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Synthesis of 3β-Hydroxy-5α-card-20(22)-enolide (14-Deoxy-14β-uzarigenin)[1]

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The synthesis of 3β-hydroxy-5α-card-20(22)-enolide (14-deoxy-14β-uzarigenin) (IX) was accomplished using 3β-acetoxy-5α,14β,17α-pregnan-20-one (I) derivable from Δ^{16}-pregnenolone acetate as the starting material. The synthesis involved an inversion of the side chain at C_{17} (I→V) by Serini–Logemann reaction, Reformatsky condensation of V with ethyl bromoacetate, selenium dioxide oxidation to the butenolide (VIII) followed by saponification with acid.

Isomerization of IX to 3β-hydroxy-5α,17α-card-20(22)-enolide (14-deoxy-14β,17α-uzarigenin) (XI) was described, which was also prepared from I.

The most distinguished structural features essential for the cardiotonic activity of cardenolides are: *cis*–fusion of the C and D rings, hydroxy group in position 14β, and a five–membered unsaturated lactone (butenolide) ring having β–configuration at C_{17}.[3] In order to examine whether the 14β-hydroxyl group is the essential structural requirement for the carditonic activity or not, an attempt has been made to synthesize the cardenolide lacking in the hydroxyl group concerned.

Preparation of a cardenolide of this type, 3β-acetoxy-15-oxo-5β-card-20(22)-enolide (14-deoxy-14β-15-oxo-digitoxigenin acetate), was reported which was derived from digitoxigenin.[4] In view of the fact, however, that the oxygen functions at C_{15} do affect markedly the cardiotonic activity of digitoxigenin,[5] the above cardenolide having 15-oxo group would not be an apt model compound anyhow for examining the structure–activity relationship of the cardenolide on the concerning subject matter. The present work describes the synthesis of 3β-hydroxy-5α-card-20(22)-enolide (14-deoxy-14β-uzarigenin) (IX), which is constructed on an entirely similar principle to that used earlier in the synthesis of 3β-hydroxy-5α,14α,17α-card-20(22)-enolide (14-deoxy-14α,17α-uzarigenin).[6] Thus, 3β-acetoxy-5α,14β,17α-pregnan-20-one[7] (I) was singled out as the starting material which is derivable from Δ^{16}-pregnenolone acetate through 3β-acetoxy-5,14,16-pregnatrien-20-one.[8]

Enol acetylation of I was carried out in the usual way with acetic anhydride and *p*-toluenesulfonic acid to give the enol acetate (II), which was not obtained crystalline. Epoxidation of II with perbenzoic acid and subsequent reduction with lithium aluminum hydride followed

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[2] Location: *Takada 3-Chome, Toshima-ku, Tokyo.*

[3] Ch. Tamm, "Proc. 1st Internat. Pharmacol. Meeting," Vol. 3, ed. by W. Wilbrandt, Pergamon Press, Oxford, 1963, p. 11.

[4] H. Ishii, T. Tozyo, and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **11**, 576 (1963); Ch.R. Engel and G. Bach, *Steroids*, **3**, 593 (1964).

[5] *a*) M. Okada and M. Hasunuma, *Yakugaku Zasshi*, **85**, 822 (1965); *b*) M. Okada and Y. Saito, *Chem. Pharm. Bull.* (Tokyo), **15**, 352 (1967); *c*) T. Shigei, M. Katori, H. Murase, and S. Imai, *Experientia*, **20**, 572 (1964); *d*) S. Imai, H. Murase, M. Katori, M. Okada, and T. Shigei, *Jap. J. Pharmacol.*, **15**, 62 (1965); *e*) T. Shigei and S. Mineshita, *Experientia*, **24**, 466 (1968).

