[Chem. Pharm. Bull. 14(5) 496~501 (1966)]

UDC 615.711-011 : 547.92

69. Masashi Okada, Atsushi Yamada, Yukio Saito, and Mieko Hasunuma : Studies on Synthesis of Epoxides of Anhydrocardenolides and on Their Cleavage. II.*1 Synthesis of Epoxides of Anhydrogitoxigenins and Their Cleavage.*2

(*Tokyo Biochemical Research Institute*3)*)

In the preceding paper[1] the preparation of epoxides of β-anhydrodigitoxigenin and their cleavage were reported. This paper is concerned with the preparation and cleavage of epoxides derivable from anhydrogitoxigenins.

Epoxidation of $\Delta^{14,16}$-dianhydrogitoxigenin (I) with 1.2 molar equivalents of perbenzoic acid yielded 3β-hydroxy-14β, 15β-epoxy-5β-carda-16, 20(22)-dienolide (III), which exhibited a band at 276 mμ (log ε 4.18) in the ultraviolet absorption spectrum, indicating the presence of a carda-16,20(22)-dienolide structure. Its acetate (IV)[2] was identical with the compound prepared from the acetate(II) of I by epoxidation. The 14β,15β-epoxide structure was assigned to III and IV on the basis of several observations[3] regarding epoxidation of steroids having a $\Delta^{14,16}$-diene system. This assignment has been established as described below.

An attempt to open the epoxide (III) reductively using sodium borohydride in slightly alkaline condition was made in order to obtain Δ^{16}-anhydrogitoxigenin, since reductive opening of the 14β,15β-epoxide of marinobufagin by sodium borohydride had been reported.[4] The product (V) obtained in this reduction, however, exhibited a band at 215 mμ (log ε 4.14) in the ultraviolet spectrum, indicating hydrogenation of the 16(17)-double bond of III. Moreover, the acetate (VI) prepared from V in the usual way did not exhibit any band due to hydroxyl group absorption in the infrared spectrum. Compounds (V and VI)*4 were found to be different from 14α,15α-epoxy-β-anhydrodigitoxigenin as well as 14β,15β-epoxy-β-anhydrodigitoxigenin and their acetates,[1,5,6] respectively. Based on these findings, the 14β,15β-epoxy-17α-card-20(22)-enolide structure was given to V. Hydrogenation of the 16(17)-double bond by sodium borohydride had been indicated previously.[7]

Hydrogenation of III over palladium-on-charcoal on the other hand, yielded a mixture which was separated into two fractions through alumina chromatography. A substance obtained from the first fraction gave a monoacetate which did not exhibit any hydroxyl band in the infrared spectrum. The same substance was obtained by hydrogenation of I over palladium-on-charcoal. Thus the 3β-hydroxy-5β,17α-card-20(22)-enolide structure (VII) was tentatively assigned[8] to this substance on the basis of some earlier observations[3] concerning hydrogenation of the steroidal $\Delta^{14,16}$-diene system. The second fraction was found to be a mixture by thin-layer chromatography, and it was

*1 Part I. M. Okada, M. Hasunuma : Yakugaku Zasshi, **85**, 822 (1965).

*2 This paper was presented at the Kanto Branch Meeting of the Pharmaceutical Society of Japan, Tokyo, December 15th, 1962.

*3 Takadaminamicho 2-593, Toshima-ku, Tokyo (岡田正志, 山田 厚, 斉藤行生, 蓮沼美恵子).

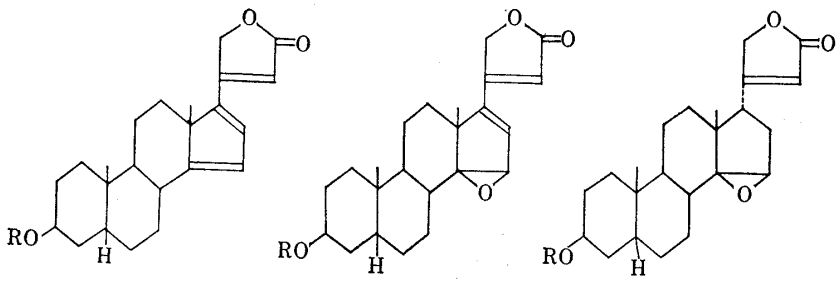
*4 Preparation of VI from IV using somewhat different conditions from those employed in the present work has been reported recently.[2]

1) M. Okada, M. Hasunuma : Yakugaku Zasshi, **85**, 822 (1965).

