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Preparation of 3-Deoxy and 17-Hydroxy Cardenolide

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All the cardenolides so far recorded possess oxygen function at C-3, mostly 3β-hydroxy group and hence its presence is regarded, without any definitive experimental evidence, as an indispensable structural requirement for the cardiotonic activity along with the distinguished structural features of the cardenolide, *i.e. cis*-fusion of the rings C and D, hydroxy group in position 14β, butenolide ring having β-configuration at C-17.[2] The introduction of a hydroxy group, on the other hand, into the ring D of a typical cardenolide digitoxigenin (I) affects differently the activity of I. Thus 15β-hydroxydigitoxigenin,[3] 16β-hydroxydigitoxigenin (= gitoxigenin) and 16α-hydroxydigitoxigenin (=16-*epi*-gitoxigenin[4]) exhibit a definite cardiotonic activity, though their activities are more or less lower than that of I, while 15α-hydroxydigitoxigenin[5] is utterly devoid of activity.

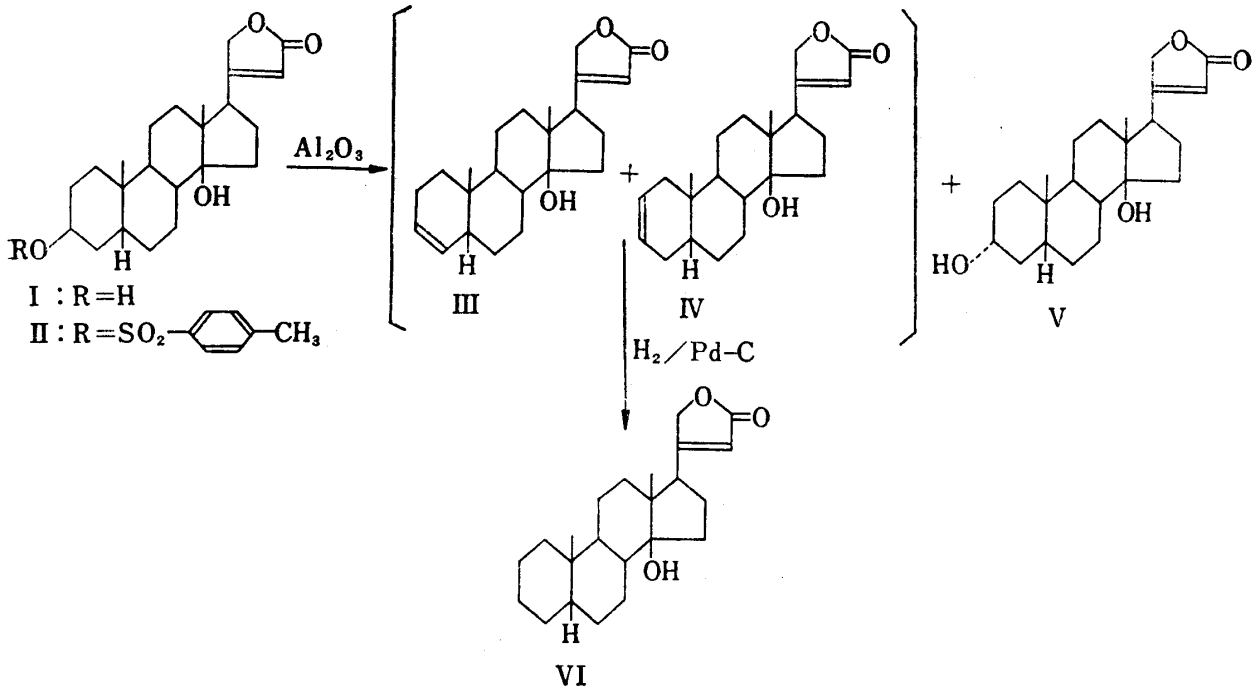

Chart 1

1) Location: *Takada 3-Chome, Toshima-ku, Tokyo.*
2) Ch. Tamm, "Proc. 1st Internat. Pharmacol. Meeting," Vol. 3, ed. by W. Wilbrandt, Pergamon Press, Oxford, 1963, p.11.
3) M. Okada and Y. Saito, *Chem. Pharm. Bull.* (Tokyo), **15**, 352 (1967); **17**, 515 (1969); T. Shigei and S. Mineshita, *Experientia*, **24**, 466 (1968). See also R. Brandt, W. Stöcklin, and T. Reichstein, *Helv. Chim. Acta*, **49**, 1662 (1966).
4) A. Kovaříková, H. Kolářová, and J. Pitra, *Experientia*, **20**, 263 (1964).
5) M. Okada and M. Hasunuma, *Yakugaku Zassi*, **85**, 822 (1965); T. Shigei, M. Katori, H. Murase, and S. Imai, *Experientia*, **20**, 572 (1964); S. Imai, H. Murase, M. Katori, M. Okada, and T. Shigei, *Jap. J. Pharmacol.*, **15**, 62 (1965).

