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THE 17α-HYDROXYLATION OF CARDENOLIDES

CONVERSION OF THE CARDENOLIDE TO THE CORTISONE SIDE-CHAIN[1,2]

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Abstract—Oxidation of digitoxigenin acetate (III) with selenium dioxide in dioxan gives rise to the corresponding 17α-hydroxy derivative II. The same substance is obtained under these conditions from the previously described ester I. Ozonolysis of II, and subsequent saponification, leads to 3β, 14β, 17α,21-tetrahydroxy-5β-pregnan-20-one 3-monoacetate (VIIa), a substance containing the cortisone side-chain; in addition, the 17-ketone IV and the cyclic hemi-acetal VIIIa are obtained. The analogous selenium dioxide oxidation of gitoxigenin diacetate (X) produces the corresponding 17α-hydroxy derivative XI.

THE oxidation of the αβ-unsaturated ester I to digitoxigenin 3-acetate (III) with selenium dioxide in boiling benzene has been described in part VI of this series.[1] We have found that III was not obtained when this oxidation was carried out in boiling dioxan. Instead, ca. 25% of a hydroxy derivative was formed, which was subsequently shown to be 17α-hydroxydigitoxigenin 3-acetate (II). The same substance was produced in ca. 60% yield from digitoxigenin acetate (III) with selenium dioxide in boiling dioxan,[4] and this cardenolide is presumably an intermediate in the conversion of I to II under these conditions. The difference in results obtained with benzene and dioxan is unusual, and may be connected with the presence of peroxides in the latter solvent.

The fact that allylic oxidation of digitoxigenin acetate (III) had occurred at the tertiary C-17 position, and not at the secondary C-21 position, was indicated by the observation that II was recovered unchanged on attempted acetylation with acetic anhydride and pyridine at 100°. This location was confirmed by the finding that dehydration of II with phosphorus oxychloride and pyridine at 100° gave ca. 70% of the known "dianhydrogitoxigenin" acetate (VI) (showing a characteristic UV maximum at 337 mμ), previously obtained from the cardenolide gitoxigenin (IX) by dehydration with conc. hydrochloric acid and subsequent acetylation at C-3.[5]

The α-configuration of the 17-hydroxyl group in II was established by ozonolysis. This type of degradation of cardenolides is generally carried out in ethyl acetate at

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[1] This is part VII in the series *Syntheses in the Cardiac Algycone Field.* For part VI, see N. Danieli, Y. Mazur and F. Sondheimer, *Tetrahedron* **22**, 3189 (1966).

[2] The work described in the present paper has been reported previously in preliminary form (N. Danieli, Y. Mazur and F. Sondheimer, *Tetrahedron Letters* 1281 (1962); see also F. Sondheimer, *Chemistry in Britain* **1**, 454 (1965)).

