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Studies on the Constituents of *Sophora* Species. XII.[1] Constituents of the Aerial Parts of *Sophora tomentosa* L. (1)[2]

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Two new benzofuran derivatives [I], mp 235—237°, $C_{15}H_{10}O_5$ and [II], mp 179—181°, $C_{16}H_{12}O_5$, together with *l*-maackiain, stigmasterol, medicagol, formononetin and 2′,4′,4-trihydroxychalone (isoliquiritigenin) were isolated from the aerial parts of *Sophora tomentosa* L., and the structures of I and II were established to be 2-(2′,4′-dihydroxyphenyl)-5,6-methylenedioxybenzofuran and 2-(2′-hydroxy-4′-methoxyphenyl)-5,6-methylenedioxybenzofuran on the bases of chemical and spectral evidence, respectively.

Keywords——*Sophora tomentosa* L.; Leguminosae; benzofuran; isoflavonoid; chalcone; C-13 NMR

In the previous papers, we reported the isolation and the structure elucidation of several new flavonoids from the root of *Sophora subprostrata* Chun et T. Chen, *S. angustifolia* Sieb. et Zucc. and *S. japonica* L.

In our further studying on this series, two new compounds (I), (II) and a few flavonoids have been isolated from the aerial parts of *Sophora tomentosa* L. This paper deals with the structure elucidation of these compounds.

Compound I and II were isolated from the ether-soluble fraction of the methanolic extract of the aerial parts of this plant, together with *l*-maackiain, stigmasterol, medicagol, formononetin and 2′,4′,4-trihydroxychalcone (isoliquiritigenin).

The compound I was obtained as colorless plates, mp 235—237°, M^+ 270.0521 (Calcd. for $C_{15}H_{10}O_5$: 270.0526), $C_{15}H_{10}O_5$, exhibiting positive ferric chloride reaction and Gibbs reaction. The infrared (IR) spectrum of I suggested the presence of hydroxyl groups (3440 cm^{-1}), aromatic ring (1620, 1600, 1520 cm^{-1}) and methylenedioxy group (1040, 940 cm^{-1}), and the ultraviolet (UV) spectrum suggested the 2-arylbenzofuran structure,[4] showing absorption maxima of 330 and 346 nm, which shifts in alkali to 348 and 358 nm respectively.

The proton magnetic resonance (PMR) spectrum of I shows one methylenedioxy group: δ 6.02 (2H, singlet), two hydroxyl groups: δ 9.58 and δ 10.15 (disappeared by the addition of D_2O) and ABX type proton signals on aromatic ring, which were confirmed by decoupling experiment, that is, irradiation of the signal at δ 7.57 (1H, d, J=8 Hz), altered the double doublet signal at δ 6.36 (1H, dd, J=8 Hz, 2 Hz) to the doublet signal, while the signal at δ 7.57 changed to singlet on irradiation of the signal at δ 6.36.

So these three protons were coupled as ABX type with each other. At the same time PMR spectrum exhibits three proton signals of olefinic or aromatic protons at δ 7.08 and δ 7.20 (Fig. 1).

On acetylation, I gave diacetate (Ia), mp 187—189°, $C_{19}H_{14}O_7$, whose PMR spectrum showed the signals due to two acetyl groups at δ 2.31 (3H), and δ 2.41 (3H) and hence I possesses two hydroxyl groups on aromatic ring.

1) Part XI: M. Komatsu, I. Yokoe, Y. Shirataki, and J. Chen, *Phytochemistry*, **15**, 1089 (1976).
2) This work was reported at the Annual Meeting of the Pharmacognostic Society of Japan, Tokyo, September, 1977.
3) Location: *Keyakidai 1-1, Sakado, Saitama, 350-02, Japan.*
4) *a*) N.W. Preston, K. Chamberlain, and R.A. Skipp, *Phytochemistry*, **14**, 1843 (1975); *b*) M. Takashina, Y. Takizawa, and T. Mitsuhashi, *Chemistry Letters*, **1974**, 869.

