GINSENOSIDE L_A, A NOVEL SAPONIN FROM THE LEAVES OF PANAX GINSENG

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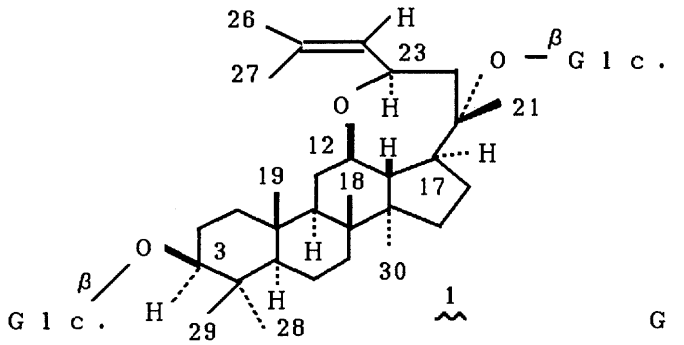
A novel minor saponin, named ginsenoside La, was isolated from the leaves of Panax ginseng and its structure was determined by means of 2D NMR spectroscopy including the HMBC technique.

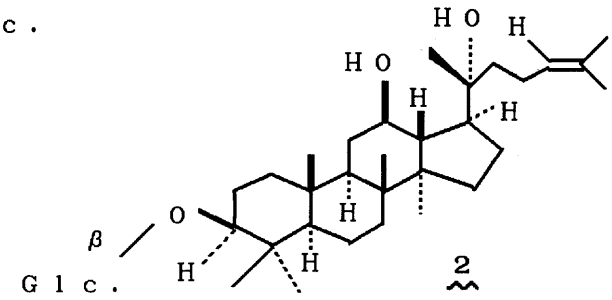
KEYWORDS ginsenoside La; ginseng leave; Panax ginseng; Araliaceae; dammarane saponin; 2D NMR; HMBC

Ginseng, one of the most famous oriental drugs, is the roots of Panax ginseng C. A. MEYER[1] (Araliaceae) and has long been used as a tonic or a drug of longevity in Chinese traditional medicine. From ginseng, a number of characteristic neutral dammarane saponins[2] and a glucuronide saponin of oleanolic acid[3] have been isolated. The constituents of the leaves, which are used to prepare ginseng tea, have also been investigated and several saponins derived from protopanaxadiol, protopanaxatriol, and oleanolic acid[4,5] and some flavonoids[6] have been isolated.

During the course of our study on the constituents of Chinese crude drugs, we isolated a new saponin from the leaves of P. ginseng and identified it as 20(R)-ginsenoside Rh_2 (**2**).[7] In continuation, we have carried out a systematic examination of their constituents and isolated a new minor saponin, named ginsenoside La, together with six known saponins. This paper deals with the structure elucidation of ginsenoside La (**1**) by the use of 2D NMR method.

Crude saponin (100 g), obtained from the air-dried leaves (2 kg) according to a procedure reported previously,[7] was chromatographed on a silica gel column (3.5 kg). Elution with $CHCl_3$-MeOH (10:1) gave ginsenoside Rh_1 (188 mg)[5] and subsequent elution with $CHCl_3$-MeOH (10:2) afforded ginsenoside La (**1**, 13 mg). Further elution with $CHCl_3$-MeOH (10:3, 10:4, and 10:6) gave ginsenoside Rg_3 (390 mg), Rg_2 (500 mg), Re (400 mg), Rd (170 mg), and Rb_1 (60 mg)[8,9] in the order of increasing polarity.





