

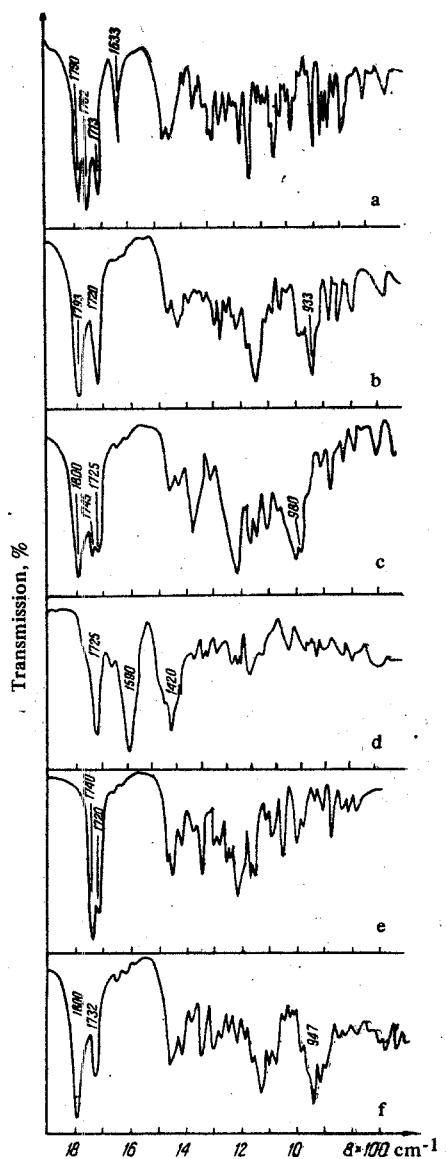
REACTION OF THE LACTONE RING OF DIFFUGENIN

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There is information in the literature that, unlike the native aglycones of cardiac glycosides, 14-anhydrocardenolides do not isomerize under the action of alkalies [1, 2].

We have studied the influence of alkalies on diffugenin (I) (14-monoanhydrostrophanthidin) [3]. The main reaction product was a crystalline substance (II) with the empirical formula $C_{23}H_{32}O_6$, i.e., with the same elementary composition as isostrophanthidin (III) but with different properties.



IR spectrum: a) diffugenin (I); b) the lactol (II ψ); c) the diacetate (VIII); d) the K salt of the aldehyde acid (II α); e) the methyl ester (IV); f) the methoxy lactone (V).

absorption of the aldehyde group at 300 m μ rises markedly (from $\log \epsilon$ 1.47 to $\log \epsilon$ 1.94), i.e., the tautomeric equilibrium is displaced in the direction of the open α form.

Substance (II) readily dissolves in dilute aqueous solutions of potassium bicarbonate. The IR spectrum of the potassium salt of compound (II) has bands at 1420 and 1590 cm^{-1} corresponding to a carboxylate ion. The spectrum of the compound (II) itself does not contain bands characteristic for a free carboxy group. Unlike the spectrum of diffugenin (II), which has the maxima of a butenolide ring at 1790 and 1762 cm^{-1} (C=O doublet) and 1633 cm^{-1} (C=C), the spectrum of compound (II) has a strong band at 1793 cm^{-1} . This band can be assigned to the C=O group of a saturated γ -lactone which, however, must contain an electronegative substituent at the γ -carbon atom (C₂₁) [4]. If one takes into account the fact that in the spectrum of compound (II) the region of absorption of hydroxy groups is narrowed (3535, 3400, and 3240 cm^{-1} in place of a broad band at 3320-50 cm^{-1} for diffugenin), and also the appearance of the band of a dioxymethylene group (933 cm^{-1}), it may be assumed that this substituent is a hydroxy group, and compound (II) is a γ -hydroxybutanolide. This assumption is in good agreement with the capacity of product (II) for forming a diacetate (VIII), with no change in the nature of the absorption band of the lactone C=O (1800 cm^{-1}), while the acid properties are lost.

Thus, substance (II) in the crystalline state is 3 β , 5 β , 21-trihydroxy-19-oxo- Δ^{14} -cardenolide (the 21-lactone of 3 β , 5 β , 21, 21-tetrahydroxy-19-oxo-24-nor- Δ^{14} -cholenic acid), the lactone ring of which is readily opened under the action of alkalies with the formation of salts of a β -substituted γ -aldehyde acid (3 β , 5 β -dihydroxy-19, 21-dioxo-24-nor- Δ^{14} -cholenic acid).

