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Chemical Studies on the Oriental Plant Drugs. XXXV.[1] The Chemical Constituents of the Heartwood of *Maackia amurensis* var. *buergeri*.

Makoto Takai, Hisashi Yamaguchi, Tamotsu Saitoh and Shoji Shibata[2]

Faculty of Pharmaceutical Sciences, University of Tokyo[3]

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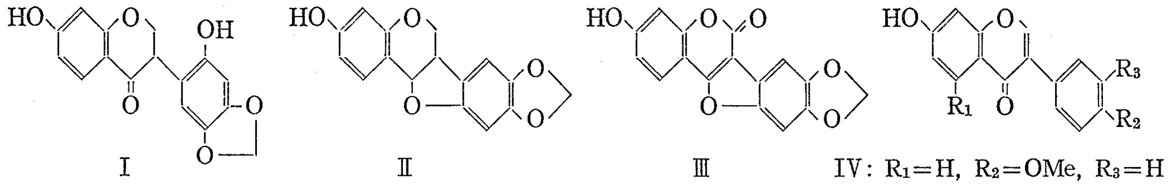
In connection with the studies on the anti-ulcer principles of licorice[4] a survey has been made on the biologically active flavonoids of Leguminosae plants.

The sodium carbonate-soluble fraction of the ethanolic extracts of the heartwood of *Maackia amurensis* Rupr. *et* Maxim. var. *buergeri* (Maxim.) C.K. Schneid. has been found to show an anti-ulcer activity reducing the gastric secretion in Shay rats.[5] The present paper deals with the chemical studies on that fraction.

As the principles of the heartwood of the same plant, Suginome[6] reported the occurrence of sophorol (2′,7-dihydroxy-4′,5 methylenedioxyflavanone) (I) and maackiain (II). The present authors isolated maackiain (II), medicagol (III), formononetin (IV), genistein (V) and a compound (VI), $C_{16}H_{12}O_5$, mp 260—263°, from the above mentioned fraction of the plant growing in the Botanic Garden of University of Tokyo.

The nuclear magnetic resonance (NMR) spectrum of VI showed a singlet signal in the low field (δ 8.30 ppm) to suggest the presence of a proton at $C_{(2)}$ in the isoflavone ring system along with a methoxyl signal at δ 3.70 ppm. The ultraviolet (UV) spectrum of VI showing a bathochromic shift by the addition of sodium acetate indicated the presence of a hydroxyl at the 7-position. The NMR spectrum (in d_6-benzene: $CDCl_3$ (9 :1)) of dimethyl ether of VI revealed the presence of three methoxyl groups (δ 3.71, 3.63 and 3.47 ppm.), whose pattern ruled out a symmetric disposition of the methoxyls, such as at 2′,6′ or 3′,5′. As the mass spectrum of dimethyl ether of VI showed the absence of methoxyl group at the 2′ or 6′ position by the absence of M-31 peak,[7] the structure of 7,3′,4′-trimethoxyisoflavone (O-methylsayanedine)[8] could be assigned to the dimethyl ether of VI, which has been confirmed by the comparison with the authentic sample. Hence the possible structure of VI is either 7,4′-dihydroxy-3′-methoxyisoflavone or 7,3′-dihydroxy-4′-methoxyisoflavone; the latter, however, has been proved to be different from VI by the melting point and the infrared (IR) spectrum.[9]



