UDC 547.918 : 615.711.5

101. Hiroshi Ishii, Takehiko Tozyo, and Daisuke Satoh : Studies on Digitalis Glycosides. XVIII.*1 Some D Ring Transformations in Digitoxigenin.*2

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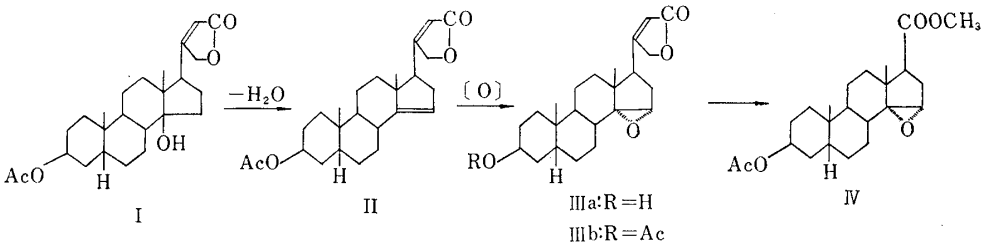
Okada[1] found that resibufogenin, 3β-hydroxy-14β,15β-epoxy-5β-bufadienolide, has a strong stimulating effect on respiratory center. With a view to see whether the corresponding cardenolide derivative would have this pharmacological activity, a series of reactions to prepare 14β,15β-epoxide starting from digitoxigenin were carried out.

Oxidation with monoperphthalic acid of β-anhydrodigitoxigenin acetate (II), obtainable from digitoxigenin acetate (I) by dehydration with thionyl chloride in pyridine, gave a 14,15-epoxide IIIb in good yield. Since this compound was converted by permanganate oxidation and esterification of an acid thus obtained into the known methyl 3β-acetoxy-14α,15α-epoxy-5β-etianate[2] (IV), the structure of IIIb was established to be 3β-acetoxy-14α,15α-epoxy-5β-card-20(22)-enolide. Hydrolysis of IIIb by microbiological means with *Mucor parasiticus* gave 3β-hydroxy compound IIIa, which was acetylated to regenerate IIIb.



I II IIIa: R=H IIIb: R=Ac IV

Chart 1.

Hydrolytic cleavage of IIIb with perchloric acid yielded a *trans*-glycol Vb,*4 which was oxidized with chromic acid to give a 14-hydroxy-15-ketone derivative VI. This substance showed a negative Cotton effect in its rotatory dispersion curve as shown in Fig. 1. Since neither presence of butenolide ring in cardiac aglycones[4] nor introduction of a hydroxyl adjacent to the ketone[5] is usually found to affect the sign of the Cotton effect, VI was considered to be a 15-oxo-14β-steroid similar to the 15-ketone of spirostane.[6] Consequently, the structure of Vb was assumed to be 15α-hydroxydigitoxigenin 3-acetate. Hydrolysis of Vb with acid afforded 15α-hydroxydigitoxigenin (Va), and both compounds Va and Vb were acetylated to the same 3,15-diacetate Vc.*5

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*1 Part XVII : This Bulletin, **11**,156 (1963).

*2 A part of this work has been published as a brief communication in this Bulletin **10**, 645 (1962).

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*4 In a previous communication,[3] one of the authors (H. I.) learned that hydrolysis of 14α,15α-epoxy derivative of progesterone yielded 14β,15α-dihydroxyprogesterone.

*5 After sending of this paper for publication, the compound Vc was changed into a corresponding methyl etianate, m.p. 112~114°, which was shown to be identical with an authentic sample of 3β, 15α-diacetoxy-14β-hydroxy-5β-etianic acid methyl ester (AL 485)[10] kindly furnished from Prof. T. Reichstein by mixed melting point determination and direct comparison of their infrared spectra in chloroform solution. This fact verified the configuration of 14β,15α-diol in Va.

1) M. Okada : Nippon Yakurigaku Zasshi, **57**, 160 § (1961).
2) A. Lardon, H. P. Sigg, T. Reichstein : Helv. Chim. Acta, **42**, 1457 (1959).
3) H. Ishii : This Bulletin, **9**, 411 (1961).
4) C. Djerrassi : "Optical Rotatory Dispersion," 46 (1960). McGraw-Hill Book Co., New York.
5) *Idem* : *Ibid.*, 111 (1960).
6) *Idem* : *Ibid.*, 58 (1960).

