



Title	Application of 1D BIRD or X-filtered DEPT long-range C-C relay for detection of proton and carbon via four bonds and measuring long-range C-13-C-13 coupling constants
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Citation	Magnetic Resonance in Chemistry, 44(4), 475-480 https://doi.org/10.1002/mrc.1768
Issue Date	2006-01-05
Doc URL	https://hdl.handle.net/2115/8469
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Type	journal article
File Information	MRC_44(4)_475-480.pdf



MRC3004 Revised manuscript.

Title : Application of 1D BIRD or X-filtered DEPT long-range C-C relay for detection of proton and carbon via four bonds and measuring long range ^{13}C - ^{13}C coupling constants.

Short title : 1D BIRD or X-filtered DEPT long-range C-C relay.

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grant number A Grant-in-Aid for Scientific Research (No. 13024205 to J.K.) from Ministry of Education, Science, and Culture of Japan.

Abstract

We propose the ^{13}C -detecting 1D DEPT long-range C-C relay to detect super long-range H-C connectivity via four bonds ($^1\text{H}-^{13}\text{C}-\text{X}-\text{X}-^{13}\text{C}$, X represents ^{12}C or heteronuclear). It is derived from the DEPT C-C relay which detects the H-C correlations via two bonds ($^1\text{H}-^{13}\text{C}-^{13}\text{C}$) by setting the delays for J_{CC} in the C-C relay sequence to the $^{\text{LR}}J_{\text{CC}}$. This sequence gives correlation signals split by small $^{\text{LR}}J_{\text{CC}}$, which is seriously suffered from residual center signal. The unwanted signal is due to long-range C-H couplings ($^{\text{LR}}J_{\text{CH}}$). The expected relayed magnetization transfer $^1J_{\text{CH}} \rightarrow ^{\text{LR}}J_{\text{CC}}$ occurs in the $^1\text{H}-^{13}\text{C}-\text{X}-(\text{X})-^{13}\text{C}$ isotopomer, whereas the unwanted signal of $^{\text{LR}}J_{\text{CH}}$ comes from $^1\text{H}-^{12}\text{C}-(\text{X})-^{13}\text{C}$ isotopomers, whose population is 100 times larger than that of the $^1\text{H}-^{13}\text{C}-\text{X}-(\text{X})-^{13}\text{C}$ isotopomer. Large dispersive line of this unwanted center signal would be a fatal problem in the case of detecting small $^{\text{LR}}J_{\text{CC}}$ couplings. This central signal could be removed by an insertion of BIRD pulse or X-filter. DEPT spectrum editing solved a signal overlapping problem and enabled accurate determination of particular $^{\text{LR}}J_{\text{CC}}$ values. We demonstrate here the examples of structure determination using connectivity between ^1H and ^{13}C via four bonds, and

the application of long-range C-C coupling constants to discrimination of stereochemical assignments.

Keywords : NMR, ^1H , ^{13}C , ^{13}C -detecting 1D X-filtered DEPT long-range C-C relay, 1D BIRD DEPT long-range C-C relay, long-range ^{13}C - ^{13}C coupling constants.

Introduction

Structure determination of natural products by heteronuclear long-range C-H correlation spectroscopies, such as HMBC¹ and COLOC², has a substantial problem. A carbon bearing no proton within three bonds cannot be correlated since these methods detect long-range J couplings between proton and carbon connected via three or less than three bonds. Hence, the structure elucidation of highly hydrogen-deficient molecules might be a problem since the carbon network of such molecules cannot be completely followed by information only from $^{2,3}J_{\text{CH}}$ connectivities. For structure elucidation of such compounds, several approaches have been reported such as D-HMBC³ which detects small $^nJ_{\text{CH}}$ ($n>3$) and ^1H detecting 2D n,n-ADEQUATE⁴ which detects ^1H magnetization transferred from $^nJ_{\text{CC}} \rightarrow ^nJ_{\text{CH}}$. In this paper, we propose a practical method for detection of super long-range H-C connectivity via four bonds ($^1\text{H}-^{13}\text{C}-\text{X}-\text{X}-^{13}\text{C}$). Although J_{CH} values over three bonds are usually negligible, utilization of long-range $^3J_{\text{CC}}$ observation enables to correlate this four-bond connectivity. We have previously proposed⁵ ^{13}C -detectinf 1D

