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Studies on Digitalis Glycosides. XXXI.[1] Stepwise Degradation of Polydigitoxosides of Cardenolides[2]

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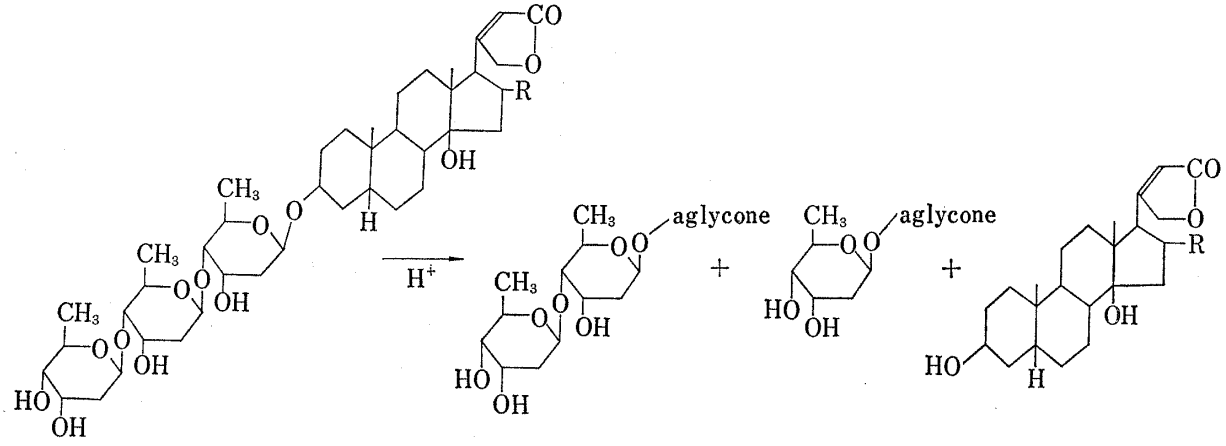
Stepwise degradation of the polydigitoxosides of cardenolides was achieved by metaperiodate oxidation, reduction and subsequent hydrolysis under the specified conditions in good yield.

As a degradation method of digitoxin (Ia) and gitoxin (Ib), the tridigitoxoside of digitoxigenin (IVa) and gitoxigenin (IVb) respectively, to the corresponding bisdigitoxoside (II) and monodigitoxoside (III) was reported the partial hydrolysis of the tridigitoxosides (I) with dilute acid.[4] However, this method produced a mixture of II, III and IV besides the intact starting material (I), and the isolation of the individual product needed the complicated procedure. Recently, one of the authors (D.S.) and co-worker[1] found a selective degradation method of the terminal digitoxose (2-deoxysugar) moiety in tridigitoxoside by metaperiodate oxidation and subsequent hydrolysis in the course of studies on the digitoxin acetate. While the controlled degradation of polysaccharides consisting of 2-hydroxysugar by metaperiodate oxidation, reduction and hydrolysis had been well known,[5] that of 2-deoxysugar has never been reported. Further studies of our degradation method showed that it is very superior to the known method[4] in the degradation of tridigitoxoside to bis- and monodigitoxoside. This paper deals with the detailed studies of this method.



Ia : R=H
Ib : R=OH

IIa, b

IIIa, b

aglycone
IVa, b

Chart 1

1) Part XXX: D. Satoh and J. Morita, *Chem. Pharm. Bull.* (Tokyo), **17**, 1456 (1969).
2) This work was reported at the 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April, 1969.
3) Location: *Sagisu, Fukushima-ku, Osaka.*
4) F. Kaiser, E. Haack, and H. Spingler, *Ann.*, **603**, 75 (1957).
5) I.J. Goldstein, G.W. Hay, B.A. Lewis, and F. Smith, "Methods in Carbohydrate Chemistry," Vol. V, Academic Press, New York and London, 1965, p. 361; T. Kubota and H. Hinoh, *Tetrahedron*, **24**, 675 (1968).

Digitoxin (Ia) was easily oxidized with sodium metaperiodate and formed a so-called dialdehyde (Va) as a homogeneous powder under cleavage of 3‴,4‴-*cis*-glycol in the terminal digitoxose moiety. As the nuclear magnetic resonance spectrum[6] (NMR) of Va exhibited only an aldehydic proton signal at 0.30 τ as a triplet (J=2.0 cps), it was assumed that 3‴-aldehyde group remains intact and 4‴-aldehyde group forms an intramolecular hemiacetal (VIa) with 3″-hydroxyl group.

