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Studies on the Constituents of Guang-Dou-Gen (the Root of *Sophora subprostrata* Chun et T. Chen). (5).[1] Isolation of Two New Flavanones and Daidzein[2]

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Two new flavanones, I and VII, and daidzein were isolated from Guang-Dou-Gen (the root of *Sophora subprostrata* Chun et T. Chen). Fom the spectral data and comparison with sophoranochromene and its derivative, the structure of I was established as 2-[{3′-hydroxy-2′,2′-dimethyl-8′-(3-methyl-2-butenyl)}chroman-6′-yl]-7-hydroxy-8-(3-methyl-2-butenyl)chroman-4-one. The structure of VII was formulated from the spectral data and biogenetic speculation as 2-[{2′-(1-hydroxy-1-methylethyl)-7′-(3-methyl-2-butenyl)-2′,3′-dihydrobenzofuran}-5′-yl]-7-hydroxy-8-(3-methyl-2-butenyl)chroman-4-one.

In previous papers, we reported the isolation and the structure elucidation of seven new flavonoids, sophoradin,[4a] sophoranone,[4a] sophoradochromene,[4b] sophoranochromene,[4b] and compounds I,[1] IV,[1] and VIII,[1] and the characterization of caffeic acid esters,[5] *l*-maackiain,[5] and genistein[5] as the constituents of the root of *Sophora subprostrata* Chun et T. Chen, a species of the Chinese drug, Shan-Dou-Gen (山豆根), Guang-Dou-Gen[6] (広豆根).

Further studies on the constituents of this drug resulted in the isolation of two new flavanones, I and VII, and daidzein from the ether-soluble fraction of the methanolic extract. The present paper deals with the structure of these flavonoids.

Compounds (I) was obtained as colorless needles, mp 196°, M^+ 476, $C_{30}H_{36}O_5$. It gave the absorption bands for hydroxyl, conjugated carbonyl, and benzene ring in its infrared (IR) spectrum. Its ultraviolet (UV) spectrum (λ_{max}^{EtOH} nm: 285) suggested the presence of a flavanone ring,[7] and also indicated the presence of a hydroxyl group in position 7, according to the significant bathochromic shift[7] ($\lambda_{max}^{EtOH\text{-}NaOH}$ nm: 345). I gave a tetrahydro compound (II), mp 207°, by catalytic hydrogenation, and gave a diacetate (III), M^+ 560, by acetylation. I afforded a chalcone (IV) by alkali cleavage.

The nuclear magnetic resonance (NMR) spectrum of III suggested the presence of a phenolic and an alcoholic hydroxyl groups, according to two singlets at δ 2.29 (3H, C_7-$COCH_3$) and at δ 2.07 (3H, $C_{3'}$-$COCH_3$). The NMR spectrum of I exhibited the presence of two isoprenyl groups[1,4] [δ 1.67, 1.72 (each 6H, s), δ 3.20—3.45 (4H, m), δ 5.10—5.53 (2H, m)], a

1) Part 4: K. Kyogoku, K. Hatayama, S. Yokomori, M. Shio and M. Komatsu, *Chem. Pharm. Bull.* (Tokyo), **21**, 1192 (1973).
2) This work was reported at the Annual Meeting of the Pharmacognostic Society of Japan, Nagasaki, November, 1972.
3) Location: *3-34-1, Takada, Toshima-ku, Tokyo, 171, Japan.*
4) *a*) Part 1: M. Komatsu, T. Tomimori, K. Hatayama and N. Mikuriya, *Chem. Pharm. Bull.* (Tokyo), **18**, 602 (1970); *b*) Part 2: M. Komatsu, T. Tomimori, K. Hatayama, Y. Makiguchi and N. Mikuriya, *ibid.*, **18**, 741 (1970).
5) Part 3: M. Komatsu, T. Tomimori, K. Hatayama, and Y. Makiguchi, *Yakugaku Zasshi*. **90**, 459 (1970).
6) Shan-Dou-Gen[1] is a general term for several crude drugs. In order to express the name of the original crude drug more clearly, *Sophora subprostrata* Chun et T. Chen will be given by its Chinese name, Guang-Dou-Gen, from this paper.
7) Y. Tomita, "Jikken Kagaku Koza, (Experimental Chemistry), Suppl. Vol.," Vol. 5, ed. by the Chemical Society of Japan, Maruzen Co., Ltd., Tokyo, 1966, pp. 940—942.

