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Abstract

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THE STRUCTURE OF KALOPANAX-SAPONIN A*

We have previously described the isolation, from the roots of *Kalopanax septemlobum* (family Araliaceae), of two glycosides of hederagenin, named kalopanax-saponins A and B. Kalopanax-saponin A (I) is a bioside of hederagenin containing arabinose and rhamnose. Its elemental composition corresponds to the gross formula $C_{41}H_{66}O_{12} \cdot H_2O$, m.p. 226–229° (decomp.), $[\alpha]_D^{20} + 13.5°$. I is not an O-acyl glycoside, since treatment with diazomethane followed by acid hydrolysis gives not hederagenin but its methyl ester. The same conclusion follows from the fact that kalopanax-saponin B, which is unchanged by the action of diazomethane and therefore is an O-acyl glycoside ([1]), is converted by alkaline hydrolysis into kalopanax-saponin A.

At present, structure I has been established for kalopanax-saponin A. Methylation of kalopanax-saponin A according to Kuhn ([2]) led to the methylated glycoside (II) of composition $C_{48}H_{80}O_{12}$, upon hydrolysis of which 3,4-di-*O*-methyl-*L*-arabinose (III) and 2,3,4-tri-*O*-methyl-*L*-rhamnose (IV) were identified. Thus, in glycoside I a disaccharide residue, having the structure *L*-Rha $1 \rightarrow 2$ *L*-Ar–, is attached to position 3 or 23 of hederagenin by an O-glycosidic bond. Along with the methylated monosaccharides III and IV, hydrolysis of II gave a neutral genin (V), differing in chromatographic behavior from the methyl ester of hederagenin (Fig. 1). In the mass spectrum of genin V there was a peak with maximum mass number 501 ± 5, which agreed well with the molecular weight 500.7 calculated for the gross formula $C_{32}H_{52}O_4$, corresponding to the methyl ester of 3- or 23-*O*-methylhederagenin. In the IR spectrum of V there were absorption bands indicating the presence in the compound of an OH group (3440 cm^{-1}) and a $COOCH_3$ group (1730 cm^{-1}). Since the constants of genin V differed from those described in the literature ([3]) for the methyl ester of 23-*O*-methylhederagenin (see the experimental part), and the methyl ester of the 3-*O*-methyl derivative had not been described up to the present, rigorous proof of the structure of genin V was necessary.

Fig. 1. Thin-layer chromatography (SiO_2/gypsum). Development system:

Reaction scheme: compound (VI) oxidized with CrO3 to compound (VII)

Figure 1: Reaction scheme: compound (VI) oxidized with CrO3 to compound (VII)

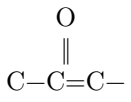
chloroform–acetone (20 : 0.4). Detection with a solution of $SbCl_3$ in chloroform. 1 –methyl ester of hederagenin; 2 –genin (V); 3 –oxidation products of (V): (VI) and (VII)

The structure of V and, consequently, the point of attachment of the carbohydrate residue in kalopanax-saponin A to hederagenin were proved as follows. Oxidation of V with chromic anhydride in acetic acid led to the formation of two neutral substances, (VI) and (VII), sharply differing in chromatographic behavior (Fig. 1). In the mass spectrum of VI there was a peak with maximum mass number 499 ± 5, consistent with the molecu-

* Communication 16 in the series "Triterpene saponins."

molecular weight 498.7, calculated for the empirical formula $C_{32}H_{50}O_4$. The IR spectrum of VI indicated the presence in the substance of a $COOCH_3$ grouping (1730 cm^{-1}), a C=O group in a six-membered ring with an α,α-gem-dialkyl grouping (1706 cm^{-1}) ([4]), and the absence of hydroxyl-containing functions. From the data given above, the structure of the methyl ester of 23-O-methylhederagenonic acid followed unambiguously for substance VI.

The data of the IR spectrum (VII) indicated the presence in the substance of a $COOCH_3$ group (1725 cm^{-1}), a C=O group in a six-membered ring with an



α,α-gem-dialkyl grouping (1705 cm^{-1}) and a grouping (1660 cm^{-1}), and, consequently, substance (VII) differed from (VI) by the presence of a carbonyl grouping conjugated with a double bond. This conclusion was also confirmed by the fact that the UV spectrum (VII) had an intense maximum λ_{max}^{EtOH}251 mμ (lg ε 3.5), corresponding, as calculation by Woodward' s rule showed ([5]), to a trisubstituted α,β-unsaturated ketone. The molecular weight of (VII), determined mass-spectrometrically (513 ± 5), agreed with that calculated for $C_{32}H_{48}O_5$ (512.7). Consequently, substance VII may be assigned the following probable structure, which is the result of further oxidation of (VI):

Thus, from the data on establishing the structures of VI and VII it follows unambiguously that the disaccharide residue in glycoside I is linked by an O-glycosidic bond with position 3 of hederagenin.

