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Studies on the Constituents of *Sophora* Species. II.[1] Constituents of *Sophora subprostrata* CHUN et T. CHEN. (2). Isolation and Structure of New Flavonoids, Sophoradochromene and Sophoranochromene[2]

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From the root of *Sophora subprostrata* CHUN et T. CHEN (Chinese drug: Shan Dou Gen (山豆根)) two new flavonoids, named sophoradochromene and sophoranochromene, have been isolated, whose structures have been established to be III and VII, respectively, by spectral and chemical data.

In the previous paper,[1] we reported the structures of sophoradin (I) and sophoranone (II) which were isolated from the root of *Sophora subprostrata* CHUN et T. CHEN (Chinese drug: Shan Dou Gen (山豆根)).

In our further studying on the constituents of this drug, two new flavonoids have been isolated. The present paper deals with the structures of these flavonoids, for which we now give the names, sophoradochromene and sophoranochromene, respectively.

Sophoradochromene (III) was obtained as yellow needles, mp 154°, $C_{30}H_{34}O_4$, with a positive ferric reaction. The infrared (IR) spectrum showed the presence of hydroxyl groups (3200 cm^{-1}) and an α,β-unsaturated carbonyl group [1625 cm^{-1} (KBr)]. The ultraviolet (UV) spectrum ($\lambda_{max}^{EtOH}=380$ mμ) suggested the presence of chalcone nucleus in III,[4a] which was also supported by the formation of a dihydrochalcone derivative (octahydrosophoradochromene) (IV), mp 134°, $C_{30}H_{42}O_4$, by catalytic hydrogenation.

On acetylation, IV gave a diacetate (V)[5] whose nuclear magnetic resonance (NMR) spectrum showed the signals due to two acetyl groups at τ 7.75 (3H) and τ 7.72 (3H).

In the NMR spectrum of III, two sharp singlets at τ −3.9 (1H) and τ 3.8 (1H, shifted to τ 3.91 at 50°) showed the presence of two hydroxyl groups. Two vinyl doublets (J=9.7 cps) at τ 3.72 (1H) and τ 4.38 (1H) in conjunction with a singlet at τ 8.56 (6H) for two methyl groups suggested a 2,2-dimethylchromene ring. Two singlets at τ 8.25 (6H) and τ 8.18 (6H) for four olefinic methyl groups and a broad triplet at τ 4.72 (2H) due to vinylic protons split by adjacent methylene groups, associated with a pair of doublets (J=7.5 cps) at τ 6.55 (2H) and τ 6.75 (2H) suggested the signals characteristic of two isopentenyl groups. Furthermore, these signals in III disappeared in IV, while a sharp singlet at τ 8.7 (6H, $\underset{-O}{\overset{C}{}}\!\!>(C\underline{H}_3)_2$), two sharp doublets ($J$=6 cps) at τ 9.05 and τ 9.07 (total intensity of 12H, $-CH(C\underline{H}_3)_2$), and broad multiplet due to methine and methylene groups appeared in IV.

1) Part I: M. Komatsu, T. Tomimori, K. Hatayama, Y. Makiguchi, and N. Mikuriya, *Chem. Pharm. Bull.* (Tokyo), **18**, 611 (1970).
2) Preliminary communication of this paper was published in *Chem. Pharm. Bull.* (Tokyo), **17**, 1302 (1969). This work was reported at the 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969.
3) Location: *No. 34-1, Takata-3-chome, Toshima-ku, Tokyo.*
4) *a*) L. Jurd, "The Chemistry of Flavonoid Compounds," ed. by T.A. Geissman, Pergamon Press, London, 1962, pp. 141—147; *b*) W.B. Whalley, *ibid.*, pp. 441—467.
5) The product was failed to be crystallized, but its purity was certified by thin-layer chromatography.