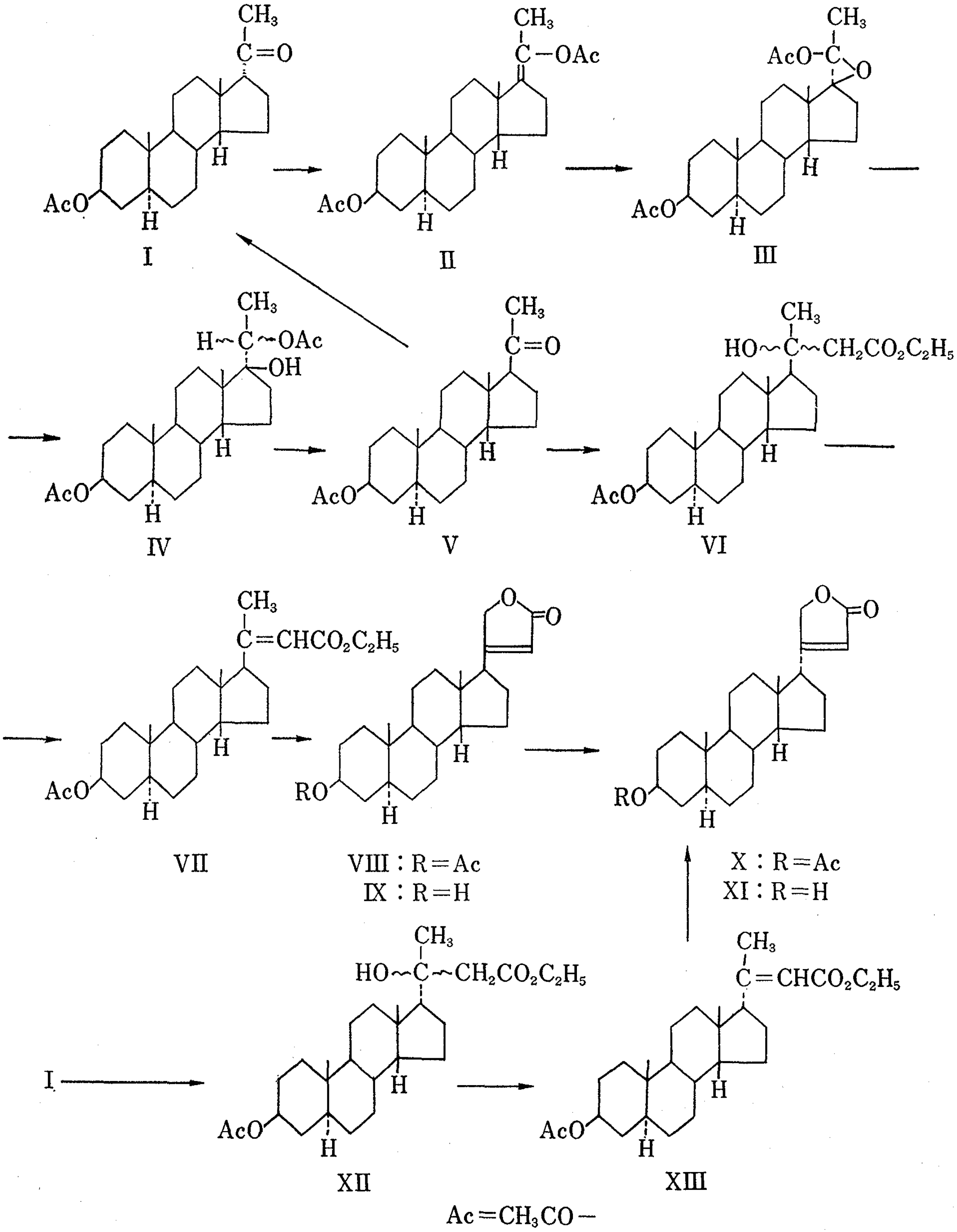
[6] M. Okada and Y. Saito, *Steroids*, **6**, 357 (1965).

[7] Pl.A. Plattner, H. Heusser, and A. Segre, *Helv. Chim. Acta*, **31**, 249 (1948).

[8] A.J. Solo and B. Singh, *J. Org. Chem.*, **30**, 1658 (1965).

by acetylation afforded crystalline 5α,14β,17α-pregnane-3β,17,20-triol 3,20-diacetate (IV), presumably a mixture of 20α- and 20β-acetoxy isomers. The intermediate compound (III) was obtained as an oil and not characterized. Since the starting material is a steroid having C/D *cis*-fusion, β-epoxide and 17β-hydroxy-17α-pregnane structures were reasonably assigned to III and IV respectively. These assignments have been confirmed on the basis of the following finding.

Thus, in accordance with expectation treatment of IV in refluxing xylene with activated zinc (Serini–Logemann reaction[9]) gave 3β-acetoxy-5α,14β-pregnan-20-one (V). The 14β,



Ac=CH_3CO−

Chart 1

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[9] *a*) M.B. Rubin and E.C. Blossey, *Steroids*, **1**, 453 (1963); *b*) M.B. Rubin, *ibid.*, **2**, 561 (1963).