Ginsenoside La (**1**) was obtained as colorless needles (from MeOH), mp 179-180°C, and showed $[\alpha]_D$ -18.4° (pyridine). It showed the quasi-molecular ion peak at m/z 781 $[C_{42}H_{70}O_{13}-H]^-$ in the negative FAB-MS and a strong hydroxyl absorption at ν3500 cm^{-1} in the IR spectrum. In the ^{1}H-NMR spectrum, **1** showed signals due to eight tert-methyls (δ0.83, 0.95, 1.00, 1.06, 1.30, 1.50, 1.66, and 1.80; 19-, 18-, 29-, 30-, 28-, 21-, 26-, and 27-H_3, respectively), an olefinic proton (δ5.51, br.d, J=9 Hz, 24-H), two anomeric protons (δ4.94 and 5.13, each d, J=8 Hz, 1'-H and 1"-H), and several protons attached to oxygen-bearing carbons. The ^{13}C-NMR spectrum of **1** showed two olefinic carbon signals [δ129.2 (d, C-24) and 131.4 (s, C-25)], two anomeric carbon signals [δ99.3 (d, C-1") and 106.9 (d, C-1')], and fourteen oxygenated carbon signals. These spectral data and the fact that **1** gave glucose on acid hydrolysis (50% aq.AcOH, reflux, 1 h) showed that **1** may be a triterpene diglucoside.

Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra with the aid of ^{1}H-^{1}H and ^{1}H-^{13}C COSY led us to postulate the partial structures shown in Fig. 1. The coupling constants of each proton were obtained from the 1D spectrum and by analysis of the fine structures of cross peaks in ^{1}H-^{1}H COSY.