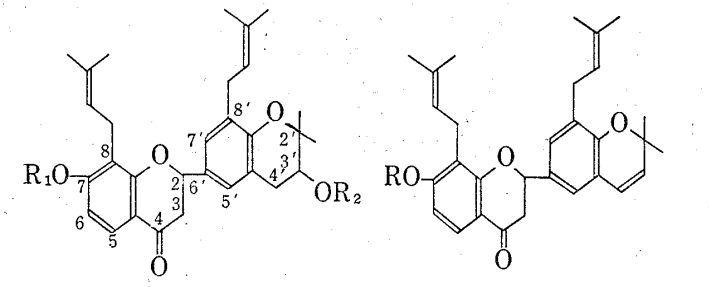
2′,2′-dimethyl-3′-hydroxychroman ring [δ 1.28, 1.29 (each 3H, s), δ 2.70—3.00 (2H, m, $C_{4'}$-H_2), δ 3.80 (1H, m, $C_{3'}$-H), δ 4.10 (1H, J=5.3 Hz, $C_{3'}$-OH; shifted to δ 4.00 at 48°)], two protons of *ortho*-coupling [δ 6.62 (1H, d, J=9.0 Hz, C_6-H), δ 7.56 (1H, d, J=9.0 Hz, C_5-H)], two protons of *meta*-coupling [δ 7.08, 7.16 (each 1H, d, J=2.2 Hz, $C_{5'}$-H, $C_{7'}$-H)], a hydroxyl group [δ 9.16 (1H, s, C_7-OH); shifted to δ 9.05 at 48°], and C-2 proton [δ 5.10—5.53 (1H, m)], two C-3 protons [δ 2.70—3.00 (2H, m)] in the flavanone ring.[1,4]

Biogenetically, the isoprenyl side chain is situated *ortho* to the hydroxyl group in many cases, and the two isoprenyl groups would be present in 8 and 8′ positions.

Treatment of I with *p*-toluenesulfonyl chloride gave a diester (V), which was converted to a chromene derivative (VI) on being heated in collidine. VI was identified with *p*-toluenesulfonyl ester of sophoranochromene[4b] from thin-layer chromatography (TLC), and IR and NMR spectra.

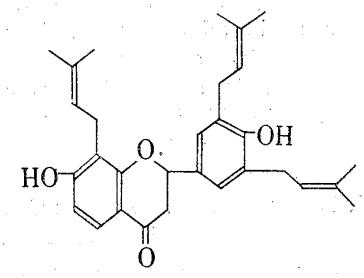
From these data, compound (I) was established as 2-[{3′-hydroxy-2′,2′-dimethyl-8′-(3-methyl-2-butenyl)}chroman-6′-yl]-7-hydroxy-8-(3-methyl-2-butenyl)chroman-4-one.

The compound (VII) was obtained as colorless needles, mp 194°, M^+ 476, $C_{30}H_{36}O_5$. It gave absorption bands for hydroxyl, conjugated carbonyl, and benzene ring in its IR spectrum. The UV spectrum (λ_{max}^{EtOH} nm: 285, $\lambda_{max}^{EtOH\text{-}NaOH}$ nm: 346) was characteristic of 7-hydroxyflavanone series.[7] By catalytic hydrogenation, VII gave a tetrahydro compound (VIII), mp 219°. On acetylation with a mixture of acetic anhydride and pyridine or acetic anhydride and sodium acetate, VII gave a monoacetate (IX), or a diacetate (X), indicating the presence of a tertiary hydroxyl group.

