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# Studies on the Constituents of *Sophora* Species. XXII.[1] Constituents of the Root of *Sophora moorcroftiana* BENTH. *ex* BAKER. (1)[2]

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A new flavanone, named sophoraflavanone G (I), mp 173—175 °C, $C_{25}H_{28}O_6$, and a new isoflavone, named sophoraisoflavone A (II), mp 235—237 °C, $C_{20}H_{16}O_6$, were isolated from the root of *Sophora moorcroftiana* BENTH. *ex* BAKER (Leguminosae), together with sophoraflavanone B, licoisoflavone B, calycosin, *l*-maackiain and medicagol. The structures of I and II were established to be (2*S*)-5,7,2′,4′-tetrahydroxy-8-lavandulylflavanone and 3-(5′-hydroxy-2′,2′-dimethyl-2*H*-benzopyran-8′-yl)-5,7-dihydroxychromone, respectively, on the basis of chemical and spectral evidence. The revision of the structures of nor-kurarinone and vexibinol is also described.

**Keywords**——*Sophora moorcroftiana*; Leguminosae; flavanone; isoflavone; sophoraflavanone G; sophoraisoflavone A; sophoraflavanone B; licoisoflavone B; calycosin; *l*-maackiain

In connection with our previous studies on the phenolics of some plants in the genus *Sophora* (Leguminosae), we have now investigated the constituents of *Sophora moorcroftiana* BENTH. *ex* BAKER.[1,2] The seed of this plant is used in China as a crude drug, which is known as "shā shēng huái" (砂生槐).[3] With regard to the constituents of this plant, matrine, oxymatrine, sophocarpine and oxysophocarpine have been reported.[4] As described in Experimental, a new flavanone (I) and a new isoflavone (II) were isolated, together with five known flavonoids, from the methanol extract of the root of this plant, which was collected in Nepal. This paper deals with the structural elucidation.

Compound I was obtained as colorless needles, mp 173—175 °C, $C_{25}H_{28}O_6$, $[\alpha]_D^{25}$ −49° ($c$=1.0, MeOH), Mg–HCl test (+). It showed hydroxyl (3400 $cm^{-1}$), conjugated carbonyl (1640 $cm^{-1}$) and benzene ring (1605, 1520 $cm^{-1}$) absorption bands in the infrared (IR) spectrum. The ultraviolet (UV) spectrum of I was characteristic of the 5,7-dihydoxyflavanone series.[5] The proton nuclear magnetic resonance ($^1$H-NMR) spectrum of I showed the signals of one lavandulyl group [$\delta$ 1.50, 1.57 and 1.65 ppm (each 3H, each s, $CH_3\times3$), 2.06—3.33 (5H, m, $>CH_2\times2$, –CH), 4.58 (2H, brs, $=CH_2$), 5.00 (1H, brt, $J$=7.0 Hz, $=CH$–$CH_2$–)], three hydroxyls [8.6 (OH × 2) and 9.5 ppm (OH × 1)], one chelated hydroxyl (12.20 ppm) and an ABX type grouping due to the C-2 (5.68 ppm) and C-3 protons (2.77 and 3.08 ppm). In the aromatic region of the spectrum, the signals of the remaining four protons appeared as a singlet ($\delta$ 6.02 ppm, 1H) due to the A-ring proton, and an ABC type grouping [6.47 ppm (1H, dd, $J$=8.1, 2.2 Hz), 6.49 ppm (1H, d, $J$=2.2 Hz) and 7.40 ppm (1H, d, $J$=8.1 Hz)] due to the C-5′, C-3′ and C-6′ protons in the B-ring. The mass spectrum (MS) of compound I showed the fragment ion $m/z$ 165 ($C_8H_5O_4$), indicating the presence of the lavandulyl group in the A-ring, and the elimination of $H_2O$ from the molecular ion showed the presence of a 2′-hydroxyl group in the flavanone nucleus.[6]

