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The Structure of Phytolaccoside G

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Abstract—The structure of phytolaccoside G(**I**), one of the minor saponins of the roots of *Phytolacca americana*, has been elucidated as 3-β-D-xylopyranosyljaligonic acid. And phytolaccoside B(**II**) is 30-methylester of phytolaccoside G.

The roots of *Phytolacca americana* (Phytolaccaceae) which are being used as a folk medicine in treating edema and rheumatism, has been described to contain a large amount of antiinflammatory saponins[1]. This paper describes the structural elucidation of phytolaccoside G.

Phytolaccoside G(**I**), $C_{35}H_{54}O_{11} \cdot 2H_2O$, mp 276-8°, $[\alpha]_D^{25}+77°$, on acid hydrolysis, gave jaligonic acid[2], mp 318-9° and D-xylose. Methylation of **I** with CH_2N_2 gave dimethylester(**III**), $C_{37}H_{58}O_{11}$, mp 181-184°. Exhaustive methylation of **I** by Hakomori's method[3] gave the permethylated product(**V**), which showed NMR signals for five tertiary methyls(δ 0.70~1.22), seven O-methyls (δ 3.31~3.62), an anomeric proton (δ 4.20, 1H, d, J=8 Hz) and an olefinic proton (δ 5.48, m, 1H). The mass spectrum showed the molecular ion peak at m/e 748 ($C_{42}H_{68}O_{11}$) and two peaks at m/e 557 and 175 which corresponded to mass of genin portion minus H^+ formed by cleavage of C-O bond at x and that of sugar portion by cleavage at y, respectively. Besides these peaks, the two retro-Diels-Alder fragmentation ions from the ion of mass 557 appeared at m/e 306 and 219 and important peaks at m/e 247, 246, 233, 187, 173, 133 formed by further fragmentation from the RDA fragment ion of mass 306 were also observed. From the above results, it was apparent that **I** was composed of one molecule each of D-xylose and jaligonic acid.

Phytolaccoside G was oxidized with HIO_4 and two moles of reagent was consumed. Hydrolysis of the oxidation product gave jaligonic acid. Formation of jaligonic acid indicated that xylose must have been attached to the C-2 or C-3 position rather than C-23 position and 2-mole-reagent consumption indicated that xylose was present as pyranose form rather than furanose form. Methanolysis of **IV** gave di-O-methyljaligonic acid dimethylester(**V**) which showed in its NMR spectrum five tertiary methyl signals (δ 0.60~1.14), two methyl ether signals (δ 3.33, s, 6H), two methylester signals (δ 3.59 and 3.71, 3H each), an AB quartet (δ 3.06 and 3.32, 1H each, J=10 Hz) and a multiplet (δ 5.38, 1H) due to olefinic proton. The presence of a doublet (1H) centered at δ 5.01 (J=3 Hz) in the NMR spectrum of

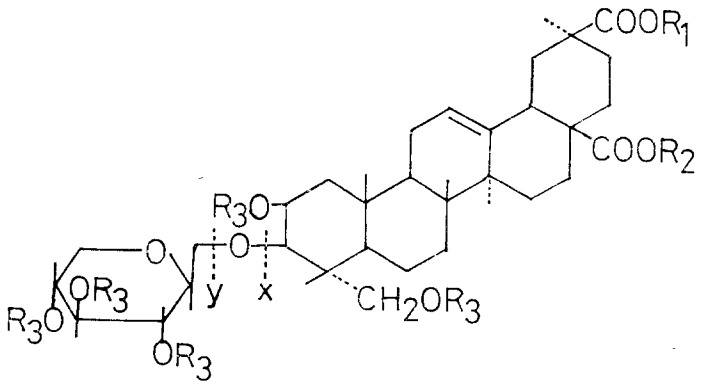
acetate of **V** clearly indicated that 3β-OH of **IV** was not methylated. This result suggests that xylose unit is linked to C-3 of jaligonic acid in the saponin.

β-Orientation of glycosidic linkage was suggested not only from the coupling constant of the anomeric proton signal of permethylated product, but also by molecular rotation difference between the saponin and the sapogenin (Table I).

Table I—Molecular rotations of phytolaccoside G and related substance.

Substance	$[\alpha]_D$	$[M]_D$
Observed value		
Phytolaccoside G	+77°	+500.5°
Jaligonic acid	+113.7°	+589°
$[M]_D$ contribution of xylose unit		−88.5°
Literature value[4]		
Methyl-α-D-xylopyranoside		+249°
Methyl-β-D-xylopyranoside		−107°

Amendment—Phytolaccoside B was earlier shown to be 3-β-D-glucopyranosyl phytolaccagenin[5]. However, further examination showed that sugar unit of the saponin was D-xylose and not D-glucose. And methylphytolaccoside B was identical to dimethylphytolaccoside G (NMR, MS, mmp, co-TLC). Therefore, the constitution of phytolaccoside B(**II**) was revised as 3-β-D-xylopyranosylphytolaccagenin.



I $R_1=R_2=R_3=H$ **II** $R_1=Me$, $R_2=R_3=H$
III $R_1=R_2=Me$, $R_3=H$ **IV** $R_1=R_2=R_3=Me$

EXPERIMENTAL

Mp's are uncorrected. NMR spectra were taken in $CDCl_3$ using TMS as internal reference at 100 MHz.