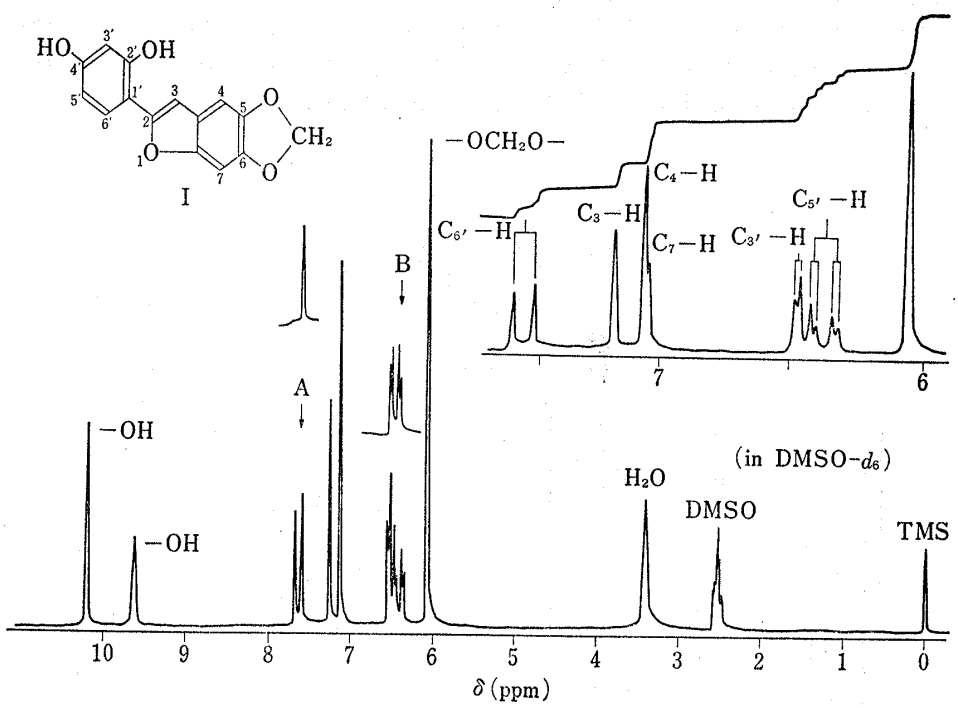


Fig. 1. PMR Spectrum of I

On methylation with diazomethane, I gave dimethyl ether (Ib), mp 168—169°, $C_{17}H_{14}O_5$. The PMR spectrum of Ib exhibited two methoxyl groups at δ 3.86 (3H) and δ 3.95 (3H), one methylenedioxy group at δ 5.97 (2H), ABX type protons on aromatic ring at δ 6.55—6.66 (2H, m), δ 7.88 (1H, d, J=8 Hz) and three protons at δ 6.93, δ 6.99, δ 7.08 (Fig. 2).