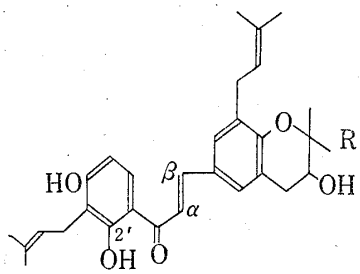


I : R_1=R_2=H
III : R_1=R_2=Ac
V : R_1=R_2=Ts

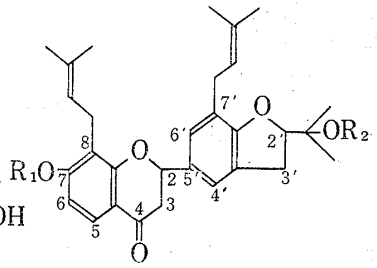
VI : R=Ts
sophoranochromene : R=H



sophoranone



IV



VII : R_1=R_2=H
IX : R_1=Ac, R_2=H
X : R_1=R_2=Ac

Chart 1

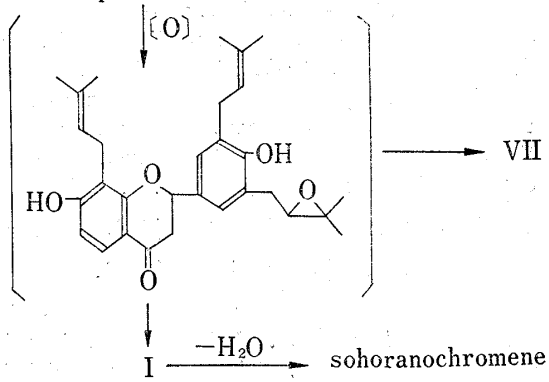


Chart 2

The NMR spectrum of VII showed, as in the spectrum of I, the presence of two isoprenyl groups[4] [δ 1.63, 1.71 (each 6H, s), δ 3.10—3.45 (4H, m), δ 5.10—5.55 (2H, m)], two protons of *ortho*-coupling [δ 6.61 (1H, d, J=9.0 Hz, C_6-H), δ 7.58 (1H, d, J=9.0 Hz, C_5-H)], two protons of *meta*-coupling [δ 7.11, 7.20 (each 1H, d, J=2.2 Hz, $C_{4'}$-H, $C_{6'}$-H)], a hydroxyl group [δ 9.20 (1H, br, C_7-OH); shifted to δ 9.00 at 48°], and C-2 proton [δ 5.10—5.55 (1H, m)], C-3 protons [δ 2.50—3.00 (2H, m)] in the flavanone ring.[1,4] It also showed the presence of C-3′

protons [δ 3.10—3.45 (2H, m)], C-2′ proton [δ 4.65 (1H, q, J=8.3 Hz, J=9.0 Hz)], and six protons [δ 1.26 (6H, s, $C_{2'}$ -C$\langle^{CH_3}_{CH_3}$)], but not the presence of the above-mentioned tertiary hydroxyl group.

The biogenetic speculation on monophenol by Bohlman and Grenz[8] would be applied for flavanones in Guang-Dou-Gen as in Chart 2, supporting the existence of VII.

From the above data and biogenetic speculation, compound VII would be formulated as 2-[{2′-(1-hydroxy-1-methylethyl)-7′-(3-methyl-2-butenyl)-2′,3′-dihydrobenzofuran}-5′-yl]-7-hydroxy-8-(3-methyl-2-butenyl)chroman-4-one.

These I and VII are the first example of the isolation of 3-hydroxy-1-chroman or 2-(1-hydroxy-1-methylethyl)-1-benzofuran derivatives as a part of flavanone ring.

