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Synthesis of 16-Substituted 14,17-*cis*-5α-Cardenolides[1]

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The attempts were made to synthesize 16-substituted 14,17-*cis*-5α-cardenolides by the method worked out by Ruzicka, *et al.* starting from 16β-methyl-14α,17α- and 16α-cyano-14β,17β-pregnan-20-one derivatives (I and VI). Condensation with ethyl bromoacetate followed by dehydration gave the unsaturated acid esters, which on selenium dioxide oxidation were led to the desired cardenolides (IV and IX). However, Reformatsky reaction with 3β-acetoxy-16α-methyl-14β,17β-pregn-5-en-20-one (V) resulted in failure probably due to the steric interaction with the methyl group at C-16.

The chemical modification of the cardiotonic steroids has recently been developed by several groups and the structure–activity relationship has been disclosed.[3] As regards the hormonal steroids the biological activity can often be enhanced, when the suitable substituent is introduced to the steroid nucleus. It appeared, therefore, to be of particular interest to examine the effect of the various substituents on the physiological potency in the field of the cardiotonic steroids, although several attempts have already been made on this problem.[4] We now wish to report the preparation of 14,17-*cis*-5α-cardenolides having cyano or methyl groups at C-16 according to the method worked out by Ruzicka, *et al.*[5]

An initial project was directed to the synthesis of 16β-methyl-5α,14α,17α-cardenolide employing 3β-acetoxy-16β-methyl-5α,14α,17α-pregnan-20-one[6] (I) as the starting compound. Reformatsky reaction of I with ethyl bromoacetate and subsequent hydrolysis gave 3β,20-dihydroxy-16β-methyl-23-nor-5α,14α,17α-cholanic acid (IIa), which on treatment with diazomethane was led to the methyl ester (IIb) and then to the 3-acetate (IIc) by usual acetylation. Dehydration of IIc with use of acetic anhydride gave a mixture of α,β- and β,γ-unsaturated acid esters, which was subjected to further elaboration without separation. When the mixture was treated with potassium carbonate, the double bond isomerization and deacetylation did take place simultaneously to yield 23–norchol–20(22)–enate (IIIa) as a sole product. Oxidation of the 3-acetat (IIIb) with selenium dioxide and intramolecular condensation resulted in formation of the butenolide ring (IVb). Hydrolytic cleavage of the acetate with

1) This paper constitutes Part VIII of the series entitled, "Studies on Cardiotonic Steroid Analogs"; Part VII: T. Nambara and K. Shimada, *Chem. Pharm. Bull.* (Tokyo), **19**, 16 (1971).
2) Location: *Aobayama, Sendai.*
3) 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); T. Shigei and S. Mineshita, *Experientia*, **24**, 466 (1968); Ch.Tamm, "Proceedings of the 1st International Pharmacological Meeting," Vol. 3, ed. by W. Wilbrandt, Pergamon Press, Oxford, 1963, p. 11.
4) W. Fritsch, U. Stache, and H. Ruschig, *Ann.*, **699**, 195 (1966); N. Danieli, Y. Mazur, and F. Sondheimer, *Tetrahedron*, **23**, 715 (1967); H.-G. Lehmann and R. Wiechert, *Angew. Chem.*, **80**, 317 (1968); M. Okada and H. Hasunuma, *Yakugaku Zasshi*, **85**, 822 (1965); M. Okada, A. Yamada, Y. Saito, and M. Hasunuma, *Chem. Pharm. Bull.* (Tokyo), **14**, 496 (1966); M. Okada and Y. Saito, *ibid.*, **15**, 352 (1967); *idem, ibid.*, **17**, 515 (1969); Y. Saito, Y. Kanemasa, and M. Okada, *ibid.*, **18**, 629 (1970).
5) L. Ruzicka, Pl.A. Plattner, and J. Pataki, *Helv. Chim. Acta*, **25**, 425 (1942).
6) T. Nambara, K. Shimada, S. Goya, and J. Goto, *Chem. Pharm. Bull.* (Tokyo), **16**, 2236 (1968); R. Mickova and K. Syhora, *Collection Czech. Chem. Commun.*, **30**, 2771 (1965).