17β-configuration of V was established by converting it into the more stable 17α-isomer (I) on acid treatment in ethanol.[10)]

Reformatsky condensation of V with ethyl bromoacetate yielded ethyl 3β, 20-dihydroxy-23-nor-5α,14β-cholanate 3-monoacetate (VI), which was obtained as an oily product after chromatographic purification. Dehydration of VI in boiling acetic anhydride followed by selenium dioxide oxidation of the resulting compound, ethyl 3β-acetoxy-23-nor-5α,14β-chol-20(22)-enate (VII), afforded the butenolide (VIII). Deacetylation of VIII with hydrochloric acid in methanol[11)] gave the desired cardenolide (IX). The assignment of β-configuration of the butenolide ring at C_{17} to IX could further be confirmed by converting IX into 3β-hydroxy-5α,17α-card-20(22)-enolide (14-deoxy-14β,17α-uzarigenin) (XI) under conditions employed for the isomerization of 17β-cardenolide to 17α-cardenolide.[12)]

The reference cardenolide (XI), on the other hand, was prepared in the unequivocal way, subjecting I to Reformatsky condensation, dehydration in boiling acetic anhydride, selenium dioxide oxidation, and deacetylation of the resulting butenolide (X) successively as described above in the synthesis of IX. In this connection, one of the two side products obtained earlier[13)] in the syntheses of uzarigenin acetate and 17α-uzarigenin acetate has now been shown to be identical as expected with 3β-acetoxy-5α, 17α-card-20(22)-enolide (X) by direct comparison with the authentic sample.

Pharmacological examinations using the isolated frog's heart (Straub's preparation) disclosed that IX possessed a definite cardiotonic activity, although it was fairly less potent than uzarigenin.[5e)]

Experimental[14)]

3β-Acetoxy-5α,14β-pregnan-20-one (V)——A solution of 3β-acetoxy-5α,14β,17α-pregnan-20-one (I) (mp 100—106°, 2 g) and *p*-toluenesulfonic acid (1.26 g) in acetic anhydride (300 ml) was distilled slowly through a short column for 4 hr, the final volume being about 100 ml. Most of the acetic anhydride was removed *in vacuo*, and the residue was dissolved in ether, which was washed with ice-cold 5% Na_2CO_3 to remove any acid present, and finally with H_2O, and dried over anhyd. Na_2SO_4. Evaporation of ether gave 2.3 g of the crude enol acetate (II) as an yellow oil, whose thin-layer chromatography (TLC) indicated the absence of the starting material (I). This oily product (II) was used for the next reaction without further purification. To a solution of II in 45 ml of $CHCl_3$ was added 45 ml of $CHCl_3$ solution of perbenzoic acid (22.1 mg/ml). The mixture was allowed to stand at room temperature for 1 hr. After addition of 100 ml of $CHCl_3$ to the reaction mixture, the $CHCl_3$ solution was washed with ice-cold 5% Na_2CO_3, H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave 2.6 g of an oily residue, which was dissolved in a mixture of anhydrous benzene (40 ml) and anhydrous ether (360 ml). To this mixture solution $LiAlH_4$ (4.4 g) was added for 30 min with stirring. After stirring for 1 hr the reaction mixture was heated to boiling for 5 min, and then AcOEt and 5% HCl were added. The organic layer was washed with H_2O, 5% Na_2CO_3 and again with H_2O, and dried over anhyd. Na_2SO_4. Evaporation of the solvent afforded a residue (1.6 g), which was acetylated in the usual way with acetic anhydride and pyridine. The resulting product was crystallized from petroleum ether containing a trace of acetone to give 1.1 g of 5α,14β,17α-pregnane-3β,17,20-triol 3, 20-diacetate (IV), mp 186—189°, IR ν_{max} cm^{-1}: 3552 (OH), 1730, 1715, 1260 (br) (acetoxyl).

A solution of IV (4 g) in distilled xylene (400 ml) was refluxed for 33 hr in a nitrogen atmosphere with freshly activated zinc[9a)] (80 g) while stirring vigorously. The solution was cooled, and the zinc filtered and washed with benzene. The combined filtrates were concentrated under reduced pressure to give 3.6 g

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10) C.W. Shoppee and T. Reichstein, *Helv. Chim. Acta*, **23**, 729 (1940).

11) N. Danieli, Y. Mazur, and F. Sondheimer, *J. Am. Chem. Soc.*, **84**, 875 (1962); *Idem*, *Tetrahedron*, **22**, 3189 (1966).

12) A. Kuritzkes, J. von Euw, and T. Reichstein, *Helv. Chim. Acta*, **42**, 1502 (1959).

13) M. Okada and Y. Saito, *Steroids*, **6**, 645 (1965).

