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# THE SYNTHESIS OF DIGITOXIGENIN[1]

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**Abstract**—Digitoxigenin (VIIIb), a typical member of the cardenolide family, has been synthesized from methyl 3β-acetoxy-14β-hydroxy-5β-etianate (II) by a seven-step sequence.

THE cardenolides are a group of steroids, occurring in Nature as glycosides, which are of importance in view of their powerful action on the heart. The group is characterized by the presence of a 14β-hydroxy and a 17β-butenolide function, as in digitoxigenin (VIIIb).[3] This substance is a typical cardenolide, which possesses much of the cardiac activity of the glycosides from which it is derived (e.g., digitoxin, a constituent of digitalis).[3] The synthesis of digitoxigenin has been reported in preliminary form.[4] We now describe the details of these experiments.

Our work was based on the pioneering investigations of Ruzicka, Plattner *et al.* in Switzerland, as well as of Elderfield *et al.* in the U.S. This earlier work has been summarized in a Tilden Lecture of the Chemical Society,[5] which also gives the background to the presently described experiments.

The starting material was methyl 3β-acetoxy-14β-hydroxy-5β-etianate (II), available from 3β-acetoxy-5β-androstan-17-one (I) by a nine-step route[6,7] (as well as from digitoxigenin 3-acetate (VIIIa) by oxidation with potassium permanganate and subsequent esterification).[8] Saponification with potassium carbonate in boiling aqueous methanol gave the hydroxy-acid IIIa, which was acetylated to the acetoxy-acid IIIb.[8] Treatment of IIIb with diazomethane regenerated the acetoxy-ester II, and inversion to the thermodynamically more stable 17α-configuration had therefore not taken place during the saponification.

Reaction of IIIb with methyl-lithium, according to Arens and van Dorp,[9] followed by re-acetylation, led to 3β,14β-dihydroxy-5β-pregnan-20-one 3-acetate (IV) in ca.

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[3] For reviews, see R. B. Turner, *Chem. Revs.* **43**, 1 (1948); H. Heusser, *Fortschr. Chem. org. Naturstoffe* **7**, 87 (1950); C. W. Shoppee and E. Shoppee in E. H. Rodd, *Chemistry of Carbon Compounds* Vol. IIB, Chapter 19. Elsevier, Amsterdam (1953); C. Tamm, *Fortschr. Chem. org. Naturstoffe* **13**, 137 (1956); **14**, 71 (1957); L. F. Fieser and M. Fieser, *Steroids* (3rd Edition), Chapter 20. Reinhold, New York (1959).

[4] N. Danieli, Y. Mazur and F. Sondheimer, *J. Amer. Chem. Soc.* **84**, 875 (1962).

[5] F. Sondheimer, *Chemistry in Britain* **1**, 454 (1965).