I II III

IV: R_1=H, R_2=OMe, R_3=H
V : R_1=OH, R_2=OH, R_3=H
VI: R_1=H, R_2=OH, R_3=OMe

Chart 1

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1) Part XXXIV: O. Tanaka, S. Mihashi, I. Yanagisawa, T. Nikaido and S. Shibata, *Tetrahedron*, **28**, 4523 (1972).
2) Author to whom inquiries should be addressed.
3) Location: *Hongo, Bunkyo-ku, Tokyo.*
4) T. Saitoh and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **17**, 729 (1969), Ref. cited therein.
5) K. Takagi, Private communication.
6) H. Suginome, *Bull. Chem. Soc. Japan*, **39**, 1525 (1966); H. Suginome and T. Kio, *ibid.*, **39**, 1529 (1966).
7) R.V.M. Campbell, S.H. Harper and A.D. Kemp, *J. Chem. Soc.* (*C*), 1787 (1969).
8) Y. Isogai, Y. Komoda and T. Okamoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 1872 (1970).
9) M.R. Parthasarathy, R.N. Puri and T.R. Seshadri, *Ind. J. Chem.*, **7**, 118 (1969).

On the other hand, 7,4′-dihydroxy-3′-methoxyisoflavone was reported by Oliveria, *et al.* in 1968[10] as a constituent of the heartwood of *Machaerium villosum* Vog. (Leguminosae), but any detailed information about this compound has not been given except UV and a negative response to Gibbs' test. Thus the synthesis of this compound has been undertaken to compare with VI.

Acetylhomovanillonitrile (VII)[11] was condensed with resorcinol by the Hoesch reaction to give 2′,4′-dihydroxy-2-(4-hydroxy-3-methoxyphenyl) acetophenone (VIII), which was then reacted with ethyl formate to afford 7,4′-dihydroxy-3′-methoxyisoflavone, mp 260—262°. This compound has been established to be identical with VI by a mixed fusion and the comparison of thin-layer chromatography (TLC), IR, UV, NMR and mass spectra.

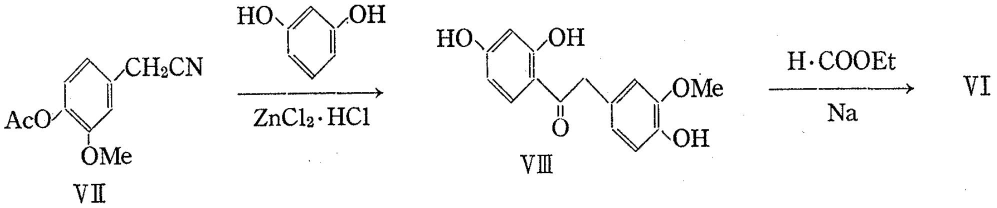
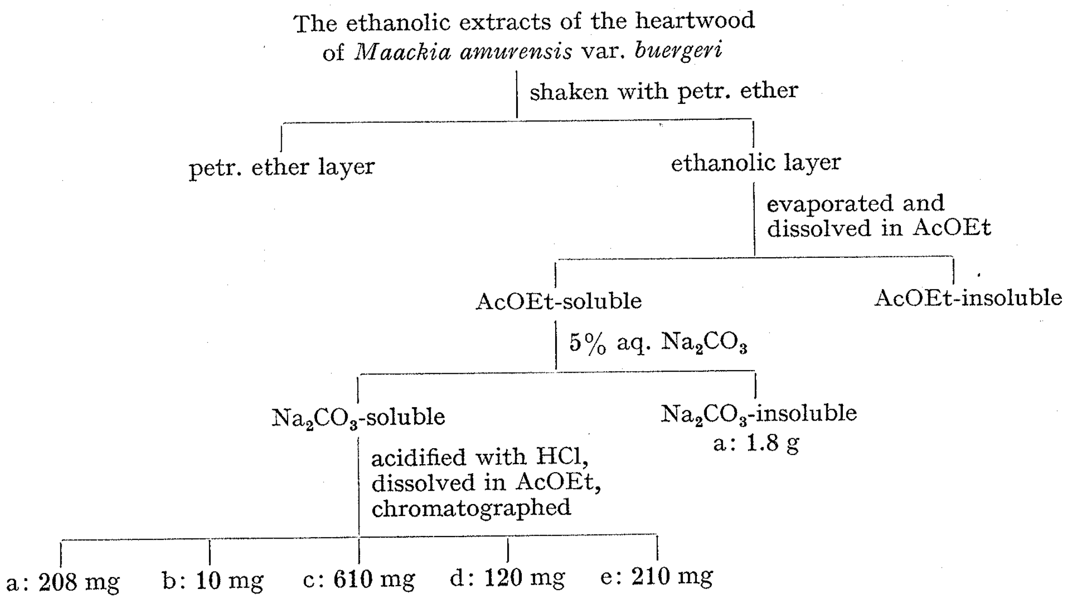


