Communications to the Editor

[Chem. Pharm. Bull. 33(3)1293—1296(1985)]

OLEANENE-SAPOGENOLS FROM PUERARIAE RADIX

Jun-ei Kinjo,[a] Izumi Miyamoto,[a] Kotaro Murakami,[b] Kiyoshi Kida,[b] Toshiaki Tomimatsu,[b] Masaki Yamasaki[c] and Toshihiro Nohara*,[a]

Faculty of Pharmaceutical Sciences, Kumamoto University,[a] 5-1 Oe-honmachi, Kumamoto 862, Japan, Faculty of Pharmaceutical Sciences, Tokushima University,[b] 1-78 Shomachi, Tokushima 770, Japan, and Department of Biochemistry, Medical School, Kumamoto University,[c] Honjo, Kumamoto 860, Japan

Seven oleanene-sapogenols (1-7) were obtained from the methanolysate of the crude saponin in Puerariae Radix. Four of them, sapogenols 1-4, were identical with sophoradiol, cantoniensistriol, and soyasapogenols B and A. The structures of three new sapogenols (5-7), named kudzusapogenols C, A and B methyl ester, were deduced by spectroscopic means.

KEYWORDS —— Puerariae Radix; Pueraria lobata; Leguminosae; kudzusaponin; oleanene-sapogenol; sophoradiol; soyasapogenols A, B; cantoniensistriol; kudzusapogenols A, B methyl ester, C

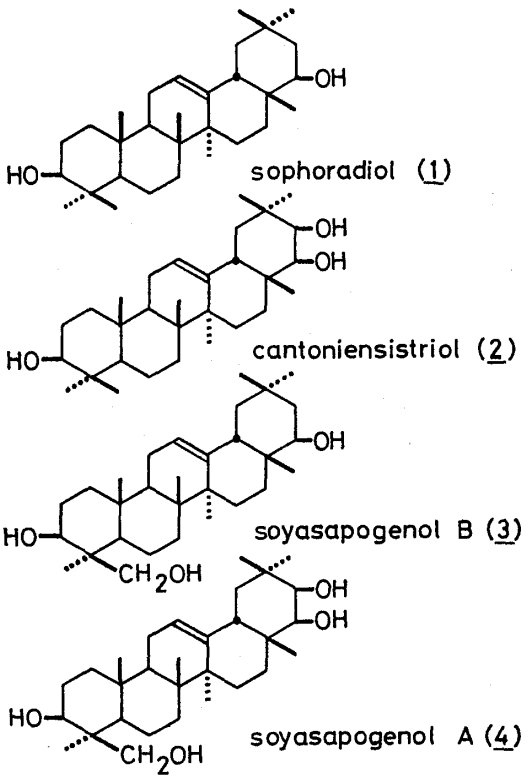
Puerariae Radix (葛根), the dried roots of Pueraria lobata (Willd.) Ohwi, is one of the most important oriental crude drugs used as a perspiration agent, antipyretic and antispasmodic. As regards the ingredients, isoflavonoids and their C-glycosides have been known.[1] In the course of our studies on Puerariae Flos (葛花), a traditional medicine for promotion of an alcoholic metabolism, we have found the occurrence of a series of the triterpenoidal saponins in Puerariae Radix. No report has been hitherto seen with respect to the triterpenoidal ingredients; however, it may be natural that the presence of the triterpenoidal saponins have been found in this plant, in view of the occurrence of the triterpenoidal saponin in leguminous plants such as soybeans[2] (the seeds of Glycine max Merrill), azuki beans[3] (the seeds of Vigna angularis (Willd.) Ohwi et Ohashi) and Astragalus membranaceus Bunge.,[4] whose ingredients have been extensively revealed by Kitagawa et al., recently.

We have now obtained seven triterpenoidal sapogenols (1-7) from the hydrolysate of crude saponin fraction (total kudzusaponin, ca. 30 g) which was obtained by Sephadex LH-20 column chromatography using MeOH as eluent of the MeOH extractive (1.4 kg) of Puerariae Radix (10 kg). Among them, four sapogenols 1-4 were identified as sophoradiol,[2] cantoniensistriol,[5] and soyasapogenols B and A,[2] respectively. This paper deals with the structural elucidation of three new sapogenols, 5-7, which were designated as kudzusapogenols C, A and B methyl ester, respectively.

