

¹H-NMR $\delta_{TMS}^{CDCl_3}$ (500 MHz): 1.097 (d, $J = 6.3$ Hz), 0.950 (d, $J = 6.1$ Hz), 0.887, 0.885, 0.880, 0.873, 0.866.

¹³C-NMR $\delta_{TMS}^{CDCl_3}$ (125 MHz): 74.6, 74.5, 47.6, 46.9, 40.2, 40.1, 39.7, 35.5, 35.4, 32.8, 32.1, 32.1, 28.0, 27.4, 26.9, 26.7, 24.1, 24.1, 22.7, 22.7, 22.6, 21.4, 21.1, 20.7, 17.4.

As carried out for 19b, (-)- α -bisabolol (94) was also hydrogenated. In this case, palladium black (30 mg) was used as a catalyst instead of platinum black. After 5 hr stirring, the 82.5 mg of starting material completely disappeared and a major product was detectable on TLC, whose response to vanillin-H₂SO₄ test was slightly but clearly distinguishable from 94. By the PTLC separation, 59.6 mg of the product was obtained (BS-PDH, yield 71 %). In the spectroscopic comparisons of FI-MS, ¹H- and ¹³C-NMR, BS-PLH were completely identical to RL-116-LAC-PLH1 (19f + 19g). The result also supported the proposed planar structure of bisaborosaol A (19) (Fig. 3-360, 361, 362 and 363). Since relative ratio of *cis* and *trans* C-7 methyl proton signal in BS-PLH also showed a good accordance to that of RL-116-LAC-PLH1, both optical rotation was measured. As mentioned above, the optical rotation of these derivatives were expected to be quite small; therefore, ORD spectra of BS-PDH and RL-116-LAC-PLH1 were taken. As shown in Fig. 3-364, both showed dexorotatory at 386 nm, which indicated that the C-4 stereochemistry of 19f and 19g was *R* being equal to that of 94. Consequently, absolute configuration of 19 was established as C-4*S*, C-8*R*.

H-EA 6:1

○ vanillin-H₂SO₄ test: +



Reaction Std. 94
mix.

Fig. 3-360 TL Chromatogram of α -Bisabolol Reduction Product

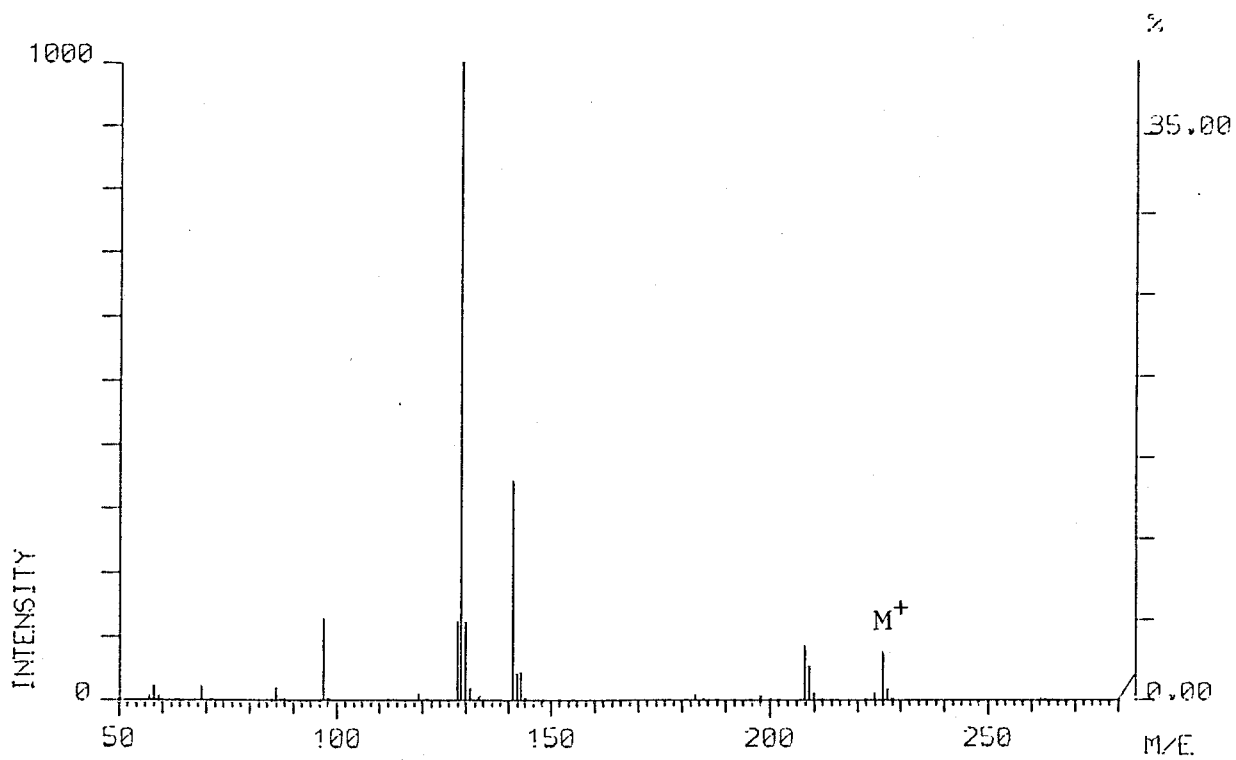


Fig. 3-361 FI-Mass Spectrum of BS-PDH

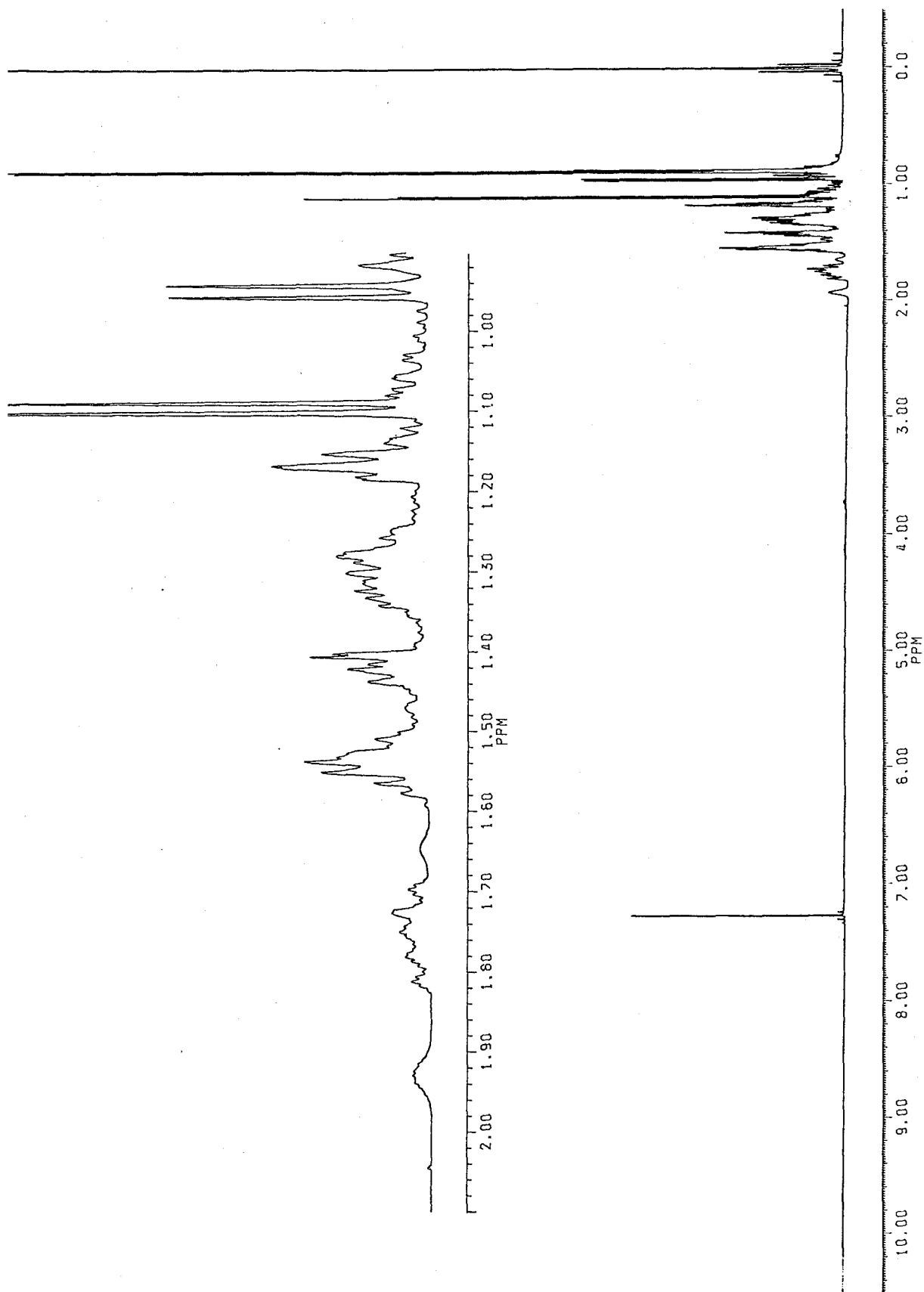


Fig. 3-362 ^1H -NMR Spectrum of BS-PDH (500 MHz, in CDCl_3)

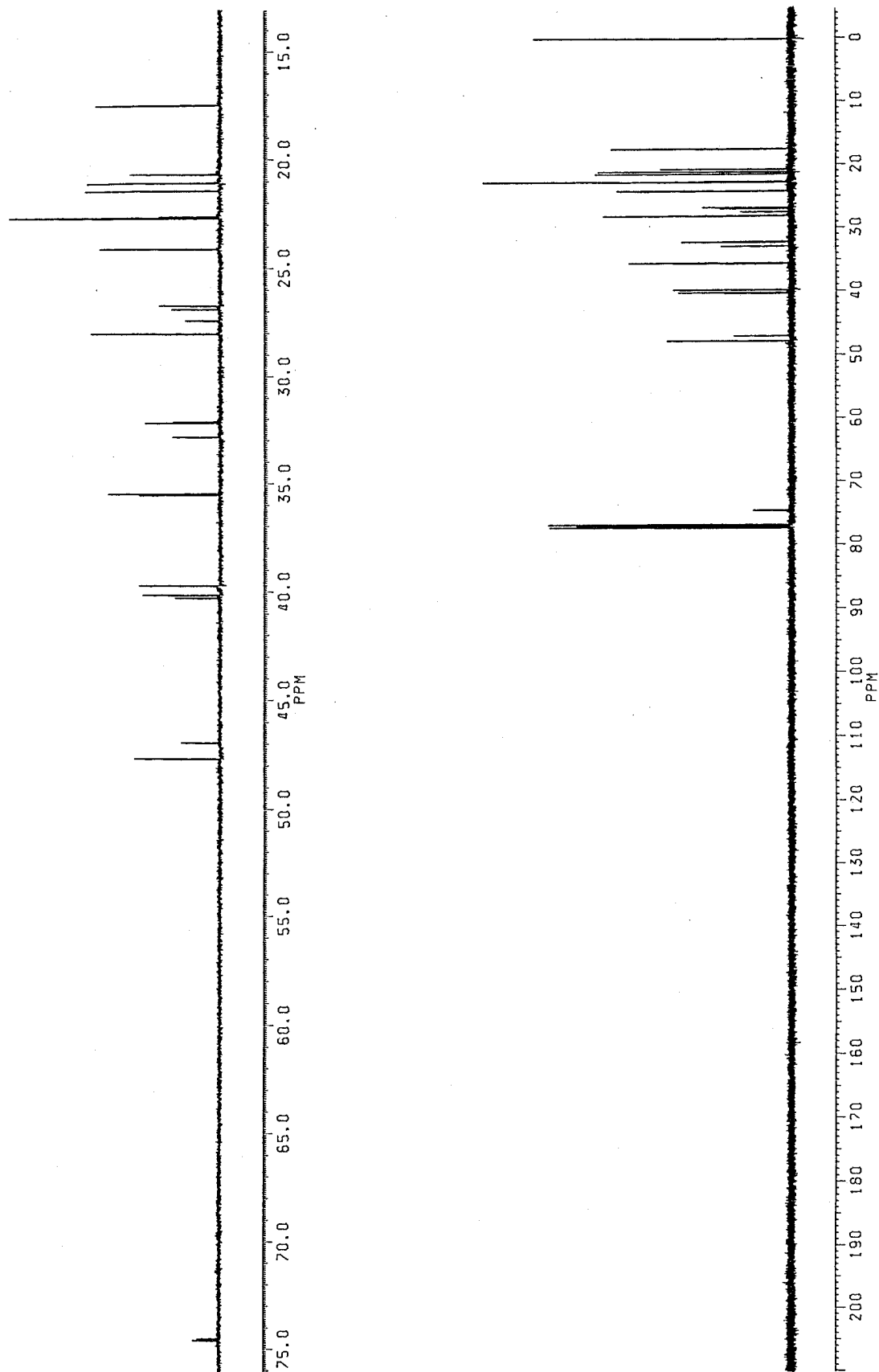


Fig. 3-363a ^{13}H -NMR Spectrum of BS-PDH (125 MHz, in CDCl_3)

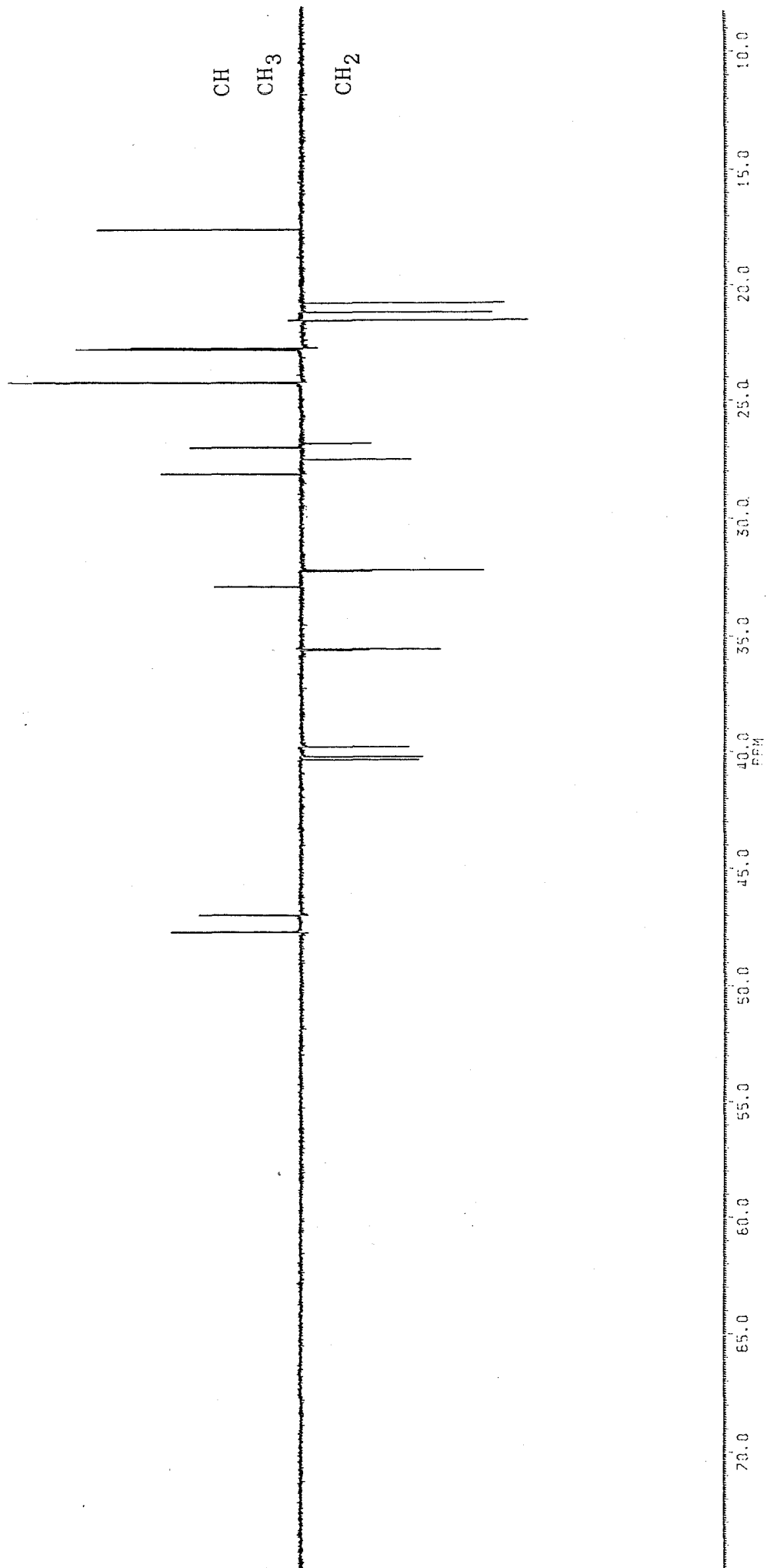
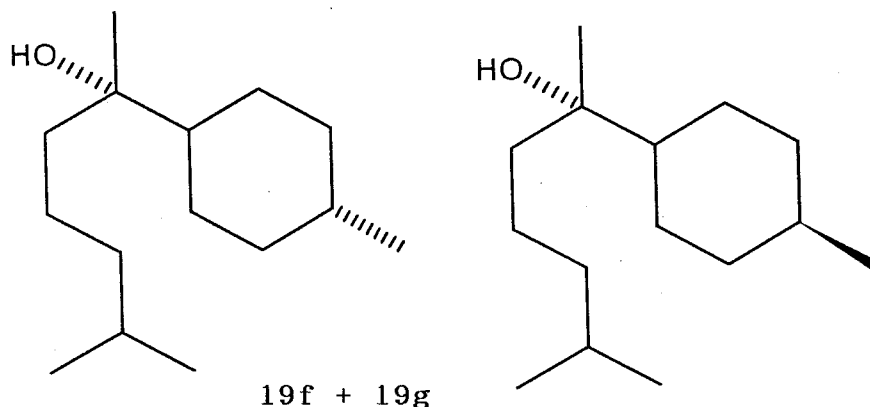


Fig. 3-363b Continued (DEPT)

Table 3-141 Physicochemical properties of BS-PDH (19f + 19g)



A colorless syrup

Vanillin-H₂SO₄ color: pinkish blue

UVλ_{max}^{MeOH}: featureless above 210 nm

FI-MS *m/z* (%); 226 (M⁺, 7.8), 209 (5.6), 208 (M⁺-H₂O, 9.4), 141 (35), 130 (13), 129 (100), 97 (13).

¹H-NMR δ_{TMS}^{CDCl₃} (500 MHz): 1.096 (d, *J*= 6.3 Hz), 0.950 (d, *J*= 7.2 Hz), 0.885, 0.879, 0.872, 0.866.

¹³C-NMR δ_{TMS}^{CDCl₃} (125 MHz): 74.6 (C), 74.5 (C), 47.6 (CH), 46.9 (CH), 40.2 (CH₂), 40.1 (CH₂), 39.7 (CH₂), 39.7 (CH₂), 35.5 (CH₂), 35.4, (CH₂), 32.8 (CH), 32.1 (CH₂), 32.1 (CH₂), 28.0 (CH), 27.4 (CH₂), 26.9 (CH), 26.7 (CH₂), 24.1 (CH₃), 24.1 (CH₃), 22.7 (CH₃), 22.7 (CH₃), 22.6 (CH₃), 21.4 (CH₂), 21.1 (CH₂), 21.1 (CH₂), 20.7 (CH₂), 17.5 (CH₃).

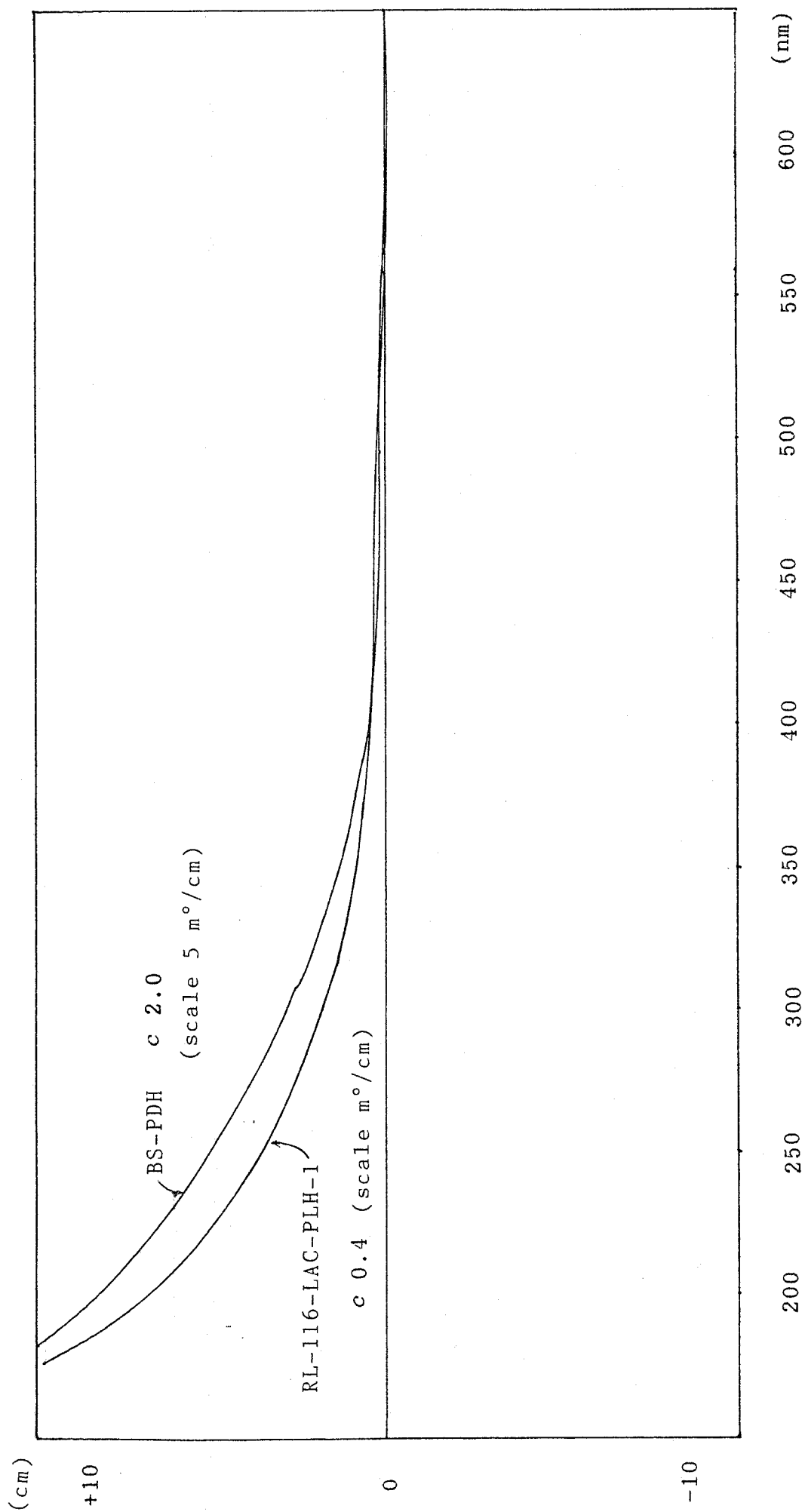
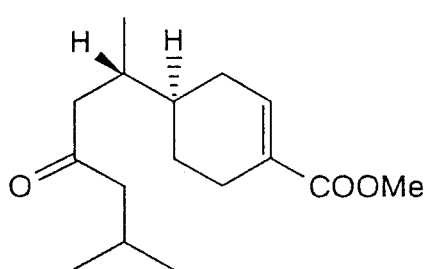


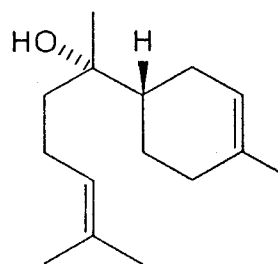
Fig. 3-364 ORD Spectrum of RL-116-LAC-PLH-1 and BS-PDH: Since both of the derivatives showed dextrorotatory, the absolute configuration of RL-116 at C-8 was determined as *R*.

Thus, the absolute configuration of bisaborosaol A (19) was established. This stereostructure, especially *R* at C-4 was, comparatively rare among naturally occurring bisabolanoids. Bisabolanoids whose stereochemistry has already been established are listed in Fig. 3-365 with their plant sources [146-150].



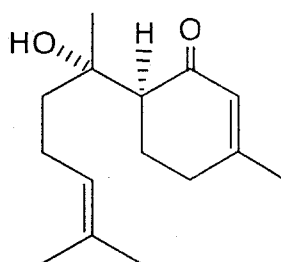
juvabione (98)

[146]



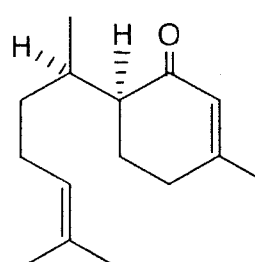
(-)-α-bisabolol (94)

[147]



(+)-hernandulcin (106)

[149, 150]



/-bisabolone (107)

[148]

Fig. 3-365 Some of Stereochemically Established Bisabolane Derivatives

3-8-3 Bisaborosaic Acid A

1) Isolation of an Acid Corresponding to Bisaborosaol A

During a survey of acidic constituents in *Rosa rugosa*, a substance showing a reddish purple color, being similar to the response of bisaborosaol A (19), was detected by vanillin-H₂SO₄ test. To isolate the focused substance, acidic constituents were prepared from the diffusates of damaged leaves (11 kg) through extraction of the crude EtOAc-solubles with 5 % NaHCO₃ solution followed by re-extraction of the aqueous layer with EtOAc after being acidified. The acidic extractives were coated on 80 ml of silica gel which was put on a silica gel column (gel volume, 1500 ml), and successively fractionated as follows: Fr-A-1; washings with 40 % EtOAc/*n*-hexane (1500 ml), Fr-A-2, 3 and 4; eluates with 60 % EtOAc/*n*-hexane (each 500 ml), Fr-A-5; eluates with 100 % EtOAc/*n*-hexane (500 ml). Fr-A-2 and 3 mainly contained the focused substance, and Fr-A-4 and 5 also involved it in small amounts (Fig. 3-366). From a part of Fr-A-3 (ca 1/5), the focused acid was isolated by PTLC developed in hexane-EtOAc-HCOOH 25:25:1 (*R_f* 0.44) to give ca 50 mg of a colorless syrup. This acid was also detected in an acidic fraction from the MeOH extract obtained of unwounded *Rosa rugosa* leaves (Sample V).

2) Structure Elucidation

The isolate RL-123A showing M^+ 252 in FD-MS (Fig. 3-367) afforded quite similar spectra to those of bisaborosaol A (16) in EI-MS, ^1H - and ^{13}C -NMR (Fig. 3-368, 369, 370 and Tables 3-142, 143). The results suggested that the isolate was an acid corresponding to 19. Accordingly, hydrocleavage of 19 was carried out as shown in Scheme 3-43, and the reaction product RL-116-HYD was successfully obtained.

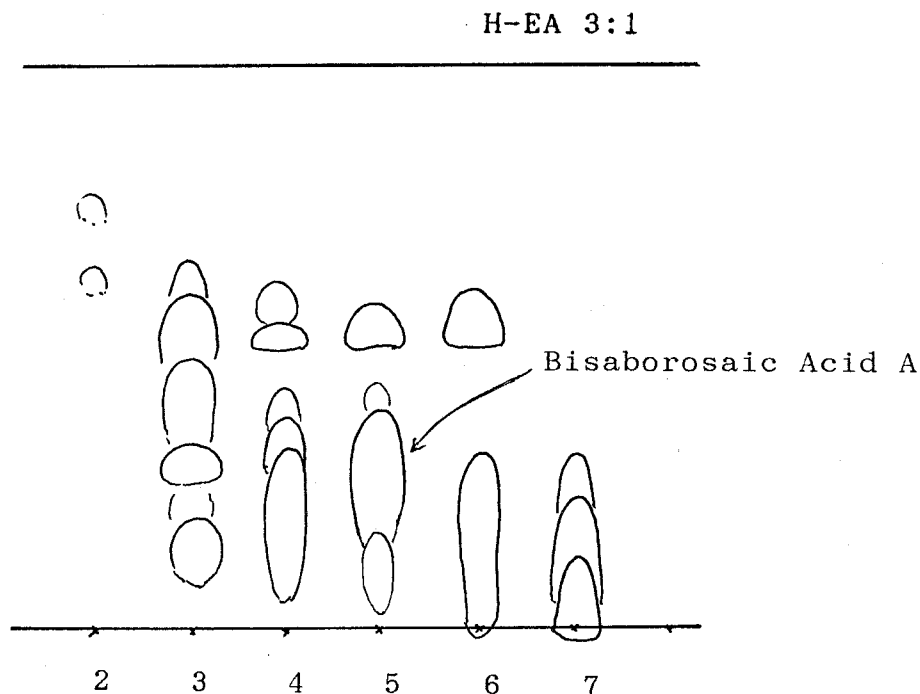


Fig. 3-366 TL Chromatogram of Coulmn Fractions of Acidic Constituents from Sample IV

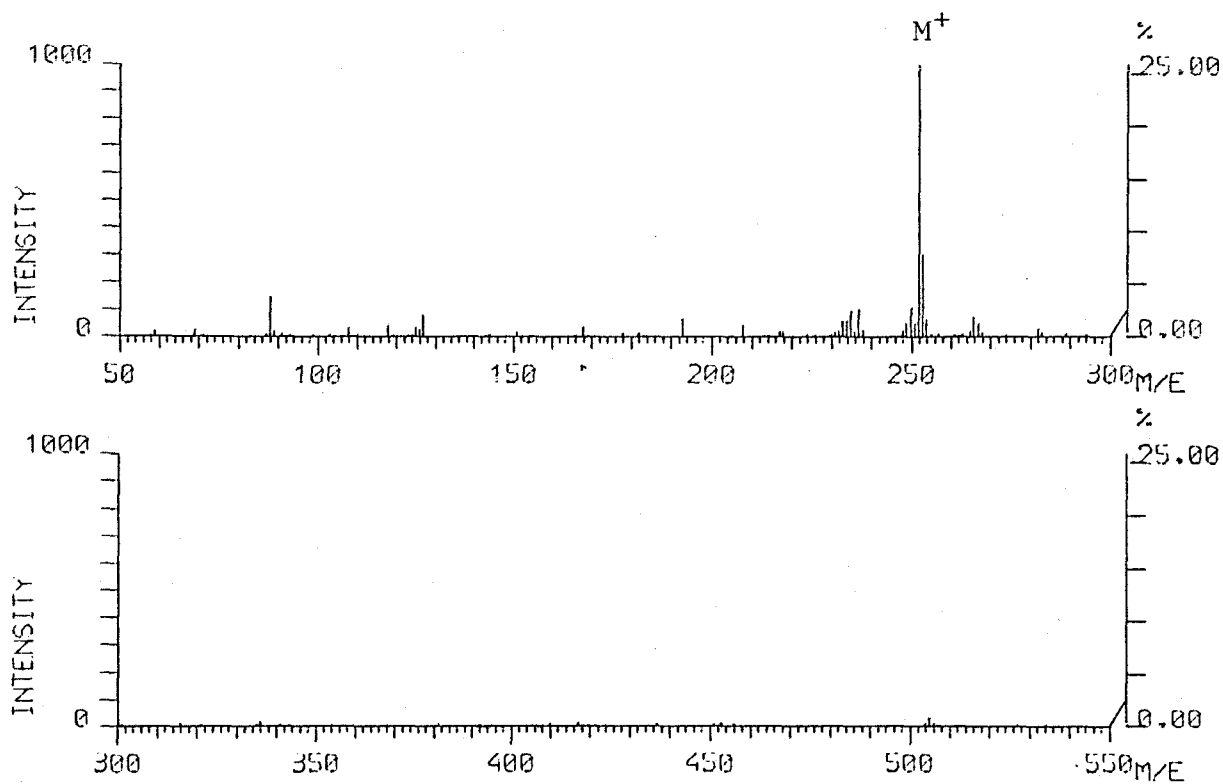


Fig. 3-367 FD-Mass Spectrum of RL-123A

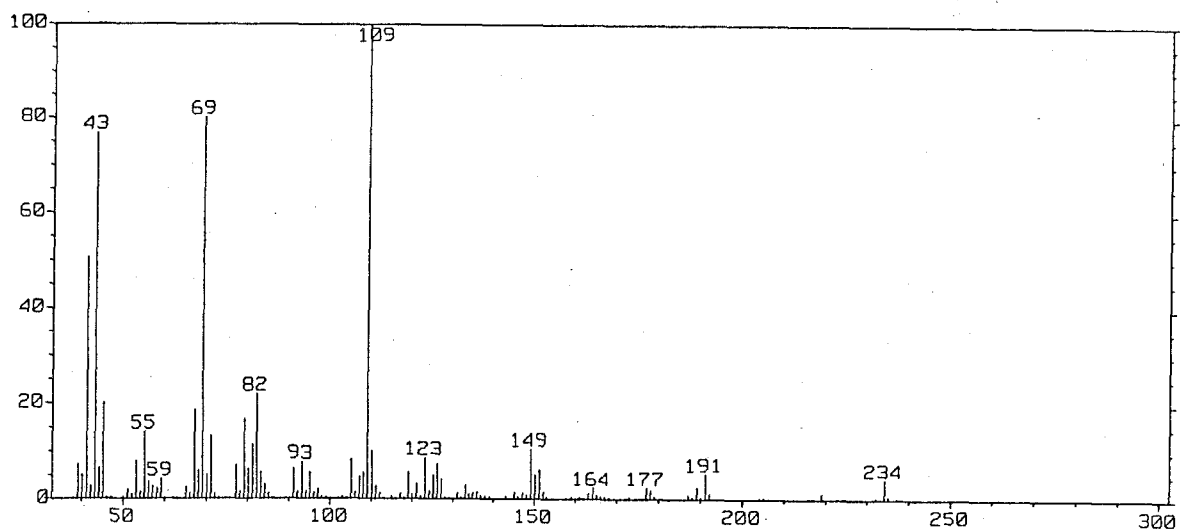


Fig. 3-368 EI-Mass Spectrum of RL-123A

Table 3-142 ^1H -NMR chemical shift values of RL-123A

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}		Coupling	Assignment
7.145	1H	s-like br. m	C-2-H
5.133	1H	t-like m $J= 7.0$ Hz	C-12-H
2.520	1H	br. d $J= 17.5$ Hz	C-6-Ha
2.377	1H	m-dvid. d* $J= 18.8$ Hz	C-3-Ha
2.165	1H	br. t-like m	C-6-Hb
2.12 (approx.)	1H	m	C-3-Hb
2.063	2H	m	C-11-H ₂
1.916	1H	m-dvid. d $J= 11.3$ Hz	C-5-Ha
1.692	3H	br. s	C-14-H ₃
1.626	3H	br. s	C-15-H ₃
1.62 (approx.)	1H	m	C-4-H
1.542	1H	t $J= 8.2$ Hz	C-9-H ₂
1.251	1H	dddd $J= 12.4, 12.3, 12.3$ and 5.4 Hz	C-11-Hb
1.167	3H	s	C-10-H ₃

* multiply divided doublet

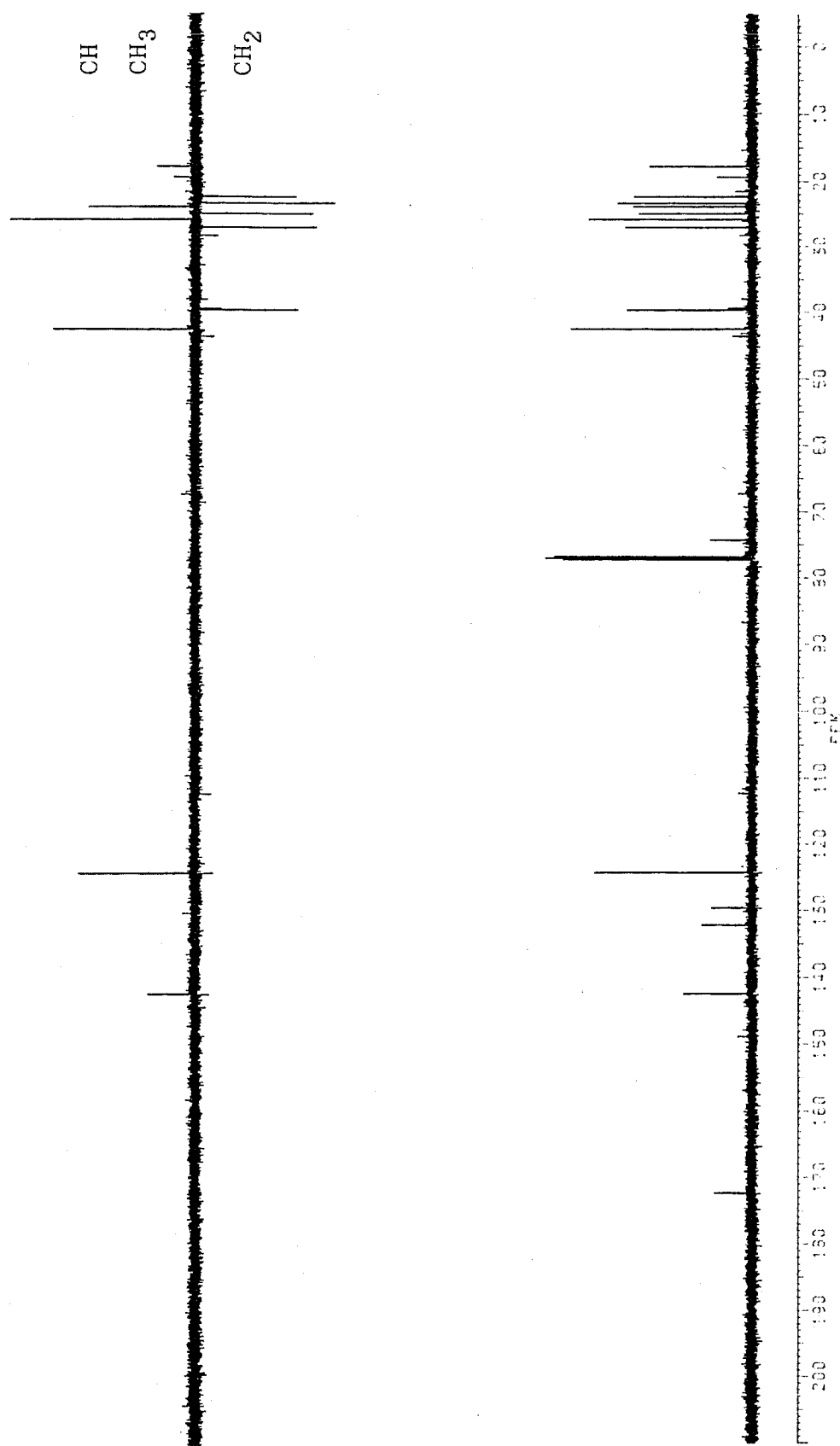


Fig. 3-370 ^{13}C -NMR Spectra of RL-123A (125 MHz, in CDCl_3)

Table 3-143 ^{13}C -NMR chemical shift values of RL-123A

(125 MHz, in CDCl_3 , TMS as an int. std.)

δ_{C}	Property	Assignment
172.4	-COO-	C-7
142.3	=CH-	C-2
132.1	=C-	C-13
129.5	=C-	C-1
124.2	=CH-	C-12
74.2	-C-O	C-8
42.3	CH	C-4
39.4	CH_2	C-9
27.0	CH_2	C-6
25.7	CH_3	C-14
24.8	CH_2	C-3
23.8	CH_3	C-10
23.2	CH_2	C-5
22.2	CH_2	C-11
17.7	CH_3	C-15

Bisaborosaol A (19.8 mg) Dissolved in 2 ml of EtOH

added 2 ml of 1 N NaOH solution
standing overnight at room temperature
diluted with 20 ml of a satd. NaCl
acidified to pH 3.0 with 2 N HCl
extracted with 15 ml of EtOAc

EtOAc Layer

dried over Na₂SO₄
removed the solvent *in vacuo*
PTLC (C-M-F 100:5:2)

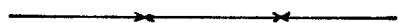
14.5 mg of the Main Product [RL-116-HYD (19h), *R_f* 0.35,
77 % yield]

Scheme 3-43 Alkali hydrocleavage of bisaborosaol A

The product (RL-116-HYD) was completely indistinguishable from RL-123A in TLC, EI-MS and ¹H-NMR (Fig. 3-371, 372 and 373). RL-123A was thus characterized to be 20. On the other hand, the methylation product of 20 with CH₂N₂, RL-123A-ME also revealed to be identical to 19 in EI-MS, ¹H- and ¹³C-NMR (Fig. 3-374, 375 and 376). The methyl ester (RL-123A-ME) was further agreeable with 19 in optical rotation ($[\alpha]_D + 68^\circ$, *c* 0.1 in acetone, *cf.* 19; $+ 78^\circ$, *c* 0.06 in MeOH). This naturally occurring bisabolane acid (20) was accordingly proved to be a carboxylic acid derivative stereochemically corresponding to 19 and was named bisaborosaic acid A.

H-EA-F 25:25:1

○ quenching under UV 254 nm



Reaction Std. 20
mix.

Fig. 3-371 TL Chromatograms of RL-116-HYD Obtained by Hydrolysis of Bisaborosaol A (19).

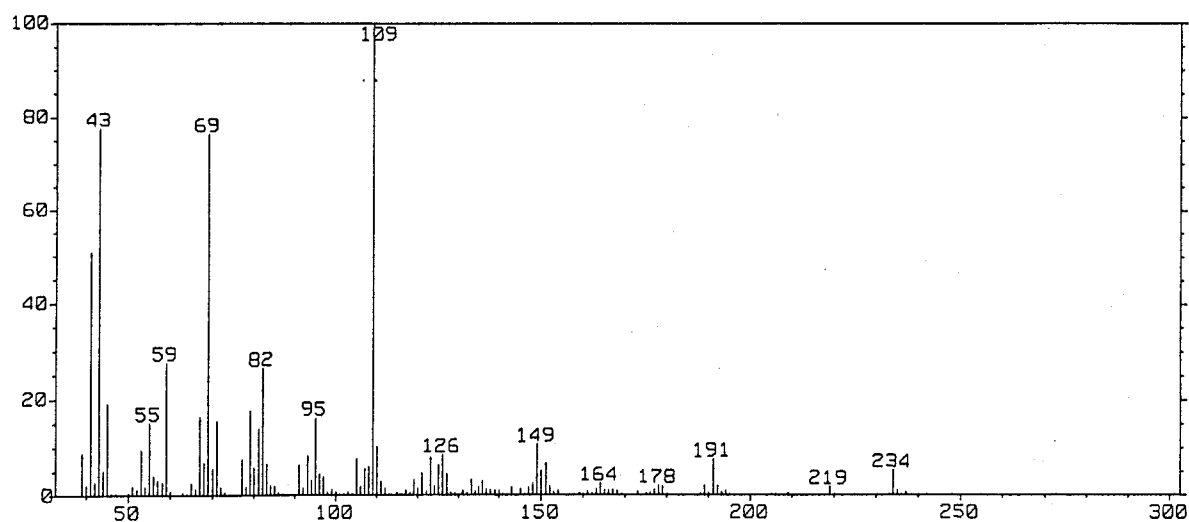


Fig. 3-372 EI-Mass Spectrum of RL-116-HYD: The fragmentation pattern is well agreeable with that of bisaborosaic acid A (20)

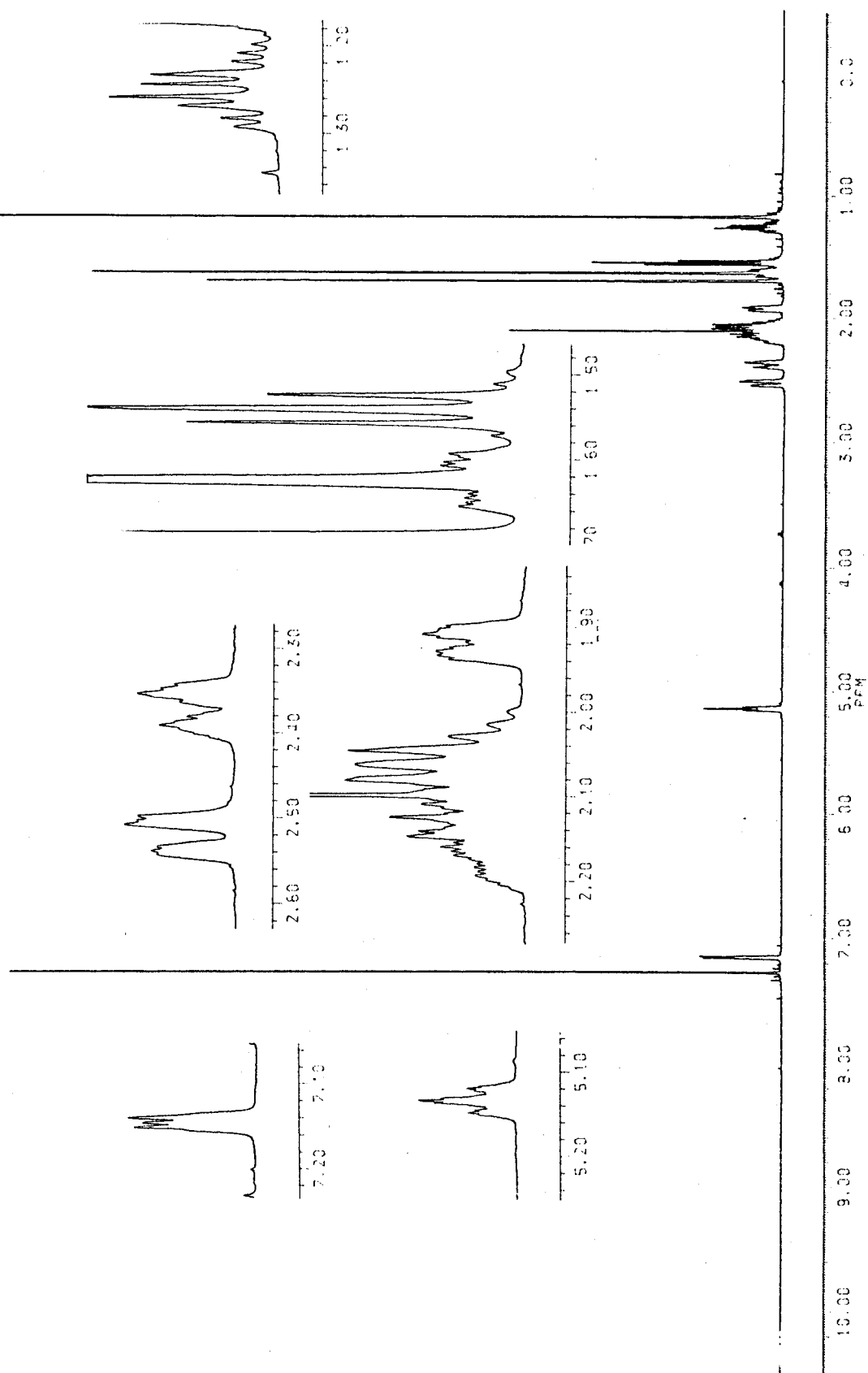


Fig. 3-373 ^1H -NMR Spectrum of RL-116-HYD (500 MHz, in CDCl_3)

