aqueous tetrahydrofuran, methanol, or ethanol gave triiodoacrylic acid (XIIIa), methyl triiodoacrylate (XIIIb) or ethyl triiodoacrylate (XIIIc), respectively.

The formation of III from 1,3-diiodopropyne (II) by the action of alkali was well explained in terms of intermediate formation of the allenic carbanion (IX) followed by the iodination of IX with the hypoiodite resulted from the ethynyl iodine of III.

A reaction mechanism involving the intermediate formation of the epoxides (XXIII) and their isomerization to the corresponding carbonyl compounds with the 1,2-iodide shift gave a good explanation proposed for the oxygenolytic solvolysis of III and IV.

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155. Daisuke Satoh and Mieko Horie: Studies on Digitalis Glycosides. XXV.*1 Preparation of 14β,15β-Epoxycardenolides from Odoroside H and Digitoxin.*2

(*Shionogi Research Laboratory, Shionogi & Co., Ltd.**3)

Several epoxycardenolides, having the epoxide ring at 7β,8β-,[1] 8β,14β-,[2] 11β,12β-[3] and 16α,17α-[4] positions were found in plants as glycosides, while those at 14α,15α- and 14β,15β-,[5~9] and 16β,17β-[10] positions were only synthesized and have never been known as glycosides. In the previous paper,[6] the preparation of 3β-hydroxy-14β,15β-epoxy-5β-card-20(22)-enolide was reported. This paper is concerned with the preparation of glycosides possessing 14β,15β-epoxide as aglycone.

Digitoxigenin-β-D-monodigitaloside diacetate (odoroside H diacetate, Ib)[11,12] was treated with thionyl chloride in pyridine to give 14-anhydrodigitoxigenin-β-D-monodigitaloside diacetate (IIc), $C_{34}H_{48}O_9$, m.p. 218~221°. A mild hydrolysis of IIc with 2% hydrochloric acid in a mixture of chloroform and methanol (1:3) afforded 14-anhydrodigitoxigenin-β-D-monodigitaloside (IIa), $C_{30}H_{44}O_7$, m.p. 209~212°, and its monoacetate (IIb), $C_{32}H_{46}O_8$, m.p. 250~255°. The compounds (IIb) and (IIc) had been prepared by Reichstein and his co-workers[11] from odoroside H monoacetate (Ia) by

*1 Part XXIV. D. Satoh, M. Horie, J. Morita: This Bulletin, **14**, 613 (1966).

*2 A part of this work has been reported in brief in the review entitled "Studies on the Constituents of *Digitalis purpurea* L Leaves" published in Ann. Rept. Shionogi Res. Lab., **14**, 14 (1964).

*3 Fukushima-ku, Osaka (佐藤大助, 堀江美恵子).