Three new sapogenols (5-7) were suggested to be the oleanene derivatives as well as 1-4 by their ^{1}H- and ^{13}C-NMR data listed in Table I and II.

Kudzusapogenol C (5), $C_{50}H_{50}O_5$, fine colorless plates,[6] mp 295-296°C, $[\alpha]_D$ +92.8°,[6] showed an analogous pattern to that of 3 with the peaks at m/z 458 (M^+), 224 (A/B ring) and 234, 219 (D/E ring) originating via retro Diels-Alder fission[7] at the C-ring of 3β-hydroxy-olean-12-ene triterpene in the MS. The ^{1}H-NMR spectrum of the triacetate (8) of 5 showed signals, 1H, dd (J=4,8 Hz) at δ 4.58 and 2H, ABq (J=12 Hz) at δ 4.13 and 4.37, ascribable to 3α-H[8] geminal to the 3β-hydroxyl group and 4β-acetoxymethyl (axial).[2,9] Another dd signal (J=11,6 Hz) at δ 4.80 could be attributable to 21α-H (axial) since the comparative study of the ^{13}C-NMR data for 5 with those of β-amyrin[10] exhibited the respective shifts by +38.0, +5.8, +10.5, -4.5 and -5.9 ppm at C-21, 20, 22, 29 and 30 in 5, suggesting the presence of the hydroxyl group at C-21. Therefore, kudzusapogenol C (5) was deduced to be 3β,21β,24 -trihydroxy-olean-12-ene.

Kudzusapogenol A (6), $C_{30}H_{50}O_5$, mp>300°C, colorless plates, $[\alpha]_D$ +104.8°, showed the peaks at m/z 490 (M^+), 224 (A/B ring) and, 266 and 235 (D/E ring) in the MS, indicating that 6 possesses two hydroxyl groups in the A/B ring and three hydroxyl groups in the D/E ring. The ^{1}H-NMR spectrum of the pentaacetate (9) of 6 showed signals due to 3α-H at δ 4.58 (1H, dd, J=4,8 Hz) and 4β-acetoxymethyl at δ 4.13, 4.37 (2H, ABq, J=12 Hz). Moreover, signals due to two methine protons adjacent to the hydroxyl groups with mutual coupling at δ 4.97 (1H, d, J=3 Hz) and 5.16 (1H, d, J=3 Hz), and an equatorial acetoxymethyl[2,9] at δ 3.55, 3.87 (2H, ABq, J=11 Hz) were most likely assumed to be assignable respectively to 22α-H, 21α-H and 20α-acetoxymethyl by comparing with those of the 21α-H (1H, d, J=2.4 Hz, at δ 4.94) and 22α-H (1H, d, J=2.4 Hz, at δ 3.44) in 3,24-dimethyl soyasapogenol A 21-monoacetate,[2] and the 29-CH_2 (2H, ABq, J=10 Hz, at δ 3.68, 3.77) in the triacetate[3] of the reductive



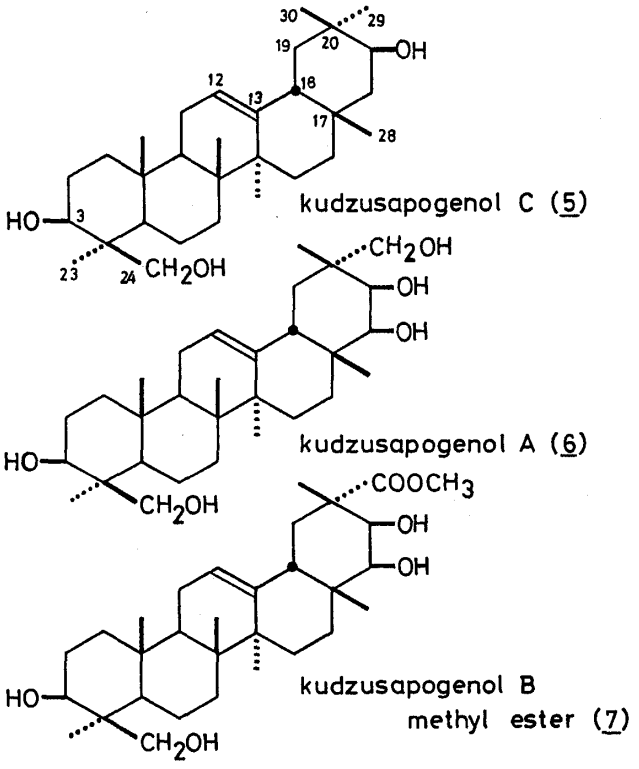


Table I. ^{1}H-NMR Data for the Acetates of 1-7 (200MHz, in $CDCl_3$)