When Va was treated with 0.0065N hydrochloric acid in methanol or 0.1% potassium hydrogen carbonate in acetone at room temperature for about 24 hours, digitoxigenin bisdigitoxoside (IIa) was obtained as a main product. Though the fragments splitted from the terminal sugar moiety were presumed to be propan-1-al-2-ol (VII) and propane-1,3-dial (VIII), they could not be proved in gas–liquid chromatography (GLC) probably due to their instabili-

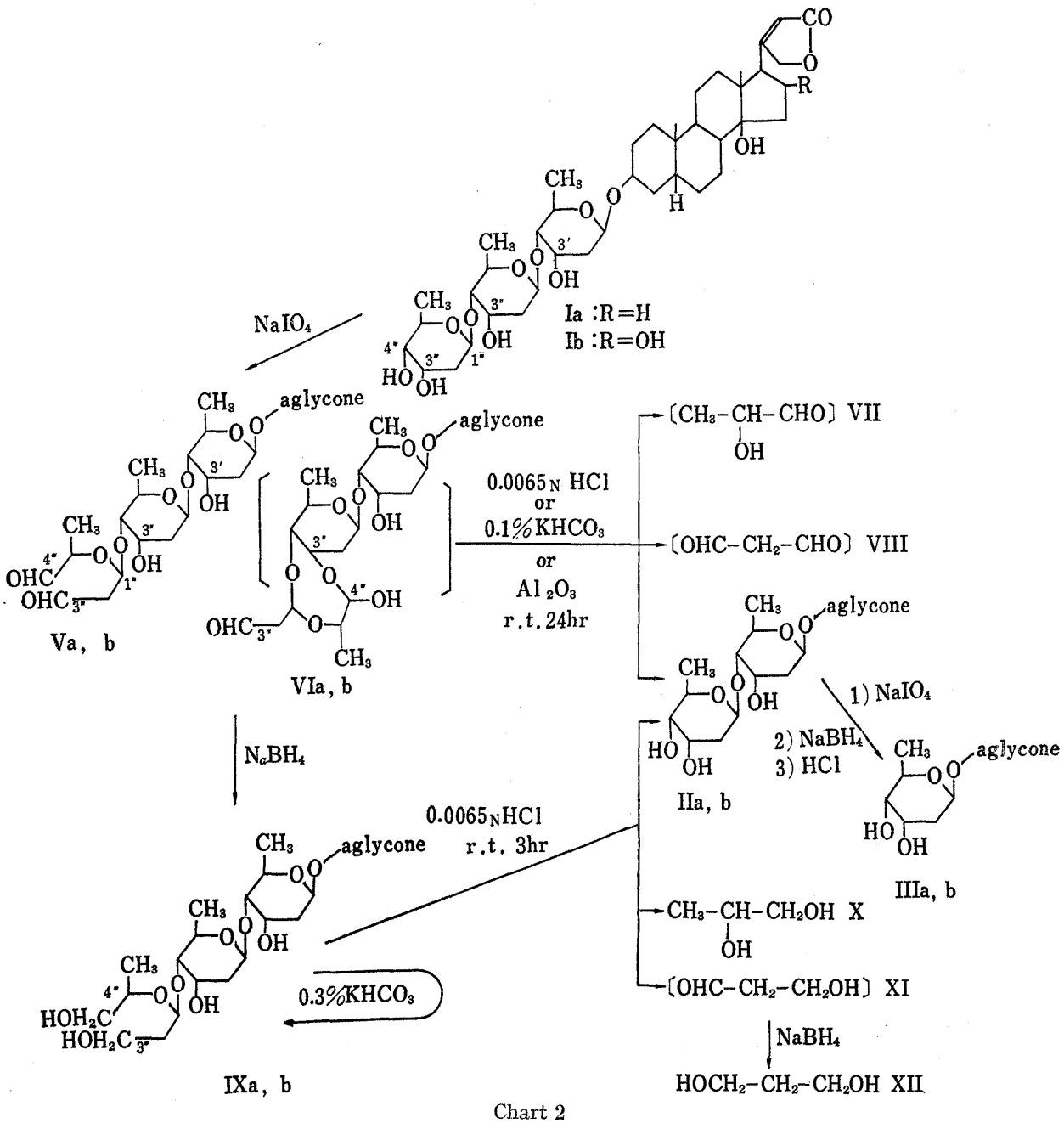


Chart 2

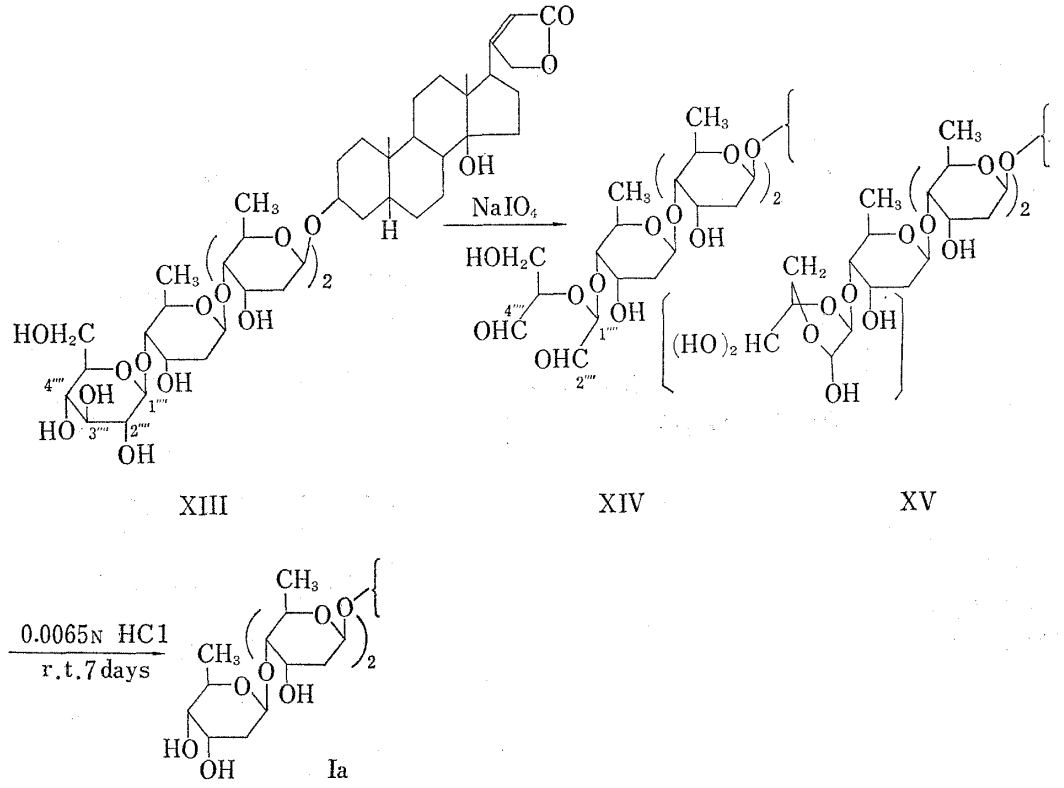
6) Chemical shifts were measured at 60 Mc in $CDCl_3$.

ties. Treatment of Va with neutral alumina at room temperature overnight also gave IIa as a major product, but the yield was inferior to the former procedures.

The best result was obtained by the following way. Thus, when Va was reduced with sodium borohydride, a dimethylol (IXa) was formed as a homogeneous product. Though IXa was inert to 0.3% potassium hydrogen carbonate, it easily underwent a fission with 0.0065N hydrochloric acid and the hydrolysis was completed at room temperature after 3 hours to afford IIa in about 80% yield. Among the fragments produced in the fission of the dimethylol moiety, propane-1,2-diol (X) was identified by GLC and the another fragment, *i.e.*, propan-1-al-3-ol (XI), was proved as propane-1,3-diol (XII) after reduction with sodium borohydride. As, contrary to IXa, its tetraacetate was found to be inert to 0.0065N hydrochloric acid, the readiness of IXa for hydrolysis to IIa was presumed to be ascribable to some participation of the hydroxyl group at 3‴- or 4‴- position.

Degradation of IIa to digitoxigenin monodigitoxoside (IIIa) and that of gitoxin (Ib) to gitoxigenin bisdigitoxoside (IIb) were achieved satisfactorily by the analogous procedure, and the further degradation of IIb to the monodigitoxoside (IIIb) and the stepwise degradation of digoxin (12β-hydroxydigitoxin) to the corresponding bis- and monodigitoxoside can be expected to proceed by the analogous way without any difficulty.