For complete proof of the structure of kalopanax-saponin A, it was necessary to decide the question of the configuration of the glycosidic bonds in I. As

calculation by Klyne' s rule showed ([6]), both glycosidic centers have the 1,2-trans configuration (see Table 1).

Table 1

Compound	$[\alpha]_D$	$M_D = [\alpha]_D \cdot$ mol. wt./100
Hederagenin ([7])	+81°	+383°
α-Methyl-*L*-arabinoside ([8])	+17°	+28°
β-Methyl-*L*-arabinoside ([8])	+245°	+402°
α-Methyl-*L*-rhamnoside ([8])	−62.5°	−117°
β-Methyl-*L*-rhamnoside ([9])	+95°	+179°
Calculated for the configurations of the bonds (arabinosidic, rhamnosidic): α, α	–	+294°
Calculated for the configurations of the bonds (arabinosidic, rhamnosidic): α, β	–	+596°
Calculated for the configurations of the bonds (arabinosidic, rhamnosidic): β, α	–	+666°
Calculated for the configurations of the bonds (arabinosidic, rhamnosidic): β, β	–	+963°
Found for kalopanax-saponin A	+13.5°	+102°

On the basis of the data presented above, kalopanax-saponin A should be assigned structure I.

(I)

Structural formula (I)

Figure 2: Structural formula (I)

Experimental Part*

IR spectra were recorded on a UR-10 spectrophotometer; UV spectra, on a Hitachi EPS-2 spectrophotometer. Mass spectra were recorded on an MX-1303 mass spectrometer. Specific rotation was determined on a Hilger M 412 polarimeter. KSK silica gel was used (fraction 0.25-0.1). Plates for thin-layer chromatography were prepared analogously to that described in ([10]).

1. Alkaline hydrolysis of kalopanax-saponin B. A solution of 1 g of kalopanax-saponin B in 20 ml of 10% NaOH was heated in an ampoule on a boiling water bath for 4 h. A voluminous precipitate separated. The reaction mixture was acidified with 10% HCl with cooling; the crystalline precipitate formed was filtered off, washed with water to neutral reaction, and dried (0.5 g).

The substance obtained was identical in its chromatographic behavior to kalopanax-saponin A (thin-layer chromatography on silica gel, system *n*-butanol–ethanol–20% NH_4OH (10 : 2 : 5)). Mp 226—229° (decomp.) (from ethanol); mp of a mixed sample with kalopanax-saponin A: 228—230° (decomp.), $[\alpha]_D^{20} + 16.1° \pm 1°$ (*C* 1.99, abs. ethanol).

After hydrolysis of the obtained substance with Kiliani mixture, paper chromatography (system *n*-butanol–benzene–pyridine–water, 5 : 1 : 3 : 3) identified arabinose and rhamnose.

2. Methylation of kalopanax-saponin A (I). 1.5 g of I in dimethylformamide was methylated with CH_3I in the presence of Ag_2O, analogously to that described in ([11]). 1 g of the resulting substance was chromatographed on Al_2O_3 (grade III, *H* 25 cm, *D* 2.7 cm), eluting with a mixture of benzene–chloroform (1 : 1)–ethyl acetate with an increase in the concentration of the latter from 1 to 50%. Yield of methylated kalopanax-saponin A (II): 0.53 g, colorless amorphous powder, $[\alpha]_D^{20} + 38° \pm 3°$ (*C* 2.9, chloroform).

Found, %: C 68.59, 67.98; H 9.64, 9.88; OCH_3 24.86, 25.13
$C_{48}H_{80}O_{12}$. Calculated, %: C 67.88; H 9.49; OCH_3 25.6

3. Hydrolysis of methylated kalopanax-saponin A (II). 0.53 g of II in 20 ml of a mixture of 72% $HClO_4$–CH_3OH (1 : 10) was heated in an ampoule for 5 h at 100°. The mixture was diluted with an equal volume of water; the precipitated genin was filtered off, washed on the filter with water, and dried. Yield 0.27 g. CH_3OH was removed from the filtrate; the aqueous acidic solution was heated in an ampoule for 4 h at 100°, neutralized with Dowex-1 (HCO_3^-), and evaporated to dryness. Yield of methylated monosaccharides 0.19 g.