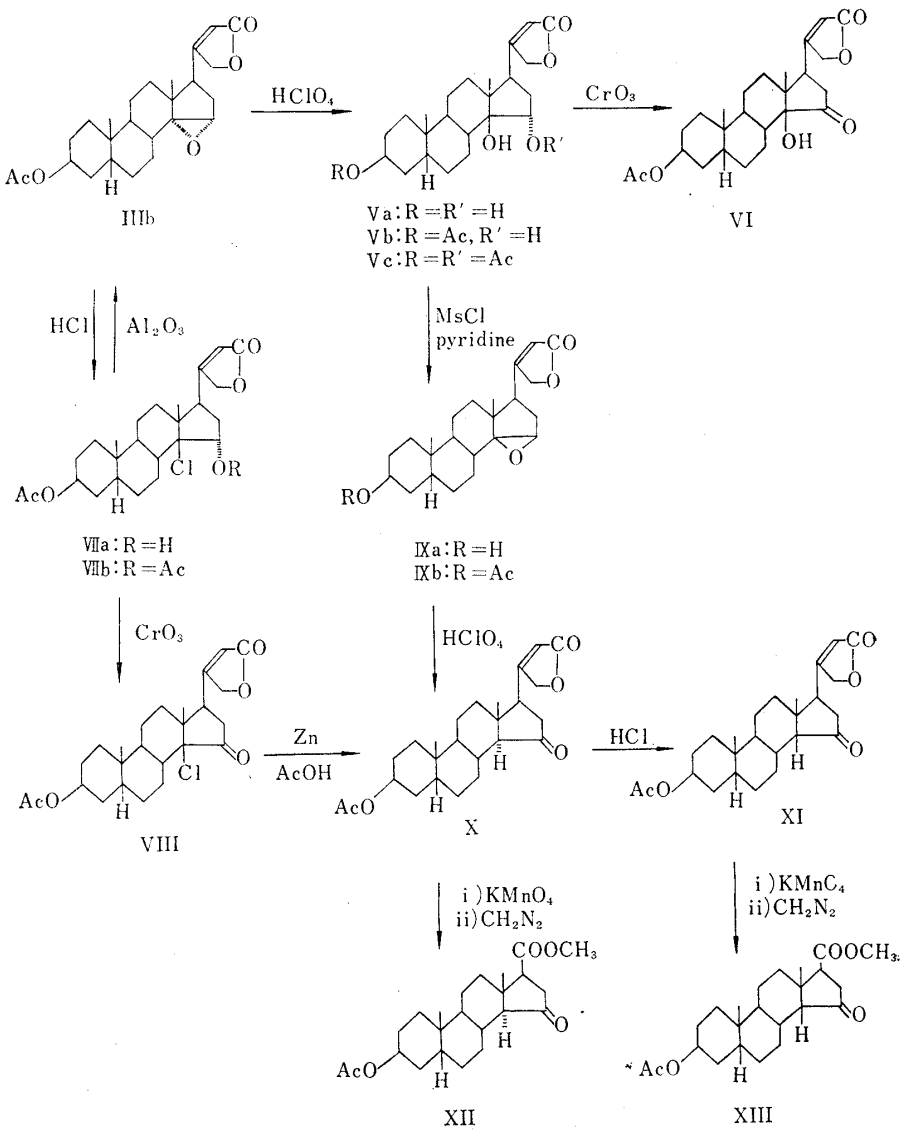


Chart 2.

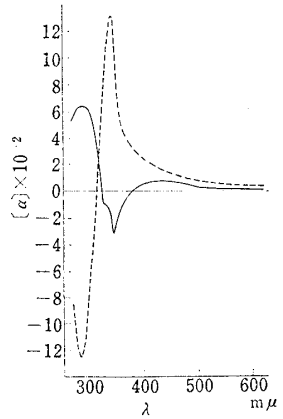


Fig. 1. Rotatory Dispersion Curves in Dioxane

--- Compound (VIII)
—— Compound (VI)

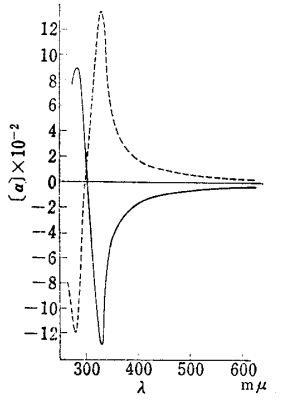


Fig. 2. Rotatory Dispersion Curves in Dioxane

---- Compound (X)
—— Compound (XI)