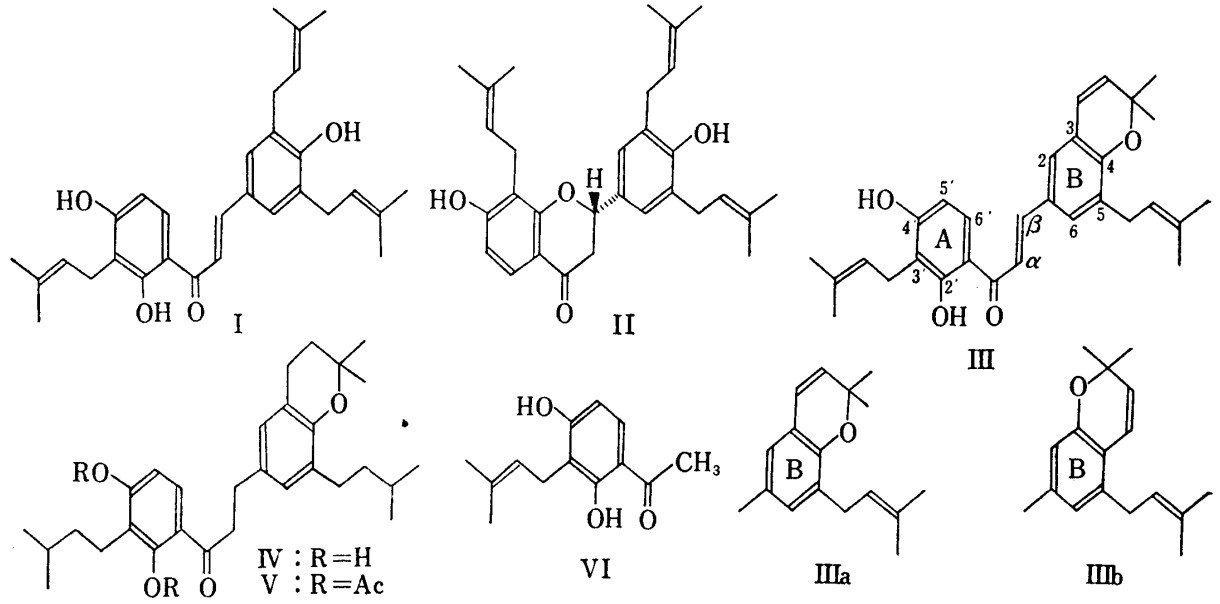
That one of the two hydroxyls in III must be placed at position 2′ is strongly suggested by indicating the presence of a hydrogen-bonded hydroxyl proton at τ −3.9 in the NMR spectrum and by giving a significant bathochromic shift of the UV absorption maximum ($\Delta\lambda_{max}$ =55 mμ) by adding alminum chloride.[4a,6-8]

On the other hand, it has generally been observed that the UV absorption maximum of chalcones containing a free 4-hydroxyl group undergoes a bathochromic shift of 70—90 mμ and an increase in intensity on the addition of sodium ethoxide, whereas in the case of chalcones containing a 4′-hydroxyl, it results in the shift of only 40—50 mμ when a 2′-hydroxyl is present.[4a]

The UV absorption maximum of III was shifted bathochromically by 40 mμ in the presence of sodium ethoxide, suggesting that the remaining a hydroxyl group must be placed at position 4′.

The 2′,4′-dihydroxy system in III was also proved by the following chemical method. Alkali fission of III with 50% potassium hydroxide gave a degradation product, mp 160°, $C_{13}H_{16}O_3$, which was a fragment corresponding to A-ring and proved to be identical with 2,4-dihydroxy-3-isopentenylacetophenone (VI) by comparison with the authentic sample from sophoradin.[1]

In the NMR spectrum of III, coupled doublets centred at τ 2.25, 2.65 (J=15 cps) and τ 2.79, 2.91 (J=2 cps) represent two pair of protons which are assigned to olefinic protons of chalcone nucleus (C-β, α-H) and *meta* proton pair of the B-ring, respectively.



6) L. Jurd and T.A. Geissman, *J. Org. Chem.*, **21**, 1395 (1956).
7) E.C. Bate-Smith and T. Swain, *J. Chem. Soc.*, **1953**, 2185.
8) T.A. Geissman, J.B. Harborne, and M.K. Seikel, *J. Am. Chem. Soc.*, **78**, 825 (1956).

These data indicated that the remaining an isopentenyl group and a 2,2-dimethylchromene ring must be placed at the B-ring, and at the same time, which led to the four possible substitution patterns (IIIa,b) (IIIc,d) for this ring.

The partial formula IIIa is presumed to be preferred on considering the co-existence of III with I. The decision was made by the transformation of I to III. Refluxing I in pyridine containing some piperidine afforded (±)-sophoranone and a small amounts of III.

On the basis of these data, sophoradochromene could be formulated as III.

