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Studies on Digitalis Glycosides. XXX.[1]

Digitoxin Acetates. (1)

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The order of acetylation of four hydroxyl groups in the sugar moieties of digitoxin (Ia) was revised to be $4'''\rightarrow3'''\rightarrow3''$- or $3'$-positions by the results of acetyl migration test and *cis*-glycol test as well as modified controlled polysaccharide degradation method using metaperiodate. Nuclear magnetic resonance (NMR) spectra of several acetates of Ia were examined, and the conformations of digitoxose moieties of Ia were confirmed to be all C1-form as formula XIa from the NMR data and reactiviteis of hydroxyl groups in acetylation.

In the previous paper,[3] the author and co-workers reported that acetyldigitoxin-α ($3'''$-monoacetyldigitoxin, III) was obtained as a main product in partial acetylation of digitoxin (Ia). Sasakawa, *et al.*[4] proposed the order of acetylation of four hydroxyl groups in sugar moieties of Ia to be $3'''\rightarrow3''\rightarrow3'\rightarrow4'''$-positions. While Kroszczynski, *et al.*[5] reported the preferential formation of acetyldigitoxin-β ($4'''$-monoacetyldigitoxin, II) in partial acetylation of Ia, Voigtländer, *et al.*[6] described that $3'''$-hydroxyl group was more reactive in acetylation than $4'''$-hydroxyl group. Moreover, the results of our study on the acetylation of digitoxin[7] (Ib) prompted the authors to reinvestigate the acetylation of Ia in detail, and the real order of acetylation was established as $4'''\rightarrow3'''\rightarrow3''$- or $3'$-positions. This paper deals with these studies.

When Ia was acetylated with 3.4 moles of acetic anhydride in pyridine at room temperature, a mixture of monoacetate and diacetate was obtained, which was separated into each component by preparative thin-layer chromatography (TLC) with silica gel avoiding prolonged contact with adsorbent. The monoacetate, mp 160°/264—267°, was identified with the known acetyldigitoxin-β[8] ($4'''$-monoacetyldigitoxin,II) by mixed melting point and comparisons of optical rotations, TLC and infrared (IR) spectra. Treatment of II with 0.1% potassium hydrogen carbonate in aqueous acetone yielded the known acetyldigitoxin-α[8] ($3'''$-monoacetyldigitoxin, III), mp 158°/245—249°, which was formed by acetyl migration from $4'''$-position to the neighbouring hydroxyl group, and the similar isomerization was also observed in prolonged contact with silica gel or alumina. From these resluts, it was clarified that III isolated previously by the author and co-workers[3] was a secondary product formed from the original product (II) by acetyl migration during column chromatography. The positions of acetyl groups of acetyldigitoxin-α (III) and -β (II) isolated from digitalis leaves had been

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1) Part XXIX: D. Satoh and S. Nishii, *Chem. Pahrm. Bull.* (Tokyo), **17**, 1401 (1969).
2) Location: *Sagisu, Fukushima-ku, Osaka.*
3) D. Satoh, Y. Oyama, and T. Okumura, *Chem. Pharm. Bull.* (Tokyo), **5**, 627 (1957).
4) Y. Sasakawa and T. Kamiya, *Yakugaku Zasshi*, **81**, 1007 (1961).
5) W. Kroszczynski, M. Lukaszewski, J. Zurkowska, H. Marciszewski, and A. Ozarowski, *Arch. Polan. Pharm.*, **20**, 121 (1963).
6) H.-W. Voigtländer and G. Balsam, *Arch. Pharm.*, **301**, 208 (1968).
7) J. Morita and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **16**, 1056 (1968).
8) A. Stoll and W. Kreis, *Helv. Chim. Acta*, **35**, 1318 (1952).
9) R. Tschesche, B. Niyomporn, and H. Machleidt, *Chem. Ber.*, **92**, 2258 (1959).

established.[9,10] As the diacetate[11] (IV), mp 263—266°, showed a negative acetyl migration test,[12] the vicinal hydroxyl groups in the terminal digitoxose should be both substituted. Accordingly, IV was designated as 3‴, 4‴-diacetyldigitoxin.