On cleavage with hydrogen chloride the ring of α-epoxide IIIb opened at C_{14} to give a chlorohydrin with structure of 14-chloro-3β,15α-hydroxy-5β-card-20(22)-enolide 3-acetate (VIIa), from which the original epoxide was easily regenerated on alumina chromatography. This structure was taken by the following two observations : (a) The chlorohydrin on acetylation in a usual way gave a diacetate VIIb and when oxidized with chromic acid yielded a chloroketone VIII, indicating that the hydroxyl group newly formed in VIIa was a secondary one. (b) The chloroketone VIII showed a positive Cotton effect curve in its optical rotatory dispersion as given in Fig. 1, in accordance with a 14-chloro-15-oxo-5β,14β-etianic acid.[2]

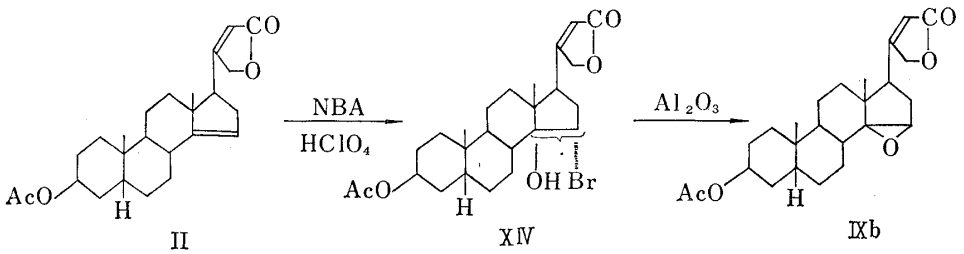
This result of ring fission agreed with those of 14α,15α-epoxy derivatives of etianic acid[2] and progesterone,[7] and supported the assumption that Vb should be a 14β-steroid.

Treatment of Vb with mesyl chloride in pyridine and purification of the product by alumina chromatography afforded a sulfur-free compound IXb, whose analytical values were in good agreement with $C_{25}H_{34}O_5$. This reaction product was regarded as a 14β,15β-epoxide because of absence of hydroxyl band in its infrared spectrum, failure in oxime formation and its chemical property described below analogous to that of resibufogenin.[8] Linde and Meyer[8] found that resibufogenin was changed readily by heating in acetone containing a trace of perchloric acid with a *cis*-hydride shift into a 15-oxo-14α-bufadienolide, which was further converted into a 15-oxo-14β-steroid by refluxing with alumina in benzene.

When treated under the condition of Linde and Meyer, IXb was isomerized to a compound X which formed a monoxime. This compound was also produced by reduction of the chloroketone VIII with zinc and acetic acid and hence X was clarified to be a 15-ketone compound. Since X was transformed into a methyl etianate XII identical with the known 3β-acetoxy-15-oxo-5β-etianic acid methyl ester,[8] the structure of X was proved to be 3β-acetoxy-15-oxo-5β,14α-card-20(22)-enolide. The substance X was further changed by treatment with hydrochloric acid into an isomer XI. As its corresponding methyl etianate XIII was shown to be identical with the known 3β-acetoxy-15-oxo-5β,14β-etianic acid methyl ester,[8] the structure of 3β-acetoxy-15-oxo-5β-card-20(22)-enolide was assigned to XI. Rotatory dispersion curves of X and XI showed positive and negative Cotton effects as given in Fig. 2, respectively. These pictures agreed well with those of 15-ketones of spirostane.[6]

Finally identification of IXb with an authentic sample of 3β-acetoxy-14β,15β-epoxy-5β-card-20(22)-enolide prepared independently by Hofer, *et al.*[9] with an alternative method established the structure of IXb.

The production of β-epoxide from Vb afforded evidence confirming the configuration of 14β,15α-diol above assigned to Vb since this reaction was considered to pro-



II XIV IXb

Chart 3.

7) H. Hasegawa, Y. Sato, K. Tsuda : This Bulletin, **9**, 409 (1961).
8) H. Linde, K. Meyer : Helv. Chim. Acta, **42**, 807 (1959).
9) P. Hofer, H. Linde, K. Meyer : *Ibid.*, **45**, 1041 (1962).

ceed through formation of 15-monomesylate. This configuration is comparable to that of the similar reaction product of etianic acid series recently reported by Lardon and Reichstein.[10]

The β-epoxide IXb was also obtained from β-anhydrodigitoxigenin acetate (II) via bromohydrin XIV: Treatment of II with N-bromoacetamide and perchloric acid gave a compound XIV which showed a positive Beilstein test and a hydroxyl band in its infrared spectrum. Chromatography of XIV on alumina afforded a halogen-free compound identical with IXb.

