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Syntheses of 5α,13α-Cardenolides[1]

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In order to examine the physiological activity C-17 epimeric 5α,13α-cardenolides have been synthesized from 3β-hydroxy-5α,13α-pregnan-20-one acetates (I, VII). Condensation with lithium ethoxyacetylide gave rise to the ethoxyacetylenic carbinols (V, VIII), respectively. Being exposed to sulfuric acid these carbinols underwent easily rearrangement to form the α,β-unsaturated esters (IVa, IXa). Subsequent treatment with selenium dioxide effected oxidation followed by intramolecular condensation resulting in formation of the butenolide ring. Hydrolytic cleavage of the 3-acetates (VIb, Xb) with methanolic hydrochloric acid afforded the desired 3β-hydroxy-5α,13α-card-20(22)-enolides (VIa, Xa).

The chemical modification of the cardiotonic steroids has recently been developed by several groups and in consequence the structure-activity relationship has been disclosed.[3,4] Of the structural features of the naturally occurring steroids, the β-configuration at both C-14

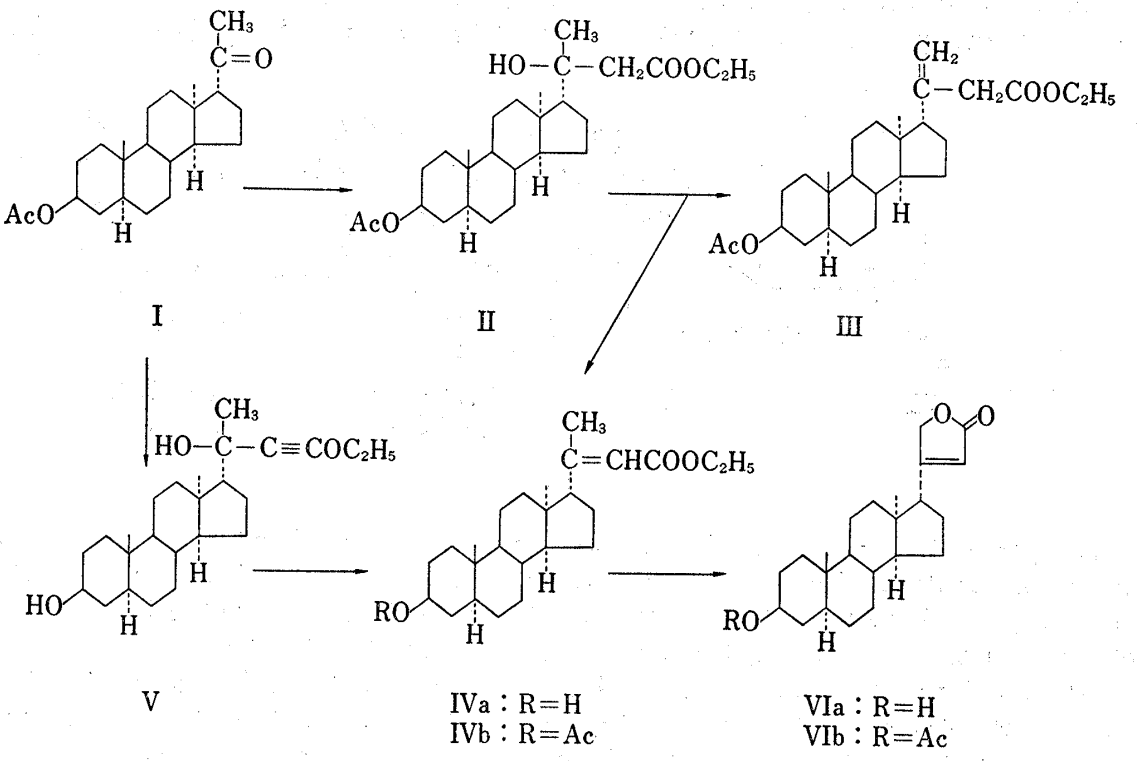

Chart 1

1) This paper constitutes Part XVI of the series entitled "Studies on Cardiotonic Steroid Analogs"; Part XV: K. Shimada, Y. Fujii, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **21**, 2183 (1973). A part of this work has been presented as a preliminary account: J. Goto and T. Nambara, *Chem. Ind.* (London), **1972**, 977.