This conclusion is satisfactorily confirmed by data relating to the properties of γ -aldehyde acids which, in solution, exist in the aldehyde acid (α -form)-lactol (ψ -form) equilibrium. As M. M. Shenyakin's investigations [5] have shown, the equilibrium in such aldo-lactol tautomeric systems is displaced completely in the direction of the cyclized (lactol) ψ -form in the crystalline substance.

The presence of such ring-chain tautomerism in the case of the lactol (II ψ) is well shown in its various reactions. Thus, the methylation of compound (II) with diazomethane leads to the methyl ester (IV) of the aldehyde acid (II α), and in the IR spectrum the maximum at 1793 cm^{-1} (C=O of a lactone) disappears and the bands of C=O (1740 cm^{-1}) and C-O-C (1210, 1165 cm^{-1}) groups of esters appear (see figure).

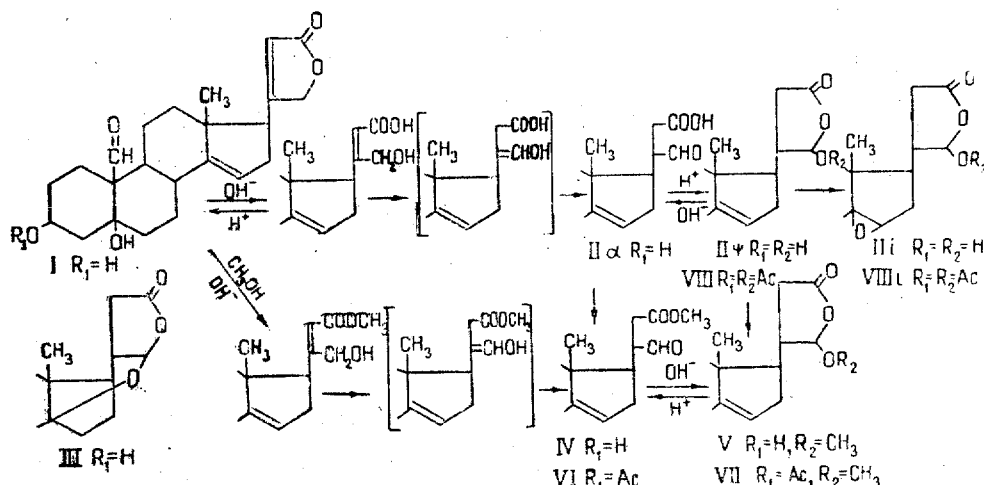
In the IR spectra of compounds (IV), (VI), and of the potassium salt of (II α), the intensity of the absorption bands of the aldehyde group at 1720 cm^{-1} is higher, which shows the appearance of a new carbonyl group (in addition to that at C₁₀). This is confirmed by the UV spectrum of substance (II) taken in a neutral medium and after the addition of a drop of 1 N caustic soda to the solution under study. On this, the intensity of the

An ether of a different type is formed from compound (II) in methanolic solution containing traces of hydrogen

chloride. In this case a quantitative yield of the methyl ether of the ψ form—the methoxy lactone (V)—is obtained. The IR spectrum of this substance has a strong lactone C=O band at 1800 cm^{-1} .

The IR spectra of compounds (II ψ), (V), (VII), and (VIII) exhibit bands in the $933\text{--}980\text{ cm}^{-1}$ region which must be ascribed to a dioxymethylene grouping (O—CH—O) at C₂₁.

An interesting fact confirming the existence of aldo-lactol tautomerism is the isomerization of the α -ester (IV) into the ψ -ether (V) that we observed. A similar isomerization has been found previously in the case of opianic acid [6]. It is curious that the methoxy lactone (V) is formed directly from diffugenin by the action of alkali in aqueous methanol on it. It must be assumed that in the first stage of this reaction (see scheme) the methanolysis of the butenolide ring with the formation of the α -ester (IV), which is well known for the bufadienolides [7], takes place, and this ester immediately isomerizes in the alkaline medium into the more stable ψ -ether (V).



In the formation of the lactol (II ψ) from diffugenin (I), two new asymmetric centers arise at C₂₀ and C₂₁. It was possible to separate two epimers, obviously corresponding to the "α" and "β" epimers at C₂₀ by thin-layer chromatography on silica gel in the ethyl acetate–hexane–benzene–methanol (12:6:2:2) system only in the case of the lactol (II ψ). In this case it is impossible to separate the epimers at C₂₁ because of the existence of aldo-lactol tautomerism.