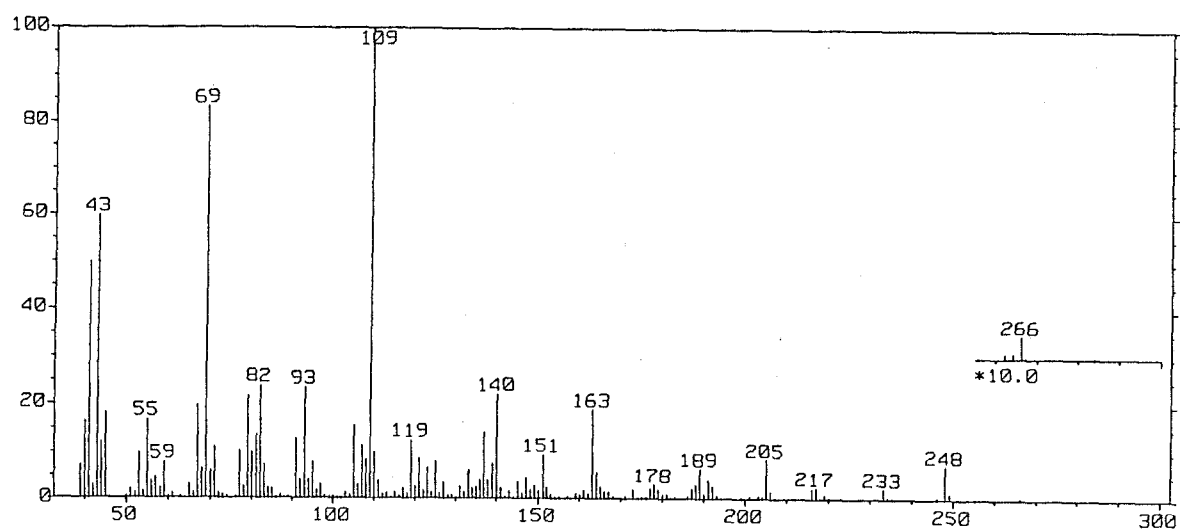


Fig. 3-374 EI-Mass Spectrum of RL-123A-ME

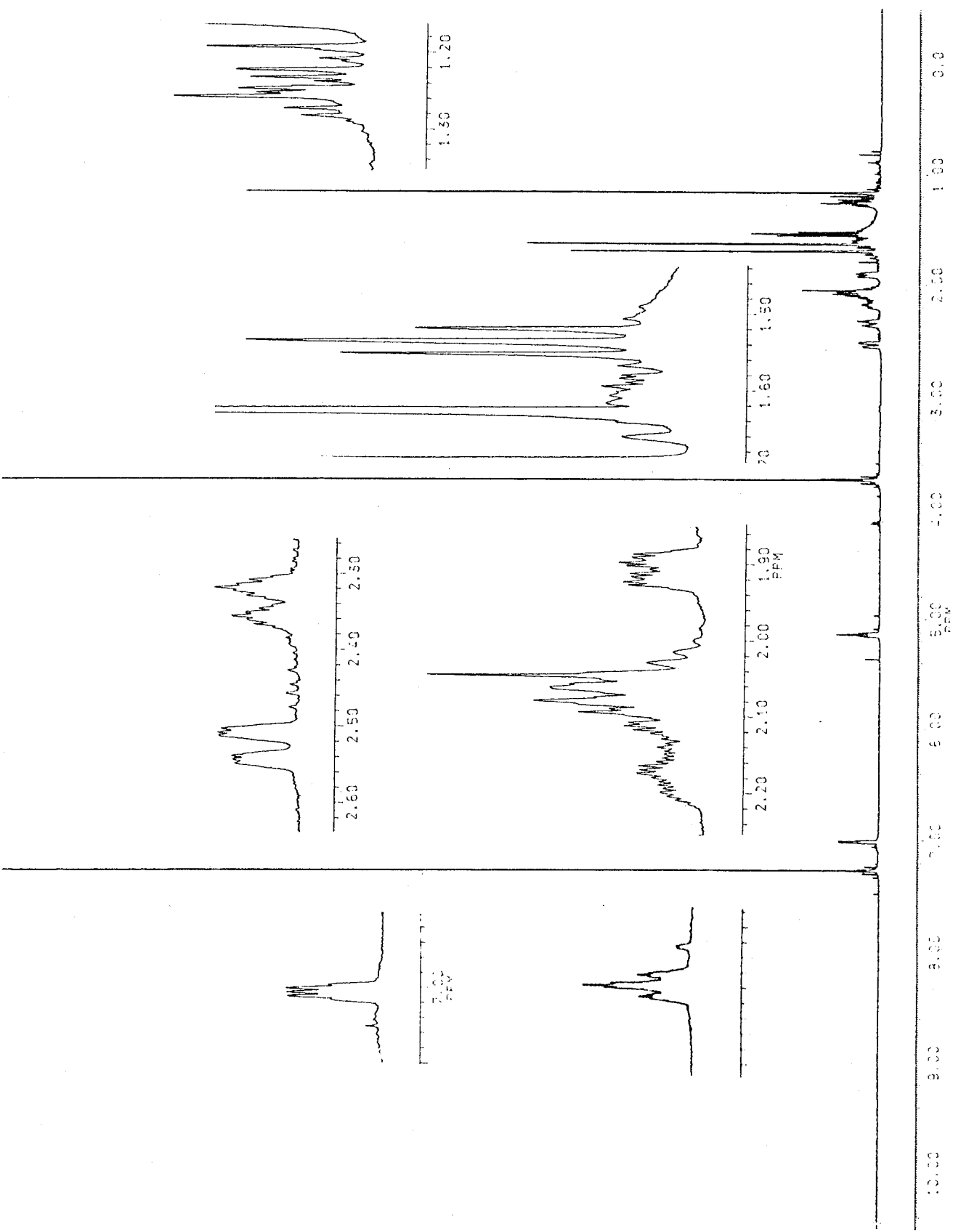


Fig. 3-375 ^1H -NMR Spectrum of RL-123A-ME (500 MHz, in CDCl_3)

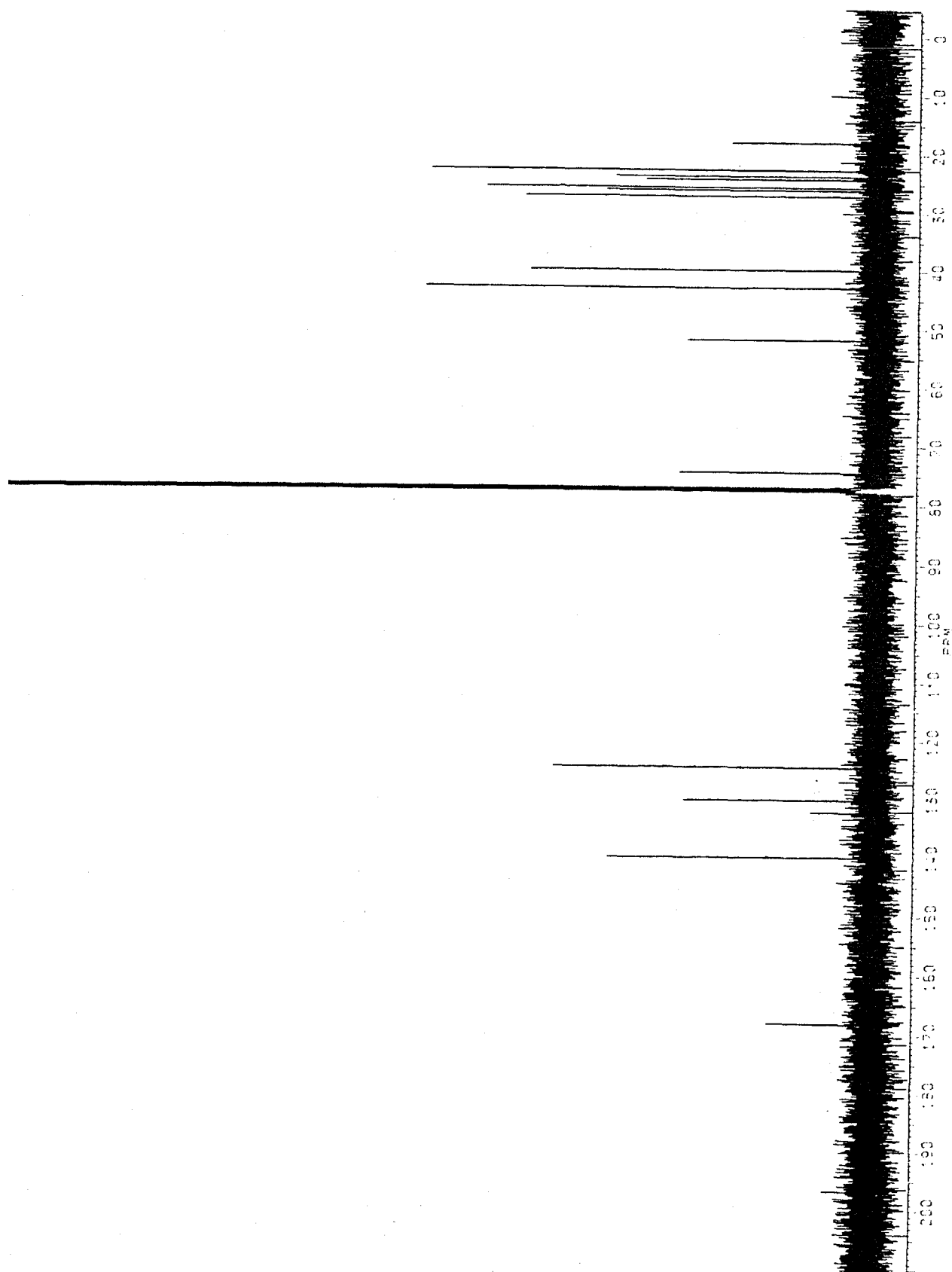
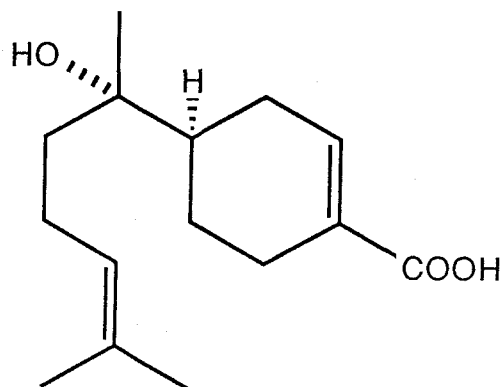


Fig. 3-376 ^{13}C -NMR Spectrum of RL-123A-ME (125 MHz, in CDCl_3)

Table 3-144 Physicochemical properties of bisaborosaic acid A (20)



20

A colorless syrup

R_f 0.35 (C-M-F 100:5:2)

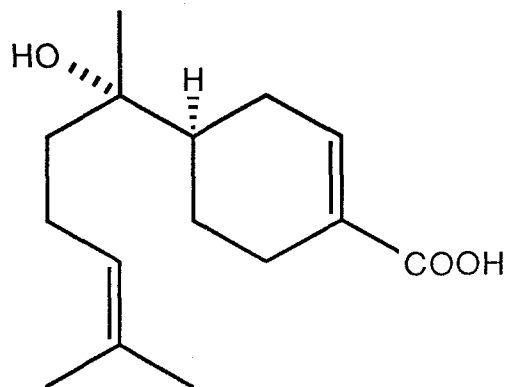
Vanillin-H₂SO₄ test: pinkish red

FI-MS *m/z* (%): 253 (M⁺+1, 30), 252 (M⁺, 100)

EI-MS *m/z* (%): 234 (M⁺-H₂, 4.5), 219 (1.3), 191 (5.6), 189 (2.7),
178 (2.0), 177 (2.6), 164 (2.6), 151 (6.4), 150 (5.3), 149
(11), 126 (7.8), 123 (9.0), 110 (10), 109 (100), 105 (8.5),
93 (7.9), 82 (22), 81 (12), 79 (17), 71 (13), 69 (80), 67
(19), 55 (14), 45 (20), 43 (77), 41 (51).

¹H- and ¹³C-NMR spectra data are shown in Tables 3-142 and
3-143, respectively.

Table 3-145 Physicochemical properties of RL-116-HYD (19h)



19h

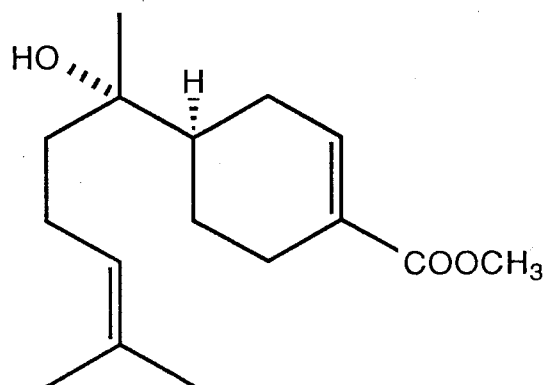
A colorless syrup

Vanillin- H_2SO_4 color: pinkish red

EI-MS m/z (%): 234 ($\text{M}^+ - \text{H}_2\text{O}$, 5.3), 219 (1.9), 191 (7.7), 189 (2.2), 179 (1.9), 178 (2.1), 164 (2.6), 151 (6.8), 149 (11), 126 (8.5), 123 (8.0), 110 (10), 109 (100), 95 (16), 82 (27), 81 (14), 79 (18), 71 (16), 69 (77), 67 (17), 59 (28), 55 (15), 45 (19), 43 (78), 41 (51).

$^1\text{H-NMR}$ $\delta_{\text{TMS}}^{\text{CDCl}_3}$ (500 MHz): 7.144 (1H, br. s, C-2-H), 2.372 (1H, m-divid. d, $J = 18.6$ Hz, C-3-Ha, *ca* 2.12 (1H, m, C-3-Hb), 1.624 (1H, m, C-4-H), 1.917 (1H, m-divid. d, $J = 12.9$ Hz, C-5-Ha), 1.250 (1H, dddd, $J = 12.5, 12.4, 12.2$ & 5.2 Hz, C-5-Hb), 2.521 (1H, br. d, $J = 17.7$ Hz, C-6-Ha), 2.162 (1H, m, C-6-Hb), 1.541 (2H, t, $J = 8.2$ Hz, C-9- H_2), 1.166 (3H, s, C-10- H_3), 2.062 (2H, m, C-11- H_2), 5.133 (1H, m-like t, $J = 7.1$ Hz, C-12-H), 1.692 (3H, br. s, C-14- H_3), 1.626 (3H, br. s, C-15- H_3).

Table 3-146 Physicochemical properties of RL-123A-ME (19)



19

A colorless syrup

$[\alpha]_D$: + 68 ° (c 0.1 in acetone)

Vanillin-H₂SO₄ color: pinkish red

EI-MS m/z (%): 266 (M^+ , 0.5), 248 ($M^+ - H_2O$, 7.4), 233 (2.4), 217 (2.5), 216 (2.2), 205 (8.7), 189 (6.3), 163 (19), 151 (9.6), 140 (22), 137 (14), 119 (13), 109 (100), 105 (16), 93 (24), 82 (24), 79 (22), 69 (83), 67 (20), 55 (17), 45 (18), 43 (60), 41 (50).

¹H-NMR $\delta_{TMS}^{CDCl_3}$ (500 MHz): 7.006 (1H, ddd, J = 5.5, 2.8 & 2.7 Hz, C-2-H), 2.337 (1H, m-divid. d, J = 19.0 Hz, C-3-Ha), *ca* 2.09 (1H, m, C-3-Hb), 1.612 (1H, m, C-4-H), 1.907 (1H, m-divid. d, J = 12.7 Hz, C-5-Ha), 1.241 (1H, dddd, J = 12.4, 12.3, 12.3 & 5.1 Hz, C-5-Hb), 2.526 (1H, br. d, J = 17.7 Hz, C-6-Ha), 2.171 (1H, m, C-6-Hb), 1.536 (2H, t, J = 8.2 Hz, C-9-H₂), 1.161 (3H, s, C-10-H₃), 2.062 (2H, m, C-11-H₂), 5.136 (1H, m-like t, J = 7.2 Hz, C-12-H), 1.694 (3H, br. s, C-14-H₃), 1.628 (3H, br. s, C-15-H₃), 3.731 (3H, s, C-7'-H₃).

¹³C-NMR $\delta_{TMS}^{CDCl_3}$ (125 MHz): 168.0 (C-7), 139.9 (C-2), 132.3 (C-1), 130.2 (C-13), 124.5 (C-12), 74.3 (C-8), 51.7 (C-7'), 42.7 (C-4), 39.6 (C-9), 26.9 (C-3), 25.9 (C-5), 25.4 (C-10), 24.0 (C-14), 23.5 (C-5), 22.4 (C-11), 17.9 (C-15).

3-8-4 Monooxygenated Derivative of Bisaborosaol A

1) Isolation and Spectroscopic Analyses

During the isolation of bisaborosaol A (19), two compounds showing a clear yellow color with vanillin- H_2SO_4 reagent on TL plates were detected under the spot of 19 (in Fr-I-8), and these were tentatively named RL-117B and RL-118B (R_f 0.38 and 0.34, respectively, *cf.* 0.47 in 19). By the re-column chromatography, RL-117B was eluted in FFr-I-3 and successive FFr-I-4 (25 % EtOAc/hexane 50 ml). The latter fraction also contained RL-118B as the major substance. With the guidance of the characteristic response to vanillin- H_2SO_4 reagent, each compound was isolated by PTLC (H-EA 3:1) (Fig. 3-377).

Both of them showed M^+ 282 in FI-MS (Fig. 3-378 and 379), although the parent ions were undetectable in EI-MS of the each compound (Fig. 3-380 and 381). The ^1H -NMR spectra were both similar to that of 19; however, two methyl signals were observed upfield to be assignable to a methyl group allocated not to an olefinic but to an oxygenated carbon (Fig. 3-382 and 383, and Tables 3-147 and 148). Furthermore, each double-doublet methine proton (δ_{H} 3.415 in RL-117B, and 3.553 in RL-118B, respectively) was newly detected instead of the C-12 olefinic proton of 19. The results suggested that the olefinic bond on the side chain was monooxygenated to contend the molecular weight 282 (16 + O). An exchangeable and broad singlet proton was also detectable in both of the ^1H -NMR spectra.

Since each ^{13}C -NMR spectrum of the compounds was quite similar, RL-117B and RL-118B were considered to be epimeric to each other (Fig. 3-384, 385 and Tables 3-149, 150). Although from 19 three monooxygenated forms (D, E and F) were possible to draw (Fig. 3-386), D having an epoxy group was neglected due to the chemical

sift values of the oxygenated carbons. These carbon shifts [e.g. δ_C 86.4 (CH), in RL-117B] were too low as a methine carbon in an epoxide ring. As a matter of fact, if an epoxy group was induced to the olefinic bond, it will be cyclized by intramolecular alcoholysis with the C-8-OH, as known in *l*-linalool (68) and (-)- α -bisabolol (94), for example [151,152]. Epoxidation of 19 was therefore carried out.

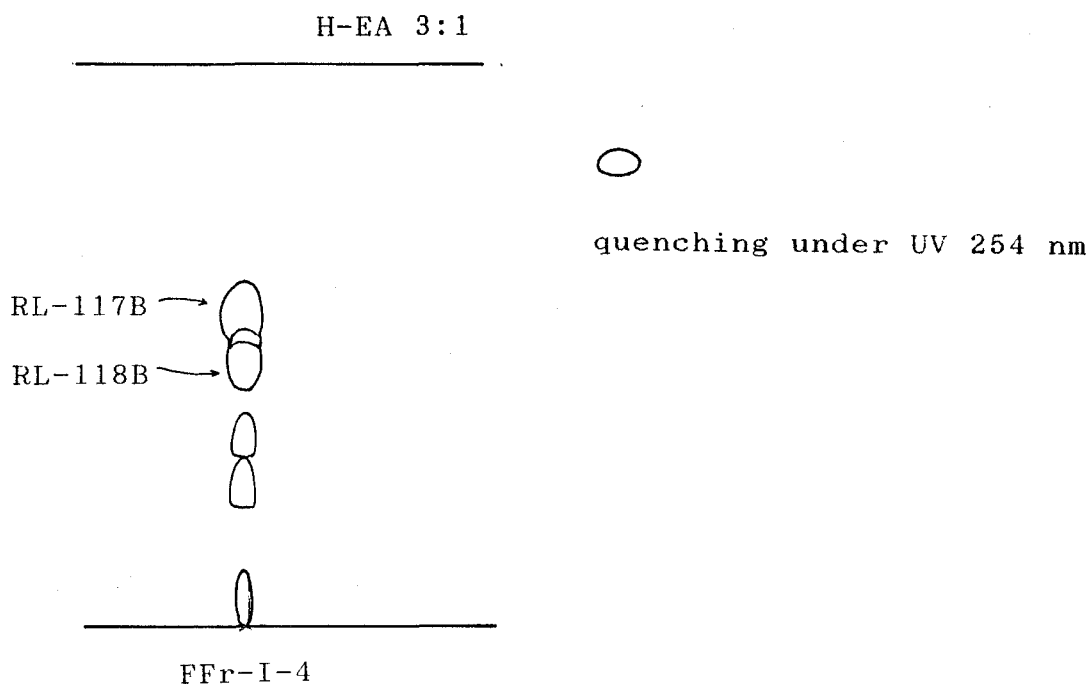


Fig. 3-377 TL Chromatogram of FFr-I-4 Containing RL-117B and RL-118B

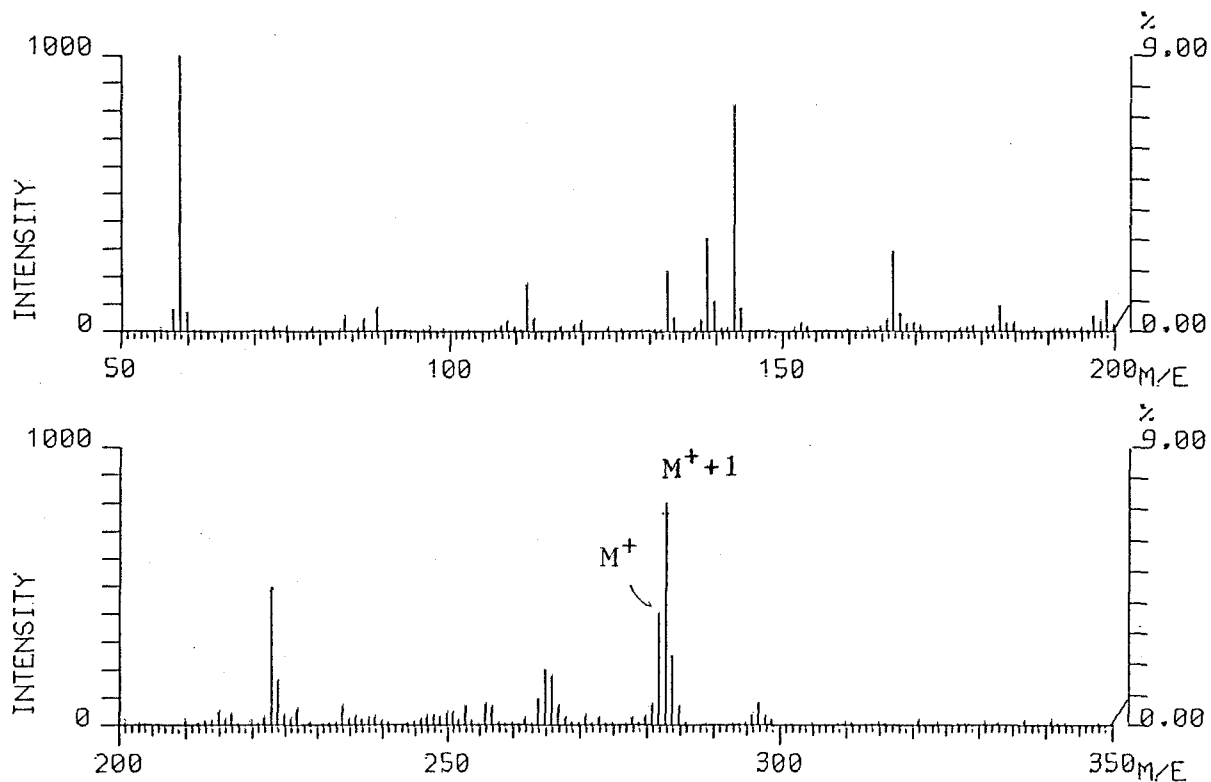


Fig. 3-378 FI-Mass Spectrum of RL-117B

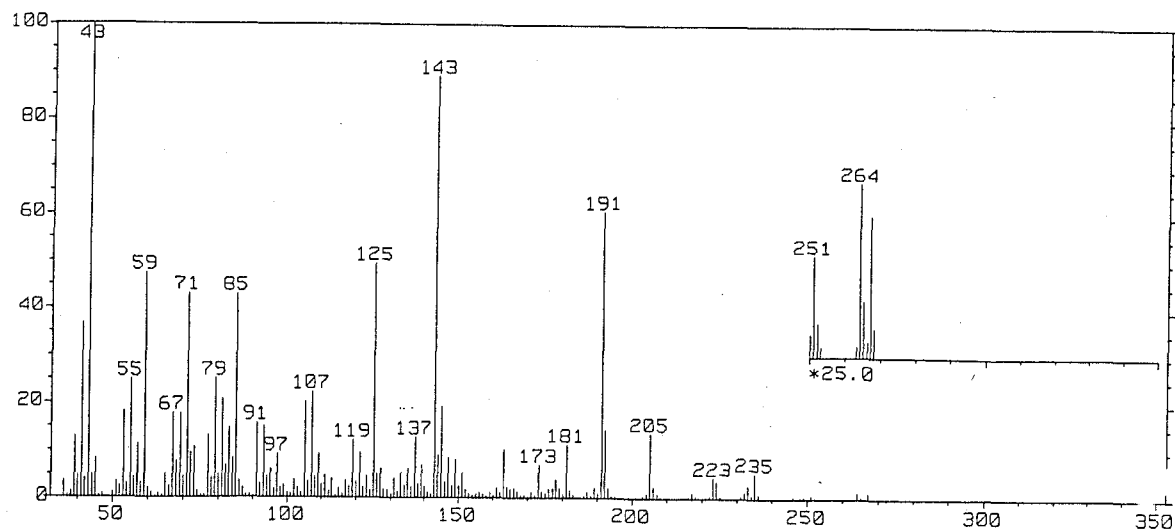


Fig. 3-379 EI-Mass Spectrum of RL-117B

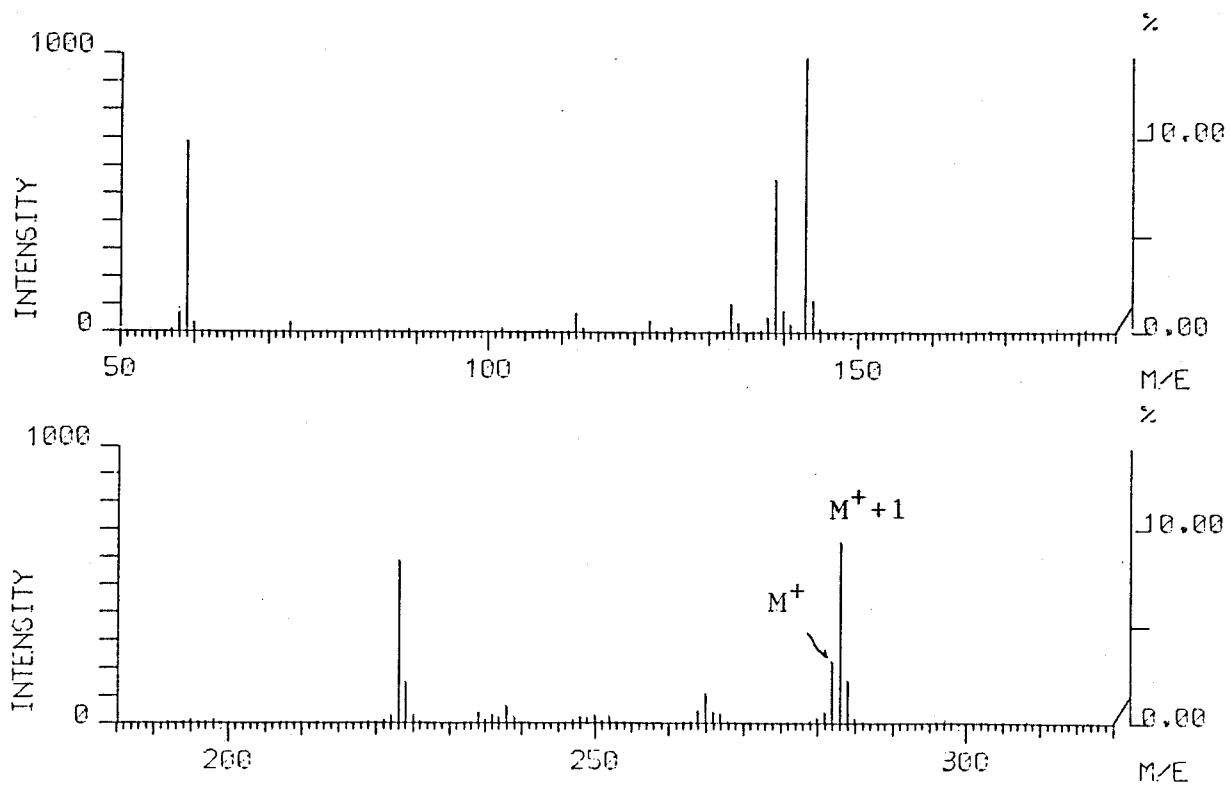


Fig. 3-380 FI-Mass Spectrum of RL-118B

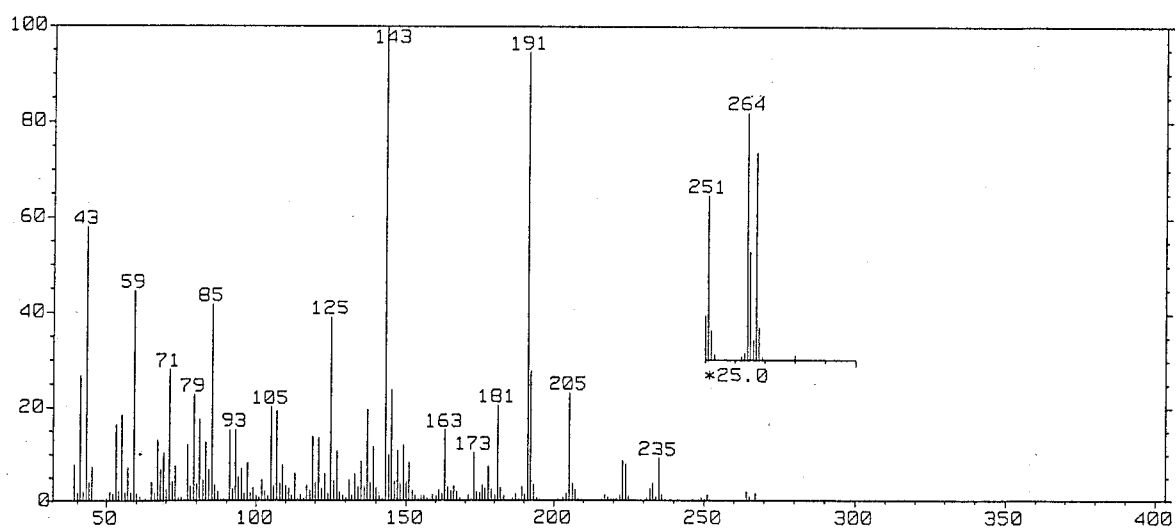


Fig. 3-381 EI-Mass Spectrum of RL-118B

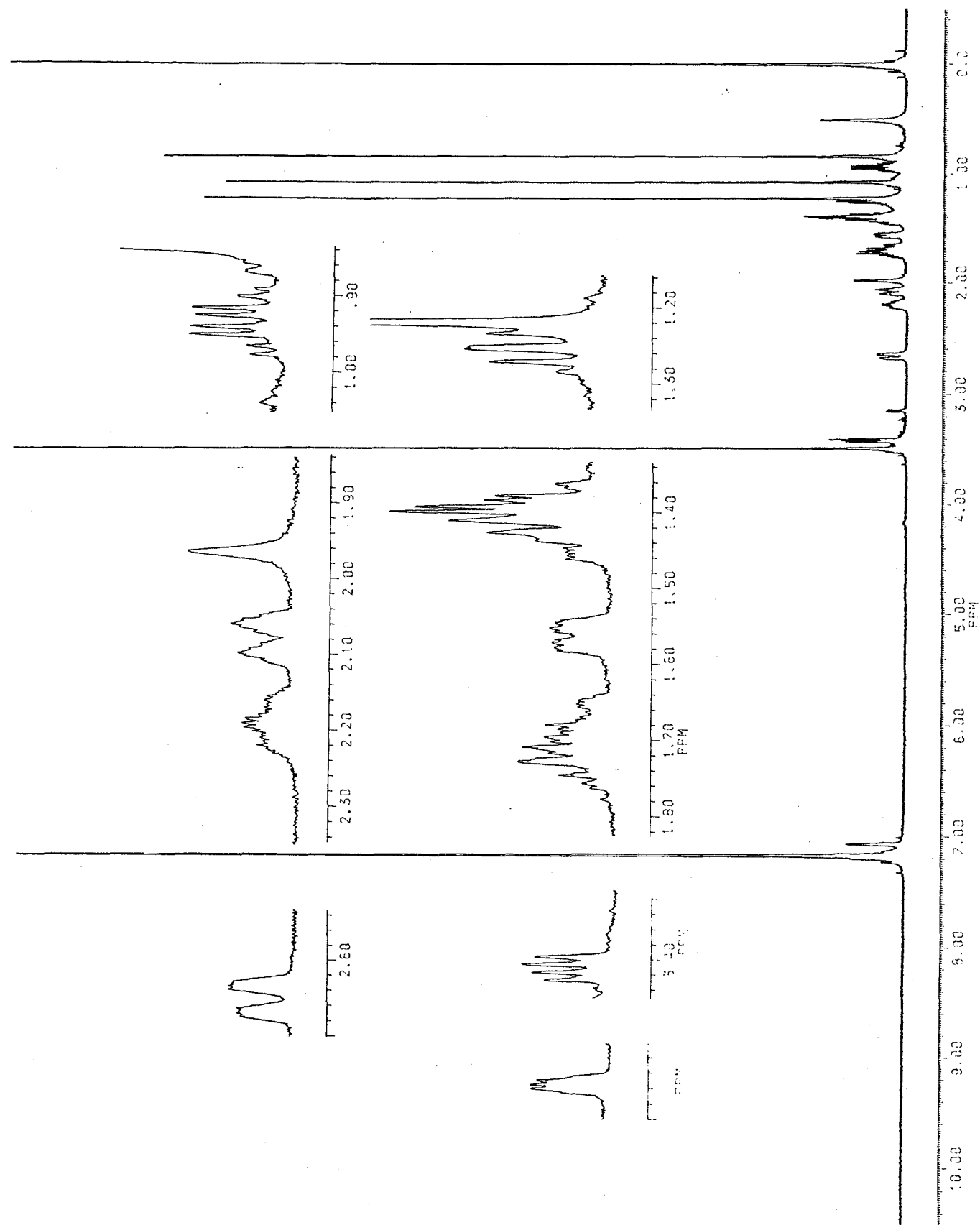


Fig. 3-382 ^1H -NMR Spectrum of RL-117B (500 MHz, in C_6D_6)

Table 3-147 ^1H -NMR chemical shift values of RL-117B

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}		Coupling	Assignment
7.057	1H	br. m	C-2-H
3.481	3H	s	C-7'-H ₃
3.405	1H	dd $J= 10.3$ and 5.2 Hz	C-12-H
2.655	1H	br. d $J= 15.0$ Hz	C-6-Ha
2.192	1H	t-like m	C-6-Hb
2.075	1H	m-dvid. d $J= 19.3$ Hz	C-3-Ha
1.975	1H	br. s (exchangeable)	C-13-OH
1.719	1H	m	C-11-Ha
1.682	1H	m	C-3-Hb
1.559	1H	d-like m	C-5-Ha
1.43 (approx.)	1H	t-like m	C-4-H
1.403	1H	dd $J= 9.8$ and 8.6 Hz	C-9-Ha
1.381	1H	m	C-11-Hb
1.249	1H	dd $J= 9.8$ and 9.0 Hz	C-12-H
1.221	3H	s	C-14-H ₃
1.074	3H	s	C-15-H ₃
0.930	1H	dddd $J= 12.5, 12.4, 12.4$ and 5.1 Hz	C-5-Hb
0.834	3H	s	C-10-H ₃

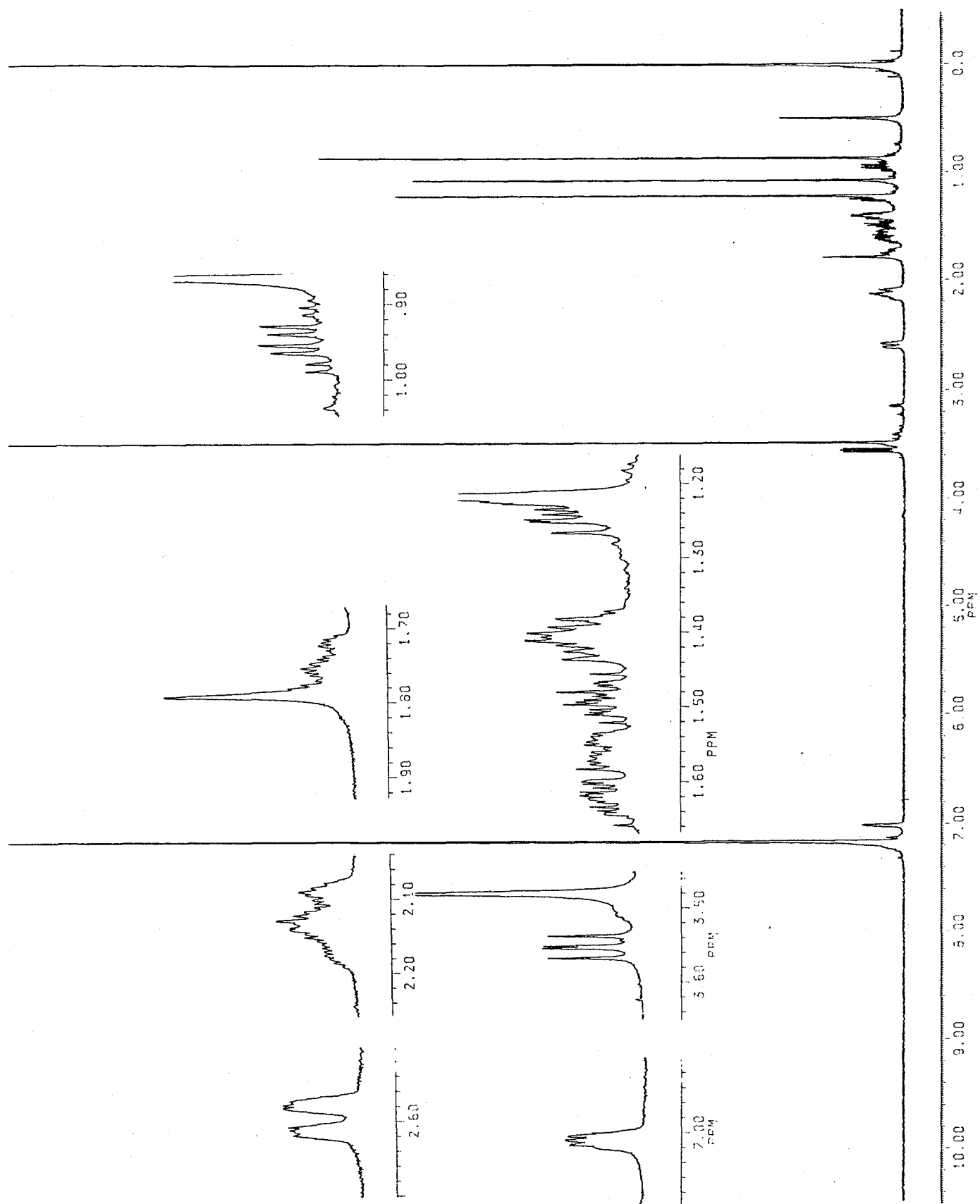


Fig. 3-383 ^1H -NMR Spectrum of RL-118B (500 MHz, in C_6D_6)

Table 3-148 ^1H -NMR chemical shift values of RL-118B

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}		Coupling	Assignment
7.010	1H	br. m	C-2-H
3.552	1H	dd $J= 8.0$ and 6.0 Hz	C-12- H_3
3.479	3H	s	C-7'- H_3
2.588	1H	br. d $J= 17.8$ Hz	C-6-Ha
2.138	1H	t-like m	C-6-Hb
2.106	1H	m-dvid. d* $J= 19.8$ Hz	C-2-Ha
1.788	1H	s (exchangeable)	C-13-OH
1.753	1H	t-like m	C-2-Hb
1.620	1H	m	C-11-Ha
1.553	1H	d-like m	C-5-Ha
1.488	1H	dddd $J= 17.7, 8.7, 6.8$ and 5.4 Hz	C-11-Hb
1.408	1H	ddd $J= 12.2, 9.7$ and 5.4 Hz	C-9-Ha
1.40 (approx.)		m	C-4-H
1.237	1H	ddd $J= 12.2, 8.6$ and 7.2 Hz	C-9-Hb
1.216	3H	s	C-14- H_3
1.070	3H	s	C-15- H_3
0.945	1H	dddd $J= 12.4, 12.4, 12.3$ and 5.1 Hz	C-5-Hb
0.863	3H	s	C-10- H_3

* multiply divided doublet

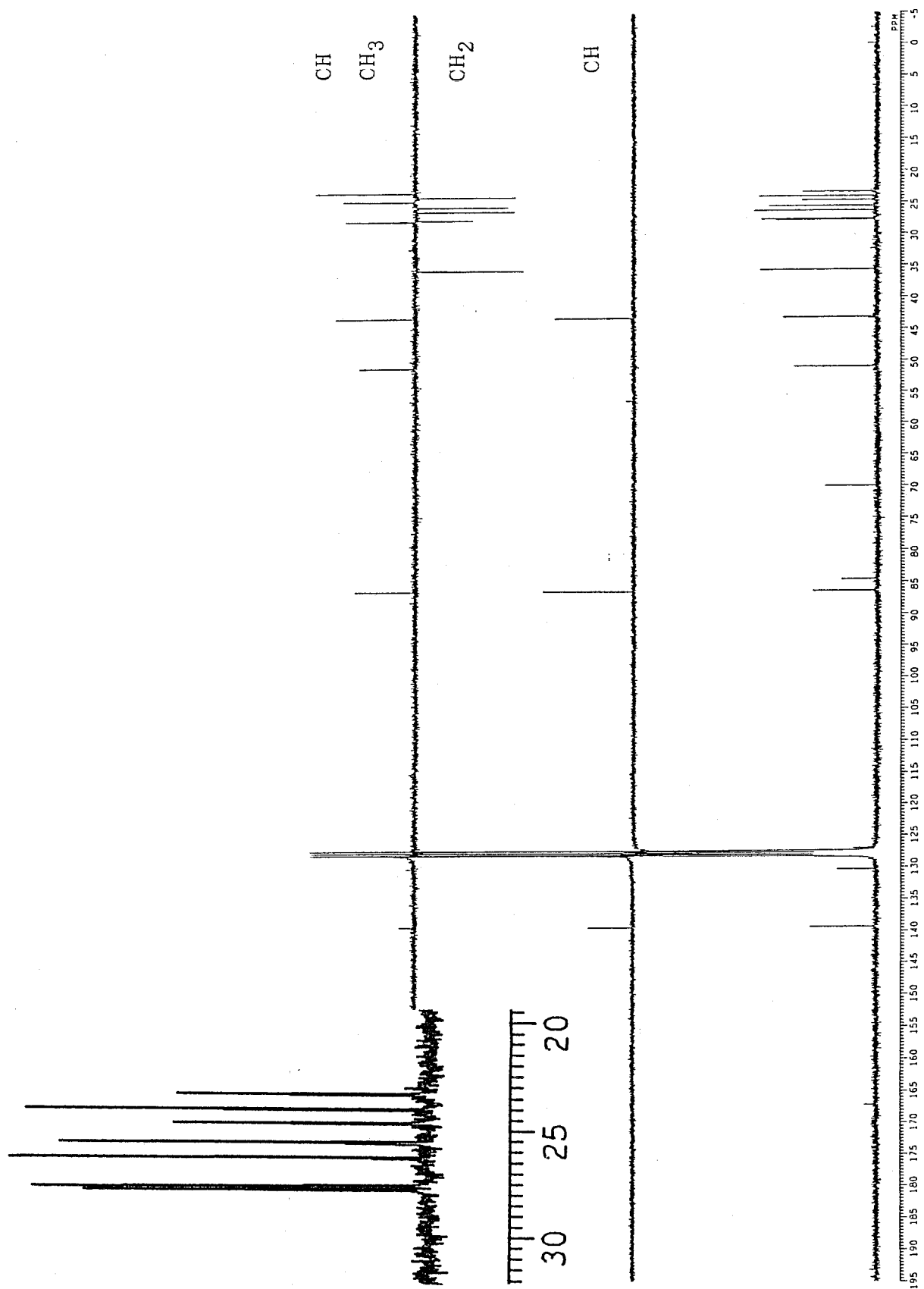


Fig. 3-384 ^{13}C -NMR Spectra of RL-117B (68 MHz, in C_6D_6 , COM and DEPT)

Table 3-149 ^{13}C -NMR chemical shift values of RL-117B

(68 MHz, in CDCl_3 , TMS as an int. std., COM and DEPT)

δ_{C}	Property	Assignment
167.3	-COO-	C-7
139.5	=CH-	C-2
130.4	=C-	C-1
86.4	-CH-O	C-12
84.6	-C-O	C-8 or C-13
70.1	-C-O	C-8 or C-13
51.1	-O-CH ₃	C-7'
43.3	CH	C-4
35.8	CH ₂	C-9
27.9	CH ₃	C-10
27.7	CH ₂	C-11 or C-3
26.4	CH ₂	C-11 or C-3
25.7	CH ₂	C-6
24.8	CH ₃	C-14
24.1	CH ₂	C-5
23.4	CH ₃	C-15

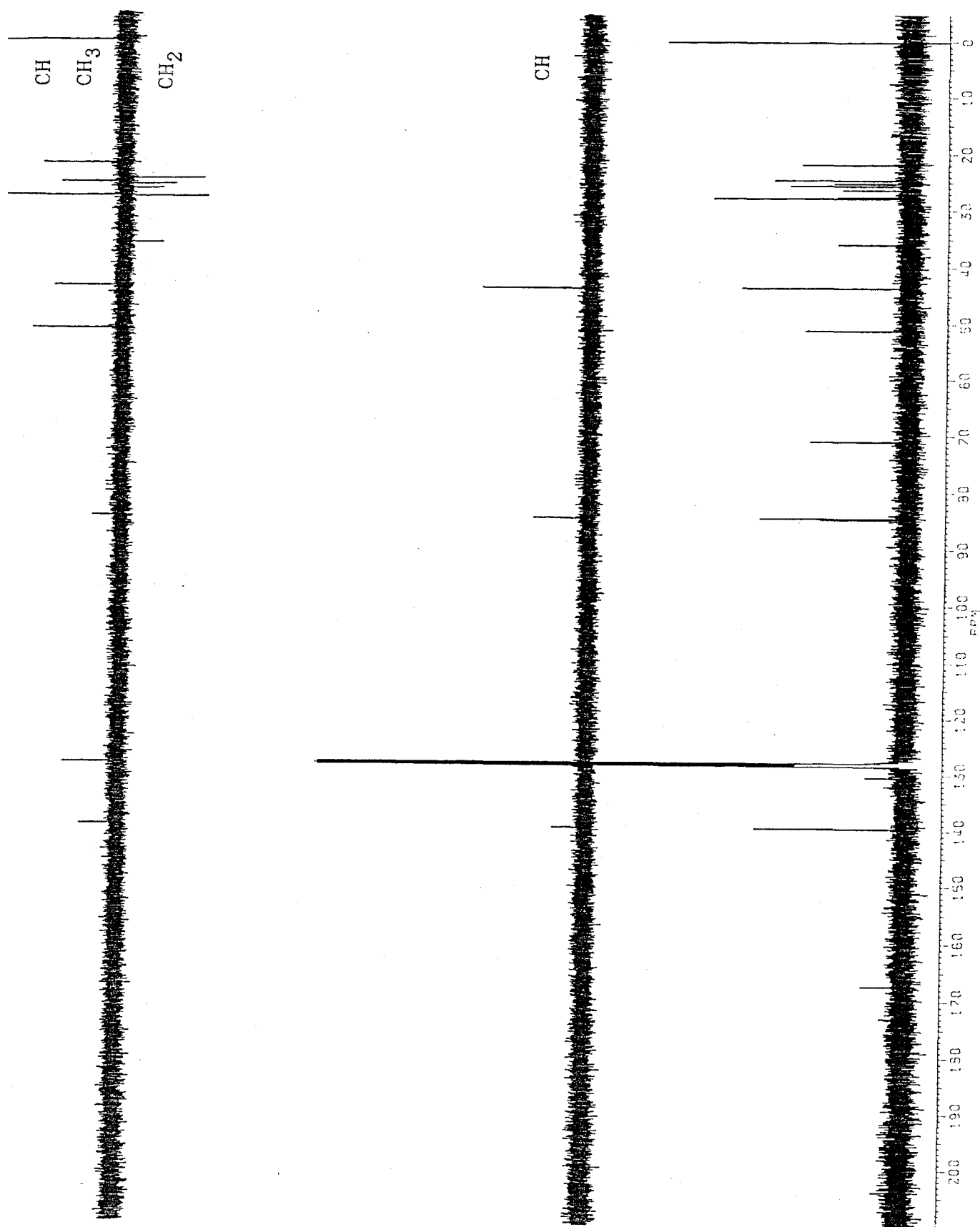


Fig. 3-385 ^{13}C -NMR Spectra of RL-118B (125 MHz, in C_6D_6 , COM and DEPT)

Table 3-150 ^{13}C -NMR chemical shift values of RL-118B

(68 MHz, in CDCl_3 , TMS as int. std.)