Sophoranochromene (VII) was obtained as colorless needles, mp 152°, $[\alpha]_D^{25}$ −63.9 (EtOH), $C_{30}H_{34}O_4$. VII gave the absorption bands of hydroxyl and conjugated carbonyl groups in the IR spectrum. The UV spectrum was characteristic of 7-hydroxyflavanone series giving the absorption maxima at 286 mμ in ethanol and at 348 mμ in the presence of sodium hydroxide.[9]

Although VII shows negative ferric reaction, it possesses a phenolic hydroxyl forming monoacetate (VIII)[5] whose NMR spectrum indicates the presence of an acetyl group at τ 7.75 (3H).

On catalytic hydrogenation, VII gave a hexahydro derivative, mp 212°, $C_{30}H_{40}O_4$ (IX), which formed a monoacetate, mp 92°, $C_{32}H_{42}O_5$ (X), by acetylation.

The NMR spectrum of VII revealed the presence of two isopentenyl groups [τ 8.29 (12H, singlet), 6.73 (2H, doublet, J=7 cps), 6.61 (2H, doublet, J=7 cps), and 4.73 (2H, triplet, J=7 cps)], a 2,2-dimethylchromene ring [τ 8.56 (6H, singlet), 4.41 (1H, doublet, J=9.7 cps), and 3.72 (1H, doublet, J=9.7 cps)], C-2-proton [τ *ca.* 4.7 (1H, ill-defined multiplet, being overlapped by another 2H absorption of olefinic protons], C-3-protons [τ 7.1 (2H, multiplet)], four aromatic protons [τ 3.46 (1H, doublet, J=8 cps), 3.12 (1H, doublet, J=2 cps), 2.97 (1H, doublet, J=2 cps), and 2.30 (1H, doublet, J=8 cps)], and a hydroxyl group [τ 2.5 (1H, singlet, shifted to 2.87 at 50°)].

From these spectral characteristics, VII was considered to be the flavanone corresponding to III. The chemical proof was attempted in the following way.

VII was readily cleaved to form III by a short treatment with hot 5% potassium hydroxide. Furthermore, refluxing III in 0.2% sodium hydroxide regenerated (±)-sophoranochromene (VII'), mp 152°, which exhibited no optical rotation and did not depress the melting point on admixture with natural sophoranochromene (VII). The spectra (IR, UV, and NMR) of VII' were also found to be superimposable with those of VII.

Since all natural (−)-flavanones generally have S-configuration at C-2,[4b] the structure VII could be given to sophoranochromene.

Experimental

All melting points were uncorrected. UV spectra were measured after Jurd,[10,11] using a Hitachi Recording Spectrophotometer EPS-2U type. IR spectra were determined on KBr disks using a JASCO DS-301 Spectrophotometer. NMR spectra were taken at 60 Mcps in $CDCl_3$ with TMS as an internal standard using a Hitachi Perkin-Elmer Spectrometer (Model R-20). The chemical shifts were given in τ values. Abbrebiations: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, and br=broad.

Isolation of Flavonoids——The crude drug "Shan Dou Gen" (山豆根), the dried root of *Sophora subprostrata* Chun et T. Chen (100 kg), was extracted three times with boiling methanol. A ether-soluble part of the methanolic extracts was chromatographed on silica gel using chloroform as an eluant, and each fractions were checked by thin-layer chromatography(TLC). The faster-moving fraction consisted of sophoradochromene (III) and sophoradin (I), and the slower-moving fraction consisted of sophoranochromene (VII) and sophoranone (II), respectively. The former was submitted to rechromatography on silica gel. Elution with acetone-hexane (1:4) yielded III (1 g) and I (12 g) in turn. The latter was treated in the same way and gave VII (10 g) and II (40 g), respectively.

9) Y. Tomita, "Zikken Kagaku Koza (Suppl. Vol.)", Vol. 5, ed. by The Chemical Society of Japan, Maruzen Co., Ltd., Tokyo, 1966, pp. 940—942.
10) L. Jurd and R.M. Horowitz, *J. Org. Chem.*, **22**, 1618 (1957).
11) L. Jurd, *Arch. Biophem. Biophys.*, **63**, 376 (1956).