2) Location: *Aobayama, Sendai.*

3) Ch. Tamm, "Proceedings of the 1st International Pharmacological Meeting," Vol. 3, ed. by W. Wilbrandt, Pergamon Press, Oxford, 1963, p. 11.

4) K. Meyer, *Planta Medica, Suppl.* 4, 2 (1971).

and C-17 has proved to be essential for the cardiotonic activity.[5] However, whether the cardenolides having an unnatural C/D-*cis* juncture, that is the 13α,14α-configuration, exhibit the physiological activity or not still remains unsolved. The present paper deals with the preparation of C-17 epimeric 3β-hydroxy-5α,13α-card-20(22)-enolides (VIa, Xa) starting from the 5α,13α-pregnane derivatives.

Building up of the butenolide ring was attempted without first introducing the 21-acetoxyl group. An initial effort was directed to the synthesis of the desired compound by the carboethoxymethyl carbinol method worked out by Ruzicka, *et al.*[6] Reformatsky reaction of 3β-hydroxy-5α,13α,17α-pregnan-20-one acetate (I)[7] with ethyl bromoacetate followed by reacetylation gave the required compound (II) in a satisfactory yield. Subsequent dehydration with acetic anhydride and potassium bisulfate gave a mixture of two isomeric α,β- and β,γ-unsaturated acid esters (IVb, III) in a ratio of *ca.* 1 to 1, judged from the nuclear magnetic resonance (NMR) spectrum. However, an attempt to isomerize III to IVb with alkali resulted in failure. Therefore, an alternative synthetic route *via* the ethoxyacetylenic carbinol as an intermediate[8] was then undertaken. Reaction of I with lithium ethoxyacetylide in ether-tetrahydrofuran (THF) gave rise to the 20-hydroxy-20-ethoxyacetylenic derivative (V), which exhibited a characteristic acetylenic band at 2270 cm^{-1} in the infrared (IR) spectrum. On brief exposure to sulfuric acid in aqueous methanol the ethoxyacetylenic carbinol (V) was isomerized with ease to yield ethyl 3β-hydroxy-23-*nor*-5α,13α,17α-chol-20(22)-enate (IVa). After reacetylation in the usual manner treatment with selenium dioxide in boiling acetic anhydride effected oxidation of the ester and intramolecular condensation to form the butenolide ring. Hydrolytic cleavage of the 3-acetate (VIb) with hydrochloric acid in methanol provided the desired 3β-hydroxy-5α,13α,17α-card-20(22)-enolide (VIa).