The whole series of compounds (I–VIII) is characterized by a high lability, and a series of compounds parallel with it—the i series—is formed in the process of preparation and crystallization in the presence of even small amounts of oxygen (air, impurity in the nitrogen). This phenomenon is apparently due to autoxidation with molecular oxygen. The assumption that here the conversion of the aldehyde group into a carboxy group takes place as in the case of strophanthidin [8] was rejected, since the IR and UV spectra of the substances of the main and derivative series are identical in the regions of carbonyl and hydroxyl absorption. The substances can be distinguished satisfactorily by chromatography and also in color reactions with concentrated sulfuric acid and antimony trichloride.

Of the substances of the derivative series, (IIIi) and its diacetate (VIIIi) have been isolated and characterized. Elementary analysis of these compounds shows that they contained one more atom of oxygen. If one takes into account the fact that in the conversion of substance (II) into (IIIi) the low-intensity band of the stretching vibrations of the C=CH group at 3060 and 1660 cm^{-1} disappears and two weak bands arise at 720 and 750 cm^{-1} , and the narrow band at 870 cm^{-1} is converted into a broader doublet at 870 and 880 cm^{-1} , it may be assumed that the double bond at C₁₄ undergoes autoxidation with the formation of an epoxide grouping. Consequently, compound (IIIi) is most probably 14,15-epoxy-3 β ,5 β ,21-tri-hydroxy-19-oxocardenolide.

The experimental material presented forces us to reconsider the accepted view of the alkaline isomerization of the cardenolides, and this will be the subject of discussion in a special paper.

Experimental

The thin-layer chromatography was carried out with a mixture of type KSK silica gel and 10% of gypsum. The following solvent systems were used: chloroform–ethyl acetate–hexane–benzene–ethanol in ratios of 1) 10:6:3:2:3; 2) 7:6:6:2:3; and 3) 6:6:7:2:3.

The reactions were carried out only with chromatographically pure samples. The melting points are uncorrected. The IR spectra were taken on a UR-10 spectrometer in tablets with potassium bromide.

3 β , 5 β , 21-Trihydroxy-19-oxo- Δ^{14} -cardenolide (21-lactone of 3 β , 5 β , 21, 21-tetrahydroxy-19-oxo-24-nor- Δ^{14} -

cholenic acid) (II ψ). A solution of 1 g of diffugenin (I) in 100 ml of 2% caustic potash in aqueous methanol (1:1) was left at room temperature for 3 hr and was then neutralized with 5% hydrochloric acid to pH 9 and evaporated to half bulk in vacuum at 20–30° C. The reaction mixture was extracted first with chloroform (3 \times 30 ml) and then, after acidification to pH 4–5, with ethyl acetate (exhaustively). After washing and evaporation in vacuum, the chloroform extracts gave 70 mg of an amorphous product consisting of a mixture of unchanged diffugenin (I), with the methoxy lactone (V) and traces of other substances. The product was chromatographed in a thin layer of silica gel with gypsum in system 2. The zone of the methoxy lactone (V), which had travelled the greatest distance, was eluted with ethyl acetate to give 20 mg of a chromatographically homogeneous amorphous product the crystallization of which from methanol gave 10 mg of the methoxy lactone (V) with mp 252–255° C.

After the corresponding treatment, the ethyl acetate extract (acid fraction) gave 1.09 g of an amorphous product consisting mainly of the lactol (II ψ) together with some epoxy lactol (III), traces of compounds (I) and (IV), and small amounts of unknown compounds with smaller R_f values. The crude product was chromatographed on a 22 \times 36 cm plate with 60 g of adsorbent in system 1 (10 hr). The zones of the lactols (II ψ) and (III) were eluted with ethyl acetate to give, respectively, 650 and 110 mg of chromatographically homogeneous substances the crystallization of which from methanol led to the following two compounds.

The lactol (II ψ) had mp 215–218° C, $[\alpha]_D^{27} + 80 \pm 2^\circ$ (c 1.54; methanol–chloroform (9:2)). The change of coloration with time in the reaction with concentrated sulfuric acid was: brown-red, orange, green yellow, yellowish green, colorless. When the chromatogram was treated with antimony trichloride it gave a yellow spot. IR spectrum: 3535, 3400, 3240 (OH), 3060, 1655 (C=C–H), 2735, 1720 (CHO), 1793 (lactone C=O), 933 (lactone O–CH–O) cm^{-1} . UV spectrum: $\lambda_{\text{max}}^{\text{C}_2\text{H}_5}$ 218, 300 m μ (log ϵ 3.09, 1.47).

