TRITERPENE GLYCOSIDE OF KOELREUTERIA PANICULATA

IV. Structure of Koelreuteria Saponins A and B

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In the course of a systematic study of plants of the family Sapindaceae for their content of triterpene glycosides, we turned our attention to Koelreuteria paniculata (panicled goldraintree) growing in Moldavia. Literature information indicates the presence in it of glycosides of a triterpene nature [1]. There is no information at all on their chemical characteritics.

Aqueous ethanolic extracts of the fruit of this tree gave a positive Liebermann-Burchard reaction [2]. A methanolic extract, after purification, was subjected to thin-layer chromatography in several solvent mixtures, as a result of which the presence of two glycosides was established. In order of increasing polarity, we have called them koelreuteria saponin A and B. When alumina was used, the substances were isolated separately.

By the acid cleavage of koelreuteria saponin A, the aglycone was identified as hederagenin, and the carbohydrate moiety was found to contain arabinose. Methylation by Hakomori's method [3] showed that the monosaccharide had a pyranose ring and that it was attached in position 3 of the aglycone. In its melting point, specific rotation, and chromatographic behavior, koelreuteria saponin A was identical to the 3-O-α-arabinoside of hederagenin which we had obtained previously by the partial hydrolysis of the glycosides from the Chinese soapberry Sapindus mukorossii [4] and in the native state from Leontice eversmannii and Caulophyllum robustum [5, 6].

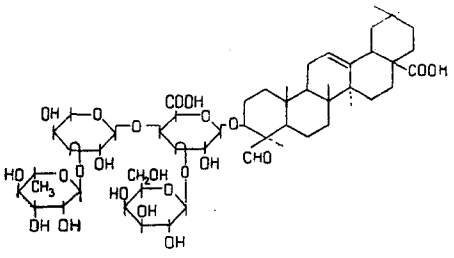
When koelreuteria saponin B was hydrolyzed with Kiliani's mixture [7], gypsogenin, glucuronic acid, galactose, arabinose, and rhamnose were identified. By analogy with other glycosides of gypsogenin [8, 9], 2% H_2SO_4 yielded the 3-O-β-glucuronoside.

The types of bond between the monosaccharides in koelreuteria saponin B were determined by methylation and periodate oxidation. The following methyl ethers were identified in the presence of reference samples in the hydrolysate of the permethylated saponin: 2-O-methyl-D-glucuronic acid, 2, 4-di-O-methyl-L-arabinose, 2, 3, 4-tri-O-methyl-L-rhamnose, and 2, 3, 4, 6-tetra-O-methyl-D-galactose. The Smith degradation of the glycosides [10] gave arabinose and glucuronic acid. Consequently, in the saponin there is branching at the glucuronic acid, and two 1 → 3 and one 1 → 4 bonds are present.

The results of the treatment of koelreuteria saponin B with alkali showed that all the sugars are attached to the hydroxyl at C_3 of the gypsogenin.

The definitive structure of the carbohydrate moiety was shown by partial hydrolysis, which led to a bioside consisting of glucuronic acid and galactose. On its degradation, by Smith's method, it was found that a 1 → 3 bond exists between them.

On the basis of what has been said above, the definitive structure of koelreuteria saponin B can be shown in the following form.



The configurations of the glycoside centers of the monosaccharides were determined by Klyne's rule [11] (table).

Glycosides, monosaccharides	$[M]_D$, deg		Glycoside	$[M]_D$, deg	ΔC,	Form of the bond
	α	β				
Methyl D-galactopyranoside [12]	+380	0	Bioside	−178	—	—
Methyl D-glucopyranuronic acid [13]	+307	−62	Monooside	−106	−284	β
			Gypsogenin	+433	−539	β
Found for the configuration of the bonds (arabinoside, rhamnoside)			Tetraoside	−227	−49	αα
			Bioside	−179	—	—
Methyl L-arabopyranoside [14]	+28	+402	—	—	—	—
Methyl L-rhamnopyranoside [15]	−111	+170	—	—	—	—

Calculated for the following bond configurations (arabinoside-rhamnoside): α,α (−83°), α,β (+198°), β,α (+291°), and β,β (+572°).

EXPERIMENTAL

Chromatography was carried out with type S paper of the Volodarskii Leningrad mill, KSK silica gel, and neutral alumina using the following solvent systems: 1) butan-1-ol–ethanol–25% ammonia (9 : 2 : 5), 2) ethyl acetate–methanol–water (10 : 2 : 3), 3) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3), 4) benzene–acetone (2 : 1), 5) chloroform–ethyl acetate (3 : 1), and 6) butan-1-ol–acetic acid–water (4 : 1 : 5).