Sophoradochromene (III)——III was recrystallized from ether–hexane to give yellow needles, mp 154°. $FeCl_3$ (+). *Anal.* Calcd. for $C_{30}H_{34}O_4$: C, 78.57; H, 7.47. Found: C, 78.62; H, 7.26. UV λ_{max}^{EtOH} mμ (log ε): 380 (4.50); $\lambda_{max}^{EtOH\text{-}AlCl_3}$ mμ (log ε): 435 (4.57); $\lambda_{max}^{EtOH\text{-}NaOEt}$ mμ (log ε): 420 (4.52). IR (cm^{-1}): 3200 (OH); 1625 (conjugated CO); 1600 (sh.), 1590 (sh.), 1550 (arom. C=C); 1385 (CH_3). NMR: 8.56 (6H, s., $\underset{-O}{\overset{C}{>}}C(C\underline{H}_3)_2$), 8.25 (6H, s., $C=C(C\underline{H}_3)_2$), 8.18 (6H, s., $C=C(C\underline{H}_3)_2$), 6.75 (2H, d., $J=7.5$ cps, Ar–$C\underline{H}_2$–CH=C), 6.55 (2H, d., $J=7.5$ cps, Ar–$C\underline{H}_2$–CH=C), 4.72 (2H, br. t., $J=7.5$ cps, $-CH_2-C\underline{H}=C<\times 2$), 4.38 (1H, d., $J=9.7$ cps, –C<u>H</u>=CH–(*cis*)), 3.80 (1H, s., C-7-OH, shifted to 3.91 at 50°), 3.72 (1H, d., $J=9.7$ cps, –CH=C<u>H</u>–(*cis*)), 3.62 (1H, d., $J=8$ cps, C-5-H), 2.91 and 2.79 (2H, d., $J=2$ cps, C-2, 6-H), 2.65 (1H, d., $J=15$ cps, C-α-H), 2.35 (1H, d., $J=8$ cps, C-6′-H), 2.25 (1H, d., $J=15$ cps, C-β-H), −3.9 (1H, s., C-2′-OH).

Octahydrosophoradochromene (IV)——III (240 mg) in EtOH (30 ml) was hydrogenated with PtO_2 (60 mg) as the catalyst at room temperature. Four moles of H_2 were absorbed during 30 min, and the solvent was removed from the reaction mixture *in vacuo*. The product obtained was recrystallized from hexane to give colorless needles, mp 134°. *Anal.* Calcd. for $C_{30}H_{42}O_4$: C, 77.21; H, 9.07. Found: C, 76.91; H, 8.93. UV λ_{max}^{EtOH} mμ: 289. IR (cm^{-1}): 3220, 3140 (OH); 1615 (conjugated CO); 1600, 1575 (arom. C=C); 1385, 1370 (CH_3). NMR: 9.07 (6H, d., $J=6$ cps, $-CH(C\underline{H}_3)_2$), 9.05 (6H, d., $J=6$ cps, $-CH(C\underline{H}_3)_2$), 8.70 $\left(6H, s., \underset{-O}{\overset{C}{>}}(C\underline{H}_3)_2\right)$, 8.2—8.6 $\left(8H, br. m., -CH_2-C\underline{H}_2-C\underline{H}< \times 2 \text{ and } (CH_3)_2C<\underset{C\underline{H}_2-CH_2-}{\overset{O-}{}}\right)$, 6.8—7.65 (10H, br. m., Ar–$C\underline{H}_2$–CH_2– ×4 and Ar–CO–$C\underline{H}_2$–CH_2–), 4.20 (1H, s., C-4′-OH, shifted to 4.50 at 50°), 3.75 (1H, d., $J=9$ cps, C-5′-H), 3.27 (2H, br. s., C-2, 6-H), 2.52 (1H, d., $J=9$ cps, C-6′-H), −3.21 (1H, s., C-2′-OH).

Octahydrosophoradochromene Diacetate (V)[5]——IV was acetylated with Ac_2O and pyridine for 2 hr at 100°. The reaction mixture was poured into ice water and allowed to harden. The amorphous acetate obtained was failed to be crystallized, but it indicated only one spot on TLC. NMR: 9.10 (12H, d., $J=6$ cps, $-CH(C\underline{H}_3)_2$), 8.72 $\left(6H, s., \underset{-O}{\overset{C}{>}}C(C\underline{H}_3)_2\right)$, 8.2—8.7 $\left(8H, br. m., -CH_2-C\underline{H}_2-C\underline{H}< \times 2 \text{ and } (CH_3)_2-C<\underset{C\underline{H}_2-CH_2-}{\overset{O-}{}}\right)$, 7.75 (3H, s., –OAc), 7.70 (3H, s., –OAc), 6.8—7.7 (10H, br. m., Ar–$C\underline{H}_2$–CH_2– ×4 and Ar–CO–$C\underline{H}_2$–CH_2–), 3.21 (2H, br. d., $J=2$ cps, C-2, 6-H), 2.95 (1H, d., $J=9$ cps, C-5′-H), 2.37 (1H, d., $J=9$ cps, C-6′-H).