TABLE I. $^{13}$C-NMR Spectral Data for Sophoraflavanone G (I), Vexibinol, Kushenol F and Nor-kurarinone

| Carbon No. | Sophoraflavanone G (I) | Vexibinol[6] | Kushenol F[7] | Nor-kurarinone |
|---|---|---|---|---|
| 2 | 75.3 | 73.7 | 75.3 | 75.3 |
| 3 | 42.8 | 41.4 | 42.8 | 42.8 |
| 4 | 198.2 | 194.4 | 197.5 | 197.8 |
| 5 | 163.0 | 160.8 | 162.9[b] | 163.0 |
| 6 | 96.2 (dd) | 106.2 | 108.4 | 108.4 |
| 7 | 165.3 | 164.4 | 165.1[b] | 165.3 |
| 8 | 107.8 | 94.9 | 95.1 | 95.2 (d) |
| 9 | 162.1 | 160.5 | 162.3[b] | 162.5 |
| 10 | 103.2 | 101.4 | 102.9 | 102.9 |
| 1′ | 117.9 | 115.7 | 117.6 | 117.6 |
| 2′ | 156.1 | 155.2 | 156.2[c] | 156.3 |
| 3′ | 103.4 | 102.2 | 103.6 | 103.5 |
| 4′ | 159.4 | 158.0 | 159.3[c] | 159.5 |
| 5′ | 107.8 | 106.1 | 107.9 | 107.9 |
| 6′ | 128.6 | 127.3 | 128.7 | 129.0 |
| 1″ | 27.7[a] | 30.7 | 27.4 | 27.4[d] |
| 2″ | 47.8 | 46.2 | 47.4 | 47.5 |
| 3″ | 31.9[a] | 26.5 | 31.5 | 32.1[d] |
| 4″ | 124.5 | 123.1 | 124.6 | 124.5 |
| 5″ | 131.6 | 130.4 | 131.3 | 131.5 |
| 6″ | 25.8 | 25.7 | 25.8 | 25.9 |
| 7″ | 17.9 | 17.6 | 17.9 | 17.9 |
| 8″ | 149.1 | 147.5 | 149.1 | 149.1 |
| 9″ | 111.2 | 110.5 | 111.1 | 111.3 |
| 10″ | 19.1 | 18.6 | 19.1 | 18.9 |

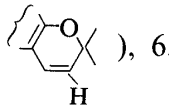
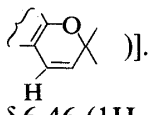
In $CD_3COCD_3$, $\delta$ (ppm).
*a*—*d*) Assignments may be interchanged in each column.

The chemical shift values excepting those of C-6 and C-8 in the carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectrum of compound I were very similar to those of kushenol F[7] (Table I). From the above data, compound I was considered to be a 6- or 8-lavandulyl-5,7,2′,4′-tetrahydroxyflavanone.

The position of the lavandulyl group on the A-ring was shown to be at C-8 by the long-range selective proton decoupling (LSPD) method in the $^{13}$C-NMR spectrum as follows. In the proton non-decoupled $^{13}$C-NMR spectrum with nuclear Overhauser effect (gated decoupling with NOE mode), a signal at $\delta$ 96.2 was observed as a double doublet ($J$=160.8, 7.7 Hz) which changed to a doublet ($J$=160.8 Hz) when the chelated hydroxyl proton at the C-5 position ($\delta$ 12.20 ppm) was selectively irradiated.[8] So, the signal of $\delta$ 96.2 ppm could be assigned to the C-6 carbon and the lavandulyl group must be located at C-8. In the circular dichroism (CD) spectrum, compound I showed a positive maximum at 333 nm ([$\theta$]+16960) and a negative maximum at 292 nm ([$\theta$]−118720), so the absolute configuration at C-2 was confirmed as *S*.[9] Thus, the structure of I was concluded to be (2*S*)-5,7,2′,4′-tetrahydroxy-8-lavandulylflavanone.