DEPT C-C relay sequences for sensitivity enhanced and clean $^1J_{CC}$ detection. The DEPT C-C relay has originally been designed by combining DEPT⁶ and C-C relay⁷. The former part enables a sensitivity enhancement by polarization transfer from connected 1H to ^{13}C and an inherent sensitivity loss by double-quantum filtration in INADEQUATE⁸ can be escaped in the latter part. The resultant spectrum gives an antiphase doublet of $^1J_{CC}$ at the chemical shift position of the observed carbon, resulting a 1H - ^{13}C - ^{13}C correlation. In the method, a phase distortion problem arising from resonance-offset variation through the wide range of ^{13}C resonance is compensated by adopting symmetric phase-alternating composite pulses⁹. This corrected phase makes it possible clean DEPT spectrum editing for discriminating CH_3 -, CH_2 - and CH -C connectivities.

Setting the delays for J_{CC} in the DEPT C-C relay to $^{LR}J_{CC}$, the sequence gives correlation signals split by small $^{LR}J_{CC}$, which can correlate super long-range 1H - ^{13}C -X-X- ^{13}C . However, detection of small splitting by $^{LR}J_{CC}$ is seriously suffered from residual center signal, which is mainly due to direct magnetization transfer through long-range C-H couplings ($^{LR}J_{CH}$) from neighboring protons. The expected relayed magnetization transfer

${}^1J_{\text{CH}} \rightarrow {}^{\text{LR}}J_{\text{CC}}$ occurs in the ${}^1\text{H}-{}^{13}\text{C}-\text{X}-(\text{X})-{}^{13}\text{C}$ isotopomer, whereas the unwanted signal of ${}^{\text{LR}}J_{\text{CH}}$ comes from the ${}^1\text{H}-{}^{12}\text{C}-(\text{X})-{}^{13}\text{C}$ isotopomers, whose population is 100 times larger than that of the desired ${}^1\text{H}-{}^{13}\text{C}-\text{X}-(\text{X})-{}^{13}\text{C}$ isotopomer. Large dispersive line of this unwanted center signal does not severely affect observation of large splitting from direct ${}^{13}\text{C}-{}^{13}\text{C}$ couplings (${}^1J_{\text{CC}}$), but could be a fatal problem in the case of detecting small ${}^{\text{LR}}J_{\text{CC}}$ couplings. This central signal could be removed by adding BIRD pulse¹⁰ followed by null delay (BIRD delay, BD) or isotope-filters such as G-BIRD^{11, 12}, zz filter¹³, and zz gradient-enhanced X-filter¹⁴ prior to starting the DEPT sequence. We first adopt the BIRD and delay prefix since it does not need a pulsed field gradient equipment, and next, among the methods using gradient pulses, the zz gradient-enhanced X-filter¹⁴. Resulting sequences are BIRD or X-filtered DEPT long-range C-C relay for clean detecting super long-range ${}^1\text{H}-{}^{13}\text{C}-\text{X}-\text{X}-{}^{13}\text{C}$ connectivities. A signal overlapping problem arising from more than one correlation at the observed carbon could be resolved by DEPT spectrum editing as has been utilized in the conventional DEPT C-C relay. The resulting rather simple CH_3- , CH_2- and CH -subspectra enabled

unambiguous assignments of each ^1H - ^{13}C -X-X- ^{13}C correlation and accurate determination of particular $^{\text{LR}}J_{\text{CC}}$ values. We demonstrate here the examples of structure determination using connectivity between ^1H and ^{13}C via four bonds, and application of long-range C-C coupling constant for determination of stereochemical assignments.

