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Conversion of Digoxigenin to 5α-Digoxigenin: Structure of Syriogenin

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3-Oxo-12β,14-dihydroxy-14β-carda-4,20(22)-dienolide (VII) derivable from digoxigenin (V) was treated with lithium borohydride in pyridine to give 5α-digoxigenin (VIa), which was demonstrated to be identical with the natural cardenolide aglycone, syriogenin. The structure of syriogenin was thus firmly established as 3β,12β,14-trihydroxy-5α,14β-card-20(22)-enolide.

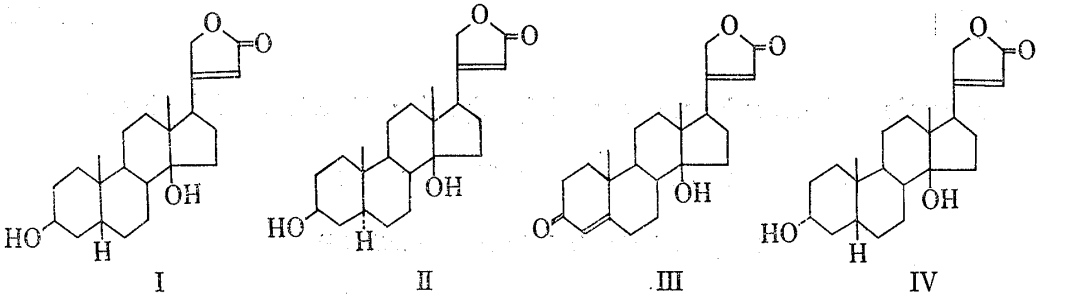
In our previous paper[2] conversion of digitoxigenin (I) to uzarigenin (5α-digitoxigenin) (II) was reported, which was achieved with the aid of the isomerization procedure of steroidal *cis*-A/B ring juncture to *trans*-A/B ring juncture. Thus anhydroperiplogenone (3-oxo-14-hydroxy-14β-carda-4,20(22)-dienolide) (III) derived from I (5β-steroid) was treated with palladium-charcoal in refluxing triglyme to give a 3-oxo-5α-steroid which was reduced by sodium borohydride to afford II (5α-steroid). In the present paper we report conversion of digoxigenin (V) to 5α-digoxigenin (3β,12β,14-trihydroxy-5α,14β-card-20(22)-enolide) (VIa). This structure had been assigned in 1962 to the cardenolide aglycone syriogenin by Masler, *et al.*,[3] without definite experimental evidences.

3-Oxo-12β,14-dihydroxy-14β-carda-4,20(22)-dienolide (VII)[4] derivable from V was treated in the entirely similar way to that reported earlier with III.[2] However, owing to the inversion of the butenolide at C-17[2] which occurred in a much greater extent than with III, the desired cardenolide VIa was obtained only in a very poor yield (less than 2% from VII) after tedious chromatographic separation procedures. Meanwhile, we have noticed that treatment of bufadienolides having 3-oxo-Δ^4-grouping with lithium borohydride in pyridine at 0° gave 3β-hydroxy-5α-bufadienolides in fairly good yields.[5,6]

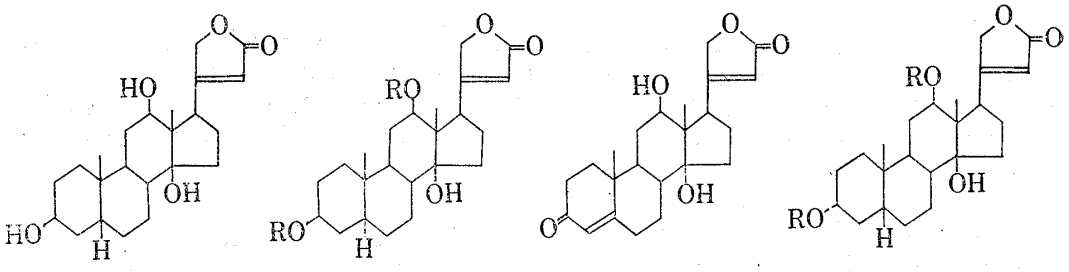
Thus III was treated with lithium borohydride in pyridine to afford II together with 3-epi-digitoxigenin (IV) after preparative thin-layer chromatography (TLC). The estimated yield (*ca.* 15%) of II from III in this procedure was found to be fairly better than that observed previously.[2] Moreover, the procedure was not accompanied by the inversion of the butenolide.

