STRUCTURES OF NEW TWO ISOFLAVONES AND ONE FLAVANONE FROM GLASSWORT (*Salicornia europaea* L.)

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Introduction

The genus *Salicornia*, which belongs to the family Chenopodiaceae, consists of a few species and only one of which, glasswort (*Salicornia europaea* L.), is distributed in the eastern region of Hokkaido, Japan. In a series of studies on the organic constituents of this plant, CHIJI *et al.* reported on the isolation and structure elucidation of a violet red pigment,[5] which was confirmed to be betanidin-5-O-[2-O-(β-D-glucopyranosyl uronic acid)]-β-D-glucopyranoside, and two chromones,[6] which were identified to be 6, 7-methylenedioxychromone and 6, 7-dimethoxychromone. By further examination, four new compounds, **I, II, III** and **IV**, have now been isolated from the methanolic extract of this plant. The present paper deals with the result of experiments carried out on the elucidation of the chemical structure of **I, II** and **III**. Detailed data of **IV** will be published.

Materials and Methods

Materials

Glasswort (*Salicornia europaea* L.) was collected at the lakeside salt march of Notoro-ko, Hokkaido, in September of 1978.

Instruments

Melting points were measured on a YANACO micromelting point apparatus and uncorrected. UV spectra were determined on a Hitachi 124 Spectrometer, and IR spectra were recorded in potassium bromide tablets on a Hitachi 285 Grating Infrared Spectrometer. NMR Spectra were taken at 100 MHz with tetramethylsilane as an internal standard using JNM PS-100 High Resolution NMR Spectrometer. The signals were given on chemical shifts in δ and following abbreviation: s=singlet, d=doublet, dd=double

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doublet, m=multiplet, br=broad. Mass spectral data were obtained with a JEOL JMS-D300. Circular dichroism (CD) and optical rotation were measured on a JASCO ORD UV 5 Type Automatic Recording Spectropolarimeter.

Isolation of compounds

Reddish leaves and stems of glasswort (fresh weight 52 kg) were extracted with methanol (200 l). After the methanolic extract was concentrated under reduced pressure at 30-40°C, the concentrate was centrifuged at 9000 rpm for 10 min. The supernatant (ca. 14.2 l) was adjusted to pH 7.0 with sodium bicarbonate and extracted with ether. After the ethereal layer was dried over anhydrous sodium sulfate, it was concentrated to give a brown sirup (38.2 g). The sirup (35 g) was chromatographed on a silica gel column (Wako gel C-200) and eluted with benzene-ethyl acetate (10 : 0, F1), (9 : 1, F2), (8 : 2, F3), (7 : 3, F4), (6 : 4, F5), (5 : 5, F6) and (0 : 10, F7). Compound **I** (81 mg) was isolated from the concentrate of F2 fraction. Compound **II** (45 mg) and **III** (20 mg) were obtained from the F3 and F4 fractions by rechromatography on silica gel columns respectively, which were eluted with petroleum ether-ether (1 : 1) for F3 and (1 : 2) for F4, monitoring with an ultraviolet lamp (365 nm). Similarly the F6 fraction was purified on a silica gel column with chloroform to afford compound **IV** (25 mg).

Physicochemical properties of isolated compounds and their derivatives.

1) *Compound* **I**. Compound **I** was recrystallized from ethylacetate and subsequently chloroform-ether mixture to give colorless needles, mp 229-230°C, grayish blue to Folin-Ciocalteu, purple to concentrated sulfuric acid and chromotropic acid for the methylenedioxy group. *Anal.* Found: C, 67.89; H, 3.60. Calcd. for $C_{16}H_{10}O_5$: C, 68.09; H, 3.57%. UV λ_{max}^{EtOH} nm (log ε): 216 (4.70), 248 (4. 28), 289 (3. 97), 318 (4.04), 328sh (4.02). IR ν_{max}^{KBr} cm^{-1}: 3430, 2900, 1625 (C=O), 1550, 925 (methylenedioxy). MS m/z: 282 (M^+), 265, 164 (base peak), 118. NMR $\delta_{Me_4Si}^{acetone\text{-}d_6}$ ppm: 9.0 (1H, s, OH), 8.39 (1H, s, H-2), 7.52 (1H, s,H-5), 7.26-7.42 (2H, m, H-4', 6,'), 7.18 (1H, s, H-8), 6.84-7.06 (2H, m, H-3', 5'), 6.28 (2H, s, methylenedioxy).