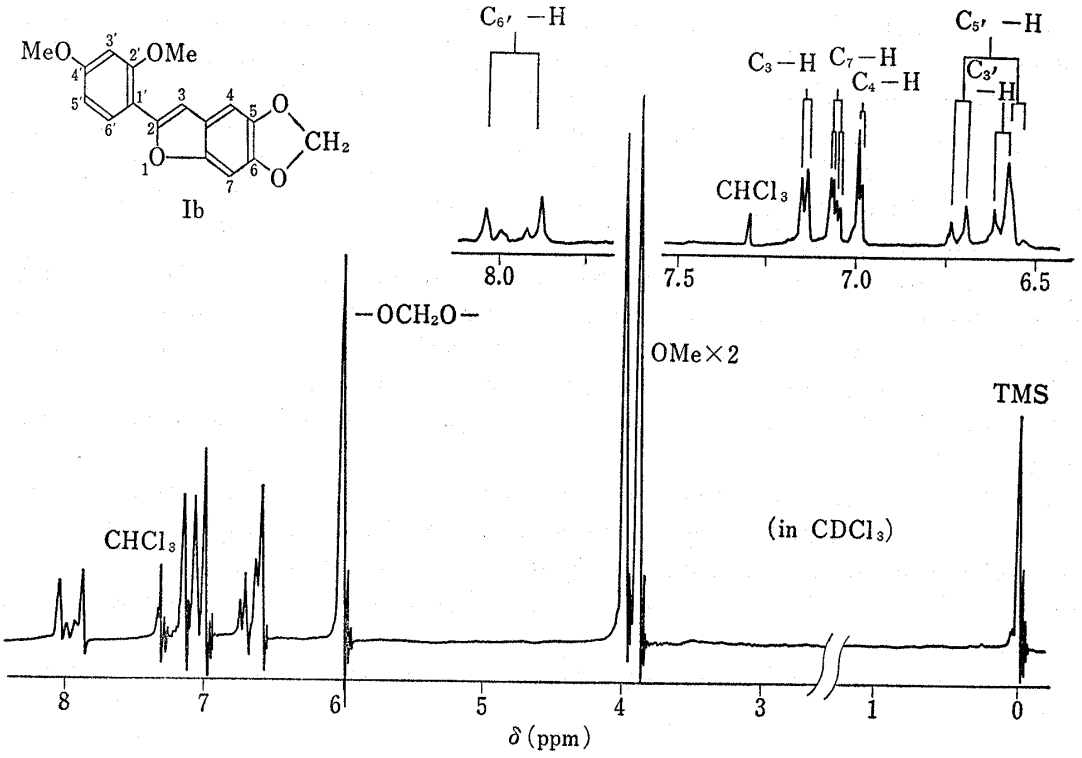


Fig. 2. PMR Spectrum of Ib

These data of Ib were in good agreement with those of medicagol methoxybenzofuran,[5] therefore Ib was established as 2-(2′,4′-dimethoxyphenyl)-5,6-methylenedioxybenzofuran, consequently I was determined to be 2-(2′,4′-dihydroxyphenyl)-5,6-methylenedioxybenzofuran. This structure was also supported by C-13 NMR spectrum of I (Fig. 3).

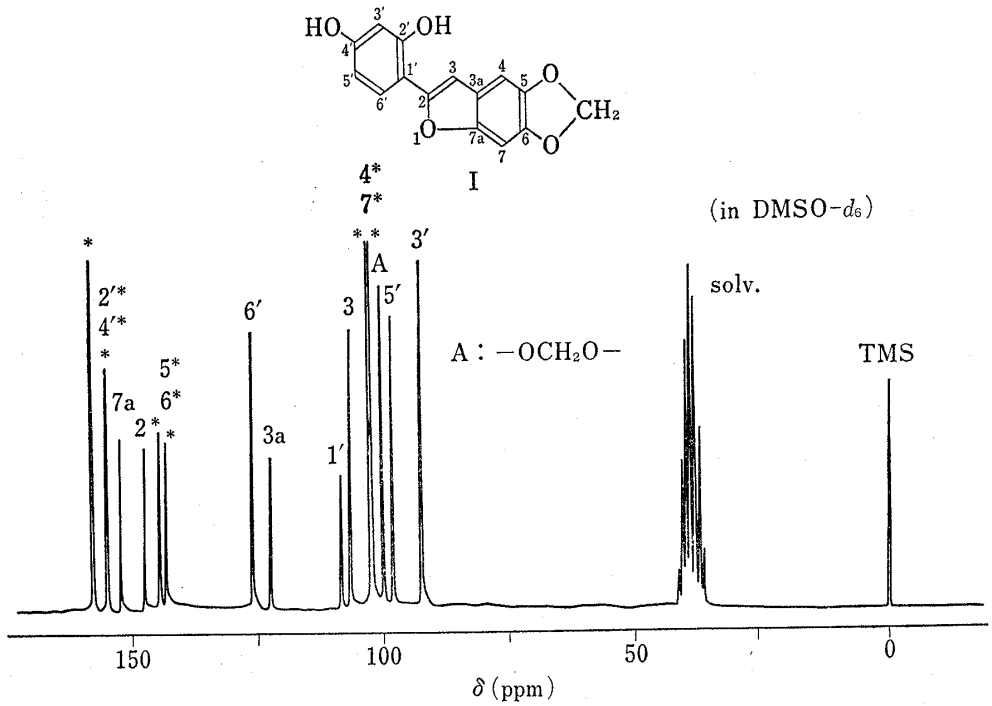


Fig. 3. C-13 NMR Spectrum of I

*Signals may be interchanged.

The compound II was obtained as colorless needles, mp 179—181°, M^+ 284, $C_{16}H_{12}O_5$, exhibiting a positive Gibbs reaction. UV and IR spectrum were similar to I. PMR spectrum was also similar to I except signal at δ 3.83 (3H, s), which was attributed to methoxyl group.

On methylation with diazomethane, II gave the dimethyl ether, the data of which were in good agreement with those of Ib. From these data, II was considered as the monomethylether of I and the position of methoxyl group is located at 2′ or 4′. II exhibited a positive Gibbs reaction, therefore the position of methoxyl group was determined at 4′ and the hydroxyl group was at 2′.