According to a method described by Meister and Murray,[11] fermentative deacetylation of IXb with *Rhizopus shanghaiensis* was carried out to give 3β-hydroxy-14β,15β-epoxy-5β-card-20(22)-enolide (IXa), from which IXb was recovered by acetylation.

The 3-hydroxy compounds (IIIa, Va and IXa) are undergoing pharmacological tests.

After completion of this manuscript, it was learned that recently Meyer[12] also prepared 3β-acetoxy compounds of 15-oxo-14α- and 14β-cardenolides. The authors' samples of X and XI were found to be identical with the corresponding specimens kindly supplied by Prof. Meyer, respectively.

Experimental*6

3β-Acetoxy-14α,15α-epoxy-5β-card-20(22)-enolide (IIIb)——To a solution of II (675 mg.) dissolved in 5 cc. of $CHCl_3$, 3.5 cc. of Et_2O solution of monoperphthalic acid (85 mg./cc.) was added and the mixture was allowed to stand for 16 hr. at room temperature, and the separated phthalic acid was removed by filtration. $CHCl_3$ was added to the filtrate, which was washed consecutively with aqueous solutions of KI, $Na_2S_2O_3$ and $NaHCO_3$, and H_2O. The $CHCl_3$ layer was dried over Na_2SO_4 and the solvent was evaporated. Crystallization of the residue (705 mg.) so obtained from MeOH-Et_2O afforded 560 mg. of IIIb as plates, m.p. 187~198°, which was recrystallized from the same solvents to give an analytical sample, m.p. 199~206°, $[\alpha]_D^{21}$ +12.0° (c=1.021, $CHCl_3$), UV: λ_{max}^{EtOH} 216 mμ (log ε 4.20). *Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27. Found: C, 72.46; H, 8.39.

3β-Acetoxy-14α,15α-epoxy-5β-etianic Acid Methyl Ester (IV)——To a solution of IIIb (200 mg.) dissolved in 12 cc. of Me_2CO, finely powdered $KMnO_4$ (360 mg.) was added portionwise during 1 hr. at room temperature with stirring. After additional stirring for 4 hr., the solvent was evaporated to dryness *in vacuo* and the residue thus obtained was extracted with H_2O. The aqueous solution was acidified and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure to afford 149 mg. of residue, which was esterified with CH_2N_2 in a usual manner. The resulting product was dissolved in benzene and submitted to chromatography on 10 g. of Al_2O_3. The fraction (87 mg.) eluted with benzene was crystallized from Et_2O-petr.ether to give 69 mg. of IV as plates, m.p. 86~89°, $[\alpha]_D^{25}$ +39.4° (c=0.999, $CHCl_3$), identical with an authentic sample of 3β-acetoxy-14α,15α-epoxy-5β-etianic acid methyl ester[2] kindly supplied by Prof. T. Reichstein through mixed melting point determination and comparison of their IR spectra.

3β-Hydroxy-14α,15α-epoxy-5β-card-20(22)-enolide (IIIa)——A nutrient solution was prepared from 6 g. of glucose, 3 g. of peptone, 0.45 g. of corn steep liquor and 1.5 L. of H_2O. After sterilization, this solution was inoculated with *Mucor parasiticus* (ATCC 6476) and incubated with shaking for 24 hr. at 26~28°. To this fermentation broths, 150 mg. of IIIb dissolved in 15 cc. of Me_2CO was added. The incubation was further continued for 4.5 hr. without aeration. The fermentation broths were extracted with $CHCl_3$ and the extract was washed with H_2O and dried over Na_2SO_4. $CHCl_3$ was evaporated *in vacuo* and 290 mg. of residue was obtained. The residue was dissolved in benzene, adsorbed on a column of Al_2O_3 (10 g.) and eluted successively with benzene, benzene-$CHCl_3$ (4:1) and (1:1). The fractions eluted with the mixtures of benzene and $CHCl_3$ were combined to yield, after removal of the solvent, 154 mg. of residue. Crystallization from MeOH-Et_2O gave 70 mg. of IIIa as plates, m.p. 242~246°, $[\alpha]_D^{25}$ +8.3° (c=1.009, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.26; H, 8.75. Acetylation of IIIa in a usual manner afforded an acetate, m.p. 199~206°, which gave no depression by admixture with a specimen of IIIb.

