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Constituents of *Hibiscus moscheutos* L. I[1)]

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Two new compounds have been isolated from the pistils and stamens of *Hibiscus moscheutos* L. and these compounds were determined to be 3,5,7,8,4′-pentahydroxyflavone 7-*O*-α-rhamnopyranoside (VIII) and 2-oxindole-3-acetylaminomethylaspartic acid (X) by chemical and spectroscopic methods. Nine known compounds, β-sitosterol (I), ikshusterol (II), *epi*-ikshusterol (III), methyl linolenate (IV), β-sitosteryl-β-D-glucoside (V), quercimeritrin (VI), kaempferol-7-*O*-α-rhamnopyranoside (VII), methyl dioxindole-3-acetate (IX) and rutin (XI), were also isolated.

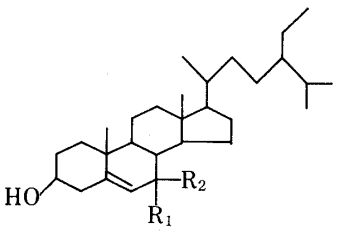
Keywords——*Hibiscus moscheutos*; Malvaceae; pistil; stamen; 3,5,7,8,4′-pentahydroxyflavone 7-*O*-α-rhamnopyranoside; 2-oxindole-3-acetylaminomethylaspartic acid

As a part of our continuing comparative studies on constituents of various parts of the plant, this paper deals with the chemical constituents of the pistils and stamens of *Hibiscus moscheutos* L. (americafuyo in Japanese) (Malvaceae). *Hibiscus moscheutos* is a perennial herb with pink or rose-colored flowers which has been cultivated widely, but there have been few reports on its constituents.[2)]

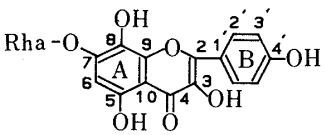
The pistils and stamens of *Hibiscus moscheutos* were extracted with methanol. The water-soluble portion of the methanol extract was successively extracted with ether, ethyl acetate and butanol. Column chromatography of the ether extract resulted in the isolation of compounds I—IV. The ethyl acetate extract, on column chromatography, yielded compounds V—X. The butanol extract, on chromatography, furnished compound XI.

Compounds I, V, VI and XI were identified as β-sitosterol (I), β-sitosteryl-β-D-glucoside (V), quercimeritrin (VI) and rutin (XI) by direct comparison [thin layer chromatography (TLC), infrared (IR) spectrum and mixed melting point determination] with authentic samples.

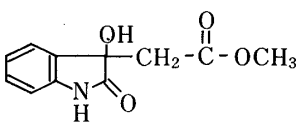
Compounds, II, III, IV, VII and IX were identified as ikshusterol, *epi*-ikshusterol,[3)] methyl linolenate, kaempferol-7-*O*-α-rhamnopyranoside,[4)] and methyl dioxindole-3-acetate[5)] respectively, by comparisons of their physical and spectral data with those reported in the literature.



II : R_1=OH, R_2=H
III : R_1=H, R_2=OH



VIII



IX

Fig. 1

TABLE I. ^{13}C-NMR Chemical Shifts of VIII and Herbacetin 7-Glucoside

Carbon No.	VIII[a)]	Herbacetin 7-glucoside[b)]	Carbon No.	VIII[a)]	Herbacetin 7-glucoside[b)]
2	148.1	147.8	3′	115.8	115.9
3	136.7	136.2	4′	160.0	159.7
4	177.0	176.7	5′	115.8	115.9
5	150.8	151.8	6′	130.5	130.1
6	98.9	99.0	Sugar-1	100.5	101.7
7	153.1	150.7	Sugar-2	71.8	73.5
8	127.9	127.9	Sugar-3	71.4	76.0
9	145.5	144.1	Sugar-4	73.5	70.1
10	105.6	105.1	Sugar-5	70.9	76.9
1′	123.3	122.3	Sugar-6	18.0	61.1
2′	130.5	130.1			

a) Run at 100 MHz in CD_3OD solution. *b*) Run at 100 MHz in DMSO-d_6 solution.

