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CAULOSIDE D A NEW TRITERPENOID GLYCOSIDE FROM *CAULOPHYLLUM ROBUSTUM* MAXIM: IDENTIFICATION OF CAULOSIDE A*

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Key Word Index—*Caulophyllum robustum*; Berberidaceae; triterpenoid glycoside; cauloside A; cauloside D; enzymatic hydrolysis, *Eulota maackii*, structure, oligosaccharide, configuration of glycoside bond, NMR, MS, GLC data.

Abstract—The structure of cauloside D, one of the main saponins isolated from *Caulophyllum robustum* roots, was shown to be 3-*O*-α-L-arabinopyranosyl hederagenin-28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside with the aid of methylation and enzymatic hydrolysis by the digestive juice of the *Eulota maackii*. Cauloside A was shown to be identical with saponin A, isolated from *C. robustum* Maxim. previously. The composition of the digestive juice of *E. maakii* was shown to include enzymes that catalyse the cleavage of α-arabinosidic, β-1,6-glucosidic and acyl-*O*-β-glucosidic linkages.

INTRODUCTION

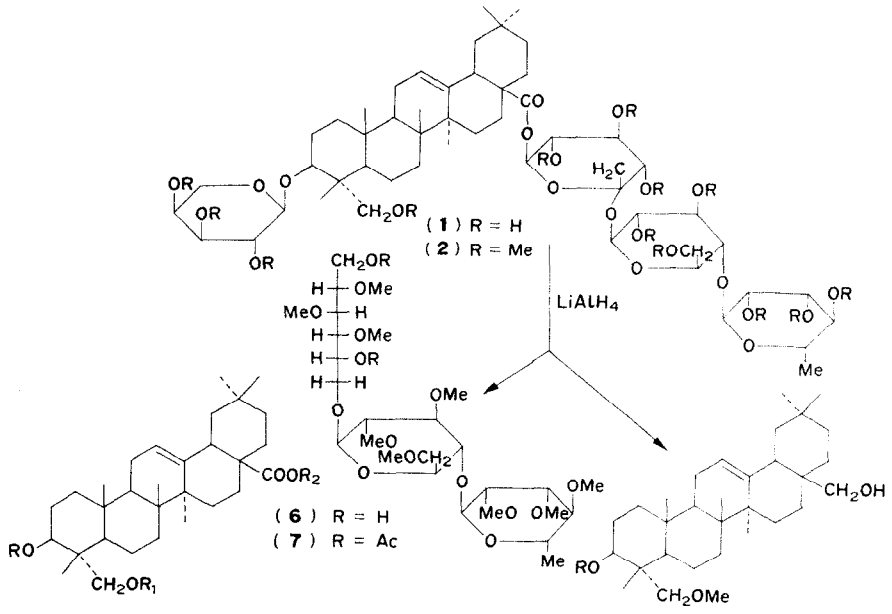
Murakami *et al.* [1] and ourselves [2–4] have reported studies on the triterpenoid glycosides of *Caulophyllum robustum* Maxim. (Berberidaceae). We now describe the results obtained in a study of causosides D (**1**) and A (**9**).

RESULTS AND DISCUSSION

Acid hydrolysis of permethylated cauloside D (**2**), synthesized by the method of Purdie [5], yielded methyl-23-*O*-methyl-hederagenin (**3**) and a mixture of methyl glycosides. The latter mixture was acetylated and assayed with the aid of GLC–MS while the methyl ethers were examined by MS using the technique reported previously [6]. The compounds identified were 2,3,4-tri-*O*-methyl-rhamnose, 2,3,4-tri-*O*-methyl-arabinose, 2,3,6-tri-*O*-methyl-4-*O*-acetylglucose and 2,3,4-tri-*O*-methyl-6-*O*-acetyl-glucose. Reductive cleavage of (**2**) with lithium aluminum hydride resulted in a methylated progenin and an oligosaccharide. Structure (**4**) corresponds to the progenin, since upon acid hydrolysis it yielded 23-methoxyerythrodiol [7] and 2,3,4-tri-*O*-methyl-L-arabinopyranoside. TLC was used to identify 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-sorbitol in the acid-hydrolysis products of the oligosaccharide. The reduction of 2,3,4-tri-*O*-methyl-D-glucose in the course of splitting (**2**) with lithium aluminum hydride indicated the direct bonding of the former with a hederagenin carboxyl group.