For the purpose to compare the above mentioned fission of digitoxose (2-deoxysugar) to that of glucose (2-hydroxysugar) in cardenolides, we examined the degradation of purpurea glycoside A (XIII) to digitoxin (Ia) by the analogous way. Though a cleavage product of XIII with sodium metaperiodate reduced the Tollen's reagent, NMR[6] of the product did not exhibited any aldehydic proton signal. Therefore, the dialdehyde (XIV) produced in the glycol cleavage of the terminal glucose moiety was presumed to form an intramolecular hemiacetal (XV).[5] Hydrolysis of XIV to Ia with 0.0065N hydrochloric acid did not proceed so easily as that of Va, and the completion of hydrolysis required 7 days at room temperature. From these results, the surpassing reactivity of V comparing to XIV for the fission of the alde-



XIII XIV XV

Ia

Chart 3

hydic fragments was considered to be due to a β-elimination,[7] because the acetal linkage at 1'''-position in V is situated at β-position to 3'''-aldehyde group while that at 1''''-position in XIV is located at α-position to both 2''''- and 4''''-aldehyde groups.

Experimental[8]

Thin-Layer Chromatography (TLC)——TLC were all performed by the following system.
SiO_2 (Merck), $CHCl_3$–acetone (1:1, v/v)

Oxidation of Digitoxin (Ia) with $NaIO_4$——To a solution of Ia (1 g) in 95% EtOH (80 ml) was added a solution of $NaIO_4$ (1 g) in H_2O (10 ml) under stirring at room temperature and the mixture was allowed to stand at the same temperature for 1 hr. After removing $NaIO_3$ precipitated by filtration, the solution was concentrated *in vacuo* under 50° and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give dialdehyde (Va, 0.99 g) as a white powder, which was proved to be homogeneous by TLC. $[\alpha]_D^{23}$ +8.4° (c=0.478, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470 (broad, OH), 1788, 1742, 1625 (butenolide), 1742 (CHO). Va reduced the Tollen's reagent.

Reduction of Va with $NaBH_4$——To a solution of Va (500 mg) in 95% MeOH (50 ml) was added $NaBH_4$ (250 mg) in portionwise at room temperature. After the mixture was set aside at the same temperature for 1 hr, the solution was exactly neutralized[9] with 5% AcOH, concentrated *in vacuo* under 50° and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give the dimethylol (IXa, 490 mg) as a white and almost homogeneous powder which was purified with CH_2Cl_2–*n*-hexane to afford a white and homogeneous powder, mp 125—130°. *Anal.* Calcd. for $C_{41}H_{66}O_{13}$: C, 64.20; H, 8.67. Found: C, 64.26; H, 8.90. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430 (broad, OH), 1781, 1740, 1620 (butenolide).

Acetylation of IXa——After a solution of IXa (100 mg) and Ac_2O (1 ml) in pyridine (1 ml) was allowed to stand at room temperature for 6 days to complete acetylation, ice–water was added and the mixture was extracted with $CHCl_3$. The $CHCl_3$ solution was washed successively with ice-cooled 3% HCl, 3% $NaHCO_3$ and H_2O to neutral, dried over Na_2SO_4 and evaporated *in vacuo* to give a crude acetate (103 mg), which was purified with AcOEt–*n*-hexane to afford a white and homogeneous tetraacetate of IXa, mp 82—87°. *Anal.* Calcd. for $C_{49}H_{74}O_{17}\cdot 1\frac{1}{2}H_2O$: C, 61.17; H, 8.07. Found: C, 60.80; H, 7.86. NMR ($CDCl_3$ τ): 7.91 (2Ac), 7.94 (Ac), 7.98 (Ac).

Oxidation of Gitoxin (Ib) with $NaIO_4$——To a solution of Ib (1 g) in a mixture of $CHCl_3$ and MeOH (1:1) (250 ml) was added a solution of $NaIO_4$ (1 g) in H_2O (10 ml) under stirring. After being allowed to stand at room temperature for 2 hr, the mixture was treated in the analogous manner to the oxidation of Ia to afford a crude dialdehyde (Vb, 980 mg) as a white and homogeneous powder. Vb reduced the Tollen's reagent. $[\alpha]_D^{27}$ +10.5° (c=1.027, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (broad, OH), 1776, 1731, 1613 (butenolide), 1731 (CHO).

Reduction of Vb with $NaBH_4$——To a solution of Vb (500 mg) in 95% MeOH (50 ml) was added $NaBH_4$ (59 mg, 5 equivalents[10]) in portionwise at room temperature. After the mixture was set aside at the same temperature for 30 min, the resulted solution was treated analogously to the reduction of Va to give a crude dimethylol (IXb, 502 mg) as a white and almost homogeneous powder. $[\alpha]_D^{27}$ +11.3° (c=0.940, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420 (broad, OH), 1788, 1742, 1623 (butenolide). The crude dimethylol could be used to the next reaction without further purification.