2) After completion of the present work the preparation of IV from II was reported. T. Wada, D. Satoh : This Bulletin, **13**, 308 (1965).

3) For reviews, see R.B. Turner : Chem. Revs., **43**, 1 (1948); H. Heusser: Fortschr. Chem. org. Naturstoffe, **7**, 87 (1950).

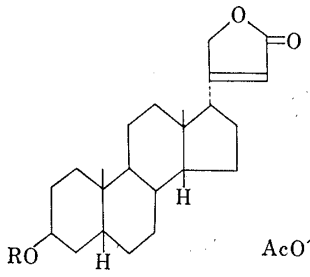
4) M. Bharucha, H. Jäger, K. Meyer, T. Reichstein, O. Schindler : Helv. Chim. Acta, **42**, 1395 (1959).



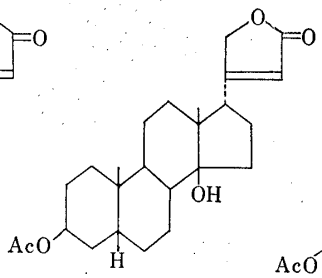
I : R=H
II : R=Ac

III : R=H
IV : R=Ac

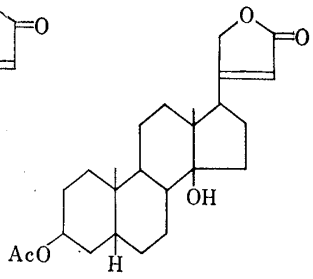
V : R=H
VI : R=Ac



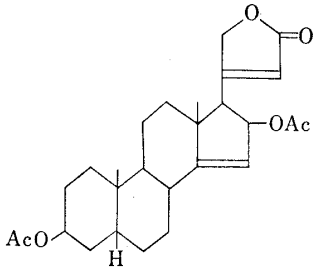
VII : R=H
VIII : R=Ac



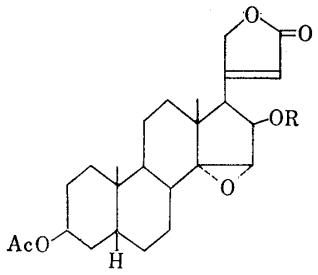
IX



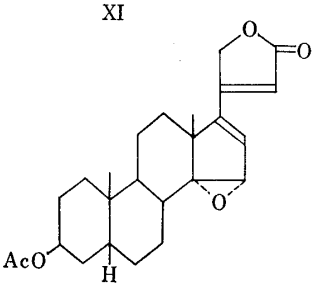
X



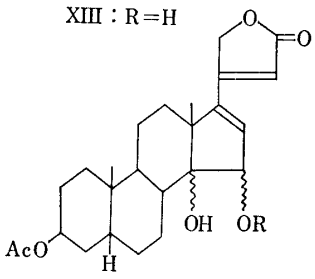
XI



XII : R=Ac
XIII : R=H



XIV



XV : R=H
XVI : R=Ac

Chart 1.

5) P. Hofer, H. Linde, K. Meyer: Helv. Chim. Acta, **45**, 1041 (1962).
6) H. Ishii, T. Tozyo, D. Satoh : This Bulletin, **11**, 576 (1963).
7) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler, W. M. McLamore : J. Am. Chem. Soc., **74**, 4223 (1952).
8) Definitive evidence for the assigned structure and configuration has been presented. T. Wada, D. Satoh : This Bulletin, **11**, 544 (1963); T. Wada; *Ibid.*, **13**, 312 (1965).

acetylated. Direct crystallization of the acetylated mixture afforded a substance which was identical with 17α-digitoxigenin acetate[9] (=menabegenin acetate[10]) (IX). From the mother liquor of IX, which showed two spots corresponding to IX and digitoxigenin acetate (X) on thin-layer chromatography, X and IX were obtained through preparative thin-layer chromatography. The formation of these 14β-hydroxylated compounds by hydrogenation of III confirmed the structure and configuration of III as indicated.