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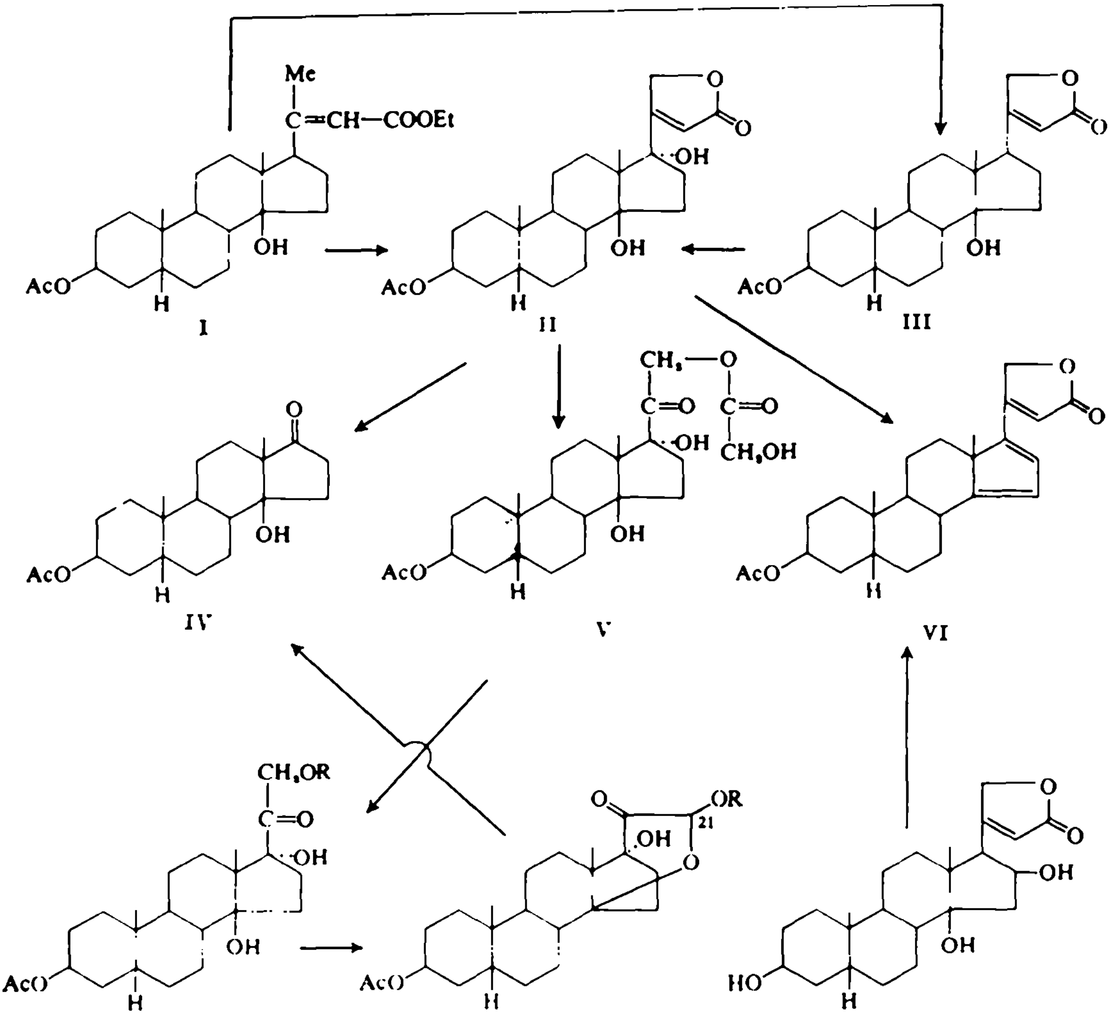
[4] However, no II was formed when digitoxigenin acetate was treated with selenium dioxide in boiling benzene.

[5] See O. Schindler and T. Reichstein, *Helv. Chim. Acta* **35**, 442 (1952), and Refs. to earlier work quoted there.



I II III IV V VI

VIIa, R = H
b, R = Ac

VIIIa, R = H
b, R = Ac

IX

−80°.[6] However, ozonolysis of II under these conditions resulted in no change, and the reaction was very slow even at −18°. After some experimentation, it was found that the ozonolysis of II proceeded satisfactorily when carried out in glacial acetic acid–ethyl acetate solution at 0°. Reduction with zinc, and subsequent saponification with potassium bicarbonate at room temperature,[6b] then led to three substances, which were separated by chromatography.

The first ozonolysis product, isolated in 30% yield, proved to be the 17-ketone IV. The structural assignment is based on the elemental composition, the IR spectrum and the fact that the same substance could be obtained from II in improved yield (ca. 55%) by oxidation with potassium permanganate.

