Notes

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Studies on Syntheses of Epoxycardenolides and on Their Cleavage. V.[1] Hydrolytic Cleavage of 3β-Hydroxy-14α,15α-epoxy-5β-card-20(22)-enolide and 3β-Acetoxy-14β,15β-epoxy-5β-card-20(22)-enolide with Hydrochloric Acid

Yukio Saito, Yoshiko Kanemasa, and Masashi Okada

Tokyo Biochemical Research Institute[2]

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In the previous paper[1] the hydrolytic cleavage of 3β-hydroxy-14β,15β-epoxy-5β,17α-card-20(22)-enolide in 50% methanol containing 5% hydrochloric acid was reported, when the epoxide was cleaved to give 14β,15β-dihydroxy- and 14β-chloro-15β-hydroxy-17α-card-enolide, thus demonstrating an unusual *cis*-opening of the epoxide ring. In connection with this interesting finding hydrolytic cleavage in the similar manner of 3β-hydroxy-14α,15α-epoxy-5β-card-20(22)-enolide (I) and 3β-acetoxy-14β,15β-epoxy-5β-card-20(22)-enolide (Vb) was performed, which is described in this paper. The hydrolytic cleavage of these epoxy-cardenolides in aqueous acetone containing sulfuric acid[3] or perchloric acid[4] was reported earlier, when 3β,14,15α-trihydroxy-5β,14β-card-20(22)-enolide (15α-hydroxydigitoxigenin) (IIa) and 3β-acetoxy-15-oxo-5β,14α-card-20(22)-enolide (VIIb) were obtained from I and Vb as the sole product, respectively.

Treatment of I in 50% methanol with 5% hydrochloric acid gave a mixture of reaction products, whose thin-layer chromatography (TLC) revealed the formation of three products as shown in Fig. 1a. Chromatography using a column of silica gel afforded three substances. The first one was identified as IIa by direct comparison with the authentic sample reported earlier.[3] The second one was found to be 3β-hydroxy-15-oxo-5β,14β-card-20(22)-enolide (IIIa), since on acetylation it gave 3β-acetoxy-15-oxo-5β,14β-card-20(22)-enolide (IIIb) reported previously.[4] This compound was also prepared in the present work from 3β,15α-diacetoxy-14-hydroxy-5β,14α-card-20(22)-enolide (IVc)[5] by Serini-Logemann reaction.[6] The third one giving a positive Beilstein test was obtained as a minor product and could not be purified. Therefore, further study to elucidate the structure was not made.

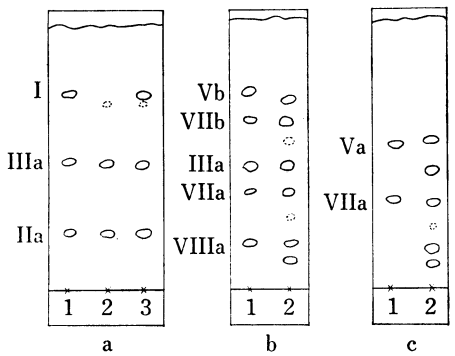

Fig. 1. Thin-Layer Chromatography

a : reaction product obtained from I after treatment with HCl in 50% MeOH: 1) I+IIa+IIIa, 2) reaction product, 3) I+2

b : reaction product obtained from Vb after treatment with HCl in 50% MeOH: 1) IIIa+Vb+VIIa+VIIb+VIIIa, 2) reaction product

c : reaction product obtained from Va after treatment with HCl in 50% MeOH: 1) Va+VIIa, 2) reaction product

1) Part IV: Y. Saito, Y. Kanemasa, and M. Okada, *Chem. Pharm. Bull.* (Tokyo), **19**, 1363 (1971).
2) Location: *Takada 3-Chome, Toshima-ku, Tokyo.*
3) M. Okada and M. Hasunuma, *Yakugaku Zasshi*, **85**, 822 (1965).
4) H. Ishii, T. Tozyo, and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **11**, 576 (1963).
5) M. Okada and Y. Saito, *Chem. Pharm. Bull.* (Tokyo), **17**, 515 (1969).
6) *a*) M.B. Rubin and E.C. Blossey, *Steroids*, **1**, 453 (1963); *b*) M.B. Rubin, *ibid.*, **2**, 561 (1963).