The stereochemistry at C-2 and C-3′ of I, and C-2 and C-2′ of VII could not be examined due to small amount of the material available.

Daidzein was obtained as light brown needles, mp 320°. Its IR spectrum was identical with that of an authentic one.

Experimental

All the melting points are uncorrected. IR spectra were measured using a JASCO DS-701 spectrophotometer. NMR spectra were taken at 60 MHz with TMS as an internal standard using a Hitachi Perkin-Elmer spectrometer, Model R-20. The chemical shifts were given in δ values. The unit (Hz) of coupling constant (J Hz) was abbreviated.

Isolation of Flavanoids——The crude drug, Guang-Dou-Gen (100 kg) were extracted three times with boiling MeOH. The ether-soluble part (1 kg) of the MeOH extract was chromatographed on silica gel (100 kg) using acetone–hexane (1: 5) as an eluent and each fraction was checked by TLC. Neutral compounds, caffeic acid esters, sophoradochromene, the mixture of sophoradin and sophoranochromene, sophoranone, the mixture of *l*-maackiain and compounds I and VII, the mixture of compounds I and IV, and VIII in previous report,[1] genistein, and daidzein were eluted in that order. The mixture of *l*-maackiain and compounds I and VII, and daidzein were submitted to rechromatography on silica gel to yield I (800 mg), VII (300 mg), and daidzein (500 mg).

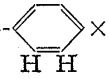
Compound (I)——I was recrystallized from benzene to colorless needles, mp 196°, M^+ 476. *Anal.* Calcd. for $C_{30}H_{36}O_5$: C, 75.60; H, 7.61. Found: C, 75.32; H, 7.50. UV λ_{max}^{EtOH} nm (log ε): 285 (4.17), $\lambda_{max}^{EtOH\text{-}NaOH}$ nm (log ε): 345 (4.46). IR ν_{max}^{KBr} cm^{-1}: 3330 (OH), 1661 (CO), 1602, 1585 (arom. C=C). NMR [$(CD_3)_2CO$]: 1.28, 1.39 (each 3H, s, $C_{2'}\langle^{CH_3}_{CH_3}$), 1.67, 1.72 (each 6H, s, Ar-CH_2-CH=C$\langle^{CH_3}_{CH_3}\times 2$), 2.70—3.00 (4H, m, C_3–H_2 and $C_{4'}$–H_2), 3.20—3.45 (4H, m, Ar-CH_2-×2), 3.80 (1H, m, $C_{3'}$–H), 4.10 (1H, d, J=5.3, $C_{3'}$–OH; shifted to 4.00 at 48°), 5.10—5.53 (3H, m, C_2–H and Ar–CH_2–C<u>H</u>=×2), 6.62 (1H, d, J=9.0, C_6–H), 7.08, 7.16 (each 1H, d, J=2.2, $C_{5'}$–H, $C_{7'}$–H), 7.56 (1H, d, J=9.0, C_5–H), 9.16 (1H, s, C_7–OH; shifted to 9.05 at 48°).

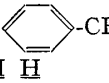
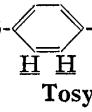
Catalytic Hydrogenation of I (Formation of II)——I (50 mg) in EtOH (20 ml) was hydrogenated over PtO_2 (30 mg) as a catalyst. Two moles of H_2 were absorbed during 1 hr. After removal of the catalyst, the solvent was evaporated *in vacuo*. Recrystallization of the residue from benzene yielded a tetrahydro compound (II), mp 207°, M^+ 480. *Anal.* Calcd. for $C_{30}H_{40}O_5$: C, 74.97; H, 8.39. Found: C, 74.96; H, 8.47. IR ν_{max}^{KBr} cm^{-1}: 3320 (OH), 1660 (CO), 1601, 1585 (arom. C=C). NMR [$(CD_3)_2CO$]: 0.88 (3H, s, –CH$\langle^{CH_3}$), 0.98 (9H, s, –CH$\langle^{CH_3}_{CH_3}$ and –CH$\langle_{CH_3}$).