2) *Monomethyl derivative of Compound* **I**. An ethereal solution of diazomethane was added to a solution of compound **I** (5 mg) in a small amount of absolute methanol. After standing overnight, the mixture was concentrated to afford monomethyl derivative (5 mg) as white needles, mp 145-147°C. UV λ_{max}^{EtOH} nm (log ε): 215 (4. 31), 240 (4.17), 281 (3.84), 318 (3.90), 328sh (3.88). IR ν_{max}^{KBr} cm^{-1}: 3040, 2920, 1647 (C=O), 1620, 1600, 1500, 933 (methylenedioxy). MS m/z: 296 (M^+), 265 (base peak), 165, 164, 132, 131.

NMR $\delta^{CCl_4}_{Me_4Si}$ ppm: 7.84 (1H, s, H-2), 7.59 (1H, s, H-5) 7.25-7.33 (2H, m, H-4′, 6′), 6.89-7.05 (2H, m, H-3′, 5′), 6.83 (1H, s, H-8), 6.10 (2H, s, methylenedioxy), 3.81 (3H, s, MeO).

3) *Compound* **II**. Compound **II** was recrystallized from chloroform-ethanol mixture to give colorless needles, mp 193-195°C, grayish blue to Folin-Ciocalteu, purple to concentrated sulfuric acid and chromotropic acid. $[\alpha]_D^{25}$ −82.4° (c=0.25, abs. MeOH). CD (c=0.0017, abs. MeOH) $[\theta]^{25}$ (nm): +18800 (350), −26800 (320), −27200 (312), +3800 (281), −5900 (270), +12500 (236), −12100 (223). HR-MS m/z: 284.0674 (M^+). Calcd. for $C_{16}H_{12}O_5$: 284.0685. UV λ^{EtOH}_{max} nm (log ε): 239 (4.21), 273 (3.94), 338 (3.84). IR ν^{KBr}_{max} cm^{-1}: 3200, 1653 (C=O), 1632, 1615, 1602, 1510, 1485sh, 1473, 1465sh, 1278, 1041, 940 (methylenedioxy). MS m/z: 284 (M^+), 266 (base peak), 165, 164, 155, 136, 120, 107. NMR $\delta^{acetone\text{-}d_6}_{Me_4Si}$ ppm: 8.70 (1H, s, OH), 7.52 (1H, dd, J=2 and 7 Hz, H-6′), 7.12-7.24 (2H, m, H-5, 4′), 6.84-7.00 (2H, m, H-3′, 5′), 6.58 (1H, s, H-8), 6.08 (2H, s, methylenedioxy), 5.79 (1H, dd, J=4.5 and 12 Hz, H-2), 2.81-2.97 (2H, m, H-3).

4) *Monomethyl derivative of Compound* **II**. An ethereal solution of diazomethane was added to a solution of compound **II** (4 mg) in a small amount of absolute methanol. After standing overnight, the mixture was concentrated to afford monomethyl derivative (4 mg) as white needles, mp 118-119°C. UV λ^{EtOH}_{max} nm (log ε): 239 (4.02), 272 (3.74), 339 (3.61). IR ν^{KBr}_{max} cm^{-1}: 1672 (C=O), 1630, 1615, 1607, 935 (methylenedioxy). MS m/z: 298 (M^+), 267, 266, 165, 164 (base peak), 136, 134, 121, 119. NMR $\delta^{acetone\text{-}d_6}_{Me_4Si}$ ppm: 7.59 (1H, dd, J=2 and 8 Hz, H-6′), 7.22-7.38 (2H, m, H-5, 4′), 7.09 (1H, m, H-5′), 6.88 (1H, d, J=8 Hz, H-3′), 6.49 (1H, s, H-8), 6.03 (2H, s, methylenedioxy), 5.76 (1H, dd, J=5 and 11 Hz, H-2), 3.93 (3H, s, MeO), 2.71-2.84 (2H, m, H-3).

5) *Compound* **III**. Compound **III** was recrystallized from petroleum ether-ether mixture to give colorless needles, mp 217-218°C, grayish blue to Folin-Ciocalteu. HR-MS m/z: 284.0692 (M^+), Calcd. for $C_{16}H_{12}O_5$: 284.0685. UV λ^{EtOH}_{max} nm (log ε): 245 (4.28), 289 (4. 03), 317 (4.04); +NaOEt 249, 264sh, 287, 362; +NaOAc 249, 287, 330, 360; +NaOAc/H_3BO_3 249, 287, 330, 360. IR^{KBr}_{max} cm^{-1}: 3500 (OH), 3180, 1625 (C=O), 1587, 1505. MS m/z: 284 (M^+), 267, 166 (base peak), 151, 123, 118. NMR $\delta^{acetone\text{-}d_6}_{Me_4Si}$ ppm: 9.20 (2H, br, OH), 8.40 (1H, s, H-2), 7.64 (1H, s, H-5), 7.27-7.42 (2H, m, H-4′, 6′), 7.10 (1H, s, H-8), 6.90-7.04 (2H, m, H-3′, 5′), 4.05 (3H, s, MeO).

