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EVONOGENIN

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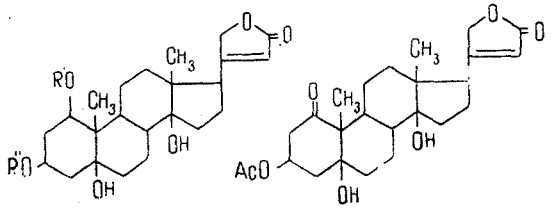
Our isolation from the seeds of Evonymus europaea auct. (central Russian race of European euonymus) of the cardiac glycosides evomonoside and evonoloside has been reported previously [1, 2].

Continuing an investigation of the cardenolide composition of this plant, we have isolated yet another substance. After its structure had been established, it was named evonogenin. This cardenolide was obtained from native seeds of euonymus and from seeds subjected to the action of an enzyme preparation from the fungus Aspergillus oryzae [3]. The yield of evonogenin was considerably higher in the second experiment. In an attempt to hydrolyze euonymus glycosides with the enzymes of the seeds, no increase in the amount of aglycone was observed. Accordingly, it may be assumed that this cardenolide is present in the plant not only in the free state but also in the form of glycosides.

Evonogenin gives positive reactions for cardenolides and, as established by M. A. Angarskaya and Zh. A. Lyubetskaya, has a cardiotonic activity approximately equal to that of periplogenin. The empirical formula of the substance is $C_{23}H_{34}O_6$. The UV spectrum has only one absorption maximum, at 218 mμ (log ε 4.16), due to the presence of a butenolide ring. The IR spectrum likewise contains bands characteristic for a butenolide ring. In the spectrum obtained from a solution of the compound in methylene chloride, in the region of the stretching vibrations of hydroxyl groups there is a maximum at 3606 cm^{-1} (ε = 146 $mole^{-1} \cdot l \cdot cm^{-1}$) the intensity of which shows the presence of three free hydroxyl groups (the intensity of one free OH group averages 50 $mol^{-1} \cdot l \cdot cm^{-1}$). A second maximum appears at 3503 cm^{-1} (ε = = 104 $mole^{-1} \cdot l \cdot cm^{-1}$), which relates to the absorption of a fourth hydroxyl group bound by an intramolecular hydrogen bond [4]. The cardiotonic activity of evonogenin shows the β-configuration of the butenolide ring and the presence of 3β,14β-hydroxyl groups.

Under the action of acetic anhydride in pyridine, evonogenin gives mono- and di-O-acetyl derivatives. The formation of the diacetate (III) shows the presence in the substance of two secondary OH groups; one of them is obviously $C_{(3)}$. A primary alcoholic group is excluded, since it would readily acetylate and impart a considerable polarity to the substance, while evonogenin is an aglycone of relatively low polarity and acetylates with difficulty. A small amount of the initial glycone is found in the reaction mixture even after acetylation for 72 hr. By evaluating the rate of acetylation in accordance with a published method [5] it may be concluded that the two secondary OH groups are axial, while, in association with the cardiotonic activity, the presence of a hydroxyl at $C_{(3)}$ shows the cis linkage of the A/B rings. Such protracted acetylation of two OH groups simultaneously has also been found for acovenosigenin [6-8], in which there is a hydroxyl in the 1β position apparently connected by an intramolecular hydrogen bond with the OH group at $C_{(3)}$.



I-III IV

I) R' = R" = H. Evonogenin. II) R' = H; R" = Ac. 3-O-acetylevonogenin. III) R' = R" = Ac. 1,3-di-O-acetylevonogenin. IV) 3-O-Acetyl-1-oxo-evonogenin.

In order to confirm the position of the 1β-OH group, we oxidized evonogenin monoacetate (II). The IR spectrum of the resulting substance, IV, exhibited, in addition to the usual absorption connected with the butenolide ring (218 mμ, log ε 4.17) a shoulder at about 296 mμ (log ε ~2.12). The latter shows the presence of a carbonyl group in the oxidation product. The optical rotatory dispersion spectrum of substance IV is characterized by a curve with a negative Cotton effect, which is typical for 1-oxo-5β-steroids [9]. The IR spectrum of this compound confirms the presence of a carbonyl group in it (shoulder at 1714 cm^{-1}) and also has an unsymmetrical absorption band in the region of the stretching vibrations of hydroxyl groups at 3623 cm^{-1} (ε = 112 $mole^{-1} \cdot l \cdot cm^{-1}$).

The intensity of the OH band shows that substance IV has two tertiary (nonacetylatable) hydroxyl groups. One of the them is at $C_{(14)}$ and the second, in our opinion, at $C_{(5)}$. The position of the two unknown OH groups at $C_{(1)}$ and $C_{(5)}$ agrees well with the molecular rotation found for evonogenin (M_D +54.8 ± 8°) and that calculated for structure I (M_D +53.3 ± ± 16°). The comparatively low polarity of evonogenin, approximately equal to that of acovenosigenin, is possibly due to

the intramolecular interaction of the three OH groups in ring A, with the formation of hydrogen bonds. Thus, evonogenin can be characterized as 1β, 3β, 5β, 14β-tetrahydroxycard-20(22)-enolide.