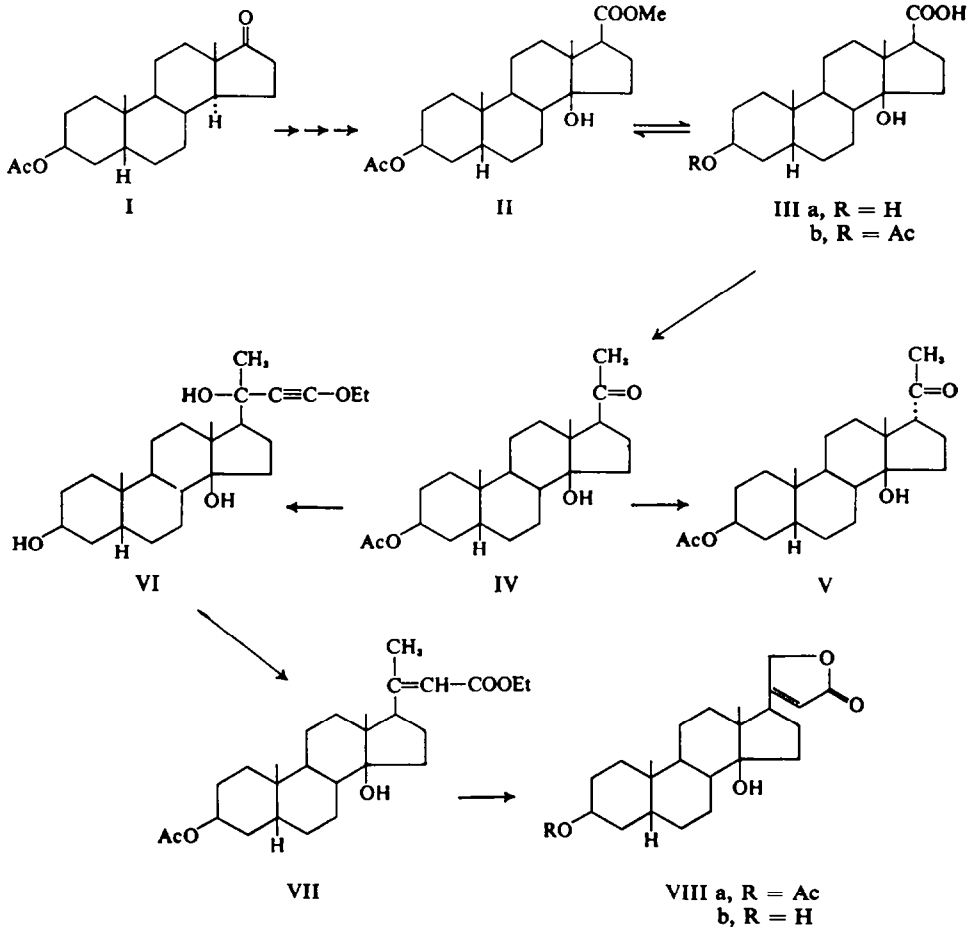
[6] K. Meyer, *Helv. Chim. Acta* **29**, 1580 (1946).

[7] L. Ruzicka, P. A. Plattner, H. Heusser and K. Meier, *Helv. Chim. Acta* **30**, 1342 (1947).

[8] F. Hunziker and T. Reichstein, *Helv. Chim. Acta* **28**, 1472 (1945).

[9] See J. F. Arens and D. A. van Dorp, *Rec. Trav. Chim.* **65**, 338 (1946).

45% yield.[10] The fact that IV still possesses a 17$\beta$-oriented side-chain was demonstrated by its ready inversion with potassium hydroxide in boiling methanol to the more stable 17$\alpha$-isomer V (after re-acetylation). The ORD curve of the 17$\beta$-pregnan-20-one IV showed a positive Cotton effect, while the curve of the 17$\alpha$-isomer V showed a negative Cotton effect (Fig. 1), a behaviour found previously for 14-unsubstituted pregnan-20-ones in which rings C and D are *trans*-fused.[11]




Treatment of IV with lithium ethoxyacetylide in tetrahydrofuran gave rise to the ethoxyacetylenic carbinol VI, which exhibited a characteristic acetylene band at 2270 $cm^{-1}$ in the IR. Reaction with 2% sulphuric acid in aqueous methanol at room temperature resulted in rearrangement of the ethoxyacetylenic carbinol grouping,[12]

[10] The conversion of II to IV via IIIa and IIIb is based on an analogous transformation in the 5$\alpha$-series, carried out previously in these Laboratories by S. Burstein, A. Meisels and F. Sondheimer (see footnote 5).

[11] See C. Djerassi, *Bull. Soc. Chim. Fr.* 741 (1957).

[12] See J. F. Arens, *Advances in Organic Chemistry, Methods and Results* (Edited by R. A. Raphael, E. C. Taylor and H. Wynberg) Vol II, pp. 159–160, and Refs. cited. Interscience, New York (1960).

but these conditions were sufficiently mild to leave the 14$\beta$-hydroxy group intact. Re-acetylation and chromatography then gave the $\alpha\beta$-unsaturated ester VII ($UV_{max}$ EtOH, 232·5 m$\mu$, $\epsilon$ 12,100),[18] the over-all yield from IV being ca. 45%.

