STUDY OF THE STRUCTURE OF AN A-SECO COMPOUND PRODUCED BY THE OXIDATION OF URSOLIC ACID

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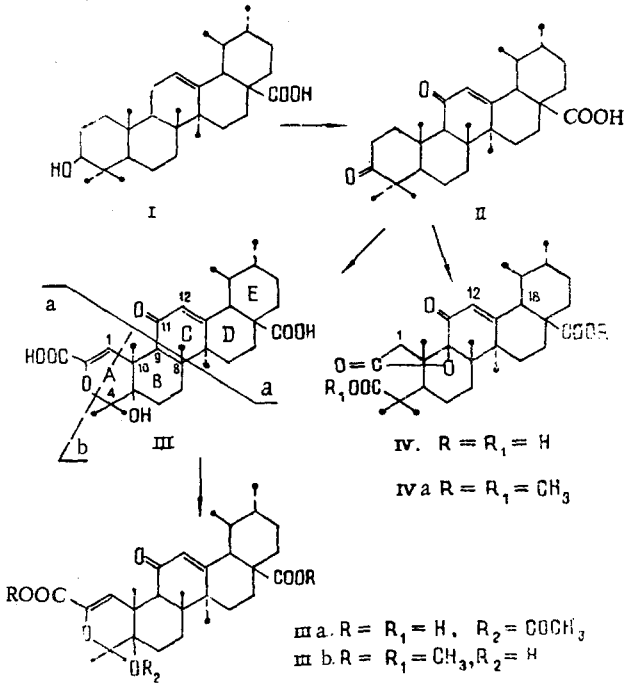
When ursolic acid was oxidized with potassium dichromate in the presence of sulfuric acid in glacial acetic acid, two new compounds were obtained. On the basis of the results of instrumental methods of analysis, structures have been suggested which correspond to 2-carboxy-5-hydroxy-11-oxo-3-oxaursa-1,12-dien-28-oic acid and 9-hydroxy-11-oxo-2,3-secours-12-en-2,3,28-trioic acid 2,9-lactone.

We have previously reported that ursolic acid (I) is oxidized by potassium dichromate in the presence of 20% sulfuric acid is glacial acetic acid at 40°C to 11-oxoursolic acid (II) [1, 2]. In the present work it has been shown that when (I) is oxidized under more severe conditions (70°C), in addition to (II) two new compounds are formed (III) and (IV), a determination of the structure of which is described below.



Compound (III) was obtained in the form of white microcrystalline powder with a molecular weight of 514 (the isotopic correction corresponds to 30 carbon atoms in the molecule). The results of high-resolution mass spectroscopy showed that the empirical formula of the molecule was $C_{30}H_{42}O_7$ (found: 514.2018; calculated: 514.2030). The IR spectrum of compound (II) showed the absorption bands of a hydroxy group (3320 cm^{-1}), a nonequivalent carbonyl groups (1770, 1720 cm^{-1}), and of an α,β-unsaturated ketone (1630, 1605 cm^{-1}).

The UV spectrum was characterized by an absorption maximum at 250 nm (log ε 3.34) which is characteristic for an α,β-unsaturated ketone. The PMR spectrum contained the signals of the protons of seven methyl groups, and singlet signals of olefinic protons at 5.64 and 6.57

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ppm. It was established by double homonuclear resonance that a doublet at 2.41 ppm with a spin–spin coupling constant of 10.8 Hz was due to the vicinal interaction of the protons at C-18 and C-19. Compound (III) was acetylated by acetic anhydride in pyridine. The PMR spectrum of the acetyl derivative (IIIa) differed from that of the initial compound by the presence of an additional three-proton signal of an acetoxy group at 2.21 ppm. The recording of the molecular ion of (IIIa) with m/z 556 also showed the presence of one hydroxy group in the compound being studied. The methylation of (III) with diazomethane at +20°C and under milder conditions (–20°C) led to the formation of the ester (IIIb). The presence of two methoxy groups was confirmed by the PMR spectrum (3.41 ppm, s, 3 H, OCH_3; 3.61 ppm, s, 3H, OCH_3).