δ_{C}	Property	Assignment
167.3	-COO-	C-7
139.5	=CH-	C-2
130.4	=C-	C-1
84.7	-CH-O	C-12
84.5	-C-O	C-8
70.9	-C-O	C-13
51.1	-O-CH ₃	C-7'
43.5	CH	C-4
35.8	CH ₂	C-9
27.7	CH ₂	C-11
27.5	CH ₃	C-10
26.2	CH ₂	C-3
25.5	CH ₂	C-6
25.1	CH ₃	C-14
24.5	CH ₂	C-5
21.7	CH ₃	C-15

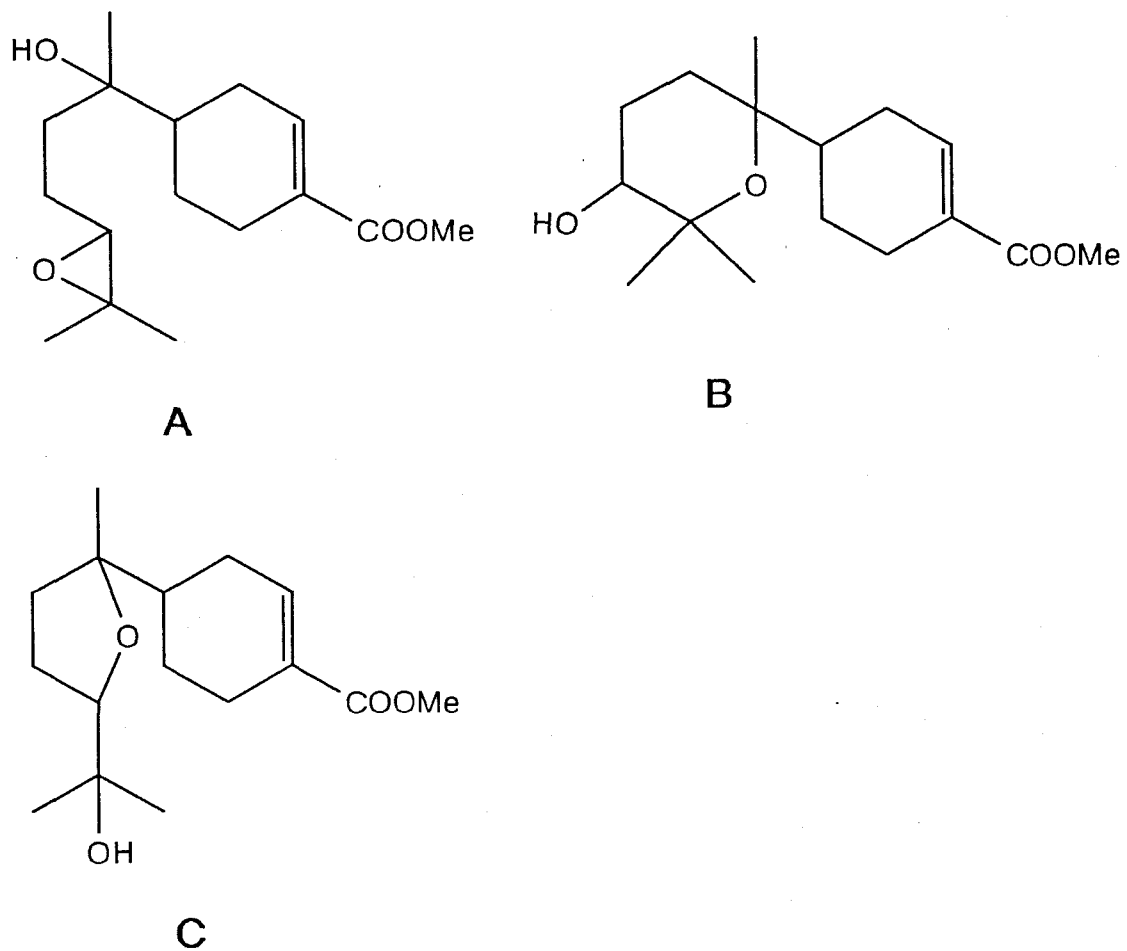


Fig. 3-386 Three Possible Monooxygenated Structures for RL-117B and RL-118B

2) Epoxidation of Bisaborosaol A

m-CPBA was used to obtain a monooxygenated product of bisaborosaol A (19). In 2 ml of ice-cold CHCl_3 , 11.5 mg of 19 and 10.0 mg of *m*-CPBA were dissolved, and the mixture was stirred for 1 hr, at which point, 25 ml of EtOAc was added to the reaction mixture. Then the mixture was washed with 5 % aq. Na_2CO_3 solution (25 ml x 2). From the organic layer, two major products 116-CPBA-1 and 116-CPBA-2 which were respectively agreeable with RL-117B and RL-118B on TL plates (*R_f* values and responses to vanillin- H_2SO_4 reagent) were obtained by PTLC (116-CPBA-1: 5.2 mg in 43 %, and 116-CPBA-2: 4.5 mg in 37 % yield, respectively) (Fig. 3-387). Successively, each product was confirmed to be identical to the authentic compound obtained as a naturally occurring sesquiterpene, in EI-MS, ^1H - and ^{13}C -NMR and the optical property (Fig. 3-388, 389, 390, 391, 392 and 393).

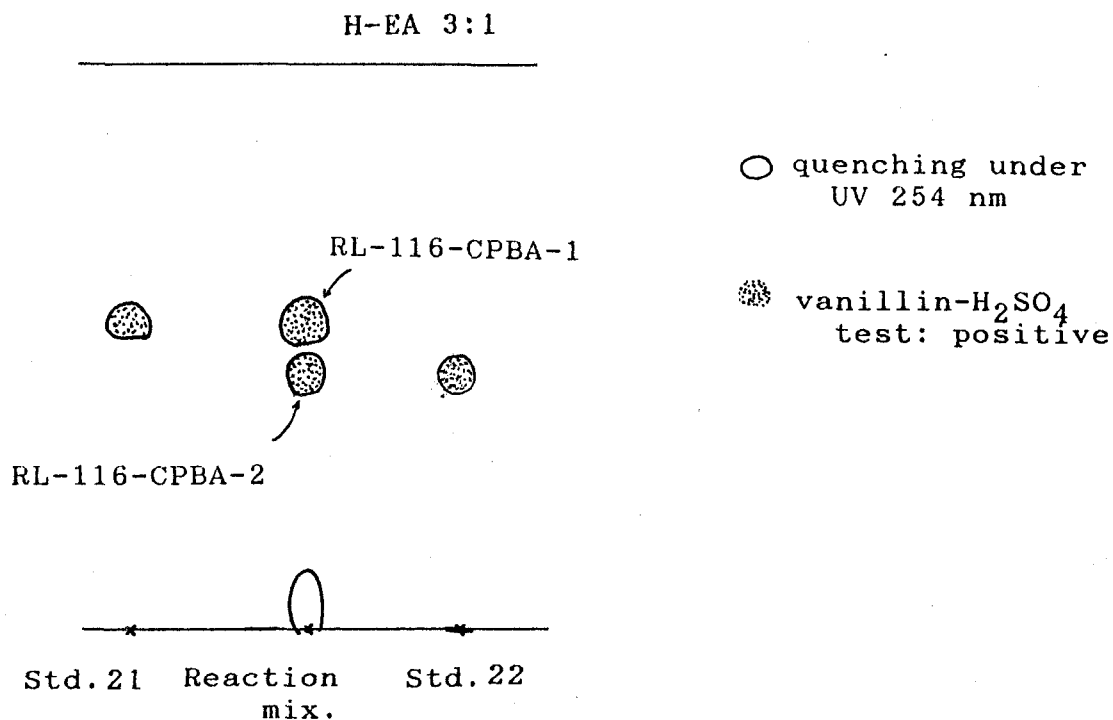


Fig. 3-387 TL Chromatogram of Reaction Product Obtained by *m*-CPBA Oxidation of Bisaborosaol A (19)

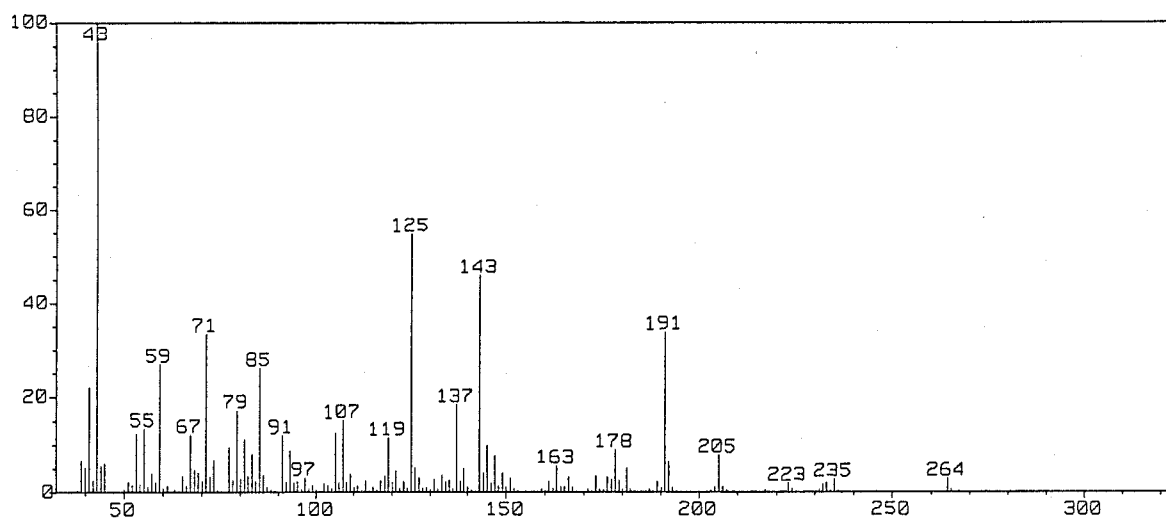


Fig. 3-388 EI-Mass Spectrum of RL-116-CPBA-1

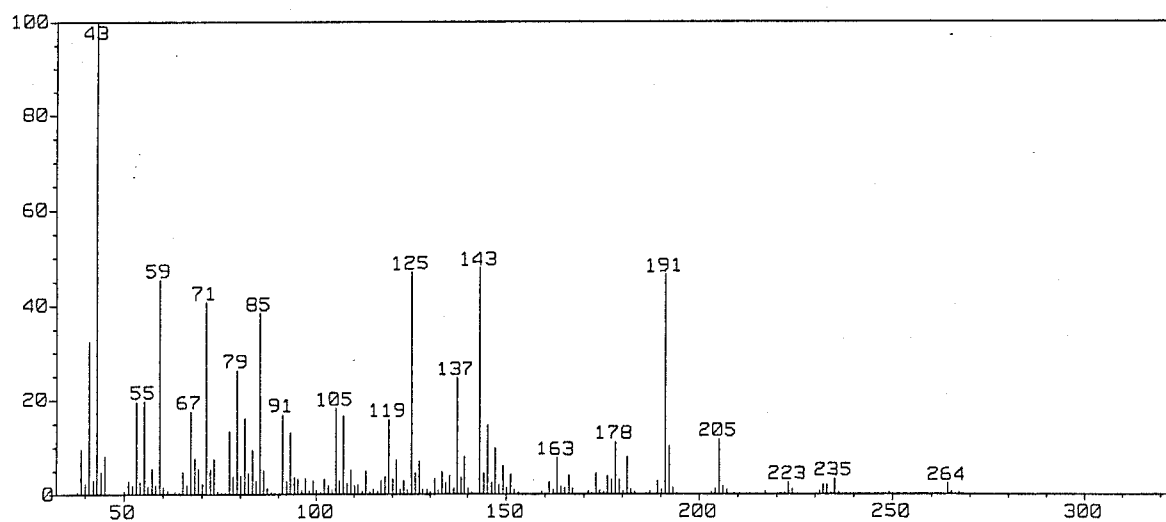


Fig. 3-389 EI-Mass Spectrum of RL-116-CPBA-2

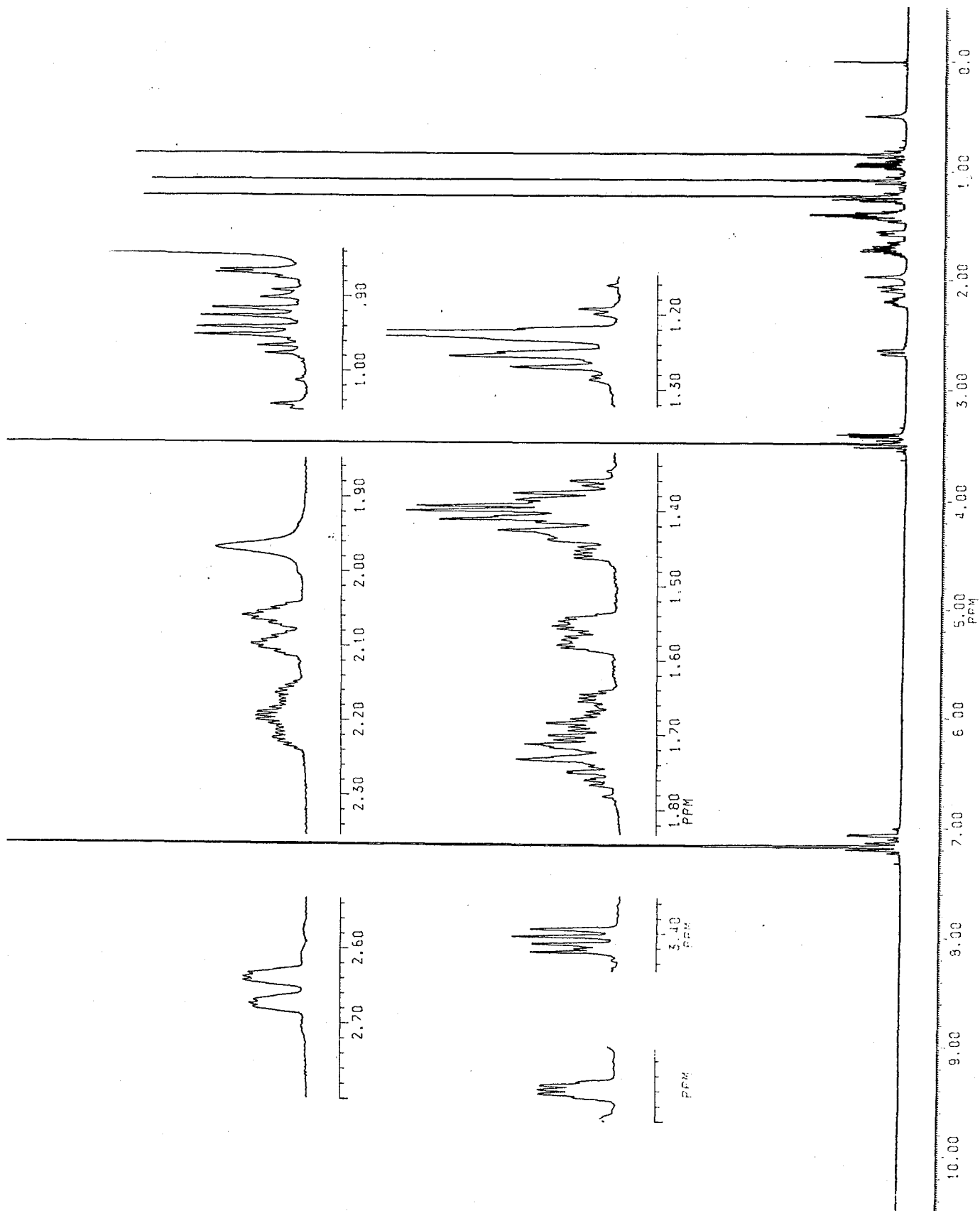


Fig. 3-390 ^1H -NMR Spectrum of RL-116-CPBA-1 (500 MHz, in C_6D_6)

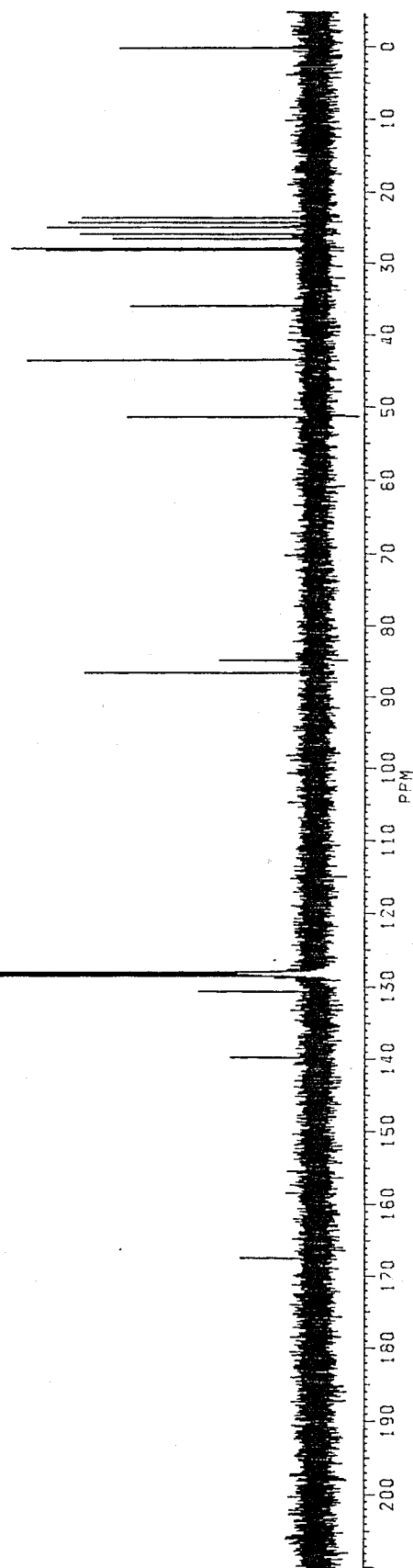


Fig. 3-391. ^{13}C -NMR Spectra of RL-116-CPBA-1 (125 MHz, in C_6D_6 , COM and DEPT)

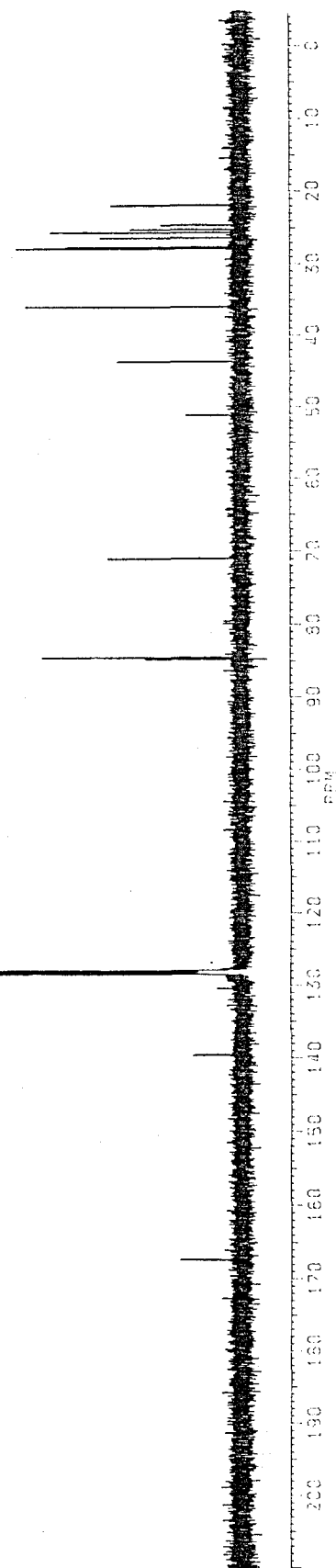


Fig. 3-393 ^{13}C -NMR Spectra of RL-116-CPBA-2 (125 MHz, in C_6D_6 , COM and DEPT)

RL-117B and RL-118B were therefore considered to be cyclization products derived from the epoxy intermediate. Since *m*-CPBA was presumed to attack the olefinic bond in the sidechain non-stereoselectively to give the epoxy intermediate, these compounds produced *via* diastereo-epoxides were regarded as the diastereoisomeric at the newly formed C-12 chiral center (Scheme 3-41). Usually, 1-hydroxy-3-monoene derivatives, for example, linalool (68) or α -bisabolol (94), give tetrahydrofuran derivatives by epoxidation with *m*-CPBA in CHCl_3 , through cyclization between the epoxy group and the γ -hydroxyl group as the result of intramolecular alcoholysis [151]. The tetrahydrofuran type structure for RL-117B or RL-118B was thus quite reasonable. The tetrahydrofuran ring structure for each compound was further supported by the presence of EI-MS fragments at m/z 223 ($M^+ - 59$) and m/z 59 ($\text{C}_3\text{H}_7\text{O}^+$) as shown in Fig. 3-394). Consequently, the tetrahydrofuran structures were proposed for these compounds.

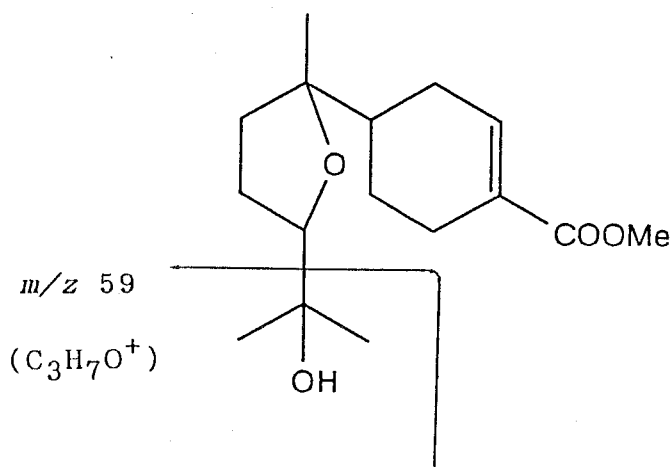
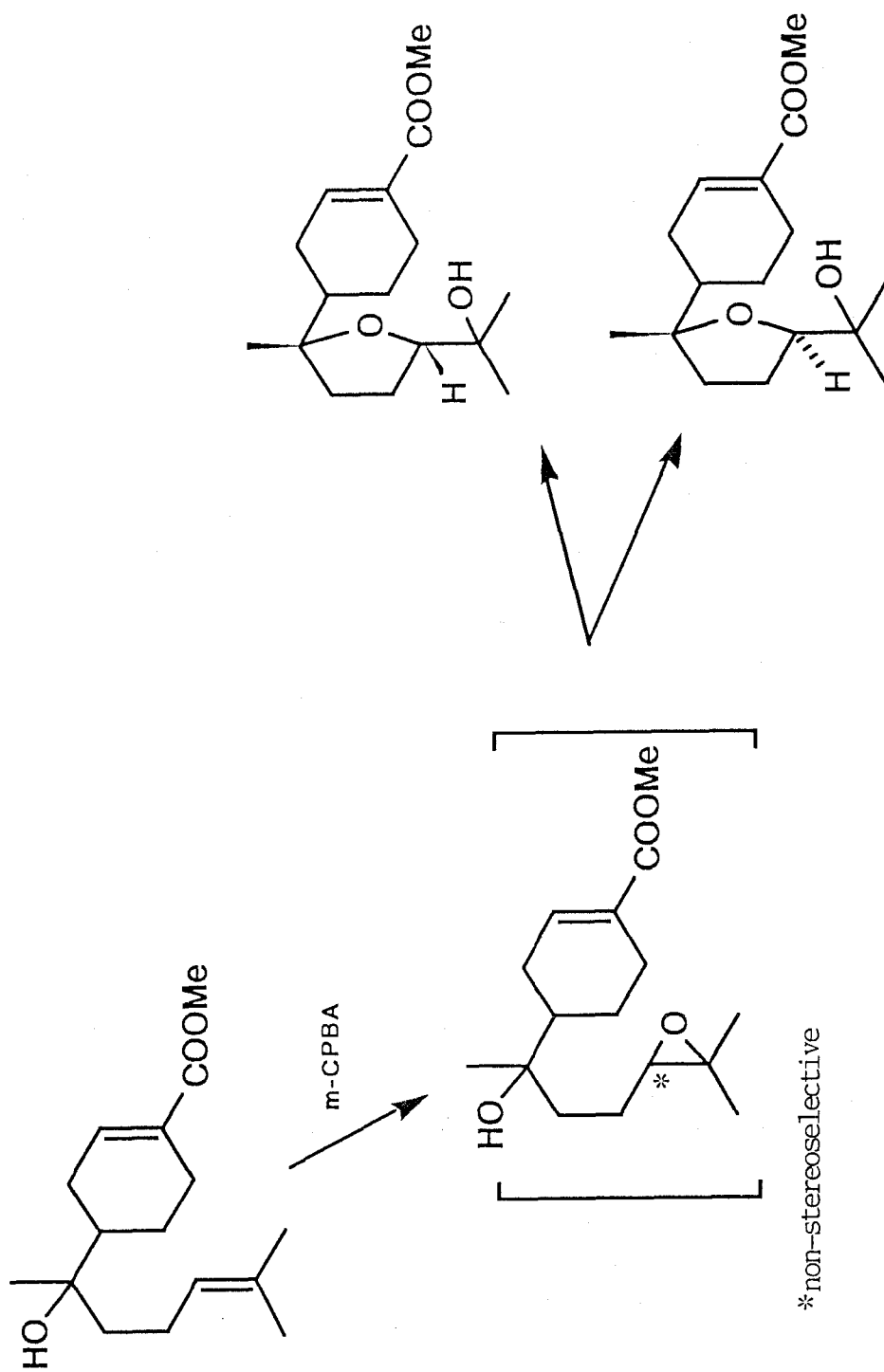
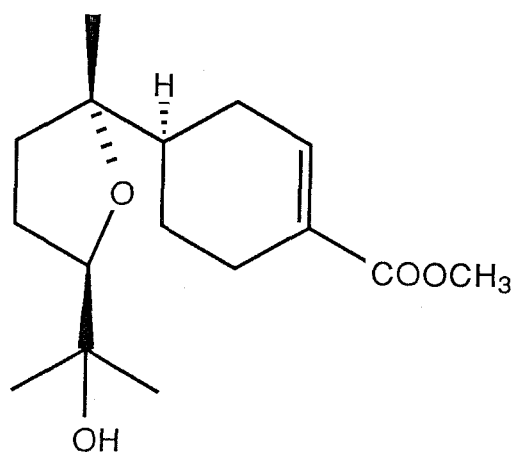


Fig. 3-394 EI-MS Fragmentation of the Tetrahydrofuran Derivative



Scheme 3-44 Epoxidation of Bisaborosaol A with *m*-CPBA

Table 3-151 Physicochemical properties of bisaborosaol B1 (21)



21

A colorless syrup

Vanillin-H₂SO₄ color: yellow

$[\alpha]_D + 63^\circ$ (c 0.19 in MeOH)

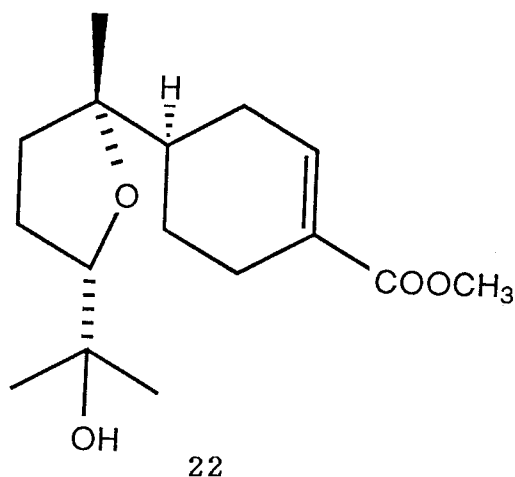
UV $\lambda_{\text{max}}^{\text{MeOH}}$: 218 nm (ϵ 14300)

FI-MS m/z (%): 283 ($M^+ + 1$, 81), 282 (M^+ , 42), 265 (21), 223 (50), 143 (82), 139 (34), 133 (22), 112 (18), 59 (100).

EI-MS m/z (%): 267 (1.2), 264 ($M^+ - \text{H}_2\text{O}$, 1.5), 251 (0.9), 235 ($M^+ - \text{H}_2\text{O} - \text{OCH}_3$, 5.4), 224 (3.7), 223 ($M^+ - \text{COOCH}_3$, 4.5), 205 ($M^+ - \text{H}_2\text{O} - \text{COOCH}_3$, 14), 192 (14), 191 (61), 181 (11), 173 (7.2), 163 (10), 145 (19), 143 (89), 137 (13), 125 (50), 119 (12), 107 (23), 105 (20), 93 (15), 91 (16), 85 (43), 83 (15), 81 (21), 79 (25), 77 (11), 71 (43), 69 (18), 67 (18), 59 (48), 57 (11), 55 (25), 53 (18), 43 (100), 41 (37).

¹H- and ¹³C-NMR data are shown in Tables 3-147 and 3-149, respectively.

Table 3-152 Physicochemical properties of bisaborosaol B2 (18)



A colorless syrup

Vanillin-H₂SO₄ color: yellow

$[\alpha]_D + 68^\circ$ (*c* 0.2 in MeOH)

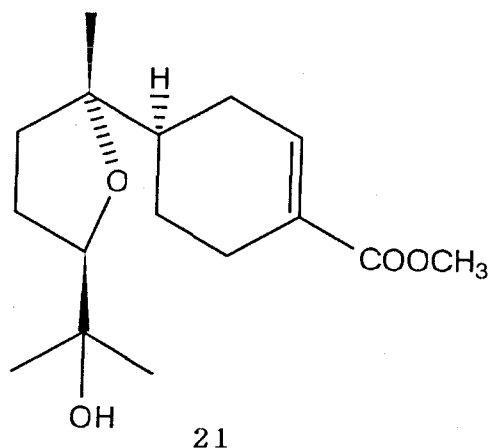
UV $\lambda_{\max}^{\text{MeOH}}$: 218 nm (ϵ 12200)

FI-MS *m/z* (%): 283 ($M^+ + 1$, 66), 282 (M^+ , 23), 265 (11), 223 (59), 143 (100), 139 (56), 59 (69).

EI-MS *m/z* (%): 267 (1.8), 264 ($M^+ - \text{H}_2\text{O}$, 2.1), 251 (1.4), 235 ($M^+ - \text{H}_2\text{O} - \text{OCH}_3$, 9.4), 233 (3.9), 224 (8.0), 223 ($M^+ - \text{COOCH}_3$, 8.9), 205 ($M^+ - \text{H}_2\text{O} - \text{COOCH}_3$, 23), 192 (28), 191 (95), 181 (21), 147 (20), 145 (24), 143 (100), 125 (39), 107 (19), 105 (20), 93 (16), 91 (15), 85 (42), 81 (18), 79 (23), 71 (28), 59 (45), 55 (18), 53 (16), 43 (58), 41 (27).

¹H- and ¹³C-NMR data are shown in Tables 3-148 and 3-150, respectively.

(= bisaborosaol B1, 21)



A colorless syrup

Vanillin-H₂SO₄ color: yellow[α]_D: + 69 ° (c 0.2 in MeOH)

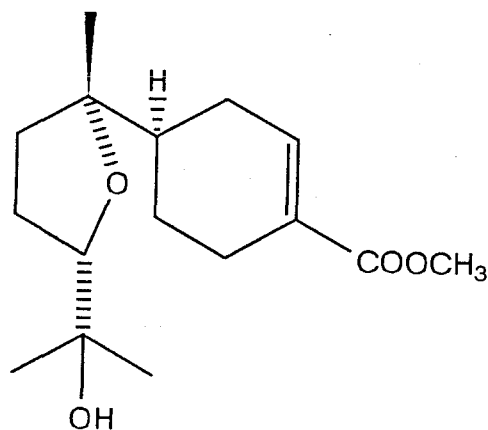
EI-MS m/z (%): 264 ($M^+ - H_2O$, 3.0), 235 ($M^+ - H_2O - OCH_3$, 2.8), 233 (2.1), 223 ($M^+ - COOCH_3$, 2.0), 205 ($M^+ - H_2O - COOCH_3$, 8.0), 192 (6.6), 191 (34), 181 (5.2), 178 (9.1), 163 (5.7), 145 (10), 143 (46), 137 (19), 125 (55), 119 (12), 107 (15), 105 (13), 91 (12), 85 (26), 81 (11), 79 (17), 71 (34), 67 (12), 59 (26), 55 (13), 53 (12), 43 (100), 41 (22).

¹H-NMR $\delta_{TMS}^{C_6D_6}$ (500 MHz): 7.058 (1H, br. s, C-2-H), 2.078 (1H, multiply divided d, J = 19.1 Hz, C-3-Ha), 1.685 (1H, m, C-3-Hb), 1.429 (1H, dddd, J = 12.2, 11.8, 4.9 and 2.5 Hz, C-4-H), 1.561 (1H, d-like m, J = 12.4 Hz, C-5-Ha), 0.933 (1H, dddd, J = 12.4, 12.4, 12.4 and 5.2 Hz, C-5-Hb), 2.655 (1H, br. d, J = 17.7 Hz, C-6-Ha), 2.193 (1H, t-like m, C-6-Hb), 1.406 (1H, dd, J = 9.3 and 7.5 Hz, C-9-Ha), 1.252 (1H, dd, J = 9.3 and 7.2 Hz, C-9-Hb), 0.836 (3H, s, C-10-H₃), ca 1.73 (1H, m, C-11-Ha), 1.378 (1H, ddd, J = 8.6, 7.7 and 3.3 Hz, C-11-Hb), 3.408 (1H, dd, J = 10.4 and 5.1 Hz, C-12-H), 1.223 (3H, s, C-14-H₃), 1.075 (3H, s, C-15-H₃), 1.966 (1H, br. s, C-13-OH), 3.483 (3H, s, C-7'-H₃).

¹³C-NMR $\delta_{TMS}^{C_6D_6}$ (125 MHz): 130.4 (C-1), 139.6 (C-2), 26.4 (C-3), 43.3 (C-4), 24.1 (C-5), 25.7 (C-6), 167.4 (C-7), 84.7 (C-8), 35.8 (C-9), 27.9 (C-10), 27.7 (C-11), 86.4 (C-12), 70.2 (C-13), 24.8 (C-14), 23.4 (C-15), 51.1 (C-7').

Table 3-154 Physicochemical properties of 116-CPBA-2

(= bisaborosaol B2, 22)



22

A colorless syrup

Vanillin-H₂SO₄ color: yellow $[\alpha]_D$: + 65 ° (c 0.2 in MeOH)

EI-MS m/z (%); 264 ($M^+ - H_2O$, 2.4), 235 ($M^+ - H_2O - OCH_3$, 3.4), 233 (2.2), 223 ($M^+ - COOCH_3$, 2.6), 205 ($M^+ - H_2O - COOCH_3$, 12), 192 (10), 191 (47), 181 (8.1), 178 (11), 163 (7.9), 145 (15), 143 (48), 137 (25), 125 (47), 119 (16), 107 (17), 105 (18), 93 (13), 91 (17), 85 (39), 81 (16), 79 (26), 77 (13), 71 (41), 67 (18), 59 (46), 55 (20), 53 (20), 43 (100), 41 (32).

¹H-NMR $\delta_{TMS}^{C_6D_6}$ (500 MHz): 7.010 (1H, br. s, C-2-H), 2.108 (1H, multiply divided d, $J = 19.0$ Hz, C-3-Ha), 1.755 (1H, m, C-3-Hb), ca 1.41 (1H, m, C-4-H), 1.557 (1H, d-like m, C-5-Ha), 0.947 (1H, dddd, $J = 12.4, 12.4, 12.3$ and 5.2 Hz, C-5-Hb), 2.595 (1H, br. d, $J = 17.8$ Hz, C-6-Ha), 2.141 (1H, t-like m, C-6-Hb), ca 1.42 (1H, m, C-9-Ha), 1.238 (1H, ddd, $J = 12.2, 8.5$ and 6.5 Hz, C-9-Hb), 0.864 (3H, s, C-10-H₃), 1.622 (1H, dddd, $J = 12.3, 9.7, 8.1$ and 7.6 Hz, C-11-Ha), 1.489 (1H, dddd, $J = 12.3, 8.6, 6.8$ and 5.4 Hz, C-11-Hb), 3.553 (1H, dd, $J = 8.1$ and 6.8 Hz, C-12-H), 1.217 (3H, s, C-14-H₃), 1.071 (3H, s, C-15-H₃), 1.780 (1H, br. s, C-13-OH), 3.480 (3H, s, C-7'-H₃).

¹³C-NMR $\delta_{TMS}^{C_6D_6}$ (125 MHz): 130.4 (C-1), 139.5 (C-2), 26.2 (C-3), 43.5 (C-4), 24.5 (C-5), 25.5 (C-6), 167.3 (C-7), 84.7 (C-8), 35.8 (C-9), 27.5 (C-10), 27.7 (C-11), 84.5 (C-12), 70.9 (C-13), 25.1 (C-14), 21.8 (C-15), 51.1 (C-7').

3) Stereochemical Analysis and Further Note

While the planar structures of RL-117B and RL-118B were thus proposed, the relative and absolute stereostructure on the tetrahydrofuran ring was elucidated by NOE experiments applied to these two compounds. When the C-9 methyl proton was each irradiated, NOE on C-12 methine proton was observed only in RL-118B. On the other hand, RL-117B showed an NOE between C-9-H₃ and C-13-OH (Fig. 3-395). The relative configuration was, according to the results, elucidated as *trans* in RL-117B (21) and *cis* in RL-118B (22). Since the absolute configuration of bisaborosaol A (19) has already been determined to be 4-*R*, 8-*S*, as mentioned above, the absolute configurations of those derivatives were also solved as *R* in 21 and *S* in 22, respectively. As both compounds were novel bisabolanoids, they were named bisaborosaol B1 and bisaborosaol B2.

Unlike 21 found as one of major constituents in MeOH extracts of *Rosa rugosa* leaves, 22 was detected only as a minor compound on TLC in the MeOH extract. Furthermore, the amount of 19 diffused into water was greatly reduced when the leaves were injured or treated with CuCl₂. These facts allowed a speculation that 19 is convertible to 22 by an oxygenase-like enzyme activated during the leaves being damaged physically or chemically.

Currently, Jaensch and his colleagues isolated a bisabolanoid possessing the same planar structure as 19 from *Podolepis rugata* [153]. From the source, the monooxygenated tetrahydrofuran derivative is also found, as well as *Rosa rugosa*. The latter compound was considered to be the *trans* form because the chemical shift value of C-12-H was resonated to δ_{H} 3.68, higher than C-7'-OCH₃ (δ_{H} 3.83) [153]. Furthermore, this compound showed laevorotatry ($[\alpha]_{\text{D}} - 29^\circ$, *c* 1 in CHCl₃), indicating that the C-4 chiral center of those isolates was *R* (structure 95 and 108), contrary to 19 and 21. Thus, the bisabolanoids of *Rosa rugosa* were confirmed to be stereochemically different group from those C-7 oxygenated

bisabolanoids originated in Compositae plants (Fig. 3-396) [144,145,153,154].

When (-)- α -bisabolol (94) was metabolized by a fungus *Aspergillus niger*, only the *cis* type of tetrahydrofuran derivative (110) can be obtained as the initial metabolite [137]. Although this report may be contradictory to the fact that only *trans* form (21) was detectable in the methanol extracts of fresh *Rosa rugosa* leaves and 108 in the *Podolepis rugata* was the *trans* form, this fungal metabolism can be a model of the selective epoxidation and successive non-biochemical cyclization.

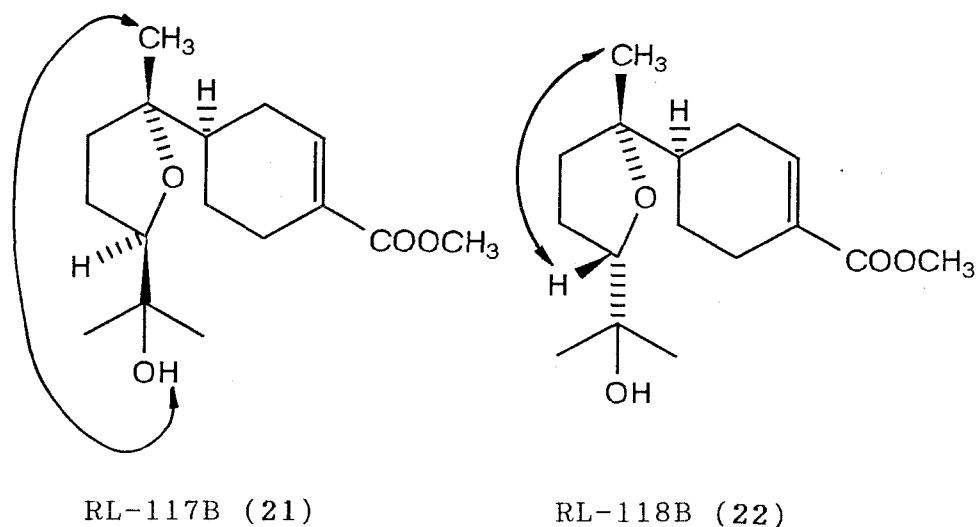
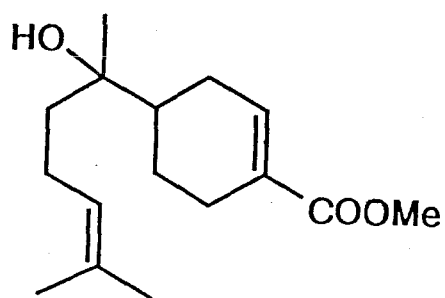
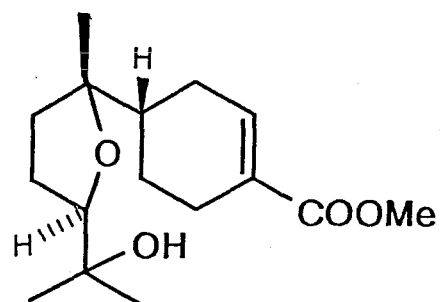


Fig. 3-395 NOEs Observed in the Two Compounds, and the
Establisd Stereostructure

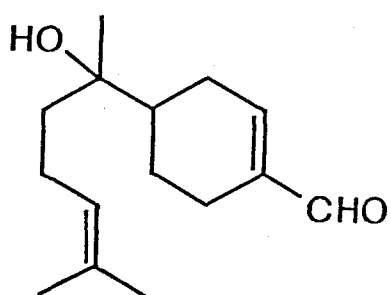


95

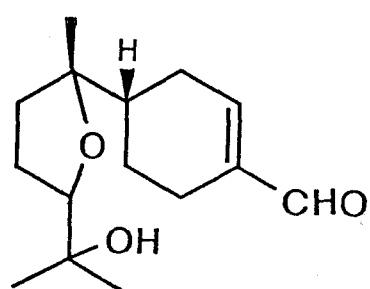


108

Podolepis rugata [153]



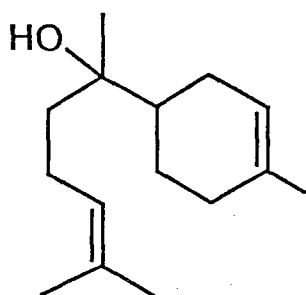
100



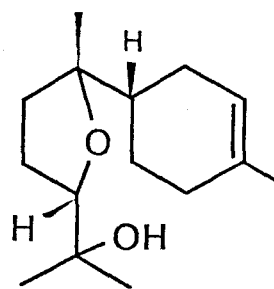
109

Pleiotaxis rugosa

[154]



94



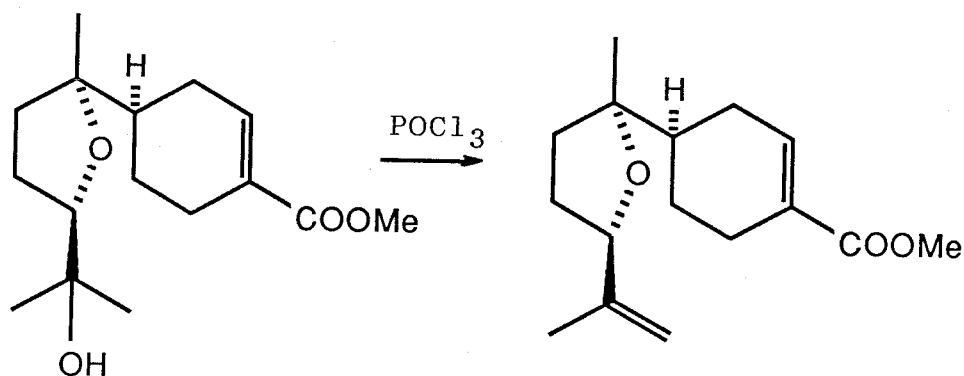
110

(-)-α-bisabolol (94) metabolite by *Aspergillus niger*

Fig. 3-396 Some Tetrahydrofuran Derivatives of Bisabolanoids and Their Precursors Found as Naturally Occurring Bisabolanoids or Fungal Metabolites

4) Dehydration

To obtain a chemical proof for the presence of tetrahydrofuran ring in bisaborosaol B1 and B2 (21 and 22), dehydration of 21 was carried out [155]. By the chemical conversion, the dehydration product containing an exomethylene protons was expected (Scheme 3-45). In 1 ml of pyridine, 12.7 mg of 21 was dissolved, and then to the mixture 60 μ l of POCl_3 was added. The mixture was kept in a freezer for 24 hr and successively left at room temperature for 1 hr. After the reaction, to the mixture was poured 5 ml of ice water, and the resulting solution was extracted with 3 ml of Et_2O . The less polar and quenching product RL-117B- H_2O (21a) was isolated by PTLC in hexane- EtOAc 10:1 to give 3.2 mg of a colorless syrup in a yield of 26 % (recovered 21, 1.0 mg, 8 %) (Fig. 3-397). This product affording M^+ 264 in EI-MS (Fig. 3-398) showed the exomethylene part (proton: δ_{H} 5.169 and 4.836, carbon: δ_{C} 109.9, $=\text{CH}_2$) in the ^1H - and ^{13}C -NMR spectra (Fig. 3-399 and 400). Consequently, structure of RL-117B- H_2O was formulated as 21a, which provided concrete evidence for the tetrahydrofuran structure 21. Since bisaborosaol B2 (22) has given some line of evidence as a diastereomer of 21, the structure of 21 and 22 were finally established.



Scheme 3-45 Dehydration of bisaborosaol B1 (20): Dhehydration product is expected to show a pair of exomethylene protons at C-14 in ^1H -NMR spectrum.

H-EA 10:1

○ quenching under
UV 254 nm

RL-117B-H₂O ○

○
Reaction Std. 21
mix.