	3_α-H	12-H	21_α-H	22_α-H	24-H_2	tert Me groups	Acetoxyl Me groups	Others
1-Diacetate	4.51 (dd, J=4,8)	5.26 (br t, J=4)	——	4.64 (t, J=4)	——	0.82,0.87,0.88, 0.90,0.97,0.98, 1.00,1.15	2.03,2.05	——
2-Triacetate	4.50 (dd, J=4,8)	5.28 (br t, J=3)	4.89 (s)	4.89 (s)	——	0.80,3x0.87, 2x0.97,1.08, 1.16	2.00,2.05, 2.07	——
3-Triacetate	4.59 (dd, J=4,8)	5.26 (br t, J=3)	——	4.64 (t, J=3)	4.14,4.37 (ABq, J=12)	0.81,0.90,0.97, 0.98,1.00,1.03, 1.14	2.03,2.04, 2.07	——
4-Tetraacetate	4.59 (dd, J=4,8)	5.28 (br t, J=3)	4.89 (s)	4.89 (s)	4.13,4.37 (ABq, J=12)	0.79,0.87,0.96, 0.98,1.03,1.08, 1.16	2.00,2.04, 2x2.07	——
8	4.58 (dd, J=4,8)	5.22 (br t, J=3)	4.80 (dd, J=11,6)	——	4.13,4.37 (ABq, J=12)	2x0.86,0.93, 0.95,0.97, 1.02,1.12	2.03,2.04, 2.06	——
9	4.58 (dd, J=4,8)	5.30 (br t, J=3)	5.16 (d, J=3)	4.97 (d, J=3)	4.13,4.37 (ABq, J=12)	0.81,0.97, 0.98,1.03, 1.10,1.16	1.97,2.04, 2x2.07, 2.08	(29-CH_2OAc) 3.55,3.87 (ABq,J=11)
10	4.58 (dd, J=4,8)	5.31 (br t, J=3)	5.60 (d, J=3)	5.01 (d, J=3)	4.13,4.37 (ABq, J=12)	0.81,0.96, 0.97,1.03, 1.15,1.43	1.95,2.04, 2.07,2.08	(29-COOMe) 3.66 (s)

Table II. ^{13}C-NMR Data of 1–7 (50MHz, in Pyridine-d_5)

	1	2	3	4	5	6	7
1	39.2	39.2	39.0	38.9	38.9	38.9	38.9
2	28.2	28.1	28.4	28.4	28.4	28.4	28.4
3	78.1	78.1	80.1	80.1	80.1	80.1	80.1
4	39.4	39.4	43.2	43.2	43.2	43.2	43.2
5	55.9	55.7	56.4	56.3	56.3	56.3	56.3
6	18.9	18.8	19.1	19.1	19.1	19.1	19.1
7	33.3	32.9	33.6	33.2	33.3	33.2	33.1
8	40.1	40.3	40.1	40.3	40.1	40.3	40.3
9	48.1	48.0	48.1	48.1	48.1	48.1	48.0
10	37.3	37.3	37.1	37.0	37.0	37.0	37.0
11	23.9	23.9	24.1	24.2	24.1	24.1	24.2
12	122.5	122.6	122.4	122.5	122.7	122.5	123.8
13	144.9	144.5	144.8	144.5	144.3	144.6	143.6
14	42.5	42.1	42.4	42.1	41.9	42.0	42.0
15	26.5	26.6	26.5	26.6	26.5	26.6	26.5
16	28.7	27.5	28.7	27.5	28.6	27.4	27.4
17	38.0	39.2	38.0	39.2	35.1	39.0	38.9
18	45.4	44.0	45.4	44.0	47.2	43.2	42.7
19	46.9	47.3	46.8	47.3	46.5	41.1	42.4
20	30.9	36.6	30.9	36.6	36.9	41.0	49.9
21	42.3	74.6	42.3	74.6	72.8	70.5	70.5
22	75.6	79.6	75.5	79.6	47.7	79.7	79.1
23	28.8	28.8	23.6	23.6	23.5	23.5	23.5
24	15.9	15.8	64.6	64.6	64.5	64.5	64.5
25	16.6	16.5	16.3	16.2	16.2	16.2	16.2
26	17.3	17.1	17.1	17.0	16.9	17.0	16.9
27	25.8	26.7	25.7	26.7	26.0	26.7	26.5
28	28.8	22.3	28.7	22.3	28.7	22.3	22.1
29	33.3	31.5	33.3	31.5	29.9	71.7	178.7
30	21.2	21.3	21.1	21.3	17.7	17.5	16.5