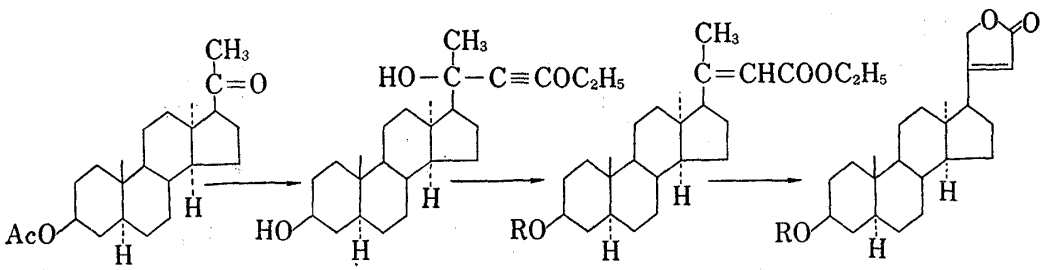

Chart 2

The preparation of 5α,13α,17β-cardenolide was then carried out by the same reaction sequence as for its C-17 epimer. First, 5α,13α,17β-pregnan-20-one (VII), which is thermodynamically much more unstable than its C-17 epimer,[7] was converted into the ethoxyacetylenic carbinol (VIII) by treatment with lithium ethoxyacetylide. Subsequent isomerization under mildly acidic condition afforded the α,β-unsaturated acid ester (IXa), which on usual acetylation was led to the 3-acetate (IXb). Oxidation of the ester followed by cyclization was effected by treatment with selenium dioxide in boiling acetic anhydride to result in formation of 5α,13α,17β-cardenolide (Xb). Hydrolysis of the acetoxyl group at C-3 was accomplished by means of methanolic hydrochloric acid to give the desired 3β-hydroxy-5α,-13α,17β-card-20(22)-enolide (Xa).

5) T. Shigei and S. Mineshita, *Experientia*, **24**, 466 (1968).
6) L. Ruzicka, Pl. A. Plattner, and J. Pataki, *Helv. Chim. Acta*, **25**, 425 (1942).
7) T. Nambara and J. Goto, *Chem. Pharm. Bull.* (Tokyo), **19**, 1937 (1971).
8) N. Danieli, Y. Mazur, and F. Sondheimer, *J. Am. Chem. Soc.*, **84**, 875 (1962); *idem*, *Tetrahedron*, **22**, 3189 (1966).

Both VIa and Xa showed a positive Kedde test characteristic of the butenolide ring. These two C-17 epimers could distinctly be differentiated each other by IR spectral comparison and mixed melting point measurement, though they exhibited the similar chromatographic behaviors.

The results of pharmacological test with these 5α,13α-cardenolides will be reported elsewhere in the near future.

Experimental[9]

Ethyl 3β-Acetoxy-20-hydroxy-23-*nor*-5α,13α,17α-cholanate (II)——To a solution of 3β-hydroxy-5α,13α,-17α-pregnan-20-one acetate (I)[7] (150 mg) in benzene (7 ml) was added activated granular Zn metal (250 mg), and the moisture was azeotropically removed by slow distillation to bring one-half of its volume. To the resulting solution was added ethyl bromoacetate (0.4 ml) and refluxed for 30 min. After removal of the precipitate by filtration the filtrate was diluted with AcOEt, washed with 5% HCl and H_2O, and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave an oily residue, which in turn was treated with Ac_2O (1 ml) and pyridine (1 ml) in the usual manner. The crude product thus obtained was chromatographed on Al_2O_3 (5 g). Elution with hexane–benzene (3: 1 to 1: 1) gave II (140 mg) as colorless oil. NMR (4% solution in $CDCl_3$) δ: 2.25 (2H, s, 22-CH_2), 4.20 (2H, q, J=13.6 Hz, -COOC$\underline{H}_2$CH$_3$). Analytical sample could not be obtained and therefore the crude product was submitted to further elaboration without purification.

Dehydration of II——To a solution of II (140 mg) in Ac_2O (6 ml) was added freshly fused $KHSO_4$ (130 mg) and heated at 90° for 1 hr. The reaction mixture was poured into ice-water and extracted with AcOEt. The organic layer was separated, washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up an oily product obtained was submitted to preparative thin-layer chromatography (TLC) using benzene–AcOEt (100: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.65) gave a mixture of ethyl 3β-acetoxy-23-*nor*-5α,13α,17α-chol-20(22)-enate (IVb) and ethyl 3β-acetoxy-23-*nor*-5α,13α,17α-chol-20(21)-enate (III) as colorless oil. NMR (4% solution in $CDCl_3$) δ: 2.17 (1.5H, s, 21-CH_3), 3.06 (1H, s, 22-CH_2), 4.16 (2H, q, J=13.5 Hz, -COOC$\underline{H}_2$CH$_3$), 4.98 (0.5H, broad s, 21-H), 5.10 (0.5H, broad s, 21-H), 5.65 (0.5H, broad s, 22-H).