6) *Dimethyl derivative of Compound* **III**. An ethereal solution of diazomethane was added to a solution of compound **III** (3 mg) in a small amount of absolute methanol. After standing overnight, the mixture was

concentrated to afford dimethyl derivative (3 mg) as white powder, mp 191–192°C. UV λ_{max}^{EtOH} nm (log ε): 216 (4.43), 237 (4.25), 282 (3.96), 316 (3.96). IR ν_{max}^{KBr} cm^{-1}: 1640 (C=O), 1600. MS m/z: 312 (M^+), 298, 281 181 (base peak), 180, 165, 137. NMR $\delta_{Me_4Si}^{DMSO\text{-}d_6}$ ppm: 8.25 (1H, s, H-2), 7.40 (1H, s, H-5), 7.19 (1H, s, H-8), 6.90–7.42 (4H, m, H-3′, 4′, 5′, 6′), 3.92 (3H, s, MeO), 3,87 (3H, s, MeO), 3.72 (3H, s, MeO).

Synthesis of 2′-hydroxy-6, 7-methylenedioxyisoflavone (**I**).

1) *2-Hydroxy-4, 5-methylenedioxyphenyl o-methoxybenzylketone* (**V**). A suspension of sesamol (2.5 g), *o*-methoxyphenylacetonitrile[1] (2.5 g) and fused zinc chloride (1.5 g) in dry ether (150 ml) was saturated with dry hydrogen chloride gas at 0°C. The reaction mixture was kept in a refrigerator overnight. The ethereal solution was decanted off and the greenish oily ketimine hydrochloride was washed twice with dry ether. The oil was then refluxed for 4 hr with water (150 ml). After cooling, the precipitates appeared in the reaction mixture were filtered and recrystallized from methanol to give (**V**) (530 mg, 10.9%) as colorless needles, mp 121–123°C. *Anal.* Found: C, 67.09; H, 4.89. Calcd. for $C_{16}H_{14}O_5$: C, 67.13; H, 4.93%. UV λ_{max}^{EtOH} nm (log ε): 213 (4.28), 240 (4.11), 277 (3.91), 350 (3.93). IR ν_{max}^{KBr} cm^{-1}: 1638 (C=O), 928 (methylenedioxy). MS m/z: 286 (M^+), 165 (base peak), 121. NMR $\delta_{Me_4Si}^{acetone\text{-}d_6}$ ppm: 12.92 (1H, s, OH), 7.44 (1H, s, phenyl H-6), 7.23 (2H, m, benzyl H-4, 6), 6.82–7.05 (2H, m, benzyl H-3, 5), 6.45 (1H, s, phenyl H-3), 6.07 (2H, s, methylenedioxy), 4.26 (2H, s, CH_2), 3.81 (3H, s, MeO).

2) *2′-Methoxy-6, 7-methylenedioxyisoflavone* (**VI**). To a solution of the ketone (**V**, 423 mg) in dry pyridine (2 ml) were added ethyl orthoformate (15 ml) and piperidine (2–3 drops). The reaction mixture was refluxed for 4 hr. After keepting in a refrigerator overnight, the precipitate were filtered and recrystallized from methanol to give (**VI**) (249 mg, 56.6%) as colorless needles, mp 156–158°C. *Anal.* Found: C, 68,80; H, 4.06. Calcd. for $C_{17}H_{12}O_5$: C, 68.92; H, 4.08%. UV λ_{max}^{EtOH} nm (log ε): 216 (4.34), 240 (4.23), 280 (3.88), 318 (3.97), 328sh (3.95). IR ν_{max}^{KBr} cm^{-1}: 1647 (C=O), 940 (methylenedioxy). MS m/z: 296 (M^+), 265 (base peak), 165, 164, 132, 131. NMR $\delta_{Me_4Si}^{CCl_4}$ ppm: 7.83 (1H, s, H-2), 7.57 (1H, s, H-5), 7.23–7.29 (2H, m, H-4′, 6′), 6.87–7.03 (2H, m, H-3′, 5′), 6.83 (1H, s, H-8), 6.10 (2H, s, methylenedioxy), 3.81 (3H, s, MeO).