The structure (**6**), suggested for the oligosaccharide, was confirmed by the MS data for the oligosaccharide acetate (**7**). The detection in the MS of (**7**) of ion peaks at *m/e* 640 (M^+-60) and 567 (M^+-60-73) confirmed that (**6**) is a trisaccharide. Ion peaks at *m/e* 627, 539 and 117 were caused by the fragmentation of 2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl-D-sorbitol [8].

The configurations of the glycosidic bonds in cauloside D were established on examining the NMR spectra of the acetates of some progenins obtained from cauloside D by enzymatic hydrolysis. Earlier, we reported that incubation of causosides D and E with the digestive juice or liver



(3) R = H, R_1 = R_2 = Me
(8) R = R_1 = H; R_2 = β-D-glucopyranosyl
(9) R = α-L-arabinopyranosyl
R_1 = R_2 = H
(10) R_1 = R_2 = Me; R = α-L-2,3,4-tri-*O*-methyl-arabinopyranosyl
(11) R = α-L-arabinopyranosyl
R_1 = H, R_2 = β-D-glucopyranosyl
(12) R = R_1 = H, R_2 = α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl

(4) R = α-L-2,3,4-tri-*O*-methyl-arabinopyranosyl
(5) R = H

homogenate of *Eulota maackii* resulted in hederagenin and several progenins [9]. Progenins (**8**), (**9**), (**11**), and (**12**) were obtained after a chromatographic separation of the mixture obtained from the enzymatic hydrolysis. Acid hydrolysis of each of the progenins (**8**), (**9**), (**11**) and (**12**) and subsequent analysis of the reaction mixture on TLC resulted in the identification of glucose and rhamnose (**12**), arabinose and glucose (**11**), L-arabinose (**9**), and D-glucose (**8**). Accordingly each of the progenins (**8**) and (**12**) had one carbohydrate chain linked to a carboxyl group. Progenin (**11**) had two carbohydrate chains.

The integral intensity (18H) of the acetyl group signal in the NMR spectrum of the acetate of progenin (**8**) showed it to be a monoglucoside at C-28 of hederagenin. The anomeric proton signal of glucose (δ 5·58 ppm, J 8 Hz) indicated a β-configuration of the glycosidic bond. The β-configuration of the D-glucopyranosyl residue was confirmed by the difference in molecular rotation of (**8**) and hederagenin: Δ $[M]_D$ [(**8**)-hederagenin] −144·15°, $[M]_D$ methyl-D-glucosides: β −62°; α +276°.

Progenin (**9**) is the 3-*O*-α-L-arabinopyranoside of hederagenin, since in the NMR spectrum of the corresponding acetate there was the anomeric proton signal of arabinose (δ 4·41 ppm, J 7 Hz), which indicated an α-configuration of the glycosidic bond. This was confirmed by the difference in molecular rotation of (**9**) and hederagenin: Δ [M] (9-hederagenin) −85·33°, $[M]_D$ methyl-L-arabinosides: α +28°; β +404° [10].

In the NMR spectrum of the acetate of progenin (**11**), the anomeric proton signals of D-glucose (δ 5·58 ppm, J 8 Hz) and arabinose (δ 4·41 ppm, J 7 Hz) indicated the β- and α-configurations respectively of the corresponding glycosidic bonds.

The integral intensity (33H) of the proton signal in the NMR spectrum of the acetate of progenin (**12**) showed it to be a triglycoside. The anomeric

proton signals of the two glucose molecules (δ 5·58 ppm, J 8 Hz and δ 4·60 ppm, J 7 Hz) indicated a β-configuration of the corresponding glycosidic bonds and a broad signal at δ 4·80 ppm, J 1 Hz, showed that rhamnose was linked by an α-glycosidic bond. An α-configuration of the L-rhamnopyranosyl residue in progenin (**12**) was confirmed by the difference in molecular rotation of cauloside D [2] and saponin D from *Akebia quinata* Decne [11]: Δ $[M]_D$ (cauloside D and saponin D) $-139°$, $[M]_D$ methyl-L-rhamnosides: α $-111°$, β $+170°$. Thus, the study of the progenins obtained by enzymatic hydrolysis allowed the determination of the configurations of all the glycosidic bonds in cauloside D, which is thus a novel glycoside of hederagenin with the structure of 3-*O*-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl (1→4) -β-D-glucopyranosyl (1→6) -β-D-glucopyranoside (**1**). The structures of the products obtained by enzymatic hydrolysis shows that the digestive juice of *E. maackii* contains enzymes that catalyze the hydrolysis of the β-1,6-glucosidic, acyl-*O*-β-glucosidic and α-arabinosidic bonds.