Fig. 3-397 TL Chromatogram of Dehydration Product from RL-117B

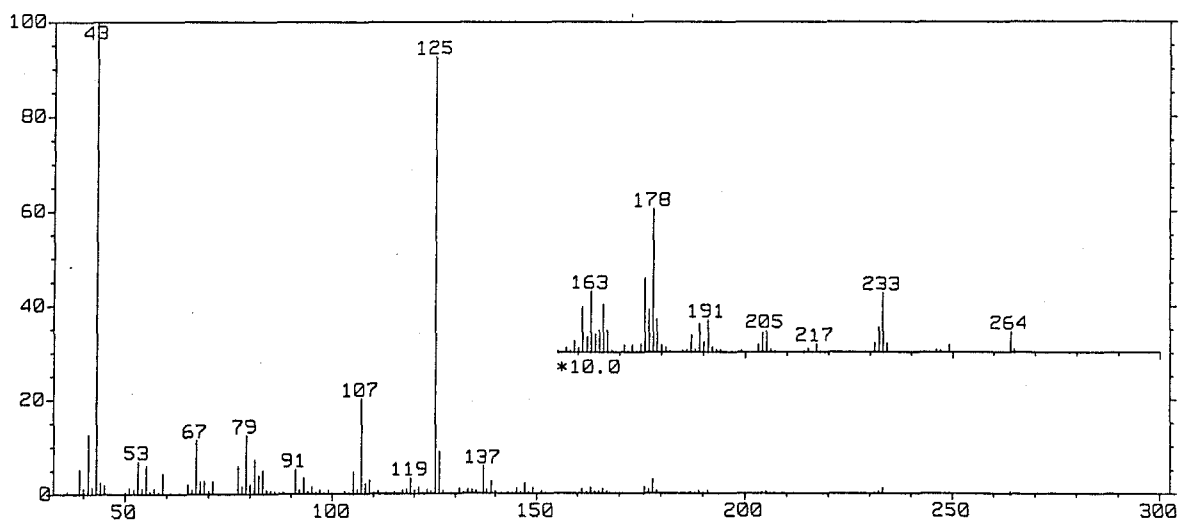


Fig. 3-398 EI-Mass Spectrum of RL-117B-H₂O

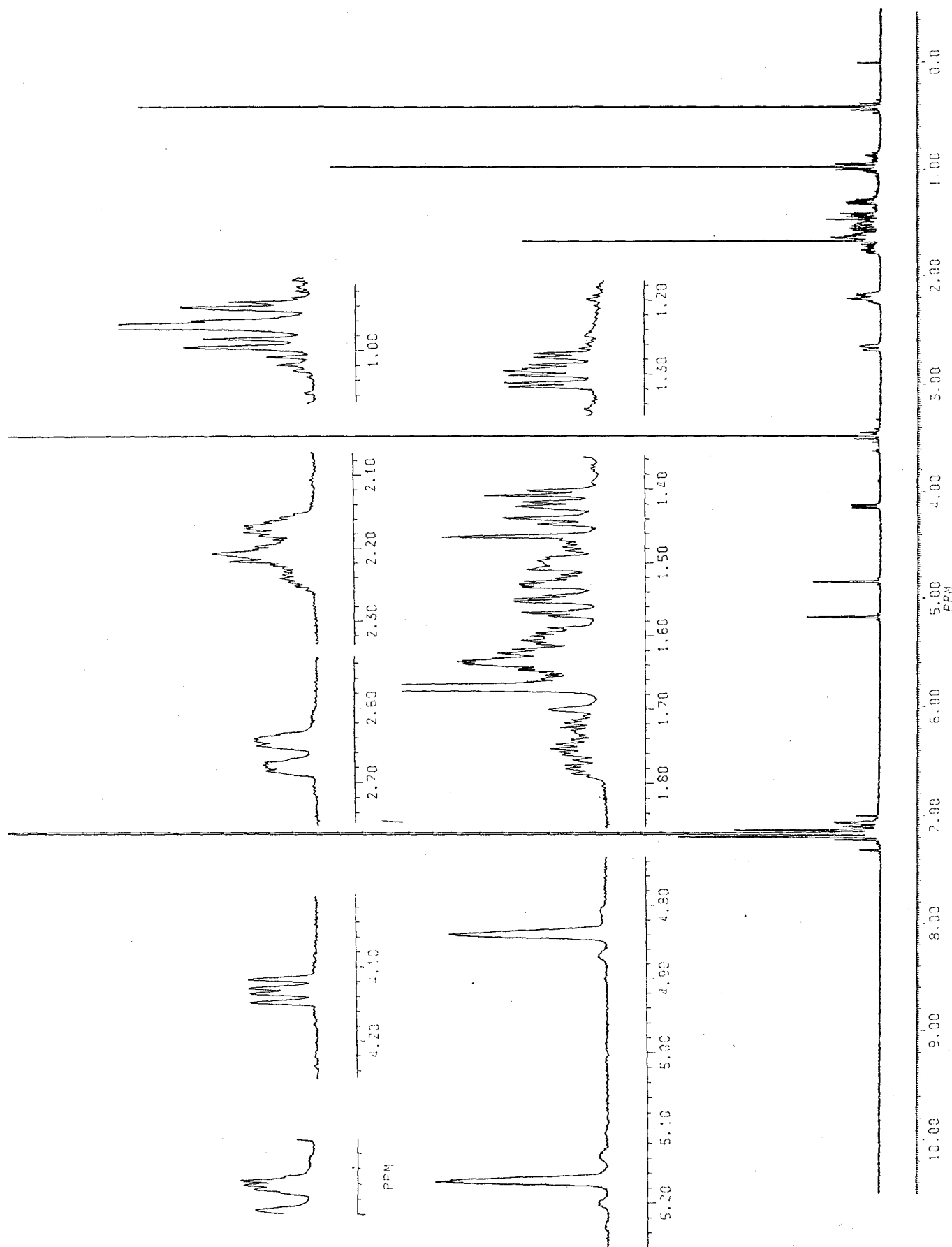


Fig. 3-399 ^1H -NMR Spectrum of RL-117B-H₂O (500 MHz, in C₆D₆)

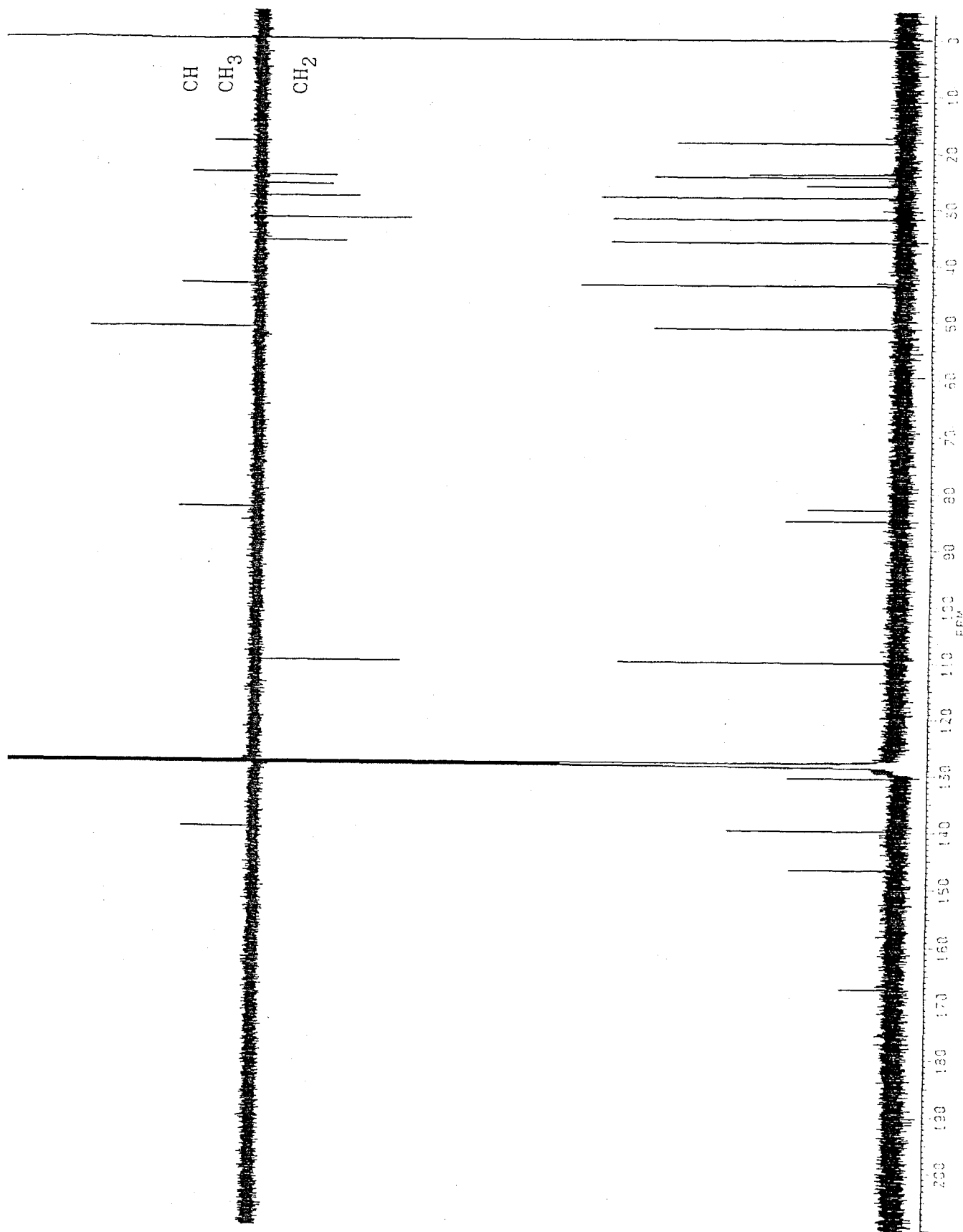
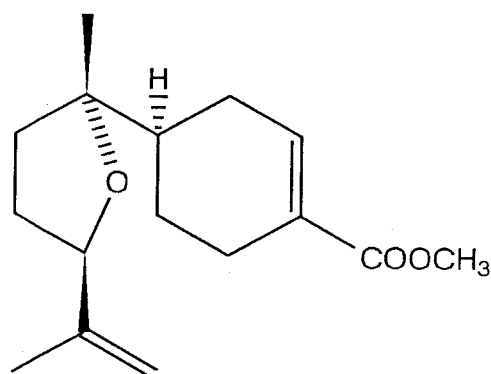


Fig. 3-400 ^{13}C -NMR Spectra of RL-117B-H₂O (125 MHz, in C_6D_6 , COM and DEPT)

Table 3-155 Physicochemical properties of RL-117B-H₂O (21a)



21a

A colorless syrup

R_f: 0.56 (H-EA 10:1)

EI-MS *m/z* (%): 264 (M⁺, 1.2), 249 (0.2), 233 (M⁺-OCH₃, 2.2), 217 (0.2), 207 (0.6), 205 (0.7), 204 (0.7), 178 (3.1), 161 (2.8), 137 (7.7), 126 (10), 125 (100), 107 (28), 91 (11), 81 (11), 79 (23), 77 (13), 67 (15), 55 (10), 53 (13), 43 (90), 41 (22).

¹H-NMR δ_{TMS}^{C₆D₆}: 7.061 (1H, br. s-like m, C-2-H), 2.190 (1H, multiply divided d, *J*= 19.8 Hz, C-3-Ha), 1.749 (1H, t-like m, C-3-Hb), 1.499 (1H, m, C-4-H), 1.614 (1H, m, C-5-Ha), 0.976 (1H, dddd, *J*= 12.3, 12.2, 12.1 and 5.3 Hz, C-5-Hb), 2.660 (1H, br. d, *J*= 17.0 Hz, C-6-Ha), 2.218 (1H, t-like m, C-6-Hb), 1.422 (1H, m, C-9-Ha), 1.294 (1H, ddd, *J*= 12.0, 7.8 and 2.6 Hz, C-9-Hb), 0.964 (3H, s, C-10-H₃), *ca* 1.64 (1H, m, C-11-Ha), 1.538 (1H, m, C-11-Hb), 4.130 (1H, dd, *J*= 9.6 and 5.9 Hz, C-12-H), 5.169 (1H, br. s, C-14-Ha), 4.836 (1H, br. s, C-14-Hb), 1.668 (3H, s, C-15-H₃), 3.471 (3H, s, C-7'-H₃).

¹³C-NMR δ_{TMS}^{C₆D₆}: 130.4 (C, C-1), 139.7 (CH, C-2), 27.9 (CH₂, C-3), 43.4 (CH, C-4), 24.2 (CH₂, C-5), 25.7 (CH₂, C-6), 167.4 (C, C-7), 84.8 (C, C-8), 35.8 (CH₂, C-9), 23.7 (CH₃, C-10), 31.6 (CH₂, C-11), 82.8 (CH, C-12), 146.6 (C, C-13), 109.9 (CH₂, C-14), 18.2 (CH₃, C-15), 51.5 (CH₃, C-7').

3-8-5 Exoperoxy Bisabolanoids

1) Isolation

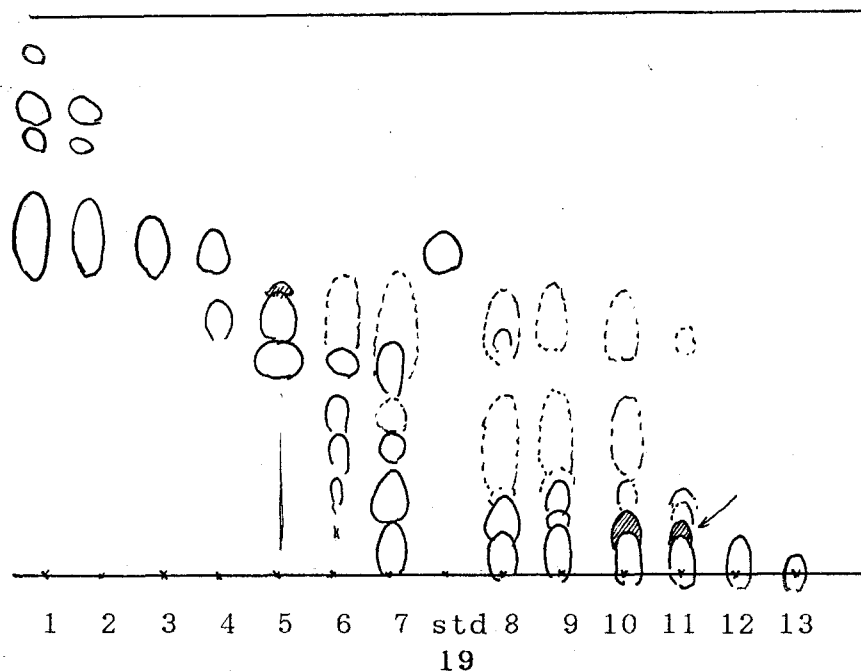
During a search for peroxy constituents in the *Rosa rugosa* leaves, a neutral fraction (Fr-VIIH-12, recovered with EtOAc) obtained as described (pp. 290) was found to contain some compounds clearly positive to the peroxide test. Those substances were more polar than rugosal A (1) and showed properties as a neutral compound. Isolation of the compounds was accordingly carried out with the guidance of the positive response to the peroxide reagent. As the result of successive PTLCs (H-EA 1:1, C-M 50:4 and multiple development PTLC in B-EA 25:10), were isolated three peroxides all showing properties as bisabolane exoperoxides. RL-PERO-5, -6 and -7 [*R_f* 0.25, 0.23 and 0.17 in B-EA 25:10] (Fig. 3-401), all showed the same molecular weight (M^+ 298 in FD-MS), which suggested that those were isomeric peroxides.

2) Structure Elucidation of RL-PERO-5 and -6

RL-PERO-5, obtained as a colorless syrup (2.1 mg) from ca 1/3 of Fr-VI-12 showed *m/z* 298 (M^+ , 45 %) and 265 (M^+ -OOH, 81 %) in FI-MS, and the latter fragment was indicative of -OOH partial structure for the isolate (Fig. 3-402). In EI-MS of the isolate, neither the parent ion nor the fragment due to -OOH fission was detectable (Fig. 3-403). On the other hand, its $^1\text{H-NMR}$ spectrum showed some signals similar to those of bisaborosaol A (19) [e.g. δ_{H} 7.012 (1H, br. s-like m) and 2.587 (1H, br. d), corresponding to C-2 and C-6 protons of 19, respectively)]. This fact indicated that RL-PERO-5 was a relative of 19.

As characteristic proton signals, a pair of exomethylene protons (δ_{H} 4.967 and 4.892), an oxygenated methine proton (δ_{H} 4.219, triplet, $J = 6.6$ Hz) and an exchangeable proton (δ_{H} 7.550, singlet) were recognizable (Fig. 3-404 and Table 3-156).

H-EA 3:1



- quenching under UV 254 nm
- vanillin- H_2SO_4 test: +
- ▨ peroxide test: +

B-EA 25:10

B-EA 5:1 x 3

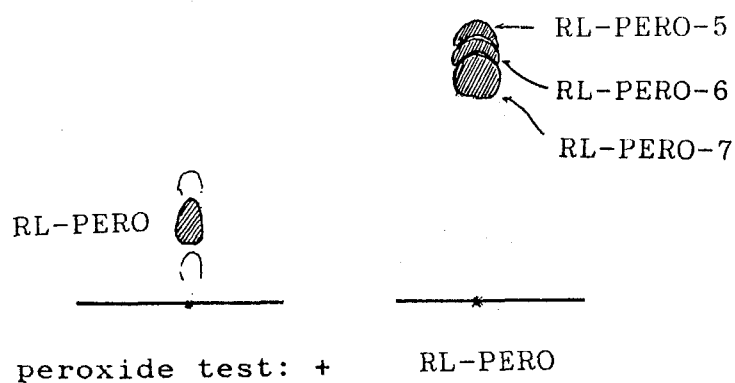


Fig. 3-401 TL Chromatograms of RL-PERO-5 ~ 7

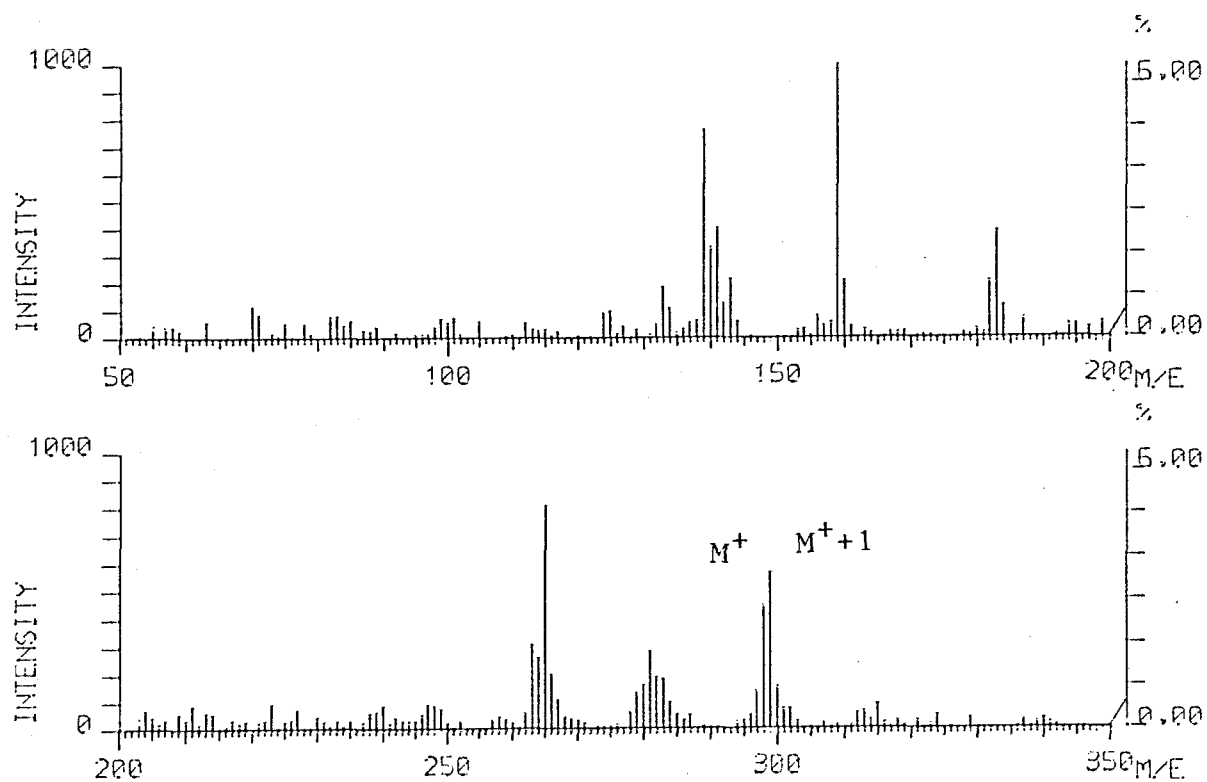


Fig. 3-402 FI-Mass Spectrum of RL-PERO-5

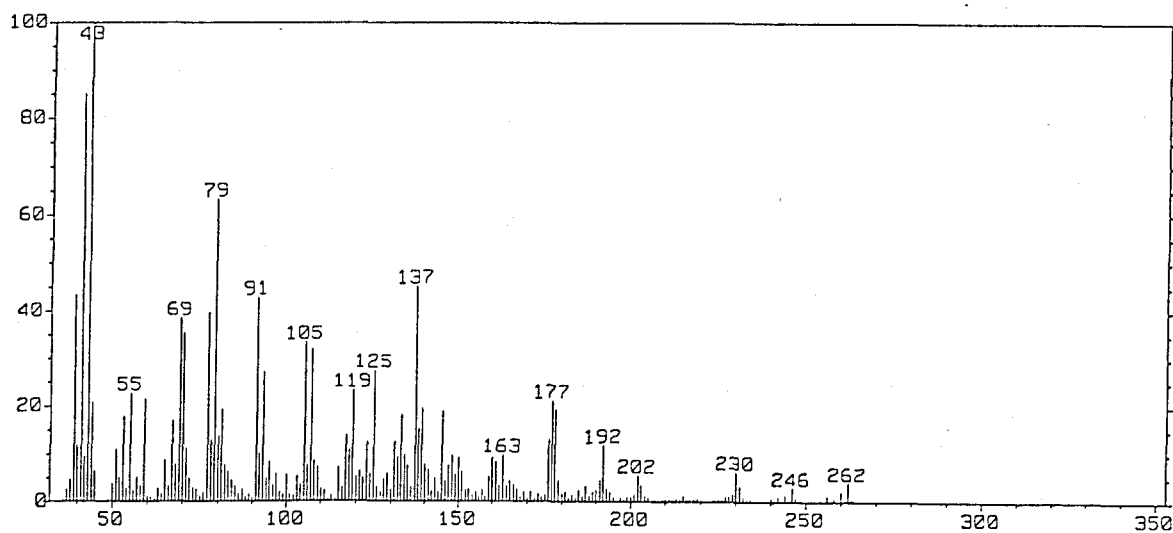


Fig. 3-403 EI-Mass Spectrum of RL-PERO-5

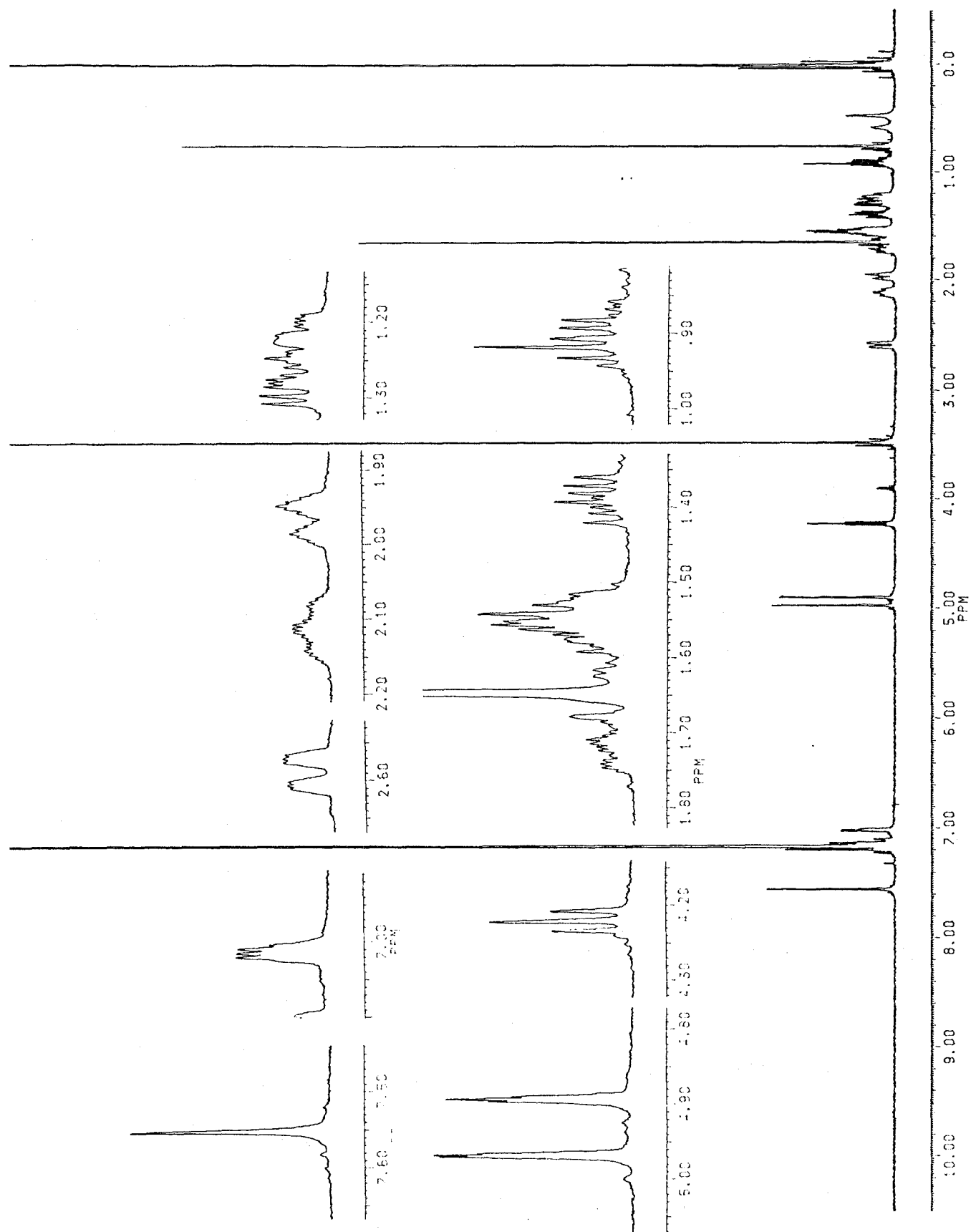


Fig. 3-404 ^1H -NMR Spectrum of RL-PERO-5 (500 MHz, in C_6D_6)

Table 3-156 ^1H -NMR chemical shift values of RL-PERO-5

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}	Coupling		Assignment
7.550	1H	s	C-13-OOH
7.012	1H	br. s-like	C-2-H
4.967	1H	d $J= 0.9$ Hz	C-14-Ha
4.892	1H	dd $J= 1.0$ and 0.9 Hz	C-14-Hb
4.219	1H	t $J= 6.6$ Hz	C-12-H
3.473	3H	s	C-7'-H ₃
2.587	1H	br. d $J= 18.0$ Hz	C-6-Ha
2.112	1H	br. t-like m	C-6-Hb
1.965	1H	m-dvid. d $J= 18.9$ Hz	C-3-Ha
1.711	1H	br. t-like m	C-3-Hb
1.646	3H	s	C-15-H ₃
1.55 (approx.)	2H	m	C-11-H ₂
1.54 (approx.)	1H	m	C-5-Ha
1.390	1H	ddd $J= 13.8, 10.8$ and 6.4 Hz	C-9-Ha
1.278	1H	ddd $J= 13.8, 11.3$ and 5.0 Hz	C-9-Hb
1.221	1H	br. t-like m	C-4-H
0.900	1H	dddd $J= 12.6, 12.4, 12.4$ and 5.2 Hz	C-5-Hb
0.752	3H	s	C-10-H ₃

Disappearance of signals attributable to the 3,3-dimethylallyl moiety of 19 suggested that hydroperoxidation at C-12 of 19 followed by the concerted olefinic bond translocation to C-13/C-14 yielded 23 as a proposed structure for RL-PERO-5. In addition to a coupling sequence on the 1,4-disubstituted cyclohexene moiety, HH-COSY spectrum of the compound (Fig. 3-405) clearly indicated a sequence $\blacksquare$ -C(OOH)H-CH₂-CH₂- $\blacksquare$ attributable to C-9, 11 and 12 (Fig. 3-406, 407). The peroxylation on the sidechain was further supported by ¹H-NMR detection of an allylic methyl group at δ_H 1.646 assignable to the C-15. Furthermore, FI-MS fragment at *m/z* 159 (100 %), which results from fission of the sidechain, was also indicative of the structure (Fig. 3-407).

¹³C-NMR analyses of the compound eventually proved this structure (Fig. 3-408 and Table 3-157). Two oxygenated sp³ carbons at δ_C 89.5 (CH) and 73.1 (C) were assignable to C-12 and C-8, respectively, and the former carbon resonating in a comparatively lower field should be reasonably assigned to a hydroperoxylated carbon as discussed in Section 4 of this chapter (See pp. 283). In addition, carbons of the sidechain part were approximately agreeable in their chemical shift values with those of isoaminobisabolanol (111) found in a sponge, *Theonella* sp. by Kitagawa *et al.* (Table 3-158) [156].

The second peroxide, RL-PERO-6 showed almost the same mass fragmentation patterns with those of 23 in FI- and EI-MS (Fig. 3-409 and 410). However, the proton signals assignable to C-9-H₂, C-11-H₂ and C-12-OOH, were slightly but clearly different from those of 23 (Fig. 3-411, 412 and Table 3-159). As the ¹³C-NMR spectrum was almost indistinguishable from that of 23 (Fig. 3-413 and Table 3-160), RL-PERO-6 was easily characterized to be an epimer of 23 at C-12 (structure 24). The stereostructure of RL-PERO-5 and RL-PERO-6 remained unsolved. However, the absolute configuration at C-12 will be determined, since the benzoate chirality methods is applicable to the compounds of this type after reduction to the diols [35,157].

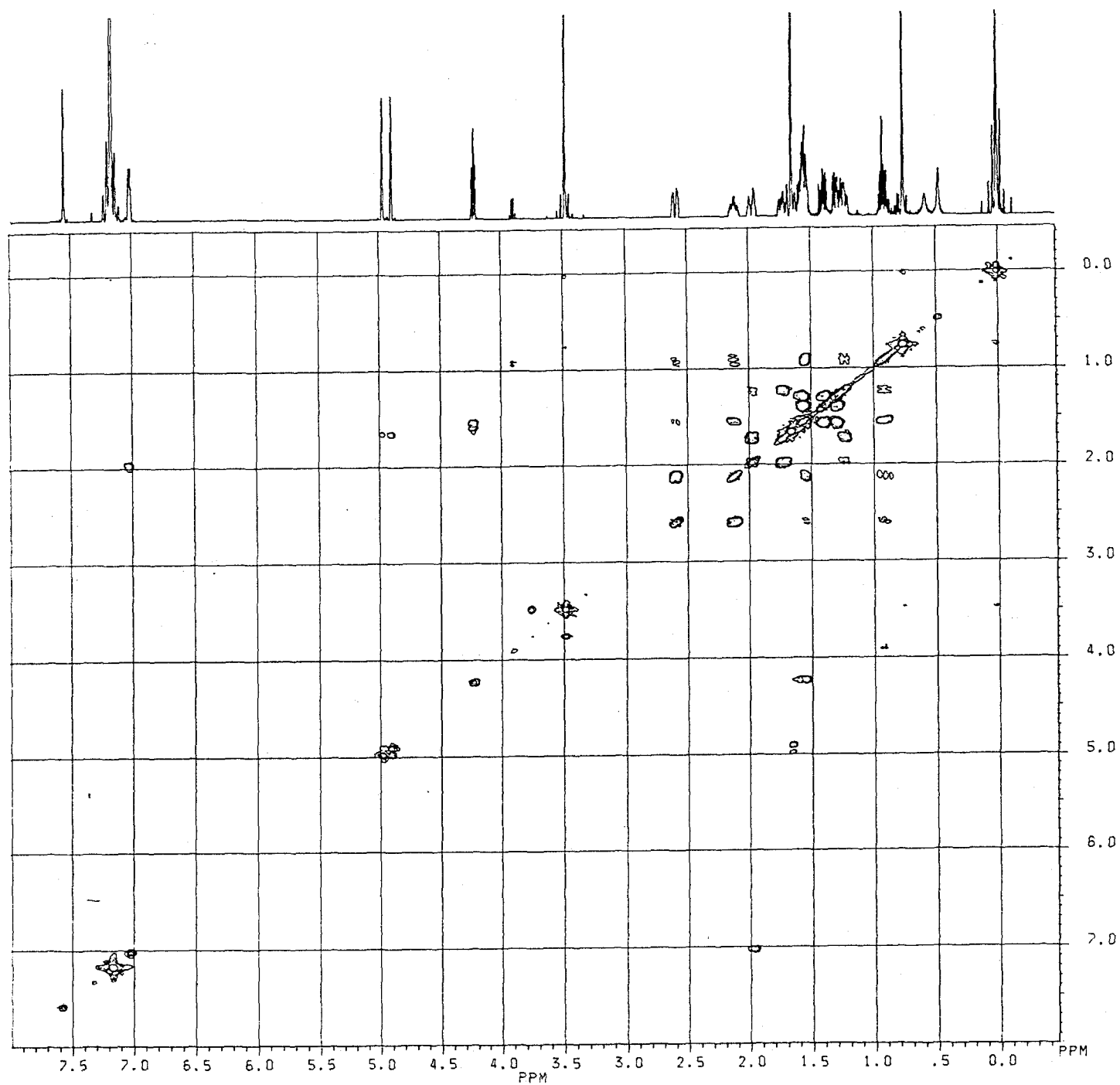


Fig. 3-405 HH-COSY Spectrum of RL-PERO-5 (500 MHz, in C_6D_6)

These two compounds were found to be a novel compound, and were named bisaborosaol C1 and bisaborosaol C2, respectively.

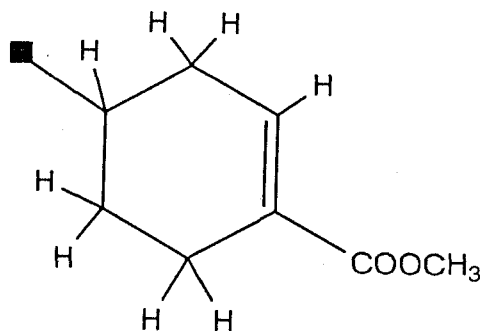


Fig. 3-406 Proton Sequence on 1,4-Disubstituted 1-Cyclohexene Moiety of RL-PERO-5

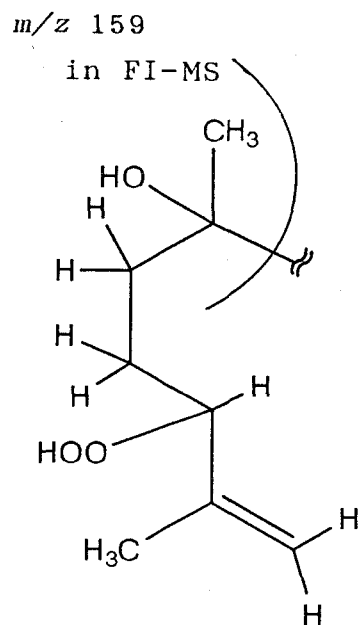


Fig. 3-407 Proton Sequence on the Sidechain Part of RL-PERO-5

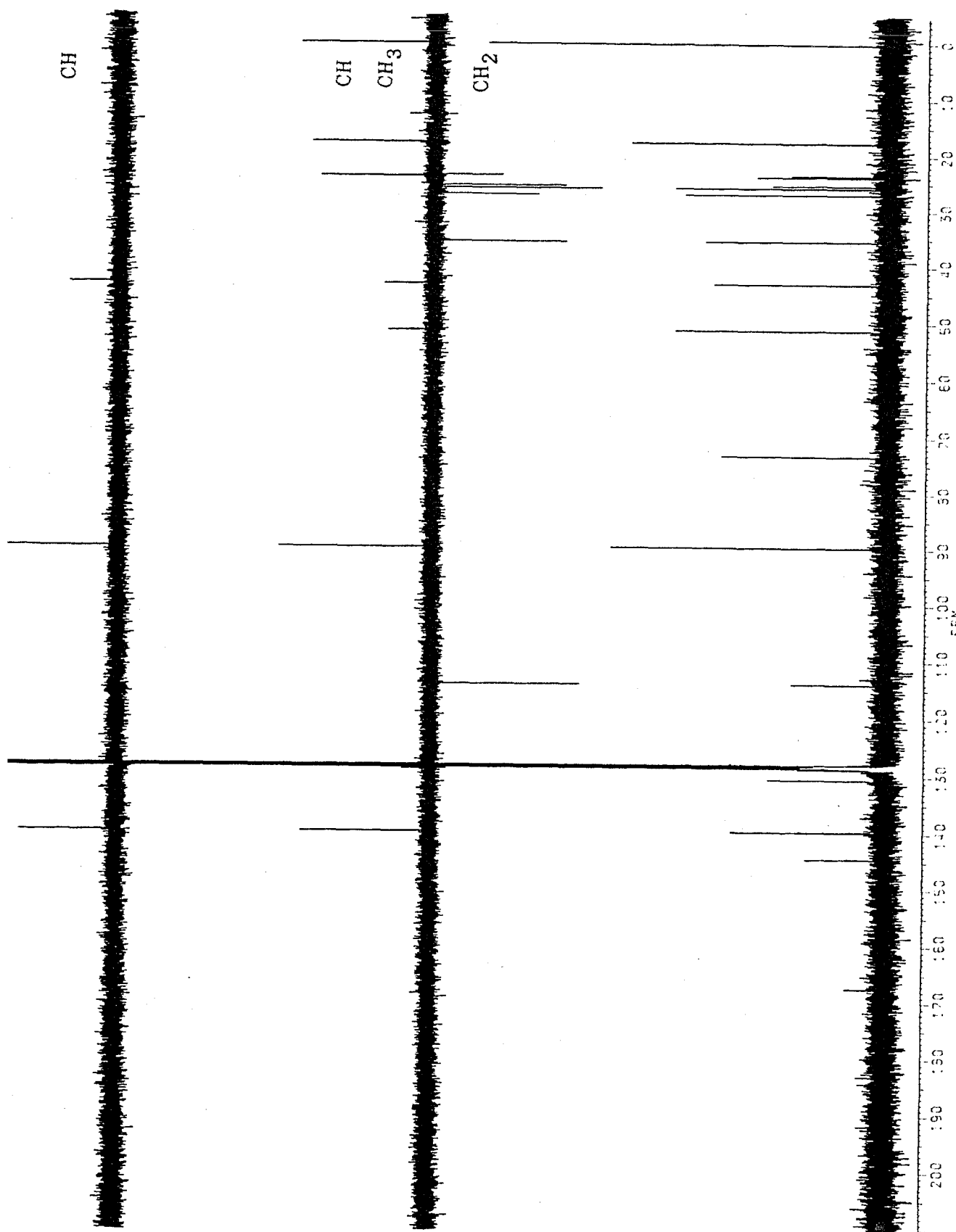


Fig. 3-408 ^{13}H -NMR Spectra of RL-PERO-5 (125 MHz, in C_6D_6 , COM and DEPT)

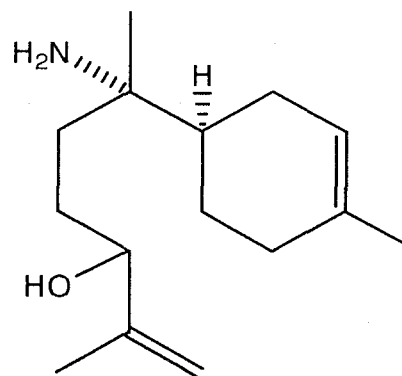
Table 3-157 ^{13}C -NMR chemical shift values of RL-PERO-5

(125 MHz, in CDCl_3 , COM and DEPT, TMS as an int. std.)

δ_{C}	Property	Assignment
167.3	-COO-	C-7
144.3	=C-	C-13
139.5	=CH-	C-2
130.4	=C-	C-1
113.8	=CH ₂	C-14
89.5	-CH-O	C-12
73.1	-C-O	C-8
51.1	CH ₃	C-7'
42.9	CH	C-4
35.3	CH ₂	C-9
26.8	CH ₂	C-3
25.7	CH ₂	C-6
25.2	CH ₂	C-11
23.6	CH ₃	C-10
23.4	CH ₂	C-5
17.6	CH ₃	C-15

Table 3-158 Comparison of RL-PERO-5 carbon chemical shift values with those of isoaminobisabolenol (111) and bisaborosaol A (19)

C-No	RL-PERO-5	111 [156]	19
1	130.4	134.0	132.0
2	139.5	120.0	139.8
3	26.8	26.2	26.8
4	42.9	42.4	42.5
5	23.4	23.7	23.3
6	25.7	30.9	25.2
7	167.3	23.2	167.9
8	73.1	57.3	74.1
9	35.3	29.2	39.5
10	23.6	22.2	23.8
11	25.2	33.7	22.3
12	89.5	75.6	124.3
13	144.3	147.3	129.9
14	113.8	110.9	25.7
15	17.6	18.2	17.7
7'	51.1	—	51.5



isoaminobisabolenol (111)

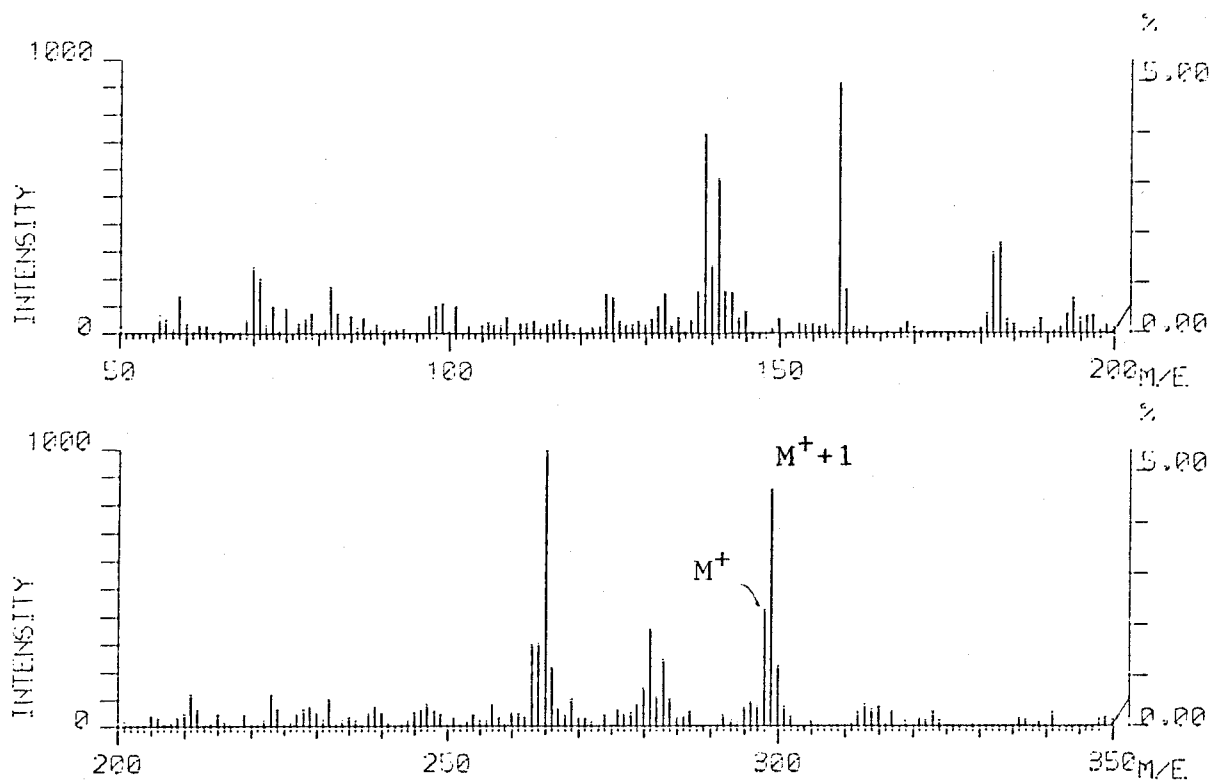


Fig. 3-409 FI-Mass Spectrum of RL-PERO-6

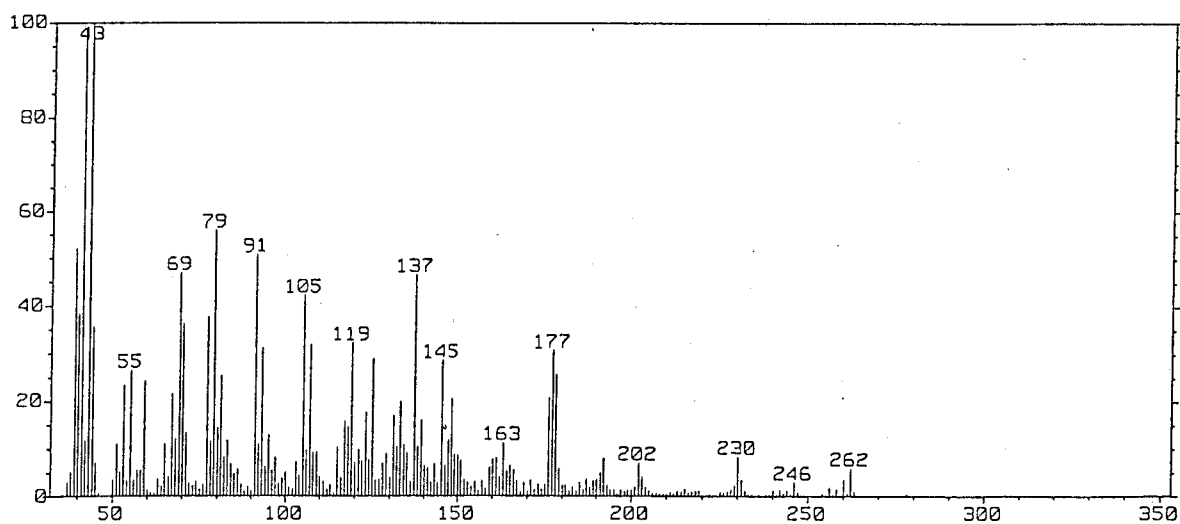


Fig. 3-410 EI-Mass Spectrum of RL-PERO-6

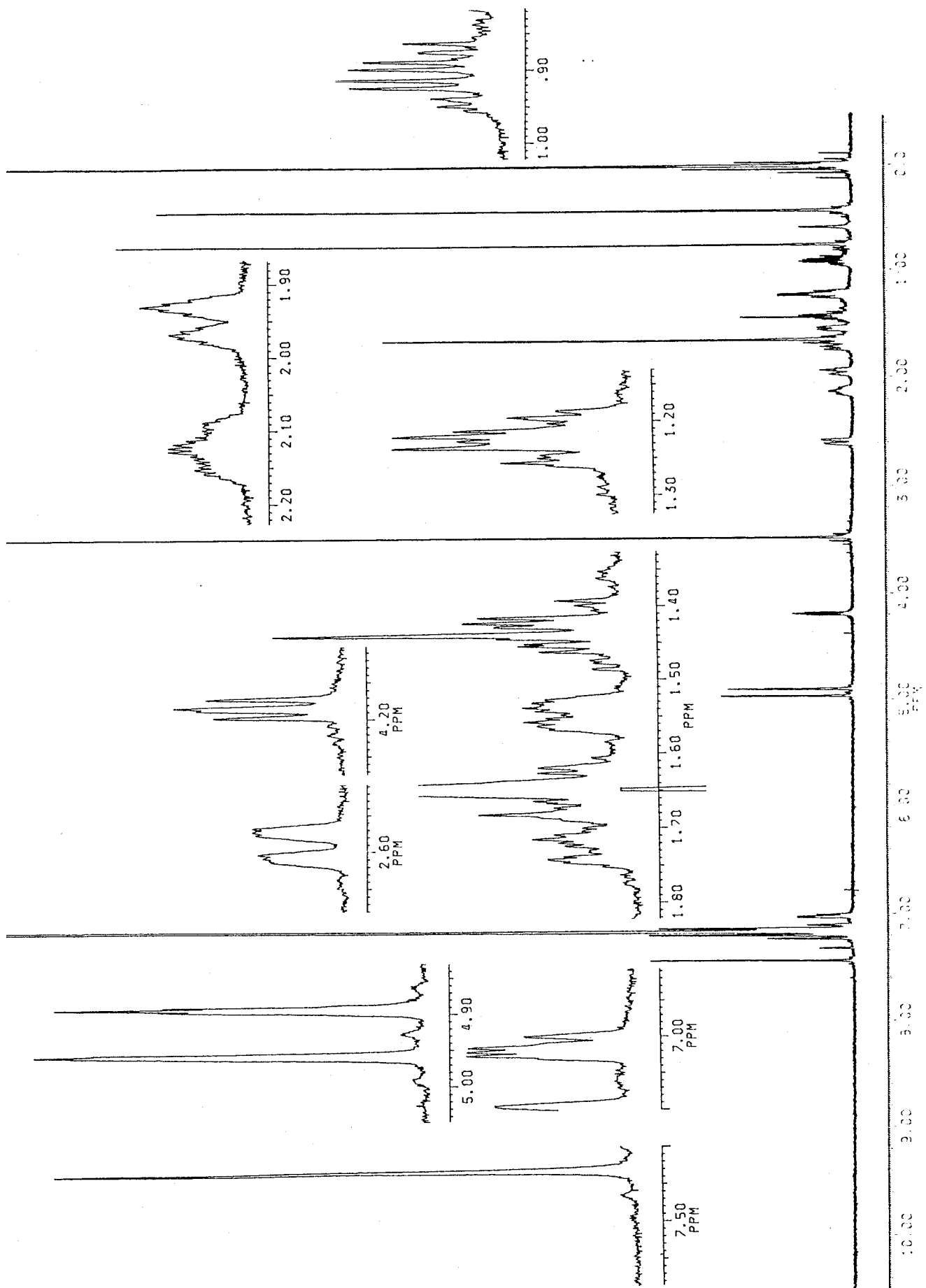


Fig. 3-411 ^1H -NMR Spectrum of RL-PERO-6 (500 MHz, in C_6D_6)

Table 3-159 ^1H -NMR chemical shift values of RL-PERO-6

(500 MHz, in CDCl_3 , TMS as an int. std.)