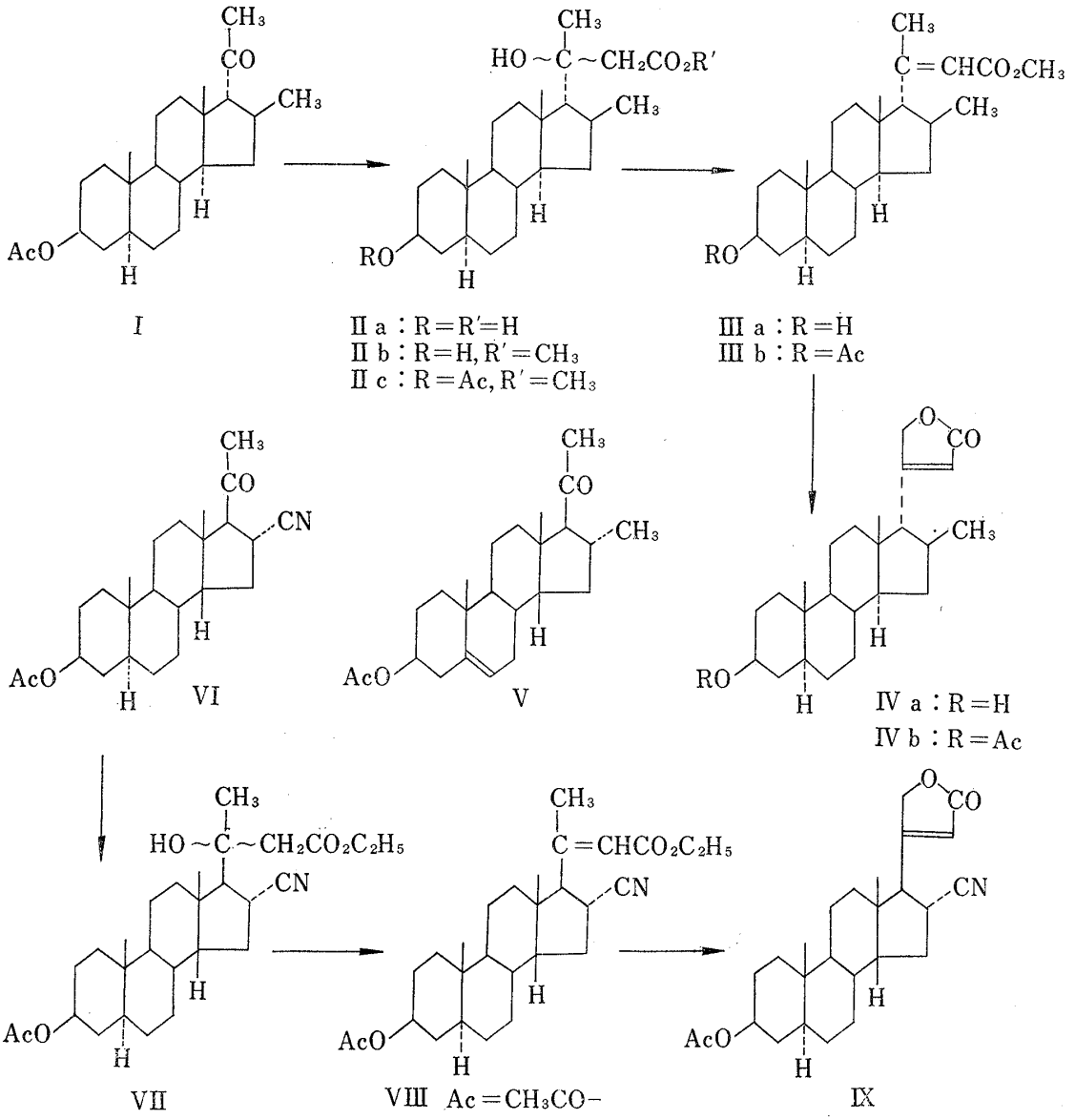

Chart 1

hydrochloric acid in methanol[7] furnished the desired 16β-methyl-5α,14α,17α-card-20(22)-enolide (IVa).

The next attempt was made to synthesize 16α-methyl-5α,14β,17β-cardenolide starting from 16α-methyl-14β,17β-pregn-5-enolone (V) by the same reaction sequence as mentioned above. Contrary to the expectations, however, Reformatsky condensation with V resulted in failure. The lack of reactivity may be ascribable to the steric interaction with the methyl group at C–16.