15α-Hydroxydigitoxigenin 3-Acetate (Vb)——To a solution of IIIb (1 g.) dissolved in 100 cc. of Me_2CO, 20 cc. of 2% $HClO_4$ was added and the mixture was allowed to stand for 4 days at room temperature.

*6 All melting points are uncorrected.

10) A. Lardon, T. Reichstein: *Ibid.*, **45**, 943 (1962).
11) P.D. Meister, H.C. Murray: U.S. Pat., 2,968,596 (1961).
12) K. Meyer: Private communication.

After neutralization with Na_2CO_3 solution, the reaction mixture was concentrated to a quater of the original volume and extracted with $CHCl_3$. The extract was washed with H_2O and dried over Na_2SO_4. The residue (978 mg.) from $CHCl_3$ extract was dissolved in a mixture of benzene-$CHCl_3$ (1:1), adsorbed on a column of Al_2O_3 (30 g.) and eluted successively with benzene-$CHCl_3$ (1:1), $CHCl_3$ and $CHCl_3$-MeOH (98:2). The fraction (314 mg.) eluted with benzene-$CHCl_3$ (1:1) was crystallized from MeOH-Et_2O to give 179 mg. of the starting material.

The $CHCl_3$ eluate (608 mg.) was crystallized from MeOH-Et_2O to yield 452 mg. of Vb as prisms, m.p. 247~250°, $[\alpha]_D^{26}$ +35.6°(c=1.034, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.84; H, 8.52.

15α-Hydroxydigitoxigenin (Va)——A mixture of Vb (200 mg.) dissolved in 20 cc. of dioxane and 20 cc. of 5% HCl was allowed to stand for 24 hr. at room temperature. After neutralization, the mixture was concentrated under reduced pressure to deposit prisms (57 mg.) of the starting material.

The mother liquor was extracted with $CHCl_3$ and the extract was washed with H_2O, dried and evaporated to dryness *in vacuo*. The residue (140 mg.) thus obtained was dissolved in a mixture of benzene-$CHCl_3$ (1:1), adsorbed on a column of Al_2O_3 (5 g.) and eluted consecutively with benzene-$CHCl_3$ (1:1), $CHCl_3$ and $CHCl_3$-MeOH (98:2). The fractions eluted with $CHCl_3$ and $CHCl_3$-MeOH (98:2) were combined to give, after removal of the solvent, 115 mg. of residue. Crystallization from MeOH-Et_2O yielded 55 mg. of plates of Va, m.p. 245~248°, $[\alpha]_D^{23}$ +34.7°(c=1.020, MeOH). *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.81; H, 8.81.

15α-Hydroxydigitoxigenin 3,15-Diacetate (Vc)——On acetylation with Ac_2O and pyridine in a usual manner, both Va and Vb afforded a diacetate Vc as needles from MeOH-Et_2O, m.p. 226~230°, $[\alpha]_D^{24}$ +40.0°(c=0.968, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07. Found: C, 68.45; H, 8.29.

15-Oxodigitoxigenin 3-Acetate (VI)——To an ice-cold solution of 100 mg. of Vb dissolved in 2.5 cc. of AcOH, 1.2 cc. of 2% solution of CrO_3 in AcOH was added and the mixture was allowed to stand for 4.5 hr. at room temperature. Excess of CrO_3 was reduced with MeOH, the mixture was diluted with H_2O and extracted with $CHCl_3$. The extract was washed with Na_2CO_3 solution and H_2O, and dried over Na_2SO_4. The residue (101 mg.) obtained by $CHCl_3$ extraction was crystallized from MeOH-Et_2O to VI as prisms, m.p. 236~240°, $[\alpha]_D^{23}$ +0.5°(c=0.997, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.98; H, 8.15. Rotatory dispersion curve of this product is shown in Fig. 1.