Alkali Fission of III (Formation of VI)——A mixture of III (300 mg) and 50% KOH solution (80 ml) was refluxed in an atmosphere of N_2 for 3 hr. After cooling and dilution with H_2O, the reaction mixture was acidified with dil. H_2SO_4 and extracted with ether. The ethereal ext. was fractionated by the usual method into a phenolic and an acidic fractions. The acidic fraction could not be investigated due to lack of the material. The phenolic fraction was chromatographed on silica gel. Elution with acetone–hexane (1:1) followed by crystallization from MeOH gave 2,4-dihydroxy-3-isopentenylacetophenone(VI) as colorless needles (20 mg), mp 160°, which was identical with authentic specimen[1] by the comparison of TLC, mixed fusion, IR, and UV.

Conversion of Sophoradin (I) to Sophoradochromene (III)——A mixture of sophoradin (460 mg), pyridine (120 ml), and piperidine (13 ml) was refluxed for 37 hr. After removal of the solvents, the yellow solid obtained was chromatographed on silica gel and eluted with acetone–hexane (1:4). The faster-moving fraction gave a crystalline compound, which was recrystallized from ether–hexane to give yellow needles (30 mg), mp 154°. Admixture with III did not depress the melting point and the IR and UV spectra were also found to be superimposable with those of III. The middle part of the fraction gave recovered material. The slower-moving fraction gave colorless needles (50 mg), mp 108°, $[\alpha]_D^{25}$ ±0 ($c=0.1$, EtOH), which was identical with (±)-sophoranone[1] by mixed fusion, UV, and IR spectra.

Sophoranochromene (VII)——VII was recrystallized from ether–hexane to give colorless needles, mp 152°. $[\alpha]_D^{25}$ −63.9 ($c=0.57$, EtOH). $FeCl_3$ (−). *Anal.* Calcd. for $C_{30}H_{34}O_4$: C, 78.57; H, 7.47. Found: C, 78.23; H, 7.22. UV λ_{max}^{EtOH} mμ (log ε): 286 (4.20); $\lambda_{max}^{EtOH\text{-}NaOH}$ mμ (log ε): 262 (4.22), 348 (4.49). IR (cm^{-1}): 3270 (OH); 1660 (conjugated CO); 1600, 1590 (arom. C=C); 1385, 1370 (CH_3). NMR: 8.56 $\left(6H, s., \underset{-O}{\overset{C}{>}}C(C\underline{H}_3)_2\right)$, 8.29 (12H, s., $C=C(C\underline{H}_3)_2 \times 2$), 7.10 (2H, m., C-3-$H_2$), 6.73 (2H, d., $J=7$ cps, Ar–$C\underline{H}_2$–CH=C), 6.61 (2H, d., $J=7$ cps, Ar–$C\underline{H}_2$–CH=C), 4.7 (3H, m., $-CH_2-C\underline{H}-C< \times 2$ and C-2-H), 4.41 (1H, d., $J=9.7$ cps, –C<u>H</u>=CH–(*cis*)), 3.72 (1H, d., $J=9.7$ cps, –CH=C<u>H</u>–(*cis*)), 3.46 (1H, d., $J=8$ cps, C-6-H), 3.12 and 2.97 (2H, d., $J=2$ cps, C-2′, 6′-H), 2.50 (1H, s., C-7-OH, shifted to 2.87 at 50°), 2.30 (1H, d., $J=8$ cps, C-5-H).

Sophoranochromene Monoacetate (VIII)[5]——VII on treatment with boiling Ac_2O–pyridine yielded an acetate which was failed to be crystallized, but it indicated only one spot on TLC. NMR: 8.60 $\left(6H, s., \underset{-O}{\overset{C}{>}}C(C\underline{H}_3)_2\right)$, 8.40 (6H, s., $C=C(C\underline{H}_3)_2$), 8.30 (6H, s., $C=C(C\underline{H}_3)_2$), 7.75 (3H, s., –OAc), 7.10 (2H, m., C-3-H_2), 6.77 (4H, br. d., $J=7$ cps, Ar–$C\underline{H}_2$–CH=C ×2), 4.8 (3H, br. m., $-CH_2-C\underline{H}-C< \times 2$ and C-2-H), 4.48 (1H, d., $J=9.7$ cps, –C<u>H</u>=CH–(*cis*)), 3.80 (1H, d., $J=9.7$ cps, –CH=C<u>H</u>–(*cis*)), 3.33 (1H, d., $J=8$ cps, C-6-H), 3.17 and 3.00 (2H, d., $J=2$ cps, C-2′, 6′-H), 2.25 (1H, d., $J=8$ cps, C-5-H).