product of azukisapogenol methyl ester. Moreover, the ^{13}C-NMR spectrum of 6 also provided information with regard to the locations of the hydroxyl substituents. Therefore, kudzusapogenol A (6) could be represented as 3β,21β,22β,24,29-pentahydroxy-olean-12-ene.

Kudzusapogenol B methyl ester (7), $C_{31}H_{50}O_6$, mp 251-253°C, colorless needles, $[\alpha]_D$ +86.4°, showed signals due to a methoxycarbonyl group at δ 51.9 and 178.7 in its ^{13}C-NMR spectrum. The fragmantation in the MS of 7 suggested the presence of the following functional groups : two hydroxyls on the A/B ring, and two hydroxyls and one methoxycarbonyl residue on the D/E ring. Moreover, the ^{1}H-NMR spectrum of the corresponding tetraacetate (10) of 7 showed signals, 1H, dd (J=4,8 Hz) at δ 4.58, 2H, ABq (J=12 Hz) at δ 4.13, 4.37, 1H, d (J=3 Hz) at δ 5.60, 1H, d (J=3 Hz) at δ 5.01 and 3H, s, at δ 3.66, which could be assigned respectively to 3α-H, 4β-acetoxymethyl, 21α-H, 22α-H and one methoxycarbonyl group. Since reduction of 7 by using $NaBH_4$ in MeOH afforded a product identical with 6, the methoxycarbonyl residue is situated to C-20α. Therefore, the structure of 7 could be concluded to be 3β,21β,22β,24-tetrahydroxy-20α-desmethyl-20α-methoxycarbonyl-olean-12-ene. It seemed that the methyl group on the ester function in the molecule was secondarily formed[3,11] during methanolic acid treatment of the total saponin mixture.

It is noteworthy that a series of the oleanene-sapogenols has been revealed in an important crude drug, Puerariae Radix. The isolation and structural elucidation of the glycosides of these sapogenols are now under investigation.

ACKNOWLEDGEMENT The authors are grateful to Prof. Isao Kitagawa, Faculty of Pharmaceutical Sciences, Osaka University, for supplying valuable specimens of sophoradiol, and soyasapogenols A and B.

REFERENCES AND NOTES

1) S.Shibata, M.Harada and T.Murakami, Yakugaku Zasshi, 79, 863 (1959); T.Kinoshita, Gendai Toyo Igaku, 3, 58 (1982).
2) I.Kitagawa, M.Yoshikawa, H.K.Wang, M.Saito, U.Tosirisuk, T.Fujiwara and K.Tomita, Chem. Pharm. Bull., 30, 2294 (1982).
3) I.Kitagawa, H.K.Wang, M.Saito and M.Yoshikawa, Chem. Pharm. Bull., 31, 664 (1983); idem,ibid., 31, 674 (1983); idem,ibid., 31, 683 (1983).
4) I.Kitagawa, H.K.Wang, A.Takagi, M.Fuchida, I.Miura and M.Yoshikawa, Chem. Pharm. Bull., 31, 689 (1983).
5) T.C.Chiang and H.M.Chang, Planta Medica, 46, 52 (1982).
6) Kudzusapogenols C (5), A (6) and B methyl ester (7) were crystallized from $CHCl_3$-MeOH and their specific rotations were measured with $CHCl_3$-MeOH=1:1, v/v.
7) H.Budzikiewicz, C.Djerassi and D.H.Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day Inc., San Francisco, 1964, p. 121.
8) I.Yosioka, T.Nishimura, A.Matsuda and I.Kitagawa, Chem. Pharm. Bull., 18, 1610 (1970).
9) A.Gansemer, J.Polonsky and E.Wenkert, Bull. Soc. Chim. Fr., 1964, 407.
10) S.Seo, Y.Tomita and K.Tori, Tetrahedron Lett., 1975, 7.
11) I.Kitagawa, K.Kitazawa, K.Aoyama, M.Asanuma and I.Yosioka, Tetrahedron, 28, 923 (1972).

(Received December 20, 1984)