14β-Chloro-3β,15α-dihydroxy-5β-card-20(22)-enolide 3-Acetate (VIIa)——A solution of IIIb (866mg.) dissolved in 15 cc. of abs. $CHCl_3$ was cooled in an ice-salt bath of ca. −10°. Into this solution, dry HCl gas was introduced for 10 min. and the mixture was allowed to react for 1 hr. at 0° and for additional 4 hr. at room temperature. The reaction solution was consecutively washed with H_2O, $NaHCO_3$ solution and H_2O, and dried over Na_2SO_4. Removal of the solvent afforded 954 mg. of residue, which was crystallized from MeOH-Et_2O to give 712 mg. of VIIb, m.p. 207~215°. The crystals were recrystallized from the same solvents to yield an analytical sample as prisms, m.p. 216~218°, $[\alpha]_D^{23}$ +7.6°(c=1.060, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{35}O_5Cl$: C, 66.57; H, 7.82; Cl, 7.84. Found: C, 66.57; H, 7.89; Cl, 7.89.

Chromatography of VIIa on Al_2O_3——Chlorohydrin VIIa (60 mg.) was dissolved in benzene, adsorbed on a column of Al_2O_3 (5 g.) and eluted with a mixture of benzene-Et_2O (1:1). The eluate was evaporated *in vacuo* to give 62 mg. of residue, which was crystallized from MeOH-Et_2O to 22 mg. of plates, m.p. 188~200°. Admixture with an authentic sample of the epoxide IIIb did not show any depression of the melting point.

3β,15α-Diacetoxy-14β-chloro-5β-card-20(22)-enolide (VIIb)——Acetylation of VIIa with Ac_2O and pyridine in a usual way gave a diacetate VIIb as plates from MeOH-Et_2O, m.p. 204~209°, $[\alpha]_D^{23.5}$ +21.1° (c=1.004, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{37}O_6Cl$: C, 65.77; H, 7.57; Cl, 7.08. Found: C, 66.06; H, 7.67; Cl, 6.50.

3β-Acetoxy-14β-chloro-15-oxo-5β-card-20(22)-enolide (VIII)——Chlorohydrin VIIa (100 mg.) was treated in the manner analogous to the CrO_3 oxidation of Vb to give 71 mg. of VIII as crystalline powder, m.p. 231°, $[\alpha]_D^{23}$ +60.5°(c=1.031, $CHCl_3$). UV: λ_{max}^{EtOH} 216 mμ (log ε 4.18). Beilstein test, positive. *Anal.* Calcd. for $C_{25}H_{33}O_5Cl$: C, 66.87; H, 7.41. Found: C, 66.77; H, 7.49. Rotatory dispersion curve of this compound is given in Fig. 1.

3β-Acetoxy-14β,15β-epoxy-5β-card-20(22)-enolide (IXb)

a) From Diol (Vb)——To an ice-cold solution of Vb (400 mg.) dissolved in 2 cc. of pyridine, 0.5 cc. of mesyl chloride was added and the mixture was allowed to stand for 28 hr. in a refrigerator. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The $CHCl_3$ layer was successively washed with dil. HCl, dil. $NaHCO_3$ solution and H_2O, and dried over Na_2SO_4. The residue (430 mg.) from the $CHCl_3$ extract was dissolved in benzene, adsorbed on a column of Al_2O_3 (25 g.) and eluted consecutively with benzene, benzene-Et_2O (9:1), (1:1), Et_2O and Et_2O-$CHCl_3$ (1:1). The fraction (305 mg.) eluted with benzene-Et_2O (1:1) was crystallized from MeOH-Et_2O to 147 mg. of IXb as scales, m.p. 180~181°, $[\alpha]_D^{24}$ +34.4°(c=1.051, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27. Found: C, 72.52; H, 8.30. This product was identical with an authentic sample[9] kindly supplied by Prof. Meyer through mixed melting point determination and direct IR spectra comparison.

b) *via* Bromohydrin (XIV)——To a mixture of II (1.19 g.) dissolved in 50 cc. of dioxane and 1.5 cc. of 70% $HClO_4$ diluted with 8.5 cc. of H_2O, a solution of N-bromoacetamide (1 g.) dissolved in 10 cc. of dioxane was added. The mixture was stirred for 10 min. at room temperature and added a solution of Na_2SO_3 (1 g.) dissolved in H_2O. The solution was concentrated to one-third of the original volume under reduced pressure and poured into ice water with stirring. The precipitate was collected by filtration and dried *in vacuo* to give 1.403 g. of crude bromohydrin XIV, which was crystallized from MeOH to give an analytical sample, m.p. 151~153°(decomp.), $[\alpha]_D^{23}$ +36.7°(c=1.007, $CHCl_3$). IR : λ_{max}^{Nujol} 2.90 μ (OH). *Anal.* Calcd. for $C_{25}H_{35}O_5Br$: C, 60.60; H, 7.12; Br, 16.13. Found : C, 60.37; H, 7.05; Br, 15.90.

