A DITERPENE GLYOSIDE – DORONICOSIDE D – FROM *Doronicum macrophyllum*

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In a methanolic extract of the roots of Doronicum macrophyllum Fisch. (family Compositae) six substances of glycosidic nature have been detected chromatographically and have been named in order of increasing polarity doronicosides A, B, C, D, E, and F.

Acid hydrolysis of the purified total glycosides showed that they contained one and the same aglycone and differed only by the qualitative and the quantitative composition of the carbohydrate moiety.

By chromatography on silica gel, doronicoside D was isolated in the individual state with the composition $C_{42}H_{66}O_{21}$ (I). It follows from an analysis of its IR spectrum that the glycoside contains a $>C=CH_2$ (3080, 1645, 900 cm^{-1}) and a free carboxy group (1745 cm^{-1}). The presence of the latter was also confirmed by the preparation of a methyl ester (II) of the glycoside (I) by the action of diazomethane on it. Analysis of the trimethylsilyl ethers of the sugars of dorinicoside D showed that it contained one molecule each of L-arabinose and D-xylose and also two molecules of D-mannose. A hydrolyzate of the permethylate of doronicoside D was found to contain tetramethyl and trimethyl derivatives of D-mannose and dimethyl derivatives of D-xylose and L-arabinose. It follows from this that doronicoside D has a straight unbranched sugar chain and the terminal sugar is D-mannose.

The acid hydrolysis of doronicoside D yielded a product with the composition $C_{20}H_{30}O_3$ which we originally called doronigenin (IV). The composition of doronigenin itself permitted the assumption that it was a diterpene. The substance contained a free carboxy group (broad band at 2600-3300 cm^{-1} and band at 1690 cm^{-1}) and a ketonic carbonyl group (1730 cm^{-1}), which was confirmed by the preparation of the methyl ester (V) and the oxime (VI). In the PMR spectrum of compound (IV), two singlets of δ 1.00 and 1.24 ppm (HMDS) showed the presence of two tertiary C-methyl groups, and a doublet at 1.14 ppm indicated the presence of a second C-methyl group.

At the same time, as the IR and NMR spectra showed, compound (IV) lacked the $>C=CH_2$ grouping present in the initial glycoside (I) and a hydroxy group. The disappearance of the hydroxyl and the exomethylene group and the appearance in exchange of a ketonic carbonyl and a $>CH-CH_3$ grouping show that when the glycoside was hydrolyzed, in addition to the splitting off of the carbohydrate chain the structure of the aglycone also changed.

In an analysis of literature information it was observed that such rearrangements are characteristic for diterpenoids of the kaurene series. Thus, for example, in the agylcone of the diterpene glycoside stevioside on acid hydrolysis the hydroxy and exomethylene groups are converted into ketone and methyl groups, respectively [1].

A detailed study of the properties of known kaurene compounds permitted the identification of the doronigenin (IV) that we had isolated as 15-oxo(–)-19-oic acid [2]. The properties of the methyl esters of the two compounds also coincided.

By reducing doronigenin (IV) with sodium tetrahydroborate we obtained a hydroxy acid (VII) which was characterized in the form of its acetate (VIII) and the methyl ester of the acetate (IX). The properties of this hydroxy acid coincided with those of 15β-hydroxy-(–)kauran-19-oic acid (VII) obtained by the acid treatment of xylopic acid (III, R_2 = Ac) isolated previously from the fruit of Xylopia aethiopica [3].

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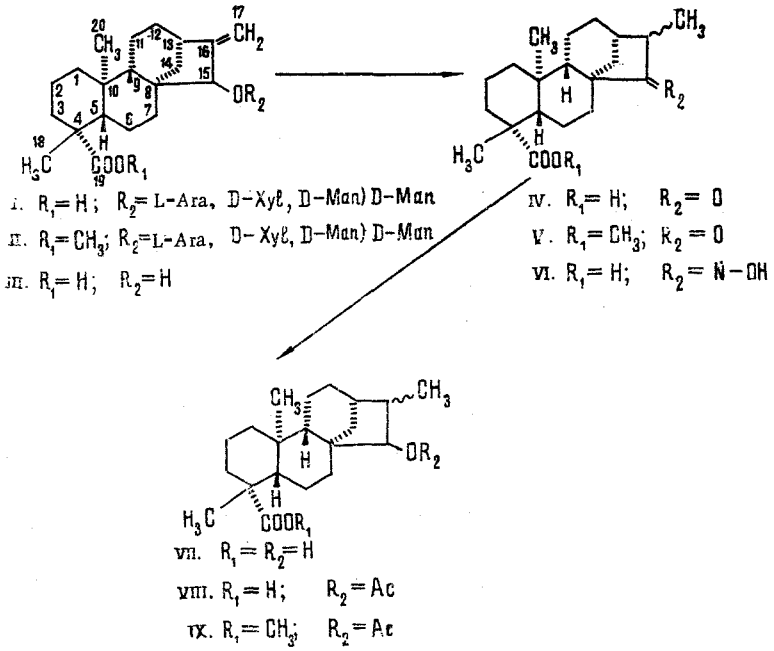