Epoxidation of Δ^{14}-anhydrogitoxigenin diacetate (XI)[11] resulted in the formation of the 14α,15α-epoxide (XII). Based on the findings[1,5,6] on epoxidation of β-anhydrodigitoxigenin, the α-epoxide structure was assigned to XII, and this assignment was confirmed by transforming XII into 3β-acetoxy-14α,15α-epoxy-5β-carda-16,20(22)-dienolide (XIV) different from the corresponding β-epoxide (IV). Hydrolysis of XII with potassium bicarbonate, which had been successfully employed in the partial hydrolysis of gitoxigenin diacetate,[12,13] yielded the expected monoacetate (XIII). Acetylation of XIII regenerated XII. On the other hand, treatment of XI with potassium bicarbonate or alumina brought about elimination of acetic acid to afford II.

Elimination of acetic acid by means of alumina from 16β-acetoxy-card-20(22)-enolide, resulting in the formation of a carda-16,20(22)-dienolide, has been reported.[14~17] Therefore we examined the elimination of acetic acid from XII using various kinds of alumina. The principal product obtained in this procedure exhibited a band at 275 mμ (log ε 4.21) in the ultraviolet absorption spectrum, indicating the presence of a carda-16,20(22)-dienolide structure, but it did not exhibit any band due to hydroxyl group absorption in the infrared spectrum. It was different from IV, and the 3β-acetoxy-14α,15α-epoxy-5β-carda-16,20(22)-dienolide structure (XIV) was assigned to it.

In addition to XIV, two crystalline substances were isolated by alumina chromatography of the product obtained from treatment of XII with alumina. Both substances were more polar than XIV on thin-layer chromatography, and they exhibited bands in their ultraviolet and infrared spectra indicating the presence of the carda-16,20(22)-dienolide structure and hydroxyl groups. Cleavage of the 14α,15α-epoxide of XIV with 0.08 *N* sulfuric acid under the conditions reported earlier,[1] afforded a glycol (XV), which was found to be identical with one of the polar substances obtained by alumina treatment of XII. The third substance isolated from the alumina treatment of XII was found to be identical with acetate (XVI) derived from XV. Although acid treatment of 14α,15α-epoxy-β-anhydrodigitoxigenin and its acetate had yielded glycols having the 14β,15α-configuration,[1,6] shortage of our material prevented elucidating configuration of the glycol (XV).

Hydration of an epoxide through alumina chromatography, resulting in the formation of an α-glycol, has been recorded.[18~20] On the other hand, the formation of XVI from XII on treatment with alumina can not be explained reasonably.

9) D. Satoh, H. Ishii : Yakugaku Zasshi, **80**, 1143 (1960).
10) M. Frèrejacque : Compt. rend., **248**, 2382, 3027 (1959).
11) H. Jäger, O. Schindler, T. Reichstein : Helv. Chim. Acta, **42**, 977 (1959).
12) K. Meyer : *Ibid.*, **29**, 1580 (1946).
13) M. Okada, A. Yamada : Yakugaku Zasshi, **72**, 933 (1952).
14) K. Meyer : Helv. Chim. Acta, **29**, 718 (1946).
15) A. Hunger, T. Reichstein : *Ibid.*, **33**, 76 (1950).
16) A. Aebi, T. Reichstein : *Ibid.*, **33**, 1013 (1950).
17) M. Okada : Yakugaku Zasshi, **73**, 86 (1953).
18) H. Heusser, E. V. Jensen, N. Frick, Pl. A. Plattner: Helv. Chim. Acta, **32**, 1326 (1949).
19) A. T. Rowland, H. R. Nace: J. Am. Chem. Soc., **82**, 2833 (1960).
20) A. A. Akhrem, I. G. Zavelśkaya : Zhur. Obshcheĭ Khim., **32**, 50 (1962).