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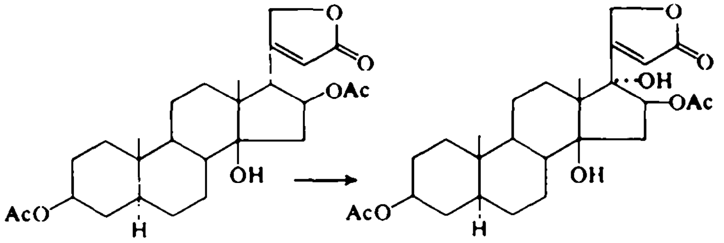
[6] *Inter al.*, [a] K. Meyer and T. Reichstein, *Helv. Chim. Acta* **30**, 1508 (1947); [b] M. Zingg and K. Meyer, *Ibid.* **43**, 145 (1960), and Refs cited there.

The second ozonolysis product (25% yield) was the 17,21-dihydroxy-20-one VIIa, possessing the cortisone side-chain. This substance, which was characterised as the 21-acetate VIIb, is the compound expected to be formed by saponification of the ozonolysis cleavage product V.[6b] The structure was confirmed by the IR and NMR spectra, as well as by the fact that the colour test with 2,3,5-triphenyltetrazolium chloride reagent[7] was positive. The presently described sequence of reactions represents a simple conversion of the cardenolide to the cortisone side-chain.

The third substance obtained from the ozonolysis (in ca. 15% yield) was the cyclic hemiacetal VIIIa, derived from VIIa by autoxidation to the 21-aldehyde and hemiacetal formation. An analogous hemiacetal (possessing a 16β-acetoxyl instead of a 17α-hydroxyl group) has been obtained previously by ozonolysis of gitoxigenin diacetate (X).[8] The structure assigned to VIIIa was confirmed by the NMR spectrum (singlet at τ5·68 due to 21-H)[9], and by the fact that acetylation with acetic anhydride and pyridine yielded the 21-acetate VIIIb (21-H singlet shifted to τ5·40). Oxidation of VIIIa with chromium trioxide in acetic acid at 0° gave the 17-ketone IV.

The formation of the hemiacetal VIIIa shows the C-17 side-chain in II to possess the β-configuration, and the 17-hydroxyl group is therefore α-oriented. This also follows from the ORD curve of VIIIa, which exhibited a positive Cotton effect (Fig. 1). This curve is very similar to that of 3β,14β-dihydroxy-5β-pregnan-20-one 3-acetate,[1] but quite different from that of the corresponding 17α-isomer[1] which showed a negative Cotton effect (Fig. 1). Similarly, the ORD curve of the cortisone derivative VIIb exhibited a positive Cotton effect.

Gitoxigenin diacetate (X) on similar oxidation with selenium dioxide in boiling dioxan yielded ca. 65% of a hydroxy derivative, to which we assign the 17α-hydroxy-structure XI, by analogy. The 17α-hydroxylation of cardenolides under these conditions therefore appears to be general.



X XI

EXPERIMENTAL

M.ps are uncorrected. Rotations were determined at room temp in chf solution, unless stated otherwise. UV spectra: in 95% EtOH soln on a Cary model 14 recording spectrophotometer. IR spectra: as KBr discs on a Perkin–Elmer Infracord recording spectrophotometer. NMR spectra: in CDCl$_3$ on a Varian V4300 spectrometer operating at 60 Mc/s, TMS being used as an internal standard. Analyses were carried out in our microanalytical laboratory under the direction of Mr. Erich Meier.

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[7] See W. J. Mader and R. R. Buck, *Analyt. Chem.* **24**, 666 (1952).

[8] M. S. Ragab, H. Linde and K. Meyer, *Helv. Chim. Acta* **45**, 152 (1962).

[9] Several 21,21-dimethoxypregnan-20-ones, in which the 21-H is in a rather similar environment to that in VIIIa, show a singlet at ca. τ 5·50 due to this proton (W. McCrae and F. Sondheimer, unpublished observations).