δ_{H}	Coupling		Assignment
7.432	1H	s	C-13-OOH
7.019	1H	br. s-like	C-2-H
4.958	1H	br. s	C-14-Ha
4.894	1H	dd $J= 1.5$ and 1.5 Hz	C-14-Hb
4.183	1H	dd $J= 6.9$ and 5.6 Hz	C-12-H
3.473	3H	s	C-7'-H ₃
2.590	1H	br. d $J= 17.7$ Hz	C-6-Ha
2.123	1H	br. t-like m	C-6-Hb
1.949	1H	m-dvid. d $J= 19.0$ Hz	C-3-Ha
1.717	1H	br. t-like m	C-3-Hb
1.65 (approx.)	1H	m	C-11-Ha
1.648	3H	s	C-15-H ₃
1.546	1H	m	C-5-Ha
1.46 (approx.)	1H	m	C-11-Hb
1.421	1H	m	C-9-Ha
1.225	1H	dd $J= 12.5$ and 5.0 Hz	C-9-Hb
1.222	1H	br. t-like m	C-4-H
0.906	1H	dddd $J= 12.5, 12.5, 12.4$ and 5.2 Hz	C-5-Hb
0.752	3H	s	C-10-H ₃

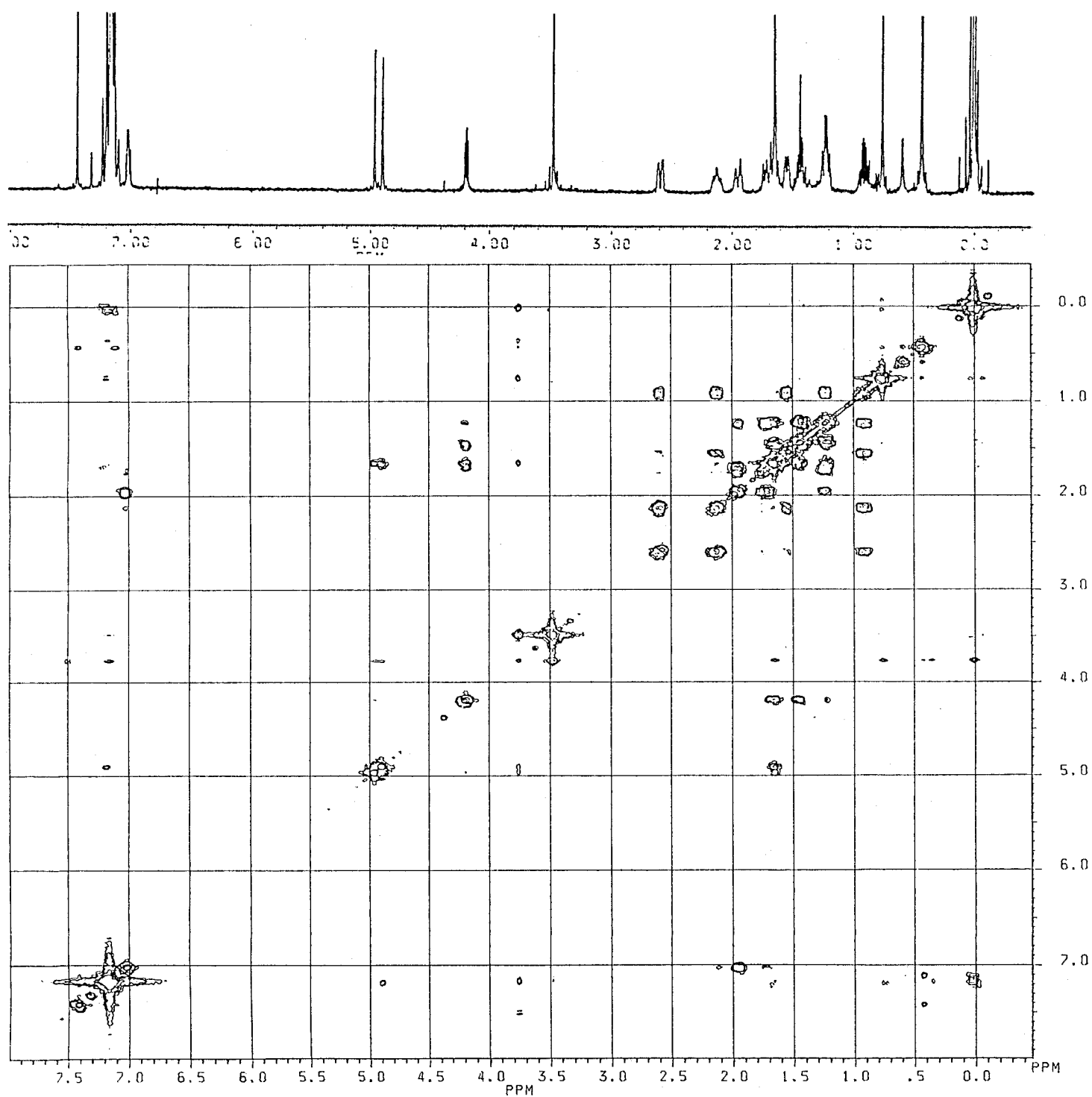


Fig. 3-412 HH-COSY Spectrum of RL-PERO-6 (500 MHz, in C_6D_6)

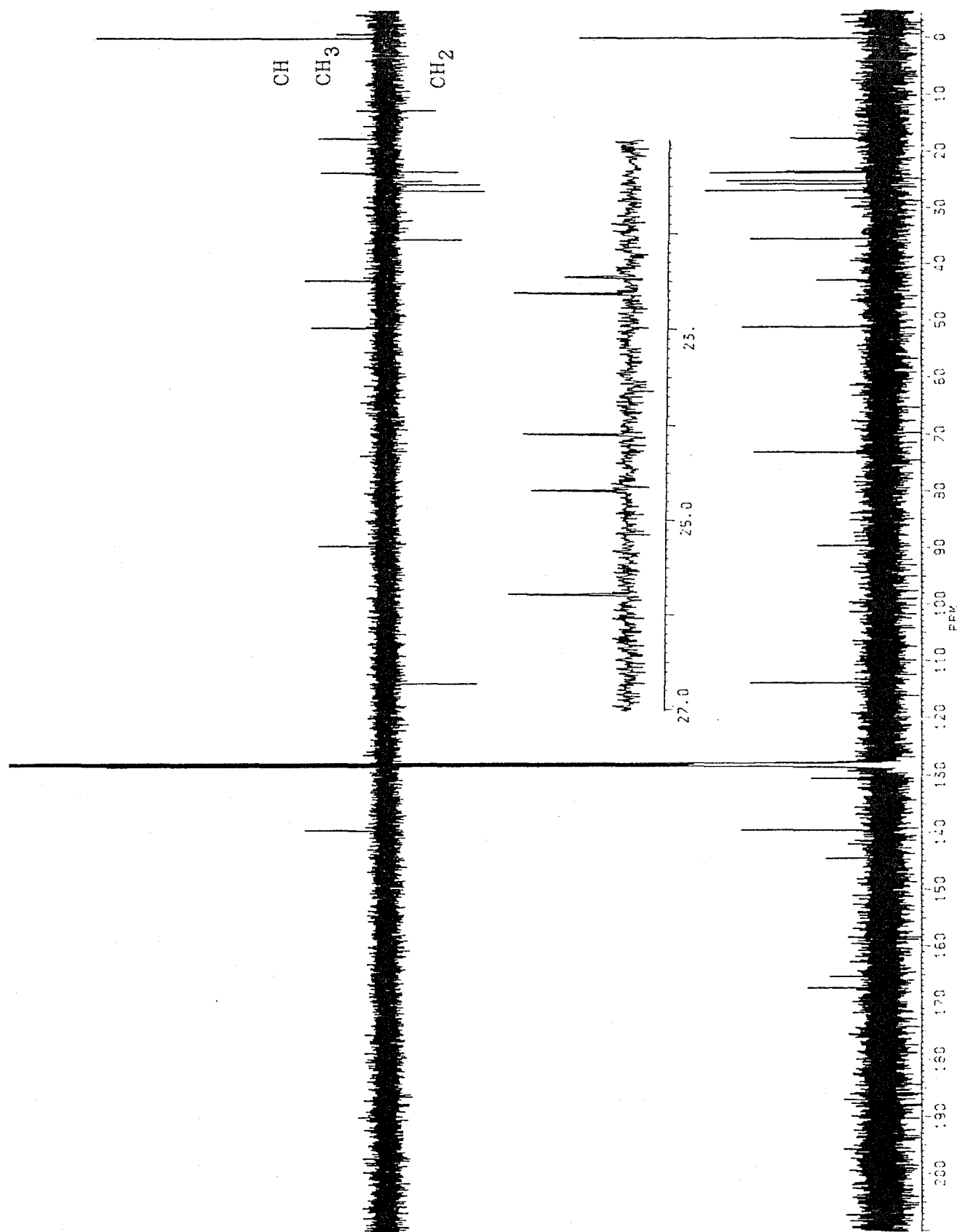


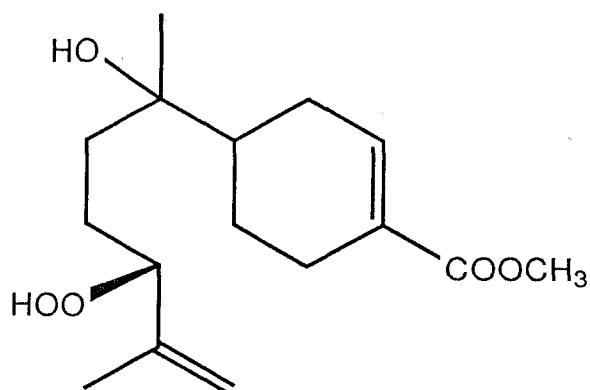
Fig. 3-413 ^{13}H -NMR Spectra of RL-PERO-6 (125 MHz, in C_6D_6 , COM and DEPT)

Table 3-160 ^{13}C -NMR chemical shift values of RL-PERO-6 and comparison of the shift values with those of RL-PERO-5 (23)

(68 MHz, in CDCl_3 , TMS as an int. std.)

δ_{C}	Property	Assignment	23
167.3	-COO-	C-7	167.3
144.4	=C-	C-13	144.3
139.4	=CH-	C-2	139.5
130.4	=C-	C-1	130.4
113.8	=CH ₂	C-14	113.8
89.5	-CH-O	C-12	89.5
73.1	-C-O	C-8	73.1
51.1	CH ₃	C-7'	51.1
42.7	CH	C-4	42.9
35.4	CH ₂	C-9	35.3
26.8	CH ₂	C-3	26.8
25.7	CH ₂	C-6	25.7
25.1	CH ₂	C-11	25.2
23.6	CH ₃	C-10	23.6
23.5	CH ₂	C-5	23.4
17.6	CH ₃	C-15	17.6

Table 3-161 Physicochemical properties of bisaborosol C1
(RL-PERO-5, 23)



23

A colorless syrup

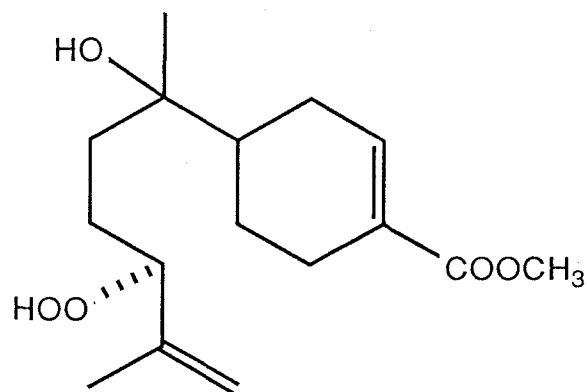
Vanillin- H_2SO_4 color: pinkish red

FI-MS m/z (%): 299 ($\text{M}^+ + 1$, 57), 298 (M^+ , 45), 281 (29), 265 (81), 183 (40), 159 (100), 141 (41), 139 (76).

EI-MS m/z (%): 262 (4.2), 246 (3.1), 230 (6.4), 202 (5.7), 192 (12), 178 (20), 177 (21), 176 (13), 145 (19), 139 (20), 137 (45), 125 (28), 119 (23), 107 (32), 105 (34), 93 (27), 91 (43), 79 (63), 77 (40), 70 (35), 69 (39), 55 (23), 43 (100), 41 (85).

^1H - and ^{13}C -NMR data are shown in Tables 3-156 and 3-157, respectively.

Table 3-162 Physicochemical properties of bisaborosaol C2
(RL-PERO-6, 24)



A colorless syrup 24

Vanillin-H₂SO₄ color: pinkish red

FI-MS *m/z* (%): 299 (M⁺+1, 86), 298 (M⁺, 43), 281 (36), 265 (100), 183 (34), 159 (92), 141 (57), 139 (73).

EI-MS *m/z* (%): 262 (6.1), 260 (3.6), 246 (3.1), 230 (8.5), 202 (7.1), 192 (8.0), 178 (26), 177 (31), 176 (21), 163 (11), 148 (21), 145 (29), 137 (47), 125 (29), 119 (32), 107 (32), 105 (42), 93 (31), 91 (51), 79 (56), 77 (38), 70 (36), 69 (47), 55 (27), 43 (100), 41 (99).

¹H- and ¹³C-NMR data are shown in Table 3-159 and 3-160, respectively.

3) Structure Elucidation of RL-PERO-7

RL-PERO-7 obtained as a colorless syrup (4.1 mg) showed a weak parent ion at m/z 298 in FI-MS together with the base fragment at m/z 183⁺ (Fig. 3-414). In EI-MS, however, the compound afforded neither the parent ion nor the fragment at m/z 183 (Fig. 3-415). On the other hand, this compound also indicated some signals characteristic of a bisabolanoid in the ¹H-NMR and HH-COSY spectra, where the 1,4-disubstituted cyclohexene structure was visible (Fig. 3-416, 417, 418 and Table 3-163). Furthermore, some signals attributable to the sidechain were indicative of its partial structure on the sidechain. In the ¹H-NMR spectrum, two olefinic protons were detected at δ_H 5.690 (1H, ddd, J = 14.8, 7.5 and 7.2 Hz) and 5.516 (1H, br. d, J = 14.8 Hz) which showed a clear *trans* coupling with each other. Since the former proton was further coupled vicinally with a pair of methylene protons with J = 7.5 and 7.7 Hz, a substructure $\blacksquare$ -CH₂-CH=CH- $\blacksquare$ became feasible. In addition, two singlet methyl groups at δ_H 1.261 and 1.250 were reasonably assigned to an oxygenated isopropyl group. The structure of the sidechain was therefore elucidated as shown in Fig. 3-419. Cleavage of the sidechain was responsible for the fragment at m/z 183.

By the ¹³C-NMR spectroscopy, this substructure was proved (Fig. 3-420 and Table 3-164). The carbon chemical shifts for the side chain carbons in RL-PERO-7 showed a good correspondence with those of a bisabolane alkaloid, aminobisabolenol (112) which has also been isolated from the sponge together with isoamino-bisabolenol (111) by Kitagawa *et al.* (Table 3-165) [156]. Furthermore, the clear deshielding effect on the C-13 carbon in RL-PERO-7 was reasonably attributed to hydroperoxylation at the of RL-PERO-7 was elucidated as 25, and this novel bisabolane exoperoxide was named bisaborosaol D.

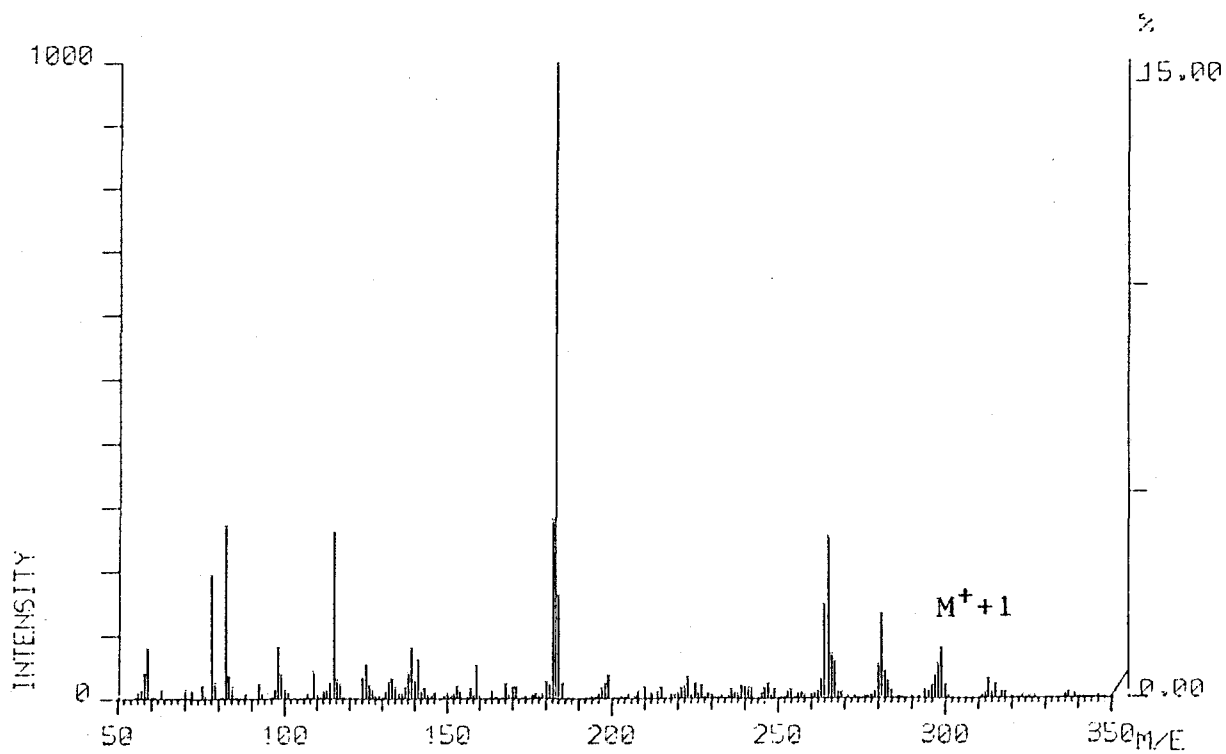


Fig. 3-414 FI-Mass Spectrum of RL-PERO-7

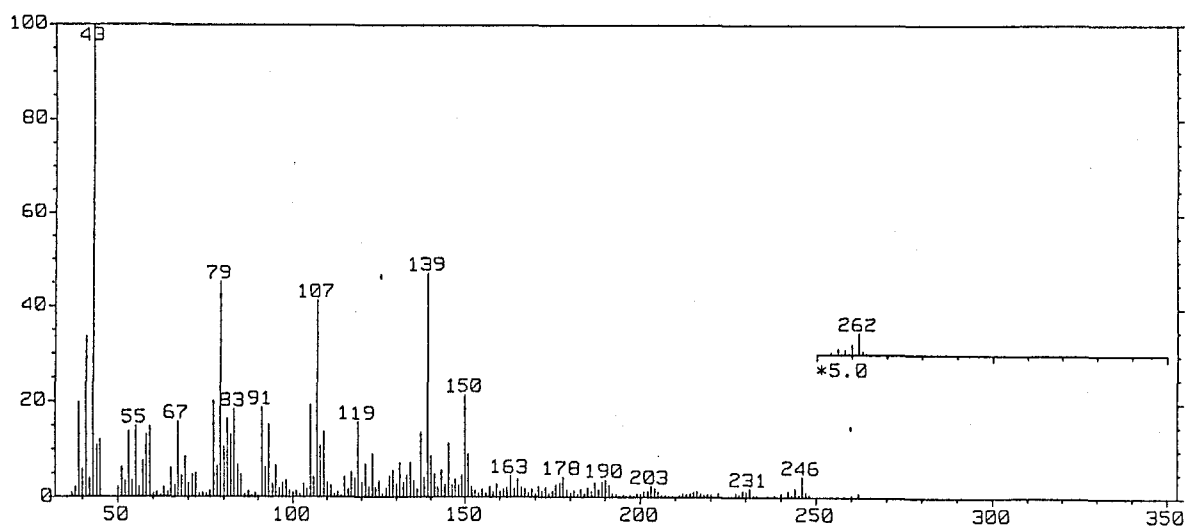


Fig. 3-415 EI-Mass Spectrum of RL-PERO-7

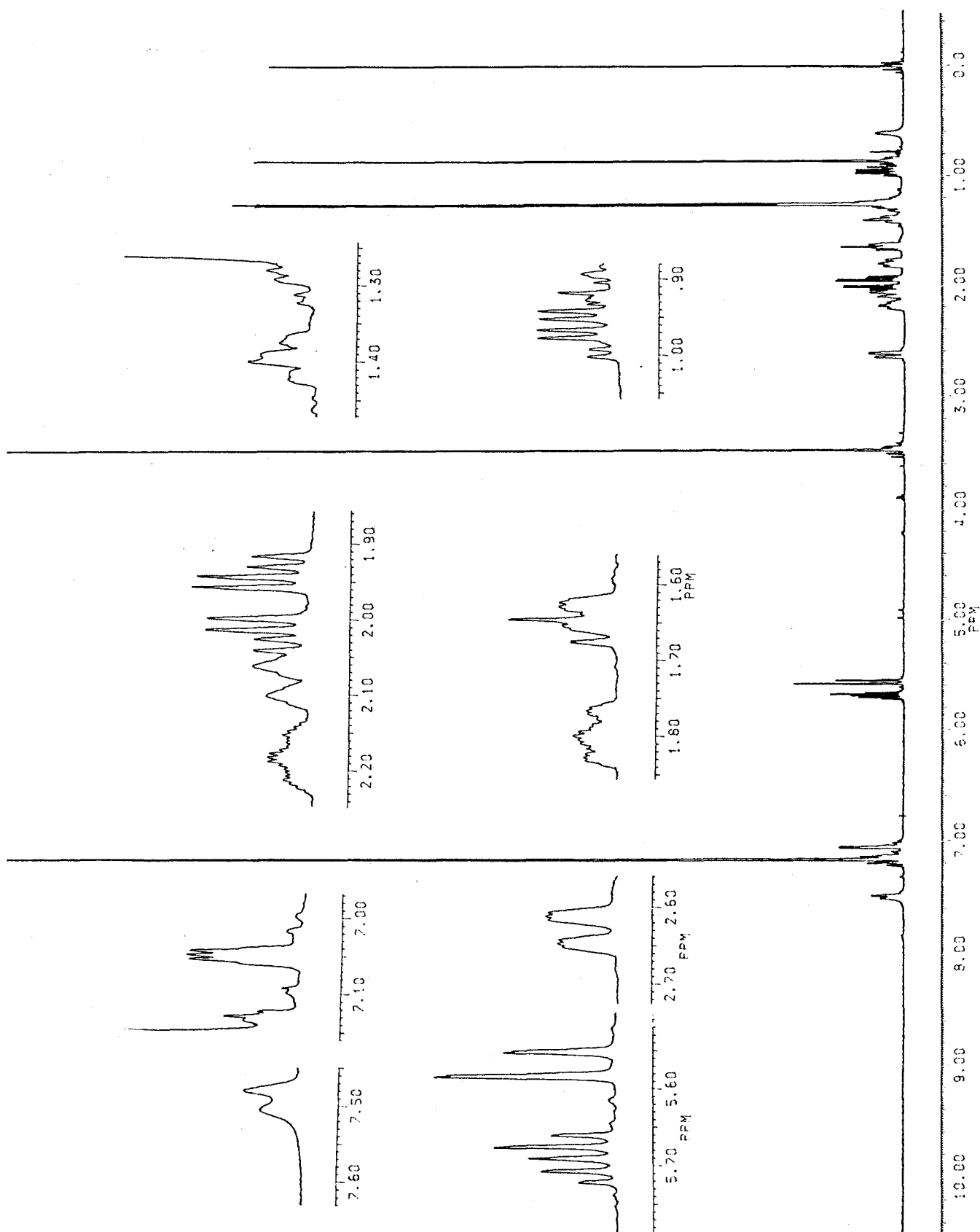


Fig. 3-416 ^1H -NMR Spectrum of RL-PERO-7(500 MHz, in C_6D_6)

Table 3-163 Physicochemical properties of bisaborosaol D (25)

(500 MHz, in C₆D₆, TMS as an int. std.)

δ_H		Coupling	Assignment
7.505	1H	s	C-13-OOH
7.135	1H	br. m	C-2-H
5.690	1H	ddd $J= 14.8, 7.5$ and 7.2 Hz	C-11-H
5.516	1H	dd $J= 14.8$ and 0.8 Hz	C-12-H
3.472	3H	s	C-7'-H ₃
2.630	1H	br. d $J= 15.3$ Hz	C-6-Ha
2.182	1H	t-like m	C-6-Hb
2.082	1H	m-dvid. d* $J= 19.4$ Hz	C-3-Ha
2.019	1H	br. dd $J= 13.7$ and 7.5 Hz	C-9-Ha
1.937	1H	br. dd $J= 13.7$ and 7.2 Hz	C-9-Hb
1.800	1H	m	C-3-Hb
1.639	1H	m-dvid. d $J= 12.3$ Hz	C-5-Ha
1.396	1H	br. t-like	C-4-H
1.261	3H	s	C-14-H ₃
1.250	3H	s	C-15-H ₃
0.958	1H	dddd $J= 12.4, 12.4, 12.4$ and 5.1 Hz	C-10-H ₂
0.860	3H	s	C-10-H ₃

* multiply divided doublet

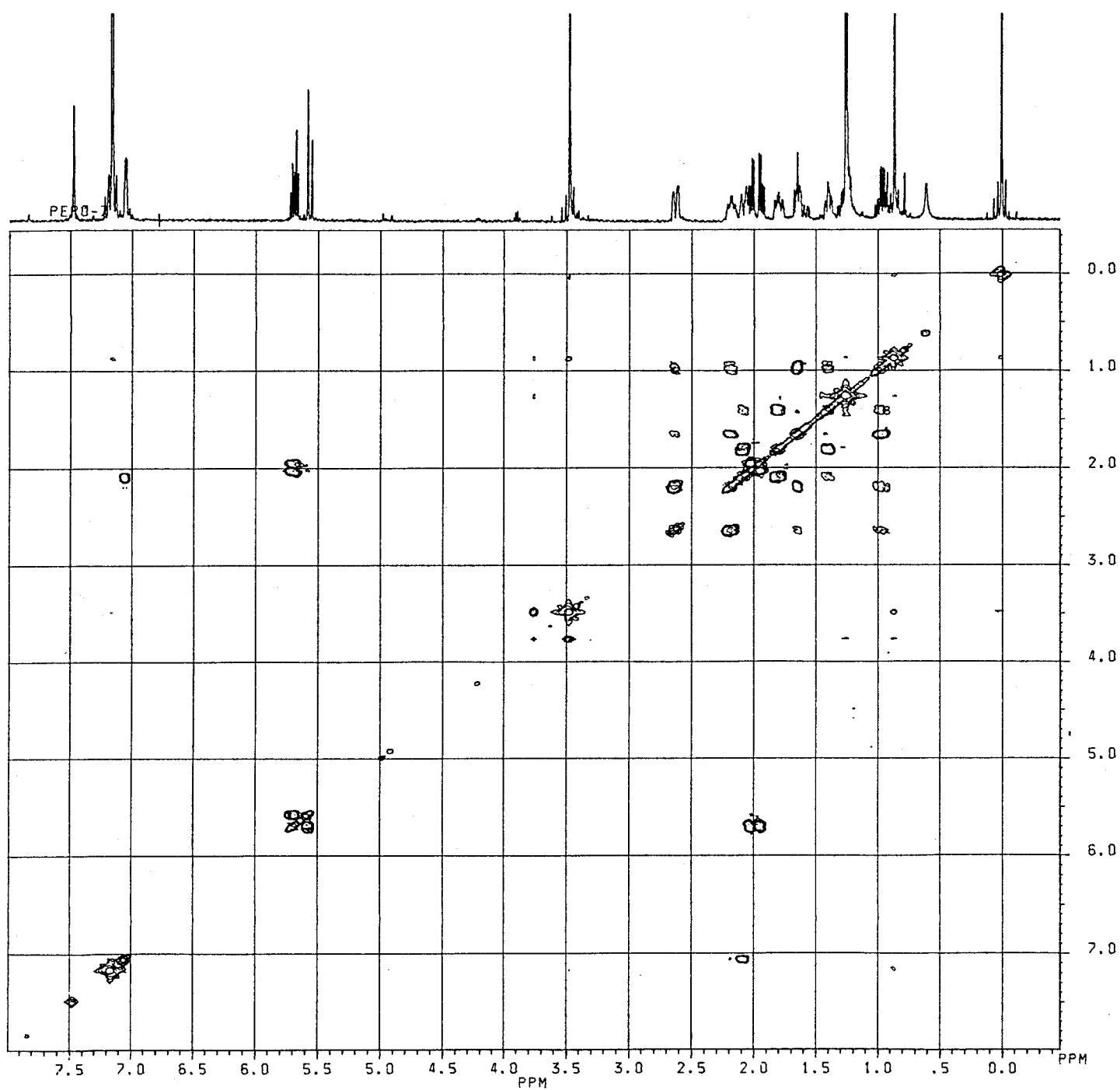


Fig. 3-417 HH-COSY Spectrum of RL-PERO-7 (500 MHz, in C_6D_6)

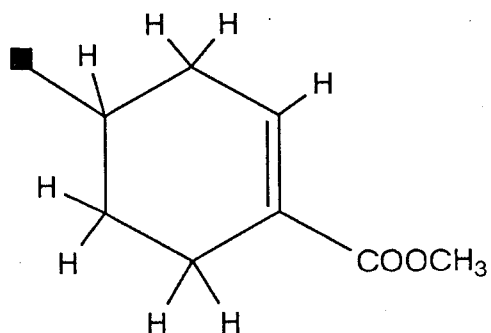


Fig. 3-418 Proton Sequence on 1,4-Disubstituted 1-Cyclohexene
Moiety of RL-PERO-7

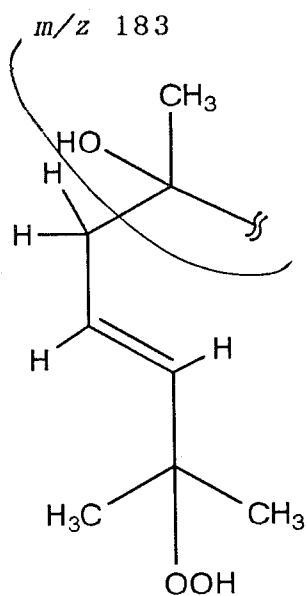


Fig. 3-419 Proton Sequence on the Sidechain Part of RL-PERO-7

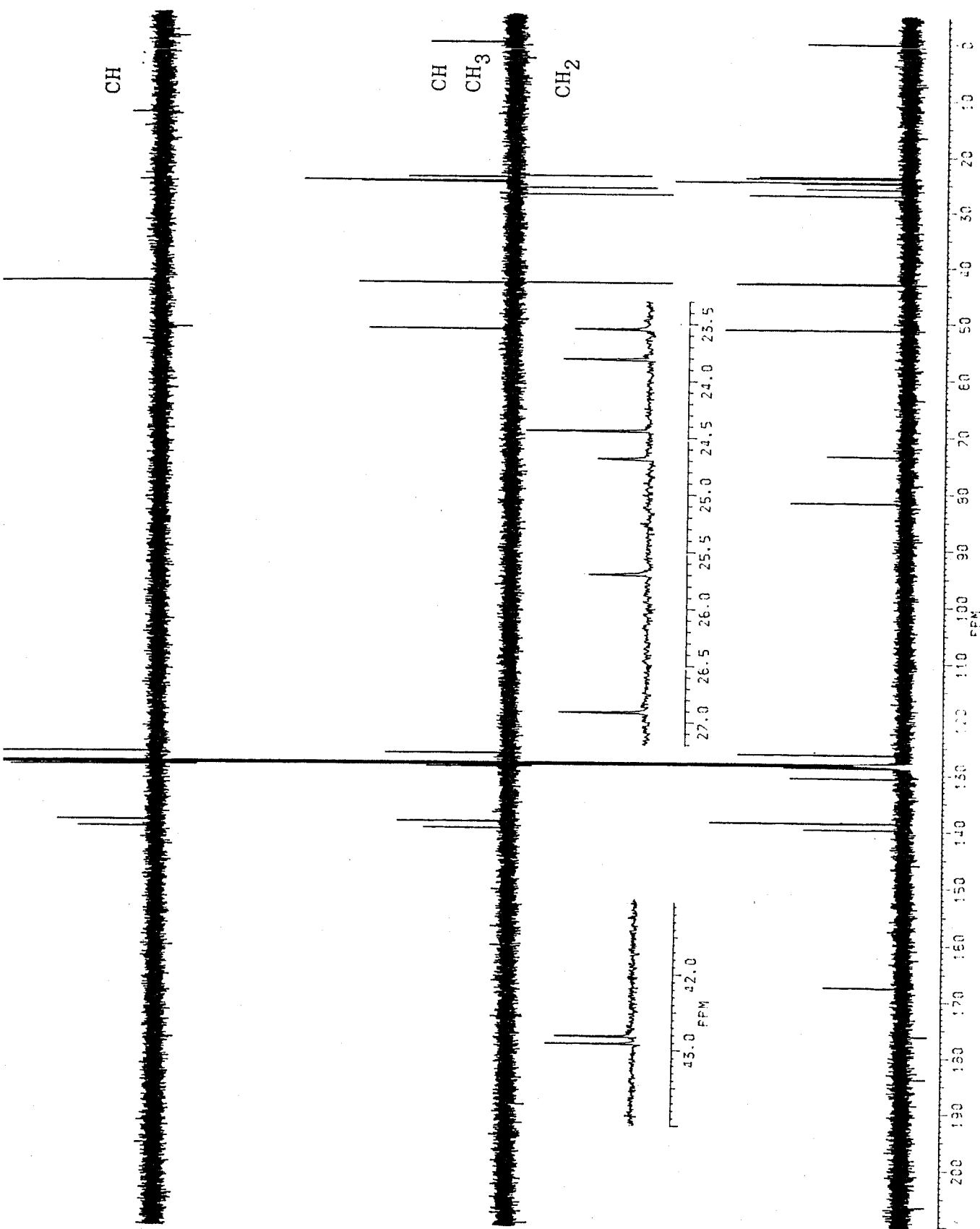


Fig. 3-420 ^{13}H -NMR Spectra of RL-PERO-7 (125 MHz, in C_6D_6 , COM and DEPT)

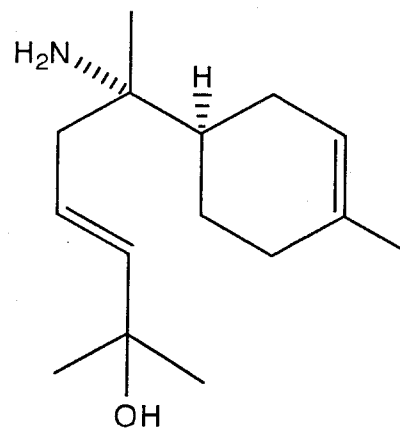
Table 3-164 ^{13}C -NMR chemical shift values of RL-PERO-7

(125 MHz, in C_6D_6 , TMS as an int. std., COM and DEPT)

δ_{C}	Property	Assignment
167.4	-COO-	C-7
139.6	=CH-	C-2
138.4	=CH-	C-12
130.4	=C-	C-1
126.2	=CH-	C-11
81.5	-CH-O	C-13
73.3	-C-O	C-8
51.1	CH_3	C-7'
42.9	CH	C-4
42.8	CH_2	C-9
26.9	CH_2	C-3
25.7	CH_2	C-6
24.7	CH_3	C-14
24.4	CH_3	C-15
23.8	CH_3	C-10
23.5	CH_2	C-5

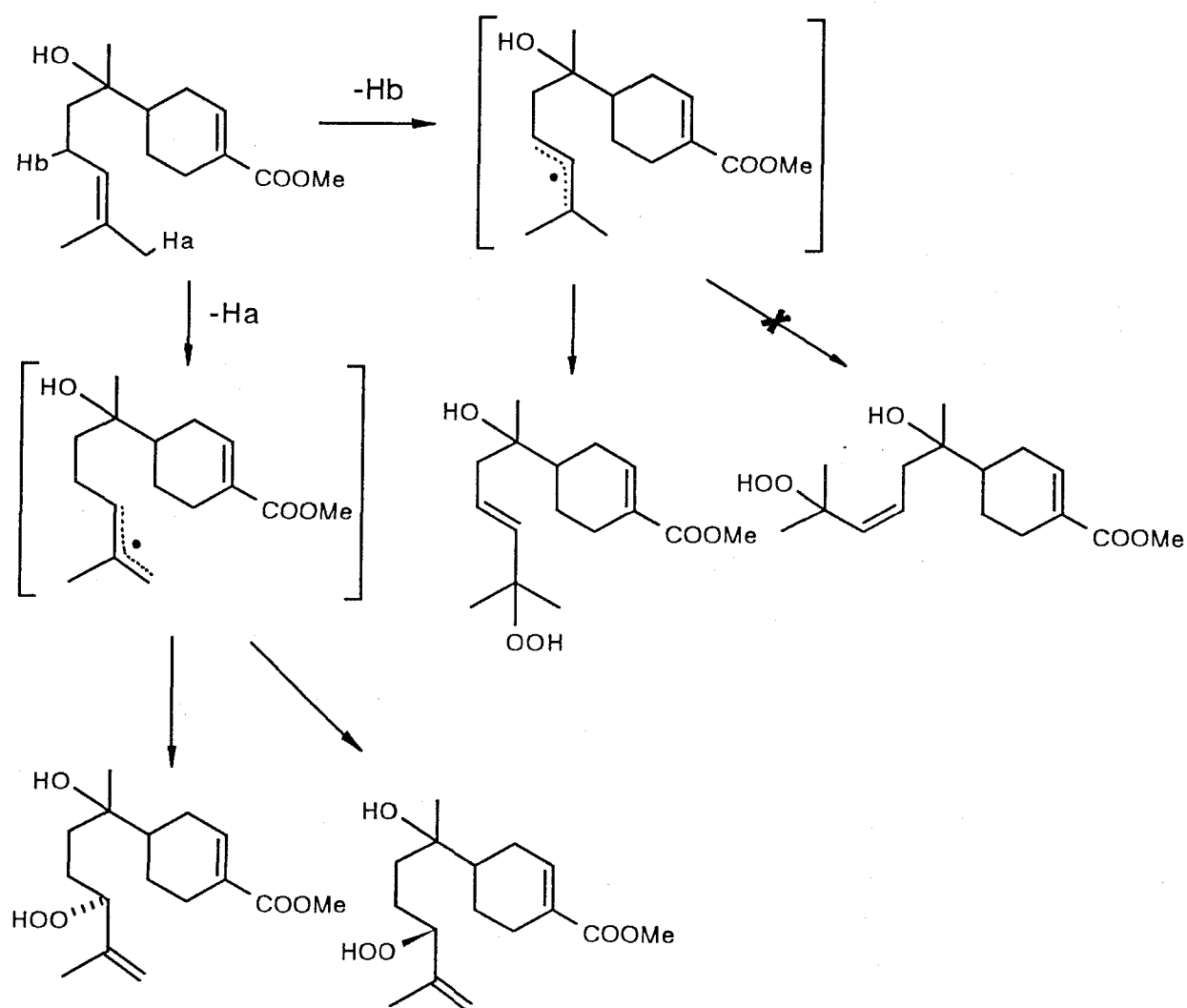
Table 3-165 Comparison of RL-PERO-7 carbon chemical shift values with those of aminobisabolenol (112) and bisaborosaol A (19)

C-No	RL-PERO-7	112 [156]	19
1	130.4	133.9	
2	139.6	119.7	
3	26.9	25.9	
4	42.9	41.1	
5	23.5	23.6	
6	25.7	30.7	
7	167.4	23.2	
8	73.3	59.0	
9	42.8	39.4	
10	23.8	21.5	
11	126.2	119.4	
12	138.4	144.7	
13	81.5	70.7	
14	24.7	29.3	
15	24.4	29.3	
7'	51.1	-	



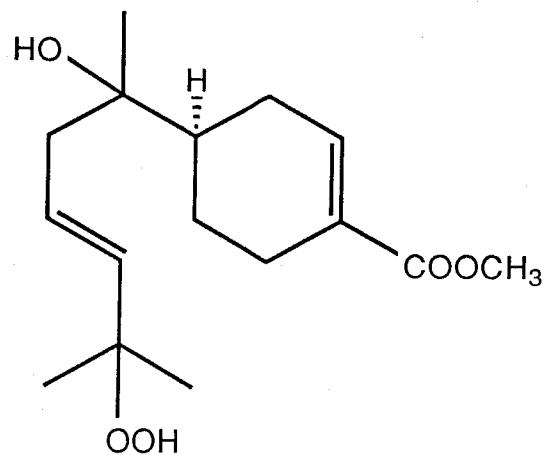
aminobisabolenol (112)

Bisaborosaol D (25) is probably formed from bisaborosaol A (19), as well as bisaborosaol C1 (23) and bisaborosaol C2 (24). Unlike 23 and 24, the derivative only gave the *trans* form which requests much less energy than the *cis* form. As it has been proved that the radical reaction on an olefine having 3,3-dimethylallyl part indicates neither stereoselectivity nor position-selectivity [126], 19 may be convertible to these hydroperoxide isomers (Scheme 3-46). The fact that only the *trans* form was isolated from the *Rosa rugosa* leaf extracts therefore seems to be compatible.



Scheme 3-46 Formation of the Bisabolane Hydroperoxides (23 ~ 25) from Bisaborosaol A (19)

Table 3-166 Physicochemical properties of bisaborosaol D
(RL-PERO-7, 25)



A colorless syrup

Vanillin-H₂SO₄ color: pinkish red

FI-MS m/z (%): 299 ($M^+ + 1$, 57), 298 (M^+ , 45), 281 (29), 265 (81), 183 (40), 159 (100), 141 (41), 139 (76).

EI-MS m/z (%): 262 (4.2), 246 (3.1), 230 (6.4), 202 (5.7), 192 (12), 178 (20), 177 (21), 176 (13), 145 (19), 139 (20), 137 (45), 125 (28), 119 (23), 107 (32), 105 (34), 93 (27), 91 (43), 79 (63), 77 (40), 70 (35), 69 (39), 55 (23), 43 (100), 41 (85).

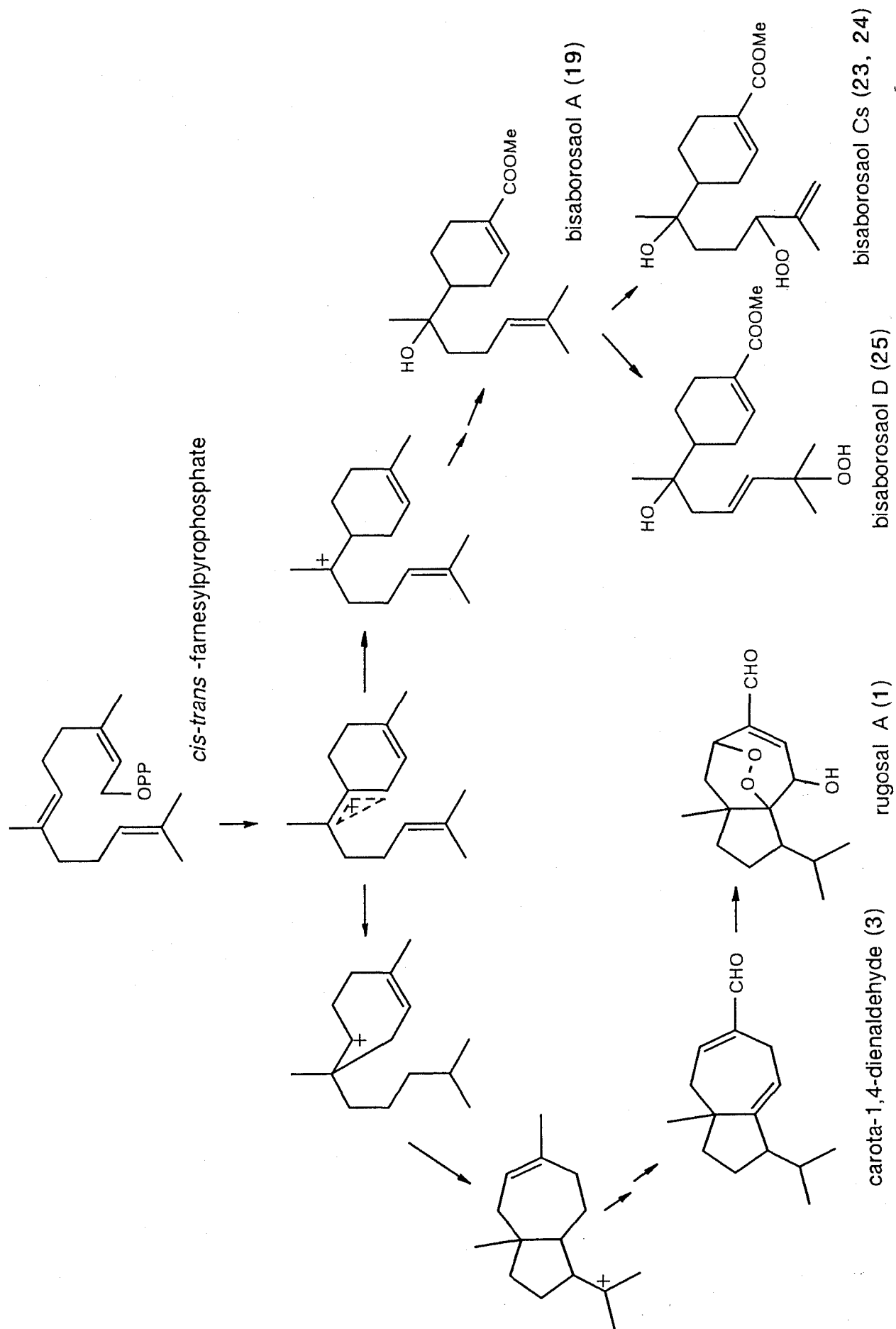
¹H- and ¹³C-NMR data are shown in Tables 3-164 and 3-165, respectively.

4) Discussion on the Exoperoxy Bisabolanoids

In the formation routes, these exoproxy bisabolanoids are considered to be yielded through a radical reaction as shown in Scheme 3-46. Firstly, hydrogen radical abstraction occurs either at C-14 or C-11. Successive allyl radicals of two type (G and H) are attacked by $^3\text{O}_2$ molecule. Since C-12 radical is more stable than C-14 in G, and C-13 than C-11 in H, those are accordingly peroxyated at C-12 and C-13, respectively, to yield 23, 24 (both from G) and 25 (from H). As mentioned above, the non-stereoselectivity in the peroxylation at the C-12 and the selective *trans*-olefinic bond formation of the C-13-exoperoxide are compatible with the radical reaction scheme. In the *Rosa rugosa* leaves, these bisabolane peroxides are contained with less amounts than those of carotane peroxides. contained in the *Rosa rugosa* leaves with less amounts. However, these compounds which are unexpectedly stable as the isolated state may provide a possibility that in the leaf tissues some oxidative condition is set up and unignorable amounts of free radicals are yielded. Since these peroxybisabolanoids show a stable property, bisaborosaol A (19) which is considered to be their precursor may function as a radical scavenger.

3-8-6 Relation between Bisaboranoids and Carotanoids of
Rosa rugosa

In addition to the carotanoids which are contained as the major sesquiterpenes in *Rosa rugosa*, bisabolanoids are also found as the major part of the constituents. In particular, some of those bisabolanoids such as bisaborosaol A (19) are involved in the leaf extractives in a high content (ca 50 ~ 100 mg/kg f.w.). Since bisabolanoids and carotanoids are closely related in their biogenesis, resemblance of those two in their regio-specific oxygenation at the methyl groups (C-14 and C-7 in the carotanoids and bisabolanoids, respectively) are reasonable. Significance or physiological roles of those bisabolanoids has not been known. However, some facts: (1) several bisabolanoids are contained in *Rosa rugosa* leaves and some of them are found with around 50 ppm concentration, (2) the oxygenated forms of them are comparatively stable, and (3) those bisabolanoids were more detectable than the carotanoids in the diffusate of the unwounded leaves soaked in tap water, may be indicative of some roles of the bisabolanoids in the tissues. They may function as a radical scavenger as mentioned above, or may take a part in an antifungal agent (See Section 10, pp. 703) (Scheme 3-47).