Results and discussion

Pulse sequence of 1D BIRD or X-filtered DEPT long-range C-C relay

Pulse sequences used contain BIRD (Fig. 1a) or X-filter (Fig. 1b) prior to the conventional DEPT C-C relay to suppress the dispersive line at the center of the observed chemical shift position. The product operator formulae¹⁵ before starting C-C relay in each 1D BIRD and X-filtered DEPT long-range C-C relay sequence is

$$\sigma = I_z - I'_z \cos(\pi J 2\Delta) - 2I'_x S_z \sin(\pi J 2\Delta)$$

where I , I' and S represent ^1H in isotopomer ^1H - ^{13}C - ^{13}C , ^1H and ^{13}C in ^1H - ^{12}C - ^{13}C , respectively. J and Δ represent $^{\text{LR}}J_{\text{CH}}$ and Δ_1 , respectively. The

term $-2I'_x S_z \sin(\pi J 2\Delta)$ is negligible since $\pi J 2\Delta$ is small, and the term $-I'_z \cos(\pi J 2\Delta)$ should effectively be eliminated by a BIRD delay (BD) or X-filter. The BIRD pulse inverts the spins that we do not wish to see, and then a BD results in them passing through a null as the pulse sequence starts. On the other hand, the X-filter selectively excites the ^1H attached to ^{13}C , by suppression of the unwanted signals by spoiler gradient. Both methods resulted on good spectra. Although another sequence using G-BIRD^{11, 12} was also examined, it did not give better results than the above two methods. Setting the delay for C-C evolution (Δ_2) to long-range coupling gave a small doublet in BIRD or X-filtered DEPT C-C relay spectrum. This doublet would be hidden under residual central signal without the aid of BIRD pulse or X-filter. The DEPT spectrum editing can be made by ordinary addition-subtraction procedures as normal DEPT. Unexpectedly, the annoying central signal was partly attenuated by the spectrum editing in case when carbon status of DEPT-excited and observed carbons is different. A replacement of the DEPT part with other ^{13}C -multiplicity editing methods such as gradient-selected multiple-quantum filter¹⁶ did not give better results probably due to

inclusion of longer delay and larger number of gradient pulses.

Structural assignments using super long range H-C correlation

To verify the validity of the BIRD DEPT long-range C-C relay method, which can detect super long-range $^1\text{H}-^{13}\text{C}-\text{X}-\text{X}-^{13}\text{C}$ correlations, we first applied the sequence for assignments of quaternary carbons in a phenolic ester. Assignments of an acylated position of phenols are in some case a problem since acyl protons locate four-bond away from the attached acyloxy carbons, which cannot be correlated by conventional heteronuclear long-range C-H correlation methods. In the model compound, orcinol monoacetate (**1**), two oxygenated benzene carbons, C-3 and C-5, resonate at δ_{C} 151.2 and 156.7 ppm. If a correlation from the phenolic hydroxyl proton is available, these two quaternary carbons can be easily assigned using the two-bond H-O-C correlation. However, an observation of exchangeable hydroxyl protons is strongly affected by environmental circumstance and measuring condition and thus unreliable. By using the BIRD DEPT long-range C-C relay, unambiguous assignments of these two

carbons were made as follows. The CH₃-subspectrum of **1** gave two doublets for the carbon of δ_C 151.2 (Fig. 2a) and one doublet for δ_C 156.7 (Fig. 2b). Methyl protons on C-1 would give correlation signals as a small doublet on both C-3 and C-5, since the protons and carbons are in a four-bond distance. In addition, methyl protons of the acetyl group could also be correlated with one of the oxygenated benzene carbons. Appearance of two doublets at δ_C 151.2 undoubtedly indicated this carbon should carry the acetoxy group. From these results, the carbon at δ_C 151.2 and 156.7 were assigned as C-3 and C-5, respectively, and $^3J_{CC}$ values of C-7/C-3, C-7/C-5 and C-9/C-3 were determined as 5.2, 5.0 and 2.2 Hz, respectively.

