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Studies on the Constituents of *Pueraria lobata*. V.[1] A Tryptophan Derivative from Puerariae Flos

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A tryptophan derivative, *N*-acyl-N_1-glucosyltryptophan, was isolated from Puerariae Flos.

Keywords——Puerariae Flos; *Pueraria lobata*; Leguminosae; tryptophan derivative; *N*-acyl-N_1-glucosyltryptophan; caproic acid

Puerariae Flos, the flowers of *Pueraria. lobata* (WILLD.) OHWI, is an important oriental crude drug used to ameliorate crapulence. In the preceding paper,[1] we described two triterpenoidal glycosides of the sophoradiol glucuronides, along with the known six aromatic compounds, kakkalide, daidzin, genistin, rutin, robinin and nicotiflorin. This paper further deals with a tryptophan derivative, tentatively named PF-P, obtained from this plant.

PF-P (**1**), an amorphous powder, $[\alpha]_D$ +19.7° (dimethyl sulfoxide (DMSO)), was isolated by MCI-gel CHP 20P (solvent, H_2O–MeOH) and silica gel (solvent, $CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5) column chromatographies of the aqueous layer. PF-P (**1**), $C_{23}H_{32}N_2O_8 \cdot 2H_2CO_3$, showed absorptions due to ν_{OH} (3400 cm^{-1}) and $\nu_{C=O}$ and δ_{N-H} (1640—1580 cm^{-1}) in the infrared (IR) spectrum, and gave a characteristic ultraviolet (UV) curve (ε 25800 at 223 nm, ε 5500 at 273, ε 5400 at 279 and ε 4300 at 290) due to an indole skeleton. It was negative to the Dragendorff, ninhydrin and diazobenzidine color reactions. Negative fast atom bombardment mass spectrometry (FAB-MS) of **1** gave a cluster ion $(M-H)^-$ at *m/z* 463. The ^1H–^1H and ^1H–^{13}C correlation spectroscopy (COSY) nuclear magnetic resonance (NMR) spectra (Figs. 1 and 2) of **1** disclosed that **1** was constituted of the following three partial structures: a caproic acid moiety, ^1H-NMR (DMSO-d_6 + D_2O): δ 2.00 (2H, t, J = 7.7 Hz, H-2″), 1.42 (2H, quintet, J = 7.1 Hz, H-3″), 1.18 (2H, m, H-4″), 1,23 (2H, m, H-5″), 0.83 (3H, t, J = 7.0 Hz, H-6″); ^{13}C-NMR (DMSO-d_6): δ 171.2 (s, C-1″), 35.5 (t, C-2″), 24.8 (t, C-3″), 30.9 (t, C-4″), 21.8 (t, C-5″), 13.7 (q, C-6″), an β-D-glucopyranosyl moiety, ^1H-NMR: δ 5.35 (1H, d, J = 9.2 Hz, H-1′), 3.78 (1H, t, J = 9.0 Hz, H-2′), 3.42—3.48 (3H, m, H-3′, 5′, $6'_a$) and 3.67 (1H, d, J = 10.2 Hz, $H\text{-}6'_b$), H-4′ (near 3.27) was overlapped with the H_2O signal; ^{13}C-NMR: δ 84.1 (d, C-1′), 70.9 (d, C-2′), 77.7 (d, C-3′), 70.1 (d, C-4′), 78.9 (d, C-5′), 61.0 (t, C-6′), and a tryptophan moiety, ^1H-NMR: δ 7.19 (1H, s, H-2), 7.45 (1H, d, J = 8.4 Hz, H-4), 6.97 (1H, t, J = 7.5 Hz, H-5), 7.08 (1H, t, J = 8.0 Hz, H-6), 7.45 (1H, d, J = 8.4 Hz, H-7), 3.01 (1H, dd, J = 5.5, 14.7 Hz, $H\text{-}10_a$), 3.20 (1H, dd, J = 9.9, 14.2 Hz, $H\text{-}10_b$), 4.26 (1H, dd, J = 6.2, 10.2, H-11); ^{13}C-NMR: δ 123.7 (d, C-2), 111.5 (s, C-3), 118.5 (d, C-4), 118.7 (d, C-5), 120.7 (d, C-6), 110.0 (d, C-7), 129.2 (s, C-8), 136.3 (s, C-9), 26.9 (t, C-10), 54.6 (d, C-11), 174.9 (s, C-12). The location of the linkage between the glucosyl moiety and the tryptophan part was determined from the ^{13}C-^1H long-range COSY spectrum. That is, a long-range coupling was observed between the anomeric proton of the glucosyl moiety and the C-2 of tryptophan. This suggested the glucosyl moiety to be linked to N-1 of tryptophan.

