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Studies on the Constituents of *Sophora* Species. XVI.[1] Constituents of the Root of *Euchresta japonica* Hook. f. ex Regel (1)[2]

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A new flavanone, named euchrestaflavanone A (I), mp 145—147°C, $C_{25}H_{28}O_5$, was isolated from the root of *Euchresta japonica* Hook. f. ex Regel (Leguminosae) together with *l*-maackiain, medicagol and trifolirhizin.

The structure of I was established to be 6,3′-di-γ,γ-dimethylallyl-5,7,4′-trihydroxyflavanone on the basis of chemical and spectral evidence.

Keywords——*Euchresta japonica*; Shan-Dou-Gen; Leguminosae; euchrestaflavanone A; *l*-maackiain; medicagol; trifolirhizin; flavonoid

Euchresta japonica (Leguminosae; Japanese name, Miyamatobera) is an evergreen and perennial shrub occurring in shady and humid places in the southern region of Japan. The roots have been used as a substitute for a Chinese drug, Shan-Dou-Gen (山豆根) which has itself been used as an analgesic, antipyretic and anti-inframmatory agent or as an anti-tumor agent. In the previous papers,[3] we reported the isolation and structural elucidation of twelve new flavonoids from the root of *Sophora subprostrata*, which is described at present as the origin of this drug in "Zhong Yao Zhi" (中薬志).[4] Therefore, we have begun studies on the constituents of *Euchresta japonica* in order to establish whether there is any phytochemical variation in these plants, and a new flavanone, named euchrestaflavanone A (I), together with *l*-maackiain, medicagol and trifolirhizin, has been isolated. This paper deals with the structural elucidation of the new flavanone.

Euchrestaflavanone A (I) (M^+=408, $[\alpha]_D^{22}$ −35° in EtOH, $C_{25}H_{28}O_5$), a major phenolic component (0.1%) was obtained as colorless needles, mp 145—147°C. It gave a greenish-brown color in the ferric chloride reaction, a dark blue color in the Gibbs reaction, and a positive Mg–HCl test. The infrared (IR) spectrum of I showed strong absorptions at 1650 cm^{-1} (chelated C=O group) and 3400 cm^{-1} (OH). The ultraviolet (UV) spectrum (λ_{max}^{EtOH}=295, $340_{(sh)}$nm) suggested a flavanone structure. It formed a triacetate (Ia) (pyridine/Ac_2O) indicating the presence of three hydroxy groups.

The proton magnetic resonance (^{1}H-NMR) spectrum of I (CD_3COCD_3) showed δ 5.42 (1H, d.d, J=12.0, 3.6 Hz) and δ 2.6—3.1 (2H, m), attributed to the C-ring protons (C_2–H, C_3–H_2) of the flavanone. It also indicated the presence of two γ,γ-dimethylallyl groups [δ 1.62, 1.72 (12H, each s, $(C\underline{H}_3)_2\times2$), δ 3.0—3.5 (4H, m, Ar–C$\underline{H}_2$–CH=$\times2$), δ 5.21 (2H, br.t, J=7.4 Hz, –CH_2–C$\underline{H}$=C$\langle\times2$)], three hydroxy groups [δ 8.4, 9.6 (each 1H, each br. s) and 12.1 (1H, s, chelated with C_4-carbonyl); both of which disappeared on the addition of D_2O] and four aromatic protons [δ 6.03 (1H, s, C_6 or C_8–H), δ 6.89 (1H, d, J=8.0 Hz, $C_{5'}$–H), δ 7.1—7.4 (2H, m, $C_{2'}$ and $C_{6'}$–H)] (Fig. 1).

The mass spectrum (MS) of I showed major ion peaks at *m/e* 353 (46%), 220 (53%) and 188 (20%). The ion peaks at *m/e* 220 and 188 were derived from a retro-Diels–Alder fragmentation. In view of the ^{1}H-NMR spectral data, the ion peak at *m/e* 220 must include the A-ring. This ion loses C_4H_7 to yield the ion peak at *m/e* 165 (90%) and therefore the A-ring contains one γ,γ-dimethylallyl group. On the other hand, the ion peak at *m/e* 188 arises from the B-ring. It loses C_4H_7 to yield the ion peak at *m/e* 133 (30%). Therefore the B-ring also contains one γ,γ-dimethylallyl group.