3) *2′-Hydroxy-6, 7-methylenedioxyisoflavone* (**I**). To a solution of the isoflavone (**VI**, 35 mg) in acetonitrile (3 ml) was added aluminium chloride (150 mg). The resulting mixture was refluxed for 3 hr. After the solvent was removed, the residue was treated with cold aqueous 10% hydrochloric acid and extracted with chloroform. The extract was washed with water

and dried over anhydrous sodium sulfate. The solvent was concentrated to give (**I**) (10 mg, 29.9%) as colorless needles, mp 229–230°C. *Anal.* Found: C, 67.94; H, 3.61. Calcd. for $C_{16}H_{10}O_5$: C, 68.09; H, 3.57%. UV λ_{max}^{EtOH} nm (log ε): 216 (4.35), 248 (4.24), 289 (3.93), 318 (3.96), 328sh (3.93). IR ν_{max}^{KBr} cm^{-1}: 1625 (C=O), 925 (methylenedioxy). MS m/z: 282 (M^+), 265, 164 (base peak), 118. NMR $\delta_{Me_4Si}^{acetone\text{-}d_6}$ ppm: 8.95 (1H, s, OH), 8.37 (1H, s, H-2), 7.49 (1H, s, H-5), 7.24–7.40 (2H, m, H-4′, 6′), 7.15 (1H, s, H-8), 6.82–7.04 (2H, m, H-3′, 5′), 6.24 (2H, s, methylenedioxy).

This synthesized isoflavone (**I**) was identical with the natural product, compound **I**, in spectral data.

Synthesis of 2′-hydroxy-6, 7-methylenedioxyflavanone (**II**).

1) *2-Hydroxy-4, 5-methylenedioxyacetophenone* (**VII**). After sesamol (6.2 g), acetonitrile (6.3 g) and fused zinc chloride (12 g) were succesively dissolved in dry ether (200 ml), the solution was saturated with dry hydrogen chloride gas at 0°C and allowed to keep overnight in a refrigerator. The ethereal solution was decanted off and the brownish oily ketimine hydrochloride was washed twice with dry ether. The oil was then refluxed for 1 hr with water (200 ml). After cooling, the precipitates appeared in the reaction mixture were filtered and chromatographed on a silica gel column. The column was eluted with chloroform. The eluate was concentrated to give (**VII**) (889 mg, 10.9%) as white needles, mp 113–114°C (Lit.[7] 113–114°C). *Anal.* Found: C, 59.99; H, 4.47. Calcd. for $C_{16}H_{14}O_5$: C, 60.00; H, 4.48%. UV λ_{max}^{EtOH} nm (log ε): 239 (4.06), 276 (3.82), 345 (3.81). IR ν_{max}^{KBr} cm^{-1}: 1640 (C=O), 925 (methylenedioxy). MS m/z: 180 (M^+), 165 (base peak), 137, 107. NMR $\delta_{Me_4Si}^{acetone\text{-}d_6}$ ppm: 12.87 (1H, s, OH), 7.04 (1H, s, H-6), 6.44 (1H, s, H-3), 6.02 (2H, s, methylenedioxy), 2.57 (3H, s, AcO).

2) *2, 2′-Dihydroxy-4′, 5′-methylenedioxychalcone* (**VIII**). After the acetophenone (**VII**, 300 mg), salicylaldehyde (450 mg) and potassium hydroxide (16 g) were dissolved in water (40 ml) and methanol (40 ml), the solution was refluxed for 24 hr. The reaction mixture was added to water (300 ml) and neutralized with 2N hydrogen chloride. The neutral solution was extracted with chloroform. After removal of the solvent, the chloroform extract was chromatographed on a silica gel column. The column was eluted with chloroform. The eluate was concentrated to give (**VIII**) (50 mg, 10.6%) as orange red crystals, mp 190–191°C. *Anal.* Found: C, 67.77; H, 4.41. Calcd. for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26%. UV λ_{max}^{EtOH} nm (log ε): 241 (4.06), 259 (4.07), 271sh (4.06), 303 (4.06), 389 (4.20). IR ν_{max}^{KBr} cm^{-1}: 1625 (C=O), 925 (methylenedioxy). MS m/z: 284 (M^+), 266 (base peak), 165, 164, 155. NMR $\delta_{Me_4Si}^{acetone\text{-}d_6}$ ppm: 13.86 (1H, s, OH-2′), 9.07 (1H, br, OH-2), 8.31 (1H, d, J=16

Hz, H-β), 7.80-7.97 (2H, m, H-6, α), 7.59 (1H, s, H-6'), 7.13 (1H, m, H-4), 6.92-7.04 (2H, m, H-3, 5), 6.47 (1H, s, H-3'), 6.08 (2H, s, methylenedioxy).