For a determination of the carbon skeleton of a highly hydrogen-deficient molecule, we chose a furobenzopyron, khellin (**2**), as a model compound, which carries only three hydrogens in its outer 13-membered ring. In this kind of highly functionalized fused heteroaromatics, determination of the direction of a fused ring by $^3J_{CH}$ detection would be a serious problem. Actually, C-8a of **2**, locating at the fused position between γ -pyron and benzene rings will give no HMBC correlation peak since it has no protons within three-bond. As for C-4a, no

correlation from furan ring is obtained, although a $^3J_{\text{CH}}$ correlation with H-6 on the pyron-ring can be available. For this oxacarbocyclic skeleton, only the connectivities represented by bold line in Fig. 3 can be traced by HMBC or COLOC, and hence, a fused direction of γ -pyron ring to benzofuran ring cannot be determined. In the two quaternary carbons, δ_{C} 113.7 and 148.3, placed between γ -pyron and benzene rings, the higher field carbon could be assigned to C-4a and the lower, C-8a, from taking their chemical shifts into account. These assignments were also supported by the HMBC correlation of H-6 with the carbon at δ_{C} 113.7, C-4a. If the orientation of γ -pyron ring to the benzofuran ring is as depicted in **2**, C-4a will be correlated with H-3 on the furan ring via four bonds by BIRD DEPT C-C relay, whereas C-8a has no methine proton on the furan ring within four bonds. In the CH-subspectrum of **2** (Fig. 3), two sets of doublets, $J=11.8$ and 3.1 Hz, were clearly observed at δ_{C} 113.7, which should be derived from two-bond coupling with C-6 and three-bond with C-3, respectively. These assignments were supported by the fact that a $^2J_{\text{CC}}$ value via a carbonyl carbon such as C-6/C-4a is exceptionally large, 11.8 Hz in this case, compared to normal $^{2,3}J_{\text{CC}}$ values¹⁷. Hence, the fused

direction of the γ -pyron to benzofuran could be unambiguously determined by the BIRD DEPT long-range C-C method.

Application of long-range C-C coupling constants for determining stereochemistry

Three-bond coupling constants between two carbons, $^3J_{CC}$, show dependency on their dihedral angle like $^3J_{HH}$ and $^3J_{CH}$ ¹⁷. However, the use of $^3J_{CC}$ values to determine the stereochemistry of natural molecules has been quite limited since the precise detection of $^3J_{CC}$ is discouraged with the inherent poor sensitivity due to low natural abundance of isotopomers carrying two ^{13}C in one molecule and with their relatively small absolute values, 0-6 Hz, compared to $^3J_{HH}$, 0-20 Hz, and $^3J_{CH}$, 0-15 Hz¹⁷. These drawbacks are circumvented by the use of the BIRD DEPT long-range C-C relay. At first, discrimination of *E-Z* isomers around a double bond was demonstrated. Two isomers of 2-methyl-2-butenoic acid, tiglic (**3**, *E*-isomer) and angelic (**4**, *Z*-isomer) acids have been tested for determination of $^3J_{CC}$ values between C-4 methyl and C-1 carbonyl carbons.

The carbonyl carbon at δ_C 169.1 in CH_3 -subspectrum of **3** (Fig. 4a) gave two set of doublets with $J=5.5$ and 3.3 Hz. In contrast, the carbonyl at δ_C 169.3 of **4** (Fig. 4b) consisted of overlapping doublets of $J=3.3$ and 2.3 Hz. Both acids have two methyl groups, C-4 and a side-chain methyl (C-5) on C-2. The C-5, which is two-bond away from C-1 carbonyl and will give a $^2J_{\text{CC}}$ signal on it, is the common structural feature in two acids. Thus, the doublet of $J=3.3$ Hz should be derived from the coupling of C-5/C-1 in both **3** and **4**. In the *E*-acid (**3**), the larger additional coupling of $J=5.5$ Hz observed must be three-bond C-4/C-1, while the corresponding $^3J_{\text{CC}}$ gave the smaller doublet of $J=2.3$ Hz in *Z*-acid (**4**). These assignments clearly indicated that a $^3J_{\text{CC}}$ value in *E*-configuration with a dihedral angle of 180° is obviously larger than that in *Z*-form of 0° , as has been well known for $^3J_{\text{HH}}$ values in *E-Z* isomers of 1, 2-disubstituted ethylene systems. To confirm this distinction, 3-methyl-2-butenic acid (**5**), which has two methyls of *E* and *Z* configuration with a carbonyl, was next examined. As expected, two set of $^3J_{\text{CC}}$ doublets of $J=7.8$ and 1.6 Hz for *E*- and *Z*-methyls, respectively, were separately observed in the CH_3 -subspectrum (Fig. 4c). From these results, it was demonstrated that an accurate determination of

$^3J_{CC}$ values enabled distinction of *E-Z* isomers by larger 5-8 Hz and smaller 1-3 Hz, respectively.