Experimental

Isolation of evonogenin. The cardenolides were extracted from the comminuted and defatted seeds with 70% ethanol, and the extracts were concentrated in vacuum to aqueous residues. The evonogenin, evomonoside, and part of the evonoloside were extracted with chloroform. The chloroform extract was distilled and the residue was chromatographed on alumina (activity grade III). The evonogenin were combined and evaporated. The residue contained impurities of noncardenolide nature as well as the aglycone. Consequently the substance was additionally purified by column partition chromatography on cellulose (ratio of substance to be purified to cellulose 1:300) in the benzene/formamide solvent system. The benzene eluates containing the evonogenin were distilled and the residue was dissolved in chloroform. The chloroform solution was treated with water to eliminate the formamide, dried with anhydrous sodium sulfate, and evaporated. The evonogenin was crystallized from methanol.

Evonogenin (I). The cardenolide melts at 273–276° C, $[\alpha]^{22}$ +13.5 ± 2° (c 0.78; chloroform); it dissolves in 84% H_2SO_4 forming a yellow coloration changing after 1 min to brown and after 3 hr 15 min to blue.

Found, %: C 67.92; H 8.83; mol. wt. 402. Calculated for $C_{23}H_{34}O_6$, %: C 68.01; H 8.43; mol. wt. 406.5.

The IR spectrum of the substance in the crystalline state contained bands at (cm^{-1}): 3480 (OH group); 3040 (CH at the double bond of a butenolide ring); 2950 and 2880 (CH, CH_2, and CH_3 groups of a steroid system); 1800, 1780, and 1738 (C=O of a butenolide ring); and 1637 (C=C of a butenolide ring).

Evonogenin mono- and diacetates (II and III). A solution of 35 mg of evonogenin in 1 ml of absolute pyridine was treated with 0.5 ml of acetic anhydride and left for 7 days. Then it was evaporated to dryness in vacuum. Paper chromatography showed the presence in the residue of two cardenolides in approximately equal amounts. The mixture of substances was chromatographed on 1.5 g of alumina (activity grade III). The diacetate (III) was eluted with benzene–chloroform (4:1) and crystallized from acetone, giving 18 mg of substance III melting at 216–219° C, $[\alpha]_D^{19}$ −46 ± 7° (c 0.15; chloroform), mol. wt. 496.

The monoacetate (II) was eluted with benzene–chloroform (2:1) and crystallized from acetone, giving 14 mg of crystals with mp 228–232° C, $[\alpha]_D^{19}$ +44.5 ± 6° (c 0.695; chloroform), mol. wt. 442.

3-O-Acetyl-1-oxoevonogenin (IV). A solution of 13 mg of 3-O-acetylevonogenin (II) in 0.15 ml of glacial acetic acid was treated with 0.7 ml of an acetic acid solution of chromic anhydride and left for 25 min. Then 0.5 ml of methanol was added to the solution and it was left for another 1 hr. After this, it was diluted with 20 ml of chloroform and was treated with 2 N H_2SO_4 (3 × 0.15 ml), water (3 × 0.2 ml), saturated $NaHCO_3$ solution (0.3 ml), and water again (4 × 0.2 ml). The purified chloroform solution was dried with anhydrous sodium sulfate and evaporated. Paper chromatography showed that the residue contained two cardenolides: 3-O-acetyl-1-oxoevonogenin (IV) and a small amount of the initial cardenolide (II). The mixture of substance was chromatographed on 0.5 g of alumina (activity grade III). The 3-O-acetyl-1-oxoevonogenin (IV) was eluted with benzene–chloroform (95:5) and crystallized from acetone. Substance IV, melting at 218–220° C (11 mg) was obtained. With concentrated H_2SO_4 it gives a coloration changing with time, min: 1) yellow; 20) orange; 60) brown; and 180) green. The optical rotatory dispersion spectrum of substance IV (methanol; c 0.11, 27° C; wavelengths in mμ): 589 (−26.3°); 500 (−38.0°); 400 (−57.8°); 325 (−71.0°); 315 (−60.0°).

The IR spectra of the substances were recorded on a UR-10 spectrophotometer (potassium bromide tablets, 2 mg of substance in 350 mg of KBr, and in solutions in methylene chloride, c 0.005 M, l 0.5 cm). The UV absorption spectra were recorded on a EPS-3 spectrophotometer with substances in ethanolic solution. The optical rotatory dispersion spectra were obtained on a SPU spectropolarimeter.

Conclusions

The structure of a new cardiac aglycone, evonogenin, isolated from the seeds of Evonymus europaea auct. has been established. Evonogenin is 1β, 3β, 5β, 14β-tetrahydroxycard-20(22)-enolide.

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