Treatment of VII with lithium borohydride in the similar way gave a mixture of three products, VIa, 3-epi-digoxigenin (VIIIa) and 3ξ,12β,14-trihydroxy-14β-carda-4,20(22)-dienolide (XIIa),[7] among which VIa predominated. Separation and purification of VIa as such from the mixture, however, encountered with difficulties due to the persistent contamination of XIIa. Since an attempt to obtain pure VIa as the acetate (VIb) from the acetylated mixture (VIb and XIIb[7]) was also unsuccessful, the acetylated mixture was treated with 1% H_2SO_4 under reflux converting XIIb into the $\Delta^{3,5}$-diene (XIII).[5,7,8] After this treatment VIb was

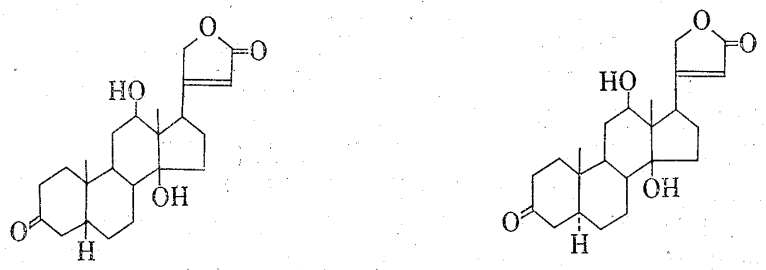
1) Location: *Takada-3-chome, Toshima-ku, Tokyo.*
2) M. Okada and T. Anjyo, *Chem. Pharm. Bull.* (Tokyo), **22**, 464 (1974).
3) L. Masler, Š. Bauer, O. Bauerová, and D. Šikl, *Collect. Czech. Chem. Commun.*, **27**, 872, 895 (1962).
4) T. Okumura, Y. Nozaki, and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **12**, 1143 (1964).
5) U. Stache, K. Radscheit, W. Fritsch, W. Haede, H. Kohl, and H. Ruschig, *Ann. Chem.*, **750**, 149 (1971).
6) E. Hauser, H.H.A. Linde, and D. Zivanov, *Helv. Chim. Acta*, **55**, 2625 (1972).
7) XIIa, XIIb, and XIII were not actually isolated and characterized, but their presence or formation were reasonably indicated by the nuclear magnetic resonance (NMR) spectrum, *etc.*
8) P. Studer, S.K. Pavanaram, C.R. Gavilanes, H. Linde, and K. Meyer, *Helv. Chim. Acta*, **46**, 23 (1963).



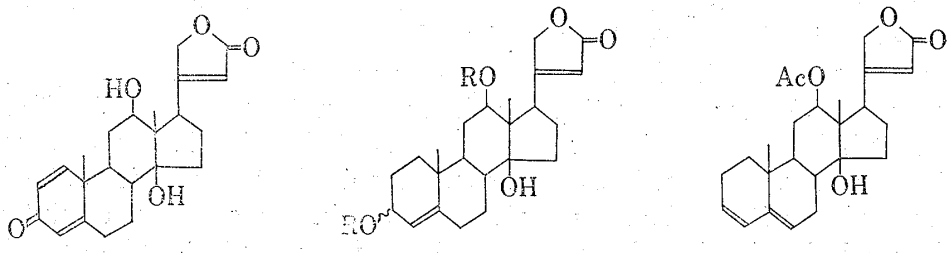
I II III IV



V VIa : R=H VIb : R=Ac VII VIIIa : R=H VIIIb : R=Ac



IX X



XI XIIa : R=H XIIb : R=Ac XIII Ac=$COCH_3$

Chart 1

obtained in pure crystalline state which was then treated with 3% HCl at room temperature for hydrolyzing to give VIa. The yield of VIa from VII was estimated to be about 20%.