The oxidation of the ester VII with selenium dioxide, in order to obtain digitoxigenin acetate (VIIIa), was studied in some detail. The conditions employed previously for an analogous transformation in the 14-unsubstituted series (selenium

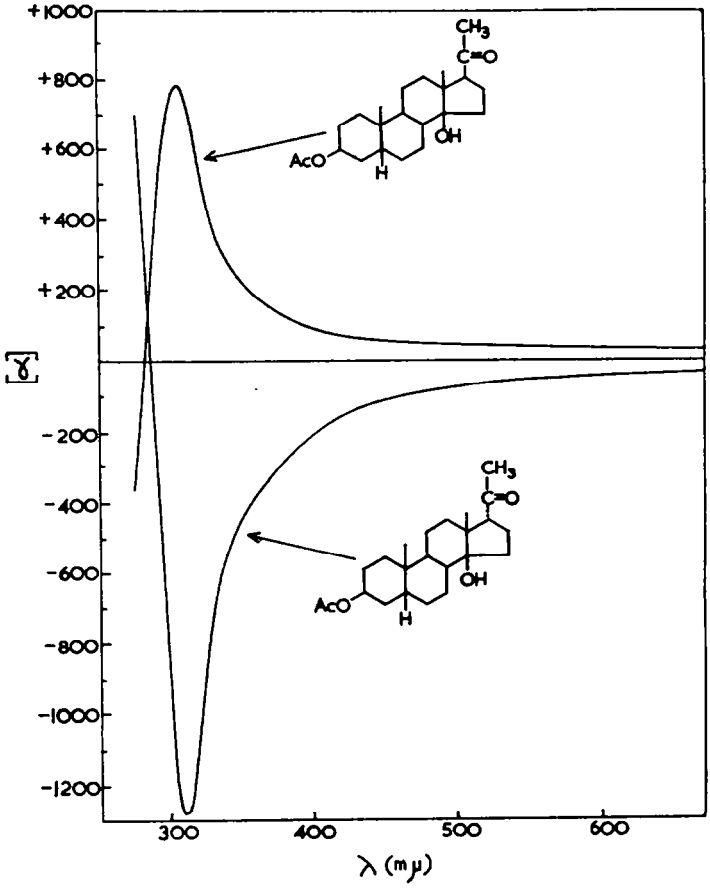



FIG. 1. ORD curves (dioxan) of 3$\beta$,14$\beta$-dihydroxy-5$\beta$-pregnan-20-one 3-acetate (IV) and 3$\beta$,14$\beta$-dihydroxy-5$\beta$,17$\alpha$-pregnan-20-one- 3-acetone (V).

dioxide in boiling acetic anhydride)[14] are of course unsuitable, since the 14$\beta$-hydroxy group would have been eliminated. After some experimentation, it was found that the desired reaction could be effected by oxidation of VII with selenium dioxide in boiling benzene (ca. 10 hr). These conditions resulted in 30% of digitoxigenin

[18] The ester VII was not obtained in crystalline form, and may be a mixture of double-bond isomers. Moreover, it has been found in subsequent experiments with other ethoxyacetylenic carbinols that treatment with methanolic sulphuric acid may give rise to mixtures of ethyl and methyl esters. It is possible that the ethyl ester VII is admixed with the corresponding methyl ester, but this was not investigated in this case.

[14] L. Ruzicka, P. A. Plattner and J. Pataki, *Helv. Chim. Acta* **25**, 425 (1942).

acetate (VIIIa), which could be saponified to digitoxigenin (VIIIb) by means of aqueous hydrochloric acid or by absorption on alkaline alumina. Both VIIIa and VIIIb were identified by direct comparison with authentic samples, and the synthesis of a natural cardenolide was complete.