Therefore, the structure of **1** could be represented as shown in the formula. PF-P (**1**) seems to be the first example of this sort of compound, *i.e.* a tryptophan derivative combined

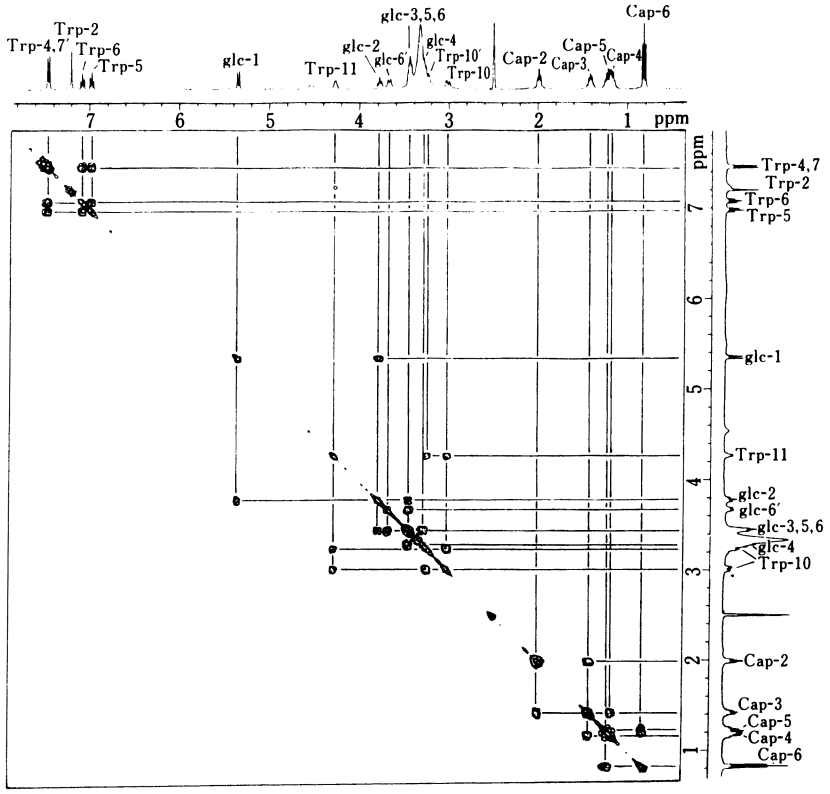


Fig. 1. ^{1}H–^{1}H COSY NMR Spectrum of **1**

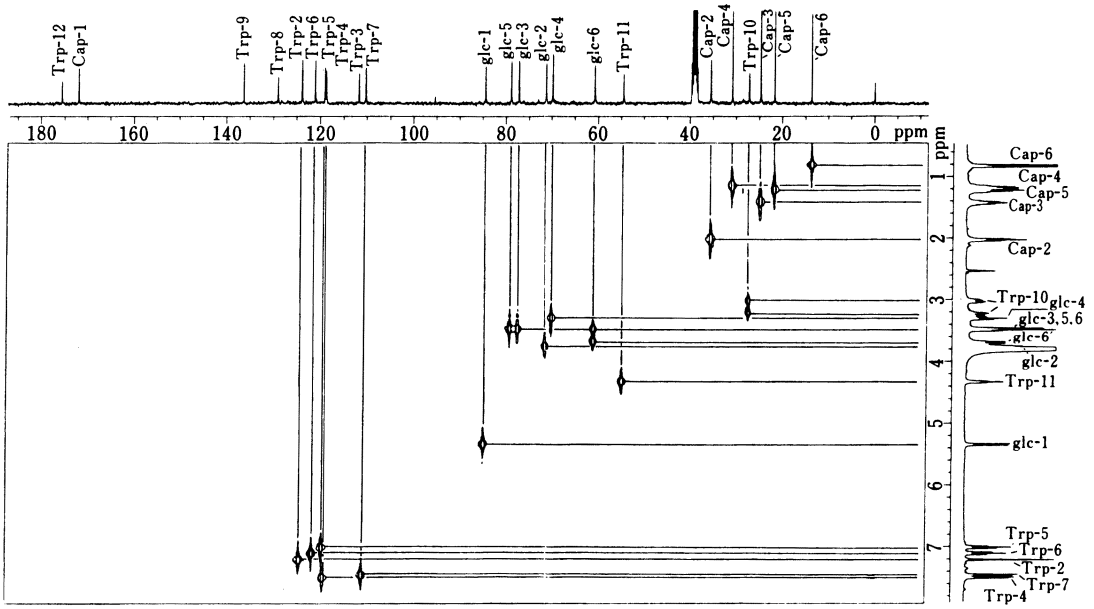
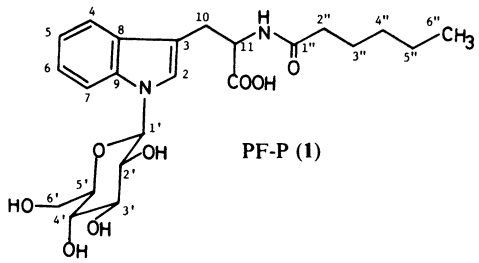


Fig. 2. ^{1}H–^{13}C COSY NMR Spectrum of **1**

with sugar and an organic acid.

Tryptophan and its derivatives have also been obtained from some other Leguminosae plants, *e.g.* the seeds of *Dolichos lablab* L., *Phaseolus radiatus* L.[2] and *Psophocarpus tetragonolobus* (L.) DC. and the whole plants of *Abrus cantoniensis* HANCE in our laboratory, so that they might be characteristic substances distributed in Lequminosae plants.



PF-P (1)

Experimental

Optical rotation was measured with a JASCO DIP-360 digital polarimeter. IR spectra were taken with a Hitachi 270—30 spectrometer, UV with a Hitachi U-3200 spectrometer, and ^{1}H- and ^{13}C-NMR with a JEOL GX-400 instrument. Chemical shifts are given on the (ppm) scale with tetramethylsilane (TMS) as an internal standard. FAB-MS were measured with a JEOL JMS DX-300/JMA 3100 spectrometer.