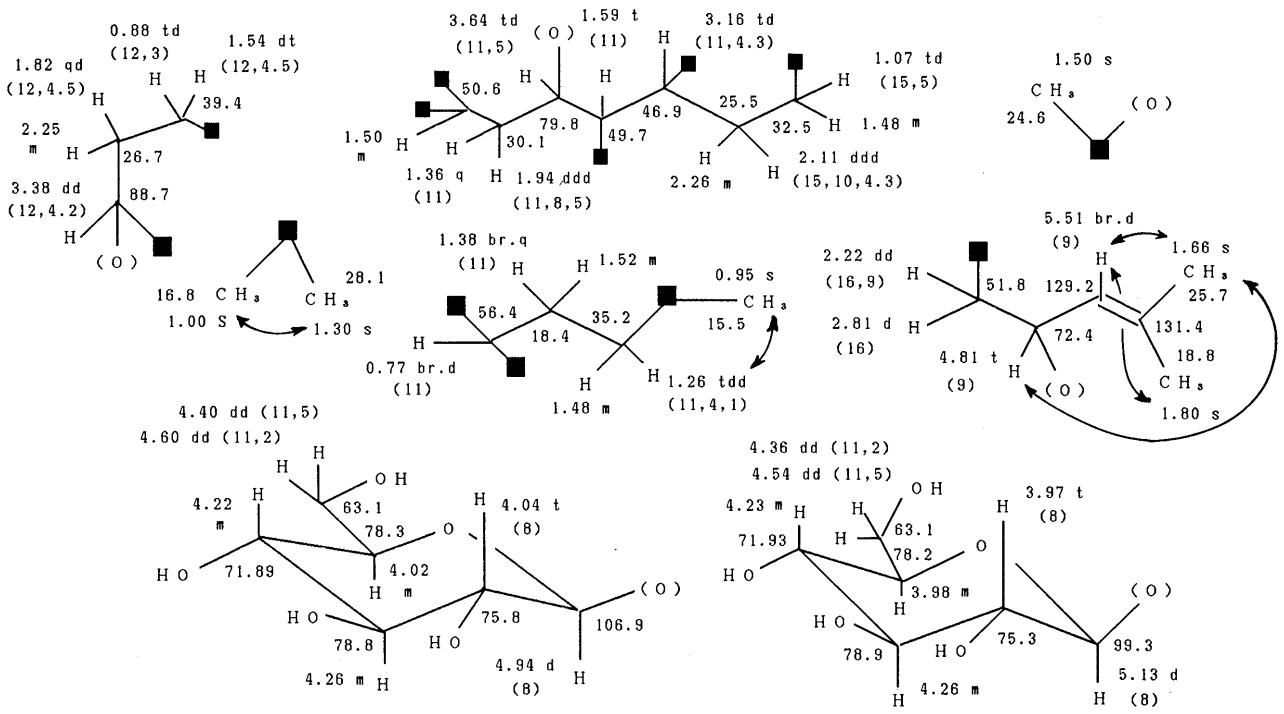


Fig. 1. Partial Structures Deduced by ^{1}H-^{1}H and ^{1}H-^{13}C COSY

: Long-range coupling observed in ^{1}H-^{1}H COSY

Next, the HMBC spectrum[10] of **1** was measured in order to clarify the connectivities of these partial structures. As shown in Fig. 2a, the methyl protons at δ0.83 (19-H$_3$) showed long-range correlations with the carbons at δ37.1 (s, C-10), 39.4 (t, C-1), 50.6 (d, C-9), and 56.4 (d, C-5). Thus, the connectivities between the quaternary carbon-10 and the carbons-1, -5, -9, and -19 were indicated. The methyl protons at δ0.95 (18-H$_3$) and 1.06 (30-H$_3$) showed long-range correlations with the carbons at δ35.2 (t, C-7), 39.8 (s, C-8), 50.6 (d, C-9), and 51.2 (s, C-14) and with the carbons at δ32.5 (t, C-15), 39.8 (s, C-8), 49.7 (d, C-13), and 51.2 (s, C-14), respectively. Thus, the connectivities between the quaternary carbon-8 and the carbons-7, -9, -14, and -18 and between the quaternary carbon-14 and the carbons-8, -13, -15, and -30 were revealed. Moreover, the methyl protons at δ1.00 (29-H$_3$) and 1.30 (28-H$_3$) both showed long-range correlations with the carbons at δ39.7 (s, C-4), 56.4 (d, C-5), and 88.7 (d, C-3, not shown in Fig. 2a), indicating the connectivities between the quaternary carbon-4 and the carbons-3, -5, -28, and -29. On the other hand, the methyl protons at δ1.50 (21-H$_3$) showed long-range correlations with the carbons at δ46.9 (d, C-17), 51.8 (t, C-22), and 81.8 (s, C-20, not shown in Fig. 2a), so that the connectivities between the oxygenated quaternary carbon-20 and the carbons-17, -21, and -22 were deduced. Based on these findings, **1** was thought to be a diglucoside of the 23-oxygenated protopanaxadiol derivative.

In the HMBC spectrum, long-range correlations were also observed between the proton at δ3.64 (1H, td, J=11, 5 Hz, 12-H) and the carbon at δ72.4 (d, C-23) and between the proton at δ4.81 (1H, t, J=9 Hz, 23-H) and the carbon at δ79.8 (d, C-12) (Fig. 2b and 2c). Thus, the presence of an ether linkage between the carbon-12 and -23 was revealed.

Positions of two glucose residues were solved also by analysis of the HMBC spectrum. Two anomeric protons at δ4.94 (1'-H) and 5.13 (1"-H) showed long-range correlations with the carbon-3 and carbon-20, respectively (Fig. 2d). Therefore, the glucose residues must be attached to C-3 and C-20, and the planar structure of **1** could be depicted as **1a**, shown in Fig. 2.

The relative stereochemistry of **1** was determined by considering the coupling constants of each proton