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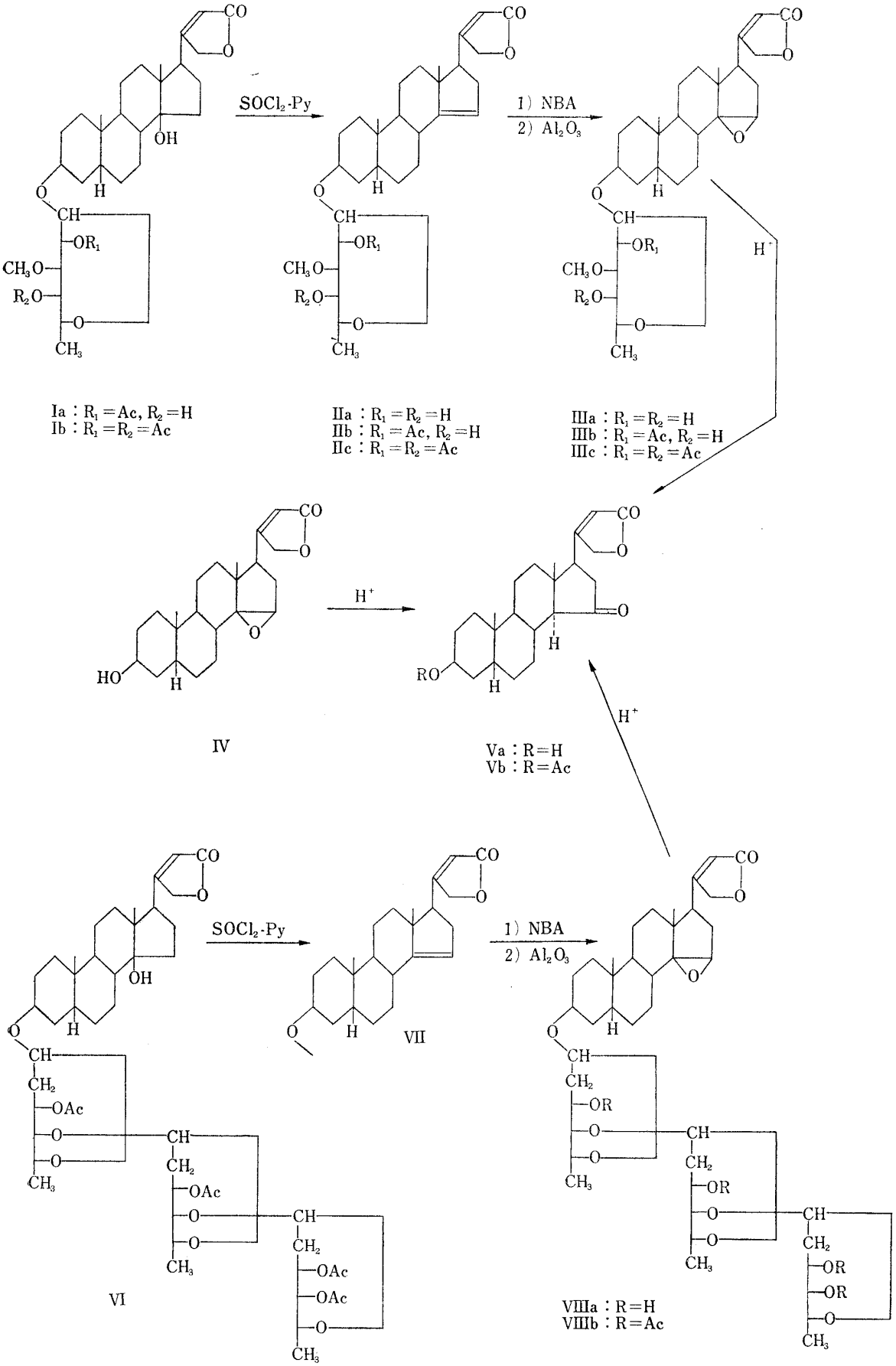

the hydrolysis with dilute hydrochloric acid in acetone and subsequent acetylation respectively.

14-Anhydro compound (IIc) was treated with N-bromoacetamide (NBA) in acetone and the crude bromohydrine thus obtained was passed through an alumia column to give 14β,15β-epoxy-5β-card-20(22)-enolide-3β-ol-monodigitaloside diacetaete (IIIc), $C_{34}H_{48}O_{10}$, m.p. 238~245°. The structrue was proved by identification of a hydrolysis product of IIIc, m.p. 271~275°, with 3β-hydroxy-15-oxo-5β,14α-card-20(22)-enolide (Va), $C_{23}H_{32}O_4$, m.p. 272~276°, which was prepared from 3β-hydroxy-14β,15β-epoxy-5β-card-20(22)-enolide (IV) by *cis* hydride shift of 14β,15β-epoxide ring with acid.[6] Submission of IIa and IIb to the epoxidation reaction analogous to that of IIc, resulted in the formation of 14β,15β-epoxy-5β-card-20(22)-enolide-3β-ol-β-D-monodigitaloside (IIIa, amorphous powder) and its monoacetate (IIIb), $C_{32}H_{46}O_9$, m.p. 205~209°, respectively. The glycoside (IIIa) was also obtained from IIIc by a microbiological deacetylation with *Absidia hyalospora*.

Dehydration of digitoxigenin-β-D-tridigitoxoside tetraacetate (digitoxin tetraacetate, VI)[13] and subsequent epoxidation of thus obtained 14-anhydrodigitoxigenin-β-D-tridigitoxoside tetraacetate (VII), $C_{49}H_{70}O_{16}$, m.p. 150~155°, gave 14β,15β-epoxy-5β-card-20(22)-enolide-3β-ol-β-D-tridigitoxoside tetraacetate (VIIb, amorphous powder). Microbiological deacetylation of VIIb with *Cunninghamella echinulata* afforded 14β,15β-epoxy-5β-card-20(22)-enolide-3β-ol-β-D-tridigitoxoside (VIIa), $C_{41}H_{62}O_{13}$, m.p. 225~230°. The proof of 14β,15β-epoxide structure of VIIa was made by identification of a hydrolysis product of VIIa with Va, analogously to that of IIIc described above.