Scheme 3-47 Relationship between Carota-1,4-dienaldehyde (3) and Bisaborosaol A (19) in the Biogenesis of *Rosa rugosa* Sesquiterpenoids

3-9 Acoranoid in *Rosa rugosa*

3-9-1 Isolation and Structure Elucidation of Rosacoranone

In RL fraction containing carota-1,4-dienaldehyde (3), daucen-aldehyde (10), isodaucenaldehyde (13), and dehydrodaucenaldehyde (16), a new compound RL-B was also involved as the fifth constituent. The fourth peak of RL-fraction in HPLC (Fig. 3-421), detected as a constituent close to isodaucenaldehyde (13) was roughly isolated and further purified next by HPLC in the same system to give ca 3 mg of a colorless syrup. EI-Mass spectrum of the isolate was agreeable with the GC-Mass spectrum of RL-B (M^+ 218, $C_{15}H_{22}O$, 218.184 in EI-HR-MS) (Fig. 3-422). Its methanolic UV absorption maximum was exhibited at 228 nm. These physicochemical properties of RL-B suggested its carotane dienaldehyde structure isomeric to 3. By the ^1H -NMR spectroscopy, however, it was revealed that RL-B possesses no formyl proton. On the other hand, an exomethylene group (δ_H 6.130 and 5.028, each d, $J= 1.5$ Hz), an allyl methyl group (δ_H 1.562, 3H, br. s) and an isopropyl group (δ_H 0.721 and 0.585, both 3H, d, $J= 6.7$ Hz, and 1.516, 1H, double sept., $J= 6.7$ and ca 6.5 Hz) became feasible to allow a speculation that RL-B was a non-carotanoide sesquiterpene (Fig. 3-423 and Table 3-167).

Further decoupling and HH-COSY experiments elucidated two coupling systems (Fig. 3-424). When the olefinic proton at δ_H 5.028 (1H, br. m) was irradiated, a pair of methylene protons at δ_H 2.180 and 1.617 (each 1H, br. d, $J= 17.8$ Hz) changed their signal patterns. In addition, the allylic methyl signal and a broad signal at δ_H 1.727 (2H) became sharpened by the irradiation. When the broad signal was irradiated, a pair of methylene proton at δ_H 1.378 and 1.123 collapsed into isolated doublets (geminal coupling, $J= 13.0$ Hz). When the δ_H 1.727 signal were regarded as that of an

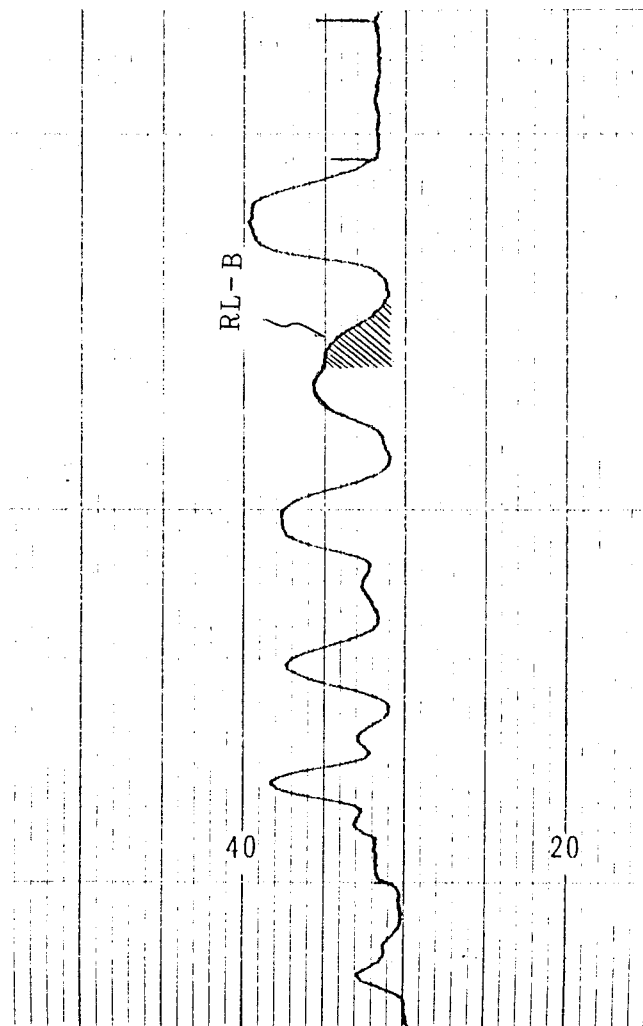


Fig. 3-421 HPL-Chromatogram of RL-B (Unisil Q 100-5, n-Hexane-EtOAc 97.5:2.5, UV 230 nm)

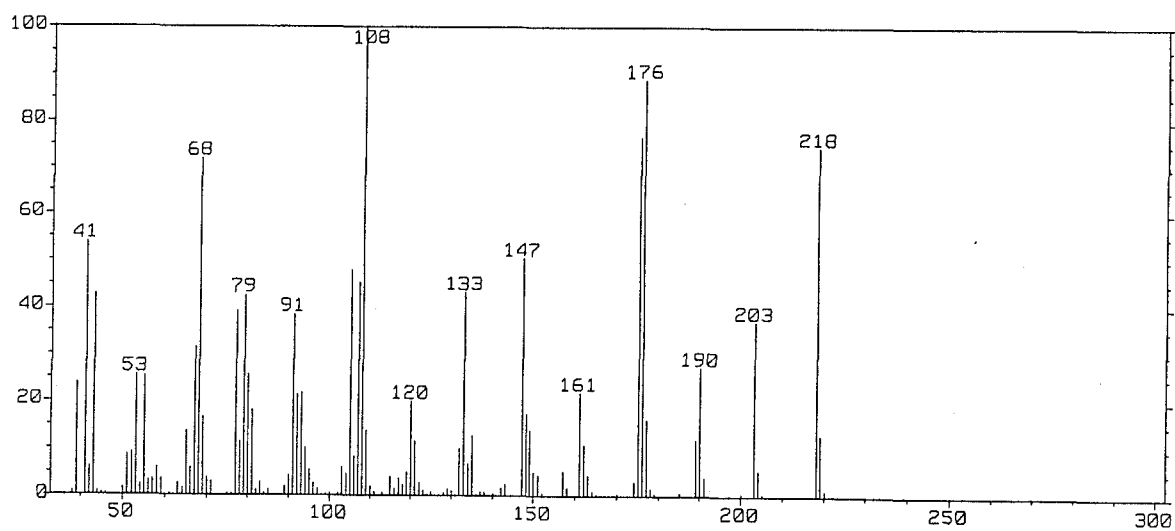
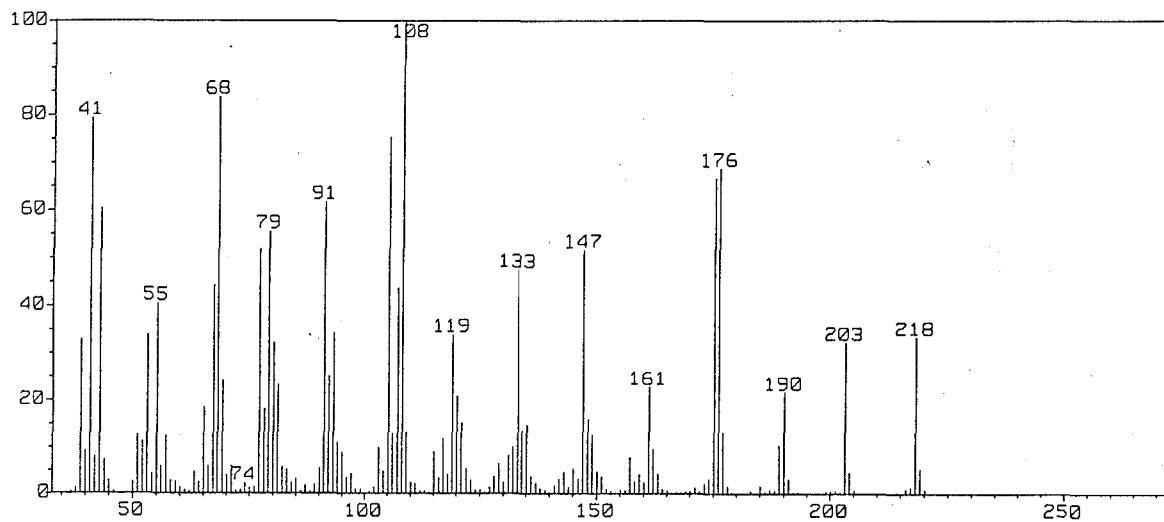


Fig. 3-422 GC-MS (top) and EI-MS (bottom) Spectrum of RL-B

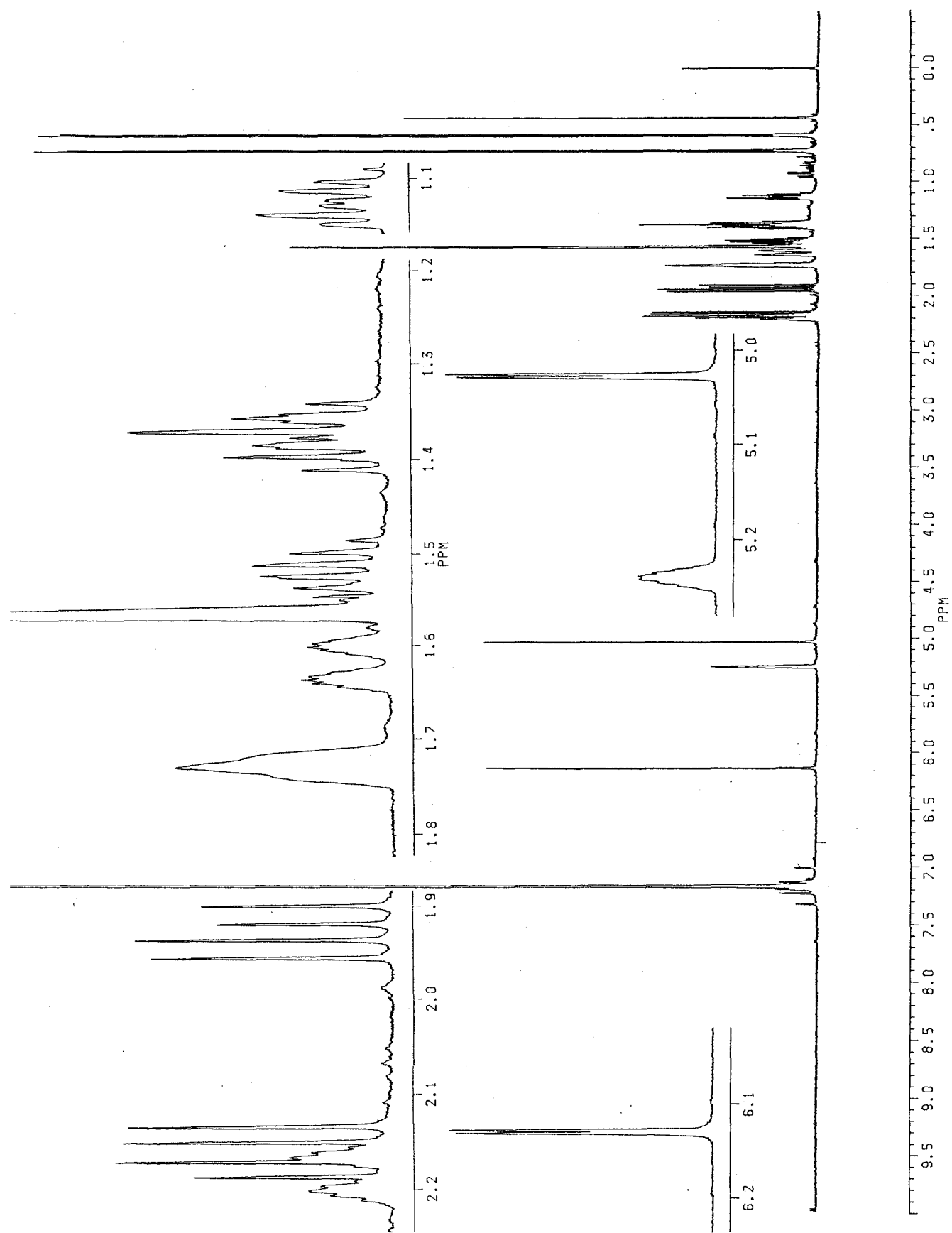


Fig. 3-423 ^1H -NMR Spectrum of RL-B (500 MHz, in C_6D_6)

Table 3-167 ^1H -NMR chemical shift values of RL-B

(500 MHz, in C_6D_6 , TMS as an int. std.)

δ_{H}		Coupling	Assignment
6.130	1H	d $J= 1.5$ Hz	C-15-Ha
5.239	1H	br. s-like m	C-3-H
5.208	1H	d $J= 1.5$ Hz	C-15-Hb
2.180	1H	m-dvid. d* $J= 17.8$ Hz	C-2-Ha
2.165	1H	dd $J= 18.2, 8.1$ Hz	C-9-Ha
1.953	1H	dd $J= 18.2, 9.7$ Hz	C-9-Hb
1.727	2H	br. m	C-5-H ₂
1.617	1H	m-dvid. d* $J= 17.8$ Hz	C-2-Hb
1.562	3H	br. s	C-14-H ₃
1.516	1H	d sept $J= 6.7, \text{ca } 6.5$ Hz	C-11-H
1.378	1H	m	C-6-Ha
1.360	1H	ddd $J= 9.7, 8.1, \text{ca } 6.5$ Hz	C-10-H
1.123	1H	ddd $J= 13.0, 5.0, 4.7$ Hz	C-6-Hb
0.721	3H	d $J= 6.7$ Hz	C-12-H ₃
0.585	3H	d $J= 6.7$ Hz	C-13-H ₃

* multiply divided doublet

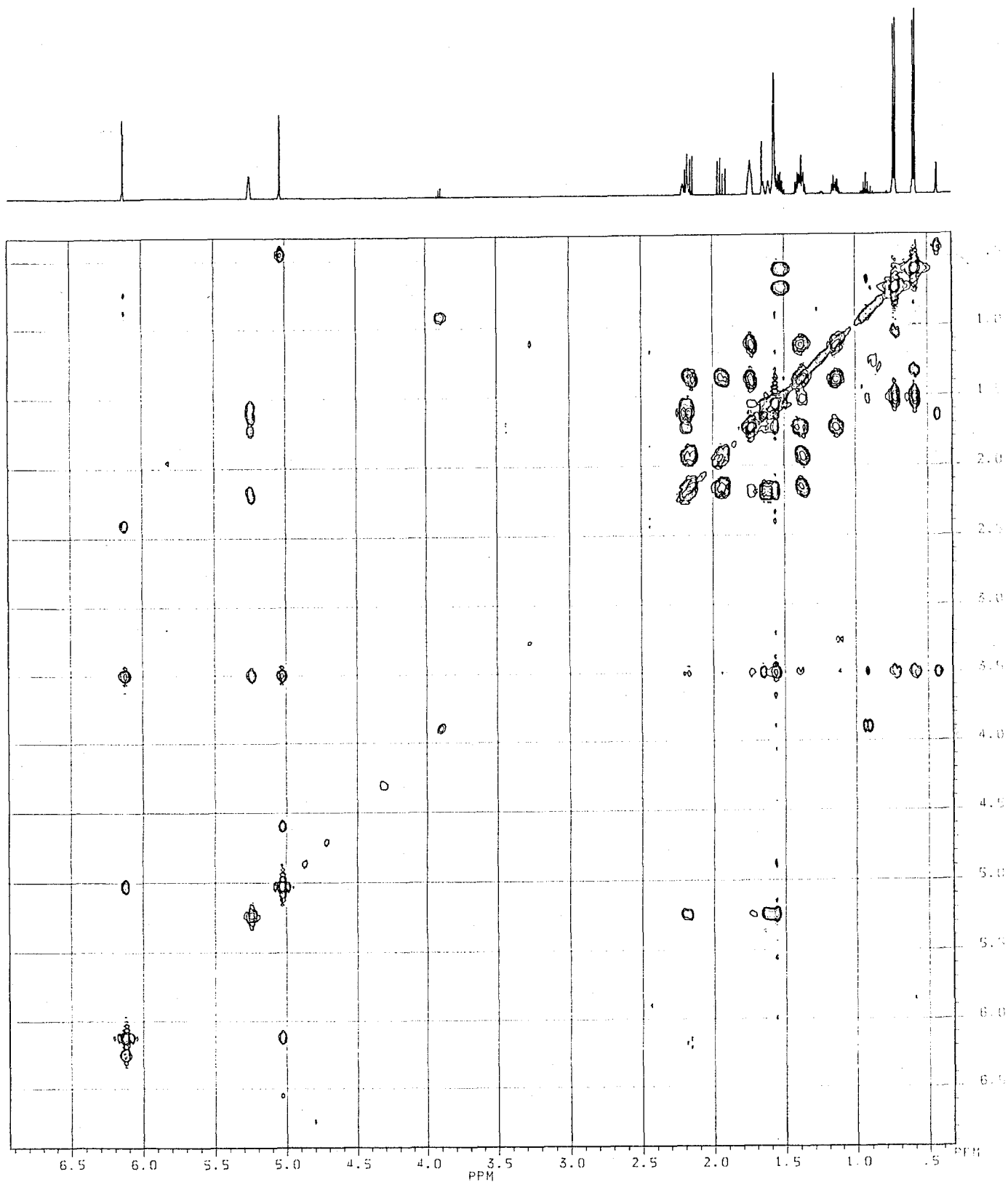


Fig. 3-424a HH-COSY Spectrum of RL-B (500 MHz, in C₆D₆)

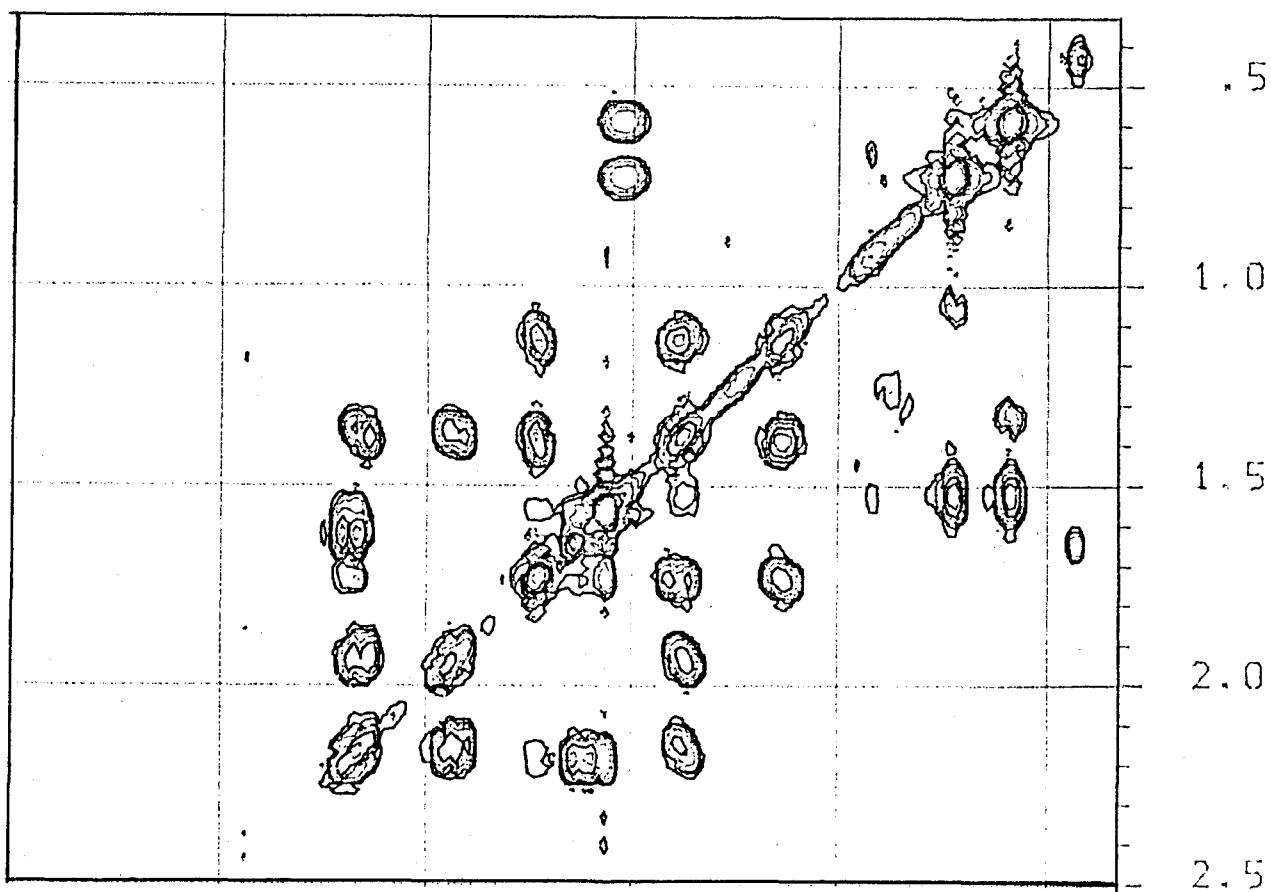
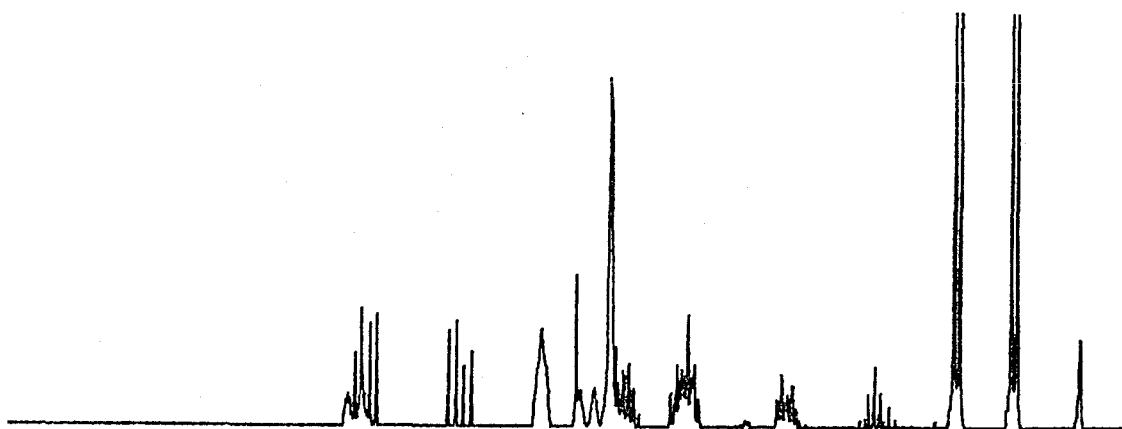


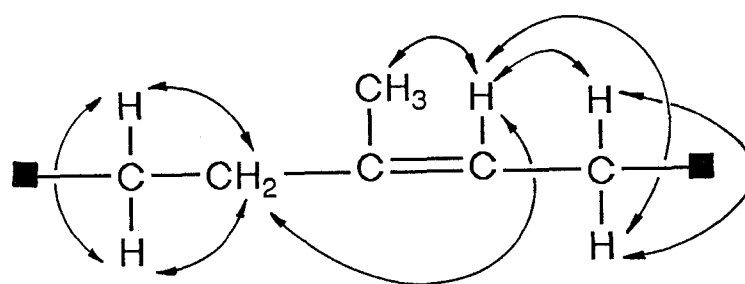
Fig. 3-424b Continued (Magnified in High Magnetic Field)

equivalent methylene protons, a substructural unit (A) became feasible (Fig. 3-425).

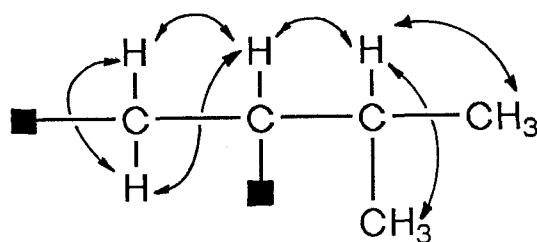
On the other hand, a signal at δ_H 1.360 (1H, ddd, $J = 9.7, 8.1$ and ca 6.5 Hz) was clearly collapsed by irradiation on the double septet signal. Therefore, the δ_H 1.360 signal must be assigned to a methine proton geminal to the isopropyl group. Furthermore, it was revealed by the HH-COSY that a pair of methylene protons each appearing as a double-doublet (δ_H 2.165, $J = 18.2$ and 8.1 Hz, and 1.953, $J = 18.2$ and 9.7 Hz) was vicinal to the methine proton. Consequently, the second part structure (B) was elucidated (See Fig. 3-425).

Those two units were proved by ^{13}C -NMR analyses (COM, DEPT and CH-COSY, Fig. 3-426, 427 and Tables 3-168, 169). The presence of five methylene carbons on RL-B suggested that the protons at δ_H 1.727 should be attributed to one of the methylene carbons. This assignment was provided a proof by the CH-COSY which revealed a correlation between δ_C 27.1 methylene carbon and the 1.727 protons.

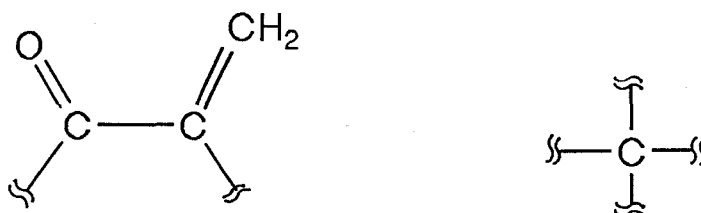
RL-B also exhibited a pair of exomethylene protons at δ_H 6.130 and 5.280 (each d, $J = 1.5$ Hz). As one of the protons was resonated in a markedly lower field, it was expected that the exomethylene protons were deshielded by something. On the other hand, the presence of a carbonyl carbon (δ_C 204.3) and a quaternary carbon (δ_C 44.5) were also revealed in the ^{13}C -NMR spectrum. The former carbon contributed to the conjugation system (UV λ_{max}^{MeOH} 228 nm) to form an α, β -unsaturated ketone group with one of olefinic bonds on the molecule. As substructure A involves the non-conjugated olefinic bond, the exomethylene part should contribute to the conjugation system to form partial structure C. Subsequently, these three substructures were connected each other via the quaternary carbon at δ_C 44.5 (See Fig. 3-425).



A



B



C

Fig. 3-425 Two Proton Sequence of RL-B Elucidated by Decoupling and COSY Experiments and Other Partial Structures

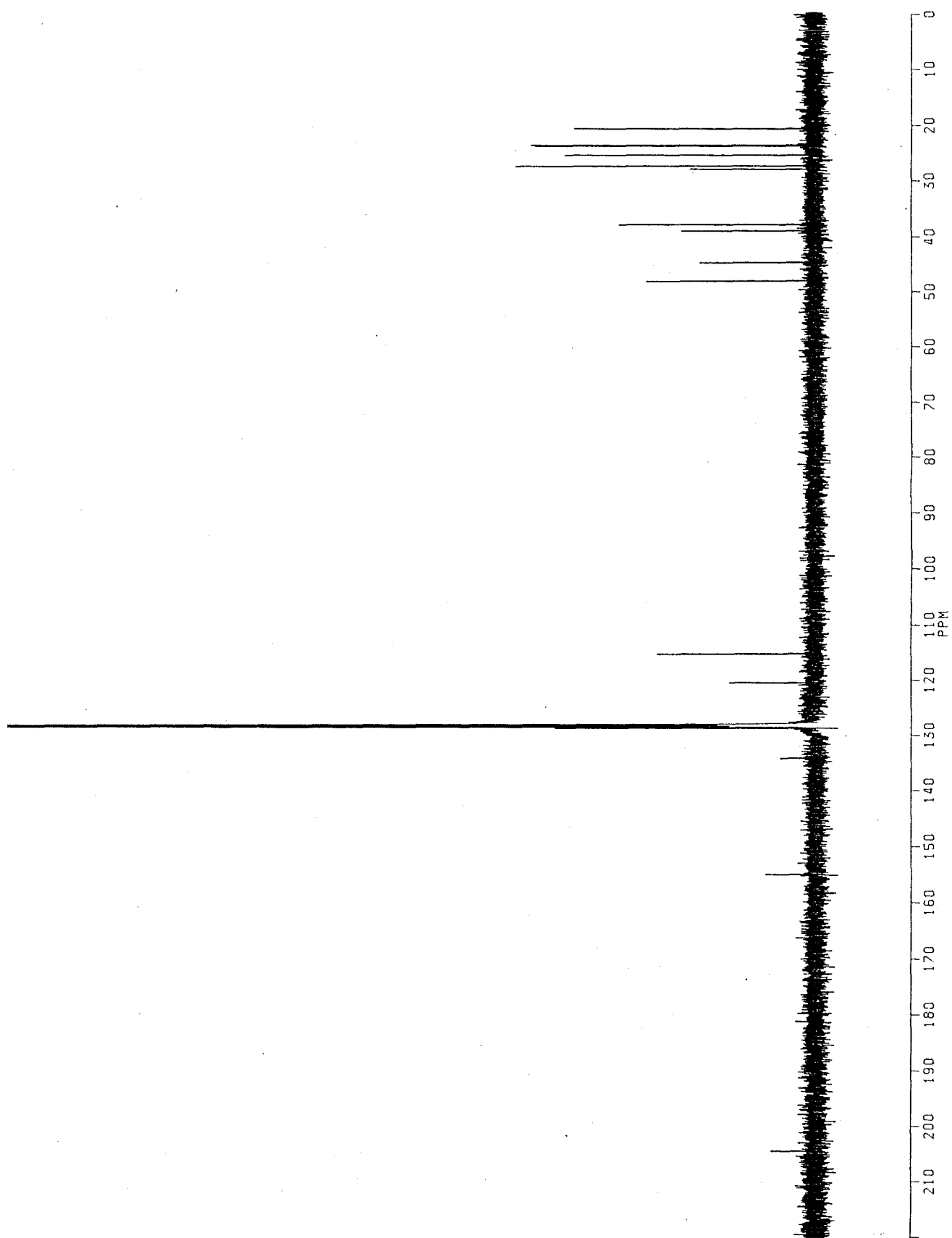


Fig. 3-426 ^{13}C -NMR Spectra of RL-B (500 MHz, in C_6D_6 , COM)

Table 3-168 ^{13}C -NMR chemical shift values of RL-B

(125 MHz, in C_6D_6 , TMS as an int. std.)

δ_{C}	Property	Assignment
204.3	C=O	C-8
154.8	=C-	C-7
134.0	=C-	C-4
120.3	=CH-	C-3
115.0	=CH ₂	C-15
47.8	CH	C-10
44.5	C	C-1
38.7	CH ₂	C-9
37.3	CH ₂	C-2
27.7	CH	C-11
27.1	CH ₂	C-5
25.2	CH ₂	C-6
23.5	CH ₃	C-12
23.4	CH ₃	C-14
20.4	CH ₃	C-15

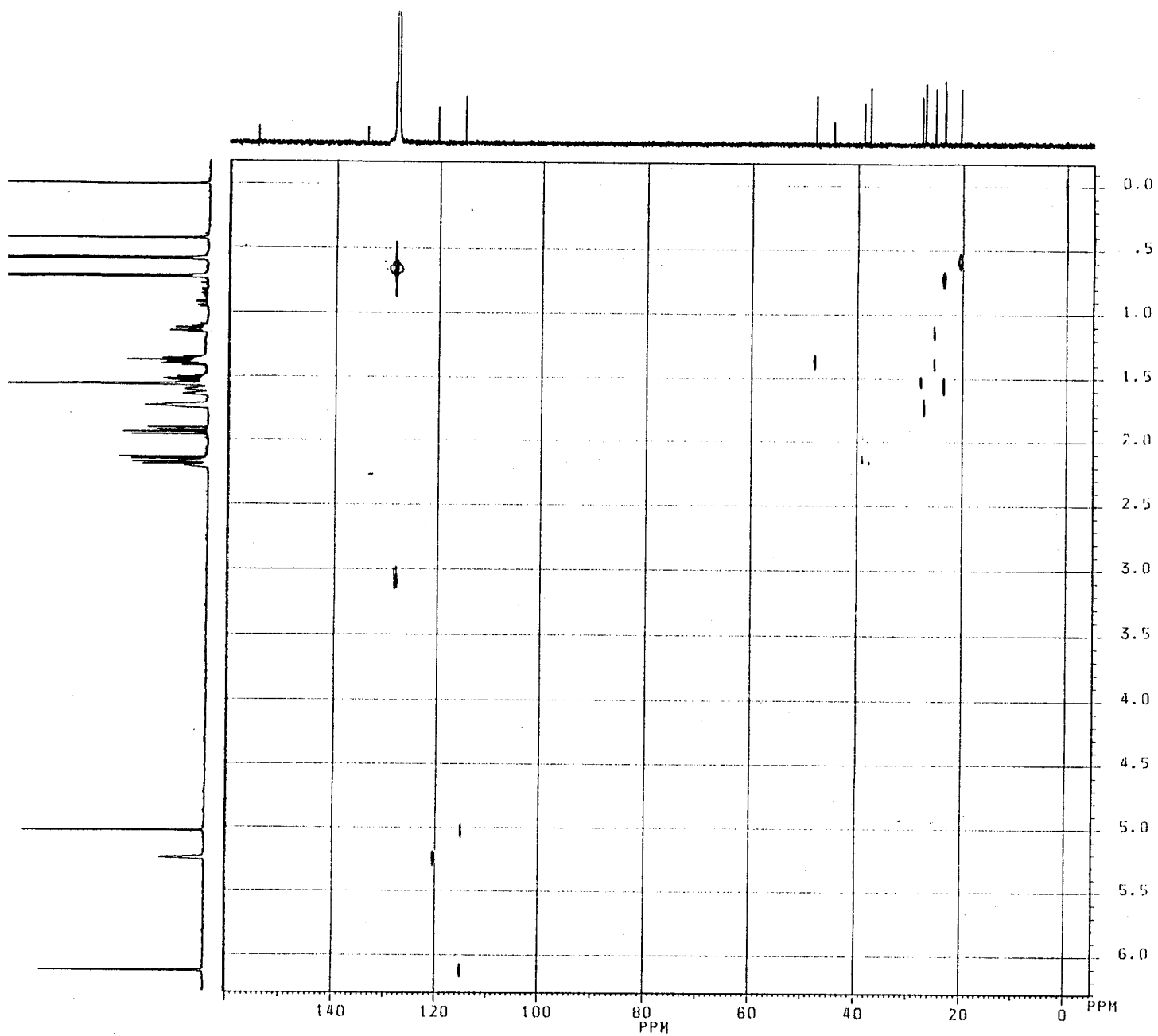


Fig. 3-427a CH-COSY NMR Spectrum RL-B (125 and 500 MHz, in C_6D_6)

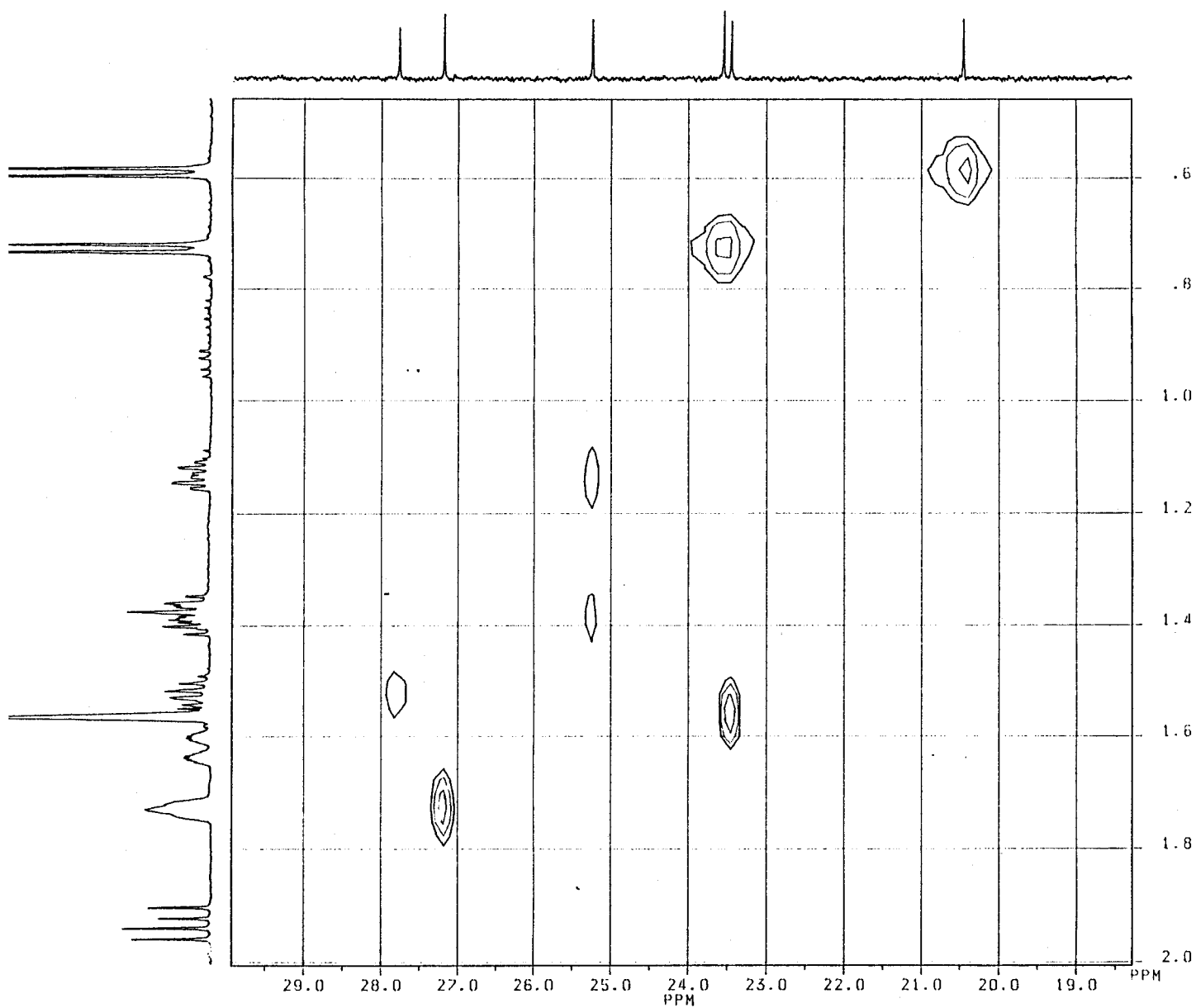


Fig. 3-427b Continued (Magnified in High Magnetic Field)

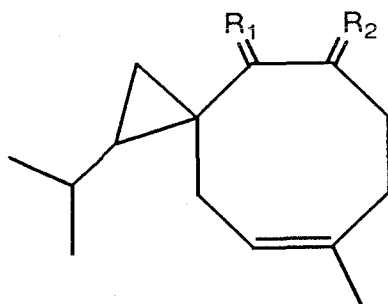
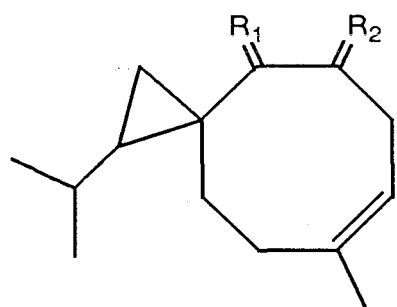
Table 3-169 Correlation between carbon and proton signals by CH-COSY of RL-B

(500 and 125 MHz, in C₆D₆, TMS as an int. std.)

Carbon (δ_C)	Position	Proton (δ_H)
37.3	C-2	2.180, 1.617
120.3	C-4	5.239
27.1	C-5	1.727
25.2	C-6	1.378, 1.123
38.7	C-9	2.165, 1.953
47.8	C-10	1.360
27.7	C-11	1.516
23.5	C-12	0.721
20.4	C-13	0.585
23.4	C-14	1.562
115.0	C-15	6.130, 5.028

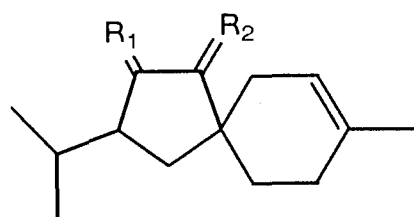
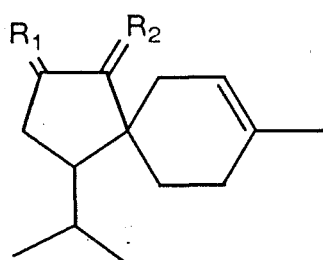
From the unsaturation number calculated as 5, the molecule must be bicyclic. As the quarternary carbon is supposed to distribute its single bonds (x 4) to each part structure, RL-B must form a spiro-ring structure through the quarternary carbon. When the third unit (C) was tentatively fixed to combine with the quarternary carbon which is compatible with an isolated geminal coupling of the exomethylene protons, 3,8- or 5,6-bicyclic structures are accordingly given (Fig. 3-428). In the case of 3,8-bicyclic structure, the methylene protons on its cyclopropane ring should have a geminal coupling around $J = 4\sim 5$ Hz [158,159]. However, the methylene protons (δ_H 2.165 and 1.953) which would be assignable to this position possessed a coupling constant $J = 18.2$ Hz as geminal coupling. The skeleton of the compound was therefore allowed to be 5,7-bicyclic. The five-membered ring can take four different structures (structure D, E, F and G as shown in Fig. 3-428). The latter structure F and G may be rejected because they do not comply with the isoprene rule.

To identify the substitution pattern, reduction of the carbonyl group was carried out. It was expected by this experiment that not only position of the isopropyl group but also the substitution pattern around an unsaturated carbonyl group would become feasible (Scheme 3-48). Accordingly, 3.2 mg of RL-B was treated with excess $LiAlH_4$ to give 2.2 mg of a non-quenching product, RL-B-LAH (Scheme 3-49) (TLC in Fig. 3-429).



3,8-bicyclic structure

$R_1 = \text{CH}_2, R_2 = \text{O}$
or $R_1 = \text{O}, R_2 = \text{CH}_2$



5,6-bicyclic structure

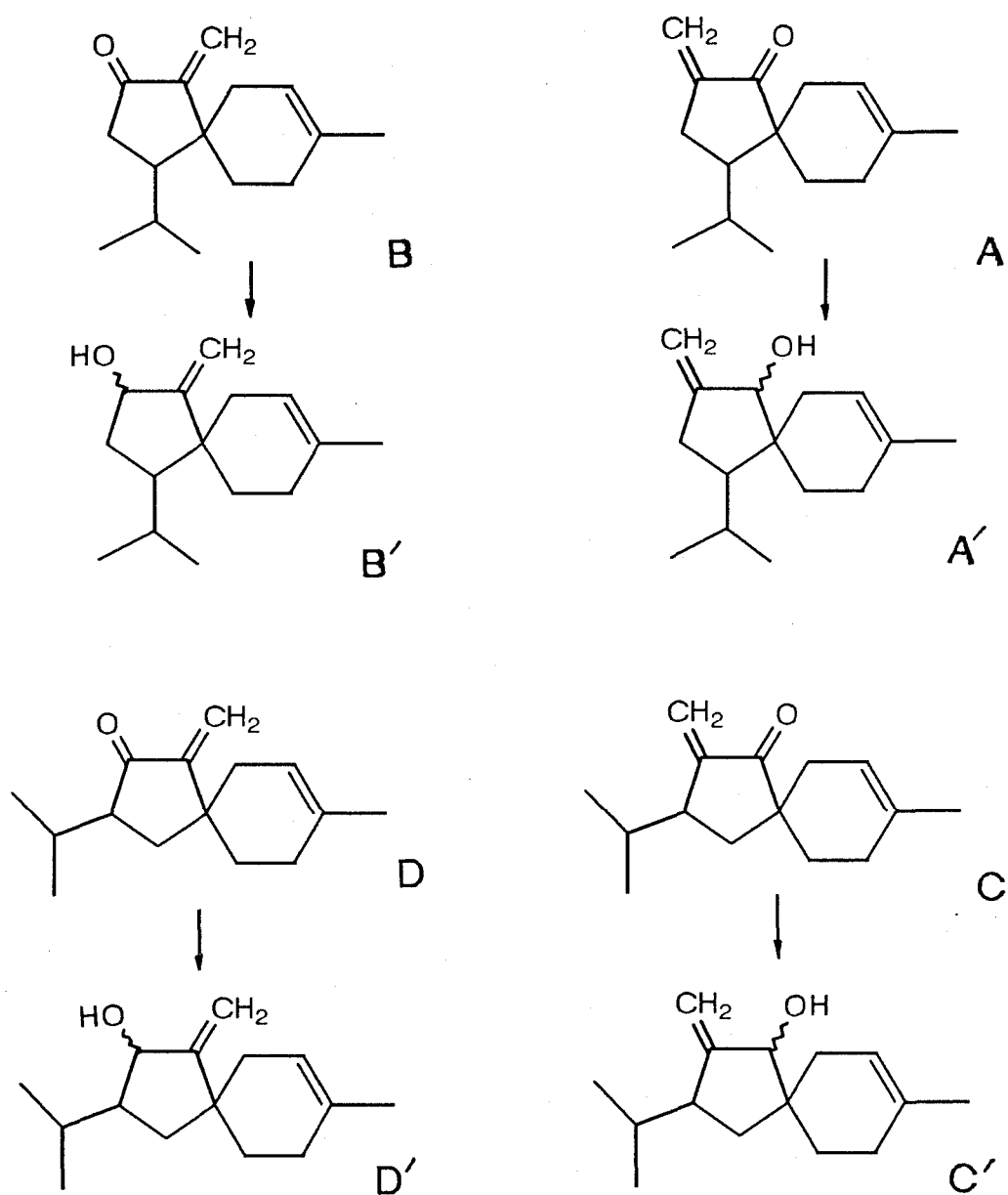
A $R_1 = \text{CH}_2, R_2 = \text{O}$

C $R_1 = \text{CH}_2, R_2 = \text{O}$

B $R_1 = \text{O}, R_2 = \text{CH}_2$

D $R_1 = \text{O}, R_2 = \text{CH}_2$

Fig. 3-428 Possible Structures for RL-B



Scheme 3-48 Newly Formed Hydroxyl Methine Proton as the Result of Reduction of the Carbonyl Group: In case the methine proton was observed as a double doublet, the structure A will be proved.

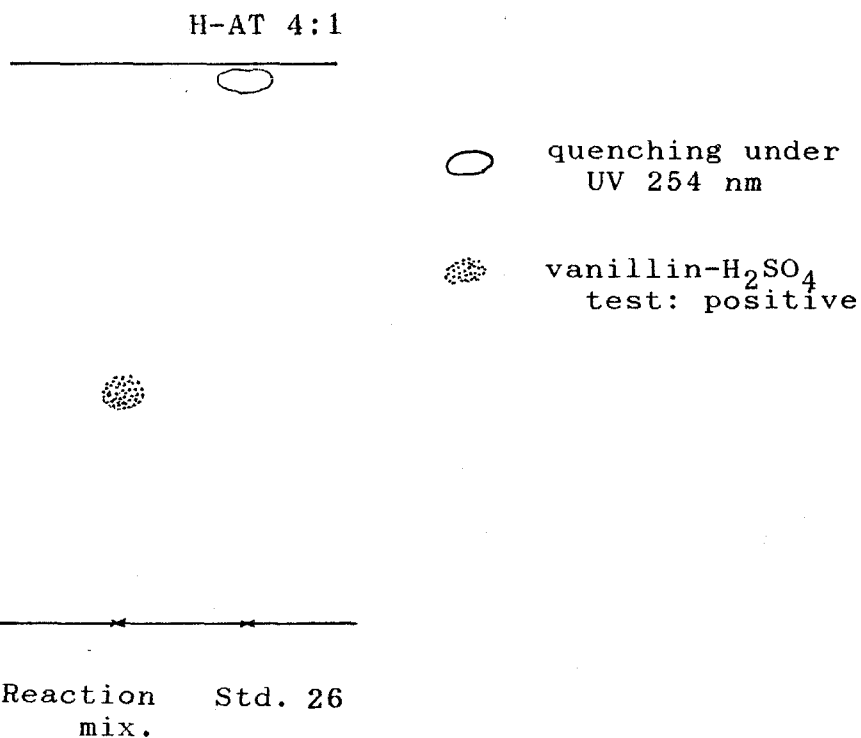


Fig. 3-429 TL Chromatogram of Reduction Product Obtained by Treatment of RL-B with LiAlH_4

RL-B (3.2 mg dissolved in 2 ml of CHCl_3)

added 20 mg of LiAlH_4
 stirred 2 hr. at room temp.
 cooled and added 5 ml of EtOAc
 diluted with 15 ml of sat. NaCl soln.
 extracted with 20 ml of EtOAc
 dried over Na_2SO_4
 concentrated *in vacuo*
 PTLC (developed in H-AT 4:1)

Product (RL-B-LAH, R_f 0.45, 2.2 mg of a colorless syrup, 68 % yield)

Scheme 3-49 Reduction of RL-B with LiAlH_4

RL-B-LAH, showing M^+ 220 in FI-MS (Fig. 3-430), was confirmed to be not a stereoisomeric mixture but a pure product in ^1H -NMR spectrum, suggesting the stereo-selective reduction at the carbonyl group (Fig. 3-431 and 432). The new methine proton on the hydroxylated carbon was observed at δ_{H} 4.449 as a broad double-doublet. Since the methylene protons on the five-membered ring, originally detected as two double-doublets, changed into two double-double-doublets on the ^1H -NMR spectrum (δ_{H} 2.224, $J = 12.7$, 8.0 and 6.5 Hz, and 1.444, $J = 12.7$, 12.3 and 7.7 Hz), the reduction product must be A' (26a). Accordingly, RL-B was elucidated as structure A (26). Other proton sequences are shown in Fig. 3-433. Thus, planar structure of RL-B was elucidated as 26 whose skeleton has been known as an acorane, and this novel acoranoid was named rosacoranone A. Interestingly, acoranoids are closely related to carotanoids as well as bisabolanoids, and some conversion schemes to obtain modified carotanoids from simple carotanoids *via* acoranoids are known, in the area of organic synthesis (Scheme 3-50) [158,159].