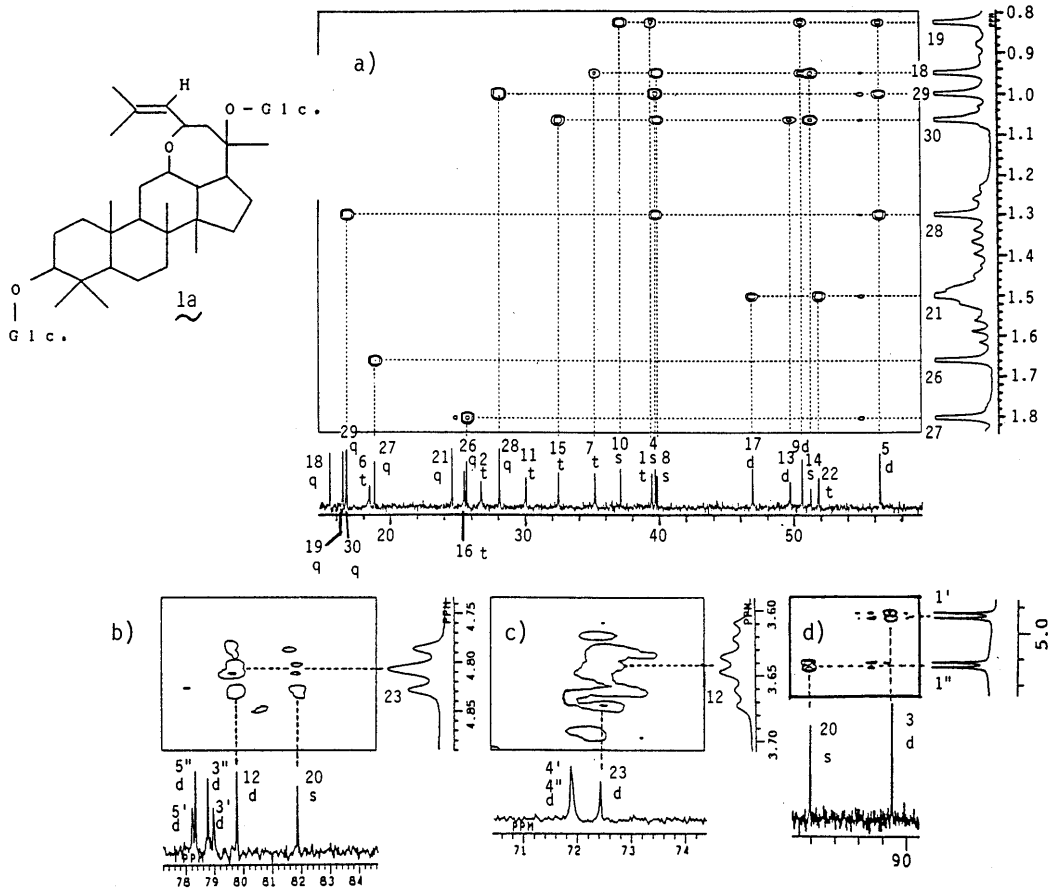


Fig. 2. HMBC Spectrum of Ginsenoside La (1) in C_5D_5N (Sample 12 mg, 36 h run)
a) High-field region. b),c) Cross peaks of 23-H and 12-H, respectively.
d) Cross peaks of 1'-H and 1"-H.

(Fig. 1) and by NOE experiments. Irradiation of 12-H decreased[11] the signal intensity of 23-H and irradiation of 23-H decreased[11] the signal intensities of 12-H and 1"-H and increased the signal intensity of 27-H_3, indicating the configuration of C-20 and C-23 as shown in the formula **1**. Our present result provided the first example of 23-oxygenated dammarane saponin, which is unique in the structural feature having an ether linkage between the ring C and the side chain.

REFERENCES AND NOTES

1) Chiang Su New Medical College ed., "Dictionary of Chinese Crude Drugs," Shanghai Scientific Technologic Publisher, Shanghai, 1977, p.29.
2) O. Tanaka and R. Kasai, "Progress in the Chemistry of Organic Natural Products," Vol.46, ed. by W. Herz, H. Grisebach, G. W. Kirby, and Ch. Tamm, Springer-Verlag, Vienna, New York, 1984, p.1.
3) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, Chem. Pharm. Bull., **22**, 421 (1974).
4) S. Yahara, O. Tanaka, and T. Komori, Chem. Pharm. Bull., **24**, 2204 (1976).
5) S. Yahara, K. Kaji, and O. Tanaka, Chem. Pharm. Bull., **27**, 88 (1979).
6) M. Komatsu, T. Tomimori, and Y. Makiguchi, Yakugaku Zasshi, **89**, 122 (1969).
7) Y. Chen, H. Xie, J. Luo, Y. Pei, S. Xu, and X. Yao, Zhang Cao Yao, **19**, 4 (1988).
8) S. Sanada, K. Kondo, J. Shoji, O. Tanaka, and S. Shibata, Chem. Pharm. Bull., **22**, 421, 2407 (1974); R. Kasai, H. Besso, O. Tanaka, Y. Saruwatari, and T. Fuwa, Chem. Pharm. Bull., **31**, 2120 (1983).
9) More efficient separation of these saponins is now under investigation.
10) A. Bax and M. F. Summers, J. Am. Chem. Soc., **108**, 2093 (1986); M. F. Summers, L. G. Marzilli, and A. Bax, ibid., **108**, 4285 (1986).
11) It was observed that some saponins showed a negative NOE; H. Wang, K. He, L. Ji, Y. Tezuka, and T. Kikuchi, Chem. Pharm. Bull., in press.

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