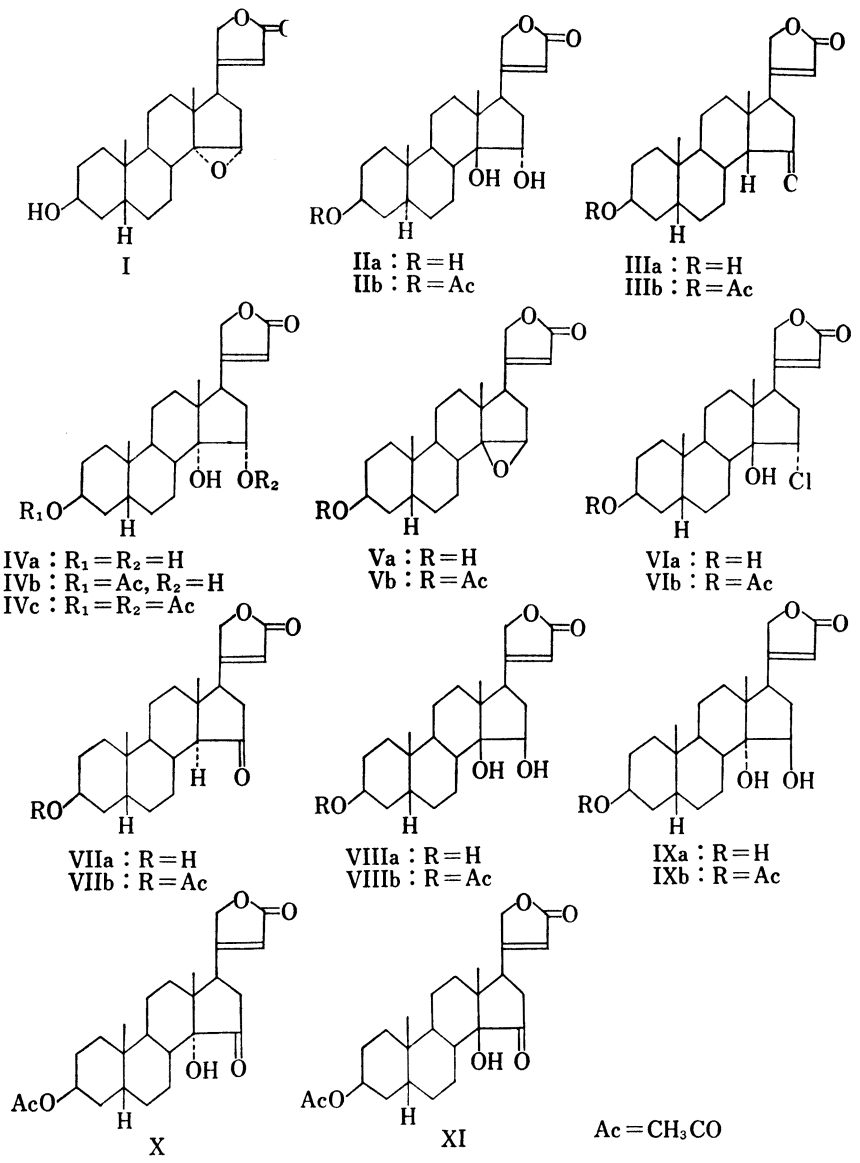

Chart 1

On the other hand, treatment of Vb in 50% methanol with hydrochloric acid gave a complex mixture of reaction products, whose TLC is given in Fig. 1b. Six compounds (IIIa, VIb, VIIa, VIIb, VIIIa, IXa) were obtained from the mixture on chromatography using silica gel. Identification of IIIa was made by direct comparison with the authentic substance described above. Concerning the structure of VIb which gave a positive Beilstein test, it is described below together with VIa. Acetylation of VIIa yielded VIIb which was recorded previously.[4] The fifth compound VIIIa was identified as 3β,14,15β-trihydroxy-5β,14β-card-20(22)-enolide (15β-hydroxydigitoxigenin) by direct comparison with the authentic one.[5]