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4. **Separation and identification of the methylated sugars.** 0.19 g of the resulting mixture of methylated sugars is chromatographed on silica gel (H 19 cm, D 2.2 cm) in the benzene–acetone–water system (1 : 1 : 0.5, v/v). Control: thin-layer chromatography on silica gel in the same system, detection of spots with H_2SO_4.

3,4-Di-*O*-methyl-*L*-arabinose (III) was detected on the chromatograms with periodate ([12]) and coincided with authentic 3,4-di-*O*-methyl-*L*-arabinose in its constants and chromatographic behavior on paper (methyl ethyl ketone saturated with 10% NH_4OH). Demethylation and subsequent hydrolysis gave 2,3,4-tri-*O*-methyl-*L*-arabinose, identical in chromatographic behavior with an authentic sample and sharply different from 2,3,5-tri-*O*-methyl-*L*-arabinose. $[\alpha]_D^{20} + 112.8 \pm 1^\circ$ (C 1.95, acetone). Literature data for 3,4-di-*O*-methyl-*L*-arabinose ([13]): $[\alpha]_D + 116^\circ$ (water).

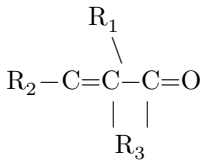
2,3,4-Tri-*O*-methyl-*L*-rhamnose (IV), in chromatographic behavior on paper, coincided with an authentic sample (methyl ethyl ketone saturated with 10% NH_4OH). Both samples were identical in their mass spectra ([14]). $[\alpha]_D^{20} -13.4^\circ \pm 1^\circ$ (c 3.4, acetone); $[\alpha]_D^{20} + 27.2 \pm 1^\circ$ (c 5.1, water). Literature data for 2,3,4-tri-*O*-methyl-*L*-rhamnose ([15]): $[\alpha]_D + 25^\circ$ (water).

5. **Methyl ester of 23-*O*-methylhederagenin (V).** 0.2 g of the genin obtained on hydrolysis of II is chromatographed on silica gel (H 19 cm, D 2.2 cm), eluting with chloroform and with a chloroform–ethyl acetate mixture with the concentration of the latter increasing from 1 to 100%. 0.15 g of a chromatographically homogeneous substance is obtained (Fig. 1), m.p. 190–192° (from CH_3OH), $[\alpha]_D^{20} + 70 \pm 1^\circ$ (C 1.9, chloroform). Molecular weight (mass spectrometric) 501 ± 5. $C_{32}H_{52}O_4$ (500.7). IR spectrum: 3440, 1730 cm^{-1}.

6. **Oxidation of the methyl ester of 23-*O*-methylhederagenin (V).** 0.1 g of (V) is oxidized with 0.03 g of CrO_3 in 3.5 ml of glacial CH_3COOH, with stirring for 14 h at room temperature. An equal volume of water is added to the reaction mixture and it is extracted with chloroform. The chloroform layer is washed with soda solution and with water. The residue after evaporation (0.09 g) is chromatographed on silica gel (H 11 cm, D 2 cm), eluting with the following mixtures: petroleum ether –benzene with an increase in the concentration of the latter from 10 to 100%, benzene–chloroform from 1 to 100%, chloroform–ethyl acetate from 1 to 100%. 0.02 g of chromatographically homogeneous methyl ester of 23-*O*-methylhederagonic acid (VI) is obtained, $[\alpha]_D^{20} - 23.1 \pm 2^\circ$ (C 1.9, chloroform). IR spectrum: 1730, 1706 cm^{-1}. Molecular weight (mass spectrometric): 499 ± 5. $C_{32}H_{50}O_4$ (498.7).

Substance (VII) (0.015 g), $[\alpha]_D^{20} + 33.6 \pm 1^\circ$ (C 1.6, chloroform); IR spectrum:

1725, 1705, 1660 cm^{-1}; UV spectrum: λ_{max}^{sp} 251 mμ ($\lg \varepsilon$ 3.5); (λ_{max}^{sp} calculated ([5]) for



249 mμ). Molecular weight (mass spectrometric) 513 ± 5. $C_{32}H_{48}O_5$ (512.7).

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