The stereochemical assignments of rigid sp^3 system were also made possible with the sequence. A mixture of *endo*- (**6**) and *exo*- (**7**) 2-benzoyl-5-norbornenes gave two carbonyl signals, δ_C 199.8 and 201.3 ppm. In the CH_2 -subspectrum, two set of doublets of $J=3.4$ and 1.1 Hz appeared at δ_C 199.8 (Fig. 5a), whereas no significant signal at δ_C 201.3 (Fig. 5b). The dihedral angles between the bridge methylene, C-7, and carbonyl C-8, were estimated from molecular modeling as 164° in **6** and 88° in **7**. It was easily expected that $^3J_{CC}$ should be larger in **6** than in **7** since dihedral angle dependency of 3J was at maxima in 0° and 180° and nearly 0 in 90° . Therefore, the carbons at δ_C 199.8 and 201.3 ppm were reasonably assigned as *endo*- (**6**) and *exo*- (**7**) isomers, respectively. The additional doublet of smaller J (1.1 Hz) in **6** seemed to be derived from $^2J_{CC}$ with C-3 methylene, which may be buried in thermal noise in **7**.

Another example for a conformationally fixed sp^3 system was a distinction of axial and equatorial isomers in cyclohexanes. Two carbonyl

carbons, δ_C 177.2 and 176.2 ppm, of CH₂-subspectrum of a mixture of *trans*- (**8**) and *cis*- (**9**) 4-*tert*-butylcyclohexanecarboxylic acids (Fig. 6a and b) gave two conspicuous set of doublets of $J=4.6$ and 1.3 Hz at δ_C 177.2 (Fig. 6a) and an ambiguous small doublet of $J=1.3$ Hz at δ_C 176.2 (Fig. 6b). The larger J_{CC} in the former should come from the three-bond coupling of C-3(5)/C-1, whose dihedral angle was 180°, in *trans* isomer. The overlapping smaller J (1.3 Hz) was thought to be derived from $^2J_{CC}$ with C-2(6). The fact that only a small coupling probably due to $^2J_{CC}$ was observed at δ_C 176.2 indicated the $^3J_{CC}$ between C-3(5) and C-1 in *cis* isomer was too small to be detected due to their dihedral angle of 70°. In the same spectrum, two quaternary carbons of *tert*-butyl residue (C-8) at 32.5 and 32.4 (Fig. 6c) showed two doublets 3.2 and 0.7, and 3.5 and 0.9 Hz, respectively. This supported the orientation of *tert*-butyl residue at C-4 in both isomers were that *tert*-butyl residues was equatorial. Thus, $^3J_{CC}$ values could also discriminate stereochemical isomers in conformationally stable sp³ systems.

In the above experiments, we usually used micro tubes of 2.5 mm in diameter which required approximately 0.09 mL of sample solution. The

best result was obtained in the CH-subpectrum of khellin (**2**) (Fig. 3), in which 0.015 mmol sample in 0.09 mL was measured for 15 hr data acquisition. This corresponds to a weekend experiment (60 h) with 0.033 mmol in 0.4 ml solution using an ordinary 5mm probe. In general, the spectrum of orcinol monoacetate (**1**) was obtained using 33 mg (0.2 mmol) in 0.09 ml for 28 min, corresponding to 26 mg (0.16 mmol) in 0.4 ml for an overnight experiment (15 h). If the use of more sensitive cryogenic NMR probehead¹⁸ will be applicable, futher reduction of sample amount will be possible. Hence, the low sensitivity problem inherent in C-C detection methods is going to be overcome by both hardware and software improvements. Application of such methods for the structural determination of natural molecules with a limited amount will come into sight.