The last compound IXa was inferred to be a 14,15-glycol from its mobility in TLC. It was different from the known glycols (14β, 15α,[3,4] 14β, 15β,[5] 14α, 15α[5]) which constitute

three out of four 14,15-glycols belonging to 3β-hydroxy-5β,17β-cardenolide. Then preparation of the unknown 14α,15β-glycol, 3β,14,15β-trihydroxy-5β,14α-card-20(22)-enolide (IXa), was made as follows. Sodium borohydride reduction of 3β-acetoxy-14-hydroxy-15-oxo-5β,14α-card-20(22)-enolide (X)[7] derivable from 3β-acetoxy-14,15α-dihydroxy-5β, 14α-card-20(22)-enolide (IVb)[5] gave IXb, which was different from IVb and afforded IXa on acid hydrolysis. This glycol was found to be different from 3β,14,15α-trihydroxy-5β,14α-card-20(22)-enolide (IVa)[5] and identical with the last compound obtained above.

In connection with this sodium borohydride reduction 3β-acetoxy-14-hydroxy-15-oxo-5β,14β-card-20(22)-enolide (XI)[3,5] was treated with the same reducing agent to give 3β-acetoxy-14,15α-dihydroxy-5β,14β-card-20(22)-enolide (IIb)[3] as the principal product. Formation of the epimer 3β-acetoxy-14,15β-dihydroxy-5β,14β-card-20(22)-enolide (VIIIb)[5] was proved only by TLC. It was thus demonstrated that sodium borohydride reduction of 14-hydroxy-15-oxo-cardenolides afforded predominantly 15β-hydroxy epimer in the 14α-cardenolide while giving 15α-hydroxy epimer in the 14β-cardenolide. In contrast with this finding it is noteworthy that 14-chloro-15-oxo-5β,14β,17α-cardenolide afforded 15β-hydroxy epimer on treatment with sodium borohydride.[1] Since it seems that stereochemistry of the sodium borohydride reduction of the 15-oxo group in cardenolides is markedly affected by the steric configuration of C_{17}-butenolide as well as of C_{14}-substituent, we are working further on this problem with several other 15-oxo cardenolides.

Hydrolytic cleavage of 3β-hydroxy-14β,15β-epoxy-5β-card-20(22)-enolide (Va) in the similar way yielded a complex mixture of reaction products (Fig. 1c), from which VIa and VIIa were obtained after repeated fractional crystallizations. The former gave a positive Beilstein test and afforded an acetate on acetylation with acetic anhydride and pyridine, which was identical with VIb described above. It was thought to be identical also with the chlorohydrin reported by Engel, *et al.*,[8] although direct comparison was not made. Treatment of VIb with alumina gave Vb. Therefore, it seemed quite reasonable to assign the structures indicated in Chart 1 (3β,14-dihydroxy-15α-chloro-5β,14β-card-20(22)-enolide and its acetate) to VIa and VIb, respectively. Based on essentially similar observations halohydrins[8,9] derived from Δ^{14}- or 14β,15β-epoxy-steroids were formulated as 14β-hydroxy-15α-halo–steroids.