Accordingly the synthesis of 16α-cyano-5α,14β,17β-cardenolide was then undertaken using 16α-cyano-5α,14β,17β-pregnanolone[8] (VI) as the starting material. Condensation of VI with ethyl bromoacetate followed by acetylation gave ethyl 3β,20-dihydroxy-16α-cyano-23-nor-5α,14β,17β-cholanate 3-monoacetate (VII). Being refluxed in acetic anhydride with potassium bisulfate, VII was readily dehydrated to yield the unsaturated product, from which ethyl 3β-acetoxy-16α-cyano-23-nor-5α,14β,17β-chol-20(22)-enate (VIII) was separated

7) N. Danieli, Y. Mazur, and F. Sondheimer, *J. Am. Chem. Soc.*, **84**, 875 (1962); *idem*, *Tetrahedron*, **22**, 3189 (1966).
8) T. Nambara, S. Goya, J. Goto, and K. Shimada, *Chem. Pharm. Bull.* (Tokyo), **16**, 2228 (1968).

by means of thin-layer chromatography (TLC). Selenium dioxide oxidation of VIII proceeded with spontaneous ring-closure to give the desired 16α-cyano-5α,14β,17β-card-20(22)-enolide (IX) in satisfactory yield. The distinct difference in reactivities between V and VI hereby observed is of interest in suggesting that the steric requirement of the cyano group is much smaller than that of the methyl group.[8,9]

Pharmacological examinations using the Straub's frog heart preparation proved that IX possessed a definite cardiotonic activity, while IVa was inactive. The result indicates that β-configuration at C-14 and C-17 is essential for physiological activity and the presence of the cyano group at C-16 does not exert any significant influence on the potency of the parent cardenolide.[10]

Experimental[11]

Methyl 3β-Acetoxy-20-hydroxy-16β-methyl-23-nor-5α,14α,17α-cholanate (IIc)——To a suspension of activated Zn (previously washed with 10% HCl, H_2O, EtOH and acetone successively and dried) (1.7 g) in benzene (10 ml) were added 3β-acetoxy-16β-methyl-5α,14α,17α-pregnan-20-one (I) (1.18 g) and ethyl bromoacetate (5 g) and refluxed for 2 hr. The precipitate was removed by filtration, and ice-cooled 2% HCl was added to the filtrate to decompose the Zn-complex. The solution was extracted with ether, washed with 5% HCl, H_2O and dried over anhydrous Na_2SO_4. On usual work-up a brown oily residue was obtained. To a methanolic solution (17 ml) of the crude product was added 50% KOH (4 ml) and refluxed for 12 hr. The resulting solution was diluted with H_2O (5 ml) and concentrated *in vacuo*. The neutral substances were removed by ether extraction and the aq. layer was then acidified with 2N HCl. The separated crystalline product was extracted with ether, washed with H_2O and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave 3β,20-dihydroxy-16β-methyl-23-nor-5α,14α,17α-cholanic acid (IIa) (820 mg) as a brown oily product. The methyl ester (IIb) was prepared from IIa with CH_2N_2 in the usual way but not obtained in the crystalline state. The 3-monoacetate (IIc) prepared from IIb in the usual manner was not crystallized and therefore was submitted to further elaboration without purification. NMR (4% solution in $CDCl_3$) δ:0.82 (6H, s, 18-CH_3, 19-CH_3), 2.00 (3H, s, 3β-$OCOCH_3$), 2.85 (2H, s, $-CH_2COO-$), 3.70 (3H, s, $-COOCH_3$), 4.65 (1H, m, 3α-H).

Dehydration of Methyl 3β-Acetoxy-20-hydroxy-16β-methyl-23-nor-5α,14α,17α-cholanate (IIc)——A solution of IIc (460 mg) in Ac_2O (7 ml) was refluxed for 35 hr. After evaporation of solvent an oily residue (400 mg) obtained was chromatographed on Al_2O_3 (15 g). Elution with hexane-benzene (4:1 to 1:1) gave a mixture of IIIb and its isomer (260 mg). These two isomers could not be separated and therefore the mixture was submitted to further step. NMR (4% solution in $CDCl_3$) δ:1.93 (3H, s, 3β-$OCOCH_3$), 2.90 (1H, s, $-CH_2COO-$), 3.60 (3H, s, $-COOCH_3$), 4.60 (1H, m, 3α-H), 4.86 (0.5H, s, $>C=C<^{H}_{H}$), 5.02 (0.5H, s, $>C=C<^{H}_{H}$), 5.63 (0.5H, s, =CHCOO-).