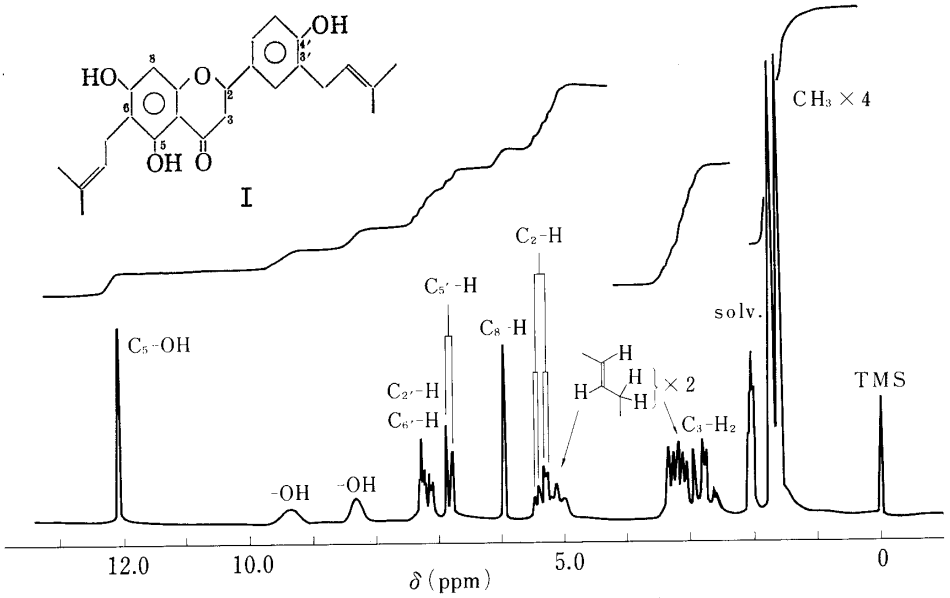


Fig. 1. ^{1}H-NMR Spectrum of I (in CD_3COCD_3)

From these data, it is clear that there are two γ,γ-dimethylallyl groups in I, one being attached to the A-ring and the other to the B-ring.

Since the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of I showed signals at δ 78.5 (d) and 42.1 (t), attributed to C-2 and C-3 of flavanones, I was determined to be a flavanone derivative. The signals of δ 107.2 (C-6) and 95.5 (C-8) are the same as those of sophoraflavanone B,[5] and the γ,γ-dimethylallyl group (A-ring) was shown to be located at C-6 (Fig. 2).

UV shifts after the addition of sodium acetate, aluminum chloride or sodium ethoxide showed that the three hydroxy groups were located at C-7, C-5 and C-4'. Since the ^{1}H-NMR spectrum (B-ring) of I showed ABX type proton signals of the aromatic ring, the γ,γ-dimethyl-

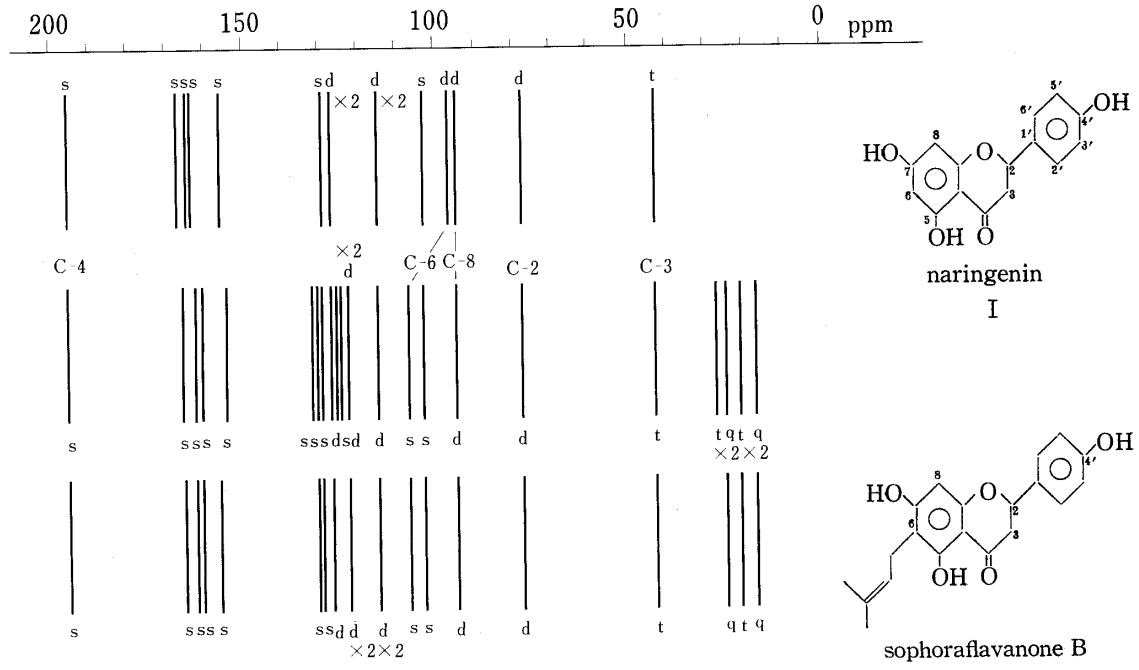
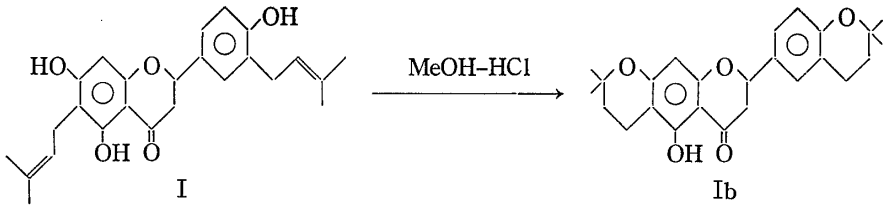