Acid hydrolysis of permethylated cauloside A resulted in methyl-23-*O*-methyl-hederagenin and 2,3,4-tri-*O*-methyl-L-arabinose identical by TLC with authentic samples. The NMR spectrum of the acetate of cauloside A showed an α-configuration for the glycosidic bond (δ 4·37 ppm, J 10 Hz). Hence, cauloside A is a 3-*O*-α-L-arabinopyranoside of hederagenin (**9**) and is identical to saponin A, isolated previously from *C. robustum* [1].

EXPERIMENTAL

All m.ps are uncorr. Column chromatography was on Si gel (150–200 mesh) with solvent systems: (1) C_6H_6–EtOAc; (2) EtOAc–MeOH; and (3) $CHCl_3$–EtOH. TLC was on Si gel or Silufol R with solvent systems: (4) toluene–EtOH (9:1); (5) $CHCl_3$–MeOH–H_2O (2:1:0, 21); (6) $CHCl_3$–MeOH (9:1); and (7) $CHCl_3$–MeOH (2:1). GLC used glass columns (1·5 × 6 mm) packed with 3% QF-1 on Chromosorb W (100–200 mesh), carier gas (Ar): 50 ml/min, temp: 120–240°.

Permethylation of (**1**). (**1**) (1·5 g) was methylated twice by the Purdie method [5]. The reaction mixture (1·45 g) was chromatographed on a Si gel column in system 1 (3:1 → 1:2). Fractions were analyzed by TLC with system 4. Yield: 0·49 g of **2**, mp 85–87° (hexane), IR: ν_{max} 1730 cm^{-1} (COOR), no OH.

Methanolysis of (**2**). (**2**) (0·07 g) was refluxed for 5 hr with 3 ml 42% $HClO_4$ in MeOH (1:5). The reaction mixture was diluted with H_2O, and the aglycone filtered, washed with H_2O, dried and methylated with CH_2N_2 in Et_2O to give (**3**) mp 190–194° (MeOH), $[\alpha]_D$ +70·4° ($CHCl_3$). (Found, C, 76·29; H, 10·62; OCH_3, 12·36. Calc. for $C_{32}H_{52}O_4$: C, 76·12; H, 10·60; OCH_3, 12·09%) The aq soln was neutralized with Dowex 1 × 2 (HCO_3^-), evaporated to a syrup, refluxed in abs. MeOH in the presence of Amberlite IR-120 (H^+), and acetylated with Ac_2O–C_5H_5N at room temp. GLC was run in the presence of synthetic standards: 2,3,4-tri-*O*-methyl-β-D-xyloside (T = 0) and 2,3,4,5,6-penta-*O*-acetyl-D-galactononitrile (T = 100). The methyl pyranosides were identified as 2,3,4-tri-*O*-methyl-rhamnose (T_β = 2, T_α = 7), 2,3,4-tri-*O*-methyl-arabinose (T_β = 11, T_α = 11·5), 2,3,4-tri-*O*-methyl-6-*O*-acetyl-glucose (T_β = 35, T_α = 42), and 2,3,6-tri-*O*-methyl-4-*O*-acetyl-glucose (T_β = 39, T_α = 44).

$LiAlH_4$ Reduction of (**2**). (**2**) (0·47 g) in THF (5 ml) and $LiAlH_4$ (0·25 g) were refluxed for 3 hr. The $LiAlH_4$ residue was decomposed with H_2O, neutralized to pH 7 by 2N H_2SO_4, evaporated and extracted, first with Et_2O and then with $CHCl_3$. The Et_2O extracts were washed with H_2O, evaporated to dryness and chromatographed in system (**1**) (6:1 → 2:1) to yield (**4**) mp 93·5°. The $CHCl_3$ extracts were washed with H_2O, evaporated to dryness and chromatographed in system 2 (50:1 → 20:1) to yield (**6**) (0·27 g) syrup, $[\alpha]_D$ $-32·3°$ ($CHCl_3$). NMR: δ 1·31 (3H, *s*, J 7·71 Hz, C′-6Me rhamnose) 4·31 (1H *d*, J 8 Hz, C′-1H glucose, 4·97 (1H, *s*, J 1·5 Hz, C′-1H rhamnose).

Methanolysis of (**4**). (**4**) (0·045 g) was hydrolyzed as described for (**2**). The residue was separated and chromatographed in system 1 (5:1 → 2:1) to yield (**5**) (0·025 g), mp 188–190° (MeOH), $[\alpha]_D$ +54·2° ($CHCl_3$). 2,3,4-Tri-*O*-methyl-L-arabinose, syrup, $[\alpha]_D$ +114° (H_2O) was identified in the aq. portion following neutralization with Dowex 1 × 2 (HCO_3^-) by TLC on Silufol in system 6.