Isolation of phytolaccoside G(I)—The saponin mixture was applied to a silica gel column and eluted with MeOH-$CHCl_3$ (gradient) to give a G fraction which was crystallized from ethylacetate saturated with water, mp 276~278°, $[\alpha]_D^{25}$+77° (c=0.5 in MeOH), IR_{KBr}^{max} cm^{-1}; 3410(OH), 1705(acid), 1000~1100(glycoside).

Anal. Calcd for $C_{35}H_{54}O_{11} \cdot 2H_2O$: C, 61.21; H, 8.51. Found: C, 61.61; H, 8.23.

The saponin (100 mg) was heated with 5% HCl-MeOH under reflex for 3hr. The product was diluted with H_2O and extracted with Et_2O. The Et_2O extract was crystallized from MeOH to give needles of jaligonic acid, mp 318~319°, which was identified by direct comparison with an authentic sample (mmp, co-TLC, IR and MS). The aqueous layer was hydrolyzed with HCl, neutralized with Ag_2CO_3 and evaporated in vacuo. The residue was found to be D-xylose by TLC (MeOH-$CHCl_3$-Acetone-NH_4OH=5 : 2 : 3 : 2, Rf 0.35; MeOH-$CHCl_3$-NH_4OH-H_2O=30 : 60 : 3 : 4, Rf 0.11).

Dimethylester(III)—The saponin (100 mg) in MeOH (20 ml) was treated with CH_2N_2 in the usual manner. The crude product was purified by PLC ($CHCl_3$-MeOH-NH_4OH-H_2O=20 : 4 : 1 : 3) and crystallized from MeOH to give needles of **III**, mp 181~184°, which is identical with methylphytolaccoside B.

HIO_4-Alkali degradation of I—A MeOH soln of **I** (32.5 mg) was added to 20 ml of 0.01 M HIO_4, the volume made up to 35 ml with MeOH and the soln kept in the dark at 4° until **I** was completely disappeared on TLC (about 10 days). An aliquot was removed and HIO_4 was estimated with 0.01 M Na_3AsO_3 in the usual way. The consumption of oxidant was 1.99 moles. To the remainder soln, 1 ml of ethyleneglycol was added, after standing at room temperature for 5hr, diluted with H_2O and extracted with BuOH. The BuOH extract was heated under reflux in 10 ml of 5% NaOH-MeOH for 5 hr. The reaction mixture was acidified and extracted with Et_2O. The Et_2O extract was crystallized from MeOH to yield needles of jaligonic acid, which was identical with an authentic sample (mmp, co-TLC).

Permethylation of I—According to the Hakomori's method, NaH (200 mg) was stirred with dimethylsulfoxide (5 ml) at 70° for 45 min under nitrogen gas flow. To this methylsulfinyl anion soln, 200 mg of **I** in 5 ml of dimethylsulfoxide was added and the mixture was stirred for 30 min at room temperature under nitrogen gas flow. CH_3I (5 ml) was added and the reaction mixture was allowed to stand at room temperature overnight with stirring. After dilution with water, the mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and concentrated. The residue was chromatographed over silica gel and eluted with $CHCl_3$ to give permethylated product (**IV**), which was purified from MeOH-H_2O to give a white powder, mp 111~4°, $[\alpha]_D^{27}$+82.1° (c=0.34 in MeOH), IR_{KBr}^{max} cm^{-1}: OH (nil) 1730 (ester).

Methanolysis of IV—A solution of **IV** (100 mg) in 20 ml of 3% HCl-MeOH was refluxed for 5 hr. The reaction mixture was concentrated, diluted with water and filtered. Ppt was crystallized from MeOH-H_2O to yield a white powder of di-O-methyljaligonic acid dimethylester (**V**), mp 105~107°.

Anal. Calcd. for $C_{34}H_{54}O_7$: C, 71.05; H, 9.47. Found: C, 69.84; H, 9.87.

The filtrate was extracted with $CHCl_3$. The $CHCl_3$ extract was found to be methyl-2, 3, 4-tri-O-methyl-D-xyloside by TLC (ethylacetate, Rf 0.65, 0.55) and GLC (column, 5% NPGS on Gas Chrom Q, 100~120 mesh, 2.2 m×4 mm; column temp., 170°; FID temp., 200°; injector temp., 190°; N_2 flow, 45 ml/min: t_R(min), 2.2, 2.8).

Acetylation of V—The sample (50 mg) was heated with Ac_2O (0.5 ml) and C_5H_5N (0.5 ml) for 2 hr. Pouring onto ice gave solids which were purified from MeOH-H_2O to yield a white powder, mp 85~89°; NMR: δ 0.73~1.21(5×CH_3), 2.12(3 H, s, CH_3CO), 3.11 and 2.87 (1 H each, AB quartet, J=10 Hz), 3.26(3 H, s, OMe), 3.30(3 H, s, OMe), 3.60(3 H, s, COOMe), 3.71(3 H, s, COOMe), 5.01(1 H, d, J=3 Hz, H-3) and 5.38 (1 H, m, H-12).

REFERENCES

1. W.S. Woo, K.H. Shin and S.S. Kang, *Kor. J. Pharmacog.*, **7**, 47 (1976); W.S. Woo and K.H. Shin, *J. Pharm. Soc. Korea*, **20**, 149 (1976).
2. W.S. Woo, *Lloydia*, **36**, 326 (1973).
3. S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
4. I. Kitagawa, A. Inada and I. Yosioka, *Chem. Pharm. Bull.*, **23**, 2268 (1975).
5. W.S. Woo and S.S. Kang, *Phytochemistry*, **15**, 1315 (1976).