This structure can be also explained by comparison with the PMR spectra of vignafuran,[4a] its monoacetate,[4a] acetates and methylethers of I and II. The acetate of II showed an acetyl methyl proton signal at δ 2.40 and a methoxyl methyl proton signal at δ 3.84. Vignafuran monoacetate exhibited an acetyl methyl proton signal at δ 2.30 and two methoxyl methyl proton signals at δ 3.87 and δ 3.97. While Ia showed two acetyl methyl proton signals at δ 2.41 and δ 2.31.

From these data, it is clear that the signal due to 2′-acetyl methyl proton appeared around at δ 2.40 and 4′-acetyl methyl proton at δ 2.30, 2′-methoxyl methyl proton at δ 3.95 and 4′-methoxyl methyl proton at δ 3.86 in these 2-arylbenzofuran (Table I).

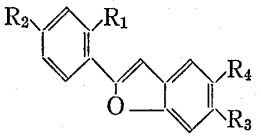
From these results II was determined as 2-(2′-hydroxy-4′-methoxyphenyl)-5,6-methylenedioxybenzofuran.

Furthermore, we have isolated some flavonoids from this plants but these structures are under investigation.

5) A.L. Livingston, S.C. Witt, R.E. Lundin, and E.M. Bickoff, *J. Org. Chem.*, **30**, 2353 (1965).

Table I. PMR Spectra Data (in $CDCl_3$) δ (ppm)

Compound	Ia	Ib	II	IIa	Vignafuran monoacetate	Vignafuran
-OAc	2.31				2.30	
	2.41			2.40		
-OMe		3.86	3.83	3.84	3.87	3.85
		3.95			3.97	3.90



I : R_1, R_2=OH, R_3, R_4=$-OCH_2O-$
Ia : R_1, R_2=OAc, R_3, R_4=$-OCH_2O-$
Ib : R_1, R_2=OMe, R_3, R_4=$-OCH_2O-$
II : R_1=OH, R_2=OMe, R_3, R_4=$-OCH_2O-$
IIa: R_1=OAc, R_2=OMe, R_3, R_4=$-OCH_2O-$
vignafuran: R_1, R_3=OMe, R_2=OH, R_4=H
vignafuran monoacetate: R_1, R_3=OMe, R_2=OAc, R_4=H

Recently, F. D. Monache and co-workers have isolated sophoronol[6] from the root of *S. tomentosa* L. but we have not yet isolated that compound from the aeral parts of same plants.

Experimental

All melting points were determined by a Shimadzu Micro Melting Point MM-2 Apparatus and are uncorrected. IR and UV spectra were recorded on a Nihon Bunko Model IRA-1 and UVIDIC-1 spectrometer, respectively. PMR and C-13 NMR spectra were measured at 100 MHz with a JNM-PS-100 spectrometer and 25 MHz with a JNM-PFT-100 NMR spectrometer, respectively, and chemical shifts are given on δ (ppm) scale with tetramethylsilane as the internal standard (s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet; br, broad). Mass spectra (MS) were taken on a Hitachi RMU-7M mass spectrometer with a direct inlet system.

GC–MS was run on a Shimadzu LKB-9000 using column 1.0 m×3 mmϕ) packed with 5% OV-1. Column chromatography was carried out with Wakogel C-200 and Polyamide C-200 (Wako Pure Chemical Ind. Ltd). Thin-layer chromatography (TLC) was conducted on Kieselgel G nach Stahl (Merck) and the spots were detected by spraying Gibbs reagent or spraying conc. H_2SO_4 followed by heating. The ratios of solvents and reagents in the mixtures are given in V/V.

Extraction and Separation——The dried aerial parts of *Sophora tomentosa* L., which were collected in Republic of China (1 kg) in 1975, was extracted three times with boiling MeOH. The methanolic extract (80 g) was extracted with ether and then with AcOEt.

The insoluble part was further extracted with *n*-BuOH. The combined etheral extract was concentrated (27 g) and chromatographed on silica gel using benzene and benzene–AcOEt as the solvents to give crude II (18 mg), *l*-maackiain (327 mg), stigmasterol (98 mg), medicagol (2 mg), I (122 mg), formononetin (22 mg) and crude 2′,4′,4-trihydroxychalcone (isoliquiritigenin) (250 mg). Crude II and 2′,4′,4-trihydroxychalcone were rechromatographed on polyamide column to afford II (3 mg) and 2′,4′,4-trihydroxychalcone (91 mg), respectively.

***l*-Maackiain**——Recrystallization from a mixture of MeOH–H_2O gave colorless needles, mp 179—181°, $[\alpha]_D^{22}$ −260° (c=1.0, acetone). This was identified by the direct comparison (mp, TLC, IR) with an authentic sample, isolated from *S. subprostrata*.