3) *2'-Hydroxy-6, 7-methylenedioxyflavanone* (**II**). The chalcone (**VIII**, 30 mg) was dissolved in methanol (15 ml) and water (5 ml). Sodium acetate (500 mg) was added, and the mixture was refluxed for 48 hr. After dilution with water (50 ml) for 1 day, the solution was extracted with chloroform. The residue obtained upon evaporation of the chloroform was chromatographed on an aluminium oxide column (Merck, activity grade II-III) employing chloroform as the eluent. The eluate was concentrated to give (**II**) (18 mg, 60.0%) as colorless needles, mp 195°C. *Anal.* Found: C, 66.90; H, 4.27. Calcd. for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25%. UV λ_{max}^{EtOH} nm (log ε): 240 (4.32), 273 (4.21), 339 (3.87). IR ν_{max}^{KBr} cm^{-1}: 1650 (C=O), 935 (methylenedioxy). MS m/z: 284 (M^+), 266 (base peak), 165, 164, 155, 136, 120, 107. NMR $\delta_{Me_4Si}^{acetone\text{-}d_6}$ ppm: 8.72 (1H, s, OH), 7.52 (1H, dd, J=2 and 7 Hz, H-6'), 7.10-7.22 (2H, m, H-5, 4'), 6.86-7.02 (2H, m, H-3', 5'), 6.58 (1H, s, H-8), 6.08 (2H, s, methylenedioxy), 5.78 (1H, dd, J=4.5 and 12 Hz, H-2), 2.84-3.02 (2H, m, H-3).

This synthesized flavanone (**II**) was identical with the natural product, compound **II**, in spectral data.

Results and Discussion

The molecular formula of compound **I** was estimated to be $C_{16}H_{10}O_5$ by elementary analysis and mass spectrometry (M^+, m/z 282). The IR spectrum of compound **I** showed the carbonyl absorption at 1625 cm^{-1}. The UV spectrum showed the absorption maxima at 216 (4.28), 289 (3.97), 318 (4.04) and 328sh (4.02) nm. In the NMR spectrum (Fig. 1), a singlet (1H) assignable to the proton at C-2 in isoflavone skelton[12] was detected at δ 8.39. From these data, compound **I** was expected to be an isoflavone. The presence of a phenolic hydroxyl group in compound **I** was proved by a grayish blue color with Folin-Ciocalteu reagent and by the formation of a crystalline monomethyl derivative. Compound **I** gave a purple color with concentrated sulfuric acid and chromotropic acid indicating the presence of methylenedioxy groups.[10] Also in the NMR spectrum, these were supported by the appearance of two singlets for a hydroxyl group (δ 9.00) and a methylenedioxy group (δ 6.28). By the mass spectrum which contained two peaks at m/z 164 and 118 arising from retro-Diels-Alder fission of the heterocyclic ring, the presence of the methylenedioxy group in ring A and the hydroxyl group in ring B was substantiated. Among the singnals appeared in aromatic region on the NMR spectrogram, two singlets at δ 7.52 (1H) and 7.18 (1H)

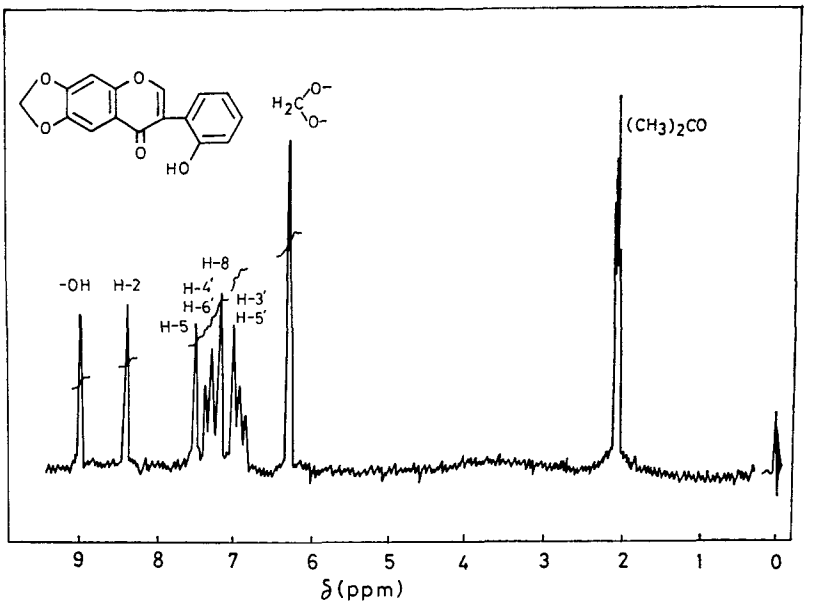
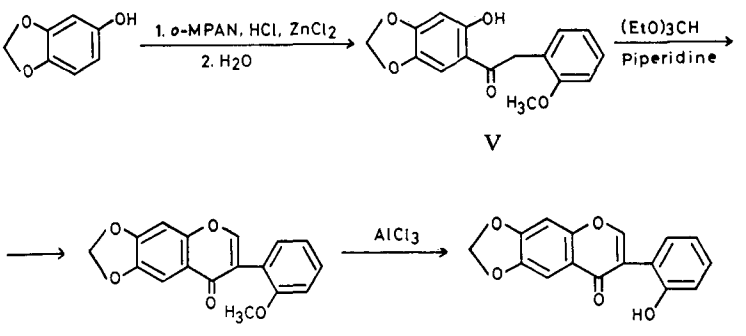