Experimental*4

14-Anhydrodigitoxigenin-β-D-monodigitaloside Diacetate (IIc)——To a solution of 200 mg. of Ib in 2 ml. of pyridine, 0.2 ml. of $SOCl_2$ was added under agitating at about −15° and the mixture was allowed to stand for 30 min. at the same temperature. Excess of $SOCl_2$ was decomposed with ice-water and the crude product was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was recrystallized from AcOEt-*n*-hexane to give 150 mg. of IIc as colorless prisms, m.p. 218~221°, $[\alpha]_D^{25}$ −7.6°(c=1.007, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{48}O_9$: C, 67.98; H, 8.05. Found: C, 67.70; H, 8.30. UV λ_{max}^{EtOH} mμ: 209 (14,15-double bond), 215 (butenolide). IR ν_{max}^{Nujol} cm^{-1}: 1799, 1768, 1638 (butenolide), 1744 (Ac).

14-Anhydrodigitoxigen-β-D-monodigitaloside (IIa) and Monoacetate (IIb) from IIc——A solution of 200 mg. of IIc in 8 ml. of 2% HCl in a mixture of $CHCl_3$ and MeOH (1:3) was allowed to stand at room temperature for 12 days, and after dilution with H_2O, neutralized with 10% Na_2CO_3, concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product (180 mg.) was separated into the following two components by a column chromatography using 10 g. of silica gel.

a) Less polar fraction eluted with $CHCl_3$–acetone (5:1) was recrystallized from AcOEt-*n*-hexane to give 32 mg. of IIb as colorless needles, m.p. 250~255°. *Anal.* Calcd. for $C_{32}H_{46}O_8 \cdot H_2O$: C, 66.64; H, 8.39. Found: C, 66.82; H, 8.35. IR ν_{max}^{Nujol} cm^{-1}: 3524, 3364 (OH), 1778, 1744, 1627 (butenolide), 1744 (Ac).

b) More polar fraction eluted with $CHCl_3$–acetone (5:1) was recrystallized from AcOEt-*n*-hexane to give 56 mg. of IIa as colorless prisms, m.p. 209~212°. *Anal* Calcd. for $C_{30}H_{44}O_7$: C, 69.74; H, 8.58. Found: C, 69.81; H, 8.70. IR ν_{max}^{Nujol} cm^{-1}: 3458, 3415 (OH), 1779, 1734, 1626 (butenolide), 1719 (Ac).

14β,15β-Epoxy-5β-card-20(22)-enolide-3β-ol-β-D-monodigitaloside Diacetate (IIIc)——To a solution of 350 mg. of IIc in 17.5 ml. of acetone, a solution of 186 mg. of NBA in 2.8 ml. of H_2O was added, and the mixture was set aside for 1 hr. at room temperature. After dilution with 20 ml. of H_2O, acetone was evaporated *in vacuo* and the crude product was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated under reduced pressure. The crude bromohydrine thus obtained (375 mg.) was dissolved in 30 ml. of benzene, poured into a column filled with 10 g. of Al_2O_3 (neutral), and eluted with benzene and benzene-$CHCl_3$ mixture (10:1 and 1:1). After evaporation of solvent, all fractions were combined (292 mg.) and recrystallized from AcOEt-*n*-hexane to afford 210 mg. of IIIc as colorless needles, m.p. 238~245°, $[\alpha]_D^{26.5}$ +22.6°(c=0.864, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{48}O_{10}$: C, 66.21; H, 7.85.

*4 All melting points are uncorrected.

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Found: C, 66.52; H, 7.86. UV λ_{max}^{EtOH} mμ (ε): 213.5 (17,060), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1787, 1741, 1626 (butenolide), 1741 (Ac).

Hydrolysis of IIIc to 3β-Hydroxy-15-oxo-5β,14α-card-20(22)-enolide (Va)——A solution of 25 mg. of IIIc in 3 ml. of 5% HCl (in 50% EtOH) was refluxed for 4 hr. and neutralized with 5% NaOH. After EtOH was removed *in vacuo*, the precipitates were extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated under reduced pressure. The residu (16 mg.) was recrystallized from MeOH–ether to give 10 mg. of Va, m.p. 271~275°. Mixed melting point and IR spectrum showed the identity with the sample prepared from 3β-hydroxy-14β,15β-epoxy-5β-card-20(22)-enolide (IV).