Experimental[10]

Treatment of 3β-Hydroxy-14α,15α-epoxy-5β-card-20(22)-enolide (I) with Hydrochloric Acid in Methanol——A solufon of I (196 mg) in a mixture of MeOH (50 ml) and 10% HCl (50 ml) was allowed to stand at 25° for 45 min. After addition of H_2O (50 ml), MeOH was removed under reduced pressure, and the product was extracted with $CHCl_3$. The organic layer was washed with H_2O and dried over anhyd.Na_2SO_4. Evaporation of the solvent yielded a crystalline residue (205 mg), whose TLC is shown in Fig. 1a. The residue was chromatographed on a column of silica gel (11 g, E. Merck AG) by successive elution with benzene–EtOAc (5:1, 3:1, 1:1, 1:3) and MeOH. The fraction (80 mg) eluted with benzene–EtOAc (5:1) was recrystallized from acetone–pet. ether to give IIIa (56 mg). mp 232—239°. $[\alpha]_D^{25}$ −42.1° (c=0.71, MeOH). UV λ_{max} mμ (log ε): 214 (4.17). IR ν_{max} cm^{-1}: 3575 (OH), 1783, 1740, 1630 (butenolide and C-15 C=O). *Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 73.96; H, 8.80.

7) W. Zürcher, E. Weiss-Berg, and Ch. Tamm, *Helv. Chim. Acta*, **52**, 2449 (1969). According to this paper X could not be obtained by the oxidation of IVb with CrO_3–pyridine complex. We could obtain X, however, by using the same agent, though in low yield.

8) Ch. R. Engel and G. Bach, *Steroids*, **3**, 593 (1964).

9) M. Heller, F. J. McEvoy, and S. Bernstein, *Steroids*, **3**, 193 (1964).

10) Melting points were determined on a Kofler block and are uncorrected. Ultraviolet (UV) spectra were measured in 99% EtOH solution. Infrared (IR) spectra were determined in KBr disks on Hitachi EPI-S2 spectrophotometer; sh=shoulder. TLC plates were prepared according to the Stahl's procedure using Silica gel H (E. Merck AG) as adsorbent unless otherwise noticed. The solvent system used was methyl ethyl ketone–heptane (1:1), and the cardenolide spots were revealed by heating plates at 110° for 10 min after spraying 95% H_2SO_4 or Kedde reagent.

The fraction eluted with benzene–EtOAc (1:1, 1:3) was recrystallized from acetone–ether to afford IIa (53 mg), mp 242—246°, which was identical with an authentic sample[3] in TLC, mixed melting point and comparison of the IR spectrum.

3β-Acetoxy-15-oxo-5β,14β-card-20(22)-enolide (IIIb)——a) Acetylation of IIIa (5 mg) in the usual way with acetic anhydride and pyridine gave IIIb (3 mg) after recrystallization from MeOH–ether, mp 178—187°, identical with an authentic specimen described below in TLC, mixed melting point and comparison of the IR spectrum.

b) A solution of IVc (100 mg) in distilled xylene (10 ml) was refluxed for 9 hr in a nitrogen atmosphere with freshly activated zinc[6a] (2 g) while stirring vigorously. The solution was cooled, and the zinc was filtered and washed with xylene. The combined filtrates were concentrated *in vacuo* to give a crystalline residue (97 mg), which was recrystallized from MeOH–ether to afford IIIb (37 mg), mp 180—188°, identical with an authentic sample prepared according to Ishii, *et al.*[4] in the mixed melting point and comparison of the IR spectrum.

Treatment of 3β-Acetoxy-14β,15β-epoxy-5β-card-20(22)-enolide (Vb) with Hydrochloric Acid in Methanol——A solution of Vb (307 mg) in a mixture of MeOH (50 ml) and 10% HCl (50 ml) was allowed to stand at 28° for 22 hr. Working up in the same way as described above yielded a crystalline product (288 mg), whose TLC (Fig. 1b) indicated the presence of seven products. It was chromatographed on a column of silica gel (14.5 g) by successive elution with hexane–benzene (1:1), benzene, benzene–EtOAc (8:1, 6:1, 4:1, 2:1, 1:1), and EtOAc. The combined fraction (96 mg) eluted with hexane–benzene (1:1) and benzene gave VIb (5.4 mg) and VIIb (11 mg) after repeated recrystallizations from acetone–pet. ether. The former, mp 188—195°, was identical with the specimen of VIb decribed below in the mixed melting point and comparison of the IR spectrum. IR ν_{max} cm^{-1}: 3400 (OH), 1792, 1740, 1725, 1632 (butenolide and acetyl C=O). The latter, mp 216—226°, was identical with the sample of VIIb prepared according to the procedure reported previously[4] in TLC, mixed melting point and comparison of the IR spectrum.