3β-Hydroxy-5α,13α,17α-card-20(22)-enolide Acetate (VIb)——To a solution of LiMe in ether (1.1M, 60 ml) was added EtOC≡CH (3 ml) and stirred under a stream of N_2 gas for 40 min. To the resulting solution was added dropwise a solution of I (300 mg) in THF (10 ml) and stirred at room temperature overnight. The reaction mixture was poured into ice-water and extracted with ether. The organic layer was separated, washed with H_2O, and dried over anhydrous Na_2SO_4. After usual work-up a yellow oily product obtained was chromatographed on Al_2O_3 (12 g). Elution with benzene gave 20-ethoxyethynyl-5α,13α,17α-pregnane-3β,20-diol (V) (210 mg) as pale yellow amorphous substance. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2260 (C≡C). NMR (4% solution in $CDCl_3$) δ: 4.04 (2H, q, J=13.5 Hz, -C$\underline{H}_2$CH$_3$). To a solution of V (210 mg) in MeOH (9 ml) was added 20% H_2SO_4 (1 ml) and stirred at room temperature for 2 hr. The resulting solution was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was submitted to preparative TLC using benzene–AcOEt (10: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.40) gave ethyl 3β-hydroxy-23-*nor*-5α,13α,17α-chol-20(22)-enate (IVa) (110 mg) as colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 (C=O), 1630 (C=C). NMR (4% solution in $CDCl_3$) δ: 2.17 (3H, s, 21-CH_3), 4.16 (2H, q, J=13.5 Hz, -COOC$\underline{H}_2$CH$_3$), 5.65 (1H, s, 22-H). Treatment of IVa with Ac_2O (1 ml) and pyridine (1 ml) in the usual manner gave IVb (85 mg). To a boiling solution of IVb in Ac_2O (6 ml) was added an aq. solution of SeO_2 (100 mg in 1 ml) and refluxed for 50 min. After removal of the precipitate by filtration the filtrate was concentrated *in vacuo*. The crude product obtained was submitted to preparative TLC using benzene as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.40) which exhibited positive Kedde test was eluted with AcOEt. Recrystallization of the eluate from acetone–hexane gave VIb (22 mg) as colorless needles. mp 200 —202°. $[\alpha]_D^{23}$ −80.3° (c=0.06). *Anal.* Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.71; H, 9.08. IR ν_{max}^{KBr} cm^{-1}: 1775, 1748 (butenolide C=O), 1725 (C=O), 1615 (C=C). NMR (4% solution in $CDCl_3$) δ: 0.79 (6H, s, 18- and 19-CH_3), 2.02 (3H, s, 3β-OCOCH_3), 2.95 (1H, t, J=8.5 Hz, 17β-H), 4.65 (1H, m, 3α-H), 4.73 (2H, d, J=1.5 Hz, 21-CH_2), 5.82 (1H, t, J=1.5 Hz, 22-H).

3β-Hydroxy-5α,13α,17α-card-20(22)-enolide (VIa)——A solution of VIb (38 mg) dissolved in MeOH (15 ml)–10% HCl (10.5 ml) was allowed to stand at 35° overnight. The resulting solution was diluted with

9) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$. IR spectra were run on JASCO Model IR-S spectrophotometer. NMR spectra were recorded on Hitachi Model R-20A spectrometer at 60 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, t=triplet, q=quartet, and m=multiplet. For preparative thin-layer chromatography (TLC) Silica gel H or HF (E. Merck AG, Darmstadt) was used as an adsorbent.

AcOEt, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product obtained from acetone–hexane gave VIa (23 mg) as colorless prisms. mp 210—212°. $[\alpha]_D^{15}$ −38.5° (c=0.39). *Anal.* Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.97; H, 9.53. NMR (4% solution in $CDCl_3$) δ: 0.79 (6H, s, 18- and 19-CH_3), 2.97 (1H, t, J=8.5 Hz, 17β-H), 3.60 (1H, m, 3α-H), 4.76 (2H, d, J=1.5 Hz, 21-CH_2), 5.83 (1H, t, J=1.5 Hz, 22-H).