Extraction and Isolation——The flowers of *Pueraria lobata* were collected at Ushiku, Ibaragi Prefecture, in September 1986, dried and extracted with MeOH. Removal of the solvent under reduced pressure afforded the methanolic extractive, which was partitioned between 1-BuOH and water. After removal of the deposited crystals of kakkalide (36 g) from the aqueous layer by filtration, the filtrate was evaporated under reduced pressure to give a syrup (84 g), which was then subjected to MCI gel CHP 20P (Mitsubishikasei Industrial Co., Ltd.) column chromatography (50% MeOH) to give a fraction (13 g) containing **1**. The fraction was subsequently subjected to silica gel column chromatography (solvent $CHCl_3$: MeOH : H_2O = 7 : 3 : 0.5) to afford **1** (2 g).

PF-P (1)——An amorphous powder, $[\alpha]_D^{20}$ +19.7° (c = 0.50, DMSO). *Anal.* Calcd for $C_{23}H_{32}N_2O_8 \cdot 2H_2CO_3$: C, 51.02; H, 6.17; N, 4.76. Found: C, 51.19; H, 6.14; N, 4.72. Negative FAB-MS (*m/z*): 965 $[2M+K-2H]^-$, 949 $[2M+Na-2H]^-$, 927 $[2M-H]^-$, 501 $[M+K-2H]^-$, 485 $[M+Na-2H]^-$, 463 $[M-H]^-$

References and Notes

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2) J. Furusawa, M. Miyagawa, S. Yahara and T. Nohara, Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Fukuoka, November 1987.