The fraction (104 mg) eluted with benzene–EtOAc (8:1, 6:1, 4:1), whose TLC indicated the presence of IIIa, VIb, and VIIb, gave IIIa (27 mg), mp 219—223°, after repeated recrystallizations from acetone–ether–pet. ether.

The fraction (30 mg) eluted with benzene–EtOAc (2:1, 1:1), whose TLC revealed the presence of IIIa, VIIa, VIIIa and IXa, afforded IIIa (11 mg) and VIIa (10 mg), mp 255—270°, after repeated recrystallizations from acetone–ether–pet. ether. The latter was acetylated in the usual way with acetic anhydride and pyridine to give VIIb, mp 218—224°, identical with the above sample of VIIb in TLC, mixed melting point and comparison of the IR spectrum.

The fraction (47 mg) eluted with benzene–EtOAc (1:1) and EtOAc, whose TLC indicated the presence of VIIIa and IXa, afforded VIIIa (3 mg) and IXa (4 mg) after repeated recrystallizations from MeOH. The former (mp 244—247°) was identical with an authentic specimen[5] in TLC, mixed melting point and comparison of the IR spectrum. The latter (mp 242—252°) was identical with the sample of IXa prepared below in TLC, mixed melting point and comparison of the IR spectrum.

Treatment of 3β-Hydroxy-14β,15β-epoxy-5β-card-20(22)-enolide (Va) with Hydrochloric Acid in Methanol——A solution of Va (193 mg) in a mixture of MeOH (30 ml) and 10% HCl (30 ml) was allowed to stand at 26° for 3 hr. Working up in the same manner as described above gave a crystalline residue (220 mg), whose TLC (Fig. 1c) revealed the presence of five products. It was recrystallized repeatedly from acetone–MeOH to give VIa (17 mg), mp 187—198° (decomp.). IR ν_{max} cm^{-1}: 3480 (OH), 1798, 1755, 1720, 1635 (butenolide). *Anal.* Calcd. for $C_{23}H_{33}O_4Cl$: C, 67.55; H, 8.13. Found: C, 67.23; H, 8.16.

The residue obtained from the mother liquor from recrystallization of VIa was repeatedly recrystallized from acetone–ether to afford VIIa (25 mg), mp 256—271°.

3β-Acetoxy-14-hydroxy-15α-chloro-5β,14β-card-20(22)-enolide (VIb)——Acetylation of VIa (10 mg) in the usual way with acetic anhydride and pyridine gave VIb (4.2 mg), mp 192—201° (decomp.), after recrystallization from acetone. It gave a positive Beilstein test.

3β-Acetoxy-14,15β-dihydroxy-5β,14α-card-20(22)-enolide (IXb)——To a solution of X[7] (25 mg) in MeOH (2 ml) was added $NaBH_4$ (25 mg) at −5° while stirring. The reaction mixture was allowed to stand further for 10 min at the same temperature. After addition of AcOH (0.5 ml) and H_2O (30 ml) the solution was concentrated *in vacuo* to a small volume, and then the product was extracted with $CHCl_3$. The organic layer was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a crystalline residue, which was crystallized from acetone to afford IXb (15 mg). mp 262—267°. $[\alpha]_D^{26}$ +3.1° (c=0.65, $CHCl_3$). UV λ_{max} mμ (log ε): 215 (4.11). IR ν_{max} cm^{-1}: 3500 (OH), 1785, 1730, 1620 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.37; H, 8.37.