Stigmasterol——Recrystallization from MeOH gave colorless plates, mp 153—157°, Liebermann Burchard reaction (+). This was proved to be a mixture of campesterol (M^+=400, $C_{28}H_{48}O$), stigmasterol (M^+=412, $C_{29}H_{48}O$), and β-sitosterol (M^+=414, $C_{29}H_{50}O$) as a fractional part of 1:20:7 by GC–MS.

Medicagol——Recrystallization from MeOH gave colorless needles, mp over 320°. This was identified by the direct comparison (mp, TLC, IR, UV) with an authentic sample, isolated from *S. japonica*.

Compound I——I was recrystallized from a mixture of MeOH–H_2O as colorless plates, mp 235—237°, violet under UV light, greenish brown to $FeCl_3$, dark blue to Gibbs reaction. TLC *Rf*: 0.04 (*n*-hexane–AcOEt=3:1) (solv. 1), 0.50 (ether–*n*-hexane=4:1) (solv. 2), 0.52 (benzene–AcOEt=1:1) (solv. 3). *Anal.* Calcd. for $C_{15}H_{10}O_5$: C, 66.67; H, 3.73. Found: C, 66.97; H, 3.60. MS *m/e*: 270.0521 (M^+, Calcd. for $C_{15}H_{10}O_5$: 270.0526) base peak, 269.0459 ($C_{15}H_9O_5$: 269.0449), 241.0531 ($C_{14}H_9O_4$: 241.0500), 213.0547 ($C_{13}H_9O_3$: 213.0551), 184.0519 ($C_{12}H_8O_2$: 184.0523), 135.0250 ($C_4H_7O_5$: 135.0292). UV λ_{max}^{EtOH} nm (log ε): 230$_{(sh)}$ (4.33), 243$_{(sh)}$ (4.19), 281 (4.15), 330 (4.53), 346 (4.56). UV $\lambda_{max}^{EtOH+1\%KOH}$ nm (log ε): 243$_{(sh)}$ (4.25), 290 (4.15), 348 (4.54), 358 (4.51). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1620, 1600, 1520 (arom. C=C), 1040, 940 (–OCH_2O–). PMR (DMSO-d_6):

6) F.D. Monache, G.D. Monache, and G.B. Marini-Bettolo, *Gazz. Chim. Ital.*, **106**, 935 (1976).

6.02 (2H, s, $-OCH_2O-$), 6.36 (1H, dd, $J=8$ Hz, 2Hz, C_5'–H), 6.47 (1H, d, $J=2$ Hz, C_3'–H), 7.08 (2H, s, $C_{4,7}$–H), 7.20 (1H, s, C_3–H), 7.57 (1H, d, $J=8$ Hz, C_6'–H), 9.58 (1H, s, OH; disappeared by the addition of D_2O), 10.15 (1H, s, OH; disappeared by the addition of D_2O).

Acetylation of I (Ia)——To a solution of I in pyridine was added acetic anhydride. After heating on a water bath for 2 hr, the reaction mixture was worked up as usual manner. Recrystallization from a mixture of $CHCl_3$–MeOH gave colorless needles, mp 187—189°, no color to $FeCl_3$. *Anal.* Calcd. for $C_{19}H_{14}O_7$: C, 64.40; H, 3.98. Found: C, 64.14; H, 4.21. MS *m/e*: 354.0728 (M^+, Calcd. for $C_{19}H_{14}O_7$: 354.0738), 312.0613 ($C_{17}H_{12}O_6$: 312.0632), 270.0486 ($C_{15}H_{10}O_5$: 270.0527) base peak, 269.0497 ($C_{15}H_9O_5$: 269.0449), 241.0535 ($C_{14}H_9O_4$: 241.0500), 43.0180 (C_2H_3O: 43.0183). IR ν_{max}^{KBr} cm^{-1}: 1760, 1220, 1190 (ester), 1500, 1460 (arom. C=C), 1040, 940 ($-OCH_2O-$). PMR ($CDCl_3$): 2.31 (3H, s, C_4'–$OCOCH_3$), 2.41 (3H, s, C_2'–$OCOCH_3$), 6.00 (2H, s, $-OCH_2O-$), 6.91 (1H, d, $J=1$ Hz, C_7–H), 6.94 (1H, s, C_4–H), 6.99 (1H, d, $J=1$ Hz, C_3–H), 7.02—7.16 (2H, m, $C_{3',5'}$–H), 7.92 (1H, d, $J=9$ Hz, C_6'–H).