Fig. 2. ^{13}C-NMR Spectrum of I (in DMSO-d_6)

Off-resonance decoupling (SFORD). s, singlet; d, doublet; t, triplet; q, quartet.

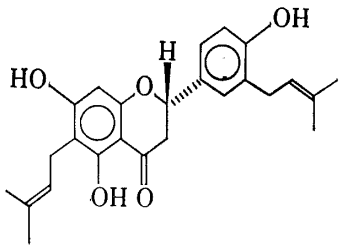


I Ib

Chart 1

allyl group in the B-ring must be located at C-3′. This was supported by the following acid-catalyzed cyclization of I (Chart 1).

On refluxing a solution of I in methanolic hydrochloric acid, the γ,γ-dimethylallyl side chain cyclized with the neighboring hydroxy group to afford only one chromane (Ib; dicycloeuchrestaflavanone A).



euchrestaflavanone A (I)

Fig. 3

Ib has the composition $C_{25}H_{28}O_5$, and gave a ^{1}H-NMR spectrum ($CDCl_3$) showing the presence of four tertiary methyl groups at δ 1.32 (12H, s) and four methylene groups of a 2,2-dimethylchromane ring at δ 1.7—1.9 (4H, m), 2.58 (2H, t, J=7 Hz) and 2.84 (2H, t, J=7 Hz). It also showed one chelated hydroxyl proton at δ 11.77 (1H, s, C_5–OH; which disappeared on the addition of D_2O).

Accordingly, the cyclized product could be formulated as Ib.

From these data, the structure of euchrestaflavanone A was concluded to be 6,3′-di- γ,γ-dimethylallyl-5,7,4′-trihydroxyflavanone.

Since the specific optical rotation of I had a minus (−) sign, like those of other natural flavanones,[6] I most probably has (*S*)-configuration at C-2 (Fig. 3).

We have also isolated some other flavonoids from this plant, and their structures are under investigation.

Experimental

All melting points were determined with a Yanagimoto MP-S3 micro melting point apparatus, and are uncorrected. IR and UV spectra were recorded on a JASCO IRA-1 spectrometer and a JASCO UVIDEC-1 spectrometer, respectively. ^{1}H-NMR and ^{13}C-NMR spectra were measured at 100 MHz with a JEOL JNM-PS-100 and at 25 MHz with a JEOL JNM-PFT-100 NMR spectrometer, respectively; chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). MS were taken on a JEOL JMS-01SG-2 mass spectrometer with a direct inlet system.

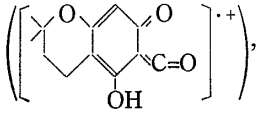
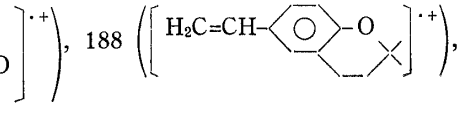
Column chromatography was carried out with Wakogel C-200 (Wako Pure Chemical Ind. Ltd.). Thin-layer chromatography (TLC) was conducted on Kieselgel G nach Stahl (Merck) and the spots were detected by spraying Gibbs reagent or conc. H_2SO_4, followed by heating. The ratios of solvents and reagents in the mixtures are given in v/v.

Extraction and Separation——The dried roots of *Euchresta japonica*, which were collected in Miyazaki prefecture (140 g) in October 1978, were extracted five times with boiling MeOH. The methanolic extract (32 g) was extracted with ether and then with AcOEt. The insoluble part was further extracted with *n*-BuOH. The combined ethereal extract was concentrated (10 g) and chromatographed on silica gel using benzene and benzene–AcOEt as solvents to give *l*-maackiain (17 mg), β-sitosterol (23 mg), I (126 mg) and medicagol (3 mg). From the AcOEt extract (0.7 g) and *n*-BuOH extract (1.6 g), trifolirhizin was also isolated.