To examine the necessity of the oxygen function at C-3 for the cardiotonic activity and the effect of hydroxy group at C-17 on the activity of I the preparation of 3-deoxydigitoxigenin (VI) and 17α-hydroxydigitoxigenin (VIII) was carried out, which is described in this paper.

The preparation of VI was done according to Chart 1. The tosylate (II) prepared from I in the usual way with *p*-toluenesulfonyl chloride and pyridine was treated with alumina[6] to give olefin, possibly a mixture of the cardadienolide III (Δ^3) and IV (Δ^2), and the epimeric alcohol, 3-*epi*-digitoxigenin (V). Without characterization of the olefin, it was hydrogenated over palladium-on-charcoal affording VI.

Danieli, *et al.*[7] prepared 17α-hydroxydigitoxigenin 3-acetate (IX) by selenium dioxide oxidation of digitoxigenin acetate (VII), which was found to have no cardiac effect even in high dose in cats.[8] In view of the fact that acetylation of the hydroxy group at C-3 generally results in a reduction of the activity of the cardenolide,[8,9] 17α-hydroxydigitoxigenin (VIII) was prepared for pharmacological examinations. Thus, VIII was obtained by the selenium dioxide oxidation of I or by acid hydrolysis of IX. In connection with this oxidation, 17α-digitoxigenin (=menabegenin) acetate (X) was also treated with selenium dioxide to give 17β-hydroxy-17α-digitoxigenin 3-acetate (XII), which on acid hydrolysis afforded 17β-hydroxy-17α-digitoxigenin (XI). XII and IX are isomeric at C-17 and XII gave dianhydrogitoxigenin acetate (XIII) by the treatment with phosphorus oxychloride in pyridine, which had also been derived from IX in the same way.[7]