* ^{13}C -NMR Spectra of RL-B-LAH are shown in Fig. 3-434.

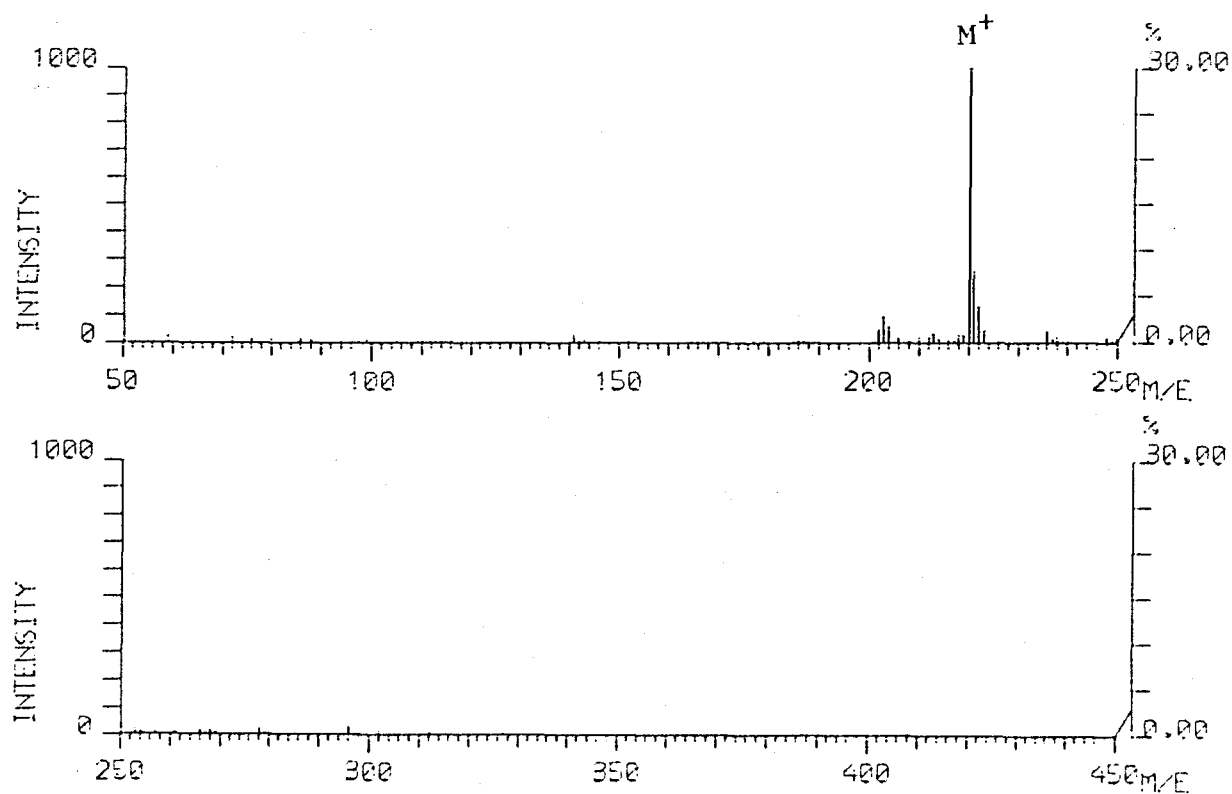


Fig. 3-430 FD-Mass Spectrum of RL-B-LAH

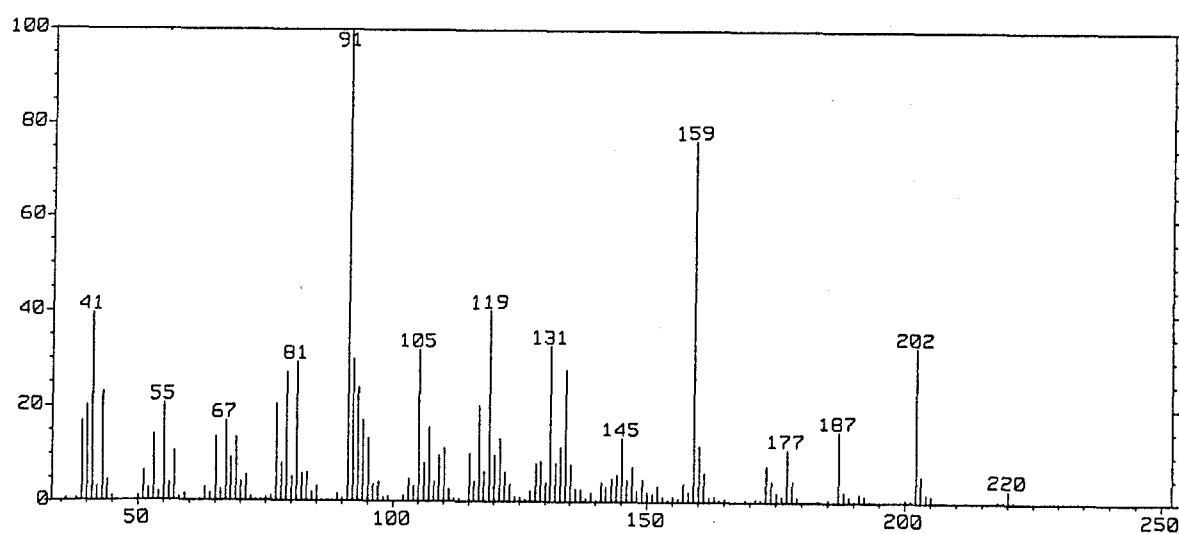


Fig. 3-431 EI-Mass Spectrum of RL-B-LAH

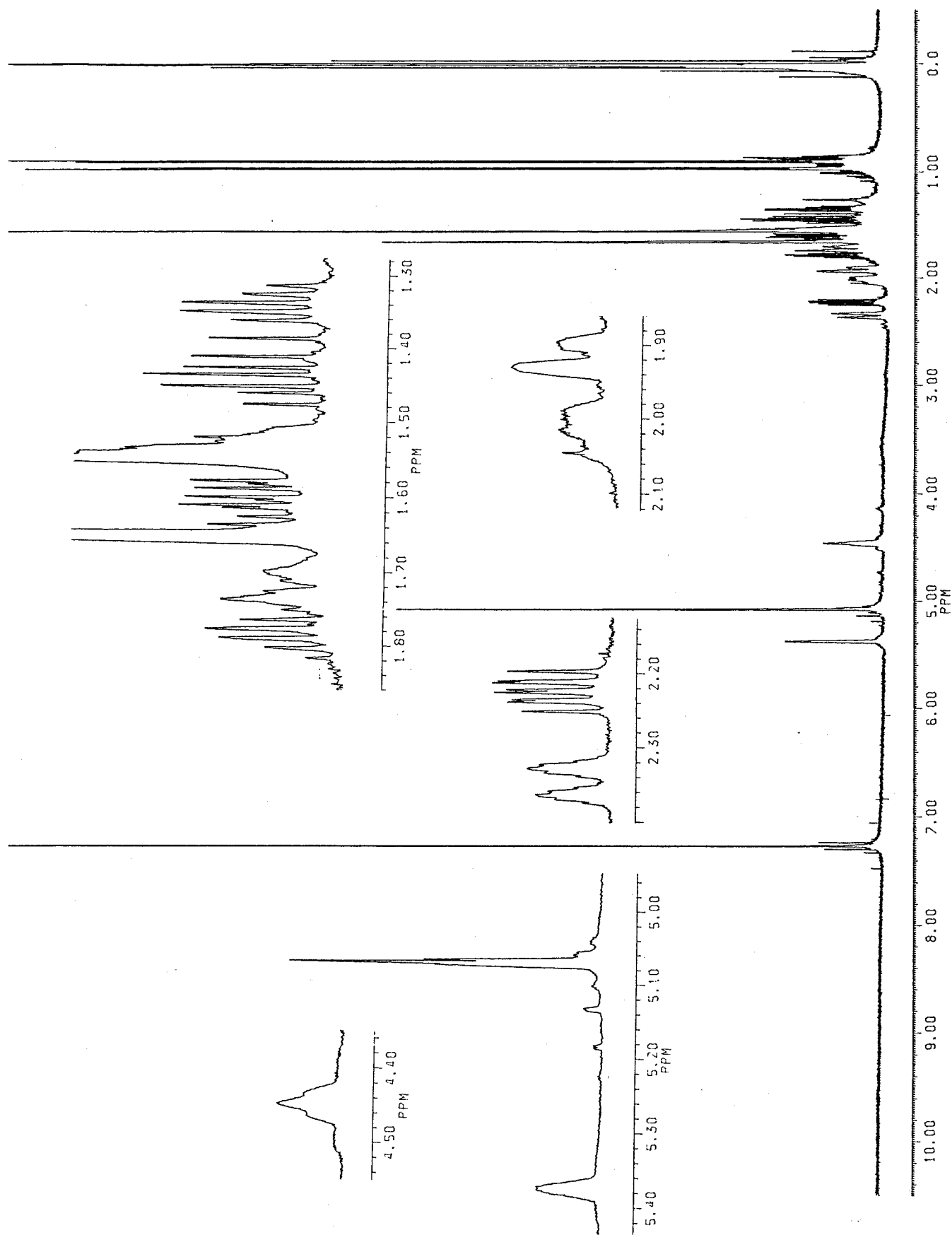


Fig. 3-432 ^1H -NMR Spectrum of RL-B-LAH (500 MHz, in CDCl_3)

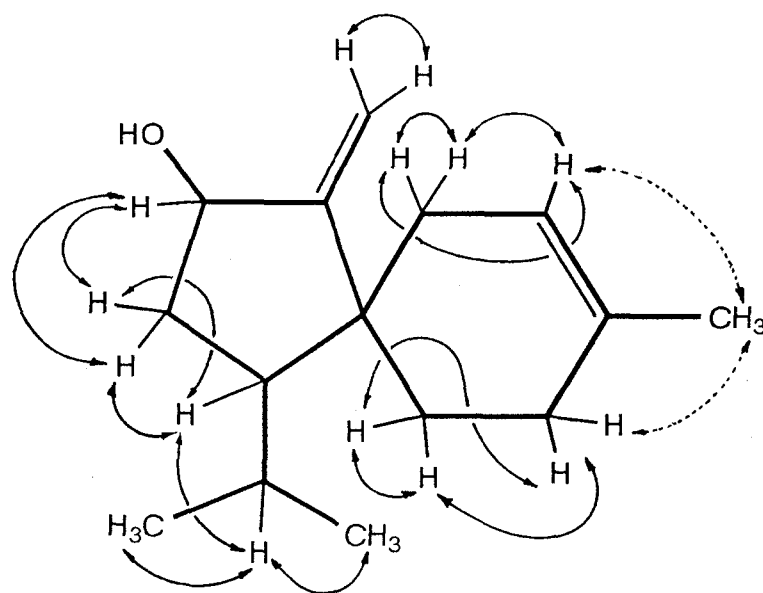


Fig. 3-433 Proton Sequences and Carbon Assignments of RL-B-LAH

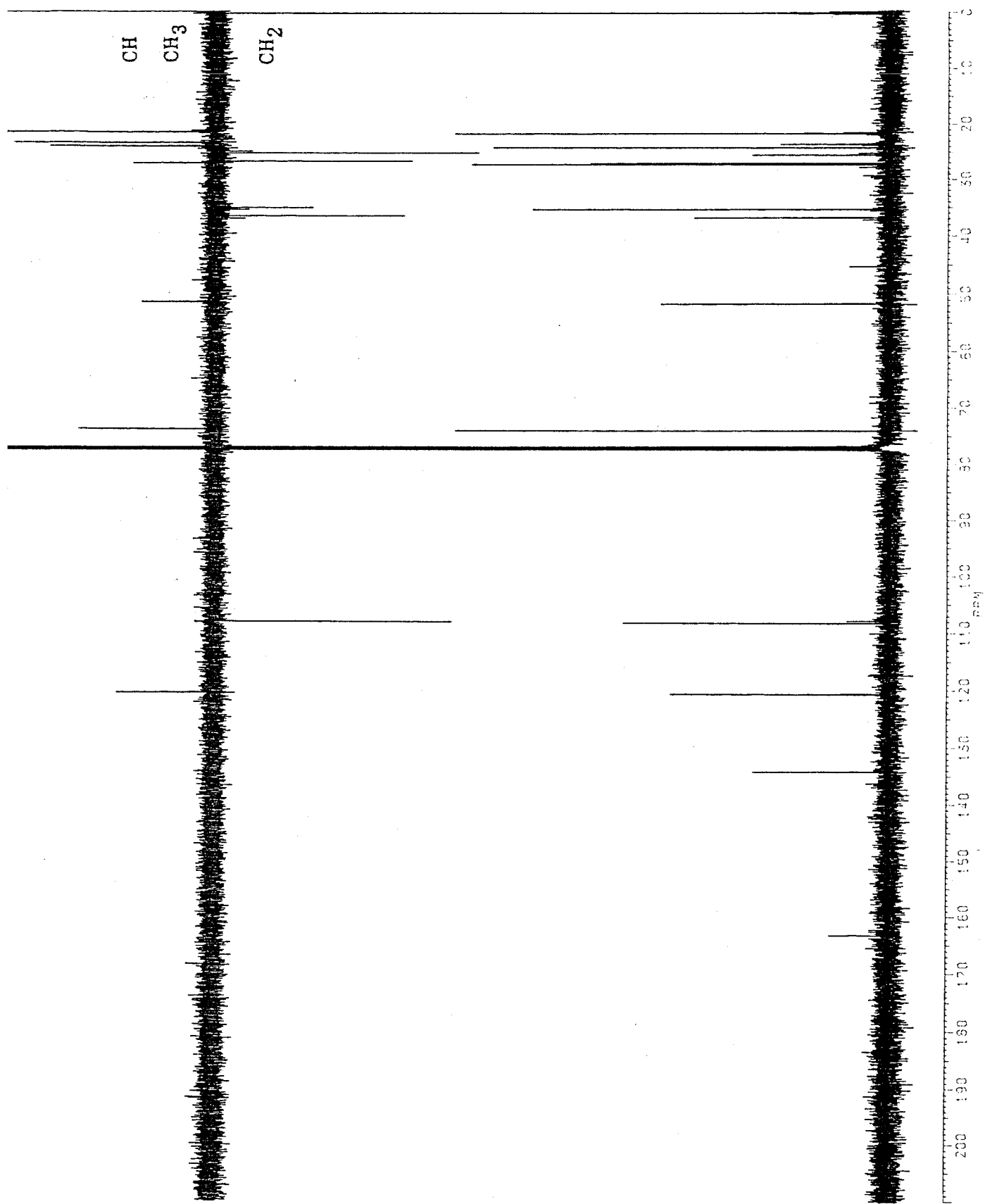
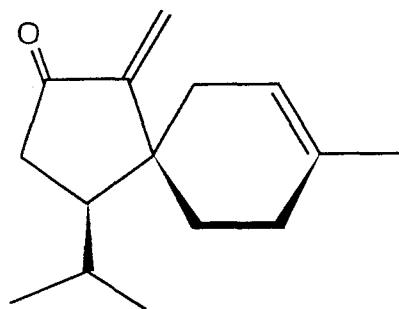


Fig. 3-434 ^{13}C -NMR Spectra of RL-B-LAH (125 MHz, in CDCl_3 , COM and DEPT)

Table 3-170 Physicochemical properties of rosacoranone (= RL-B,
26)



26

A colorless syrup

Vanillin-H₂SO₄ color: brown

FI-MS m/z (%): 219 (M^+ , 19), 218 (M^+ , 100)

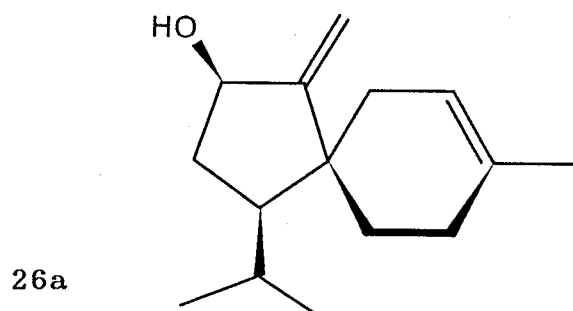
EI-HR-MS: 218.184 (C₁₅H₂₂O, calcd. 218.188)

GC-MS m/z (%): 219 (M^++1 , 13), 218 (M^+ , 75), 203 (37), 190 (27),
177 (16), 176 (89), 175 (77), 161 (22), 147 (51), 133
(43), 120 (20), 108 (100), 107 (45), 105 (48), 93 (22), 92
(21), 91 (39), 80 (26), 79 (43), 77 (39), 68 (72), 67
(31), 55 (25), 53 (25), 43 (43), 41 (54).

EI-MS m/z (%): 218 (M^+ , 34), 203 (32), 190 (22),
176 (69), 175 (67), 161 (23), 147 (52), 133 (48), 120
(21), 119 (34), 108 (100), 107 (44), 105 (75), 93 (34), 92
(25), 91 (62), 80 (32), 79 (56), 77 (52), 68 (84), 67
(44), 55 (41), 53 (34), 43 (60), 41 (79).

¹H- and ¹³C-NMR data are shown in Table 3-167 and 3-168,
respectively.

Table 3-171 Physicochemical properties of RL-B-LAH (26a)



A colorless syrup

Rf: 0.45 (H-EA 4:1)

Vanillin-H₂SO₄ color: pinkish purple

EI-MS *m/z* (%): 218 (M⁺, 34), 203 (32), 190 (22),

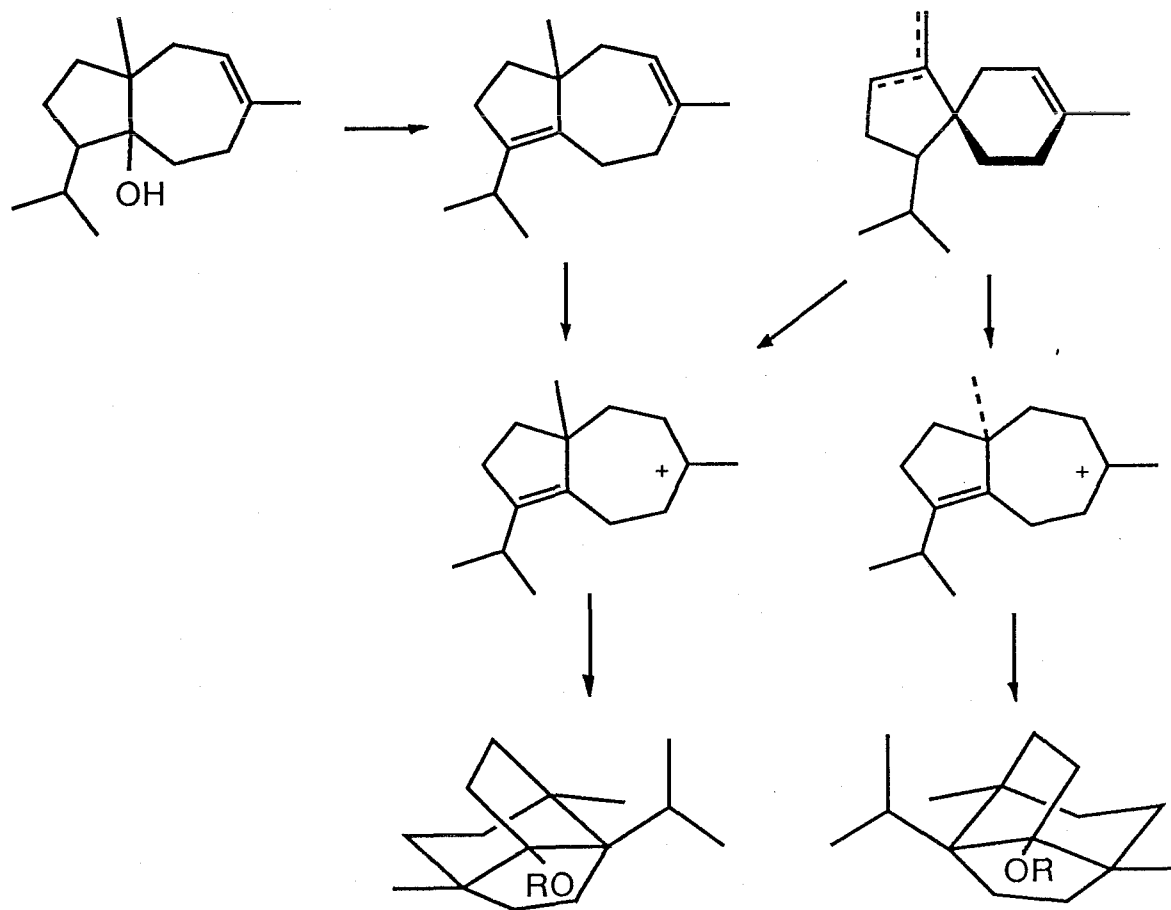
176 (69), 175 (67), 161 (23), 147 (52), 133 (48), 120

(21), 119 (34), 108 (100), 107 (44), 105 (75), 93 (34), 92

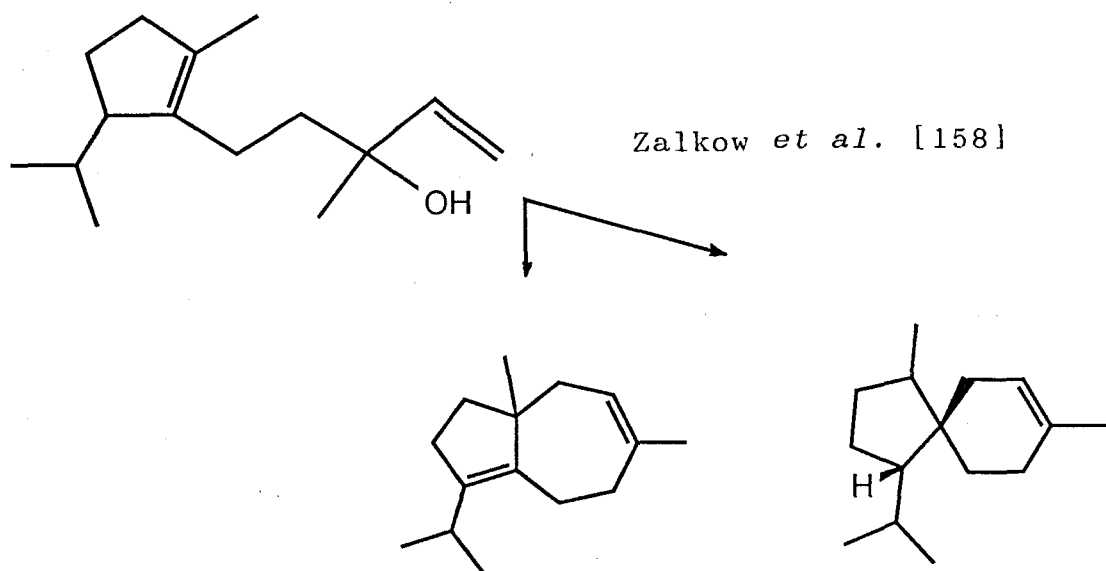
(25), 91 (62), 80 (32), 79 (56), 77 (52), 68 (84), 67

(44), 55 (41), 53 (34), 43 (60), 41 (79).

¹H-NMR δ_{TMS}^{C₆D₆} (500 MHz): 1.608 (1H, ddd, *J*= 12.9, 10.8 and 5.3 Hz, C-2-Ha), *ca* 1.53 (1H, partly overlapped, C-2-Hb), *ca* 2.02 (1H, br. m, C-3-Ha), 1.913 (1H, br. d, *J*= *ca* 19 Hz, C-3-Hb), 5.375 (1H, br. m, C-5-H), 2.345 (1H, br. d, *J*= 17.9 Hz, C-6-Ha), 1.720 (1H, d, *J*= 17.9 Hz, C-6-Hb), 4.449 (1H, br. t-like, *J*= *ca* 8 Hz, C-8-H), 2.224 (1H, ddd, *J*= 12.7, 8.0 and 6.5 Hz, C-9-Ha), 1.444 (1H, ddd, *J*= 12.7, 12.1 and 7.7 Hz, C-9-Hb), 1.338 (1H, ddd, *J*= 12.1, 6.5 and *ca* 6 Hz, C-10-H), 1.784 (1H, d sept, *J*= 6.8 and *ca* 6 Hz, C-11-H), 0.961 (3H, d, *J*= 6.8 Hz, C-12-H₃), 0.897 (3H, d, *J*= 6.8 Hz, C-13-H₃), 1.653 (3H, br. s, C-14-H₃), 5.072 (1H, d, *J*= 1.8 Hz, C-15-Ha), 5.069 (1H, d, *J*= 1.8 Hz, C-15-Hb).



Naegeli and Kaizer [159]



Zalkow *et al.* [158]

Scheme 3-50 Some examples for chemical conversions to show carotane-acorane relationships

3-9-2 Relative Configuration of Rosacoranone

Acoranoids are known to be difficult to determine their absolute or even relative configurations. When a NOESY experiment was carried out on rosacoranone (26), some NOEs were observed (Fig. 3-435). However, those were not informative in relation to the relative configuration of 26. RL-B-LAH (26a) was therefore used for the stereostructure determination. In C_5D_5N , protons coplanar to the C-8 hydroxyl group are expected to be deshielded [160]. As a matter of fact, C-8-H, C-15-Ha and C-9-Hb were strongly ($\Delta\delta_H$ 0.4~0.3), and C-15-Hb, C-3-Ha and C-2-Hb were slightly ($\Delta\delta_H$ 0.2~0.1) deshielded in C_5D_5N (Fig. 3-436). As C-10 methine proton was utterly unchanged its chemical shift, it was considered as antiplanar (Table 3-172). NOEs between C-12- H_3 and C-2-Ha and C-13- H_3 and C-9-Hb were, together with the deshielding of C-2-Hb and C-3-Hb, suggestive their coplanar position. Consequently, relative configurations of 26a and 26 were elucidated as shown in Fig. 3-437.

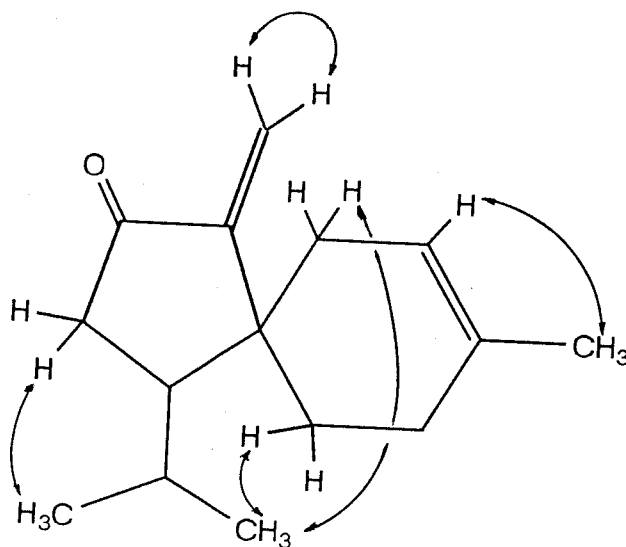


Fig. 3-435 NOEs Observed in RL-B (NOESY in C_6D_6 , 500 MHz)

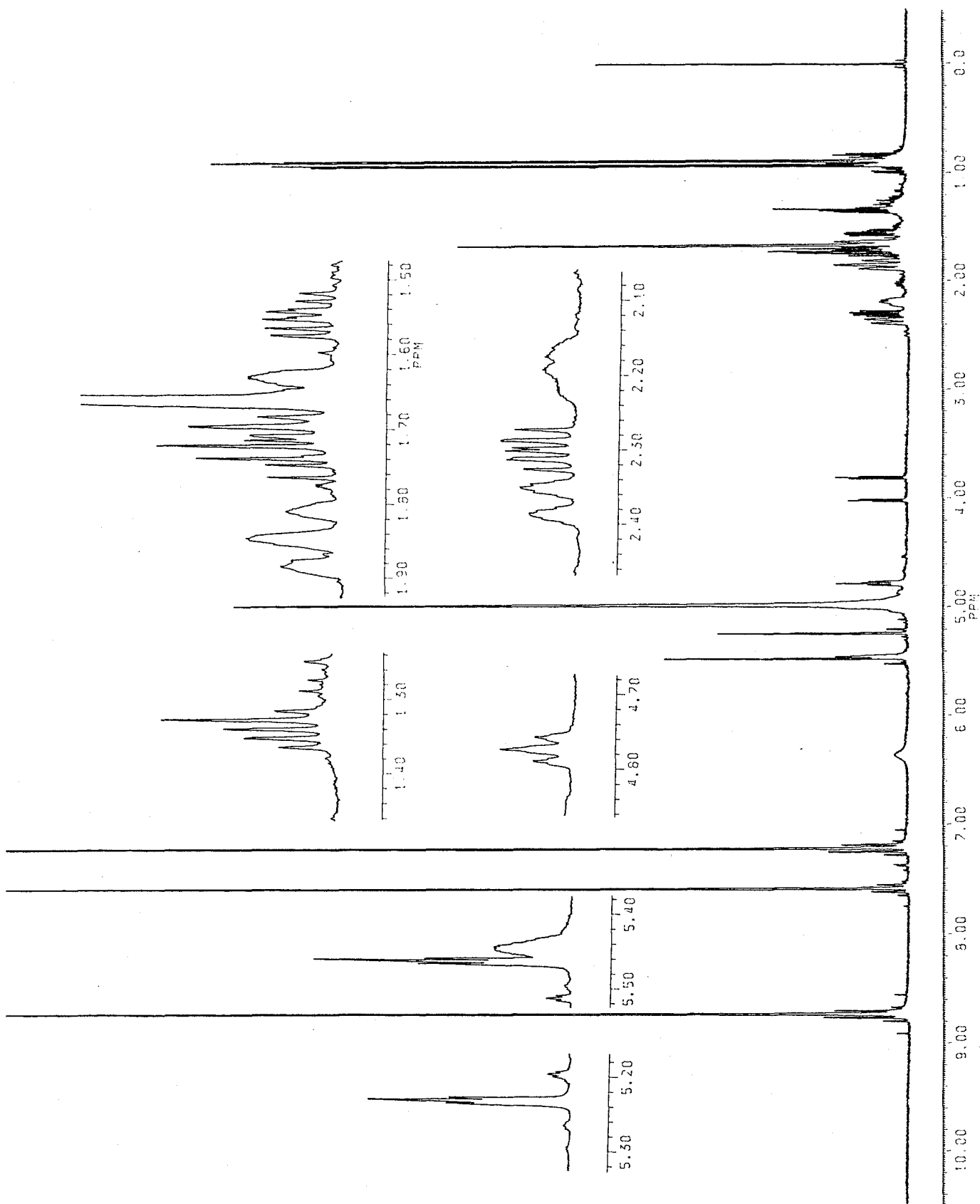
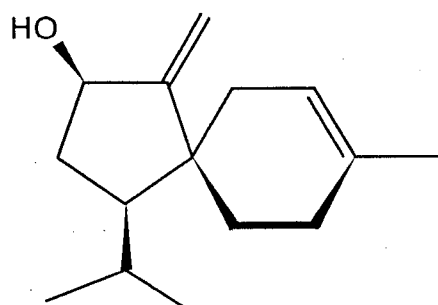


Fig. 3-436 ^1H -NMR Spectrum of RL-B-LAH (500 MHz, in $\text{C}_5\text{H}_5\text{N}$)

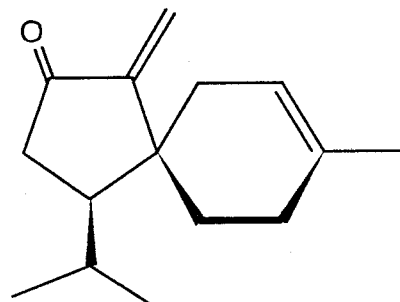
Table 3-172 Deshielding effect of RL-B-LAH in C_5D_5N

in C_5D_5N	C-No	in $CDCl_3$	$\Delta\delta_H$ (ppm)*
2.369 br. d (17.9)	2Ha	2.345	+ 0.02
1.828 br. d (17.9)	2Hb	1.720	+ 0.09
5.445 br. m	3H	5.375	+ 0.07
2.19 (approx.) m	5Ha	2.02	+ 0.17
1.87 (approx.) m	5Hb	1.913	+ 0.04
1.547 ddd (12.6, 11.0, 5.9)	6Ha	1.608	- 0.06
1.65 (approx.) m	6Hb	1.53	+ 0.12
4.773 br. dd (8.1, 7.9)	8H	4.449	+ 0.32
2.300 ddd (12.5, 7.6, 6.8)	9Ha	2.224	+ 0.08
1.732 ddd (12.3, 12.3, 8.4)	9Hb	1.444	+ 0.31
1.340 ddd (12.0, 6.0, 6.0)	10H	1.338	$\pm$ 0
1.704 d sept (6.8, ca 6)	11H	1.784	- 0.08
0.925 d (6.8)	12H ₃	0.961	- 0.03
0.883 d (6.8)	13H ₃	0.897	- 0.01
1.664 br. s	14H ₃	1.653	+ 0.01
5.464 d (1.8)	15Ha	5.072	+ 0.39
5.233 d (1.8)	15Hb	5.069	+ 0.16
6.36 (approx.) br	8-OH	-	-

$$* \Delta\delta = \delta_{H_{TMS}}^{C_5D_5N} - \delta_{H_{TMS}}^{CDCl_3}$$



RL-B-LAH



RL-B

Fig. 3-437 Relative Stereostructures RL-B and Its Derivative
 $rel.1R^*$, $10R^*$ for RL-B and $rel.1R^*$, $8R^*$, $10R^*$ for RL-B-LAH

3-9-3 Relation between Bisaborosaol A and Rosacoranone

Acoranoids are found in some monocots as their common sesquiterpenoids [161,162]. On the other hand, in dicots the sesquiterpenoids are comparatively rare, and those sources are mostly primitive dicot such as *Chloranthus* plants [163] (Fig. 3-438). Thus, the presence of an acoranoid in Rosaceae which belonging to a rather evolved dicot is an interesting fact from the view point of chemotaxonomy. Although acoranoids are biochemically synthesized from *cis-trans*-farnesylpyrophosphate via bisabolanoids [63], 26 has a different oxygenation pattern from that of bisabolanoids originating in *Rosa rugosa*. Carotanoids from *Rosa rugosa* are, without exceptions, resio-specifically oxygenated at C-14 as well as the bisabolanoid C-7 carbon which is biogenetically equivalent to C-14 in carotanoids and acoranoids. In *Rosa rugosa* leaves, sesquiterpenoid is considered to take the biogenesis as shown in Scheme 3-51. Significance and function of this minor sesquiterpene are not known.

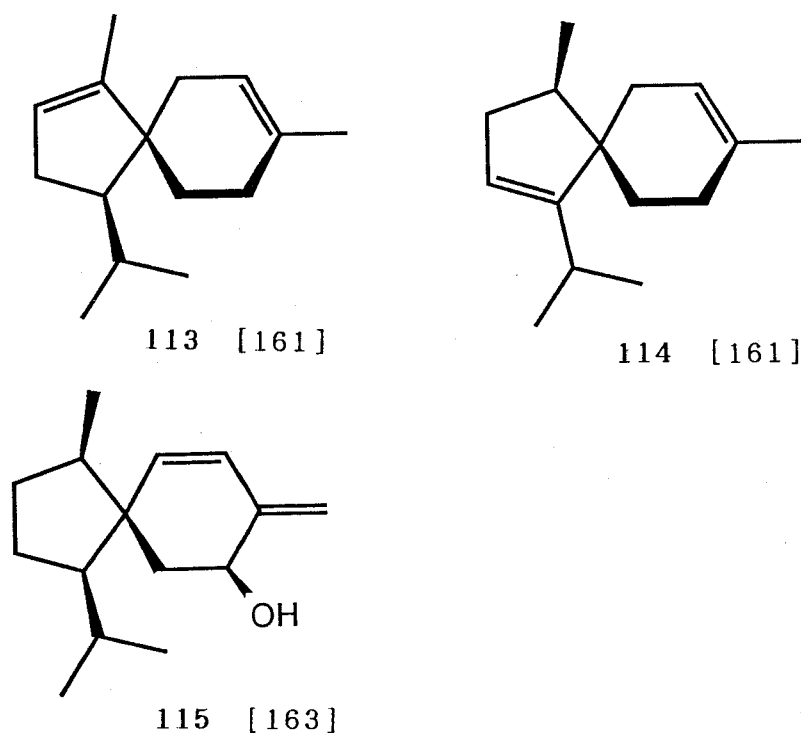
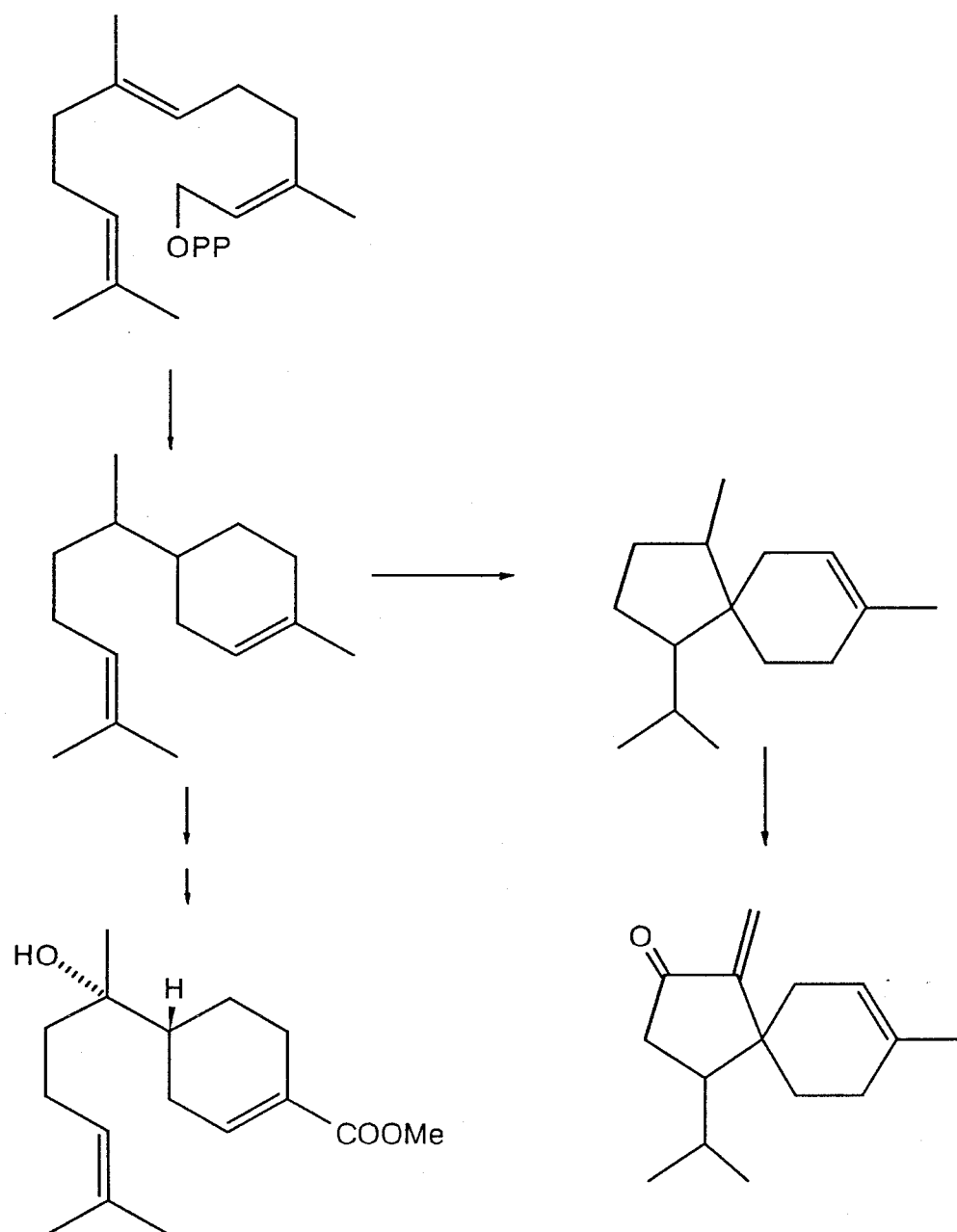


Fig. 3-438 Some Typical Naturally Occurring Acoranoids



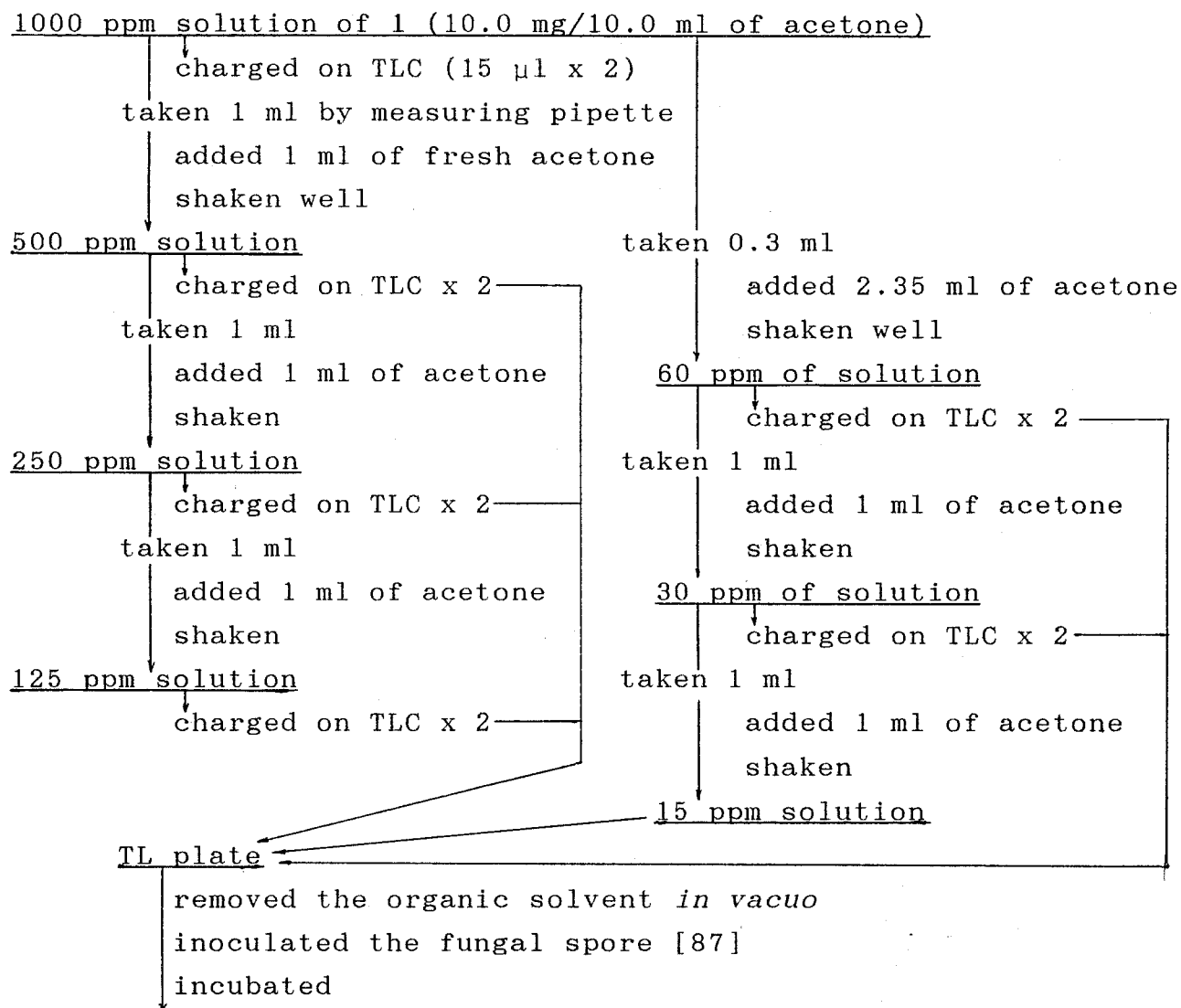
Scheme 3-51 Biochemical Relationship between Bisaboranoids and Acoranoid

3-10 Bioassay

3-10-1 Antimicrobial Activity of Rugosal A

1) TLC Bioautography

The antifungal activity of rugosal A (1) was tested by TLC bioautography using *Cladosporium herbarum* HU 9262. Pure crystallines of 1 were dissolved in acetone to be 1000 ppm (10.0 mg/10.0 ml), and 15 μ l of the solution was taken by a 50 μ l microsyringe and charged onto silica gel thin-layer plates (0.25 mm thickness) to form a circular zone ca 10 mm in diameter (ca 0.8 cm²). Successively, 1 ml of the original solution was steadily taken in a test tube by measuring pipette, and an equal volume of fresh acetone was added to the solution. The diluted solution (500 ppm) was similarly charged on the plate. Accordingly, several solutions ranged from 1000 ppm to 15 ppm of 1 was prepared as shown in Scheme 3-52. The amount of 1 charged on the area was calculated as listed on Table 3-173. Charges of the test solution and acetone as a control experiment were duplicated.



Scheme 3-52 Procedures for TLC-bioautography

Table 3-173 Prepared concentrations of rugosal A solution and each calculated weight/charged area

concn. (ppm)	1000	500	250	125	60	30	15	0
weight (µg)	15	7.5	3.8	1.9	0.9	0.5	0.2	0

After the charged TLC plates were dried enough *in vacuo*, the spore suspension (See Chapter 2-1) was uniformly sprayed on the test plate, which were kept at 25 °C in the dark under a moistured condition. After 3 days incubation, 1 exhibited clear inhibitory zones in the range from 15 µg to 0.9 µg/ 0.8 cm², and even with 0.45 µg (15 µl of 15 ppm solution), this substance showed a marked retardation of the fungal growth (Fig. 3-439 and Table 3-174).

Table 3-174 Antifungal activity of rugosal A in TLC
bioautography

weight (µg)	15	7.5	3.8	1.9	0.9	0.5	0.2	0
activity*	+++	+++	++	+	+	±	-	-

* +++ : a severe inhibition of growth (the antifungal zone is wider than the charged area), ++ : a clear inhibition of growth (the fungal area is corresponded to the charged area), + : a clear retardation of growth, ± : ambiguous retardation of growth, - : negative effect.

2) Paper Disc Method

Successively, the antimicrobial activity of rugosal A (1) was tested by the paper disc method, using *Aspergillus flavus*, *Cladosporium herbarum*, *Saccharomyces cereviciae*, *Staphylococcus aureus* and *Echerichia coli* as test microorganisms. The result was, as indicated in Table 3-175, not clear. It was obvious that under a nutrient-rich condition 1 is not toxic against *Echericha coli*. On the basis of this consideration, agar plates only containing defatted extracts of *Rosa rugosa* fruits were prepared, and antimicrobial assay was again tested using this rugosa rose-medium.

As shown in Table 3-176, the growth inhibition of the test microbe was markedly heightened.

In this series of assay, a gram-positive bacterium *Staphylococcus aureus* showed a clear but only thin inhibitory zone on PYG-agar culture (glucose:pepton:yeast extract= 5:1:0.1). However, in another assay carried out in Japan Tobacco Industry K.K., 1 showed a clear toxicity against *Bacillus subtilis* in the concentration of 25 µg.

3) Spore Germination Assay

According to the result of antimicrobial test against several phytopathogenic microbials (Table 3-177 by Hokko Kagaku Kogyo K.K.), 1 exhibited remarkable toxicity against the following fungus; *Pyricularia oryzae*, *Botrytis cinerea*, *Colletotrichum lagenarium* and *Aspergillus niger*, and bacterium *Xanthomonas campestris* pv. *oryzae*.

