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A FUROSTANOL GLYCOSIDE FROM *HELLEBORUS MACRANTHUS*

RUDOLF TSCHESCHE, ROSEMARIE WAGNER and HEM CHANDRA JHA*

Institut für Organische Chemie und Biochemie der Universität Bonn, Gerhard-Domagk-Straße 1, D-5300 Bonn 1, F.R.G.; *Physiologisch-Chemisches Institut der Universität Bonn, Nußallee 11, D-5300 Bonn 1, F.R.G.

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Key Word Index—*Helleborus macranthus*; Ranunculaceae; macranthogenin; furostanol glycoside; macranthoside I; 25(27)-dehydro-5β-furostan-3β,22,26-triol-3-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside, 26-*O*-β-D-glucopyranoside.

Abstract—From the roots and rhizomes of *Helleborus macranthus* a new glycoside, macranthoside I, has been isolated and shown to have the structure 25(27)-dehydro-5β-furostan-3β,22,26-triol-3-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside, 26-*O*-β-D-glucopyranoside.

INTRODUCTION

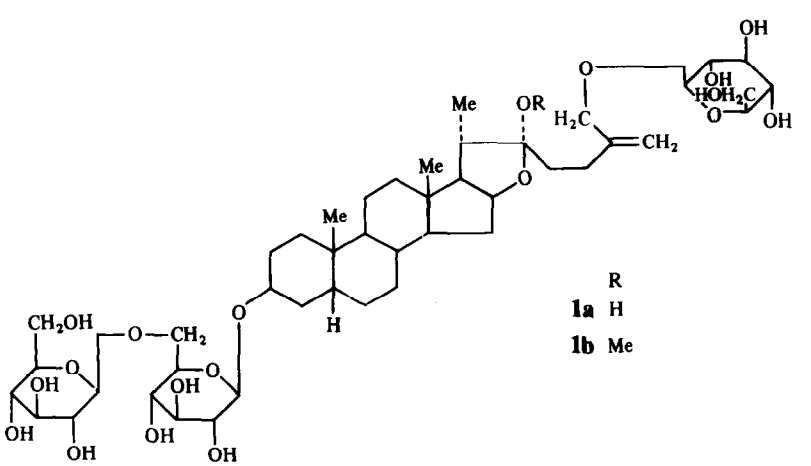
The plants of the *Helleborus* genus are known for their cardiac glycoside contents [1]. In 1969, a mixture of furostanol glycosides was isolated from *Helleborus macranthus* Freyn, a plant growing in the Slovenian and Kroatian hilly areas of Yugoslavia [2]. Acid hydrolysis afforded macranthogenin, a sapogenin with the structure 25(27)-dehydro-5β-spirostan-3-β-ol [3]. This paper describes the isolation, separation and elucidation of the structure of the main saponin, macranthoside I (**1a**).

RESULTS AND DISCUSSION

The main saponin, macranthoside I (**1a**) was isolated from the crude extract by repeated column chromatography on silica gel and sephadex. With methanol-containing solvents, macranthoside (**1**) showed two spots; each spot yielding again two fresh spots in two-dimensional TLC which indicated the presence of a mixture of 22-hydroxy (**1a**)- and 22-methoxy (**1b**)-furostanol glycosides [4]. The furostanol character was further indicated by the low hemolytic activity and IR spectrum (broad absorption at 900 cm^{-1}) [4] of the glycoside.

Partial hydrolysis of macranthoside I with β-glucosidase yielded D-glucose and a mixture of two partial glycosides with increased hemolytic activity. Each glycoside gave one spot on TLC and had a characteristic IR-absorption of spirostanol glycosides [5].

Hydrolysis of macranthoside I (**1a** plus **1b**) with 2N HCl afforded D-glucose and macranthogenin. For MW determination the glycoside was methylated by Hakomori's method [6] and the product was examined by mass spectrometry. The highest identifiable peak with *m/z* 1054 may be assigned to the ion produced by loss of water or methanol from the molecular ion $[M]^+$. This molecular ion $[M]^+$ contained three moles of methylated glucose. Loss of terminal methyl glycosyl units gave the ions at *m/z* 835 and *m/z* 615 and the corresponding tetramethyl glycosyl ion at *m/z* 219 and an ion produced by loss of methanol at *m/z* 187.



Permethylated macranthoside I was subjected to methanolysis. The products, two methylated sugars, were investigated by mass spectrometry [7] and GC and identified as methyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl-2,3,4-tri-*O*-methyl-D-glucopyranoside.

To further establish the structure of macranthoside I it was hydrogenated with hydrogen–Adam's catalyst in methanolic solution [8]. Hydrolysis of the product with HCl yielded tetrahydromacranthogenin which was identical with the hydrogenation product of the authentic macranthogenin [9] (mmp, TLC).

Based on the above data, it was concluded that macranthoside I (**1a**/**1b**) has one molecule of glucopyranose at the 26-position and a disaccharide consisting of two glucose units at the 3-position; thus the structure is 25(27)-dehydro-5β-furostan-3β,22,26-triol-3-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside, 26-*O*-β-D-glucopyranoside.