Found, %: C 68.7; 68.6; H 8.09; 7.89. Calculated for $\text{C}_{23}\text{H}_{32}\text{O}_6$, %: C 68.29; H 7.97.

The epoxy lactol (III) had mp 235–237° C, $[\alpha]_D^{20} + 68 \pm 2^\circ$ (c 0.76; methanol). The time reaction with concentrated sulfuric acid: yellowish-brown, orange, pink, colorless. On the chromatograms, antimony trichloride led to the formation of a pink color. IR spectrum, 3535, 3390, 3230 (OH), 2760, 1720 (CHO), 1798 (lactone C=O), 937 (lactone O–CH–O), 880, 750, 720 cm^{-1} (epoxy group). UV spectrum: $\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$ 212, 299 m μ (log ϵ 3.19, 1.60).

Found, %: C 65.7; 65.6; H 8.31; 7.78. Calculated for $\text{C}_{23}\text{H}_{32}\text{O}_7$, %: C 65.69; H 7.67.

3 β , 21-Diacetoxy-5 β -hydroxy-19-oxo- Δ^{14} -cardenolide (diacetate of II ψ) (VIII). Fifty milligrams of the lactone (II ψ) was acetylated with a mixture of 1 ml of acetic anhydride and 1 ml of pyridine at 37° C for 20 hr. After the appropriate working up, 40 mg of crystals was obtained with mp 233–235° C (from methanol) $[\alpha]_D^{21} + 24 \pm 2^\circ$ (c 2.3; chloroform).

Time variation of the coloration with concentrated sulfuric acid: pink, brown-orange, orange-yellow, greenish yellow, yellowish green, colorless. Treatment of chromatograms (systems 3) with antimony trichloride gave a yellow spot. IR spectrum: 3590 cm^{-1} (OH), 3050, 1655 (C=C–H), 2750, 1725 (CHO), 1800 (lactone C=O), 1745, 1220, 1000 (C–O–CO–CH₃), 980 (lactone O–CH–O) cm^{-1} .

Found, %: C 70.3; 70.5; H 8.04; 7.98. Calculated for $\text{C}_{27}\text{H}_{36}\text{O}_8$, %: C 70.20; H 7.43.

Diacetate (VIII) from the epoxy lactol (III). Fifty milligrams of the lactol (II) was acetylated and treated as in the preceding experiment. Crystallization from methanol gave 45 mg of crystals with mp 240–242° C (from ethanol), $[\alpha]_D^{20} + 7.6 \pm 2^\circ$ (c 1.84; chloroform).

The time reaction with concentrated sulfuric acid: yellow-brown, orange, pink, colorless. On chromatograms (systems 3) antimony trichloride yielded a pink spot. IR spectrum: 3560–90 (OH), 2740, 1725 (CHO), 1800 (lactone C=O), 1745, 1220, 1000 (C–O–COCH₃), 975 (lactone O–CH–O) cm^{-1} .

Found, %: C 65.50; 65.40; H 7.61; 7.83. Calculated for $\text{C}_{27}\text{H}_{36}\text{O}_9$, %: C 65.57; H 7.36.

Potassium 3 β , 5 β -dihydroxy-19, 21-dioxo-24-nor- Δ^{14} -cholenate (potassium salt of the aldehyde acid II α). A solution of 20 mg of the lactol (II ψ) in 5 ml of ethyl acetate was treated with 0.1 ml of 1% caustic potash in methanol. The reaction mixture was evaporated to half bulk in vacuum without heating. The potassium salt, depositing in the form of flocs, was filtered off with suction, washed with ethyl acetate, and dried at 20° C and 4 mm for 2 hr. This gave 10 mg of an amorphous powder. IR spectrum: 2750, 1725 (CHO), 1590, 1420 (carboxylate ion) cm^{-1} .

Methyl ester (IV) of 3 β , 5 β -dihydroxy-19, 21-dioxo-24-nor- Δ^{14} -cholenic acid (II α) from the lactone (II ψ). One hundred milligrams of the lactol (II ψ) was methylated with diazomethane in benzene, the solution was evaporated in vacuum at room temperature, and the residue was crystallized from acetone. Chromatography in system 2 yielded 90 mg of crystals with mp 203–205° C (from acetone), $[\alpha]_D^{20} + 52 \pm 2^\circ$ (c 2.17; chloroform). IR spectrum: 3350–70 (OH), 3050, 1660 (C=C–H), 2730, 1725 (CHO), 1740, 1165, 1210, 990 (C–O–C) cm^{-1} .

