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^{13}C Nuclear Magnetic Resonance Spectral Studies on Gleditsia Saponin C (GS-C)

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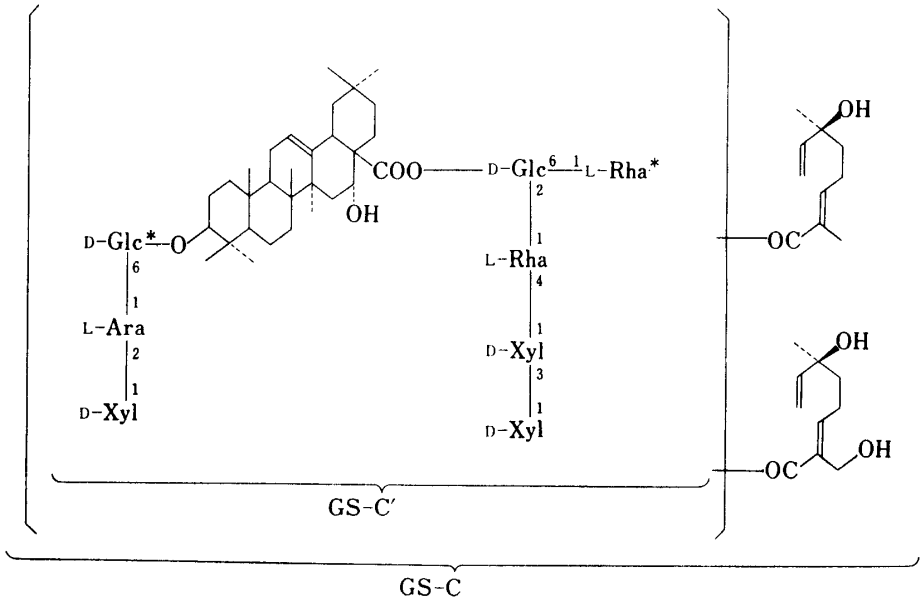
A major triterpenoid saponin (GS-C) of the fruit capsule of *Gleditsia japonica* Miq. (Leguminosae) contains eight sugar units and two monoterpenyl moieties in the molecule. It has been proved that at least one of the monoterpenyl groups is attached to the C-2 hydroxyl of the terminal L-rhamnosyl group in the C-28 oligoside portion of GS-C on the basis of upper-field shifting of the ^{13}C nuclear magnetic resonance signal of the anomeric carbon, which was refined by means of a partially relaxed Fourier transform (PRFT) experiment.

Keywords——Saponin; *Gleditsia japonica*; Leguminosae; Gleditsia saponin C (GS-C); echinocystic acid; ^{13}C NMR; PRFT experiment; bismonoterpenyl triterpenoid saponin

A major triterpenoid saponin of the fruit of *Gleditsia japonica* Miq. (Leguminosae) was isolated and designated as gleditsia saponin C (GS-C).[1,2] This saponin is a rare example of a triterpenoid saponin containing eight sugar units and two monoterpene moieties in the molecule.[2] A desmonoterpenyl compound, GS-C′, was obtained from GS-C by mild alkaline hydrolysis.

Konoshima *et al.*[1] independently reported the structure of GS-C′ as being 3-*O*-β-D-xylopyranosyl(1→2)α-L-arabinopyranosyl(1→6)β-D-glucopyranosyl-28-*O*-β-D-xylopyranosyl-(1→3)β-D-xylopyranosyl(1→4)α-L-rhamnopyranosyl(1→2)[α-L-rhamnopyranosyl(1→6)]β-D-glucopyranosyl echinocystic acid.



The structures of monoterpenyl moieties attached to GS–C′ were reported by Okada *et al.*[2] as being (+)-2,6-dimethyl-6(*S*)-hydroxy-2-*trans*-2,7-octadienoic acid and (+)-2-hydroxymethyl-6-methyl-6(*S*)-hydroxy-2-*trans*-2,7-octadienoic acid.