Experimental*5

3β-Hydroxy-14β,15β-epoxy-5β-carda-16,20(22)-dienolide (III)——To a solution of 318 mg. of $\Delta^{14,16}$-dianhydrogitoxigenin (I) in 2.0 ml. of $CHCl_3$ was added 2.0 ml. of a $CHCl_3$ solution of perbenzoic acid (1.2 molar equivalents). The mixture was allowed to stand at 21° for 18 hr. After addition of 400 ml. of $CHCl_3$ to the reaction mixture, the $CHCl_3$ solution was washed with ice-cold 5% sodium carbonate and water, and dried. The solvent was evaporated and the residue was recrystallized from acetone-ether to yield 253 mg. of III, m.p. 213~214°, $[\alpha]_D^{23}$ +206.4° (MeOH), UV λ_{max} mμ (log ε): 276 (4.18), IR ν_{max} cm^{-1}: 3500, 1786, 1740, 1623. *Anal.* Calcd. for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.55; H, 7.98.

3β-Acetoxy-14β,15β-epoxy-5β-carda-16,20(22)-dienolide (IV)——a) From III: The acetate of III prepared in the usual way was recrystallized from acetone-ether, m.p. 185~189°, $[\alpha]_D^{23}$ +159°($CHCl_3$), UV λ_{max} mμ (log ε): 276 (4.19), IR ν_{max} cm^{-1}: 1783, 1754, 1733, 1623. *Anal.* Calcd. for $C_{25}H_{32}O_5$: C, 72.79; H, 7.82. Found: C, 72.63; H, 7.69.

b) From $\Delta^{14,16}$-dianhydrogitoxigenin acetate (II): To a solution of 397 mg. of II in 2.2 ml. of $CHCl_3$ was added 2.8 ml. of a $CHCl_3$ solution of perbenzoic acid (1.2 molar equivalents). Treatment of the reaction mixture in the same manner as described above afforded 273 mg. of IV (m.p. 186~188°) after recrystallization from acetone-ether-petr. ether. The melting point of the mixture with the sample prepared in a) showed no depression, and the IR spectra of the two samples were identical in all respects.

3β-Hydroxy-14β,15β-epoxy-5β,17α-card-20(22)-enolide (V) and Its Acetate (VI)——To a solution of 59 mg. of III in 2.0 ml. of diglyme (diethylene glycol dimethyl ether) was added 1.5 ml. of a diglyme solution of sodium borohydride (15 mg.) from a dropping funnel. During the addition the temperature of the reaction mixture was kept at 0°, and the pH was kept at 8.4~8.6 by adding acetic acid. After the addition was complete, the reaction mixture was allowed to stand at 0° for 1.5 hr. Water and $CHCl_3$ were then added, and the organic layer was washed with water and dried. The solvent was evaporated and the residue was recrystallized from acetone-ether to give 21 mg. of V, m.p. 180°/210~216°, $[\alpha]_D^{23}$ +101°(MeOH), UV λ_{max} mμ(log ε): 215 (4.14), IR ν_{max} cm^{-1}: 3460, 1781, 1710 (sh), 1620. *Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 73.95; H, 8.55.

Acetylation of V in the usual way gave VI, which was recrystallized from MeOH, m.p. 184~187°, $[\alpha]_D^{23}$ +94.3° ($CHCl_3$), IR ν_{max} cm^{-1}: 1785, 1750, 1731, 1635. *Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27. Found: C, 72.29; H, 8.27.

Hydrogenation of 3β-Hydroxy-14β,15β-epoxy-5β-carda-16,20(22)-dienolide (III) over Palladium-on-charcoal——A solution of 240 mg. of III in 40 ml. of EtOH was hydrogenated over 5% palladium-on-charcoal (200 mg.). After 30 min. the catalyst was removed by filtration and the solvent was evaporated to dryness. The residue was chromatographed on a column of acid-washed alumina by elution with a mixture of benzene and $CHCl_3$ (19:1); this afforded two principal fractions which gave a positive Kedde reaction.