Methylation of I (Ib)——To a solution of I in MeOH, an ether solution of CH_2N_2 was added at 0°. After standing at room temp. for 24 hr, the solvent was removed and residue was recrystallized from MeOH as colorless needles, mp 168—169°, no color to $FeCl_3$. TLC *Rf*: 0.49 (solv. 1), 0.87 (solv. 2), 0.74 (solv. 3). *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.35; H, 5.01. MS *m/e*: 298.0838 (M^+, Calcd. for $C_{17}H_{14}O_5$: 298.0839) base peak, 283.0588 ($C_{16}H_{11}O_5$: 283.0604), 255.0633 ($C_{15}H_{11}O_4$: 255.0656), 240.0402 ($C_{14}H_8O_4$: 240.0422). UV λ_{max}^{EtOH} nm (log ε): $230_{(sh)}$ (4.29), $240_{(sh)}$ (4.16), 282 (4.17), 330 (4.57), 347 (4.61). IR ν_{max}^{KBr} cm^{-1}: 1610, 1580, 1510 (arom. C=C), 1040, 950 ($-OCH_2O-$). PMR ($CDCl_3$): 3.86 (3H, s, C_4'–OCH_3), 3.95 (3H, s, C_2'–OCH_3), 5.97 (2H, s, $-OCH_2O-$), 6.55—6.66 (2H, m, $C_{3',5'}$–H), 6.93 (1H, s, C_4–H), 6.99 (1H, s, C_7–H), 7.08 (1H, s, C_3–H), 7.88 (1H, d, $J=8$ Hz, C_6'–H).

Formononetin——Recrystallization from a mixture of benzene–MeOH gave colorless needles, mp 257—259°. This was identified by the direct comparison (mp, TLC, UV, IR, PMR and MS) with an authentic sample.

2′,4′,4-Trihydroxychalcone (isoliquiritigenin)——Recrystallization from a mixture of MeOH–H_2O gave yellow needles, mp 198—200°. This was identified by the direct comparison (mp, TLC, UV, IR, PMR and MS) with a synthetic sample.[7]

Compound II——II was recrystallized from MeOH as colorless needles, mp 179—181°, violet under UV light, blue to Gibbs reaction.

TLC *Rf*: 0.24 (solv. 1), 0.76 (solv. 2), 0.66 (solv. 3). UV λ_{max}^{EtOH} nm: $230_{(sh)}$, $244_{(sh)}$, 283, 331, 348. UV $\lambda_{max}^{EtOH+1\%KOH}$ nm: 257, 280, $332_{(sh)}$, 349, 365. IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1620, 1600, 1520 (arom. C=C), 1040, 940 ($-OCH_2O-$). PMR ($CDCl_3$): 3.83 (3H, s, C_4'–OCH_3), 6.00 (2H, s, $-OCH_2O-$), 6.52—6.62 (2H, m, $C_{3',5'}$–H), 6.81 (1H, s, C_4–H), 6.94 (1H, s, C_7–H), 7.01 (1H, s, C_3–H), 7.15 (1H, br, OH; disappeared by the addition of D_2O), 7.50 (1H, d, $J=9$ Hz, C_6'–H). MS *m/e*: 284 (M^+) base peak, 269 (M^+-CH_3).

Acetylation of II (IIa)——The same procedures described for acetylation of I were carried out to give colorless needles, mp 177—179°, no color to Gibbs reaction. PMR ($CDCl_3$): 2.40 (3H, s, C_2'–$OCOCH_3$), 3.84 (3H, s, C_4'–OCH_3), 6.00 (2H, s, $-OCH_2O-$), 6.70—6.85 (3H, m, $C_{3',5',4\text{ or }7}$–H), 6.92 (1H, s, $C_{7\text{ or }4}$–H), 6.98 (1H, s, C_3–H), 7.82 (1H, d, $J=8$ Hz, C_6'–H). MS *m/e*: 326 (M^+), 284 (M^+-CH_2CO) base peak, 269 [$M^+-(CH_2CO+CH_3)$], 255, 240, 43.

Methylation of II——The same procedures described for methylation of I were carried out and this was identified with Ib by the GC–MS and TLC.

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7) T. Kubota and T. Hase, *Nippon Kagaku Zasshi*, **87**, 1201 (1966).