Crude bromohydrin (1.4 g.) was chromatographed on 30 g. of Al_2O_3. The products (807 mg.) eluted with benzene and benzene-$CHCl_3$ (9:1~1:1) were collected and crystallized from MeOH-Et_2O to give 363 mg. of scales, m.p. 177~179°, identical with the above β-epoxide IXb.

3β-Hydroxy-14β,15β-epoxy-5β-card-20(22)-enolide (IXa)——A sterilized nutrient solution (6 L.) containing 1% glucose and 2% corn steep liquor was inoculated with *Rhizopus shanghaiensis* (ATCC 10329) and incubated for 24 hr. at 26~28° with agitation. To this fermentation broths, 600 mg. of IXb dissolved in 60 cc. of Me_2CO was added. The incubation was continued with shaking for 2.5 hr. The broths were extracted with $CHCl_3$ and the extract was washed with H_2O and dried over Na_2SO_4. The solvent was distilled off *in vacuo* to give 603 mg. of residue. The residue was dissolved in benzene, adsorbed on a column of Al_2O_3 (25 g.) and eluted with benzene and mixtures of benzene and $CHCl_3$. The fraction (346 mg.) eluted with benzene-$CHCl_3$ (1:1) was crystallized from MeOH-Et_2O to 236 mg. of IXa as prisms, m.p. 227~231°, $[\alpha]_D^{22}$ +34.6°(c=1.108, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found : C, 74.00; H, 8.60. Acetylation of IXa in a usual manner yielded scales of m.p. 177~179°, identical with IXb.

3β-Acetoxy-15-oxo-5β,14α-card-20(22)-enolide (X)

a) From IXb——To a solution of IXb (50 mg.) dissolved in 3 cc. of Me_2CO, 0.1 cc. of a $HClO_4$ solution (0.1 cc. of 70% $HClO_4$ in 2 cc. of Me_2CO) was added and the mixture was refluxed for 5 min. The solution was diluted with H_2O, concentrated *in vacuo* to remove Me_2CO and extrated with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried and evaporated to give 49 mg. of residue, which was crystallized from MeOH-Et_2O to 20 mg. of X as needles, m.p. 225~229°. Recrystallization from the same solvents afforded an analytical sample of m.p. 232~235°, $[\alpha]_D^{24}$ +36.9°(c=1.023, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27. Found : C, 72.10; H, 8.33. Rotatory dispersion curve is shown in Fig. 2.

b) From VIII——To a solution of VIII (377 mg.) dissolved in a mixture of 8 cc. of MeOH and 8 cc. of AcOH, AcONa·$3H_2O$ (375 mg.) and Zn dust (400 mg.) were added and the mixture was refluxed for 2 hr. The reaction mixture was concentrated under reduced pressure to a small amount, diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed consecutively with H_2O, dil. $NaHCO_3$ solution and H_2O, and dried over Na_2SO_4. Removal of the solvent afforded 367 mg. of residue, which was crystallized from MeOH-Et_2O to give 195 mg. of needles, m.p. 230~233°, identical with a sample of X.

Oxime : To a solution of X (50 mg.) in 2 cc. of MeOH, a solution of NH_2OH·HCl (100 mg.) and AcONa·$3H_2O$ (140 mg.) in 0.2 cc. of H_2O was added and the mixture was refluxed for 3 hr. The crude product, deposited after dilution with H_2O, was collected by filtration and recrystallized form MeOH-Et_2O to yield a monoxime of X, m.p. 200~207°. *Anal.* Calcd. for $C_{25}H_{35}O_5N\cdot\frac{1}{2}H_2O$: C, 68.47; H, 8.28; N, 3.19. Found : C, 68.12; H, 8.42; N, 3.43.

3β-Acetoxy-15-oxo-5β-etianic Acid Methyl Ester (XII)——The crude ester (180 mg.), obtained from 200 mg. of X by a method similar to the formation of methyl etianate IV, was chromatographed on 5 g. of silica gel. The eluate (157 mg.) from $CHCl_3$ was crystallized from MeOH-Et_2O to 50 mg. of scales, m.p. 175~183°, $[\alpha]_D^{24.5}$ +59.9°(c=0.760, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found : C, 70.78; H, 8.92. Admixture with an authentic specimen of 3β-acetoxy-15-oxo-5β-etianic acid methyl ester[8] kindly supplied by Prof. Meyer did not show any depression of the melting point.