It is of interest that a different result was obtained when the oxidation of the ester VII was carried out with selenium dioxide in boiling dioxan, as described elsewhere.[1,15]

Since this work was first reported,[4] other syntheses of natural cardenolides have been described.[16,17]

## EXPERIMENTAL

M.ps are uncorrected. Rotations—room temp in chf solution, unless stated otherwise. UV spectra—95% EtOH soln, Cary model 14 recording spectrophotometer. IR spectra—chf soln, Perkin–Elmer Infracord recording spectrophotometer. Analyses, microanalytical under the direction of Mr. Erich Meier.

*3β,14β-Dihydroxy-5β-etianic acid* (IIIa)

A soln of $K_2CO_3$ (2·5 g) in $H_2O$ (25 ml) was added to a soln of II (300 mg; m.p. 154–156°, $[\alpha]_D$ +36°)[6–8] in MeOH (100 ml), and the mixture was boiled under reflux for 16 hr. Water was added, the mixture extracted with AcOEt, the aqueous layer was acidified with HClaq and again extracted with AcOEt. The latter extract was washed with $H_2O$, dried, evaporated, and the residue was crystallized from $CH_2Cl_2$–ether. The resulting IIIa (225 mg; 87%) showed m.p. 221–223°, $[\alpha]_D$ −9° (EtOH) (reported:[18] m.p. 234–238° dec).

*3β-Acetoxy-14β-hydroxy-5β-etianic acid* (IIIb)

A soln containing IIIa (220 mg), $Ac_2O$ (10 ml) and pyridine (10 ml) was allowed to stand at room temp for 16 hr. Water (ca. 1 ml) was added, and the mixture heated at ca. 60° for 2 hr in order to hyrolyse any mixed anhydride. Isolation with AcOEt, followed by crystallization from $CH_2Cl_2$–hexane, yielded IIIb (205 mg; 83%), m.p. 226–228°, $[\alpha]_D$ +36° (reported:[8] m.p. 232–235° dec).

Treatment of a soln of IIIb in $CH_2Cl_2$ with excess diazomethane in ether for 1 hr at 0° smoothly led to II, m.p. 153–155,° identified by direct comparison with an authentic sample.

*3β,14β-Dihydroxy-5β-pregnan-20-one-3-acetate* (IV)

A soln of IIIb (500 mg; 1·32 mmoles) in dry THF (8 ml) was added dropwise to a solution of Me–Li (8·5 mmoles; prepared from Li and MeI)[9] in ether (10 ml), with stirring and ice-cooling under N. The mixture was stirred for a further 1 hr with continued cooling, and was then poured into ice-water. The product was extracted with ether, washed with satd NaClaq, dried over $MgSO_4$ and evaporated. The residue (335 mg) was re-acetylated with $Ac_2O$ and pyridine (16 hr, room temp). The product, isolated as usual, was then chromatographed on $Al_2O_3$ (25 g; Merck, acid-washed). Benzene–ether (9:1) eluted IV (230 mg; 46%), m.p. 146–149°. Crystallization from ether–hexane gave a pure sample, m.p. 150–151°, $[\alpha]_D$ +25°; ORD curve, see Fig. 1; IR bands at 1727 $cm^{-1}$ (acetate) and 1697 (ketone). (Found: C, 73·16; H, 9·52. $C_{23}H_{36}O_4$ requires: C, 73·36; H, 9·64%.)

*Inversion of* IV *to the* 17α*-isomer* V

A soln of IV (50 mg) in 3% methanolic KOH was boiled under reflux for 1 hr. The product was isolated with ether and re-acetylated with $Ac_2O$ and pyridine (16 hr, room temp). Isolation as previously, and crystallization from ether–hexane, yielded V (38 mg; 76%), m.p. 177–178°, $[\alpha]_D$ −44°;

[15] N. Danieli, Y. Mazur and F. Sondheimer, *Tetrahedron* in press.

[16] R. Deghenghi, A. Phillip and R. Gaudry, *Tetrahedron Letters* 2045 (1963); C. R. Engel and G. Bach, *Steroids* **3**, 593 (1964); M. Okada and Y. Saito, *ibid.* **6**, 645 (1965).