Digitoxigenin Bisdigitoxoside (IIa)——i) From Va with Acid: To a solution of Va (300 mg) in MeOH (30 ml) was added 0.05N HCl (4.5 ml) and the mixed solution was allowed to stand at room temperature for about 24 hr. The solution was neutralized with 5% $KHCO_3$, concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give a crude product (225 mg) which was shown to consist of a main product together with two by-products by TLC. The main product (124 mg) was separated by preparative TLC and recrystallized[11] from AcOEt–*n*-hexane to give IIa (101 mg) as colorless prisms, mp 228—230°, $[\alpha]_D^{23}$ +7.3° (c=0.833, MeOH). *Anal.* Calcd. for $C_{35}H_{54}O_{10}$: C, 66.22; H, 8.57. Found: C, 65.96; H, 8.53. UV λ_{max}^{EtOH} mμ (ε): 217.5 (14200). Mixed melting point and comparisons of *Rf* values and IR spectra with the authentic sample proved the structure of IIa.

ii) From Va with Alkali: Va (200 mg) was dissolved in 0.1% $KHCO_3$ in acetone (20 ml) and the solution was allowed to stand at room temperature for about 24 hr. The resulted solution was neutralized with

7) J.J. Dugan and P. de Mayo, *Can. J. Chem.*, 43, 2033 (1965); R.D. Guthrie, "Advances in Carbohydrate Chemistry," Vol. 16, Academic Press, New York and London, 1961, p. 105.
8) All melting points are uncorrected.
9) When the solution was kept in acidic condition, hydrolysis to bisdigitoxoside may proceed.
10) pH value of the reaction mixture was about 8.2 (T.B.). When pH value was over 9.0, a side reaction owing to a formation of isogitoxigenin (16,21-epoxycardanolide) could not be avoided.
11) When acetone was used in recrystallization, it combined with 11a persistently as a solvent of crystallization.

5% AcOH, concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give a crude product (166 mg) which was submitted to preparative TLC to isolate the main product (83 mg) which was recrystallized from AcOEt–*n*-hexane to afford IIa (66 mg), mp 227—230°.

iii) From Va with Al_2O_3: To a solution of Va (200 mg) in a mixture of $CHCl_3$ (2 ml) and benzene (6 ml) was added neutral Al_2O_3 (Merck, 8 g) and the mixture was set aside at room temperature for 20 hr. Then the mixture was extracted with a mixture of $CHCl_3$ and MeOH (1:1) and the extract was evaporated *in vacuo* to give a crude product (137 mg) which was shown to consist of a major product together with three minor products. The major product (76 mg) isolated by preparative TLC was recrystallized from dilute MeOH to afford IIa (63 mg), mp 228—230°.

iv) From IXa with Acid: A solution of IXa (450 mg) in a mixture of MeOH (30 ml) and 0.05N HCl (4.5 ml) was allowed to stand at room temperature for 3 hr and the solution was neutralized with 5% $NaHCO_3$, concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give a crude product (367 mg) which was shown to be almost homogeneous. Recrystallization from AcOEt–*n*-hexane afforded IIa (290 mg), mp 228—230°.

Detection of Fragments in Hydrolysis of IXa to IIa——A part of the aqueous layer which separated from the $CHCl_3$ extract in the hydrolysis of IXa to IIa was submitted to a GLC on a 4 mm×2 m column containing DEGS (10% on Gas Chrom Q (60—80 mesh)) at 140° with a flow rate of 50 ml/min of N_2 using Shimazu GC–4APF instrument, and the existence of propane-1,2-diol (X) was proved by a comparison of Kováts-index[12] (2096.5) with that (2096.5) of the authentic sample.

Another part (1 ml) of the aqueous layer was reduced with $NaBH_4$ (100 mg) and, after neutralization with 5% AcOH and deionization with Amberite IR–120A and Dowex I, was submitted to a GLC under the analogous conditions to the above procedure and the formation of propane-1,3-diol (XII) was proved by a comparison of Kováts-index (2121.8) with that (2122.7) of the authentic sample.