Hexahydrosophoranochromene (IX)——VII (200 mg) in EtOH (30 ml) was hydrogenated with PtO_2 (50 mg) as the catalyst at room temperature. Three moles of H_2 were absorbed during 30 min, and the reaction mixture was worked up as usual and recrystallized from MeOH to give colorless needles, mp 212°.

Anal. Calcd. for $C_{30}H_{40}O_4$: C, 77.55; H, 8.68. Found: C, 77.31; H, 8.26. UV λ_{max}^{EtOH} mμ: 287. IR (cm^{-1}): 3280 (OH); 1660 (conjugated CO); 1600, 1585 (arom. C=C); 1385, 1370 (CH_3). NMR: 9.10 (12H, d., J=6 cps, $-CH(C\underline{H}_3)_2\times 2$), 8.68 ((6H, s., $_{-O}^{C}\!\!>C(C\underline{H}_3)_2$), 8.1—8.65 (8H, m., $-CH_2-C\underline{H}_2-C\underline{H}<$ ×2 and $(CH_3)_2-C<^{O-}_{C\underline{H}_2-CH_2-}$), 7.0—7.6 (8H, m., Ar–$C\underline{H}_2$–$CH_2$– ×3 and C-3-$H_2$), 4.72 (1H, q., J_{cis}=6 cps, J_{trans}=10.5 cps, C-2-H), 4.00 (1H, s., C-7-OH), 3.55 (1H, d., J=9 cps, C-6-H), 3.02 (2H, br. d., J=2 cps, C-2′, 6′-H), 2.33 (1H, d., J=9 cps, C-5-H).

Hexahydrosophoranochromene Monoacetate (X)——Ac_2O–pyridine treatment of IX yielded an acetate, which was recrystallized from MeOH to give colorless needles, mp 92°. *Anal.* Calcd. for $C_{32}H_{42}O_5$: C, 75.85; H, 8.36. Found: C, 76.15; H, 8.02. IR (cm^{-1}): 1750 (OAc); 1685 (conjugated CO); 1595 (arom. C=C); 1380 (sh.), 1375 (CH_3). NMR: 9.05 (12H, d., J=6 cps, $-CH(C\underline{H}_3)_2$), 8.66 (6H, s., $_{-O}^{C}\!\!>C(C\underline{H}_3)_2$), 8.1—8.7 (8H, br. m., $-CH_2-C\underline{H}_2-C\underline{H}<$ ×2 and $(CH_3)_2C<^{O-}_{C\underline{H}_2-CH_2-}$), 6.95—7.56 (8H, m., Ar–$C\underline{H}_2$–CH_2– ×3 and C-3-H_2), 4.58 (1H, q., J_{cis}=6 cps, J_{trans}=10.5 cps, C-2-H), 3.18 (1H, d., J=9 cps, C-6-H), 2.90 (2H, br. d., J=2 cps, C-2′, 6′-H), 2.08 (1H, d., J=9 cps, C-5-H).

Conversion of VII to III——VII (500 mg) was dissolved in 5% KOH/EtOH (20 ml) and refluxed for 30 min. After cooling and dilution with H_2O, the reaction mixture was acidified with dil. HCl to separate yellow powder. Recrystallization from ether–hexane gave yellow needles, mp 154°, which was identical with III by mixed fusion, TLC, UV, and IR.

Conversion of III to (±)-Sophoranochromene (VII′)——A mixture of III (300 mg), EtOH (15 ml), and 0.2% NaOH (25 ml) was refluxed for 5 hr and then allowed to stand overnight at room temperature. After dilution with H_2O, the reaction mixture was acidified with dil. H_2SO_4 and extracted with ether. The ethereal ext. was submitted to chromatography on silica gel. Elution with acetone–hexane (1:4) gave recovered material (100 mg) and a product (40 mg). The product was recrystallized from ether–hexane to give colorless needles, mp 152°. $[\alpha]_D^{25}$ ±0 (c=0.2, EtOH). Admixture with (−)-sophoranochromene (VII) did not depress the melting point, and the IR, UV, and NMR spectra were also found to be superimposable with those of VII.

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