Experimental

Chemical reagents

Orcinol monoacetate (**1**) prepared from orcinol was provided by the Laboratory of Food Biochemistry, Graduate School of Agriculture, Hokkaido University. Other chemicals were of reagent grade.

NMR instrument

NMR spectra were recorded at 300 K with a Bruker AMX 500 spectrometer (^1H 500 MHz, ^{13}C 125 MHz) equipped with a 5 mm or 2.5 mm diameter C/H dual probe. Chemical shifts (δ) in ppm were determined relative to the solvent signal.

BIRD DEPT C-C relay spectra

BIRD DEPT C-C relay spectra were obtained using the pulse sequence of Fig. 1 a. Delays, number of transients, and spectral width were mentioned in each figure legend.

X-filtered DEPT C-C relay spectra

X-filtered DEPT C-C relay spectra were obtained using the pulse sequence of Fig. 1 b. Strength of field gradient pulses were 3.5 and -2.5

G/cm.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 13024205 to J.K.) from Ministry of Education, Science, and Culture of Japan.

References

1. Bax A, Summers MF. *J. Am. Chem. Soc.*, 1986; **108**: 2093.
2. Kessler H, Griesinger C, Zarbock J, Loosli HR. *J. Magn. Reson.*, 1984; **57**, 331.
3. Furihata K, Seto H. *Tetrahedron Lett.*, 1995; **36**: 2817.
4. Reif B, Köck M, Kerssebaum R, Kang H, Fenical W, Griesinger C. *J. Magn. Reson.*, 1996; **A 118**: 282.
5. Kawabata J, Fukushi E. *J. Magn. Reson.*, 1994; **A 107**: 173.
6. Bendall MR, Doddrell DM, Pegg DT. *J. Am. Chem. Soc.* 1981; **103**: 4603.
7. Kessler H, Bermel W, Griesinger C. *J. Magn. Reson.*, 1985; **62**: 573.

8. Bax A, Freeman R, Frenkiel TA. *J. Am. Chem. Soc.*, 1981; **103**: 2102.
9. Shaka AJ, Pines A. *J. Magn. Reson.*, 1987; **71**:495.
10. Garbow JR, Weitekamp DP, Pines A. *Chem. Phys. Lett.*, 1982; **93**: 504.
11. Emetarom C, Hwang T-L, Mackin G, Shaka AJ. *J. Magn. Reson.*, 1995; **A 115**: 137.
12. Mackin G, Shaka AJ. *J. Magn. Reson.*, 1996; **A 118**: 247.
13. John BK, Plant D, Hurd RE. *J. Magn. Reson.*, 1992; **A 101**: 113.
14. Uhrín D, Batta G, Hruby VJ, Barlow PN, Kövér KE. *J. Magn. Reson.* 1998; **130**: 155.
15. Sørensen OW, Eich GW, Levit MH, Bodenhausen G, Ernst RR. *Prog. NMR Spectrosc.* 1983; **16**: 163.
16. Parella T, Sanchez-Ferrando F, Virgili A. *J. Magn. Reson.*, 1995; **A117**: 78.
17. J. Marshall, "Carbon-carbon and Carbon-proton NMR Couplings: Applications to Organic Stereochemistry and Conformational Analysis", *Verlag Chemie Int. Deerfield Beach, FL*, 1983, p14, 68, and 206.
18. Kovacs H, Moskau D, Spraul M. *Prog. Nucl. Magn. Reson. Spectrosc.*, 2005; **46**:131.