The structure of (III) was also confirmed by the results of molecular mass spectrometry. We have shown previously [1] that for ursolic acid derivatives containing oxygen functions in rings C and D, cleavage of C_9–C_{11} and C_8–C_{14} bonds and the predominant recording of the ion *a*, indicate, rings C–E are characteristic. In actual fact, in the mass spectrum of compound (III) we observed an intense peak of the ion *a* with m/z 262 amu having the empirical composition $C_{16}H_{22}O_3$ (found: 262.1553; calculated 262.1569). Its breakdown generated a group of fragmentary ions the appearance of which did not contradict the structure of rings C–E: m/z 247 – $[a–CH_3]^+$, 234-$[a–CO_2]^+$, 218 – $[a–CO_2]^+$, 217 – $[a–COOH]^+$, 216 – $[(a–CO_2)–H_2]^+$, 201 – $[(a–CO_2)–H_2–CH_3]^+$, 189 – $[(a–COOH)–CO]^+$. The elimination of the particles CO, CO_2, and COOH showed the presence of one keto and one carboxy group in fragment *a* for the molecule under study (we have observed similar ions in a fragment of analogous structure for compound (II), 11-oxourosolic acid, and 3-O-acetyl-11-oxoursolic acid [1]). In the mass spectrum of the methylated derivatives, a fragmentary ion with m/z 279 was recorded, which confirmed the presence of one carboxy group in the structural fragment a. Thus deeper changes took place in rings A and B, in which the remaining oxygen functions were present. The ion corresponding to *a'* was not recorded in the mass spectrum of compound (III). The course of the fragmentation of M^+ for substance (III) is connected with the splitting out of the particles CH_3 – m/z 499 (predominantly from the quaternary C_4 atom), CO – m/z 486 (presence of a keto group) – CO_2 – m/z 470, $COOCH_3$ – m/z 469, and $C_3H_9O_3$ – m/z 427 (fragment b – $C_{27}H_{39}O_4$; found: 427.2841; calculated: 427.2849). The recording of ion b showed the presence of a carboxy group in ring A (cleavage of the C_1–C_{10} and C_3–C_4 bonds). This fact was additionally confirmed by a recording of M^+ with m/z 542 for the dimethyl ester (two carboxy groups). The presence in the mass spectrum of (III) of the peak of an ion with m/z 468 arising on the elimination of a molecule of water from the $[M–CO]^+$ ion indicated the presence of a hydroxy group. Thus, the proposed structure for (III) corresponds to 2-carboxy-5-hydroxy-11-oxy-3-oxaursa-1,12-dien-28-oic acid.

Compound (IV), like (III), has a molecular weight of 514 amu and the empirical formula $C_{30}H_{42}O_7$. The presence in the IR spectrum of absorption bands in the 1785, 1740, 1720, 1640, and 1605 cm^{-1} regions permitted the assumption that the compound contained a lactone ring, an α,β-unsaturated ketone, and two carboxy groups. The UR spectrum showed an absorption maximum at 257 nm (log ε 3.3). Compound (IV) was methylated with diazomethane to form the dimethyl ester (IVa) (M^+ 542). The PMR spectrum of (IV) had the singlet signal of an olefinic proton at 5.73 ppm (H-12). The mass spectrum contained the intense peak of fragmentary ion *a* with m/z 262. The fragmentation of the latter generated a group of fragmentary ions as in the case of substance (III), which indicated the identity of the structures of rings C–E in compounds (IV) and (III). Unlike (III), compound (IV) was not acetylated by acetic anhydride. The PMR spectrum of (IV) was characterized by two doublet signals of geminal protons of a CH_2 group in a six-membered ring with a spin-spin-coupling constant of 17.5 Hz (2.91 and 3.32 ppm). The absence of the signal of a proton at C-9 showed the presence of a substituent in this position. Thus, the structure of (IV) corresponds to 9-hydroxy-11-oxy-2,3-secours-12-en-2,3,28-trioic acid 2,9-lactone.

EXPERIMENTAL

The course of the processes was monitored and the purity of the compounds obtained was determined by chromatography in a thin layer of Silufol UV-254 silica gel using the chloroform–methanol (9:1) system. The reaction products were detected with a 3% ethanolic solution of tungstosilicic acid at 100-110°C. The preparative separation of the substances was performed on a column of silica gel L 100/250 (Czechoslovakia).

Mass spectra were recorded on a Varian MAT-311A spectrometer under the following conditions: energy of the ionizing electrons 70 eV, cathode emission current 300 μA, accelerating voltage 3 kV. The temperature of the ion source and the temperature of the sample inlet were 180-200°C. PMR spectra were taken in $CDCl_3$ on a Bruker WH-360 spectrometer with a working

frequency of 360 MHz (internal standard TMS) under the regime of quadrature phase detection, the volume of memory used amounting to 16 K points, the signals being assigned with the aid of double homonuclear resonance. IR spectra were obtained on a UR-20 spectrometer (GDR), with the substances in the form of mulls in paraffin oil, and UV spectra of a Hitachi spectrometer (with ethanol as the solvent). Elementary analysis was carried out on a Hewlett-Packard model 185 B CHN analyzer (United Kingdom). The results of the analyses corresponded to the calculated figures for the structures given. Melting points were determined on a Boetius heated microscope stage.