In order to confirm the 5α-configuration of VIa it was converted to the 3-oxo compound (X) by the dehydrogenation procedure using platinum as catalyst.[9] The optical rotatory dispersion curves of X and 3-dehydrodigoxigenin (IX)[9] were determined and compared as shown in Fig. 1. A definite positive Cotton effect observed with X clearly indicated the 5α-configuration of VIa.[10]

9) Ch. Tamm and A. Gubler, *Helv. Chim. Acta*, **42**, 239 (1959).
10) C. Djerassi, O. Halpern, V. Halpern, O. Schindler, and Ch. Tamm, *Helv. Chim. Acta*, **41**, 250 (1958); C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co. Inc., New York, 1960; P. Crabbé, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," Holden-Day, Inc., San Francisco, 1965.

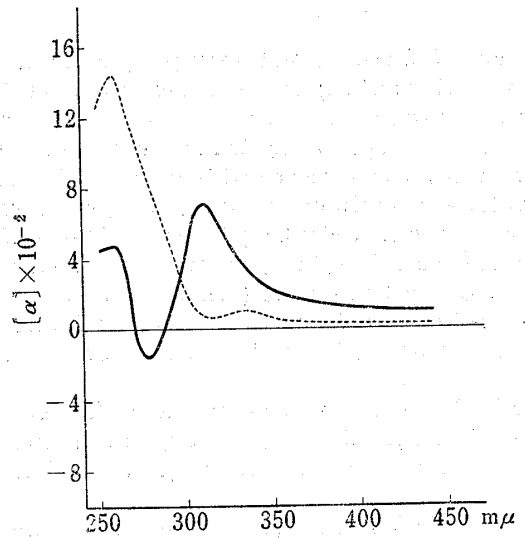


Fig. 1. Optical Rotatory Dispersion Curves of IX and X in $CHCl_3$–MeOH (1: 1)
..... : IX —— : X

Masler, *et al.*[3] assigned to the cardenolide aglycone syriogenin isolated from *Asclepias syriaca* L. the structure (VIa) of 3β,12β,14-trihydroxy-5α,14β-card-20(22)-enolide (5α-digoxigenin) essentially on the basis of ultraviolet (UV) and infrared (IR) spectra and of molecular rotation differences. However, Reichstein, *et al.*[11] isolated the same compound from *Calotropis procera* R. Br., and excluded the location at C-12 of the second hydroxyl group of syriogenin on the basis of the mass spectral data.

Professor Reichstein was kind enough to compare our synthetic 5α-digoxigenin (VIa) directly with the authentic sample of natural syriogenin. It was proved that the synthetic 5α-digoxigenin was identical with natural syriogenin on the basis of paper chromatography (PC), mixed melting point, and mass spectrum.[12]

Quite recently Casagrande, *et al.*[13] have isolated syriogenin from *Dorstenia contrajerva* and demonstrated the structure of 3β,12β,14-trihydroxy-5α,14β-card-20(22)-enolide by unequivocal chemical proof.[14] Indenpendently of our present synthetic work of 5α-digoxigenin (syriogenin), they also have prepared syriogenin from digoxigenin.[15]

Cardiotonic activities of V, VIa, VII, VIIIa, IX and 3-oxo-12β,14-dihydroxy-14β-carda-1,4,20(22)-trienolide (XI), which was obtained as a by-product in the preparation of VII from IX by selenium dioxide oxidation, were tested by using the Straub's preparation and compared with those of the corresponding compounds derived from I.[16] It was demonstrated that 5α-digoxigenin (syriogenin) was much less active than digoxigenin as is the case with uzarigenin and digitoxigenin, and the introduction of 12β-hydroxyl group into the digitoxigenin derivatives resulted in a marked fall of the cardiotonic activity.