Compound VIII was obtained as a yellow powder, which was positive to the Mg–HCl test. The ultraviolet (UV) spectrum showed absorption maxima at 270, 328 and 380 nm, indicating the presence of a flavonol skeleton. The IR spectrum indicated the presence of hydroxyl and conjugated carbonyl groups and a benzene ring. The field desorption mass spectrum (FD-MS) exhibited an intense molecular ion peak at *m/z* 448. Acid hydrolysis of VIII with 5% H_2SO_4 afforded a sugar in the filtrate which was identified as rhamnose by gas liquid chromatography (GLC) in comparison with an authentic sample. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum exhibited a doublet signal due to the rhamnosyl methyl (δ 1.27, 3H, J=7 Hz) and a doublet signal due to an anomeric proton (δ 5.53), and its chemical shift and coupling constant (J=2 Hz) suggested that the α-rhamnosyl residue was bound to the aromatic hydroxyl group. In the aromatic region of the spectrum, a 1H singlet (δ 6.65) and two 2H doublets (δ 6.91, 8.21) attributable to the A- and B-ring protons, respectively, were seen. Furthermore, the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of VIII showed rather similar chemical shifts to those of herbacetin 7-glucoside[6)] (Table I). These results suggested a flavone structure oxygenated at the 3,5,7,8,4′-positions. The position of one aromatic proton in the A-ring and the position of the rhamnosyl moiety were determined from the ^{13}C-NMR spectrum by the long-range selective proton decoupling (LSPD) method as follows. In the ^{1}H- non-decoupling ^{13}C-NMR spectrum of VIII, the signals of C-8 and C-10, which each appeared as a doublet (δ 127.9 and 105.6), were transformed into singlets by irradiation of C-6. The signal of C-7 was observed at δ 153.1 in the form of a doublet, which changed to a singlet when the anomeric proton was selectively irradiated, indicating that the rhamnosyl residue was linked to the C-7 hydroxyl group. On the basis of those observations, the structure of VIII was concluded to be 3,5,7,8,4′-pentahydroxyflavone 7-*O*-α-rhamnopyranoside.

Compound X was obtained as colorless prisms, mp 187—189 °C, and had the composition $C_{15}H_{16}N_2O_6$ on the basis of high-resolution MS (M^+ at *m/z* 320.1007). The UV spectrum was characteristic of oxindole, being similar to that of IX. The presence of a carboxylic acid function was deduced from the IR (1708 cm^{-1}) and ^{13}C-NMR (δ 173.4) spectra, and also from the electron impact-mass spectrum (EI-MS), which exhibited a prominent peak at *m/z* 44 due to CO_2. The ^{1}H- and ^{13}C-NMR spectra of this compound revealed the presence of the *ortho*-disubstituted benzene ring at δ 7.00 (1H, d, J=7.5 Hz), 7.07 (1H, t, J=7.5 Hz), 7.22 (1H, t, J=7.5 Hz) and 7.72 (1H, d, J=7.5 Hz) and the C-2 carbonyl at δ 179.7. The ^{1}H- and ^{13}C-NMR and two-dimensional proton–proton chemical shift cor-

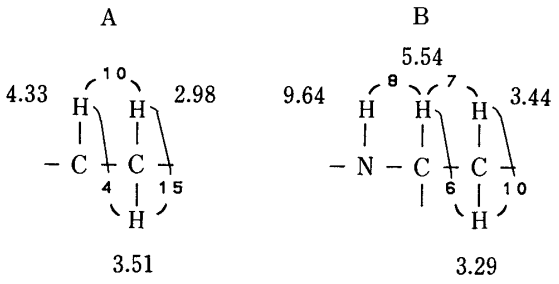


Fig. 2. The Partial Structures and Coupling Networks Derived from *J*-Resolved Two-Dimensional ^{1}H-NMR Spectroscopy (400 MHz, in C_5D_5N): δ Values and *J*/Hz