3β-Hydroxy-5β,17α-card-20(22)-enolide (VII) and Its Acetate (VIII)——a) The first fraction of the above chromatography was concentrated to dryness. The residue (61.2 mg.) was recrystallized from acetone-ether-petr. ether to afford 26 mg. of VII, m.p. 196~199°. Its acetate (VIII) was prepared in the usual way and recrystallized from acetone-ether-petr. ether, m.p. 169~170°. Identification of VII and VIII with respective samples prepared as described in b) were made by mixed melting point and IR spectra comparisons.

b) A solution of 240 mg. of $\Delta^{14,16}$-dianhydrogitoxigenin (I) in 40 ml. EtOH was hydrogenated over 5% palladium-on-charcoal (200 mg.) for 30 min. The catalyst was removed and the solvent evaporated to dryness. The residue was recrystallized from acetone-ether-petr. ether to yield VII, m.p. 197~199°, $[\alpha]_D^{17}$ +84.3° (MeOH), UV λ_{max} mμ (log ε): 217 (4.18), IR ν_{max} cm^{-1}: 3460, 1786; 1754, 1626. *Anal.* Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.84; H, 9.35.

The acetate (VIII) prepared in the usual way was recrystallized from acetone-ether-petr. ether, m.p. 169~170°, IR ν_{max} cm^{-1}: 1786, 1754, 1730, 1629. *Anal.* Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 74.97; H, 8.88.

17α-Digitoxigenin Acetate (IX) and Digitoxigenin Acetate (X)——The second fraction of the above chromatography of the hydrogenation product of III was concentrated to dryness. The residue (90 mg.) was acetylated in the usual way. The acetylated product showed two spots (Rf 0.38, 0.48), staining with Kedde reagent on thin-layer chromatography of silica gel G with methyl ethyl ketone and *n*-heptane (1 : 1). Direct crystallization of the acetylated product from acetone-ether-petr. ether afforded 12 mg. of IX, Rf 0.38, m.p. 231~235°. The melting point of the mixture with an authentic sample (m.p. 232~238°) prepared according to the procedure described by Frèrejacque[12] showed no depression, and the IR spectra of the two samples were identical in all respects.

The mother liquor from recrystallization of IX, which contained IX and X, was subjected to a preparative thin-layer chromatography. The two areas were eluted from the thin-layer chromatogram with acetone.

*5 Melting points were determined on a Kofler block and are uncorected. Ultraviolet spectra were measured in 95% ethanol solution. Infrared spectra were determined in potassium bromide disks on Hitachi EPI-S2 spectrophotometer; br=broad, sh=shoulder.

The substance of Rf 0.48 was recrystallized from acetone-ether-petr. ether to afford 5.2 mg. of X, m.p. 216 ~222°. The melting point of the mixture with an authentic specimen showed no depression, and the IR spectra of the two samples were identical in all respects. On the other hand, recrystallization of the substance of Rf 0.38 from acetone-ether-petr. ether gave 6.5 mg. of IX, m.p. 230~235°.

3β,16β-Dihydroxy-14α,15α-epoxy-5β-card-20(22)-enolide 3,16-Diacetate (XII)——To a solution of 230 mg. of Δ^{14}-anhydrogitoxigenin diacetate (XI) [11] (m.p. 152~154°) in 1.5 ml. of $CHCl_3$ was added 2.5 ml. of a $CHCl_3$ solution of perbenzoic acid (1.2 molar equivalents). The mixture was allowed to stand at 18° for 17 hr. After the addition of 200 ml. of $CHCl_3$ to the reaction mixture, the $CHCl_3$ solution was washed with ice-cold 3% sodium carbonate and water, and dried. The solvent was evaporated and the residue was recrystallized from acetone-ether-petr. ether to yield 165 mg. of XII, m.p. 181~192°, $[\alpha]_D^{23}$ +75.7° ($CHCl_3$), IR ν_{max} cm^{-1}: 1786, 1755, 1734, 1635. *Anal.* Calcd. for $C_{27}H_{36}O_7$: C, 68.62; H, 7.68. Found: C, 68.44; H, 7.56.

3β,16β-Dihydroxy-14α,15α-epoxy-5β-card-20(22)-enolide 3-Monoacetate (XIII)——A solution of 50 mg. of potassium bicarbonate in 0.5 ml. of water was added to a solution of 50 mg. of II in 5 ml. of MeOH and the mixture was allowed to stand for 15 hr. at 18°. AcOEt was then added and the organic layer was washed with water, and dried. The solvent was evaporated and the residue was recrystallized from acetone-ether to yield 31 mg. of XIII, m.p. 195~205°, $[\alpha]_D^{23}$ +44.9° ($CHCl_3$), IR ν_{max} cm^{-1}: 3485, 1785, 1753, 1632. *Anal.* Calcd. for $C_{25}H_{34}O_6$; C, 69.74; H, 7.96. Found: C, 69.82; H, 7.75.