11) F. Brüschweiler, W. Stöcklin, K. Stöckel, and T. Reichstein, *Helv. Chim. Acta*, **52**, 2086 (1969).
12) Concerning the mass spectral data which led Professor Reichstein and his co-workers to raise doubt on the location at C-12 of the second hydroxyl group of syriogenin, he wrote to the auther (M.O.) as follows (November 20, 1974): "The mass spectrum of your sample of 5α-digoxigenin is very close to the spectrum of syriogenin (Fig. 6 in *Helv. Chim. Acta*, **52**, 2098 (1969)) only the little peaks at *m/e* 402, 388, 370, 352 and 149 are ±missing which probably came from slight impurities in the natural syriogenin. The important peaks at *m/e* 219 and 201 are also present albeit the latter one is of distinctly lower intensity. It is therefore possible that the relatively strong peak at *m/e* 201 in syriogenin which made me believe that the formula is not correct might have partly originated from an impurity of mass 388 producing this 201 peak in great amount and in spite of small quantities may contribute a measurable portion to its intensity. I therefore see no reason to doubt that your 5α-digoxigenin is identical with natural syriogenin. It is of course still remarkable that the mass spectrum is so different from the mass spectrum of digoxigenin, Fig. 17 in *Helv. Chim. Acta*, **52**, 2295 (1969). To find out the reason one would have to make deuterium labeled models."
13) C. Casagrande, F. Ronchetti, and G. Russo, *Tetrahedron*, **30**, 3587 (1974).
14) We have compared our synthetic samples of 5α-digoxigenin (VIa) and its diacetate (VIb) with the natural samples of syriogenin and its diacetate which were kindly supplied from Professor Russo. They were not very pure, but their principal components have been proved to be identical with our synthetic samples on the basis of TLC.
15) C. Casagrande, F. Ronchetti, and G. Russo, *Tetrahedron Letters*, **1974**, 2369.
16) N. Ishikawa, H. Tsuru, T. Shigei, T. Anjyo, and M. Okada, *Experientia*, **30**, 1308 (1974).

Experimental[17]

3-Oxo-12β,14-dihydroxy-14β-carda-4,20(22)-dienolide (VII) and 3-Oxo-12β,14-dihydroxy-14β-carda-1,4,-20(22)-trienolide (XI)——To a solution of 3-dehydrodigoxigenin (IX)[9] (2.15 g) in a mixture of *tert*-BuOH (183 ml) and AcOH (32 ml) was added SeO_2 (605 mg) and the solution was refluxed for 2 hr under a stream of N_2 gas. After removal of the precipitated Se by filtration, the filtrate was concentrated *in vacuo* to a small volume and the product was extracted with CH_2Cl_2. The organic layer was washed with 5% $NaHCO_3$ and water, and dried over anhydrous Na_2SO_4. After evaporation of the solvent the oily residue (1.78 g) was chromatographed on a column of acid-washed alumina (75 g) by successive elution with CH_2Cl_2 and CH_2Cl_2–MeOH mixtures. The fraction eluted with CH_2Cl_2–MeOH (100: 1, 50: 1) gave VII (217 mg) as colorless plates after recrystallization from acetone. mp 215—230° and 274°. UV λ_{max} mμ (log ε): 231 (4.36). IR ν_{max} cm^{-1}: 3500 (sh), 3430 (OH), 1810, 1733, 1655, 1615 (butenolide and 3-oxo-Δ^4). *Anal.* Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.26; H, 7.74. VII gave a positive reaction for 3-oxo-Δ^4 steroids with isonicotinic acid hydrazide reagent.[18]

The fraction eluted further with CH_2Cl_2–MeOH (50: 1) afforded XI (26 mg) as colorless prisms after recrystallization from acetone. mp 235—245°. $[\alpha]_D^{24}$ +18° (c=0.11, MeOH). UV λ_{max} mμ (log ε): 222 (4.35). IR ν_{max} cm^{-1}: 3480 (sh), 3400 (OH), 1796, 1740, 1657, 1618, 1600 (butenolide and 3-oxo-$\Delta^{1,4}$). *Anal.* Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.91; H, 7.42.