Methyl 3β-Hydroxy-16β-methyl-23-nor-5α,14α,17α-chol-20(22)-enate (IIIa)——The above-mentioned mixture (260 mg) was dissolved in MeOH (5 ml)-10% K_2CO_3 (1 ml) and refluxed for 30 min. On usual work-up the crystalline product was obtained. Recrystallization from acetone gave IIIa (230 mg) as colorless needles. mp 145—146°. $[\alpha]_D^{18}$ +39.7° (c=0.29). *Anal.* Calcd. for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.07; H, 10.21. NMR (4% solution in $CDCl_3$) δ: 0.75 (3H, s, 19-CH_3), 0.87 (3H, s, 18-CH_3), 3.57 (4H, s, 3α-H, $-COOCH_3$), 5.50 (1H, s, =CHCOO-).

Methyl 3β-Acetoxy-16β-methyl-23-nor-5α,14α,17α-chol-20(22)-enate (IIIb)——Usual treatment of IIIa with pyridine and Ac_2O gave the 3-acetate (IIIb) as yellow oil. The crude product was submitted to further step without purification. NMR (4% solution in $CDCl_3$) δ:0.80 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3), 1.93 (3H, s, 3β-$OCOCH_3$), 2.13 (3H, s, 21-CH_3), 3.62 (3H, s, $-COOCH_3$), 4.50 (1H, m, 3α-H), 5.53 (1H, s, =CHCOO-).

3β-Acetoxy-16β-methyl-5α,14α,17α-card-20(22)-enolide (IVb)——To a refluxing solution of IIIb (200 mg) in Ac_2O (10 ml) was added dropwise an aq. solution (1 ml) of SeO_2 (200 mg) over a period of 30 min and further refluxed for 2 hr. After removal of the precipitate by filtration the filtrate was concentrated *in vacuo*. The residue thus obtained was submitted to the preparative TLC using benzene as developing sol-

9) N.L. Allinger and W. Szkrybalo, *J. Org. Chem.*, **27**, 4601 (1962); C. Djerassi, R.A. Schneider, H. Vorbruggen, and N.L. Allinger, *ibid.*, **28**, 1632 (1963).

10) M. Okada and Y. Saito, *Chem. Pharm. Bull.* (Tokyo), **16**, 2223 (1968).

11) All melting points were taken on a micro hot-stage apparatus and are uncorrected. The optical rotations were measured in $CHCl_3$ unless otherwise specified. The infrared spectral measurements were run on JASCO Model IR-S spectrophotometer. The nuclear magnetic resonance spectra were obtained on Hitachi Model H-60 spectrometer at 60 Mc using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, q=quartet, and m=multiplet.

vent. The adsorbent corresponding to the spot (*Rf* 0.30) exhibiting positive reaction with Kedde reagent was eluted with AcOEt. Recrystallization of the eluate from MeOH gave IVb (63 mg) as colorless plates. mp 160—161°. $[\alpha]_D^{28}$ +40.7° (c=0.11). *Anal.* Calcd. for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 75.07; H, 9.35. NMR (4% solution in $CDCl_3$) δ:0.80 (3H, s, 19-CH_3), 0.95 (3H, s, 18-CH_3), 1.99 (3H, s, 3β-OCOCH_3), 4.65 (2H, d, J=2 cps, 21-CH_2), 4.65 (1H, m, 3α-H), 5.79 (1H, s, 22-H).

3β-Hydroxy-16β-methyl-5α,14α,17α-card-20(22)-enolide (IVa)——A solution of IVb (40 mg) dissolved in MeOH (10 ml)–10% HCl (7 ml) was allowed to stand at 30° for 48 hr. The resulting solution was diluted with ether, washed with H_2O and dried over anhydrous Na_2SO_4. On usual work–up a crystalline product was obtained. Recrystallization from AcOEt gave IVa (20 mg) as colorless prisms. mp 222.5—225°. $[\alpha]_D^{23}$ +23.6° (c=0.15). *Anal.* Calcd. for $C_{24}H_{36}O_3$: C, 77.37; H, 9.74. Found: C, 77.32; H, 9.89. NMR (4% solution in $CDCl_3$) δ:0.80 (3H, s, 19-CH_3), 0.95 (3H, s, 18-CH_3), 3.50 (1H, m, 3α-H), 4.65 (2H, d, J=2 cps, 21-CH_2), 5.75 (1H, s, 22-H).