3β-Acetoxy-15-oxo-5β-card-20(22)-enolide (XI)——A solution of X (280 mg.) dissolved in 30 cc. of Me_2CO containing 1 drop of conc. HCl was allowed to stand for 70 hr. at room temperature. The solution was diluted with H_2O, concentrated *in vacuo* to remove Me_2CO and extracted with $CHCl_3$. The extract was washed with H_2O, dried and evaporated to dryness. The residue (302 mg.) thus obtained was crystallized from MeOH-Et_2O to give 88 mg. of needles of the starting material. The mother liquor was cooled in a refrigerator to deposit 81 mg. of XI as plates, m.p. 192~194°, $[\alpha]_D^{24}$ −56.8° (c=0.988, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27. Found : C, 72.73; H, 8.39. Rotatory dispersion curve of this product is given in Fig. 2,

3β-Acetoxy-15-oxo-5β,14β-etianic Acid Methyl Ester (XIII)——In the same manner as shown in the formation of IV, 115 mg. of XI was transformed by oxidation with $KMnO_4$ (220 mg.), esterification with CH_2N_2 and chromatography on silica gel into 35 mg. of XIII as plates, m.p. 173~178°, $[\alpha]_D^{24}$ −17.9° (c=1.021, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found : C, 70.94; H, 8.87. This was identified as 3β-acetoxy-15-oxo-5β,14β-etianic acid methyl ester[8] by mixed melting point determination with an authentic sample kindly supplied from Prof. Meyer.

The authors express their deep gratitude to Prof. T. Reichstein of the Organisch-chemische Anstalt der Universität Basel for his supply of valuble samples, and to Prof. K. Meyer of the Pharmazeutische Anstalt der Universität Basel for his donation of precious specimens and his favor for information on his recent investigation before publication. They are grateful to Dr. K. Takeda, Director of this Laboratory, for his unfailing guidance. They are also indebted to Dr. Y. Nozaki for his help in microbiological work, to the members of the Analysis Room for elemental analytical data, and to the members of the Section of Physical Chemistry for measurements of optical rotation, and ultraviolet and infrared spectra.

Summary

Fissions of 14α,15α-epoxy ring in a cardenolide with perchloric acid and with hydrogen chloride were found to afford 14β-steroids. With the use of these reactions, some D-ring-transformed compounds of digitoxigenin, e.g. 15α-hydroxydigitoxigenin, and 14-chloro-3β,15α-dihydroxy- and-3β-hydroxy-14β,15β-epoxy-cardenolides, were synthesized.

(Received September 14, 1962)

UDC 615.779.931-011

102. Makoto Suzuki, Yoshiyuki Egawa, and Tomoharu Okuda : Studies on Streptomyces Antibiotic, Cycloheximide. XV.[1] Hydroxycarbonylation of Optically Active 2,4-Dimethylcyclohexanones with Glutarimide-β-acetaldehyde. (Synthesis of Isocycloheximide and its Isomers.)*[1]

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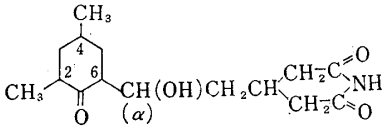
It is a recent tendency in an antibiotic field to synthesize a compound of the proposed structure of an antibiotic to confirm its molecular architecture and to find a key to elucidate the structure and activity relationships. However, notwithstanding the fact that the plane structure of cycloheximide had been proposed as (1) by Kornfeld, *et al.*[2] in 1949, papers concerning the synthesis had not been published until Phillips, *et al.*[3] suggested the way to synthesize this antibiotic by aldol condensation of 2,4-dimethylcyclohexanone with glutarimide-β-acetaldehyde in 1959. Soon later, Lawes[4] tried the condensation of 6-formyl-2,4-dimethylcyclohexanone with glutarimide-β-acetaldehyde and obtained successfully anhydrocycloheximide identical with the dehydration product



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*1 Presented before the 81st Annual Meeting of the Pharmaceutical Society of Japan (July 20, 1961). Preliminary Note : T. Okuda, M. Suzuki, Y. Egawa : J. Antibiotics, **14A**, 158 (1961).

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