EXPERIMENTAL

Mps are uncorr. GC was performed on a FID chromatograph; column: OV 101 (0.3 mm × 25 m); N_2; temp.: programmed from 100° to 250° at 1.5° min. Column chromatography was performed on silica gel (Kraemer & Martin) and Sephadex G25 (Pharmacia Fine Chemicals AB, Uppsala), PC on paper type 2043a, Selecta (Schleicher & Schüll) and TLC on TLC-plates, silica gel $F_{254+366}$, 2 mm (Merck). On TLC the glycosides were detected by 40% H_2SO_4 and subsequent charring; sugars were located on PC by Partridge's reagent [10] (aniline phthalate). The following solvent systems were employed for chromatography: A, $CHCl_3$–MeOH–H_2O (65:38:10); B, $CHCl_3$–MeOH–H_2O (65:35:10) [11]; C, $CHCl_3$–MeOH–H_2O (65:25:10); D, CH_2Cl_2–MeOH (10:0.15); E, cyclohexane–Me_2CO (1.3:1); F, cyclohexane–Me_2CO (1.3:1.1); G, C_6H_6–MeOH (7:1); H, C_6H_6–MeOH (5:1); I, petrol–Me_2CO (3:1); K, petrol–Me_2CO (2:1); L, EtOAc–*n*-BuOH–H_2O (3.6:1:1.5) [12].

Isolation of macranthoside I. Powdered roots and rhizomes of *Helleborus macranthus* (760 g) were extracted with MeOH (80%). After removal of MeOH the aq. residue was treated with *n*-BuOH. From the *n*-BuOH extract 164 g of crude brown material was obtained. Repeated CC on silica gel (solvent systems A, B) and Sephadex G25 (solvent system H_2O–MeOH, 4:1) afforded 3.1 g macranthoside I as a colourless amorphous solid; R_f: two spots at 0.27 and 0.20, system B; IR ν_{max}^{KBr} cm^{-1}: 1640, 900, 875. The glycoside had no hemolytic activity.

Enzymatic hydrolysis of macranthoside I. To a soln of 460 mg macranthoside I in 60 ml H_2O was added 20 mg β-glucosidase (Röhm & Haas). The mixture was incubated at 39° for 24 hr. After adding MeOH the soln was filtered; MeOH was evaporated and the aq. residue extracted with *n*-BuOH. Prep. TLC on silica gel plates (solvent system C) afforded two partial glycosides from the *n*-BuOH extract; PG 1 (30 mg) colourless needles from MeOH, mp 228–231°; IR ν_{max}^{KBr} cm^{-1}: 1645, 912, 890, 875, 843; R_f: 0.70 (solvent system B) and PG 2 (10 mg) colourless needles from MeOH, mp 163–166°, IR ν_{max}^{KBr} cm^{-1}: 1645, 913, 893, 875, 846, R_f: 0.51 (solvent system B). PG 1 and PG 2 possessed high hemolytic activity.

Methanolysis of macranthoside I. Macranthoside I (100 mg) was refluxed with 5% methanolic HCl soln for 5 hr. Adding H_2O, evaporation of MeOH and extraction of the aq. phase with $CHCl_3$ yielded crude macranthogenin which was purified on TLC (silica gel, solvent system D). Colourless needles from Me_2CO, mp 177–178°, no depression by admixture with authentic material, kindly provided by Prof. Dr. J. Petričič; IR ν_{max}^{KBr} cm^{-1}: 1643, 1443, 1371, 912, 872, 841, MS *m/z*: 414 $[M]^+$ (3.9%), 137 (base peak); R_f: 0.46 (solvent system I). The aq. phase was refluxed with 2N HCl, neutralized (Dowex 3) and subjected to PC (solvent system L); the presence of D-glucose was revealed.

Methylation and identification of methylated sugars. Macranthoside I (960 mg) was methylated by Hakomori's method [6]. The crude permethylated product was purified by CC (silica gel, solvent system D) to yield 570 mg of pure product. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH absorption, MS *m/z*: 1054 (2.5%), 835, 615, 219, 187 (base peak). Permethylated macranthoside I was methanolysed with 5% methanolic HCl soln for 5 hr. The mixture of glucopyranosides was analysed by GC; the proportion of methyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside to methyl-2,3,4-tri-*O*-methyl-D-glucopyranoside was 1.88:1 (theoretical 2:1). After separation on TLC (silica gel, solvent system E) the methylated glucopyranosides were identified by MS, GC and TLC comparison with authentic samples. Methyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside: MS *m/z*: 149, 101, 88 (base peak), 75, 73, 71; GC: RR_t 1.00; R_f 0.61 (system F), 0.64 (system H); methyl-2,3,4-tri-*O*-methyl-D-glucopyranoside: MS *m/z*: 176, 173, 101, 88 (base peak), 75, 73, 71; GC: RR_t 1.05; R_f 0.31 (system E), 0.24 (system G).

Preparation of tetrahydromacranthogenin. A soln of 50 mg macranthogenin in 50 ml HOAc was hydrogenated at room temp. for 60 hr in the presence of PtO_2. Filtration, evaporation and purification of the crude product on a silica gel column (solvent system K) yielded 28 mg pure tetrahydromacranthogenin. (Colourless crystals from Me_2CO; mp 167–168°, $[\alpha]_D^{20}$ = +123° (*c* 1, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 1448, 1375, 1033, MS *m/z*: 418 $[M]^+$, 273 (base peak), R_f 0.23 (system I).

Reduction of macranthoside I. Macranthoside I (400 mg) and 250 mg PtO_2 in MeOH were hydrogenated at room temp. for 60 hr [8]. After filtration from the catalyst the soln was evaporated to dryness and the residue hydrolysed with 5% methanolic HCl soln. Separation of the crude genin by TLC (silica gel, solvent system K) yielded 18 mg tetrahydromacranthogenin which was identical with authentic material (mmp, IR, TLC). Macranthogenin was also identified in the mixture.

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