19. Sparks SW, Ellis PD. *J. Magn. Reson.*, 1985; **62**: 1.

20. Levitt MH, Ernst RR. *Mol. Phys.*, 1983; **50**: 1109.

Figure Legends

Fig.1. Pulse sequences for 1D BIRD DEPT C-C relay (a) and X-filtered DEPT C-C relay (b). Narrow and wide bars represent 90° and 180° pulses, respectively. All carbon pulses except for presaturation are composite pulses consisting of $\{58^\circ -140^\circ 172^\circ\}$ (90° pulse of the DEPT), $\{24^\circ -152^\circ 346^\circ -152^\circ 24^\circ\}$ (other 90° pulses), and $\{58^\circ -140^\circ 344^\circ -140^\circ 58^\circ\}$ (180° pulses)⁹. RD and BD represent repetition and BIRD delays, respectively. In (a), RD contains ^{13}C presaturation composed of sixteen 90° pulses separated by 0.1 s intervals¹⁹. Delays Δ_1 , Δ_2 , and Δ_3 are $1/2J_{\text{CH}}$, $1/4J_{\text{CC}} - 1/2J_{\text{CH}}$, and $\Delta_1/2$ respectively. DEPT magnetization transfer was performed by phase shifting of ^1H pulses instead of changing a flip angle of the variable ^1H pulse (β) in basic DEPT²⁰. Phase cycling used is as follows: $\phi_1 = x$, $\phi_2 = x^{16}, y^{16}, -x^{16}, -y^{16}$, $\phi_3 = -x^{16}, x^{16}$, $\phi_4 = -x$, $\phi_5 = \beta, 2\pi-\beta$, $\phi_6 = x^4, y^4, -x^4, -y^4$, $\phi_7 = x, -x, y, -y, -x, x, -y, y$, $\phi_8 = y$, $\phi_9 = x^2, -x^2, y^2, -y^2, -x^2, x^2, -y^2, y^2$, and $\phi_{10} = x, -x^2, x, y, -y^2, y, -x, x^2, -x, -y, y^2, -y$. CH-subspectrum is obtained by setting $\beta = 90^\circ$. CH_2 - and $\text{CH} + \text{CH}_3$ subspectra are obtained by phase cycling of $(\beta = 45^\circ) - (\beta = 135^\circ)$ and $(\beta = 45^\circ) + (\beta = 135^\circ)$, respectively. CH_3 -subspectrum is obtained by subtracting CH-subspectrum

from (CH + CH₃)-subspectrum.

Fig.2. Part of CH₃-subspectrum of BIRD-DEPT C-C relay of orcinol monoacetate (**1**) (33mg (0.2mmol) / 0.09 ml chloroform-*d*). Two quaternary carbons, δ_C 151.2 ppm (a) and 156.7 ppm (b) are shown. Delays, RD, BD, Δ_1 and Δ_2 , were 2.5 s, 0.7 s, 3.62 ms and 58.9 ms ($J_{CH} = 138$ Hz, $J_{CC} = 4$ Hz), respectively. Each spectrum was accumulated using 256 transients in the spectral width of 10204 Hz in 65 K data points. The total measuring time was ca. 28 min each.

Fig.3. Part of CH-subspectrum of BIRD DEPT C-C relay of khellin (**2**) (4mg (0.015 mmol) / 0.09ml methanol-*d*₄). Carbon at δ_C 113.7 ppm is shown. Delays, RD, BD, Δ_1 and Δ_2 , were 1.7 s, 1.0 s, 3.62 ms and 58.9 ms ($J_{CH} = 138$ Hz, $J_{CC} = 4$ Hz), respectively. The spectrum was accumulated using 9728 transients in the spectral width of 22727 Hz in 128 K data points. The total measuring time was ca. 15 hr.

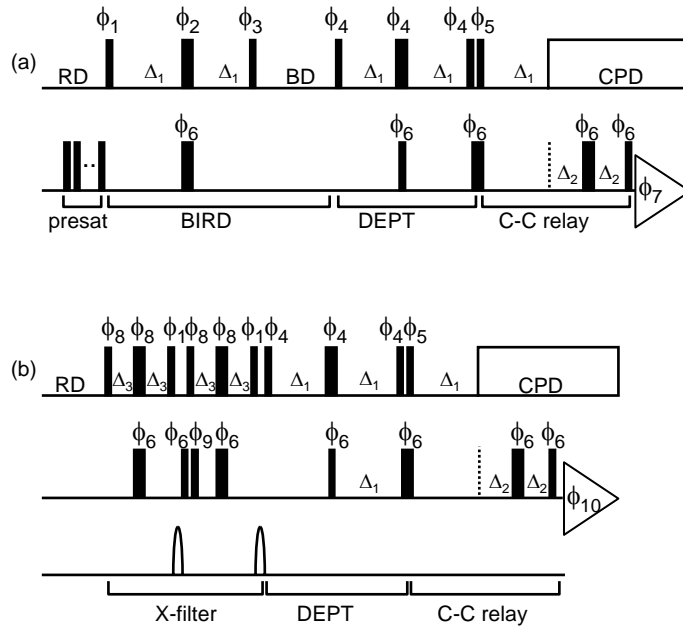
Fig. 4. Part of CH₃-subspectrum of BIRD DEPT C-C relay of tiglic (**3**) (a),