Euchrestaflavanone A (I)——I was recrystallized from benzene as colorless needles, mp 145—147°C, brown under UV light, greenish-brown to $FeCl_3$, dark blue in the Gibbs reaction. Mg–HCl (+). $[\alpha]_D^{22}$ −35° (c=0.2, EtOH). MS m/e: 408.1899 (M^+, Calcd for $C_{25}H_{28}O_5$: 408.1934) base peak (100%), 393.1672 ($C_{24}H_{25}O_5$: 393.1699) (24%), 353.1354 ($C_{21}H_{21}O_5$: 353.1386) (46%), 221.0787 ($C_{12}H_{13}O_4$: 221.0812) (39%), 220.0713 ($C_{12}H_{12}O_4$: 220.0733) (53%), 205.0492 ($C_{11}H_9O_4$: 205.0498) (86%), 192.0761 ($C_{11}H_{12}O_3$: 192.0784) (53%), 188.1164 ($C_{13}H_{16}O$: 188.1199) (20%), 165.0184 ($C_8H_5O_4$: 165.0185) (90%), 133.0604 (C_9H_9O: 133.0651) (30%). UV λ_{max}^{EtOH} nm (log ε): 295 (4.29), 340$_{(sh)}$ (3.80). UV $\lambda_{max}^{EtOH+EtONa}$ nm (log ε): 252 (4.70), 337 (4.52). UV $\lambda_{max}^{EtOH+AlCl_3}$

nm (log ε): 297 (4.29), 340$_{(sh)}$ (3.70),[7] UV $\lambda_{max}^{EtOH+NaOAc}$ nm (log ε): 295 (4.24), 338 (4.22). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (C=O), 1610, 1520 (arom. C=C), 1390 (CH_3). ^{1}H-NMR (CD_3COCD_3):[8] 1.62 (6H, s, $=C(\underline{CH_3})_2$, A-ring), 1.72 (6H, s, $=C(\underline{CH_3})_2$, B-ring), 2.6—3.5 (6H, m, C_3–H_2 and Ar–C$\underline{H_2}$–CH=$\times$2), 5.21 (2H, br.t, J=7.4 Hz, –CH_2–C$\underline{H}$=C< $\times$2), 5.42 (1H, d.d, J=12.0 Hz, 3.6 Hz, C_2–H), 6.03 (1H, s, C_8–H), 6.89 (1H, d, J=8.0 Hz, C_5'–H), 7.1—7.4 (2H, m, C_2' and C_6'–H), 8.4, 9.6 (each 1H, each br.s, OH; disappeared on the addition of D_2O), 12.1 (1H, s, C_5–OH; disappeared on the addition of D_2O). ^{13}C-NMR (DMSO-d_6): 17.6 (q, $CH_3\times2$, A,B-ring), 21.3 (t, –$\underline{C}H_2$–CH=C<, A-ring), 25.5 (q, $CH_3\times2$, A,B-ring), 28.2 (t, –$\underline{C}H_2$–CH=C<, B-ring), 42.1 (t, C-3), 78.5 (d, C-2), 95.5 (d, C-8), 102.1 (s, C-4a), 107.2 (s, C-6), 114.9 (d, C-5′), 123.0 (d, –$\underline{C}$H=C< $\times$2), 125.3 (d, C-6′), 127.7 (s, C-3′), 128.1 (d, C-2′), 129.5 (s, C-1′), 130.4 (s, –CH=$\underline{C}$<, A-ring), 131.7 (s, –CH=$\underline{C}$<, B-ring), 155.5 (s, C-4′), 160.0 (s, C-8a), 161.6 (s, C-5), 164.8 (s, C-7), 197.1 (s, C-4).