17α-*Hydroxydigitoxigenin* 3-*acetate* (II)

(a) *From digitoxigenin acetate* (III). A soln of digitoxigenin acetate (500 mg; m.p. 224–226°, $[\alpha]_D$ +20°)[10] and SeO_2 (500 mg) in dry dioxan (100 ml) was boiled under reflux for 16 hr. The pptd Se was removed, the filtrate was poured into water, and the product was extracted with chf. The organic extract was washed repeatedly with satd NaClaq, dried over $MgSO_4$, and evaporated. Crystallization from acetone–hexane (after treatment with charcoal) yielded II (310 mg; 60%), m.p. 241–244°, $[\alpha]_D$ +155° (EtOH); UV λ_{max} 216 mμ (ε 12,300); IR bands at 2·76 μ (OH), 5·73 (butenolide), 5·78 (acetate) and 6·15 (double bond). (Found: C, 69·37; H, 8·45. $C_{25}H_{36}O_6$ requires: C, 69·42; H, 8·39%.) The substance reacted positively in the "Legal" test.

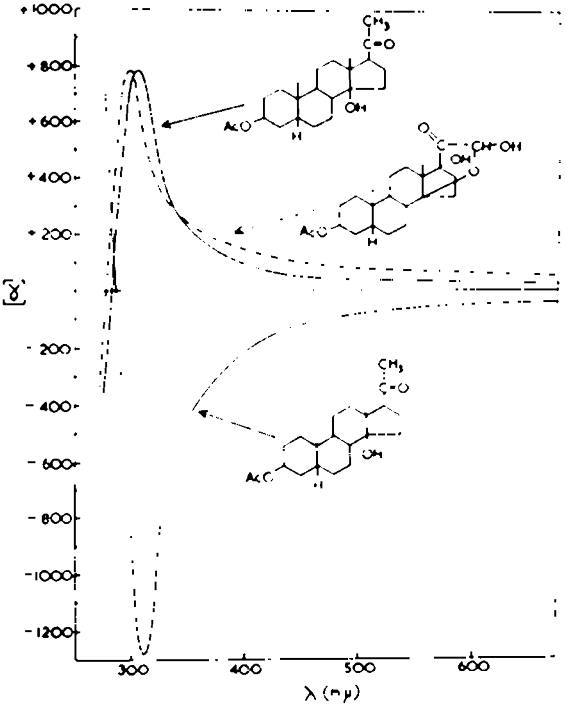

FIG. 1. ORD curves (dioxan) of hemiacetal VIIIa, 3β,14β-dihydroxy-5β-pregnan-20-one 3-acetate and 3β,14β-dihydroxy-5β, 17α-pregnan-20-one 3-acetate.

When the selenium dioxide reaction was carried out for only 3 hr, 30% of II was isolated and 55% of digitoxigenin acetate was recovered (after chromatography on silica gel). Treatment of digitoxigenin acetate with SeO_2 in benzene (16 hr boiling) gave no trace of II, and starting material was recovered in high yield.

(b) *From unsaturated ester* I. A soln of I (40 mg)[1] and SeO_2 (60 mg) in dry dioxan (10 ml) was boiled under reflux for 16 hr. Isolation with ether as above gave an oil, which was chromatographed on silica gel (10 g). Elution with ether yielded II (9 mg; 23%) as sole crystalline product, m.p. 239–242°. It was identified with the above described II by mixture m.p. determination and IR comparison.

Attempted acetylation of II

A soln of II (50 mg) in AcO_2 (2 ml) and pyridine (2 ml) was allowed to stand at room temp for 16 hr. Isolation with ether in the usual way yielded unchanged starting material, no trace of acetylated product being detected. Essentially the same result was obtained in another experiment, in which the solution was heated at 100° for 2 hr.

[10] Prepared from commercial digitoxin as described by F. Hunziker and T. Reichstein, *Helv. Chim. Acta* **28**, 1476 (1945).