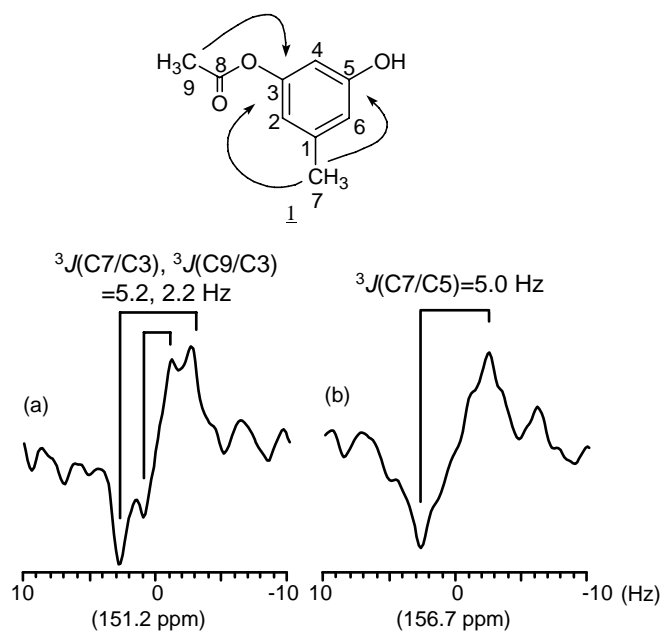
angelic (**4**) (b), and 3-methylcrotonic (**5**) (c) acid. Each sample was prepared as 30 mg (0.3 mmol) / 0.1 ml DMSO- d_6 . Carbonyl Carbons of each compound are shown. Delays, RD, BD, Δ_1 and Δ_2 , were 3.2 s, 0.8 s, 3.62 ms and 55.9 ms ($J_{CH} = 138$ Hz, $J_{CC} = 4.2$ Hz), respectively. Each spectrum was accumulated using 256 transients in the spectral width of 20833 Hz in 128 K data points. The total measuring time was ca. 30 min each.

Fig. 5. Part of CH₂-subspectrum of BIRD-DEPT C-C relay of the mixture of *endo*- (**6**) and *exo*- (**7**) 2-benzoyl-5-norbornen (148mg (0.75mmol) each / 0.5ml DMSO- d_6). Two carbonyl carbons, δ_C 199.8 ppm (a) and 201.3 ppm (b) are shown. Delays, RD, BD, Δ_1 and Δ_2 , were 2.0 s, 0.4 s, 3.57 ms and 58.9 ms ($J_{CH} = 140$ Hz, $J_{CC} = 4$ Hz), respectively. The spectrum was accumulated using 384 transients in the spectral width of 33333 Hz in 128 K data points. The total measuring time was ca. 1 hr.

Fig. 6. Part of CH₂-subspectrum of X-filtered DEPT C-C relay of the mixture of *trans*- (**8**) and *cis*- (**9**) 4-*tert*-butylcyclohexanecarboxylic

acid (27 μg (0.15 μmol) each / 0.15 μL DMSO- d_6). Two carbonyl carbons, δ_{C} 177.2 ppm (a) and 176.2 ppm (b), and two quaternary carbons at δ_{C} 32.5 ppm and 32.4 ppm (c) are shown. Delays, RD, Δ_1 and Δ_2 , are 2.0 s, 3.57 ms and 79.8 ms ($J_{\text{CH}} = 140$ Hz, $J_{\text{CC}} = 3$ Hz), respectively. The spectrum was accumulated using 2688 transients in the spectral width of 20833 Hz in 128 K data points. The total measuring time was ca. 2 h.





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