14) Melting points were determined on a Kofler block and are uncorrected. UV spectra were measured in 99% ethanol solution. IR spectra were determined in KBr disks on Hitachi EPI-S2 spectrophotometer; br=broad, sh=shoulder. TLC plate was prepared according to the Stahl's procedure using aluminum oxide G (E. Merck AG) as adsorbent. The solvent system used was benzene-MeOH (25:1) and the steroids spots were revealed by heating plate at 110° for 10 min after spraying it with 95% H_2SO_4.

of an oily residue, which was crystallized from petroleum ether containing a trace of acetone to give 1.8 g of V. mp 102—103°, $[\alpha]_D^{25}$ +45.8° (c=1.18, $CHCl_3$), IR $\nu_{\max}$ cm^{-1}: 1724 (acetyl C=O), 1700 (20-ketone), 1234 (C–O). *Anal.* Calcd. for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found; C, 76.58; H, 10.30.

Isomerization of 3β-Acetoxy-5α,14β-pregnan-20-one (V) to 3β-Acetoxy-5α,14β,17α-pregnan-20-one (I)——A solution of V (12 mg) in a mixture of 35% HCl (0.05 ml) and EtOH (0.5 ml) was heated under reflux for 30 min. After adding H_2O (1 ml), EtOH was removed *in vacuo* to afford crystalline precipitate, which was acetylated in the usual way with acetic anhydride and pyridine. The acetate was recrystallized from MeOH to give 7 mg of I, mp 101—106°, identical with the starting material (I) in the mixed melting point and comparison of the IR spectrum.

3β-Acetoxy-5α-card-20(22)-enolide (VIII)——To powdered zinc (2.7 g) (previously washed with 2% HCl, H_2O, EtOH, acetone, anhydrous ether, and dried) and several crystals of iodine in benzene (6 ml) were added 1.8 g of V and 6.8 g of ethyl bromoacetate. After the mixture was refluxed with stirring for 10 min, further addition of a few crystals of iodine was made, and the stirring and refluxing were continued for 30 min. The mixture was then cooled to room temperature, and zinc was removed by filtration. To the filtrate was added ice-cold 2% HCl for hydrolyzing. Ether was then added for extracting reaction product and the organic layer separated was washed with 5% Na_2CO_3 and H_2O, and dried over anhyd. Na_2SO_4. Evaporation of solvent gave a brown oily residue (3.1 g), which was chromatographed on a column of acid-washed alumina by successive elution with petroleum ether and petroleum ether–benzene mixtures. Without further purification the oily fraction (1.2 g) eluted was dissolved in acetic anhydride (24 ml) and the solution was heated under reflux for 2.5 hr. Evaporation of the solvent yielded an oily residue, which was chromatographed on a column of acid-washed alumina. Elutions with petroleum ether and petroleum ether–benzene mixtures gave 0.5 g of crude ethyl 3β-acetoxy-23-nor-5α,14β-chol-20(22)-enate (VII) as an oil, whose IR spectrum exhibited an absorption band at 1628 cm^{-1} indicative of a double bond ($\Delta^{20(22)}$).

To a solution of VII (0.47 g) in acetic anhydride (30 ml) was added dropwise a solution of 0.47 g of SeO_2 in 2.3 ml of H_2O for 30 min under refluxing. After the addition was complete, the refluxing was continued for 2 hr. After filtration of deposited selenium, the filtrate was concentrated under reduced pressure to dryness. The residue was chromatographed on a column of acid-washed alumina by successive elution with petroleum ether and petroleum ether–benzene mixtures (4:1, 2:1, and 1:1). The fraction eluted with petroleum ether–benzene (1:1), giving a positive Kedde reaction, was concentrated to afford about 100 mg of crystalline residue, which was recrystallized from MeOH to give 60 mg of VIII. mp 223—228°, $[\alpha]_D^{23}$ +20.6° (c=0.73, $CHCl_3$), UV $\lambda_{\max}$ mμ (log ε): 216.5 (4.19), IR $\nu_{\max}$ cm^{-1}: 1780, 1740 (butenolide C=O), 1725 (sh) (acetyl C=O), 1624 (C=C), 1236 (C–O). *Anal.* Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.68; H, 9.33.

3β-Hydroxy-5α-card-20(22)-enolide (14-Deoxy-14β-uzarigenin) (IX)——A solution of VIII (55 mg) in a mixture of MeOH (58 ml) and 10% HCl (58 ml) was allowed to stand for 18 hr at room temperature. After addition of H_2O (60 ml), MeOH was removed *in vacuo* to yield crystalline precipitate. Recrystallization from MeOH gave IX (36 mg). mp 237—240°, $[\alpha]_D^{25}$ +25.7° (c=0.70, MeOH), UV $\lambda_{\max}$ mμ (log ε): 216.5 (4.19), IR $\nu_{\max}$ cm^{-1}: 3530 (OH), 1780, 1730, 1625 (butenolide). *Anal.* Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.32; H, 9.65.