Ethyl 3β-Acetoxy-16α-cyano-23-nor-5α,14β,17β-chol-20(22)-enate (VIII)——To a solution of 3β-acetoxy-16α-cyano-5α,14β,17β-pregnan-20-one (VI) (600 mg) in benzene (10 ml) was added activated Zn (900 mg) and the moisture was azeotropically removed by slow distillation. To this solution was added ethyl bromoacetate (2.5 ml) and refluxed for 2 hr. The reaction mixture was extracted with ether, washed with 5% HCl, H_2O and dried over anhydrous Na_2SO_4. Evaporation of solvent gave an oily residue, which in turn was treated with Ac_2O and pyridine. After usual work–up the crude product was chromatographed on Al_2O_3 (20 g). Elution with hexane–benzene (1:2 to 1:3) gave ethyl 3β-acetoxy-20-hydroxy-16α-cyano-23-nor-5α,14β,17β-cholanate (VII) (230 mg) as colorless oil. NMR (4% solution in $CDCl_3$) δ:2.53 (2H, s, –CH_2COO–), 4.17 (2H, q, J=6.9 cps, –COO$\underline{CH_2}CH_3$). To a solution of VII (230 mg) in Ac_2O (10 ml) was added freshly fused $KHSO_4$ (200 mg) and heated at 90° for 2.5 hr. The reaction mixture was poured into ice–water and extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent an oily product was submitted to preparative TLC using benzene–AcOEt (50:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.65) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave VIII (90 mg) as colorless plates. mp 164.5—166°. $[\alpha]_D^{26}$ −26.5° (c=0.17). *Anal.* Calcd. for $C_{28}H_{41}O_4N$: C, 73.81; H, 9.07; N, 3.07. Found: C, 73.56; H, 8.83; N, 3.20. IR ν_{max}^{KBr} cm^{-1}: 2227 (C≡N), 1733 (C=O), 1715 (C=O), 1637 (C=C). NMR (4% solution in $CDCl_3$) δ:0.80 (3H, s, 19-CH_3), 0.91 (3H, s, 18-CH_3), 1.93 (3H, s, 3β-OCOCH_3), 2.12 (3H, s, 21-CH_3), 2.54 (1H, d, J=6 cps, 17α-H), 2.75 (1H, m, 16β-H), 4.05 (2H, q, J=6.9 cps, –COO$\underline{CH_2}CH_3$), 4.55 (1H, m, 3α-H), 5.55 (1H, s, =CHCOO–).

3β-Acetoxy-16α-cyano-5α,14β,17β-card-20(22)-enolide (IX)——To a refluxing solution of VIII (90 mg) in Ac_2O (7 ml) was added dropwise an aq. solution (0.5 ml) of SeO_2 (100 mg). After 2 and 4 hr an aq. solution (0.5 ml) of SeO_2 (100 mg) was added, respectively and further refluxed for 2 hr. The precipitate was removed by filtration and the filtrate was concentrated *in vacuo*. A colorless oil thus obtained was submitted to preparative TLC using benzene–AcOEt (20:1) as developing solvent. The adsorbent corresponding to the spot exhibiting the positive reaction with Kedde reagent was eluted with AcOEt. Recrystallization of the eluate from MeOH gave IX (22 mg) as colorless plates. mp 212—213°. $[\alpha]_D^{28}$ −33.3° (c=0.09). *Anal.* Calcd. for $C_{26}H_{35}O_4N$: C, 73.38; H, 8.29; N, 3.29. Found: C, 73.28; H, 8.13; N, 3.48. IR ν_{max}^{KBr} cm^{-1}: 2232 (C≡N), 1776, 1745 (butenolide C=O), 1730 (C=O), 1629 (C=C). NMR (4% solution in $CDCl_3$) δ:0.80 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3), 2.00 (3H, s, 3β-OCOCH_3), 2.58 (1H, d, J=6 cps, 17α-H), 2.60 (1H, m, 16β-H), 4.60 (1H, m, 3α-H), 4.73 (2H, d, J=1.7 cps, 21-CH_2), 5.84 (1H, s, 22-H).

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