Fig. 1. NMR Spectrum of Compound **I** (Acetone-d_6).

were assigned to the protons at C-5 and C-8 in ring A, respectively. Therefore, the methylenedioxy group was estimated to be placed at the C-6 and C-7 positions in ring A. In the mass spectrum of the monomethyl derivative of compound **I**, the two peaks characteristic of isoflavones containing a 2'-methoxy group[3,4,11] were observed at m/z 265 and 165. Consequently, the hydroxyl group in ring B was estimated to be placed at the C-2' position.

It was thus evidenced that compound **I** was 2'-hydroxy-6, 7-meth-



Scheme 1. Synthetic Pathway of Compound **I**.

ylenedioxyisoflavone.

In order to assure the structure assignment, this compound was synthesized according to Scheme 1. Its spectral data were identical with those of the natural product, so compound **I** was evidenced to be 2'-hydroxy-6, 7-methylenedioxyisoflavone.

The molecular formula of compound **II** was estimated to be $C_{16}H_{12}O_5$ by high resolution mass spectrometry (M^+, m/z 284.0674, Calcd. for $C_{16}H_{12}O_5$, 284.0685). Compound **II** gave positive color tests for Folin-Ciocalteu and for concentrated sulfuric acid and chromotropic acid. The IR spectrum of compound **II** exhibited the carbonyl absorption at 1653 cm^{-1}. The UV spectrum showed the absorption maxima at 239 (4.21), 273 (3.94) and 338 (3,84) nm. These properties were similar to those of compound **I**. However, the NMR spectrum of compound **II** showed a doublet at δ 5.79 (1H) and a multiplet at δ 2.81–2.97 (2H), which were respectively assigned to the protons at C-2 and C-3 in a flavanone nucleus[12], instead of the C-2 proton (δ 8.39) found in compound **I**. Thus, flavanone structure for compound **II** may be reasonably considered. By the mass spectrum which contained two peaks at m/z 165 and 120 arising from retro-Diels-Alder fission of hetero-