Digitoxigenin-monodigitoxoside (IIIa) from IIa——To a solution of IIa (150 mg) in 95% EtOH (10 ml) was added a solution of $NaIO_4$ (150 mg) in H_2O (2 ml) and the mixture was allowed to stand at room temperature for 1 hr. After $NaIO_3$ deposited was removed by filtration and the filtrate was treated in the analogous procedure to the oxidation of Ia described above to give a crude dialdehyde (152 mg) which was shown to be homogeneous by TLC.

The dialdehyde (150 mg) was dissolved in 95% MeOH (15 ml) and $NaBH_4$ (75 mg) was added under stirring at room temperature. After allowing to stand for 2 hr, the solution was neutralized and treated in the usual manner to afford a crude dimethylol (135 mg) as an homogeneous powder.

Then the dimethylol (134 mg) was dissolved in a mixture of MeOH (12 ml) and 0.05N HCl (1.8 ml), and the solution was set aside at room temperature for 3 hr, and treated in the usual manner to give a crude IIIa (118 mg) as an almost homogeneous powder which was recrystallized from acetone–*n*-hexane to afford IIIa (90 mg) as colorless needles, mp 197—200°, $[\alpha]_D^{23}$ −5.2° (c=0.327, MeOH). *Anal.* Calcd. for $C_{29}H_{44}O_7$: C, 69.02; H, 8.79. Found: C, 68.70; H, 8.68. UV λ_{max}^{EtOH} mμ (ε): 218 (15090). Mixed melting point and comparisons of *Rf* values and IR spectra with the authentic sample proved the identity of the both substances.

Gitoxigenin Bisdigitoxoside (IIb)——i) From Vb with Acid: To a solution of Vb (200 mg) in MeOH (20 ml) was added 0.05N HCl (3 ml) and the solution was treated in the analogous manner to the hydrolysis of Va to IIa, and the crude product (139 mg) was submitted to preparative TLC to separate a main product (85 mg) which was recrystallized from acetone–*n*-hexane tc afford IIb (62 mg) as colorless needles, mp 199—201°, $[\alpha]_D^{23}$ +18.6° (c=0.591, MeOH). *Anal.* Calcd. for $C_{35}H_{54}O_{11}$: C, 64.59; H, 8.36. Found: C, 64.34; H, 8.47. UV λ_{max}^{EtOH} mμ (ε): 219 (15300). Mixed fusion and comparisons of *Rf* values and IR spectra with the authentic sample proved the identity of the both substances.

ii) From Vb with Alkali: A solution of Vb (200 mg) in 0.1% $KHCO_3$ in acetone (20 ml) was treated by the similar procedure and the crude product (148 mg) was submitted to preparative TLC to isolate a main product (90 mg) which was recrystallized from acetone–*n*-hexane to afford IIb (69 mg), mp 198—201°.

iii) From Vb through IXb: To a solution of Vb (410 mg) in 95% MeOH (41 ml) was added $NaBH_4$ (48 mg) under stirring at room temperature and, after being set aside for 1 hr, the solution was acidified to pH 2.8 and was allowed to stand at room temperature for 5 hr to complete hydrolysis of IXb there formed to IIb. The acidic solution was neutralized, concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give a crude product (363 mg) which was submitted to preparative TLC to isolate a main product (258 mg). Recrystallization of the main product from acetone–*n*-hexane afforded IIb (232 mg), mp 198—201°.

Oxidation of Purpurea Glycoside A (XIII) with $NaIO_4$——After a mixture of a solution of XIII (25 mg) in 95% EtOH (2 ml) and a solution of $NaIO_4$ (25 mg) in H_2O (0.25 ml) was allowed to stand at room temperature overnight, the reaction mixture was concentrated *in vacuo* under 50°, extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give a crude dialdehyde

12) E. Kováts, *Helv. Chim. Acta*, **41**, 1915 (1958).

(XIV, 26 mg) as a white powder which was shown to be homogeneous by TLC. XIV reduces the Tollen's reagent.

Hydrolysis of XIV to Digitoxin (Ia)——To a solution of XIV (25 mg) in MeOH (10 ml) was added 0.05N HCl (0.3 ml) and the mixture was set aside at room temperature for 7 days for complete hydrolysis. The resulted solution was neutralized and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give a crude product (18 mg) which was purified by preparative TLC and recrystallization from acetone–*n*-hexane to afford Ia (8 mg) as a colorless crystalline powder, mp 247—250°. Mixed fusion and comparisons of *Rf* values and IR spectra with the authentic sample proved the identity of the both substances.

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