The mother liquor from recrystallization of XIII showed two spots after staining with Kedde reagent or sulfuric acid on a thin layer chromatogram using a mixture of acid-washed alumina and silica gel (Merck) (1 : 1) with $CHCl_3$ and MeOH (96 : 4). These spots, Rf 0.21 and 0.65, corresponded to XIII and 3β-acetoxy-14α,15α-epoxy-5β-carda-16,20 (22) -dienolide (XIV), respectively.

Acetylation of XIII by the usual method gave XII, m.p. 180~190°. The melting point of the mixture with the sample described above showed no depression, and the infrared spectra of the two samples were identical in all respects.

Treatment of Δ^{14}-Anhydrogitoxigenin Diacetate (XI) with Potassium Bicarbonate or Alumina——To a solution of 5 mg. of XI in 0.5 ml. of MeOH was added a solution of 5 mg. of potassium bicarbonate in 0.05 ml. of water, and the mixture was allowed to stand for 15 hr. at 18°. Treatment of the reaction mixture in the same way as described above afforded II, which was recrystallized from MeOH-water, m.p. 208~212°. The melting point of the mixture with an authentic specimen showed no depression, and the infrared spectra of the two samples were identical in all respects.

A $CHCl_3$ solution of 5 mg. of XI was added to 0.5 g. of neutral alumina[21] and the mixture was allowed to stand at room temperature for 10 days with occasional shaking. $CHCl_3$ was added, and the alumina was filtered and washed with $CHCl_3$. The combined filtrates were concentrated to dryness. The residue was recrystallized from MeOH-water to give II, m.p. 206~211°.

Treatment of 3β,16β-Dihydroxy-14α,15α-epoxy-5β-card-20 (22)-enolide 3,16-Diacetate (XII) with Alumina; Formation of 3β-Acetoxy-14α,15α-epoxy-5β-carda-16,20(22)-dienolide (XIV), 3β,14ξ,15ξ-Trihydroxy-5β-carda-16,20(22)-dienolide 3-Monoacetate (XV) and 3,15-Diacetate(XVI)——a) To a solution of 70 mg. of XII in 15 ml. of $CHCl_3$ was added 14 g. of alumina ("Merck" standardisiert nach Brockmann). The mixture was allowed to stand for 70 hr. at room temperature with occasional shaking. The alumina was filtered and washed with $CHCl_3$. The combined filtrates were concentrated to dryness. The residue (55 mg.) showed three spots (Rf 0.07, 0.28, 0.65), after treatment of the thin layer chromatogram with Kedde reagent or sulfuric acid. The substance of Rf 0.65 was the principal product. Recrystallization of the residue from acetone-ether-petr, ether afforded 35 mg. of XIV, Rf 0.65, m.p. 197~204°, $[\alpha]_D^{23}$ +92.2° ($CHCl_3$), UV λ_{max} mμ (log ε): 275 (4.21), IR ν_{max} cm^{-1}: 1786, 1755, 1734, 1636. *Anal.* Calcd. for $C_{25}H_{32}O_5$: C, 72.79; H, 7.82. Found: C, 72.97; H, 7.84. The melting point of the mixture of XIV with the sample of 3β-acetoxy-14β, 15β-epoxy-5β-carda-16,20 (22) -dienolide (IV) showed a depression, and the infrared spectra of the two samples were different in the region from 1400 to 650 cm^{-1}.

Further extraction of the alumina with a mixture of $CHCl_3$ and MeOH (3 : 1) yielded a residue, after evaporation of the solvent, which showed two spots (Rf 0.07, 0.28) on thin layer chromatography. No crystalline substance, however, could be obtained through alumina chromatography of the residue.

b) To a solution of 150 mg. of XII in 30 ml. of $CHCl_3$ was added 30 g. of reactivated alumina.*6

The mixture was allowed to stand with occasional shaking for 40 hr. at room temperature. The alumina was filtered and washed with $CHCl_3$. It was further stirred for 30 min. with a mixture of $CHCl_3$ and MeOH (3 : 1). The $CHCl_3$ and $CHCl_3$-MeOH extracts were combined and evaporated to dryness. The residue (115 mg.), which showed three spots (Rf 0.07, 0.28, 0.65) on thin layer chromotography, was recrystallized from acetone to afford 17 mg. of XV, Rf 0.07, m.p. 240~245°. The melting point of the mixture with the sample prepared from XIV as described below showed no depression, and the infrared spectra of the two samples were identical in all respects.