Reduction of Anhydroperiplogenone (III) with $LiBH_4$——To a solution of III (265 mg) in abs. pyridine (8 ml) was added portionwise $LiBH_4$ (180 mg) under ice-cooling and the solution was stirred for 7 hr at 0°. The reaction mixture was poured into 5% NaCl solution, acidified to pH 3 with 2N HCl, and the product was extracted with CH_2Cl_2. The organic layer was washed with 5% $NaHCO_3$ and water, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was submitted to preparative TLC using hexane–AcOEt (1: 2) as solvent. The adsorbent corresponding to the spot (*Rf*: 0.45) was eluted with CH_2Cl_2–MeOH (4: 1). Recrystallization of the eluate from MeOH–benzene gave II (38 mg) as colorless needles. mp 230—242°. Mixed mp on admixture with an authentic sample of uzarigenin showed no depression and IR spectra of the two samples were identical in all respects. The adsorbent corresponding to the spot (*Rf*: 0.38) was eluted with CH_2Cl_2–MeOH (4: 1) and the eluate was recrystallized from MeOH to give IV (33 mg) as colorless needles, mp 285—300°, which was identical with an authentic specimen of 3-epi-digitoxigenin in the mixed melting point, TLC, and comparison of the IR spectrum.

Reduction of 3-Oxo-12β,14-dihydroxy-14β-carda-4,20(22)-dienolide (VII) with $LiBH_4$——To a solution of VII (382 mg) in abs. pyridine (12 ml) was added portionwise $LiBH_4$ (260 mg) under ice-cooling. The reaction mixture was allowed to stand at 0° for 7 hr under stirring. A crystalline product (234 mg) which showed three spots (*Rf*: VIIIa, 0.38; VIa, 0.43; XIIa, 0.45) in TLC using $CHCl_3$–iso-PrOH (5: 1) as solvent was obtained after working up in the way described above. The last spot stained bright pink immediately after spraying 95% H_2SO_4 indicating the presence of 3-hydroxy-Δ^4-grouping. The product was chromatographed on a column of acid-washed alumina (21 g) by successive elution with CH_2Cl_2 and CH_2Cl_2–MeOH mixtures. The fraction eluted with CH_2Cl_2–MeOH (100: 1) gave a crystalline mixture (33 mg) of VIIIa, VIa, and XIIa, whose principal component was VIIIa. Preparative TLC of the mixture using $CHCl_3$–iso-PrOH (5: 1) gave a fraction corresponding to the spot (*Rf*: 0.38) which gave VIIIa (15 mg) as colorless needles after recrystallization from acetone–ether. mp 258—264°. VIIIa (10 mg) was acetylated in the usual way with acetic anhydride and pyridine to afford the diacetate (VIIIb) as colorless needles after recrystallization from acetone–hexane. mp 250—255°. VIIIa and VIIIb were identical with respective authentic samples prepared from IX[9] by $NaBH_4$ reduction and acetylation in TLC, mixed melting point, and comparison of the IR spectrum.

The fraction eluted with CH_2Cl_2–MeOH (100: 1, 50: 1, 25: 1) afforded a crystalline product (156 mg) which was found to be a mixture of VIa and XIIa by TLC. Although the former far predominated over the latter, separation and purification of VIa from the mixture by several chromatographic procedures including preparative TLC were unsuccessful. Then the mixture was acetylated in the usual way with acetic anhydride and pyridine to give an acetylated mixture[19] which showed two spots (*Rf*: VIb, 0.29; XIIb, 0.30) in TLC using hexane–AcOEt (1: 1) as solvent. The latter spot stained bright pink immediately after spraying 95%

17) Melting points were determined on a Kofler block and are uncorrected. UV spectra were measured in 99% EtOH solution. IR spectra were determined in KBr disks on Hitachi EPI-S2 spectrophotometer; sh=shoulder. NMR spectra were measured at room temperature at 60 MHz, using Hitachi Model R-20A 60 MHz spectrometer. Chemical shifts are expressed in δ (parts per million) with tetramethylsilane as internal standard: s, singlet; d, doublet; m, multiplet. TLC plates were prepared according to the Stahl's procedure using Silica gel GF_{254} or HF_{254} (E. Merck AG) as adsorbents. Spots were revealed by heating plates at 110° for 10 min after spraying 95% H_2SO_4 or by Kedde reagent.