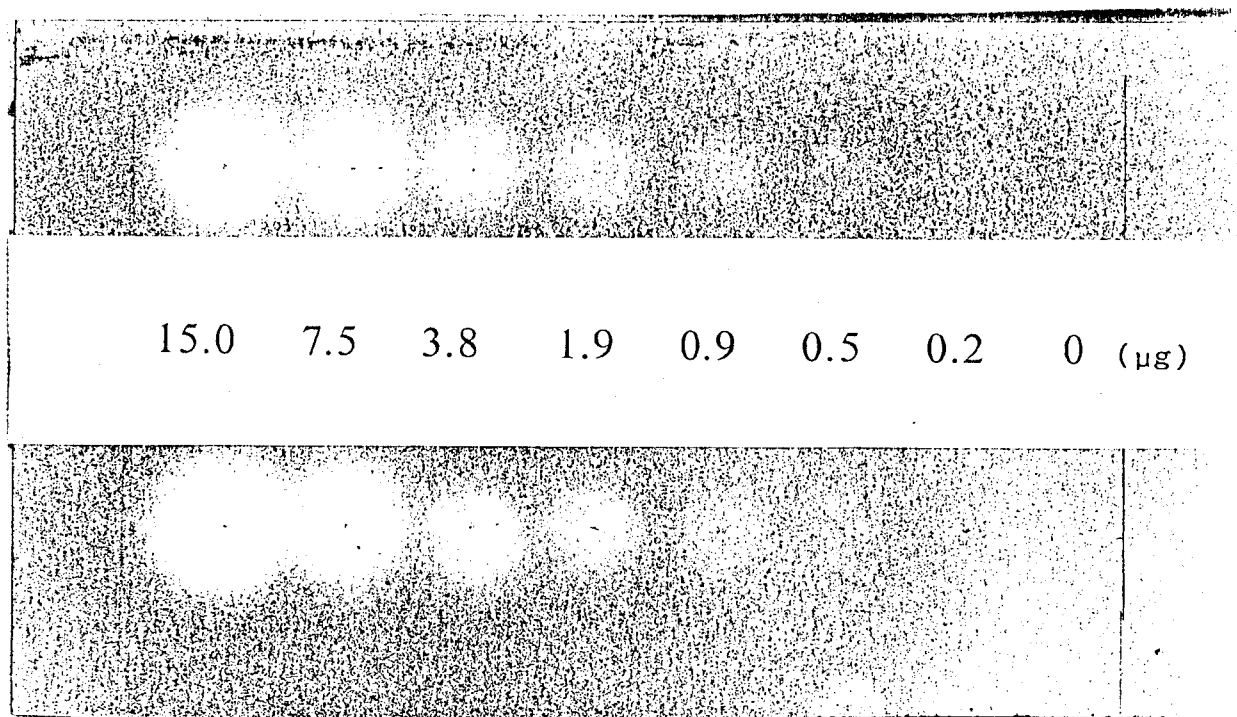


Fig. 3-435 TLC-bioautography for Several Concentrations of Rugosal

A

Table 3-175 Rugosal A antimicrobial test with paper disc method

Microbe	Concentration ($\mu\text{g}/\text{disc}$)	200	50	12.5	3	0
<i>Cladosporium herbarum</i>	+++	++	+	-	-	-
<i>Saccharomyces cerevisiae</i>	+	-	-	-	-	-
<i>Staphylococcus aureus</i>	++	+	-	-	-	-
<i>Escherichia coli</i>	+	-	-	-	-	-

Table 3-176 Rugosal A antimicrobial test on rugosa rose fruit culture.

Microbe \ Concentration(ug)	100	50	25	12.5	6.3	3.1	1.6	0
<i>Aspergillus flavus</i>	++		++		-		-	-
<i>Cladosporium herbarum</i>	+++	++	++	+	+	-	-	-
<i>Saccharomyces cerevisiae</i>	++++	+++	++	++	+	-	-	-
<i>Staphylococcus aureus</i>			no growing					
<i>Escherichia coli</i>			no growing					
+ glucose								
<i>Cladosporium herbarum</i>	++		+		-		-	-
<i>Saccharomyces cerevisiae</i>	++++		++		+		-	-
+ peptone								
<i>Staphyrococcus aureus</i>			poor growing					
<i>Escherichia coli</i>			poor growing,					

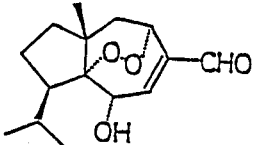
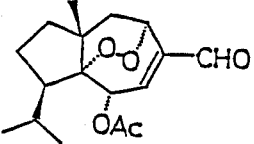
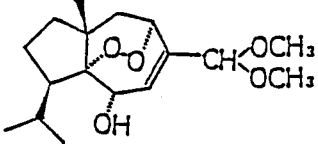
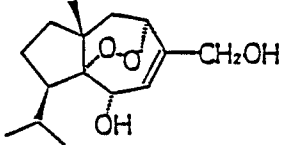
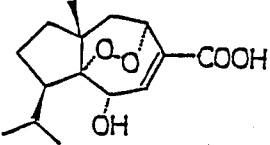
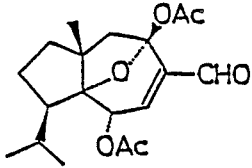
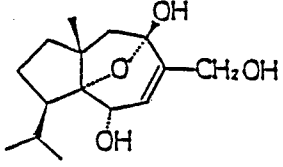
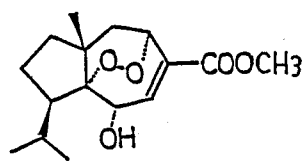
Table 3-177 Inhibitory activity of rugosal A on spore germination
of several phytopathogens

	spore germination						MIC (ppm)
	50	25	12.5	6.3	3.1	1.56	
<Fungi>							
Alternaria kikuchiana	+	+~++	+++	+++	+++	+++	>50
Aspergillus niger	-	-	--~±	±	+~++	+++	25
Botrytis cinerea	-	±	±~+	+~++	++~+++	+++	50
Cercospora beticola	±	±	±~+	+++	+++	+++	>50
Cladosporium cucumerinum	-	+~++	+++	+++	+++	+++	50
Cochliobolus miyabeanus	±	+	+++	+++	+++	+++	>50
Colletotrichum lagenarium	-	-	±~+	+++	+++	+++	25
Fusarium oxysporum f. sp. cucumerinum	+	++	+++	+++	+++	+++	>50
F.roseum	-~±	+~++	+++	+++	+++	+++	>50
Glomerella cingulata	±~+	++	+++	+++	+++	+++	>50
Gibberella fujikuroi	+	++	+++	+++	+++	+++	>50
Mycosphaerella melonis	±	++	+++	+++	+++	+++	>50
Pyricularia oryzae	-	-	-	-	+++	+++	6.3
Penicillium digitatum	±	±	+++	+++	+++	+++	>50
Rhizopus nigricans	±	+	+	++	++	+++	>50
<Bacteria>							
Pseudomonas avenae	+++	+++	+++	+++	+++	+++	>50
P. glumae	+++	+++	+++	+++	+++	+++	>50
P. solanacearum	+++	+++	+++	+++	+++	+++	>50
P. syringae pv. phaseolicola	++~+++	++~+++	+++	+++	+++	+++	>50
P. syringae pv. lachrymans	++~+++	++~+++	++~+++	+++	+++	+++	>50
Erwinia carotovora subsp. carotovora	++~+++	++~+++	++	+++	++~+++	++~+++	>50
Xanthomonas campestris pv. citri	++~+++	+++	+++	+++	+++	+++	>50
X. campestris pv. oryzae	-	-	-	±	++~+++	++~+++	12.5
Corynebacterium michiganense	+++	+++	+++	+++	+++	+++	>50
A. tumefaciens	+++	+++	+++	+++	+++	+++	>50

3-10-2 Structure-Activity Correlation of Rugosal A

Some derivatives of rugosal A (1) was also tested its antifungal activity by TLC-bioautography (Table 3-178). As the result, requirement of the fundamental structure of 1 became feasible. Inactivity of rugosic acid A (2) was suggestive that the polarity is important in the exhibition of antifungal activity. The endoperoxy group seems necessary, since peroxide rearranged one lost its activity at least the test derivatives. On the other hand, without α,β -unsaturated aldehyde group, RSA-NBH-1 (1c) and RSA-HCL (1g) were able to exhibit the activity, while RSA-AC-1 (1a) was almost inactive suggesting C-2-OH is also necessary to the activity. Those results were informative to plan the search of precursors and metabolites.

Table 3-178 Antifungal activities of some rugosal A derivatives

Dose Derivative	(μ g)	100	25	10	2
		+++	+++	++	+
		+	-	-	-
		+++	++	+	+
		+++	++	+	+
		±	-	-	-
		++	+	±	-
		+	-	-	-
		+++	++	+	+

Each derivatives dissolved in 15 μ l of EtOH was charged on 0.25 mm silica gel plates.

3-10-3 Antifungal Activity of Bisaborosaols

As tested in rugosal A (1), antifungal activity of bisaborosaol A (19), B1 (21) and B2 (22) was also examined by the TLC-bioautography. The results were showed in Table 3-179. Not only 17 but also 19 and 21 exhibited an antifungal activity; however, the values of the three were 10 more time lower than that of 1 in this assay.

Table 3-179 Prepared concentrations of bisaborosaol A, B1 and B2 solutions and each antifungal activity

concentration (ppm)	6600	3300	1650	830	410	210	0
weight (μg)	100	50	25	13	6	3	0
activity* 19	++	++	+	±	±	-	-
21	++	++	+	±	±	-	-
22	++	++	+	±	±	-	-
cf. 1	++++	++++	+++	+++	++	++	-

* activity is according to Table 3-174

3-10-4 Cytotoxic Assay of Rugosal A and Its Related Compounds

Cytotoxicity of 1 and 2 was also tested, using the P-388 ADR^S mouse cell. ID₅₀ of 1 against the cell was 0.54 (µg/ml). On the other hand, 1 exhibited ID₅₀ 0.21 against another cell line resistant to adriamycin (P-388^r). Those results suggested that 1 possesses non-selective cytotoxicity, although the activity was not so remarkable compared with some antibiotics. Compound 3 was comparatively less active.

3-10-5 Antifeedant Assay of Rugosal A

Rugosal A (1) did not showed any acute toxicity nor anti-feedant activity against test larva (See pp. 43). The test worms tended to avoid foods containing 1 when they are not starved; however, under a starved condition they fed the foods and did not show any physiological changes. Compound 1 was therefore considered to be a passive-typed retardant.

Chapter 4 Summary and Conclusion

4-1 Summary of the Thesis

Rosa rugosa is known to be a rose strongly resistant to diseases. From the leaves of *Rosa rugosa*, an antifungal sesquiterpene, rugosal A (1) was isolated and elucidated its structure including the absolute configuration. Rugosal A (1) initially isolated from damaged leaves of *Rosa rugosa* exhibited its structure as a carotane type of sesquiterpene aldehyde which possessed a unique 1,4-endoperoxide bridge whose oxygen had intramolecular hydrogen-bond with an allylic hydroxyl group. This α,β -unsaturated aldehyde showed a remarkable antifungal activity on TLC bioautography using *Cladosporium herbarum*. Several derivatives of 1 were also prepared. As the related carotane acid, rugosic acid A (2) was also found. This acid was accumulated in the tissues in a considerable amount, although it was non-fungitoxic. This acid provided some important informations for structural elucidation of 1. These carotane peroxides showed some characteristic chemical rearrangements at the peroxide bonds, under a basic or an acidic condition. Their reaction mechanisms were further discussed.

In the course of searching the precursors of 1, carota-1,4-dienaldehyde (3) was isolated. This compound was quite unstable to air, and gave 1 and 2 through an intermediate, endo-exoperoxy carotane aldehyde 3e. By the structural analyses of the intermediary compound and some byproducts in the oxygenation reaction of 3, conversion scheme of 3 into 1 was proposed. On the other hand, metabolic pathway of 1 through 2 was also examined. As the result, some peroxy-bond rearranged carotane acids were isolated in the mature leaves, and it was suggested that peroxide rearrangement of 2 was the main pathway in the metabolism of the

carotane peroxides. Furthermore, several minor carotanoids were also isolated and their structures were elucidated. Thus, biogenesis of the carotanoids in *Rosa rugosa* leaves was also suggested.

Not only carotanoids, but also bisabolanoids were contained in the leaves. Bisaborosaol A (19) and its oxygenated forms were found as a major constituents of the plant. These bisabolanoids gave an evidence showing a correlation between bisabolanoids and carotanoids in the biosynthesis from *cis-trans*-farnesyl pyrophosphate. In addition, an acoranoid was also isolated as a minor constituent of *Rosa rugosa*.

Rugosal A (1) and rugosic acid A (2) exhibited a drastic change in amount according to the seasons. In the flowering season, the amount of the carotane peroxides became the highest. It was calculated that 2 and 1 should exist in the most active tissues at a concentration of more than 500 ppm (1, 200 ppm). As an antifungal activity of 1 against *Cladosporium herbarum* was clearly visible at the concentration of 125 ppm on TLC plate, it seemed that 1 could function as a defensive agent in *Rosa rugosa* leaves.



5-2 Conclusion

When *Rosa rugosa* leaf was mechanically damaged (and also chemically damaged by NaCN solution), a typical browning is observed on the damaged part. This browning, due to hypersensitive cell disruption and accompanying accumulation of polyphenols, is known to be a defense system for some plants. In the case of *Rosa rugosa*, this drastic browning probably contributes to its resistance against several pathogens. Indeed, some non-resistant roses show only a slight browning by these kinds of damaging. However, the author dearly proposes that the hypothetical disease resistance factor of *Rosa rugosa* is mostly due to rugosal A (1) which is markedly rich in the leaves and exhibits a considerably antifungal activity. In addition, other sesquiterpenes in the leaves may also be regarded as the resistance factor.

One of the experimental evidence for the proposal is that other roses, particularly some rose cultivars, were quite poor in sesquiterpenoids. In another series of studies, the author examined sesquiterpenoids of some other *Rosa* plants [69]. In TLC, GC and GC-MS analyses, it revealed that *Rosa acicularis*, *Rosa woodsii* and *Rosa acicularis* var. *nipponesis* contained some bisabolanoids but no carotanoid, and some other wild roses were rich in monoterpenoids but very poor in sesquiterpenoids. On the other hand, cultivated roses examined so far were very poor in these terpenoids, suggesting that the resistance of wild roses are due to those chemicals. Some *Rosa rugosa* hybrids examined so far (such as Hansa) were all contained rugosal A in a marked amount.

The antifungal activity of the *Rosa rugosa* leaf could be found, so far as the author has tested, apparently less than 10 mg of fresh leaves. When the EtOAc extract from fresh leaves was diluted in various concentrations and each of the extracts was developed on TLC in a specific solvent, the compound 1 in the

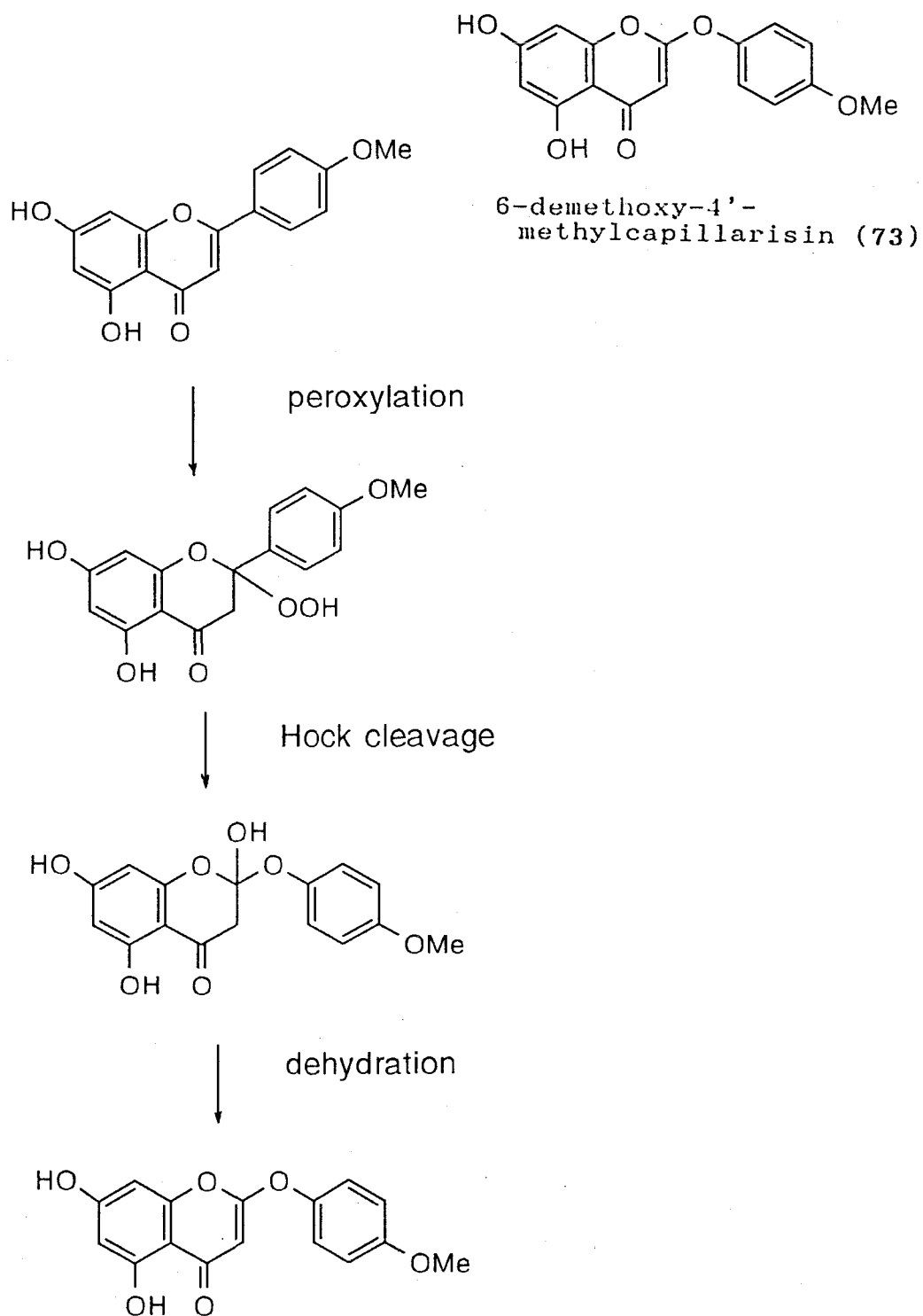
sample showed the clearest antifungal zone on TLC-plate at the concentration of ca 2 μ g/10 mg leaves. On the other hand, exudates from the damaged leaves (25 mg damaged leaves) exhibited a clear antifungal zone for 1, but no other antifungal spot. In contrast, polyphenolics from the damaged leaves rarely showed any activity in the fungus test. As the result, it was suggested that 1 was relatively concentrated in exudates of the damaged leaves. Since mechanical damage is considered to cause pathogenic infections, this effective diffusion of the antifungal agent from the wounded part may be significant in the defense system of *Rosa rugosa*.

The antifungal substance was accumulated in the leaf tissues as the result of oxygenation of carota-1,4-dienaldehyde (3) and further oxygenated to rugosic acid A (2) to be pooled in the tissues. As 3, exhibiting an easily oxidized nature, was present in the leaf tissues in a noticeable amount, it was speculated that 3 functioned as an antioxidant in the leaves. Since *Rosa rugosa* grows under strong light in a coastal area during summer season, it may need a protection system against peroxidation of the thylacoid membrane. For the protection, 3 must be used as an antioxidant. As the plant must store energy by photosynthetic reaction and regeneration during a short summer season in the northern area, it may sacrifice some membrane lipids for the effective photoreaction. The fact that *Rosa rugosa* contains a marked amount of β -carotene, α -tocopherol and ascorbic acid [79] (See Fig. 1-3, pp. 43), all known as common antioxidants and radical scavengers, fully supports this hypothesis. Furthermore, its large root system also should be taken into account in the energy resource. *Rosa rugosa* must have a large and deep root system due to dry and nutrient-poor sandy soil on the coast. To support this root system, its leaves should have enough glucose production capacity with the photosynthetic reaction during the

short summer season. This, almost overworked photosynthesis may cause a peroxidation of membrane without such an antioxidant.

Indeed, during the survey of these sesquiterpenoids in *Rosa rugosa*, it was unraveled that the leaves are under a quite strong oxidative condition due to the presence of bisaborosaol C1, C2 and D (23 ~ 25). Moreover, the presence of 6-demethoxy-4'-methylcapillarisin (73) in *Rosa rugosa* [78] suggests the oxidative condition. It is likely that 73 is formed through hydroperoxide intermediate to result in oxygen migration between C-2 and C-1'. This Hock cleavage-like pathway may be possible because in carotane peroxide, similar reaction are considerable (Scheme 4-1). Therefore, the author expects as follows. From this oxidative condition, *Rosa rugosa* protects its tissues by β -carotene, ascorbic acid, carota-1,4-dienaldehyde (3) and bisaborosaol A (19). On the other hand, when 3 was oxygenated, oxidized metabolites, mainly 1 were then re-utilized as antifungal substances. This seems very effective because the plant does not consume any other energy and carbohydrates to produce the defense compounds against microbial invasion.

The resistance of *Rosa rugosa* is probably explicable with some complex factors, one of which is the antifungal sesquiterpenes as discussed above. As the other factor, hypersensitive cell disruption with a severe browning conducting to a severe oxidative condition in the tissues induced by a chemical or a mechanical damage is also proposed. Furthermore, surprisingly high contents of polyphenolics in the rhizome may contribute to the resistance of *Rosa rugosa*. Indeed, *Rosa rugosa* root extract shows very clear antimicrobial activity. Thus, not only the aerial part but also underground part should contribute to the highly resistant nature of *Rosa rugosa* against pathogens. A model of dynamic roles of the carotanoids in the aerial part are shown in Fig. 4-1.



Scheme 4-1 Possible formation pathway for the phenoxychromone, *via* peroxylation of a flavonoid

The author has been encountered several physiological aspects of the carotane peroxides, although these were not described in this thesis. When a leaf-let of *Rosa rugosa* were slightly pressed between two sheets of filter paper which previously got wet with *n*-hexane or MeOH, and after that, the paper was sprayed with the peroxide reagent, the shape of the leaf-let clearly appeared as a red color according to the peroxide reaction. As an interesting observation, only underside of the leaf-let showed the response, as shown in Fig. 4-2. By this simple experiment, it became understandable the localization of the peroxides. In short, it was suggested that the peroxides localized in the tissue of the underside of the leaf. In fact, when a flake of tissue on surface of the upside and underside of the leaf were each separated carefully and suspended in a small volume of a EtOH/peroxide reagent mixture in test tubes, only the test tube in which underside tissue was suspended turned a slight pink. With chlorophyll, the same result was obtained.

To obtain further aspect, the tissue stained with the peroxide reagent were directly observed with a microscope. Under the microscopic observation, numerous numbers of trichomes are recognizable on the underside of the leaf, and some of them seemed to be stained to a red color as shown in Fig. 4-3. Although some effect of red light cannot be excluded, it is likely that the trichomes contained carotane peroxides in a high concentration as those of tomato plant which is known as a secretory and storage cell (or organ) for sesquiterpenoid of the plant [9]. These observations are an interesting object, as with the enhancement of ratio in diffusion of carotane peroxides from the wounded part. Further investigation is requested to reveal a function of the trichomes and the carotane peroxides in *Rosa rugosa*.

From aerial part of *Rosa rugosa*, callus can be obtained easily. Using a piece of callus, sesquiterpenes in the callus were

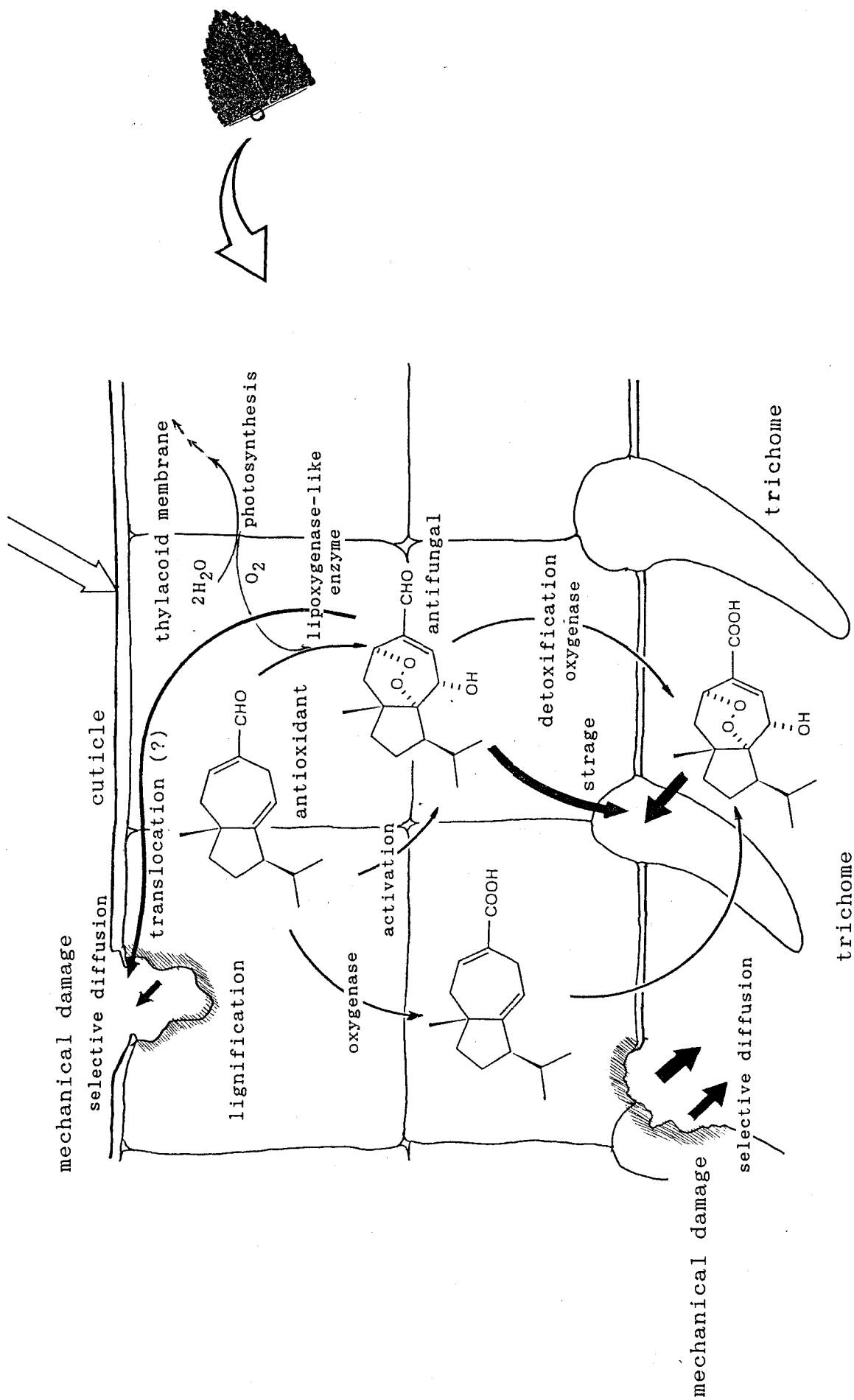


Fig. 4-1 A Model of Drastic Change and Role of Carotenoids in the Leaf Tissue of *Rosa rugosa*

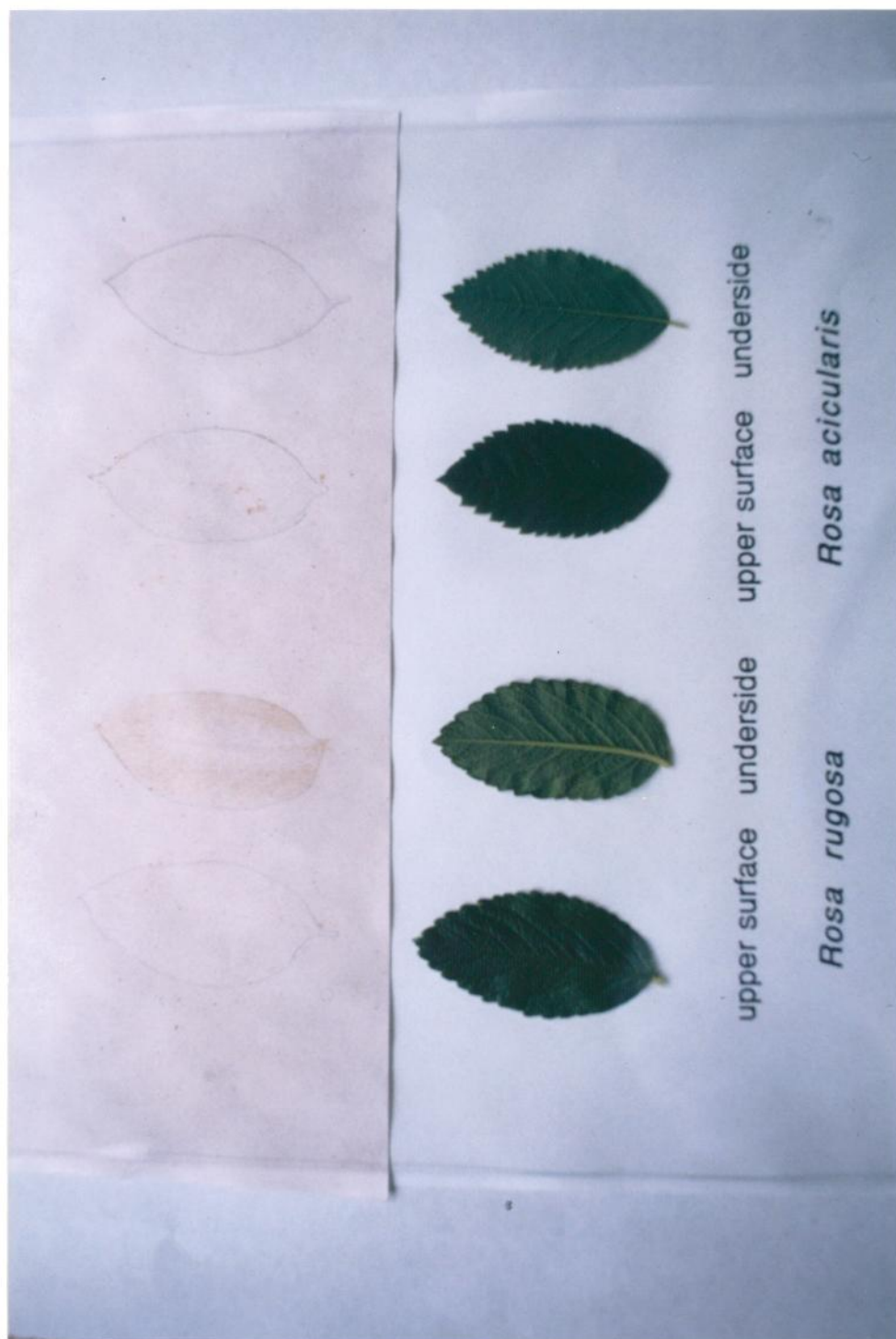


Fig. 4-2 Peroxide Test to Filter Paper on Which Leaflet of *Rosa rugosa* Was Washed with a Organic Solvent: Only the paper pressed from the underside of the leaflet showed a positive response. On the other hand, leaf of *Rosa acicularis* did not show any response.

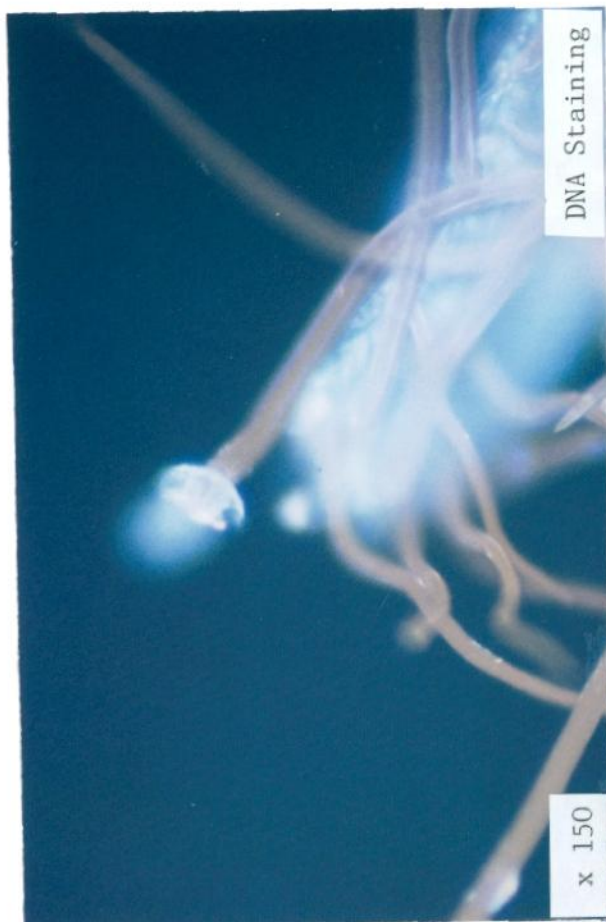


Fig. 4-3 Trichome of *Rosa rugosa*: The trichomes are present only on the underside of the leaflet and the veins.

preliminarily examined. Unlike leaf-tissues, callus of this plant did not contained any marked terpenoid. However, tissue culturing is expected to be available in the near future, in studies on sesquiterpenes of *Rosa rugosa*.

Chemotaxonomically, these constituents in *Rosa rugosa* are characteristic among Rosaceae plants. This sesquiterpene phase in this Rosaceae plant is quite similar to those of some Compositae and Umberifellae plants. Furthermore, the phenoxychromone (73) whose skeleton is quite rare as a naturally occurring compound was also reported from a Compositae plant, *Artemisia capillaris* [164]. As shown in Fig. 4-4, Rosaceae and Compositae are, however, not closely related to each other, while Umberiferae are somehow close to Rosaceae [165]. Further studies and discussion should also be carried out in this theme from the chemotaxonomical view point.

Rosa rugosa has successfully dispersed its descendants as important hybrid races to crossbreed with western roses, due to its resistant nature, and will bloom out further as an ancestor of excellent rose cultivars in the gardens all over the world. *Rosa rugosa* is commonly distributed along coastal area of Hokkaido and was chosen as the flower of Hokkaido. We are proud of this excellent wild rose, and of cause we have responsibility to conserve its presence for the next generations.

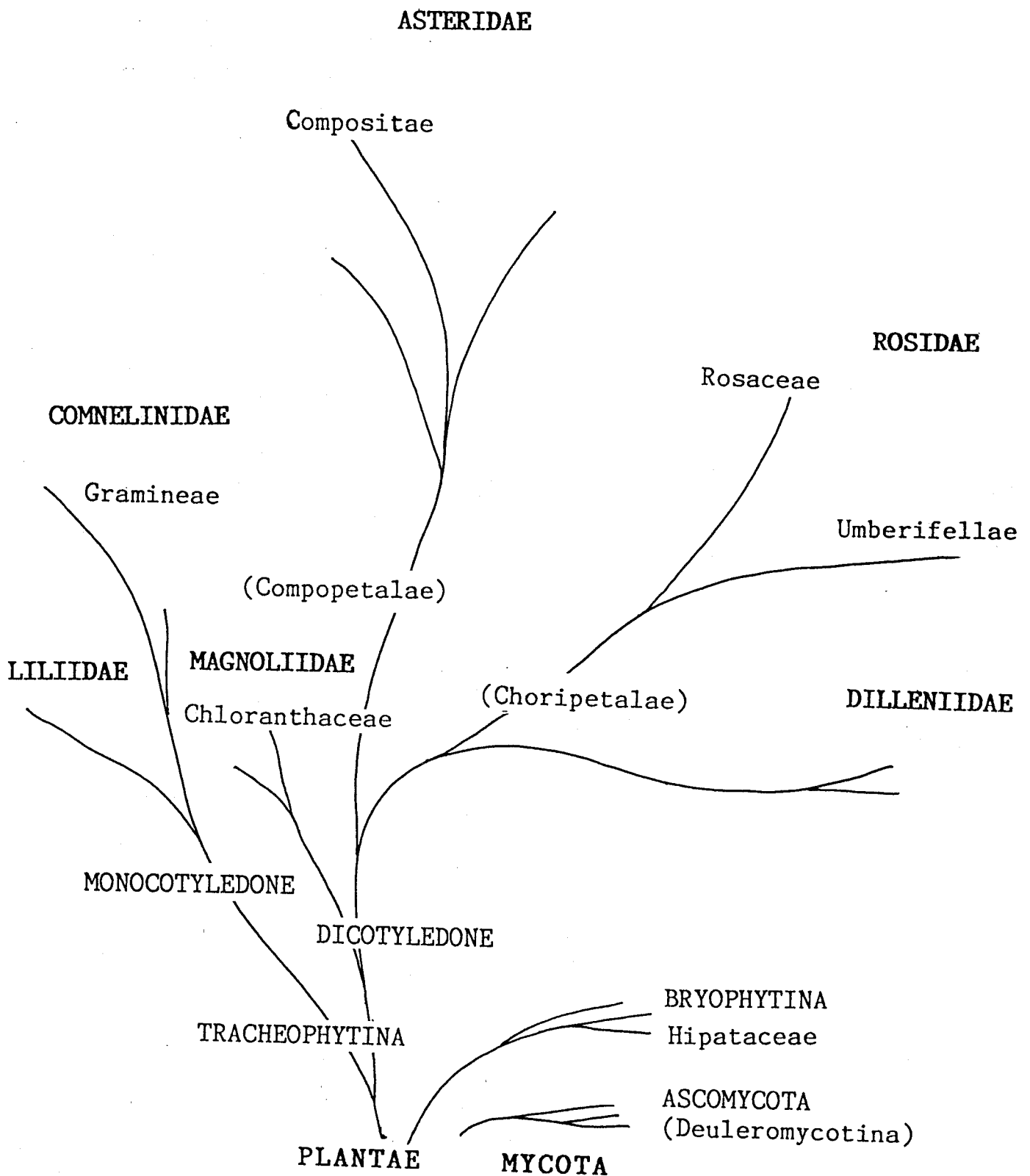


Fig. 4-4 A Possible Genealogical Tree of Plant Kingdom: The genealogical tree is based on and rearranged from the reference [165]. Rosaceae and Umberifellae are on the same branch, while Compositae forms a branch for another group.

北海道の沿岸地に自生するハマナス (*Rosa rugosa* Thunb.) は、その可憐な花と芳香により、古くから鑑賞用に用いられてきた。しかしながらその園芸的価値はわが国よりもむしろヨーロッパあるいはアメリカにおいて大きく評価され、高められた。その理由のひとつに、ハマナスが、西洋の園芸バラで問題となっていた幾つかの病害虫に対して顕著な抵抗性を有していたことが挙げられる。特にバラ黒斑病は鑑賞用品種の商品価値を落とすことから大きな問題となっていた。ハマナスはこの病気に対する抵抗性に優れていたため、ヨーロッパへ導入された後台木あるいは交配種として急速に広まり、一部の地域では野生化するまでに至った。ハマナスに認められる病虫害抵抗性因子の化学的あるいは生化学的研究は、1987年に Mashchenko らが羅病性バラとハマナスを含めた抵抗性バラの葉中 cholesterol 含量差に注目しこれを抵抗性因子であるとした報告 [81] が出されるまで行われていなかった。

著者は、各種植物の葉部を機械的傷害処理したのち水抽出し、その水層の有機溶媒転溶物の抗菌活性をスクリーニングした結果、本植物傷害葉抽出物中に極めて強い抗菌活性成分を検出した。次いで活性化合物本体の構造を決定する目的で、この活性成分の単離精製を試み、各種クロマトグラフィーにより 1.3 kg の傷害処理葉から 120 mg の活性化合物を無色針状結晶として得た。この化合物は、 $M^+ 266$ ($C_{15}H_{22}O_4$) を示し、各種機器分析により、特徴的な部分構造として、 α, β -不飽和アルデヒド基、1,5 位にかかるエンドパーオキシド架橋、および一方のパーオキシド酸素原子と分子内水素結合したアリル水酸基が存在することが判り、文献未知のカロタン構造を有するセスキテルペンアルデヒドであることが推測された。最終的には、本化合物の各種化学変換および対応するカルボン酸メチルエステルの INADEQUATE 法による C-C 相関の測定により本平面構造を証明し、これをルゴサール A と命名した。その立体化学については、NOESY 法で相対配置を、励起子キラリティー法 [106] で絶対配置を決定した。

本化合物の骨格であるカロタン型セスキテルペンは、高等植物における分布がセリ科および小数のキク科に限られており、バラ科では例の無いものであった。また、C-14 位における酸化はセリ科あるいはキク科ではほとんど知られておらず、これがバラ科由来のカロタンにおける特徴のひとつになると考えられる。ハマナス中には、更にルゴサール A に対応するカルボン酸 (ルゴス酸) が含まれており、その含有量は最多で 500 mg/kg にものぼった。ルゴス酸はその安定性、非抗菌活性および含有量から、ルゴサール A の主解毒代謝産物であると考えられ、実際に空気酸化によりルゴサール A からルゴス酸が生じることを確認した。

ルゴサール A の生成過程においては、その 1,5 エンドパーオキシド架橋が、低分子天然物パーオキシドとして一般に見出される 1,3 ジエン体と一重項酸素間におこる Diels-Alder 付加反応型の 1,4 エンドパーオキシド架橋と異なり、1,4-ジエン構造をもつ不飽和脂肪酸の酸化様式 (エキソパーオキシドラジカル体を中間体にとる) をとることが予想された。同様に 1,5-パーオキシド架橋を

有するハナルピノールの生成経路 [100] を比較して予想前駆体を想定しその検索を行った結果、低極性部よりカロタ-1,4-ジエンアルデヒドを見出した。この、前駆体と考えられたセスキテルペンアルデヒドは空气中で極めて不安定な性質を有し、幾つかのパーオキシド試薬陽性化合物へと酸化された。そのうちの二化合物は比較的安定な結晶として得られ、それらはその立体化学も含めてルゴサール A、ルゴス酸に一致した。これにより、カロタ-1,4-ジエンアルデヒドはルゴサール A の直接の前駆体で有ることを確認した。その酸化経路については、中間体の捕足による解明を試み、1,5-パーオキシド架橋をもち、C-2 位にヒドロパーオキシドの置換した中間体を高収率で得ることができたことから、C-3 位の活性メチレンから水素の引き抜きを引金として三重項酸素二分子付加を伴うラジカル反応を提出した。

ハマナス葉組織中におけるカロタンパーオキシドの代謝分解は、それらの葉の成長熟成に伴うドラステイックな消長から、酵素レベルでの制御が働くと予想された。ハマナス葉中カロタノイドの最大プールであるルゴス酸の代謝産物はそのパーオキシド架橋の変換、修飾が主な経路であると考えられたため、幾つかの化学変換物を標準として酸性画分を検索した結果、ルゴス酸の酸処理物、アルカリ処理物および還元体に対応する成分を天然物として単離することができた。特に、酸処理で生成するケタール体は完全に老化あるいは黄化した葉に比較的高濃度で存在し、これが組織中で安定な物であることが示唆された。

ルゴサール A 型のパーオキシド（アルデヒド基の還元によるアルコール誘導体を含む）では、それぞれ酸あるいはアルカリによりエンドパーオキシド基の変換が容易に起こった。その反応生成物は、従来知られているエンドパーオキシドの変換反応と異なり、その主原因はこれらカロタンパーオキシドのコンフォメーションによると考えられた。

ハマナス葉中にはこれらの他に多様なカロタンセスキテルペンが微量成分として含まれており、それらはカロタ-1,4-ジエンアルデヒドの異性体がさまざまな修飾（酸素化、脱水素等）を受けて生成したものと考えられる。

ハマナス葉にはまた、カロタン以外のセスキテルペンも比較的高含有量で含まれていた。そのなかで 100mg/kg 以上の含有量を示すピサボラン型セスキテルペンについてその構造を絶対配置を含めて決定し、これをピサボロザオール A と命名した。この化合物は、天然物としては珍しい C-7 が酸化されたピサボランであり、最近 Bohlmann らのグループによって相次いで見出されているキク科のピサボランとは C-4 位の絶対配置が異なり、従って右旋性を示す。本化合物は二種の酸化物群を葉中代謝産物として与え、オレフィン側鎖に生じたエポキシ環の γ -位のヒドロキシル基とのアルコールシスによるテトラヒドロフラン誘導体およびオレフィン部分に一重項酸素分子が攻撃して生成するヒドロパーオキシド誘導体として葉抽出物中に見出される。特に後者のグループは、生成量こそ微量ではあるがハマナス組織が強い酸化条件下にあることを示すものであった。

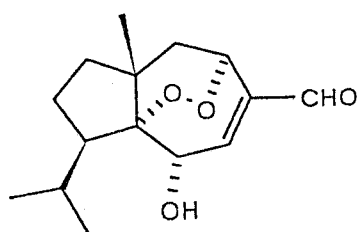
さらに、カロタンとピサボランの関係も興味をひいた。これらは共に *cis*-

trans-ファルネシルピロフوسفエートを生合成体として生合成される。ともにファルネソールの 13 位に由来するメチル基が酸化されている事実は、これら二種のセスキテルペンの密接な関連性を示すものと考えられる。

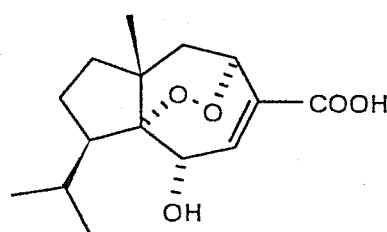
ビスボラン型に加えて、アコラン型セスキテルペンも見いだされた。この骨格は、さきのセスキテルペン類と同じく *cis-trans*-ファルネシルピロフوسفエートから生合成されるが、ファルネソールの C-13 メチル由来の炭素が酸化されておらず、ハマナス成分としては希な修飾様式を示した。

ルゴサール A の抗菌活性は、TLC バイオオートグラフィーあるいはペーパードイスク法で詳しく検討した。本化合物は、一部の糸状菌 (*Cladosporium herbarum* およびイネイモチ病菌を含む)、酵母あるいはグラム陽性菌にある程度の抗菌活性を認め、特にイモチ病菌に対しては市販農薬の EDDP に匹敵する抗菌活性を示した。細胞毒性は P-388^r を使った試験で $LD_{50} = 0.21 \mu g/ml$ を示した。いくつかの誘導体についての構造活性相関では、パーオキシド架橋およびアリル水酸基が活性発現に必須であること、不飽和アルデヒド基は必ずしも活性発現には必要無いがこれがカルボキシル基へ酸化されると活性が完全に消失すること、この活性はメチル化によって回復することがわかった。この結果は、生体内でのカロタンの代謝、生成および意義を考察するうえで非常に有用な情報となった。ビスボロザオール A は TLC バイオオートグラフィーでルゴサール A の 1/10 程度の抗菌性を示したにすぎなかった。

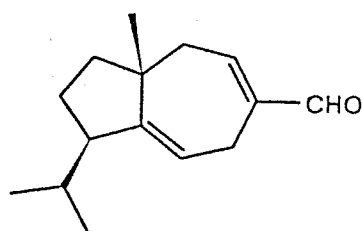
一連の化学的知見に加えて、いくつかの生理的知見も得られた。最も活性の高い開花期には 1 kg ハマナス葉中におよそ 700 mg ものカロタンパーオキシドが含まれるが、これらのほとんどが葉裏面のトリコム（突起様構造）に含まれている実験事実をいくつか見出している。また、傷害部から浸出するルゴサール A の相対量が著しく高いことも示唆された。これらの観察は、ルゴサール A が実際に葉組織中で糸状菌感染に対する防御因子となっている可能性を示しており [9]、更に詳しい検証が望まれる。また、カロタ-1,4-ジエンアルデヒドがハマナス葉組織中、特にシラコイド膜上で抗酸化剤として機能していることも考えられる。この場合、ルゴサール A は酸化老廃物にしか過ぎず、ある部分へ集積されたのち糸状菌に対する防御物質として転用されることが考えられる。これは極めて効率の良い仕組みであり、ハマナスが夏季の短い高緯度で自生している事実を考え併せれば、炭化水素の効率的利用型防御機構という点で興味深い例であるかもしれない。



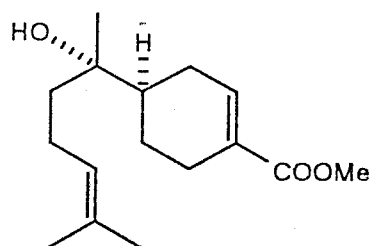
ルゴサール A



ルゴス酸



カロタ-1,4-ジエンアルデヒド



ビスボロザオール A

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