Hatayama and Komatsu[10] reported that nor-kurarinone [mp 133°C, colorless needles, $[\alpha]_D^{17}$ +8.0° ($c$=1.0, EtOH)] has the structure 5,7,2′,4′-tetrahydroxy-8-lavandulylflavanone, which is the same as that of sophoraflavanone G (I). Wu *et al.*[7] also reported that nor-kurarinone has an 8-lavandulyl group, but did not give detailed evidence, and assigned kushenol F [pale yellow amorphous, $[\alpha]_D^{25}$ −67° ($c$=0.11, MeOH)] the structure (2*S*)-5,7,2′,4′-tetrahydroxy-6-lavandulylflavanone. Thus, nor-kurarinone isolated by Hatayama and

Komatsu was investigated by $^{13}$C-NMR spectroscopy. It was concluded that the structure of their nor-kurarinone should be revised to 5,7,2′,4′-tetrahydroxy-6-lavandulylflavanone on the basis of gated decoupling with NOE and LSPD experiments (Table I). Further, Batirov *et al.*[6] reported vexibinol [mp 174—176°C, $[\alpha]_D^{20}$ $-36.5\pm2°$ ($c=1.1$, MeOH)] to have the structure (2*S*)-5,7,2′,4′-tetrahydroxy-6-lavandulylflavanone, which is the same as that of kushenol F. Since they did not carry out gated decoupling with NOE and LSPD experiments, and the spectral data and melting point were very similar to those of I, the structure of their vexibinol might have to be revised to be the same as that of sophoraflavanone G (I).

Compound II was obtained as colorless needles, mp 235—237°C, $C_{20}H_{16}O_6$. The UV absorption at 259 nm and the IR absorption at 1650 cm$^{-1}$ (C=O) indicated an isoflavone structure. The $^1$H-NMR spectrum (DMSO-$d_6$) showed signals of one 2,2-dimethyl chromene ring [$\delta$ 1.32 (6H, s, $CH_3\times2$), 5.64 (1H, d, $J=10$ Hz,  ), 6.64 (1H, d, $J=10$ Hz,  )]. The aromatic region showed signals due to *ortho* and *meta*-coupled protons at $\delta$ 6.46 (1H, d, $J=8.3$ Hz), 6.96 (1H, d, $J=8.3$ Hz) and $\delta$ 6.26 (1H, d, $J=2.2$ Hz), 6.41 (1H, d, $J=2.2$ Hz), along with a singlet at $\delta$ 8.14, characteristic of an isoflavone C-2 proton. Moreover, the spectrum also showed signals of three hydroxyls exchangeable with $D_2O$ at $\delta$ 9.84, 10.92 and 13.04. The lowest field signal was assigned to 5-OH, which was strongly chelated with the 4-carbonyl group. Acetylation with acetic anhydride and pyridine yielded a triacetate (IIa), mp 199—201°C, whose $^1$H-NMR spectrum exhibited signals of three acetoxyls at $\delta$ 2.30, 2.33 and 2.34. These results indicated that compound II is a trihydroxyisoflavone having a 2,2-dimethylchromene ring. The UV spectrum in the presence of shift reagents proved the presence of free 5- and 7-hydroxyls, since marked bathochromic shifts were observed on the addition of $AlCl_3$ and NaOAc, respectively. Therefore, the *meta*-coupled protons were assigned as C-6 and C-8 protons on the A-ring.

The remaining hydroxyl and 2,2-dimethylchromene were located in the B-ring, along