Oxidation of Ursolic Acid. At 30°C with constant stirring, 3.93 g of potassium dichromate in 60 ml of 30% solfuric acid was added to a solution of 3.02 g of ursolic acid in 250 ml of glacial acetic acid. The temperature of the reaction mixture was raised to 70°C and the reaction was continued for 3 h. After the end of the reaction, the excess of chromic acid was decomposed with sodium metabisulfite, and the reaction mixture was poured into ice water. The white precipitate that deposited was separated off, washed, dried, and chromatographed. After elution with petroleum ether–chloroform (3:2), 1.05 g (33.95%) of a substance with mp 280-283°C, identified as 11-oxoursolic acid, was obtained.

Petroleum ether–chloroform (1:1) eluted a mixture of (III) and (IV). The eluate was evaporated in vacuum, and the residue was heated with the mimimum volume (15-20 ml) of chloroform. The insoluble residue was separated off, washed with chloroform, and dried. This gives 0.86 g (25.3%) of substances (III), $C_{30}H_{42}O_7 \cdot {}^1/_2H_2O$, with mp 278-280°C (anicular crystals from aqueous methanol), $[\alpha]_D^{20}$ +160° (c 0.31 methanol, λ_{max} 250 nm (log ε 3.34), $\nu_{max}^{CHCl_3}$ 3320, 1770, 1720, 1630, 1605 cm^{-1}. PMR spectrum (δ, ppm): 2.41 d (J = 10.8 Hz, 1 H, 18-H; 2.65 s (1 H, 9-H); 5.64 s (1 H, 12-H); 6.57 s (1 H, 1-H). Mass spectrum, m/z (%): 514 (M^+, 96), 499(89), 486(27), 470(23), 469(22), 468(40), 427(29), 262(96), 247(32), 234(47), 218(29), 217(62), 216(44), 201(23), 189(93).

The chloroform filtrate was evaporated in vacuum and the residue was crystallized from aqueous acetone. This gave 0.52 g (15.3%) of substance (IV), $C_{30}H_{42}O_7$, in the form of a white microcrystalline powder with mp 275-276.5°C, λ_{max} 257 nm (log ε 3.3), ν_{max} 1785, 1740, 1720, 1640, 1605 cm^{-1}. PMR spectrum (δ, ppm): 2.44 d (J = 10.8 Hz, 1 H, 18-H); 2.91 d (J = 17.5 Hz, 1 H, 1-H); 3.32 d (J = 17.5 Hz, 1 H, k-H); 5.73 s (1 H, 12-H). Mass spectrum; m/z (%): 514 (M^+, 30), 499(26), 486(6), 470(11), 469(10), 455 (7), 454(4), 437(24), 290(35), 262(36), 247(12), 234(21), 218(12), 217(29), 216(23), 201(11), 189(42).

Acetylation of Compound (III). A solution of 0.42 g of substance (III) in 10 ml of pyridine was treated with 6 ml of acetic anhydride and the mixture was heated in the water bath (90-100°C) for 1 h and was then left at room temperature for 12 h. The reaction product was crystallized from methanol. This gave 0.38 g of (IIIa), $C_{32}H_{44}O_8$, with mp 289-293°C. M^+ 556, ν_{max} 1780, 1750, 1720, 1630, 1220 cm^{-1}. PMR spectrum (δ, ppm): 2.21 s (3 H, $OCOCH_3$).

Methylation of Substance (III). A solution of 0.11 g of (III) in 15 ml of methanol was treated with an ethereal solution of diazomethane, and after 2 h the solvent was distilled off in vacuum and the residue was crystallized from aqueous methanol. This gave 0.091 g of (IIIb), $C_{32}H_{46}O_7$, with mp 305-307°C. M^+ 542. $\nu_{max}^{CHCl_3}$ 3360, 1760, 1710, 1650 cm^{-1}. PMR spectrum (δ, ppm): 3.41 s (3H, OCH_3); 3.61 s (3H, OCH_3).

Methylation of Substance (IV). The reaction was performed in a similar manner to the preparation of the dimethyl ester (IIIb). The dimethyl ester (IVa) was obtained: $C_{32}H_{46}O_7$, mp 227-229°C (aqueous ethanol). M^+ 542. PMR spectrum (δ, ppm): 3.59 s (3H, OCH_3); 3.64 s (3 H, OCH_3).

CONCLUSIONS

The oxidation of ursolic acid with potassium dichromate in the presence of sulfuric acid in glacial acetic acid takes place with the formation of seco derivatives: 2-carboxy-5-hydroxy-11-oxo-3-oxaursa-1,1-dien-28-oic acid and 9-hydroxy-11-oxo-2,3-secours-12-en-2,3, 28-trioic acid 2,9-lactone.

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