Chart 2

Experimental

Separation of Ethanolic Extracts of the Heartwood of *Maackia amurensis*——The heartwood of *Maackia amurensis* Rupr. *et* Maxim. var. *buergeri* (3.5 kg) was extracted twice with ethanol (13 liter) at room temperature for 3 days. The combined ethanolic extracts were concentrated to 400 ml, then shaken with petroleum ether (400 ml × 4). The ethanolic layer was evaporated, dissolved in AcOEt (2 liter) and extracted with 5% aq. Na_2CO_3 (500 ml × 4). The combined aqueous layer was acidified to pH 2.5 by the addition of 5% HCl. The precipitates formed were dissolved in AcOEt (2 liter), washed with water (500 ml × 4) and evaporated to afford a brown tar (28 g), which was chromatographed over silica gel using a mixed solvent system of benzene and acetone (4 : 1) to give five compounds (a—e). The compound (a) was obtained also from Na_2CO_3-insoluble fraction.



Compound a = Maackiain (II)——Colourless needles, mp 178—179°. UV λ_{max}^{EtOH} nm: 282, 288, 312. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1623, 1603, 1477, 1463. NMR (d_6-DMSO) δ ppm: 7.28 (1H, d, $J=8$ Hz), 6.99 (1H, s), 6.51 (1H, d.d, $J=8$, 2 Hz), 6.30 (1H, d, $J=2$ Hz), 5.95 (2H, d), 5.53 (1H, d, $J=7$ Hz), 4.27 (1H, d, $J=7$ Hz), 3.65 (2H, m).

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10) A. Braga de Oliveira, O.R. Gottlieb and W.D. Ollis, *An. Acad. Brasil. Cienc.*, **40**, 147 (1968).
11) H.E. Fischer and H. Hibbert, *J. Am. Chem. Soc.*, **69**, 1208 (1947).

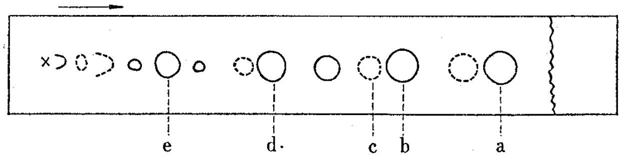

Fig. 1. Thin–Layer Chromatogram of The Na_2CO_3-soluble Fraction (Silica gel GF-254; Solvent: benzene:acetone (4:1))

Compound b = Medicagol (III)——mp 290°. M^+ 296. UV λ_{max}^{EtOH} nm: 246, 257 (sh), 264 (sh), 298 (sh), 305, 312, 348. UV $\lambda_{max}^{EtOH+NaOAc}$ nm: 246, 265 (sh), 298 (sh), 305, 312, 364. IR ν_{max}^{KBr} cm^{-1}: 1742, 1712, 1630, 1610, 1596, 1500, 1267, 947. NMR (d_6-DMSO) δ ppm: 6.14 (2H(-O-CH_2-O-)), 6,88 (1H, d, $J=2$ Hz), 6.93 (1H, d.d, $J=2$, 8 Hz), 7.26 (1H, s), 7.50 (1H, s), 7.77 (1H, d, $J=8$ Hz).

Compound c = Formononetin (IV)——mp 263—265°. UV λ_{max}^{EtOH} nm: 250, 305. UV $\lambda_{max}^{EtOH+NaOAc}$ nm: 268, 310, 340. NMR (d_6-DMSO) δ ppm: 6.85 (1H, d, $J=2$ Hz), 6.91 (1H, d.d, $J=2$, 9 Hz), 6.96 (2H, d, $J=9$ Hz), 7.50 (2H, d, $J=9$ Hz), 7.97 (1H, d, $J=9$ Hz), 8.26 (1H, s).