18) Th.E. Weichselbaum and H.W. Margraf, *J. Clin. Endocrinol. Metab.*, **17**, 959 (1957).

19) The presence of XIIb was demonstrated by the NMR spectrum of the acetylated mixture. Thus it showed peaks at δ 0.92 (shoulder, 18-CH_3), 1.06 (19-CH_3), 2.04 (3-$OCOCH_3$) and 5.29 (4-H), besides those of VIb indicated.

H_2SO_4. The acetylated mixture was submitted to preparative TLC, but VIb could not be obtained in pure crystalline state.

Purification of VIb was finaly achieved as follows: the above acetylated mixture (150 mg) in a mixture of of MeOH (55 ml) and 2% H_2SO_4 (55 ml) was refluxed for 20 min. After neutralization with 5% Na_2CO_3, MeOH was removed under reduced pressure and then $CHCl_3$ was added for extraction of the product. The organic layer was washed with water and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was subjected to preparative TLC using hexane–AcOEt (1 : 1) as solvent. Elution of the adsorbent corresponding to the spot (*Rf*: 0.29) with $CHCl_3$ and recrystallization of the eluate from acetone–hexane gave VIb (135 mg) as colorless needles. mp 175—178°. $[\alpha]_D^{24}$ +37° (c=0.10, $CHCl_3$). IR ν_{max} cm^{-1}: 3475 (OH), 1808 (sh), 1770 (sh), 1740, 1728, 1620 (butenolide and acetyl C=O). NMR (5% solution in $CDCl_3$) δ: 0.83 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3), 2.00, 2.08 (3H, 3H, s, s, 3- and 12-$OCOCH_3$), 2.88 (1H, d, 17α-H), 4.37—4.92 (4H, 3α-H, 12α-H, 21-CH_2), 5.84 (1H, m, 22-H). *Anal.* Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07. Found: C, 68.56; H, 8.22. Mixed mp on admixture with an authentic sample of syriogenin diacetate (original preparation of Masler, *et al.*[3]) showed no depression.

3β,12β,14-Trihydroxy-5α,14β-card-20(22)-enolide (5α-Digoxigenin, Syriogenin) (VIa)——A solution of VIb (43 mg) in a mixture of MeOH (20 ml) and 6% HCl (20 ml) was allowed to stand for 45 hr at room temperature. After neutralization with 5% Na_2CO_3, H_2O (20 ml) was added and the solution was concentrated *in vacuo* to afford a crystalline precipitate which was recrystallized from MeOH–benzene to give VIa (32 mg) as colorless needles. mp 264—270°. $[\alpha]_D^{24}$+16° (c=0.13, MeOH). UV λ_{max} mμ (log ε): 217 (4.21). IR ν_{max} cm^{-1}: 3550 (sh), 3460 (OH), 1800, 1730, 1620 (butenolide). *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.99; H, 8.93.

3-Oxo-12β,14-dihydroxy-5α,14β-card-20(22)-enolide (X)——To a solution of VIa (16 mg) in a mixture of acetone (30 ml) and redistilled water (2 ml) was added Pt catalyst which had been prepared from 100 mg of $PtO_2 \cdot H_2O$ by hydrogenation, and the solution was stirred at room temperature for 17 hr. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure to give a crystalline precipitate which was recrystallized from MeOH affording X (12 mg) as colorless short needles. mp 297—302°. IR ν_{max} cm^{-1}: 3450 (sh), 3420 (OH), 1805, 1725, 1615 (butenolide and 3 C=O). *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.86; H, 8.37. Optical rotatory dispersion curve of X is shown in Fig. 1 together with that of IX.

Acknowledgements The authors wish to express their gratitude to Prof. T. Reichstein and Dr. J. von Euw, Institute of Organic Chemistry, University of Basel, Basel, for comparing their synthetic samples of syriogenin and its diacetate with natural ones, and to Prof. G. Russo, Institute of Organic Chemistry, University of Milan, Milano, for providing them natural samples of syriogenin and its diacetate.