*6 Alumina ("Merck" standardisiert nach Brockmann) was heated at 170° for 5 hr. *in vacuo*.

21) J. von Euw, A. Lardon, T. Reichstein: Helv. Chim. Acta, **27**, 1292 (footnote 2) (1944).

The mother liquor from recrystallization of XV was evaporated to dryness. The residue (95 mg.) was chromatographed on a column of 5 g. of acid-washed alumina by successive elution with benzene, and benzene-$CHCl_3$ mixtures. The fraction (41 mg.) eluted with benzene was recrystallized from acetone-petr. ether to give 25 mg. of XIV, Rf 0.65, m.p. 195~200°. The fraction (15 mg.) eluted with benzene–$CHCl_3$ mixtures (2 : 1, 1 : 1) was recrystallized from acetone-petr. ether to afford 5 mg. of XVI, Rf 0.28, m.p. 247~251°. The melting point of the mixture with the sample prepared from XV by acetylation as described below showed no depression, and the infrared spectra of the two samples were identical in all respects. The fraction (27 mg.) eluted with benzene–$CHCl_3$ mixtures (1 : 2, 1 : 4) and $CHCl_3$ was recrystallized from acetone to give 15 mg. of XV, Rf 0.07, m.p. 241~246°.

3β,14ξ,15ξ-Trihydroxy-5β-carda-16,20(22)-dienolide 3-Monoacetate (XV) from 3β-Acetoxy-14α,15α-epoxy-5β-carda-16,20(22)-dienolide (XIV)——A solution of 20 mg. of XIV in 10 ml. of a mixture of acetone, 1 *N* sulfuric acid and water (60 : 5.4 : 1.6) was allowed to stand at room temperature for 20 hr. To the solution was added 20 ml. of water, and acetone was removed under reduced pressure. AcOEt was then added and the organic layer was washed with water, and dried. Evaporation of the solvent yielded a crystalline residue which was recrystallized from acetone to give 11 mg. of XV, Rf 0.07, m.p. 242~248°, UV λ_{max} mμ (log ε) : 269 (4.25), IR ν_{max} cm^{-1} : 3360 (br), 1783, 1732, 1623. *Anal.* Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found : C, 69.72; H, 7.99.

3β,14ξ,15ξ-Trihydroxy-5β-carda-16,20(22)-dienolide 3,15-Diacetate (XVI)——Acetylation of XV in the usual way yielded XVI. It was recrystallized from acetone, Rf 0.28, m.p. 248~252°, IR ν_{max} cm^{-1} : 3470, 1783, 1741 (br), 1627. *Anal.* Calcd. for $C_{27}H_{36}O_7$: C, 68.62; H, 7.68. Found : C, 68.41; H, 7.55.

The authors wish to express their appreciation to Dr. M. Ishidate, Director of the Institute, for his interest and support throughout this investigation. Thanks are due to Dr. D. Satoh of Shionogi Research Laboratory, Shionogi & Co., Ltd., for his generous gift of the gitoxin fraction obtained from *Digitalis purpurea* leaves.

Summary

Epoxidation of $\Delta^{14,16}$-dianhydrogitoxigenin (I) with 1.2 molar equivalents of perbenzoic acid gave 3β-hydroxy-14β,15β-epoxy-5β-carda-16,20(22)-dienolide (III) which was hydrogenated over palladium-on-charcoal to afford 17α-digitoxigenin and digitoxigenin, both being obtained and identified as their acetates (IX, X). The preparation of 3β,16β-diacetoxy-14α,15α-epoxy-5β-carda-16,20 (22)-dienolide (XII) from Δ^{14}-anhydrogitoxigenin diacetate (XI) and some reactions of XII were described.

(Received October 9, 1965)