Acetylation of IX in the usual way gave VIII, mp 223—228°, which was identical with the above sample of VIII in the mixed melting point and comparison of the IR spectrum.

Isomerization of 3β-Hydroxy-5α-card-20(22)-enolide (IX) to 3β-Hydroxy-5α,17α-card-20(22)-enolide (XI)——To a solution of IX (20 mg) in dimethylformamide (6 ml, freshly redistilled under reduced pressure) were added anhyd. AcONa (25 mg) and sodium tosylate(62 mg). The mixture was heated for 23 hr at 115°, and then poured into ice-water. The product was extracted with a mixture of ether–$CHCl_3$ (3:1). The organic layer was washed with 2% HCl, H_2O and dried over anhyd. Na_2SO_4. After evaporation of solvent the residue (15.7 mg)was chromatographed on a column of acid-washed alumina by successive elution with benzene and benzene–AcOEt (4:1). The fraction eluted with benzene–AcOEt (4:1) was concentrated to give a crystalline residue (10.7 mg), which was recrystallized repeatedly from MeOH. Approximately 1 mg of XI, mp 235—242°, was obtained besides 1.5 mg of the pure starting material (IX). The melting point of the mixture with the sample prepared from I as described below showed no depression, and the IR spectra of the two samples were identical in all respects.

3β-Acetoxy-5α,17α-card-20(22)-enolide (X)——To powdered zinc (0.5 g) (activated as described above) and several crystals of iodine in benzene (1.5 ml) were added 340 mg of I and 1.3 g of ethyl bromoacetate. After working up in the same way as described above, the resulting oily product was chromatographed on a column of acid-washed alumina by successive elution with petroleum ether, petroleum ether–benzene (1:1), and benzene to afford 330 mg of XII as an oil, which was dissolved in 7 ml of acetic anhydride. The solution was heated under reflux for 47 hr. Evaporation of the solvent afforded 352 mg of a brown oily residue, which was chromatographed on a column of acid-washed alumina. Elutions with petroleum ether, petroleum ether–benzene (1:1), and benzene gave 256 mg of crude ethyl 3β-acetoxy-23-nor-5α,14β,17α-chol-20(22)-enate (XIII) as an oil, whose IR spectrum exhibited an absorption band at 1627 cm^{-1} indicative of a double bond ($\Delta^{20(22)}$).

To a solution of XIII (256 mg) in acetic anhydride (20 ml) was added dropwise a solution of 257 mg of SeO_2 in 1.3 ml of H_2O for 30 min under refluxing. After working up in the same way as described above, the resulting product was chromatographed on a column of acid–washed alumina. The column was eluted successively with petroleum ether, petroleum ether–benzene (2:1, 1:1 and 1:2). The fraction eluted with petroleum ether–benzene (1:1 and 1:2), giving a positive Kedde reaction, was concentrated to give 73 mg of crystalline product, which was recrystallized from MeOH to afford 45 mg of X. mp 192—196°, $[\alpha]_D^{25}$ +74.5° (c=0.93, $CHCl_3$), UV λ_{max} mμ (log ε): 217.5 (4.19). IR ν_{max} cm^{-1}: 1774, 1738 (br), 1624 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 75.19; H, 9.29.

A side product (mp 194—196°) reported earlier[13)] was shown to be identical with X in the mixed melting point and comparison of the IR spectrum.

3β-Hydroxy-5α,17α-card-20(22)-enolide (14-Deoxy-14β,17α-uzarigenin) (XI)——A solution of X (35 mg) in a mixture of MeOH (40 ml) and 10% HCl (40 ml) was allowed to stand for 18 hr at room temperature. After working up in the way described above, the saponified product was recrystallized from MeOH to give 16 mg of XI. mp 240—245°, $[\alpha]_D^{25}$ +71.6° (c=0.29, MeOH), UV λ_{max} mμ (log ε): 217.5 (4.20), IR ν_{max} cm^{-1}: 3500 (OH), 1780, 1742, 1619 (butenolide). *Anal.* Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.77; H, 9.53.

Acetylation of XI in the usual way afforded X, mp 192—195°, which was identical with the above sample of X in the mixed melting point and comparison of the IR spectrum.

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