It follows from the facts given that the native aglycone of doronicoside D is the deacetyl derivative of xylopic acid, namely: 15β-hydroxy-(−)-kaur-16-en-19-oic acid (III). It has been isolated in the free form previously [2]. In the hydrolytic cleavage of the glycoside, the acid (III) is converted into 15-oxo-(−)-kauran-19-oic acid (IV). Doronicosides A, B, C, E, and F contain the same aglycone.

EXPERIMENTAL

For chromatography we used paper of types FN-7 and FN-14, silica gel of type KSK, and alumina (activity grade II) and the following solvent systems: 1) chloroform–methanol–water (65 : 35 : 8); 2) chloroform–ethanol–water (65 : 35 : 8); 3) benzene–acetone (2 : 1); 4) chloroform–methanol (9 : 1); 5) butan-1-ol–pyridine–water (6 : 4 : 3); 6) butan-1-ol–methanol–water (5 : 3 : 1); and 7) chloroform–methanol (a, 25 : 1; b, 50 : 1). The glycosides and aglycones were revealed on the chromatograms with a 25% solution of tungstophosphoric acid. The sugars and their derivatives were revealed with o-toluidine salicylate and aniline phthalate.

For analysis, the substances were dried in vacuum over calcium chloride at the boiling of toluene or benzene for 8 h. The infrared spectra were determined in KBr on a UR-10 spectrophotometer. The NMR spectra were recorded on a JNM-4H-100 instrument (100 MHz, HMDS, δ, ppm), and the mass spectra on an MKh-1303 instrument fitted with a system for the introduction of the sample directly into the ion source at an energy of the ionizing electrons of 40 eV and a temperature of 120°C.

The GLC of the silylated methyl glycosides was performed on a UKh-1 chromatograph using a copper column (1 × 4 mm) containing 5% of G-30-M silicone phase on Diaforit (0.2–0.315 mm); column temperature 176°C; carrier gas hydrogen at the rate of 55 ml/min.

Extraction of the Glycosides. The air-dry comminuted roots of Doronicum macrophyllum (1 kg), collected in the stage of the withering of the epigeal part in the Shakhbuz region of the Nakhichevan ASSR were extracted with hot methanol. The methanolic extract was evaporated to dryness in vacuum, the residue was dissolved in water, the solution was washed with chloroform, and the glycosides were extracted with ethyl acetate and with n-butanol.

According th TLC on silica gel in systems 1 and 2, the isolated total glycosides contained six substances of glycosidic nature (doronicosides A, B, C, D, E, and F).

Acid Hydrolysis of the Total Glycosides. The total glycosides (10 g) were dissolved in 100 ml of 5% aqueous methanolic (1 : 1) sulfuric acid and were hydrolyzed with heating for 8 h. White tubular crystals of doronigenin (IV) deposited with the composition $C_{20}H_{30}O_3$, M^+ 318, mp 223–225°C (from aqueous methanol), $[\alpha]_D^{20}$ – 108.4°(c 1.014; chloroform). IR spectrum: 2600–3300 cm^{-1} (OH of a carboxyl), 1730 cm^{-1} (C=O of a ketone), and 1690 cm^{-1} (C = O of an acid).

NMR spectrum ($CDCl_3$): s 1.24 and 1.00 ppm (3 H at C-18 and 3 H at C-20), and d 1.14 ppm (3 H at C-17, J = 7 Hz). According to the literature [2]: mp 227-231°C (from aqueous ethanol), $[\alpha]_D^{20}$ −126°. IR spectrum: 1735, 1690 cm^{-1}; NMR spectrum: 1.24 and 1.02 ppm.