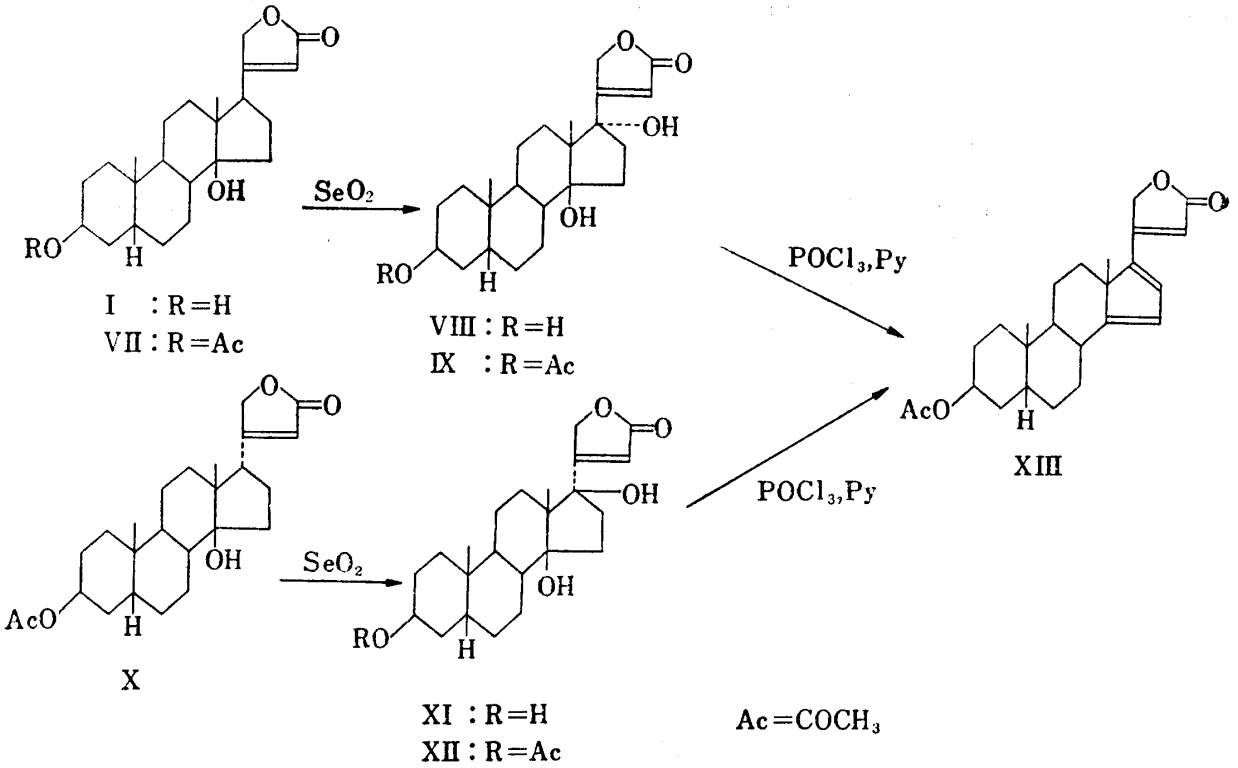

Chart 2

Pharmacological examinations[10] using the isolated frog's heart (Straub's preparation) disclosed that VI and VIII possessed a definite cardiotonic activity and moreover the activity

6) C.H. Douglas, P.S. Ellington, G.D. Meakins, and R. Swindells, *J. Chem. Soc.*, **1959**, 1720.
7) N. Danieli, Y. Mazur, and F. Sondheimer, *Tetrahedron Letter*, **1962**, 1281; *Tetrahedron*, **23**, 715 (1967).
8) F.G. Henderson and K.K. Chen, *J. Med. Chem.*, **8**, 577 (1965).
9) A. Yamada, *Chem. Pharm. Bull.* (Tokyo), **8**, 18 (1960); B.T. Brown, A. Stafford, and S.E. Wright, *Brit. J. Pharmacol.*, **18**, 311 (1962); F.G. Henderson and K.K. Chen, *J. Med. Chem.*, **5**, 988 (1962).
10) Private communication from Prof. T. Shigei, Department of Pharmacology, Institute for Cardiovascular Diseases, Tokyo Medical and Dental University.

of VI was comparable with that of I, while XI was devoid of activity as expected. Thus, it has been demonstrated that 3β-hydroxy group is not an indispensable structural requirement for the activity of the cardenolide.

Experimental[11]

3-Deoxydigitoxigenin (VI)——Treatment of I (450 mg) in the usual way with *p*-toluenesulfonyl chloride (525 mg) and pyridine gave the tosylate (II) (435 mg) which was used for the next reaction without purification. II in benzene (11 ml) was adsorbed on a column of neutral alumina (15 g, activity grade III—IV[12]) which was then stoppered for 44 hr. Elution with hexane–benzene (1:1) and benzene afforded 103 mg of olefin (mixture of III and IV) after recrystallization from acetone–ether–petroleum ether. It gave a positive tetranitromethane test. mp 162—171°. $[\alpha]_D^{28}$ 0° (c=0.83, MeOH). UV λ_{max} mμ (log ε): 218 (4.18). IR ν_{max} cm^{-1}: 3580 (OH), 1785, 1755, 1730 (sh), 1630 (butenolide). *Anal.* Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.26; H, 9.21.

Further elution of the column with benzene–EtOAc (1:1) afforded 3-*epi*-digitoxigenin (V) (5 mg), mp 274—282°, which was shown to be identical with an authentic sample in the mixed melting point and comparison of the IR spectrum.

A solution of the above olefin (100 mg) in EtOH (20 ml) was hydrogenated over 5% palladium–on-charcoal (50 mg). After 30 min the catalyst was removed by filtration and the solvent was evaporated to dryness. The residue was recrystallized from acetone–ether–petroleum ether to give VI (80 mg). mp 178.5—180.5°. $[\alpha]_D^{28}$ +24.4° (c=1.3, MeOH). UV λ_{max} mμ (log ε): 217.5 (4.15). IR ν_{max} cm^{-1}: 3580 (OH), 1785, 1755, 1740 (sh), 1630 (butenolide). *Anal.* Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.01; H, 9.53.