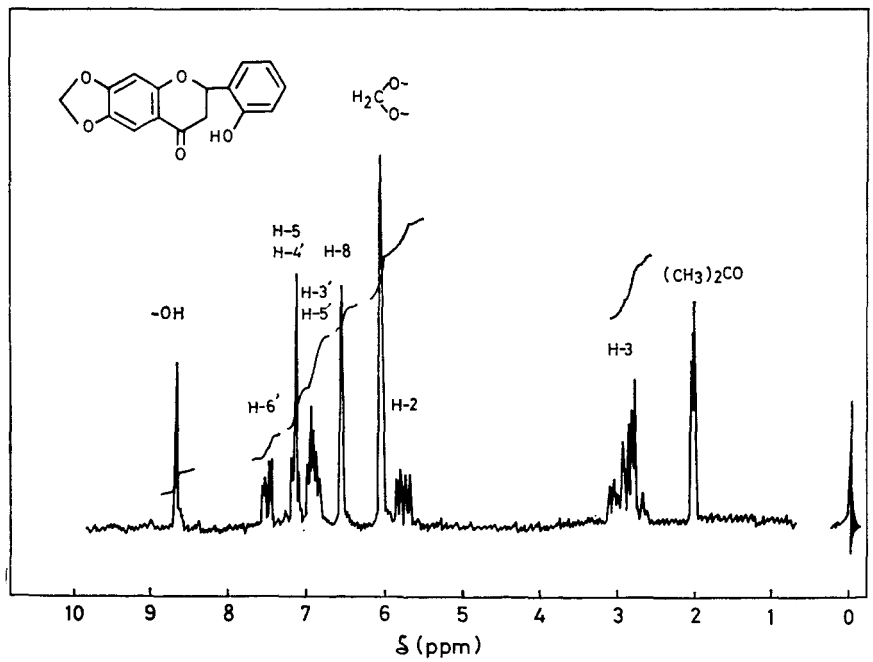
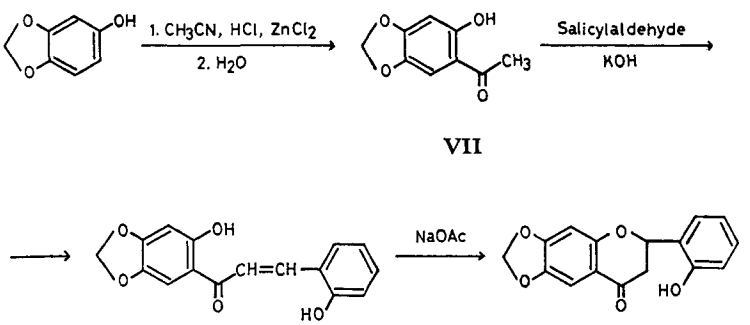


Fig. 2. NMR Spectrum of Compound **II** (Acetone-d_6).

cyclic ring, the presence of the methylenedioxy group in ring A and the hydroxyl group in ring B was substantiated. In the NMR spectrum (Fig. 2), two singlets at δ 7.16 (1H) and 6.58 (1H) were assigned to the protons at C-5 and C-8 in ring A, respectively. Therefore, the methylenedioxy group (δ 6.08) was estimated to be placed at the C-6 and C-7 positions in ring A. The mass spectrum showed the base peak (m/z 266) arising from loss of water from molecular ion, that was characteristic of flavanones containing a 2′-hydroxyl group[13]. Consequently, the hydroxyl group in ring B appeared to be placed at the C-2′ position. From the above results, the structure of compound **II** was estimated to be 2′-hydroxy-6, 7-methylenedioxyflavanone. To verify the estimated structure, this compound was synthesized according to Scheme 2. Its spectral data were identical with those of the natural product, compound **II**.



VII **VIII** **II**

Scheme 2. Synthetic Pathway of Compound **II**.

In oder to establish the absolute configuration of natural compound **II**, optical rotation and CD were examined. Its value of $[\alpha]_D^{25}$ was $-82.4°$ in absolute methanol. The CD curve (Fig. 3) showed a positive Cotton effect near 350 nm and a negative Cotton effect near 320 nm confirming the 2*S* configuration.[8]

Thus, the natural product, compound **II**, was evidenced to be (−)-(2*S*)-2′-hydroxy-6, 7-methylenedioxyflavanone.

The molecular formula of compound **III** was estimated to be $C_{16}H_{12}O_5$ by high resolution mass spectrometry (M^+, m/z 284.0674, Calcd. for $C_{16}H_{12}O_5$, 284.0685). The IR spectrum of compound **II** showed the carbonyl absorption at 1625 cm^{-1}. The UV spectrum showed the absorption maxima at 245 (4.28), 289 (4.04) and 317 (4.04) nm. The NMR spectrum (Fig. 4) revealed a singlet (1H) at δ 8.40 assignable to the proton at C-2 in isoflavone skelton[12].