3β-Hydroxy-5α,13α,17β-card-20(22)-enolide Acetate (Xb)——To a solution of LiMe in ether (1.1M, 60 ml) was added EtOC≡CH (3 ml) and stirred under a stream of N_2 gas for 40 min. To the resulting solution was added dropwise a solution of 3β-hydroxy-5α,13α,17β-pregnan-20-one acetate (VII)[7] (280 mg) in THF (10 ml) and stirred at room temperature overnight. The reaction mixture was poured into ice-water and extracted with ether. The organic layer was separated, washed with H_2O, and dried over anhydrous Na_2SO_4. After usual work-up a yellow oily product obtained was submitted to preparative TLC using benzene–AcOEt (50: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.45) with AcOEt gave 20-ethoxyethynyl-5α,13α,17β-pregnane-3β,20-diol (VIII) (150 mg) as pale yellow oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2250 (C≡C). To a solution of VIII (140 mg) in MeOH (9 ml) was added 20% H_2SO_4 (1 ml) and stirred at room temperature for 2 hr. The resulting solution was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was submitted to preparative TLC using benzene–AcOEt (20: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.50) with AcOEt gave ethyl 3β-hydroxy-23-*nor*-5α,13α,17β-chol-20(22)-enate (IXa) (100 mg) as pale yellow oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 (C=O), 1630 (C=C). Treatment of IXa (90 mg) with Ac_2O (1 ml) and pyridine (1 ml) in the usual manner gave ethyl 3β-acetoxy-23-*nor*-5α,13α,17β-chol-20(22)-enate (IXb) (100 mg). To a boiling solution of IXb (90 mg) in Ac_2O (6 ml) was added an aq. solution of SeO_2 (100 mg in 1 ml) and refluxed for 30 min. After removel of the precipitate by filtration the filtrate was concentrated *in vacuo*. The crude product obtained was submitted to preparative TLC using benzene as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.40) which exhibited positive Kedde test was eluted with AcOEt. Recrystallization of the eluate from acetone–hexane gave Xb (26 mg) as colorless needles. mp 208—210°. $[\alpha]_D^{23}$ −70.3° (c=0.08). *Anal.* Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.76; H, 9.19. IR ν_{max}^{KBr} cm^{-1}: 1780, 1745 (butenolide C=O), 1725 (C=O), 1625 (C=C). NMR (4% solution in $CDCl_3$) δ: 0.74 (3H, s, 19-CH_3), 1.13 (3H, s, 18-CH_3), 2.01 (3H, s, 3β-$OCOCH_3$), 2.52 (1H, m, 17α-H), 4.65 (1H, m, 3α-H), 4.73 (2H, d, J=1.5 Hz, 21-CH_2), 5.80 (1H, t, J=1.5 Hz, 22-H).

3β-Hydroxy-5α,13α,17β-card-20(22)-enolide (Xa)——A solution of Xb (32 mg) dissolved in MeOH (12.5 ml)–10% HCl (8.5 ml) was allowed to stand at 35° overnight. The resulting solution was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product obtained from acetone–hexane gave Xa (19 mg) as colorless plates. mp 188—190°. $[\alpha]_D^{15}$ −25.9° (c=0.27). *Anal.* Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.55; H, 9.35. NMR (4% solution in $CDCl_3$) δ: 0.74 (3H, s, 19-CH_3), 1.13 (3H, s, 18-CH_3), 2.54 (1H, m, 17α-H), 3.60 (1H, m, 3α-H), 4.73 (2H, d, J=1.5 Hz, 21-CH_2), 5.81 (1H, t, J=1.5 Hz, 22-H).

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