17α-Hydroxydigitoxigenin (VIII)——a) From I: A solution of I (119 mg) and SeO_2 (119 mg) in dry dioxane (20 ml) was boiled under reflux for 7 hr. The precipitated Se was removed, the filtrate poured into water, and the product was extracted with $CHCl_3$. The organic extract was washed repeatedly with saturated aqueous solution of NaCl, dried over anhyd. Na_2SO_4, and evaporated. Crystallization from EtOAc yielded VIII (71 mg). mp 215—219°. $[\alpha]_D^{26}$ −22.8° (c=0.31, MeOH). UV λ_{max} mμ (log ε): 217 (4.02). IR ν_{max} cm^{-1}: 3400 (sh), 3350, 3200 (sh) (OH), 1750, 1725, 1625 (butenolide). *Anal.* Calcd. for $C_{23}H_{34}O_5 \cdot H_2O$: C, 67.62; H, 8.88. Found: C, 67.91; H, 8.67.

Acetylation of VIII (10 mg) in the usual way with acetic anhydride and pyridine gave IX, mp 228—234°, identical with an authentic sample prepared from digitoxigenin acetate (VII) according to the procedure as described by Danieli, *et al.*[7] in the mixed melting point and comparison of the IR spectrum.

b) From IX: A solution of IX (16 mg) in a mixture of MeOH (14 ml) and 10% HCl (14 ml) was allowed to stand for 18 hr at room temperature. After addition of H_2O (10 ml), MeOH was removed *in vacuo* to yield a crystalline precipitate which was recrystallized from EtOAc to give VIII (15.7 mg), mp 214—218°.

17β-Hydroxy-17α-digitoxigenin 3-Acetate (XII)——A solution of X (190 mg) and SeO_2 (190 mg) in dry dioxane (50 ml) was boiled under reflux for 13 hr. Working up in the way as described above yielded XII (100 mg) after recrystallization from EtOAc. mp 237—245°. $[\alpha]_D^{22}$ +46.3° (c=0.82, $CHCl_3$). UV λ_{max} mμ (log ε): 219 (4.04). IR ν_{max} cm^{-1}: 3350, 3200 (OH), 1785, 1748, 1732, 1628 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.33; H, 8.10.

17β-Hydroxy-17α-digitoxigenin (XI)——A solution of XII (47 mg) in a mixture of MeOH (50 ml) and 10% HCl (50 ml) was allowed to stand for 18 hr at room temperature. Working up in the way as described above yielded XI (35 mg) after recrystallization from EtOAc. mp 227—237°. $[\alpha]_D^{23}$ +52.6° (c=0.78, MeOH). UV λ_{max} mμ (log ε): 218.5 (4.05). IR ν_{max} cm^{-1}: 3460 (sh), 3385, 3310 (sh) (OH), 1788, 1753, 1738 (sh), 1628 (butenolide). *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 71.05; H, 9.08.

Acetylation of XI in the usual way with acetic anhydride and pyridine gave XII, mp 237—245°, which was identical with the above sample of XII in the mixed melting point and comparison of the IR spectrum.

Dehydration of XII to Dianhydrogitoxigenin Acetate (XIII)——A solution of XII (15 mg) in pyridine (1 ml) was added to a solution containing $POCl_3$ (0.3 ml) and pyridine (1 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% HCl, aqueous solution of $NaHCO_3$ and saturated aqueous solution of NaCl. Drying over anhyd. Na_2SO_4 and evaporation gave a yellow oil, which was reacetylated in the usual way with acetic anhydride and pyridine. Crystallization from acetone-petroleum ether gave XIII (8 mg), mp 206—211°, identical with an authentic sample in the mixed melting point and comparison of the IR spectrum.

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11) Melting points were determined on a Kofler block and are uncorrected. Ultraviolet (UV) spectra were measured in 99% ethanol solution. Infrared (IR) spectra were determined in potassium bromide disks on Hitachi EPI-S2 spectrophotometer; sh=shoulder.

12) S. Heřmánek, V. Schwarz, and Z. Čekan, *Collection Czech. Chem. Commun.*, **26**, 3170 (1961).