3β-Hydroxy-15-oxo-5β,14α-card-20(22)-enolide (Va) from 3β-Hydroxy-14β,15β-epoxy-5β-card-20(22)-enolide (IV)——After a solution of 100 mg. of IV in 10 ml. of 5% HCl (in 50% EtOH) was refluxed for 1.5 hr., the solution was neutralized with 5% NaOH, concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and $CHCl_3$ was evaporated under reduced pressure. The residue was recrystallized from MeOH–ether to give 53 mg. of Va as colorless plates, m.p. 272~276°. *Anal.* Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.39; H, 8.68. IR ν_{max}^{Nujol} cm^{-1}: 3520 (OH), 1797, 1748, 1631 (butenolide), 1733 (five membered ring ketone).

Acetylation of this product with Ac_2O and pyridine gave Vb, m.p. 232~235°.[6]

14β,15β-Epoxy-5β-card-20(22)-enolide-3β-ol-β-D-monodigitaloside (IIIa) from IIIc——*Absidia hyalospora* was grown for 28 hr. with shaking at 26° on a nutrient medium (10 L.) containing 4% glucose, 2% peptone and 0.3% corn steep liquor. To this fermentation broth, a solution of 100 mg. of IIIc dissolved in 100 ml. of MeOH was added and incubation was further continued for 94 hr. at 26°. The mycelium was filtered and washed with H_2O. The filtrate was extracted with $CHCl_3$ and $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The crude product (130 mg.) was dissolved in $CHCl_3$ and purified by a column chromatography with 4 g. of SiO_2. Evaporation of $CHCl_3$ from eluate gave 30 mg. of IIIa as amorphous powder. TLC (SiO_2, $CHCl_3$–acetone=1:1) showed the homogeneity of this product. $[\alpha]_D^{27.5}$ +14.1° (c=1.053, $CHCl_3$), UV λ_{max}^{EtOH} mμ (ε): 215 (16,400), IR ν_{max}^{Nujol} cm^{-1}: 3570 (OH), 1785, 1744, 1627 (butenolide).

Acetylation of IIIa to IIIc——A mixture of 30 mg. of IIIa, 0.3 ml. of Ac_2O and 0.3 ml. of pyridine was allowed to stand overnight at room temperature. After dilution with ice-water, the precipitates were collected by filtration, washed with H_2O and recrystallized from acetone–*n*-hexane to give 20 mg. of IIIc as colorless needles, m.p. 236~243°.

14β,15β-Epoxy-5β-card-20(22)-enolide-3β-ol-β-D-monodigitaloside (IIIa) from IIa——To a solution of 200 mg. of IIa in 10 ml. of acetone, a solution of 119 mg. of NBA in 2 ml. of H_2O was added and the mixture was set aside at room temperature for 15 min. After dilution with H_2O, acetone was evaporated *in vacuo* and the residual mixture was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and $CHCl_3$ was distilled off under reduced pressure. The crude bromohydrine (250 mg.) was dissolved in $CHCl_3$ and was adsorbed on 8 g. of Al_2O_3 (neutral) in a column, and then eluted with the same solvent. Evaporation of $CHCl_3$ from the eluate under reduced pressure gave 126 mg. of IIIa as a white powder. TLC (SiO_2, $CHCl_3$–acetone=1:1) showed the homogeneity of this product, and IR spectrum exhibited the identity with IIIa prepared from IIIc.

14β,15β-Epoxy-5β-card-20(22)-enolide-3β-ol-β-D-monodigitaloside Monoacetate (IIIb) from IIb——A solution of 45 mg. of NBA in 0.8 ml. of H_2O was added to a solution of 75 mg. of IIb in 4 ml. of acetone, and the mixture was set aside at room temperature for 15 min. After dilution with H_2O, acetone was evaporated *in vacuo* and the precipitates were collected by filtration. The crude bromohydrine (70 mg.) obtained was dissolved in $CHCl_3$ and the $CHCl_3$ solution was treated with 2 g. of Al_2O_3 (neutral) for epoxidation analogously to that of IIa described above. The crude eluate (50 mg.) was recrystallized from AcOEt–*n*-hexane to afford 40 mg. of IIIb as colorless needles, m.p. 205~209°, $[\alpha]_D^{23}$ +23.1°(c=1.035, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{46}O_9$: C, 66.87; H, 8.07. Found: C, 66.56; H, 8.26. UV λ_{max}^{EtOH} mμ (ε): 215 (16,020), IR ν_{max}^{Nujol} cm^{-1}: 3420 (OH), 1784, 1742, 1628 (butenolide), 1742 (Ac).