Dehydration of II *to dianhydrogitoxigenin acetate* (VI)

A soln of II (150 mg) in dry pyridine (5 ml) was added to a soln containing $POCl_3$ (3 ml) and pyridine (3 ml). The mixture was heated at 100° for 10 min, cooled, and poured into ice water. The product was extracted with EtOAc, and the extract was washed successively with 5% HClaq, $NaHCO_3$aq and satd NaClaq. Drying over $MgSO_4$ and evaporation gave material, which was re-acetylated (Ac_2O, pyridine, 16 hr at room temp) and then chromatographed on silica gel (20 g). Elution with benzene led to VI (97 mg; 71%), m.p. 198–202°. Crystallization from ether–hexane gave a pure sample, m.p. 203–204°, UV λ_{max} 223 and 337 (ε 13,500 and 20,600); IR bands at 5·62 and 5·74 μ (butenolide), 5·77 (acetate) and 6·20 (double bond). The very characteristic IR spectrum was identical to that of an authentic sample (m.p. 203–204°) prepared from gitoxigenin,[4] and there was no m.p. depression on admixture.

Treatment of II with 50% MeOHaq containing 2% H_2SO_4 (1 hr boiling), or with $POCl_3$ and pyridine (16 hr at room temp) resulted in no appreciable amount of VI after re-acetylation.

Ozonolysis of II *to* IV, VIIa *and* VIIIa

An excess of ozonized O_2 (ca. 3% O_3) was passed through a soln of II (1 g) in glacial AcOH (50 ml) and EtOAc (50 ml) at 0°. Evaporation of the solvents at room temp under red. press. yielded an oil, which was dissolved in glacial AcOH (10 ml), and shaken with a large excess Zn dust until an aliquot no longer produced a blue colour with KI-starch paper. The metal was removed, and washed with EtOAc. The filtrate was washed with satd NaClaq, dried over $MgSO_4$ and evaporated. The oily residue was dissolved in methylcellosolve (50 ml) and MeOH (30 ml), $KHCO_3$ (0·55 g) in H_2O (12 ml) was added, and the solution was allowed to stand for 40 hr (conditions of Zingg and Meyer).[10] The soln was acidified with 0·1N HClaq, diluted with H_2O, and extracted with EtOAc. The product was chromatographed on silica gel (100 g), whereby 3 substances were obtained.

Elution with benzene ether (4:1) gave IV (240 mg; 30%), m.p. 220-224°. Crystallization from ether–hexane led to the analytical sample, m.p. 226–228°, $[\alpha]_D$ +20°; IR bands at 2·74 μ (OH) and 5·79 (acetate and 17-one). Found: C, 72·35; H, 9·33. $C_{21}H_{32}O_4$ requires: C, 72·38; H, 9·26%).

Elution with benzene ether (3:1 to 2:1) yielded VIIIa (160 mg; 17%), which on crystallization from CH_2Cl_2–hexane showed m.p. 173–175°, $[\alpha]_D$ +18°; ORD curve, see Fig. 1; IR bands at 2·76 μ (OH), 5·74 (acetate) and 5·84 (20-one); NMR signal at τ 5·68 (1H, singlet, assigned to 21-H). (Found: C, 68·02; H, 8·42. $C_{23}H_{34}O_6$ requires: C, 67·95; H, 8·43%.) The colour test with 2,3,5-triphenyltetrazolium chloride reagent[7] was negative. Acetylation (Ac_2O, pyridine, 16 hr at room temp) led to VIIIb, m.p. 202–203° (from CH_2Cl_2–hexane), $[\alpha]_D$ +30°; IR bands at 2·79 μ (OH), 5·72 (21-acetate) and 5·79 (3-acetate and 20-one); NMR signal at τ 5·40 (1H, singlet, assigned to 21-H). (Found: C, 66·80; H, 7·80. $C_{25}H_{36}O_7$ requires: C, 66·94; H, 8·09%.)