Isolation of Doronicoside D (I). The total glycosides (6 g) were chromatographed on a column of silica gel in system 2. The individual glycoside doronicoside D (I) was isolated with mp 116-118°C (from aqueous methanol), $[\alpha]_D^{24}$ −36.3 ± 2° (c 1.129; methanol). Treatment with diazomethane yielded the methyl ester (II).

Acid Hydrolysis of Doronicoside D (I). Doronicoside D (100 mg) was dissolved in 60 ml of aqueous methanol (1 : 2) and was hydrolyzed with 5% sulfuric acid for 8 h. The white crystals that deposited were separated off and were recrystallized from aqueous methanol. The compound obtained was identical with the substance isolated from the total glycosides (TLC on silica gel in system 7; a mixture of the two substances gave no depression of the melting point).

In a hydrolyzate of doronicoside D that had been neutralized with barium carbonate and purified, TLC on silica gel impregnated with a 0.3 M solution of sodium dihydrogen phosphate, in system 6, and PC, in system 5, showed the presence of D-mannose, L-arabinose, and D-xylose. The GLC of the trimethylsilyl derivatives showed that these sugars were present in a ratio of 2 : 1 : 1.

Methylation of Doronicoside D (I). Doronicoside D (50 mg) was methylated by Hakomori's method [4]. The permethylate obtained was heated in 5 ml of 5% aqueous methanolic (1 : 1) sulfuric acid for 5 h. Then 3 ml of water was added to the reaction mixture, the methanol was distilled off, and it was heated for another 5 h. After this, it was diluted with water, the aglycone that deposited was separated off, and the solution was neutralized with barium carbonate and filtered, and the filtrate was evaporated in vacuum.

TLC on "Silufol" plates in systems 3 and 4 with markers showed the presence of 2,3,4,6-tetra-O-methyl-D-mannose, dimethyl derivatives of D-xylose and L-arabinose, and a trimethyl derivative of D-mannose.

Methyl Ester of Doronigenin (V). To 40 mg of compound (IV) was added 3 ml of an ethereal solution of diazomethane. The methyl ester was recrystallized from aqueous ethanol (1 : 1); composition $C_{21}H_{32}O_3$, mp 120-121°C; $[\alpha]_D^{20}$ −136.5 ± 2° (c 1.245; chloroform). IR spectrum: 1720 cm^{-1} (C=O) of carboxy and ketone groups), and 1240 cm^{-1} (C−O−C). NMR spectrum ($CDCl_3$), ppm: s 1.2 (3 H at C-18); s 0.91 (3 H at C-20); d 1.12 (3 H at C-17, J = 7 Hz); and s 3.64 (3H, $-C(=O)-OCH_3$). According to the literature [2]: mp 119.5-120.5°C; $[\alpha]_D^{20}$ −138°.

Oxime of Doronigenin (VI). A solution of 40 mg of compound (IV) in 0.5 ml of dry pyridine was treated with 4 mg of hydroxylamine hydrochloride. The mixture was heated at 100°C for 20 h and was then cooled and poured onto ice. The precipitate that deposited was filtered off and washed with water; composition $C_{20}H_{31}O_3N$, mp 230-232°C. IR spectrum, cm^{-1}: 1690 (C=O of an acid); 1730 (C=O of a ketone), 3150-3300 (OH), 1600 (C=N−).

Reduction of Doronigenin (IV) with Sodium Tetrahydroborate. In small portions, 400 mg of sodium tetrahydroborate was added to a solution of 200 mg of compound (IV) in 40 ml of aqueous methanol (1 : 2). The reaction mixture was left as room temperature for 48 h. Finally, the mixture was heated at 100°C for 30 min. Then it was cooled and was neutralized with dilute sulfuric acid. The precipitate that deposited was again reduced with sodium tetrahydroborate. This gave 15β-hydroxykauran-19-oic acid (VII), composition $C_{29}H_{32}O_3$, M^+ 320, mp 235-237°C; $[\alpha]_D^{20}$ −66.9 ± 2° (c 0.89 methanol). IR spectrum, cm^{-1}: 1690 (C=O of a carboxyl); 3410 (OH). According to the literature [3]: mp 236-239°C.