Compound d = Genistein (V)——mp 290°, UV λ_{max}^{EtOH} nm: 262, 330. IR ν_{max}^{KBr} cm^{-1}: 3425, 3150, 1652, 1617, 1574, 1505. NMR (d_6-DMSO) δ ppm: 6.21 (1H, d, $J=2$ Hz), 6.36 (1H, d, $J=2$ Hz), 6.82 (2H, d, $J=9$ Hz), 7.38 (2H, d, $J=9$ Hz), 8.19 (1H, s), 12.95 (1H, s).

Compound e = 7,3′-Dihydroxy-4′-methoxyisoflavone (VI)——mp 260—263°. M^+ 284, $C_{16}H_{12}O_5$. UV λ_{max}^{EtOH} nm: 250, 263, 283, 310. UV $\lambda_{max}^{EtOH+NaOAc}$ nm: 260, 290, 310, 340. IR ν_{max}^{KBr} cm^{-1}: 3527, 1619, 1587, 1568, 1512. NMR (d_6-DMSO) δ ppm: 3.70 (3H, s (OCH_3)), 6.75—7.20 (5H), 7.92 (1H, d, $J=9$ Hz), 8.30 (1H, s).

7,3′,4′-Trimethoxyisoflavone——Prepared by permethylation of 7,3′-dihydroxy-4′-methoxyisoflavone (VI) with CH_2N_2. mp 156—158°. M^+ 312. IR ν_{max}^{KBr} cm^{-1}: 1633, 1602, 1568, 1520, 1446, 1369. NMR (C_6D_6: $CDCl_3=9:1$) δ ppm: 3.71 (3H, s), 3.63 (3H, s), 3.47 (3H, s), 6.58—7.30 (5H), 7.60 (1H, s), 8.20 (1H, d, $J=9$ Hz).

Synthesis of 7,4′-Dihydroxy-3′-methoxyisoflavone (VI)

2′,4′-Dihydroxy-2-(4-hydroxy-3-methylphenyl)acetophenone (VIII)——To a solution of VII[11] (8 g) and resorcinol (10 g) in ether (150 ml), dry HCl gas was passed for 2 hr at 0° and the mixture was kept at 0° for 5 days. Dried ether was added and the ethereal layer was decanted to remain a heavy oil which was heated for 2 hr with 2N HCl (100 ml). The precipitates formed were collected and recrystallized from MeOH to give XI, colourless granules. *Anal.* Calcd. for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15. Found: C, 65.77; H, 5.10. IR ν_{max}^{Nujol} cm^{-1}: 3300, 3238, 1632. NMR (d_6-acetone) δ ppm: 6.42 (1H, d.d, $J=2$, 9 Hz 5′-H), 7.94 (1H, d, $J=9$ Hz, 6′-H), 6.75 (2H, s, 5, 6-H), 6.93 (1H, s, 2-H), 12.96 (1H, s, 2′-OH).

7,4′-Dihydroxy-3′-methoxyisoflavone (VI)——To Na dispersion (2 g) was dropped a solution of VIII (1.3 g) and HCOOEt (100 ml) during 30 min, and the mixture was refluxed for 6 hr. 2N HCl was added and excess of HCOOEt was evaporated *in vacuo*. After cooling the precipitates formed were collected and chromatographed over silica gel using a mixture of benzene and acetone (9: 1). The elute afforded VI (75 mg) was recrystallized from MeOH to give colourless plates, mp 262° (Yield: 6%). *Anal.* Calcd. for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26. Found: C, 66.85; H, 4.21.

This product (VI) was proved to be identical with the naturally occurring VI by the comparison of TLC, IR, UV, NMR, and mass spectra and by mixed fusion.

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