Isolation of koelreuteria saponins A and B. The fruit of Koelreuteria paniculata, 500 g, was exhaustively extracted with aqueous methanol. The extract was evaporated to dryness, dissolved in water, and washed with diethyl ether. The yield of the saponin fraction was 88 g (17% of the weight of the initial raw material). The combined glycosides (2 g) were transferred to a column of alumina (60 × 3 cm) and eluted with mixture 1. This gave 0.3 g of koelreuteria saponin A (I) with mp 226–228° C, $[\alpha]_D^{20}$ +43° (*c* 0.5, pyridine) and +59° (*c* 1.0, methanol), and also 1.2 g of koelreuteria saponin B (II) with mp 160–162° C, $[\alpha]_D^{20}$ −20° (*c* 1.0, methanol).

Structure of koelreuteria saponin A (I). A mixture of 300 mg of I and 15 ml of Kiliani's mixture was heated in a sealed tube at 110° C for 5 hr. Then the hydrolysate was treated with 20 ml of water and the solution was extracted with ether. After concentrating the organic layer, the residue was recrystallized from methanol. This gave 150 mg of a substance with mp 226–228° C, $[\alpha]_D^{20}$ +79° (*c* 2, chloroform). Literature data [16]: mp 232–234° C, $[\alpha]_D^{20}$ +81°. From its chromatographic mobility in a thin layer of silica gel in system 5, the compound was identical with hederagenin.

By paper chromatography in systems 3 and 6 after neutralization with EDE-10P resin (OH^- form), the aqueous layer was shown to contain arabinose.

Substance I (50 mg) was heated with 10 ml of 10% water–ethanol solution of caustic potash at 80° C for 5 hr, and then extracted with isopentyl alcohol. The organic extract yielded the original substance.

Compound I (100 mg) was methylated by Hakamori's method. This gave 100 mg of permethylated substance I. This product, 50 mg, was treated with 0.1 ml of 72% perchloric acid in 1 ml of methanol and the mixture was heated in a sealed tube at 100° C for 5 hr. Then it was diluted and the resulting precipitate was shown in the presence of reference materials of contain the methyl ester of 23-O-methylhederagenin, and the filtrate was shown in a mixture of solvents to contain 2, 3, 4-tri-O-methylarabopyranoside.

The structure of koelreuteria saponin B (II). A) A 100-mg quantity of II was hydrolyzed as described above. The residue was shown in system 4 in the presence of reference samples to contain gypsogenin (III); glucuronic acid, galactose, arabinose, and rhamnose were found in the filtrate.

B) A mixture of 0.5 g of II and 20 ml of 2% H_2SO_4 was heated at 80° C for 5 hr. The precipitate of IV was filtered off and recrystallized from ethanol. In its melting point and specific rotation, the substance was identical with gypsogenin 3-O-β-glucuronoside.

When IV was heated with Kiliani's mixture, chromatography on silica gel showed the presence of III, and paper chromatography showed the presence of glucuronic acid.

C) One gram of II was methylated as described above. After cleavage, the hydrolysate was shown by thin-layer and and gas-liquid chromatography in the presence of markers to contain 2, 3, 4-tri-O-methyl-L-rhamnose; 2, 3, 4, 6-tetra-O-methyl-D-galactose; 2, 4-di-O-methyl-L-arabinose; and methyl 2-O-methyl-D-glucuronate.

D) Periodate oxidation of II. Compound II (500 mg) was dissolved in 20 ml of aqueous methanol and treated with 100 mg of $NaIO_4$, and the reaction mixture was kept in the dark at room temperature. After the consumption of periodate had become constant, the excess was destroyed by the addition of a few drops of ethylene glycol. Then the reaction mixture was neutralized with ion-exchange resins and reduced with sodium borohydride. After a day, the solution was again deionized and hydrolyzed as described previously. Glucuronic acid and arabinose were identified by paper chromatography.

E) A mixture of 20 g of substance II and 200 ml of 10% oxalic acid was heated at 75° C for 10 hr, and then extracted with butanol. The organic extracts were evaporated to dryness, and the residue was chromatographed in system 2 on a column of silica gel. This gave 0.25 g of IV, 0.15 g of II, and 0.5 g of substance V with mp 103–105° C and $[\alpha]_D^{20}$ −21° (*c* 1.9, methanol).

After the acid cleavage of 20 mg of V with Kiliani's mixture, glucuronic acid and galactose were found by paper chromatography. After the periodate oxidation of 100 mg of V as described above, glucuronic acid was identified.

CONCLUSIONS

It has been established that koelreuteria saponin A is O-α-arabopyranosyl-(1 → 3)hederagenin, and koelreuteria saponin B is O-α-rhamnopyranosyl-(1 → 3)-O-α-arabopyranosyl-(1 → 4)
O-β-galactopyranosyl-(1 → 3)> β-glucuronopyranosyl- (1 → 3)-gypsogenin.

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