The Acetate (VIII) of the Acid (VII). A solution of 40 mg of substance (VII) from the preceding experiment in 3 ml of dry pyridine was treated with 3 ml of acetic anhydride. The mixture was left at room temperature for two days and was then poured onto ice. The precipitate that deposited was filtered off and was crystallized from methanol. The acetate (VIII) had the composition $C_{22}H_{34}O_4$; M^+ 362, mp 217-220°C, $[\alpha]_D^{20}$ −90.2 ± 2° (c 1.0; chloroform). IR spectrum, cm^{-1}: 1730 (C=O of an acetate), 1690 (C=O of a carboxyl), 3300 (OH of a carboxyl).

Methyl Ester (IX) of the Acetate (VIII). The acetate from the preceding experiment (30 mg) was covered with a solution of dizaomethane in ether and the mixture was left at room temperature for three days. This gave a yellow oily compound with the composition $C_{23}H_{36}O_4$, M^+ 376, $[\alpha]_D^{20}$ −74.7 ± 2° (c 5.49; chloroform).

SUMMARY

Six new diterpene glycosides – doronicosides A, B, C, D, E, and F – have been detected in the roots of Doronicum macrophyllum Fisch. The qualitative and quantitative composition of the sugar chain of doronicoside D has been established. It has been shown that the negative aglycone of all the doronicosides is 15β-hydroxy-(–)-kaur-16-en-19-oic acid, which, in the process of hydrolysis, is converted into 15-oxo-(–)-kauran-19-oic acid.

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PRODUCTION OF PHENOLIC ALKALOIDS OF THE ISOCHONDODENDRINE SERIES

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We have previously reported the demethylation of cycleanine with hydrobromic acid [1]. Continuing work in this direction we have selected pyridine hydrochloride as a demethylating reagent; it has been used successfully by a number of workers in the production of phenol derivatives [2-5]. By keeping the optimum reaction temperature and time but varying the ratio of alkaloid and demethylating reagent we have demethylated one, two, or four methoxy groups. For monodemethylation about three molecules of pyridine hydrochloride per molecule of alkaloid must be used since two molecules of the reagent are consumed in forming the cycleanine salt. With a twofold excess of pyridine hydrochloride (ratio 4:1) norcycleanine and isochondodendrine are formed. A further increase in the excess of pyridine hydrochloride leads to the appearance in the reaction mixture of compounds with three and four hydroxy groups, while at a ratio of 7.2:1 the last-mentioned compound predominates (Table 1).

Analysis of the PMR spectra of the substance obtained showed that the formation of mono- and diphenolic reaction products is due to the splitting out of the methyl groups at C-7 and C-7'. Their PMR spectra lack the signals of the protons of,respectively, one and two methoxy groups in the strong field screened by the aromatic nucleus of the benzyl group. At the same time, no compounds isomeric with isochondodendrine and norcycleanine with hydroxy groups at C-6 and C-6' were detected. Tri-O-demethylcycleanine (IV) was not isolated in the pure form, but its presence in the minor components of the reaction products was detected mass spectrometrically.

Tetra-O-demethylcycleanine (V), like cycleanine (I) and isochondodendrine (III), has an axis of symmetry. Because of this all the fragments of its formula coincide on rotation through 180°, and the corresponding signals of the protons of the two benzylisoquinoline halves are superposed in the PMR spectra (solvent C_5D_5N, internal standard TMS). In norcycleanine (II) there are three methoxy groups and a hydroxy group, and these signals are not superposed. In the PMR spectrum of (V) the following signals are observed: a six-proton singlet at 2.47 ppm of two methylimino groups, a broadened doublet of the two protons at C-1 and C-1' (J = 10 Hz), a two-proton singlet at 6.92 ppm (C-5 and C-5'), and the signals of the protons of methoxy groups are absent. Since the macrocycle acquires the boat-shaped configuration, the protons at C-10 and C-11 (and at C-10' and C-11', respectively) undergo screening and because of this their signals are present in a stronger field than the signals of the protons at C-13 and C-14 (and C-13' and C-14', respectively): 6.53 ppm (q, J_1 = 8 Hz, J_2 = 2.5 Hz, 2 H, C-10 and C-10'), 6.33 ppm (q J_1 = 8 Hz, J_2 = 2.5 Hz, 2 H, C-11 and C-11'), and 6.75 ppm (q, J_1 = 8 Hz, J_2 = 2.5 Hz, 2 H, C-13 and C-13'). The signal of the protons at C-14 and C-14' is masked by the corresponding signal of the pyridine protons and is therefore not observed in the spectrum.

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