14-Anhydrodigitoxigenin-β-D-tridigitoxoside Tetraacetate (VII)——To a solution of 300 mg. of VI in 6 ml. of pyridine, a solution of 0.3 ml. of $SOCl_2$ in 3 ml. of pyridine was added in dropping wise under agitating at about −15° and the mixture was kept at the same temperature for 1 hr. The reaction mixture was poured into ice-water, the procipitates were extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated under reduced pressure. Recrystallization of the crude product (273 mg.) from MeOH to give 156 mg. of VII as colorless needles, m.p. 150~155°. *Anal.* Calcd. for $C_{49}H_{70}O_{16}\cdot H_2O$: C, 63.07; H, 7.78. Found: C, 62.94; H, 7.59.

14β,15β-Epoxy-5β-card-20(22)-enolide-3β-ol-β-D-tridigitoxoside Tetraacetate (VIIIb)——A solution of 65 mg. of NBA in 2 ml. of 50% acetone was added to a solution of 130 mg. of VII in 5 ml. of acetone, and the mixture was set aside at room temperature for 1 hr. After dilution with H_2O, acetone was evaporated *in vacuo* and the precipitates were extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4, and adsorbed on 4 g. of Al_2O_3 (neutral) in a column and then eluted with $CHCl_3$. After evaporation of $CHCl_3$ from the eluate, the crude product (110 mg.) was purified by a preparative TLC (SiO_2, $CHCl_3$–acetone=5:1) to give 75 mg. of VIIIb as a homogeneous powder.

14β,15β-Epoxy-5β-card-20(22)-enolide-3β-ol-β-D-tridigitoxoside (VIIIa)——Three and half liters of nutrient solution containing 4% glucose, 2% peptone, and 0.3% corn steep liquor was inoculated with

Cunninghamella echinulata and incubated under shaking for 44 hr. at 26°. To this fermentation broth, 350 mg. of VIIIb dissolved in 17.5 ml. of MeOH was added and incubation was further continued for 98 hr. at 26°. The fermentation broths were extracted with $CHCl_3$–acetone (10:1) and $CHCl_3$–MeOH (2:1) and the extracts were combined and evaporated *in vacuo*. The crude product (420 mg.) was dissolved in $CHCl_3$ and submitted to column chromatography using 5 g. of Al_2O_3(neutral). The $CHCl_3$–MeOH (10:1) fraction (130 mg.) was recrystallized from MeOH to give 70 mg. of VIIIa as colorless needles, m.p. 225~230°, $[\alpha]_D^{26}$ +26.9° (c=0.934, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{62}O_{13}$: C, 64.55; H, 8.19. Found: C, 64.17; H, 8.11. UV λ_{max}^{EtOH} mμ(ε): 214 (17,180), IR ν_{max}^{Nujol} cm^{-1}: 3570, 3400 (OH), 1785, 1755, 1627 (butenolide).

Hydrolysis of VIIIa to Va——A solution of 30 mg. of VIIIa in 3.5 ml. of 5% HCl (in 50% EtOH) was refluxed for 30 min. and neutralized with 5% NaOH. After EtOH was removed under reduced pressure, the precipitates were extracted with $CHCl_3$. The $CHCl_3$ solution washed with H_2O, dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The residue (14 mg.) was recrystallized from MeOH–ether to afford 7 mg. of Va, m.p.271~274°.

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Summary

Odoroside H (Ia) was converted into 14β,15β-epoxy-5β-card-20(22)-enolide-3β-ol--β-D-monodigitaloside (IIIa) in the following reaction sequence: Dehydration of odoroside H diacetate (Ib) with thionyl chloride in pyridine to give 14-anhydrodigitoxigenin-monodigitaloside diacetate (IIc). Mild hydrolysis of IIc with acid afforded the corresponding acetyl-free glycoside (IIa) together with monoacetate (IIb). These 14-anhydro compounds (IIa, IIb and IIc) were treated with N-bromoacetamide followed by alumina chromatography to yield 14β,15β-epoxy-5β-card-20(22)-enolide-3β-ol-β-D-monodigitaloside (IIIa), and its mono- and diacetate (IIIb and IIIc) respectively. Microbiological deacetylation of IIIc furnished IIIa.

In the same manner, digitoxin tetraacetate (VI) was changed into 14β,15β-epoxy-5β-card-20(22)-enolide-3β-ol-β-D-tridigitoxoside (VIIIa).

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