3β, 14, 15β-Trihydroxy-5β,14α-card-20(22)-enolide (IXa)——A solution of IXb (10 mg) in a mixture of MeOH (2 ml) and 10% HCl (2 ml) was allowed to stand at 26° for 18 hr. After neutralization with 5% Na_2CO_3, H_2O (20 ml) was added and the solution was concentrated *in vacuo* to a small volume, and then the product was extracted with $CHCl_3$. The organic layer was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a crystalline residue (10.8 mg), which was subjected to a preparative TLC on Aluminum oxide G (E. Merck AG) using methyl ethyl ketone–heptane (1:1) as solvent, affording IXa (6 mg) after recrystallization from acetone–ether. mp 260—262°. $[\alpha]_D^{23}$ −6.1° (c=0.66, MeOH). UV λ_{max}

mμ (log ε): 220 (4.19). IR ν_{max} cm^{-1}: 3500 (OH), 1780, 1720, 1620 (butenolide). *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.56; H, 8.92.

Reduction of 3β-Acetoxy-14-hydroxy-15-oxo-14β-card-20(22)-enolide (XI) with $NaBH_4$——To a solution of XI[3,5] (52 mg) in MeOH (3 ml) was added $NaBH_4$ (53 mg) at 0° while stirring. The reaction mixture was allowed to stand for 1.5 hr at the same temperature. Working up in the same way as described above yielded a crystalline product (46 mg) whose TLC revealed the presence of IIb and VIIIb, the former predominating. It was recrystallized from MeOH–ether to give IIb (27 mg), mp 243—248°, identical with the authentic specimen[3] in TLC, mixed melting point and comparison of the IR spectrum.

Conversion of VIb into Vb by Treatment with Alumina——To a solution of VIb (2 mg) in a mixture of MeOH (0.5 ml) and $CHCl_3$ (0.5 ml) was added neutral alumina (100 mg, activity grade III—IV[11]), and the mixture was allowed to stand at room temperature for 66 hr. After filtration of the alumina, it was washed with acetone. The combined filtrates were concentrated to dryness giving a crystalline residue (1.5 mg) which was recrystallized from acetone–pet. ether to afford Vb (*ca.* 1 mg), mp 177—178°, identical with an authentic sample in TLC, mixed melting point and comparison of the IR spectrum.

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Studies on Heterocyclic Compounds. XV.[1] Synthesis of Furo[2,3-*d*]pyridazine Derivatives. (4).[2] Synthesis of 2-[2-(5-Nitro-2-furyl)vinyl]-4,7-dichlorofuro-[2,3-*d*]pyridazine and Related Compounds by the Wittig Reaction[3]

Shigetaka Yoshina and Isamu Maeba

Faculty of Pharmacy, Meijo University[4]

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For several years 5-nitro-2-furyl derivatives have been of marked biological interest, since it was shown that particularly 5-nitro-2-furylvinyl heterocyclic compounds[5] are highly antibacterial.

The biological activity of some of these compounds encouraged us to prepare 2-[2-(5-nitro-2-furyl)vinyl]-4,7-dichlorofuro[2,3-*d*]pyridazine (IV) by the Wittig reaction.

For the synthesis of IV, 2-methyl-4,7-dichlorofuro[2,3-*d*]pyridazine(Ia)[6] served as the starting material. Reaction of Ia with N-bromosuccinimide (NBS) in anhydrous benzene solution in the presence of α,α′-azobisisobutyronitrile (AIBN)[7] as the catalyst gave 2-bromomethyl-4,7-dichlorofuro[2,3-*d*]pyridazine (IIa) in yield 45%, mp 158—160°.

Similarly, reaction of 2-methyl-7-chloro-4-methoxyfuro[2,3-*d*]pyridazine (Ib) with NBS gave 2-bromomethyl-7-chloro-4-methoxyfuro[2,3-*d*]pyridazine (IIb) and reaction of 2-methyl-

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4) Location: *Tenpaku-cho, Showa-ku, Nagoya.*
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6) S. Yoshina, I. Maeba, and K. Hirano, *Chem. Pharm. Bull.* (Tokyo), **17**, 2158 (1969).
7) J.D. Prugh, A.C. Huitric, and W.C. McCarthy, *J. Org. Chem.*, **29**, 1991 (1964).