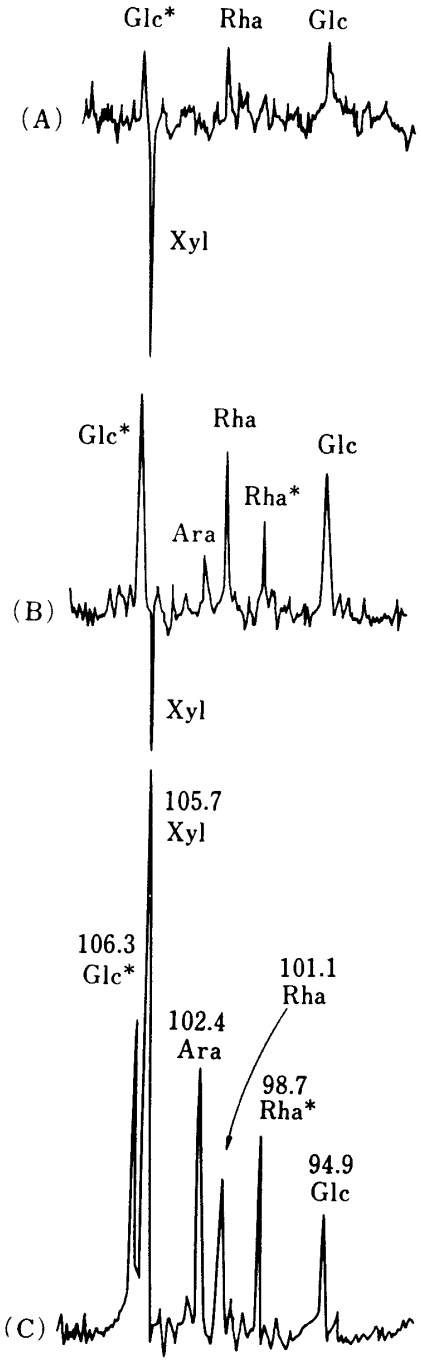


Fig. 1. Proton-decoupled ^{13}C FT-NMR spectrum and PRFT spectra of GS-C in the anomeric carbon region (ppm from internal TMS in C_5D_5N at 90°)

(A, B) PRFT spectra—intervals (s): (A) 0.1, (B) 0.15, with a recycle time of 1.6 s.
(C) Normal Fourier Transform spectrum using a recycle time of 1.0 s.

These monoterpene moieties were readily detached from the saponin, accompanied by some structural transformations, on treatment even with a weak base, such as potassium carbonate or potassium bicarbonate. This paper describes a ^{13}C nuclear magnetic resonance (NMR) spectral analysis to identify the location of one of the monoterpenyl moieties in the molecule of GS–C.

The ^{13}C NMR spectra of GS–C and GS–C′ were taken in d_5-pyridine at 90°C. All the signals in the anomeric carbon region of GS–C and GS–C′ were assigned as shown in Table I.

The anomeric carbon signals of sugar moieties of GS–C were almost superimposable on those of GS–C′ with the exception of a signal at 98.7 ppm. The signal at 94.9 ppm of GS–C, which corresponds to that at 95.0 ppm of GS–C′, was assigned to the anomeric carbon of D-glucose linked to the 28-carboxyl of the sapogenin, echinocystic acid, since the higher shifts are produced due to the esterification effect.[3]

An up-field shift of an anomeric carbon signal would also be caused by the substitution of a monoterpenyl moiety at a hydroxyl attached to the neighboring C-2 of one of the sugar components.

Table I. ^{13}C NMR Chemical Shifts of Anomeric Carbon Atoms of the Sugar Moieties of GS-C and GS-C′

GS-C′	GS-C	
106.5	106.3	Glc
105.8	105.7	Xyl
102.5[a]	98.7	Rha (terminal)
102.1[a]	102.4	Ara
101.2	101.1	Rha (inner)
95.0	94.9	Glc

a) The assignments of signals might have to be reversed.

A signal at 102.4 ppm given by GS–C was assigned to the anomeric carbon of L-arabinose, which is shifted *ca.* 2.5 ppm higher than usual due to the linkage with terminal D-xylose at the neighboring position.[4,5] Consequently, the signals at 101.1 and 98.7 ppm given by GS–C could be assigned to the anomeric carbons of two L-rhamnosyl moieties. On the other hand, the anomeric carbon signals at 102.5 and 102.1 ppm given by GS–C′ were assigned to L-rhamnosyl and L-arabinosyl moieties or *vice versa*. In relation to these data, the substitution of a monoterpenyl

group at the 2-hydroxyl of either the terminal or the inner L-rhamnosyl moiety would cause an up-field shift of the anomeric carbon signal by *ca.* 4 ppm. Hence the partially-relaxed Fourier transform (PRFT) method[6] was adopted for the NMR-spectral analysis of GS–C to establish which rhamnosyl moiety, terminal or inner, may carry a monoterpenyl group. As the terminal L-rhamnose is linked with a D-glucosyl group by an $\alpha 1 \rightarrow 6$ linkage and has a larger freedom of movement than the inner-located L-rhamnosyl group, a longer average spin-lattice relaxation time should be observed in the terminal one. The order of signal recovery in the spectra of the two anomeric carbons, 98.7 and 101.1 ppm, assigned to the respective L-rhamnosyl groups in the molecule of GS–C was measured, and it was confirmed that the former is slower than the latter, as shown in Fig. 1. Accordingly, one of the monoterpenyl groups is attached to the C-2 hydroxyl of the terminal L-rhamnosyl moiety. Experiments to determine the location of the other monoterpenyl group in the molecule of GS–C are in progress.

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References and Notes

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