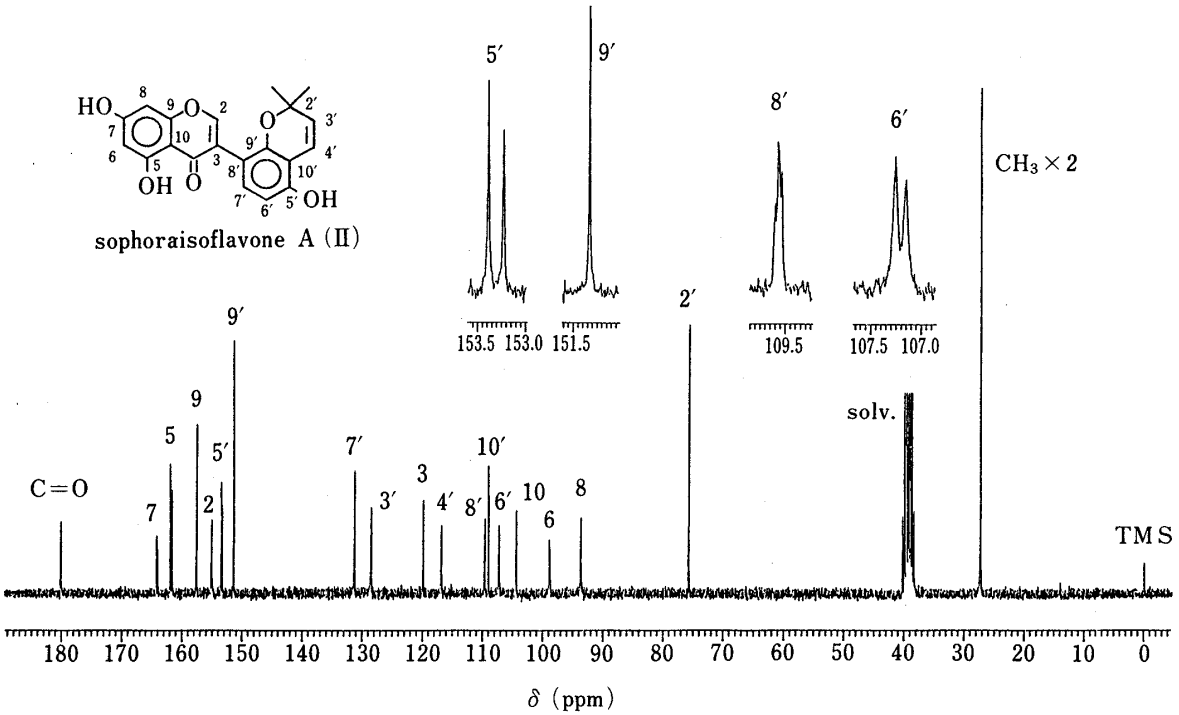
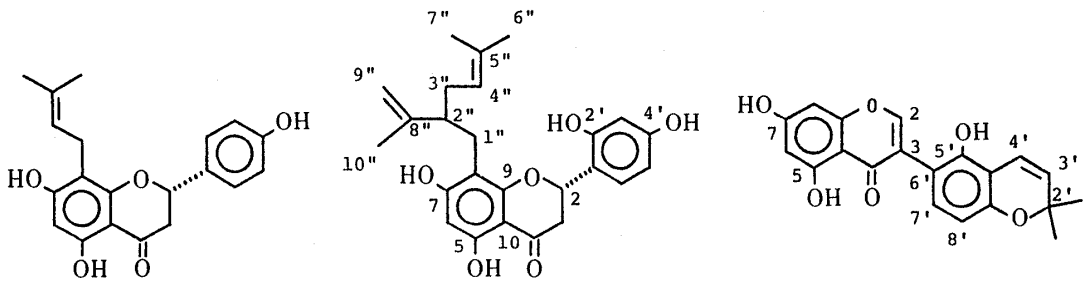



Fig. 1. $^{13}$C-NMR Spectrum of II (14°C, DMSO-$d_6$+Small Amount of $D_2O$)



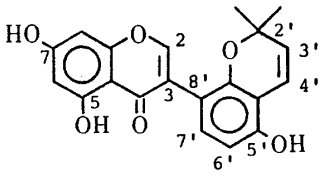
sophoraflavanone B



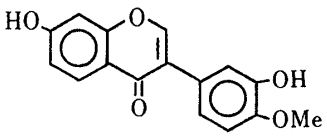
sophoraflavanone G (I)