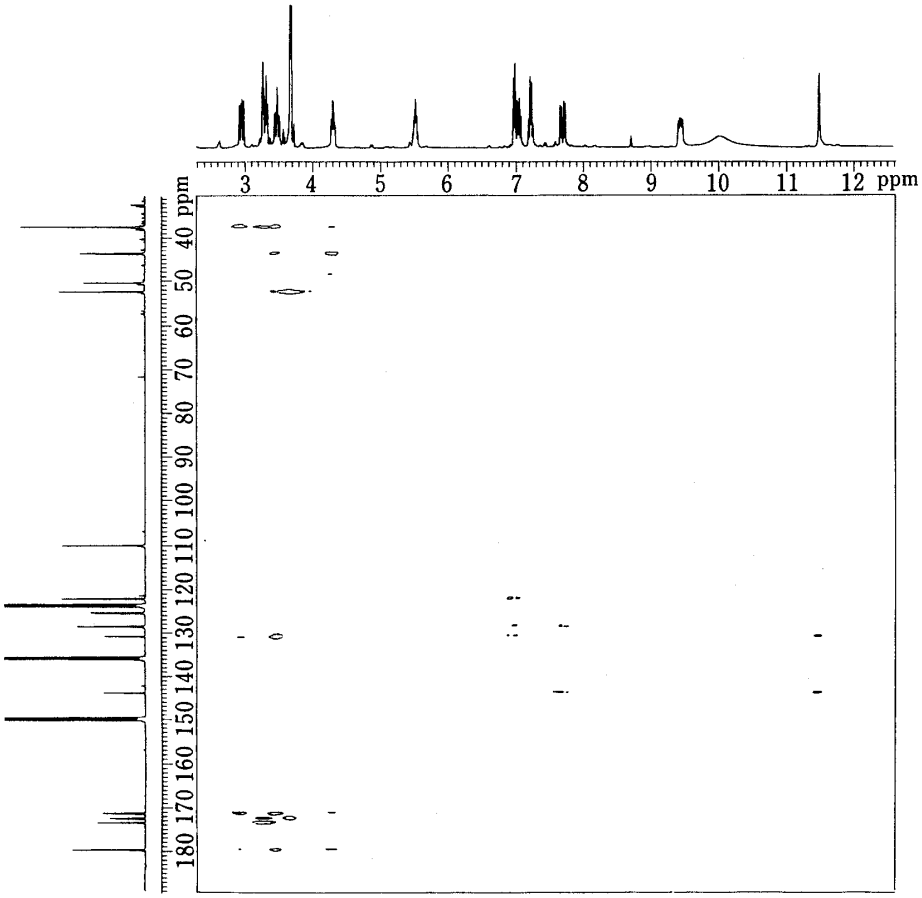


Fig. 3. Contour Map of the Heteronuclear (C/H) Long-Range Shift-Correlation Spectrum of X

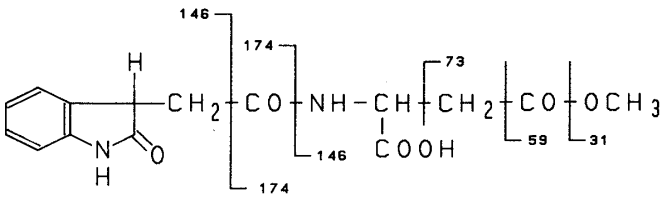


Fig. 4. Mass Spectral Fragmentations of X

relation (2-D COSY) experiments suggested the presence of partial structures A and B (Fig. 2) in addition to ten sp^2 carbons [CO × 4, =C– × 2, =CH– × 4] and five sp^3 carbons [–CH_2– × 2, –CH– × 2, –OCH_3]. Each carbon signal, except for a quaternary one, was assigned based on ^{1}H–^{13}C shift correlation spectroscopy (Fig. 3). On the basis of these observations and the fragmentation pattern in the MS, 2-oxindole-3-acetic acid might be linked by an acid amide bond to methyl aspartic acid. Thus, compound X was concluded to be 2-oxindole-3-acetylaminomethylaspartic acid.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The UV and IR spectra were recorded with Hitachi 139 and 295 spectrophotometers, respectively. The FD-MS and MS were run on JEOL JMS-01-SG-2 and JEOL JMS-D-300 mass spectrometers, respectively. The ^{1}H- and ^{13}C-NMR spectra were measured with JEOL GX-400 spectrometers. Chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane as an internal standard, and coupling constants in Hz. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. GLC was carried out on a Hitachi 063 gas liquid chromatograph using a stainless steel column (3 mm × 1 m) packed with 2% and 10% SE-30 on Chromosorb-W (60—80 mesh) with N_2 carrier gas at a flow rate of 30 ml/min. Silica gel (Fuji-Davison BW-820MH), polyamide (Wako, C-200) and Sephadex (Pharmacia Fine Chemicals, LH-20) were used for column chromatography. TLC was performed on precoated silica gel plates (Merck) and the spots were detected by spraying of 5% $FeCl_3$ or 10% H_2SO_4 following by heating.