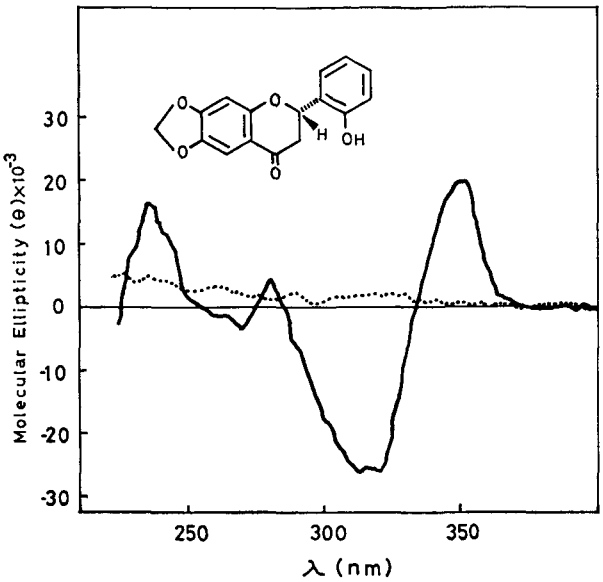


Fig. 3. CD curve of Compound **II**.
——: Compound **II**, ·····: Abs. **MeOH**

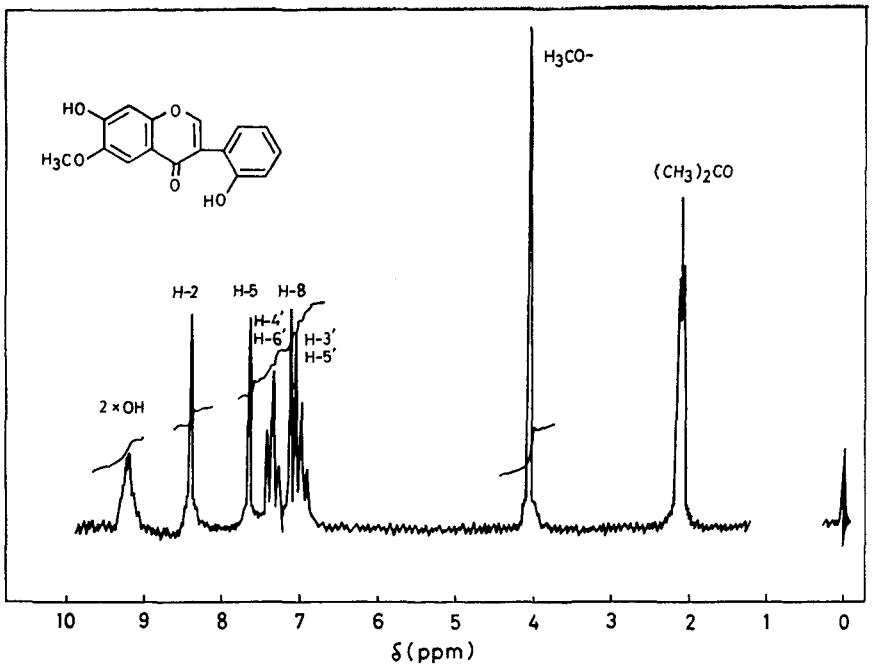


Fig. 4. NMR Spectrum of Compound **III** (Acetone-d_6).

Compound **III**, as well as compound **I**, was expected to be an isoflavone. The presence of two phenolic hydroxyl group in compound **III** was proved by color test for Folin-Ciocalteu and by the formation of a crystalline dimethyl derivative. The NMR showed a broad hydroxyl signal (2H) at δ 9.2 and a methoxy signal (3H) at δ 4.05. By the mass spectrum which contained two peaks at m/z 166 and 118 arising from retro-Diels-Alder fission of heterocyclic ring, the presence of the methoxy group and one hydroxyl group in ring A, and the other hydroxyl group in ring B was substantiated. The UV maxima exhibited the shift to longer wavelength on addition of anhydrous sodium acetate, so the hydroxyl group in ring A was estimated to be placed at the C-7 position. In the NMR spectrum, two singlets at δ 7.64 (1H) and 7.16 (1H) were assigned to the protons at C-5 and C-8 in ring A, respectively. Moreover, nuclear Overhauser effect was observed between the C-5 proton and the methoxy group signal, +32.7% CH {OCH_3}. The result suggested the presence of methoxy group at the C-6 position. In the mass spectrum of the dimethyl derivative of compound **III**, the two peaks characteristic of isoflavones containing 2′-methoxy group[3,4,11] were detected at m/z 281 and 181, so the hydroxyl group in ring B was estimated to be placed at the C-2′ position.

From the data described above, the structure of the natural product, compound **III**, was deduced to be 2′, 7-dihydroxy-6-methoxyisoflavone.

In the previous reports concerning the organic constituents in the *Salicornia* Genus plants,[2,9,14,15,16] the isolation of flavonoids has never been announced. Therefore, this paper is the first communication showing the occurrence of flavonoids in *Salicornia* Genus plant.

Summary

The new two isoflavones (compound **I** and **III**) and one flavanone (compound **II**) were isolated from the methanolic extract of glasswort (*Salicornia europaea* L.). On the basis of chemical and spectral evidences and synthetic methods, compound **I**, **II**, and **III** were shown to be 2′-hydroxy-6, 7-methylenedioxyisoflavone, (−)-(2*S*)-2′-hydroxy-6, 7-methylenedioxyflavanone, and 2′, 7-dihydroxy-6-methoxyisoflavone respectively.

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