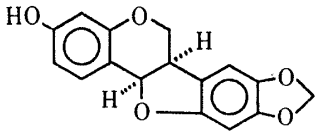
licoisoflavone B



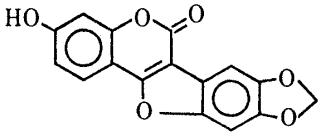
sophoraisoflavone A (II)



calycosin



*l*-maackiain



medicagol

Fig. 2

with *ortho*-coupled aromatic protons. From these results, compound II was considered to be 3-(5′-hydroxy-2′,2′-dimethyl-2*H*-benzopyran-8′-yl)-5,7-dihydroxychromone or 3-(5′-hydroxy-2′,2′-dimethyl-2*H*-benzopyran-6′-yl)-5,7-dihydroxychromone (licoisoflavone B[11]).

Finally, the $^{13}$C-NMR spectrum of compound II favored the former structure, because the signals of C-5′ ($\delta$ 153.4 ppm) and C-6′ ($\delta$ 107.2 ppm) were observed to show a $^{2}$H (D)-induced upfield shift on the addition of a small amount of $D_2O$.[12] Therefore, the hydroxyl group in the B-ring is located at C-5′ in the structural formula of II (Fig. 1).

From the above data, the structure of II was concluded to be 3-(5′-hydroxy-2′,2′-dimethyl-2*H*-benzopyran-8′-yl)-5,7-dihydroxychromone.

Furthermore, sophoraflavanone B,[8] licoisoflavone B, calycosin, *l*-maackiain and medicagol were also isolated. These are new components of this plant.

## Experimental

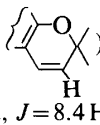
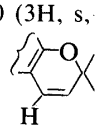
All melting points were determined on a Yanagimoto MP-S3 micro melting point apparatus and are uncorrected. IR and UV spectra were taken on Nihon Bunko IR-810 and UVIDEC-430 machines, respectively. $^{1}$H- and $^{13}$C-NMR spectra were obtained on a JEOL JNM GX-270 FT NMR spectrometer at 270 and 67.9 MHz, respectively, and chemical shifts are given in $\delta$ (ppm) with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). The multiplicities (s, d, t, q) of carbon signals were determined by means of gated decoupling with NOE. MS were taken on a JEOL JMS DX-300 mass spectrometer with a direct inlet system. The CD was recorded on a JASCO J-20A spectrometer. Column chromatography was performed on silica gel (Merck, Kieselgel 60 Art. 7734). Thin layer chromatography (TLC) was conducted on Kieselgel 60 F254 (Merck, Art. 5715) and the solvent systems were benzene : EtOAc = 1 : 1 and *n*-hexane : acetone = 2 : 1. The spots were detected by spraying concentrated $H_2SO_4$, followed by heating.

**Extraction and Separation**——The dried roots of *Sophora moorcroftiana*, which were collected in Jomsom, Nepal in September 11, 1986 (700 g), were extracted five times with boiling MeOH. The methanol extract (135 g) was shaken with $Et_2O$ and $H_2O$. The $Et_2O$ extract was concentrated (51 g) and chromatographed on silica gel using benzene and benzene–EtOAc (9 : 1—1 : 1) as solvents (each fraction was checked by TLC) to give *l*-maackiain (340 mg), licoisoflavone B (400 mg), sophoraflavanone B (220 mg), medicagol (15 mg), sophoraisoflavone A (410 mg), sophorafla-

vanone G (350 mg) and calycosin (86 mg) in that order.

**Sophoraflavanone G (I)**——Colorless needles (benzene), mp 173—175 °C. MS *m/z*: 424.1901 ($M^+$, Calcd for $C_{25}H_{28}O_6$: 424.1886), 406 ($C_{25}H_{26}O_5$), 301 ($C_{16}H_{13}O_6$), 283 ($C_{16}H_{11}O_5$), 165 ($C_8H_5O_4$), 136 ($C_8H_8O_2$). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 292 (4.41), $337_{(sh)}$ (3.70). UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 291, 335. UV $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 255, 313, 350. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 1640 (C=O), 1605, 1520 (arom. C=C). $^1$H-NMR ($CD_3COCD_3$); see text. $^{13}$C-NMR ($CD_3COCD_3$); see Table I. CD ($c=5.0\times10^{-4}$, MeOH) $[\theta]^{25}$; see text.