Methanolysis of (**6**). (**6**) (0·05 g) was hydrolyzed as described for (**4**). 2,3,4-Tri-*O*-methyl-L-rhamnose, syrup, $[\alpha]_D$ +28·5° (H_2O); 2,3,6-tri-*O*-methyl-D-glucose, syrup, $[\alpha]_D$ +58·4° (MeOH); and 2,3,4-tri-*O*-methyl-D-sorbite, syrup, were obtained after chromatography in system 3 (100:1 → 100:15).

Enzymatic hydrolysis of (**1**). (**1**) (1·3 g) was dissolved in H_2O (200 ml) and incubated at 38° for 10 days with *E. maackii* digestive juice, periodically adding new portions of the juice. The reaction was checked by TLC in system (5). The hydrolyzate was extracted with *n*-BuOH, and the extracts were evaporated to dryness. After chromatography in system (3) (10:1 → 1:2), the following products were obtained: (**12**) (0·05 g), mp 198–205° (MeCOMe + MeOH), $[\alpha]_D$ 0° (MeOH); acetate of (**12**), amorphous substance, $[\alpha]_D$ 0° ($CHCl_3$); (**11**) (0·062 g), mp 210–212° $[\alpha]_D$ +34° (MeOH); acetate of (**11**), mp 118–120°, $[\alpha]_D$ +11·2° ($CHCl_3$); (**9**) (0·1 g), mp 226–228° (MeOH), $[\alpha]_D$ +46·8° (MeOH–$CHCl_3$); (**8**), mp 222–225°, $[\alpha]_D$ +35·3° ($CHCl_3$); acetate of (**8**), mp 110–114° (EtOH), $[\alpha]_D$ +32·1° ($CHCl_3$).

Permethylation of (**9**). (**9**) (0·27 g) was methylated by the Hakomori method [12]. The reaction product was extracted with $CHCl_3$, evaporated to a foamy state, treated with CH_2N_2 in Et_2O, and chromatographed system (**1**) (50:1 → 20:1) to give (**10**) (0·17 g). IR ($CHCl_3$): ν_{max} 1730 cm^{-1}, no OH.

Methanolysis of (**10**). (**10**) (0·049 g) was hydrolyzed as described for (**2**). The mixture was diluted with H_2O, and the aglycone filtered off to obtain (**3**), mp 190–192° (MeOH). The aq. residue was neutralized, evaporated to syrup and chromatographed on Silufol R in system 6 to identify 2,3,4-tri-*O*-methyl-L-arabinose, $[\alpha]_D$ +114° (H_2O).

REFERENCES

1. Murakami, T., Nagasava, M. and Uroyama, S. (1968) *Yakugaku Zasshi* **88**, 321.

2. Strigina, L. I., Chetyrina, N. S. and Elyakov, G. B. (1970) *Khim. Prir. Soedin.* 552.
3. Strigina, L. I., Chetyrina, N. S., Isakov, V. V., Dzizenko, A. K. and Elyakov, G. B. (1974) *Phytochemistry* **13,** 479.
4. Strigina, L. I., Chetyrina, N. S., Isakov, V. V., Dzizenko, A. K. and Elyakov, G. B. (1974) *Khim. Prir. Soedin.* 733.
5. Purdie, T. and Irvine, J. C. (1964) *J. Chem. Soc.* 1021.
6. Elkin, Yu. N., Kalinovsky, A. I., Rosynov, B. V., Vakorina, G. I., Shulga, N. I. and Dzizenko, A. K. (1974) *Khim. Prir. Soedin.* **4,** 451.
7. Mzhelskaya, L. G. and Abubakirov, N. K. (1968) *Khim. Prir. Soedin.* **3,** 153.
8. Karkainen, J. (1971) *Carbohyd. Res.* **17,** 11.
9. Strigina, L. I. (1972) *Tezisy doklada na V konferentsii po khimii i biokhimii uglevodov*, Nauka 139.
10. Mzhelskaya, L. G., Abubakirov, N. K. (1967) *Khim. Prir. Soedin.* **2,** 101.
11. Higuchi, P. and Kawasaki, T. (1972) *Chem. Pharm. Bull.* **20,** 2143.
12. Hakomori, S. (1965) *J. Biochem.* **55,** 205.