Acetylation of I (Formation of III)——A mixture of I (80 mg), pyridine (1 ml), and Ac_2O (1 ml) was heated for 2 hr at 100°. When cooled, the mixture was poured into ice water (300 ml) and the mixture was extracted with ether. The extract was dried over Na_2SO_4 and evaporation of ether left a residue, which was chromatographed over silica gel with acetone–hexane (1: 7), giving a diacetate (III), M^+ 560. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1771, 1745 (OAc), 1697 (CO), 1599 (arom. C=C). NMR ($CDCl_3$): 2.07 (3H, s, $C_{3'}$-$COCH_3$), 2.29 (3H, s, C_7-$COCH_3$), 4.80—5.50 (4H, m, C_2-H and Ar-CH_2-C<u>H</u>=×2 and $C_{3'}$-H).

Alkali Cleavage of I (Formation of IV)——A mixture of I (100 mg) and 5% EtOH–KOH (300 ml) was refluxed for 2 hr. After addition of H_2O, EtOH was evaporated. The mixture was acidified to pH 2 with dil. HCl, extracted with ether, and the ether layer was washed with H_2O and dried over Na_2SO_4. Ether was evaporated and the residue was chromatographed over silica gel with acetone–hexane (1: 3), giving a chalcone (IV), M^+ 476. UV λ_{max}^{EtOH} nm: 376. NMR ($CDCl_3$): 7.41 (1H, d, J=15.0, C_α-H), 7.75 (1H, d, J=15.0, C_β-H), 13.85 (1H, s, $C_{2'}$-OH).

8) F. Bohlmann and M. Grenz, *Chem. Ber.*, **103**, 90 (1970).

Tosylation of I (Formation of V)——A mixture of I (200 mg), pyridine (5 ml), and *p*-toluenesulfonyl chloride (200 mg) was allowed to stand overnight at room temperature. After pyridine was evaporated, H_2O was added and the mixture was extracted with ether. The ether layer was washed with H_2O, dried over Na_2SO_4, and evaporated. The residue was chromatographed over silica gel with acetone–hexane (1: 9), giving a tosylate (V), which was recrystallized from MeOH to colorless needles, mp 127°. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1696 (CO), 1597 (arom. C=C), 1190, 1179 (SO_2). NMR ($CDCl_3$): 2.43 (6H, s, S--$CH_3\times2$), 7.30, 7.72 (each 4H, d, $J=8.5$, S-  $\times2$).

Treatment of V in Collidine (Form
was refluxed for 3 hr at 180—190°. After evaporation of the solvent *in vacuo*, the resulting residue was chromatographed over silica gel with acetone–hexane (1: 7). TLC[5] (*Rf*): 0.40 ($CHCl_3$), 0.46 (acetone–hexane=1: 7). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1695 (CO), 1599 (arom. C=C), 1192, 1180 (SO_2). NMR ($CDCl_3$): 2.42 (3H, s, S-  -CH_3), 5.59 (1H, d, $J=9.8$, C_3'-H), 6.23 (1H, d, $J=9.8$, C_4'-H), 7.28, 7.73 (each 2H, d, $J=8.5$, S-  -CH_3).

Tosylation of Sophoranochromene (Formation of VI)——A mixture of sophoranochromene (100 mg) in pyridine (3 ml) and *p*-toluenesulfonyl chloride (100 mg) was allowed to stand overnight at room temperature. Conventional work up gave its tosylate, which was identified with VI by TLC, and from IR and NMR spectral comparisons.