Acetylation of I (Ia)——A solution of I (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. Ia (32 mg) was obtained as an oily product. $FeCl_3$ (−), Gibbs R. (−). IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 1770, 1260, 1190 (ester), 1650 (C=O), 1610, 1520 (arom. C=C). ^{1}H-NMR ($CDCl_3$): 1.59, 1.66, 1.70, 1.76 (each 3H, each s, $CH_3\times$4), 2.31, 2.32, 2.37 (each 3H, each s, –OAc $\times$3), 2.8—3.0 (2H, m, C_3–H_2), 3.26 (4H, br. d, J=7.3 Hz, Ar–C$\underline{H_2}$–CH=$\times$2), 5.0—5.3 (2H, m, –CH_2–C$\underline{H}$=C< $\times$2), 5.45 (1H, d.d, J=12.5 Hz, 3.4 Hz, C_2–H), 6.52 (1H, s, C_8–H), 7.08 (1H, d, J=9 Hz, C_5'–H), 7.25—7.34 (2H, m, C_2' and C_6'–H). MS m/e: 534 (M$^+$), 492 (M$^+-CH_2CO$), 450 (M$^+-CH_2CO\times2$), 408 (M$^+-CH_2CO\times3$), 43.

Acid-catalyzed Cyclization of I (Formation of Ib)——A solution of I (50 mg), conc. HCl (3 ml) and MeOH (15 ml) was refluxed for 2 h. The reaction mixture was diluted with water and extracted with ether. The ether extract was evaporated to dryness *in vacuo*, and the residue was purified by chromatography on silica gel with benzene to give a colorless solid, which was recrystallized from MeOH to yield colorless needles of Ib (28 mg) mp 192—194°C. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (C=O), 1600, 1510 (arom. C=C), 1380, 1390 (CH_3). ^{1}H-NMR ($CDCl_3$): 1.32 (12H, s, $\underset{O}{\overset{C}{\rangle}}C(C\underline{H_3})_2\times2$), 1.7—1.9 (4H, m, Ar–$CH_2$–C$\underline{H_2}$–$\times$2), 2.58 (2H, t, J=7 Hz, Ar–C$\underline{H_2}$–CH_2–, A-ring), 2.84 (2H, t, J=7 Hz, Ar–C$\underline{H_2}$–CH_2–, B-ring), 2.6—3.3 (2H, m, C_3–H_2), 5.32 (1H, d.d, J=12.6 Hz, 3.5 Hz, C_2–H), 5.96 (1H, s, C_8–H), 6.83 (1H, d, J=8 Hz, C_5'–H), 7.1—7.4 (2H, m, C_2' and C_6'–H), 11.77 (1H, s, C_5–OH; disappeared on the addition of D_2O). MS m/e: 408 (M$^+$), 353 (M$^+-C_4H_7$), 220  , 188  , 165 (220$-C_4H_7$), 133 (188$-C_4H_7$).

***l*-Maackiain**——Recrystallization from a mixture of MeOH–H_2O gave colorless needles, mp 180—181°C, $[\alpha]_D^{22}$ −255° (c=0.2, acetone). This was identified by direct comparison (mp, TLC, IR) with an authentic sample isolated from *S. tomentosa*.

Medicagol——Recrystallization from MeOH gave colorless needles, mp over 300°C. This was identified by direct comparison (mp, TLC, IR, UV) with an authentic sample isolated from *S. tomentosa*.

Trifolirhizin——Recrystallization from MeOH gave colorless needles, mp 145°C (dec.), $[\alpha]_D^{22}$ −180° (AcOH). This was identified by direct comparison (mp, TLC, IR) with an authentic sample isolated from *S. subprostrata*.

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References and Notes

1) Part XV: M. Komatsu, I. Yokoe, and Y. Shirataki, *Chem. Pharm. Bull.*, **29**, 2069 (1981).
2) This work was presented at the 27th Annual Meeting of the Pharmacognostic Society of Japan, Nagoya, September, 1980.
3) M. Komatsu, I. Yokoe, Y. Shirataki, and J.-L. Chen, *Phytochemistry*, **15**, 1089 (1976) and references cited therein.
4) "Zhong Yao Zhi (中葯志)," Vol. 1, ed. by the Pharmaceutical Institute, Chinese Academy of Medical Science, Peking, 1959, p. 52.
5) M. Komatsu, I. Yokoe, and Y. Shirataki, *Chem. Pharm. Bull.*, **26**, 3863 (1978).
6) B.A. Bohm, Flavanones and dihydroflavonols in "The Flavonoids," edited by J.B. Harborne, T.J. Mabry, and H. Mabry, Chapman and Hall, London, 1975, p. 594.
7) Recently, Sherif *et al.* have reported that there is no bathochromic $AlCl_3$-induced shift when a γ,γ-dimethylallyl group is located at C-6 of flavonoids having a hydroxy group at C-5. (E.A. Sherif, R.K. Gupta, and M. Krishnamurti, *Tetrahedron Lett.*, **21**, 641 (1980)).
8) The ^{1}H-NMR spectral data of the γ,γ-dimethylallyl groups in the two rings of I were assigned by comparison with those of sophoraflavanone B.[5]