**Sophoraisoflavone A (II)**——Colorless needles (benzene), mp 235—237 °C. MS *m/z*: 352.0945 ($M^+$, Calcd for $C_{20}H_{16}O_6$: 352.0947), 337 ($C_{19}H_{13}O_6$), 185 ($C_{12}H_9O_2$), 153 ($C_7H_5O_4$). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 259 (4.78), $291_{(sh)}$ (4.39), $330_{(sh)}$ (3.84). UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 270, 322. UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 271, $312_{(sh)}$, 374. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 1650 (C=O), 1620, 1500 (arom. C=C). $^1$H-NMR (DMSO-$d_6$) $\delta$: see text. $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 27.4 (q, $CH_3\times2$), 75.8 (s, C-2′), 93.6 (d, C-8), 98.9 (d, C-6), 104.3 (s, C-10), 107.2 (d, C-6′), 109.0 (s, C-10′), 109.5 (s, C-8′), 116.8 (d, C-4′), 119.8 (s, C-3), 128.5 (d, C-3′), 131.2 (d, C-7′), 151.3 (s, C-9′), 153.4 (s, C-5′), 155.0 (d, C-2), 157.5 (s, C-9), 161.9 (s, C-5), 164.1 (s, C-7), 180.1 (s, C-4).

**Acetylation of II (IIa)**——A solution of II (50 mg) in a mixture of $Ac_2O$ (2 ml) and pyridine (2 ml) was allowed to stand at room temperature overnight, and the reaction mixture was worked up in the usual manner. IIa (42 mg) was obtained as colorless needles (MeOH), mp 199—201 °C, no color to $FeCl_3$. MS *m/z*: 478 ($M^+$). $^1$H-NMR ($CD_3COCD_3$) $\delta$: 1.38 (6H, s, $CH_3\times2$), 2.30 (3H, s, OAc), 2.33 (3H, s, OAc), 2.34 (3H, s, OAc), 5.79 (1H, d, $J$=9.9 Hz,  ), 6.45 (1H, d, $J$=9.9 Hz,  ), 6.70 (1H, d, $J$=8.4 Hz, 6′-H), 6.98 (1H, d, $J$=2.2 Hz, 6-H), 7.13 (1H, d, $J$=8.4 Hz, 7′-H), 7.35 (1H, d, $J$=2.2 Hz, 8-H), 8.13 (1H, s, 2-H).

**Sophoraflavanone B**——Colorless needles (benzene), mp 189 °C. $[\alpha]_D^{25}$ −26° ($c$=1.0, MeOH). This was identified by direct comparison (mixed melting point, TLC, IR, MS, $^1$H- and $^{13}$C-NMR) with an authentic sample isolated from *S. tomentosa*.[8]

**Licoisoflavone B**——Colorless needles (benzene), mp 180—183 °C. The $^{13}$C-NMR spectral data for this compound were identical with those reported for licoisoflavone B.[13] Triacetate; colorless needles (MeOH), mp 191—193 °C.

**Calycosin**——Colorless needles (benzene–MeOH), mp 245—248 °C. This was identified by direct comparison (mixed melting point, TLC, IR, MS and $^1$H-NMR) with an authentic sample.

***l*-Maackiain**——Colorless needles (MeOH–$H_2O$), mp 181—183°C, $[\alpha]_D^{25}$ −255° ($c$=1.0, acetone). This was identified by direct comparison (mixed melting point, TLC, IR) with an authentic sample isolated from *S. tomentosa*.[14]

**Medicagol**——Colorless needles (MeOH), mp over 300 °C. This was identified by direct comparison (mixed melting point, TLC and IR) with an authentic sample isolated from *S. tomentosa*.[14]

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## References and Notes

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