Found, %: C 68.80; 69.00; H 8.40; 8.44; CH₃O 8.75. Calculated for $\text{C}_{24}\text{H}_{34}\text{O}_6$, %: C 68.87; 8.19; CH₃O 7.12.

Methyl ester (VI) of 3 β -acetoxy-5 β -hydroxy-19-oxo-24-nor- Δ^{14} -cholenic acid from (IV). 50 milligrams of the

methyl ester (IV) was acetylated with a mixture of 1 ml of acetic anhydride and 1 ml of pyridine at 20° C for 20 hr, after which 15 ml of benzene was added to the reaction mixture. The solution was washed with water (3 × 10 ml), 1% hydrochloric acid (10 ml), and with water again (2 × 10 ml), and then the solvent was distilled off in vacuum. The reaction product was chromatographed in system 3 and crystallized from methanol with the addition of water (9:1). This gave 25 mg of crystals with mp 190–192° C [α]_D²⁰ +36.4 ± 2° (c 2.3; chloroform). IR spectrum: 3510 (OH), 2740, 1720 (CHO), 1235, 1035 (C–O–C) cm⁻¹.

Found, %: C 66.40; 66.10; H 8.35; 8.05. Calculated for C₂₆H₃₆O₇ · 1/2 H₂O, %: C 66.45; H 7.94.

3β, 5β-Dihydroxy-21-methoxy-19-oxo-Δ¹⁴-cardenolide (IV) from (IIψ). One hundred milligrams of the lactol (IIψ) was dissolved in 15 ml of methanol containing traces of hydrochloric acid (pH about 6). After 1.5 hr, the reaction mixture no longer contained compound (II). The solution was diluted with 45 ml of benzene and was washed with water to neutrality, after which the benzene was evaporated off in vacuum and the residue was crystallized from methanol. After purification by chromatography in system 2, 90 mg of the crystalline methoxy lactone was obtained with mp 257–260° C (from a mixture of acetone and ether); [α]_D²⁰ +40.5 ± 2° (c 2.17; chloroform); IR spectrum: 3360–90 (OH), 3055, 1660 (C=C–H), 2770, 1732 (CHO), 1800 (lactone C=O), 920, 947 cm⁻¹ (lactone O–CH–O).

Found, %: C 68.50; 68.70; H 8.34; 8.43. Calculated for C₂₄H₃₄O₆, %: C 68.50; H 8.19.

3β-Acetoxy-5β-hydroxy-21-methoxy-19-oxo-Δ¹⁴-cardenolide (VII) from (V). 50 milligrams of the ether (V) was acetylated and treated in just the same way as in the acetylation of the ester (IV). Crystallization from methanol-heptane yielded 30 mg of the acetate (VII) with mp 227–230° C, [α]_D²⁰ +33.4 ± 2° (c 1.86; chloroform). IR spectrum: 3590 (OH), 3055, 1650 (C=C–H), 1790 (lactone C=O), cm⁻¹.

Found, %: C 67.40; 67.40; H 8.06, 8.16. Calculated for C₂₆H₃₆O₇, %: C 67.66; H 7.86.

Isomerization of the ester (IV) of the aldehyde acid into the methoxy lactone (VI). 20 mg of the ester (IV) in 5 ml of ethanol was treated with 0.1 ml of a 1% solution of caustic potash in methanol and left to stand at room temperature for 15 hr. Then the solution was diluted with 25 ml of benzene and washed with water to neutrality. After the benzene had been distilled off, 15 mg of an amorphous product was obtained, the crystallization of which from methanol yielded 5 mg of chromatographically homogeneous (system 2) crystals with mp 253–256° C, the IR spectrum of which was identical with that of the methoxy lactone (V).

Summary

Under the influence of caustic alkalies, diffugenin (14-anhydrostrophanthidin) undergoes a complex irreversible change with the opening of the butenolide ring. The main product is a γ-hydroxy-γ-lactone (lactol)—the stable tautomeric form of a γ-aldehyde acid. The existence of aldo-lactol tautomerism is confirmed by the preparation of derivatives of the α and ψ forms. The hypothesis has been put forward that the double bond at C₁₄ in diffugenin and its derivatives readily undergoes autoxidation with the formation of epoxy compounds.

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