Compound VII——VII was recrystallized from benzene to colorless needles, mp 194°, M^+ 476. *Anal.* Calcd. for $C_{30}H_{36}O_5$: C, 75.60; H, 7.61. Found: C, 75.41; H, 7.48. UV λ_{max}^{EtOH} nm (log ε): 285 (4.21), $\lambda_{max}^{EtOH\text{-}NaOH}$ nm (log ε): 346 (4.46). IR ν_{max}^{KBr} cm^{-1}: 3288 (OH), 1660 (CO), 1597, 1582 (arom. C=C). NMR [$(CD_3)_2CO$]: 1.26 (6H, s, C_2'-C ), 1.63, 1.71 (each 6H, s, CH=  $\times2$), 2.50—3.00 (2H, m, C_3-H_2), 3.10—3.45 (6H, m, Ar-C$\underline{H}_2$-CH-$\times2$ and C_3'-H_2), 4.65 (1H, q, $J=8.3$, $J=9.0$, C_2'-H), 5.10—5.55 (3H, m, Ar-CH_2-C$\underline{H}$-$\times2$ and C_2-H), 6.61 (1H, d, $J=9.0$, C_6-H), 7.11, 7.20 (each 1H, d, $J=2.2$, C_4'-H and C_6'-H), 7.58 (1H, d, $J=9.0$, C_5-H), 9.20 (1H, br, C_7-OH; shifted to 9.00 at 48°).

Catalytic Hydrogenation of VII (Formation of VIII)——VII (50 mg) in EtOH (20 ml) was hydrogenated over PtO_2 (30 mg) as a catalyst. Two moles of H_2 were absorbed during 40 min. After removal of the catalyst, the solvent was evaporated *in vacuo*, and the residue was recrystallized from benzene to colorless needles (VIII), mp 219°, M^+ 480. *Anal.* Calcd. for $C_{30}H_{40}O_5$: C, 74.97; H, 8.39. Found: C, 74.94; H, 8.26. IR ν_{max}^{KBr} cm^{-1}: 3330 (OH), 1662 (CO), 1601, 1585 (arom. C=C). NMR [$(CD_3)_2CO$]: 0.87, 0.97 (each 6H, d, $J=1.5$, -CH  $\times2$), 1.25 (6H, s, C_2' -C ), 1.30—1.80 (6H, m, Ar-CH_2-C$\underline{H}_2$-C$\underline{H}\times2$), 2.40—3.00 (6H, m, Ar-C$\underline{H}_2$-$CH_2\times2$ and C_3-H_2).

Acetylation of VII (Formation of IX)——A solution of VII (50 mg), pyridine (1 ml), and Ac_2O (1 ml) was heated for 2 hr at 100°. Conventional work up gave its monoacetate (IX), which was recrystallized from acetone–hexane to colorless needles, mp 123°, M^+ 518. *Anal.* Calcd. for $C_{32}H_{38}O_6$: C, 74.10; H, 7.39. Found: C, 73.82; H, 7.28. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1770 (OAc), 1696 (CO), 1598 (arom. C=C). NMR ($CDCl_3$): 2.28 (3H, s, $OCOCH_3$).

Acetylation of VII (Formation of X)——A mixture of VII (50 mg), Ac_2O (2 ml), and AcONa (200 mg) was heated for 3 hr at 100°. The reaction mixture was treated as described in acetylation of I, giving a diacetate (X), M^+ 560. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1772, 1742 (OAc), 1697 (CO), 1600 (arom. C=C). NMR ($CDCl_3$): 1.98, 2.30 (each 3H, s, $OCOCH_3$), 4.80—5.50 (4H, m, C_2'-H, and C_2-H, and Ar-C$\underline{H}_2$-CH=$\times2$).

Daidzein——Recrystallization from MeOH gave light brown needles, mp 320°, M^+ 254. *Anal.* Calcd. for $C_{15}H_{10}O_4$: C, 70.86; H, 3.96. Found: C, 70.78; H, 3.92. Fluorescence (+). UV λ_{max}^{EtOH} nm: 249, 257 (sh), 305 (sh). Its IR spectrum was identical with that of an authentic sample.

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