Extraction and Isolation——Pistils and stamens (6.9 kg) of *Hibiscus moscheutos* collected in August, 1984 and 1985, at Toho University, were extracted with methanol for 6 h. An aqueous suspension of methanolic extract was extracted with ether, ethyl acetate and *n*-butanol. The ether extract (19 g) was chromatographed on silica gel and eluted successively with *n*-hexane, benzene, chloroform and chloroform–methanol. The ethyl acetate extract (6 g) was repeatedly chromatographed on Sephadex LH-20 with methanol to give VIII (16 mg) and X (35 mg).

3,5,7,8,4′-Pentahydroxyflavone 7-*O*-α-Rhamnopyranoside (VIII)——Yellow powder. UV λ_{max}^{EtOH} nm: 270, 328, 380; $\lambda_{max}^{EtOH\text{-}NaOEt}$ nm: 265, 406; $\lambda_{max}^{EtOH\text{-}NaOAc}$ nm: 270, 328, 380; $\lambda_{max}^{EtOH\text{-}AlCl_3}$ nm: 258, 360, 435; $\lambda_{max}^{EtOH\text{-}AlCl_3\text{-}HCl}$ nm: 268, 359, 430. IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1610, 1565, 1510, 1450. FD-MS *m/z*: 448 (M^+). ^{1}H-NMR (CD_3OD): 1.27 (3H, d, J=7 Hz), 3.50 (1H, m), 3.70 (1H, m), 4.00 (1H, dd, J=3, 9.5 Hz), 4.15 (1H, dd, J=2, 3 Hz), 5.53 (1H, d, J=2 Hz), 6.65 (1H, s), 6.91 (2H, d, J=9 Hz), 8.21 (2H, d, J=9 Hz). ^{13}C-NMR: Table I.

Hydrolysis of (VIII)——A solution of VIII (3 mg) in EtOH (1 ml) containing 10% H_2SO_4 (1 ml) was refluxed for 2 h. Rhamnose was identified by comparison with an authentic sample on GLC.

2-Oxindole-3-Acetylaminomethylaspartic Acid (X)——Colorless prisms, mp 187—189 °C. High-resolution MS: Found 320.1007. Calcd for $C_{15}H_{16}N_2O_6$: 320.1008. UV λ_{max}^{EtOH} nm: 206, 248, 280. IR ν_{max}^{KBr} cm^{-1}: 3290, 1750, 1708, 1650, 1230. EI-MS *m/z*: 320 (M^+), 302, 270, 191, 174, 146, 145 (base), 128, 73, 59, 44, 31. ^{1}H-NMR (C_5D_5N): 2.98 (1H, dd, J=10, 15 Hz), 3.29 (1H, dd, J=6, 10 Hz), 3.44 (1H, dd, J=7, 10 Hz), 3.51 (1H, dd, J=4, 15 Hz), 3.69 (3H, s), 4.33 (1H, dd, J=4, 10 Hz), 5.54 (1H, quintet, J=6, 7, 8 Hz), 7.00 (1H, d, J=7.5 Hz), 7.07 (1H, t, J=7.5 Hz), 7.22 (1H, t, J=7.5 Hz), 7.72 (1H, d, J=7.5 Hz), 9.64 (1H, d, J=8 Hz, NH), 11.64 (1H, s, NH). ^{13}C-NMR (C_5D_5N): 37.2 (C-1′,5′), 43.4 (C-3), 50.2 (C-4′), 52.3 (–O<u>C</u>H$_3$), 109.8 (C-7), 122.0 (C-5), 125.3 (C-4), 128.3 (C-6), 130.6 (C-4a), 143.7 (C-7a), 171.2 (C-2′, <u>C</u>=O), 172.4 (–<u>C</u>OOCH$_3$), 173.4 (–<u>C</u>OOH), 179.7 (C-2).

References and Notes

1) A part of this study was presented at the 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, April 1986.
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