Elution with MeOH led to VIIa (240 mg; 25%), which was difficult to purify. It was therefore acetylated (Ac_2O, pyridine, 16 hr at room temp). The resulting VIIb, after purification by chromatography on silica gel (elution with benzene–ether, 4:1) and crystallization from CH_2Cl_2–ether, showed m.p. 190–192°, $[\alpha]_D$ +25°; ORD, positive Cotton effect peak at $[\alpha]_{312}$ +225° (dioxan); IR bands at 2·85 μ (hydroxyl), 5·69 (21-acetate) and 5·77 (3-acetate and 20-one). In the NMR spectrum, the 2 C-21 protons appear as an AB pattern, the doublets (J = 18 c/s) being centred at τ 4·51 and 4·98;[11] singlets at τ 5·50 (1H) and 7·25 (1H) are due to the 14β- and 17α-hydroxyl groups, respectively, and disappear on addition of CF_3COOH. (Found: C, 66·61; H, 8·49. $C_{25}H_{38}O_7$ requires: C, 66·64; H, 8·50%.) The colour test with 2,3,5-triphenyltetrazolium chloride reagent[7] was positive.

Oxidation of II *to* IV *with potassium permanganate*

A soln of II (200 mg) in acetone (20 ml; freshly distilled from $KMnO_4$) was shaken with powdered $KMnO_4$ (500 mg) for 3 hr at room temp. The mixture was evaporated to dryness, and the residue was extracted with chf. The mixture was filtered, the filtrate was washed with satd NaClaq, and was then dried and evaporated. The oily product was chromatographed on silica gel. The only crystalline substance, eluted with benzene–ether (9:1), proved to be IV (85 mg; 53%), m.p. 218–220°. Crystallization from ether-hexane gave a specimen, m.p. 224–227°, identified with the ozonolysis product by IR comparison and mixture m.p. determination.

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[11] See N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry, Illustrations from the Steroid Field* pp. 90–92. Holden-Day, New York (1964).

Oxidation of VIIIa *to* IV *with chromium trioxide*

A soln of CrO_3 (150 mg) in AcOH (5 ml) and H_2O (1 ml) was added to VIIIa (100 mg) dissolved in AcOH (5 ml) with ice-cooling, and the soln was allowed to stand at ca. 0° for 1 hr. The excess reagent was decomposed by the addition of a few drops MeOH, and the product was isolated with EtOAc. The resulting oil (80 mg) was chromatographed on silica gel. Elution with benzene–ether (9:1) led to IV (27 mg), m.p. 215–220°, raised to 223–226° on crystallization from ether–hexane. Identity with IV from the ozonolysis was established in the usual way. Elution with benzene–ether (4:1) furnished unchanged VIIIa (40 mg). The yield of IV based on unrecovered VIIIa is 52%.

Substance VIIIa was recovered largely unchanged on attempted oxidation with 8N CrO_3 in acetone and H_2SO_4aq (Jones' reagent).

Selenium dioxide oxidation of gitoxigenin diacetate (X) *to 17α-hydroxygitoxigenin diacetate* (XI).

Gitoxigenin diacetate (X, m.p. 250–252°) was prepared from oleandrin by hydrolysis with HClaq to oleandrigenin (gitoxigenin 16-monoacetate), followed by acetylation at C-3.[18]

A soln of X (500 mg) and SeO_2 (1 g) in dry dioxan (75 ml) was boiled under reflux for 16 hr. Isolation as previously led to XI (325 mg; 63%), which after crystallization from acetone–hexane showed m.p. 242–243°, $[\alpha]_D$ −8° (pyridine); UV λ_{max} 216 mμ (ε 13,500); IR bands at 2·75 μ (OH), 5·60 sh and 5·74 (butenolide), 5·79 (acetate) and 6·14 (double bond). (Found: C, 66·15; H, 7·90. $C_{27}H_{38}O_8$ requires: C, 66·10; H, 7·81%.)

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[18] W. Neumann, *Ber. Dtsch. Chem. Ges.* 70, 1547 (1937).