[17] A. synthesis of digitoxigenin has also been claimed in a patent [P. D. Meister and H. C. Murray, U.S. Patent 2,930,791 (March 29, 1960); *Chem. Abstr.* **54**, 17471 (1960)], but no further details have appeared in the scientific literature.

[18] K. Meyer and T. Reichstein, *Helv. Chim. Acta* **30**, 1508 (1947).

ORD curve, see Fig. 1; IR bands at 1727 $cm^{-1}$ (acetate) and 1712 (ketone). (Found: C, 73·08; H, 9·48. $C_{23}H_{34}O_4$ requires: C, 73·36; H, 9·64%.)

*Ethoxyacetylenic carbinol* VI

A soln of IV (110 mg; 0·3 mmole) in dry THF (20 ml) was added dropwise under N at room temp to a stirred soln of Li ethoxyacetylide (ca. 6 mmoles, prepared from Me–Li and ethoxyacetylene)[18,19] in ether (10 ml) and THF (20 ml). The mixture was stirred at room temp for 16 hr, and was then poured into ice-water. The product extracted into ether was washed with satd NaClaq, dried over $MgSO_4$, and evaporated. The resulting crude VI (115 mg) was a yellow oil, which was used directly for the next step; IR bands at 3380 $cm^{-1}$ (hydroxyl) and 2270 (acetylene), no strong bands in 1750–1650 $cm^{-1}$ region.

*αβ-Unsaturated ester* VII

The crude VI (115 mg) was dissolved in MeOH (8 ml), 2N $H_2SO_4$ (2 ml) was added, and the soln allowed to stand at room temp for 1 hr. The product, isolated with ether, was then acetylated with $Ac_2O$ and pyridine (16 hr, room temp). Chromatography on silica gel (20 g) and elution with benzene to benzene–ether (49:1) led to VII[18] (61 mg; 47% based on IV) as a faintly yellow oil; UV max 232·5 mμ (ε 12,100); IR bands at 1724 $cm^{-1}$ (acetate and αβ-unsatd ester) and 1642 (double bond).

*Digitoxigenin acetate* (VIIIa)

A soln containing VII (60 mg) and $SeO_2$ (75 mg) in benzene (50 ml) was boiled under reflux for 10 hr. The precipitated Se was removed, the filtrate poured into water, and the product isolated with ether. Chromatography on silica gel (30 g) and elution with benzene–ether (9:1 to 4:1) yielded digitoxigenin acetate (16·5 mg; 30%), m.p. 221–224°. Crystallization from acetone–ether gave a pure sample, m.p. 224–225°, $[\alpha]_D$ +21°. The IR spectrum was identical with that of an authentic specimen (m.p. 224–226°, $[\alpha]_D$ +20°), and there was no m.p. depression on admixture.

*Digitoxigenin* (VIIIb)

(a) *Acid hydrolysis.* A solution of synthetic digitoxigenin acetate (7 mg) in MeOH (7 ml) and 10% HClaq (7 ml) was allowed to stand at room temp for 20 hr. Water was added, followed by $NaHCO_3$aq, and the product extracted with AcOEt. Trituration of the residue with ether gave digitoxigenin (5 mg), m.p. 246–249°, $[\alpha]_D$ +19° (EtOH). Identity with an authentic sample (m.p. 247–250°, $[\alpha]_D$ +20° (EtOH)) was established by mixture m.p. determination and IR comparison.

(b) *Hydrolysis on alumina.* A solution of synthetic digitoxigenin acetate (5 mg) in ether (5 ml) was adsorbed on a column (ca. 5 × 1·5 cm) of $Al_2O_3$ ("Alcoa" F-20). After 16 hr, the column was washed with ether, and the solvent was evaporated. Trituration with ether gave digitoxigenin (3 mg), m.p. 247–250°, identified with an authentic sample as before.

*Acknowledgement*—We are indebted to Syntex S.A., Mexico City, for the determination of the ORD spectra.

[19] The ethoxyacetylene, purchased from Pfister Chemical Works Inc., Ridgefield N.J., was freshly distilled before use.