When acetylation was performed with 7.7 moles of acetic anhydride, a mixture of diacetate, triacetate and tetraacetate was obtained, which was separated into each component by preparative TLC. The diacetate was identical with IV. Though the triacetate fraction seemed to be homogeneous by the usual TLC, a continuous development TLC[13] or multiple development TLC[14] revealed that triacetate fraction was composed of two components in about 3:1 ratio. Recrystallization of this fraction from aqueous methanol afforded a triacetate (V),[15] mp 232—235°, and preparative continuous development TLC[13] or preparative multiple development TLC[14] of the mother liquor of V yielded another triacetate (VI), mp 142—145°. As V and VI did not show an acetyl migration test similarly to IV, the both triacetate were thought to be formed by a further acetylation of one of the two hydroxyl groups in the inner digitoxose moieties of IV, *i.e.*, 3′-or 3″-position respectively, which was clarified by the following modified controlled polysaccharide degradation method[16] under avoiding acetyl migration.

Thus, when V was treated with dilute potassium hydrogen carbonate (*e.g.* 0.3%) in methanol at room temperature, two acetyl groups in the terminal digitoxose were selectively eliminated to give a monoacetate (VIII), mp 158—163°. VIII was readily oxidized with sodium metaperiodate to give a dialdehyde acetate (IX) whose structure was supported by a positive Tollen's test and nuclear magnetic resonance (NMR) spectrum (in $CDCl_3$) assigned as 0.22 τ (1H, t, J=2.0 cps, aldehyde), 0.44 τ (1H, d, J=1.5 cps, aldehyde) and 8.00 τ (3H, s, acetyl group). Further mild acid hydrolysis of IX (*e.g.* 0.0065 N HCl) yielded a digitoxigenin–bisdigitoxoside monoacetate (X), mp 143—147°, by eliminating the terminal digitoxose moiety of VIII. The fact that *cis*-glycol test[12] was negative with X showed that the acetyl group of X located at the terminal sugar moiety. As 1,4-linkages of sugar moieties of digitoxin (Ia) had been established[9,17] and acetyl migration was not observed in a blind test under the condition employed in the hydrolysis of IX to X, the acetyl group of X should be located at 3″-position. From these results, the structure of VIII and X should be 3″-monoacetyldigitoxin[18] and digitoxigenin-bisdigitoxoside 3″-monoacetate, respectively. Consequently, V was established as 3″, 3‴, 4‴-triacetyldigitoxin. As acetyl migration test[12] of the another triacetate (VI) was also negative, VI was thought to be formed by acetylating the other hydroxyl group of inner sugar moieties of IV, *i.e.*, at 3′-position. Accordingly, VI was designated as 3′,3‴,4‴-triacetyldigitoxin. Further confirmation by the sugar fission

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10) M. Kuhn, H. Lichti, and A. v. Wartburg, *Helv. Chim. Acta*, **45**, 881 (1962).
11) Melting point and optical rotation of IV are comparable to those of the diacetate of Sasakawa, *et al.*[4]
12) See the experimental section of this paper.
13) *a*) Y. Hashimoto, "Thin–Layer Chromatograph," Hirokawa–shoten, Tokyo, 1962, p. 126; *b*) N. Zöllner and G. Wolfram, *Klin. Wochschr.*, **40**, 1098 (1962); *c*) R. D. Bennett and E. Heftmann, *J. Chromatog.*, **12**, 245 (1963); *d*) E. Heftmann (ed.), "Chromatography," 2nd Ed. Reinhold Publishing Corp., New York, 1967, p. 175; *e*) N. Tsuji and K. Nagashima, *Tetrahedron*, in press.
14) E. Stahl (ed.), "Thin–Layer Chromatography," Springer-Verlag, Berlin·Heiderberg·New York Academic Press, 1965, p. 34.
15) Melting point and optical rotation are comparable to those of the triacetate of Sasakawa, *et al.*[4]
16) The controlled degradation of polysaccharide consisting of 2-deoxysuger may be the first instance. This method will be described in another paper in detail.
17) H. Lichti, M. Kuhn, and A.v. Wartburg, *Helv. Chim. Acta*, **45**, 868 (1962).
18) 3″-Monoacetyldigitoxin can be designated as acetyldigitoxin-δ to distinguish from 3′-monoacetyldigitoxin (acetyldigitoxin-γ),[19] 3‴-monoacetyldigitoxin (acetyldigitoxin-α)[8] and 4‴-monoacetyldigitoxin (acetyldigitoxin-β).[8]
19) K. Hoji, *Chem. Pharm. Bull.* (Tokyo), **9**, 296 (1961).

method is in progress.[20] The tetraacetate is the completely acetylated product, *i.e.*, 3′,3″,3‴,4‴-tetraacetyldigitoxin (VII).[4,21]

From the above mentioned results, the order of acetylation of the four hydroxyl groups in digitoxin (Ia) was established as 4‴→3‴→3″- or 3′-positions. Incorrect assignments

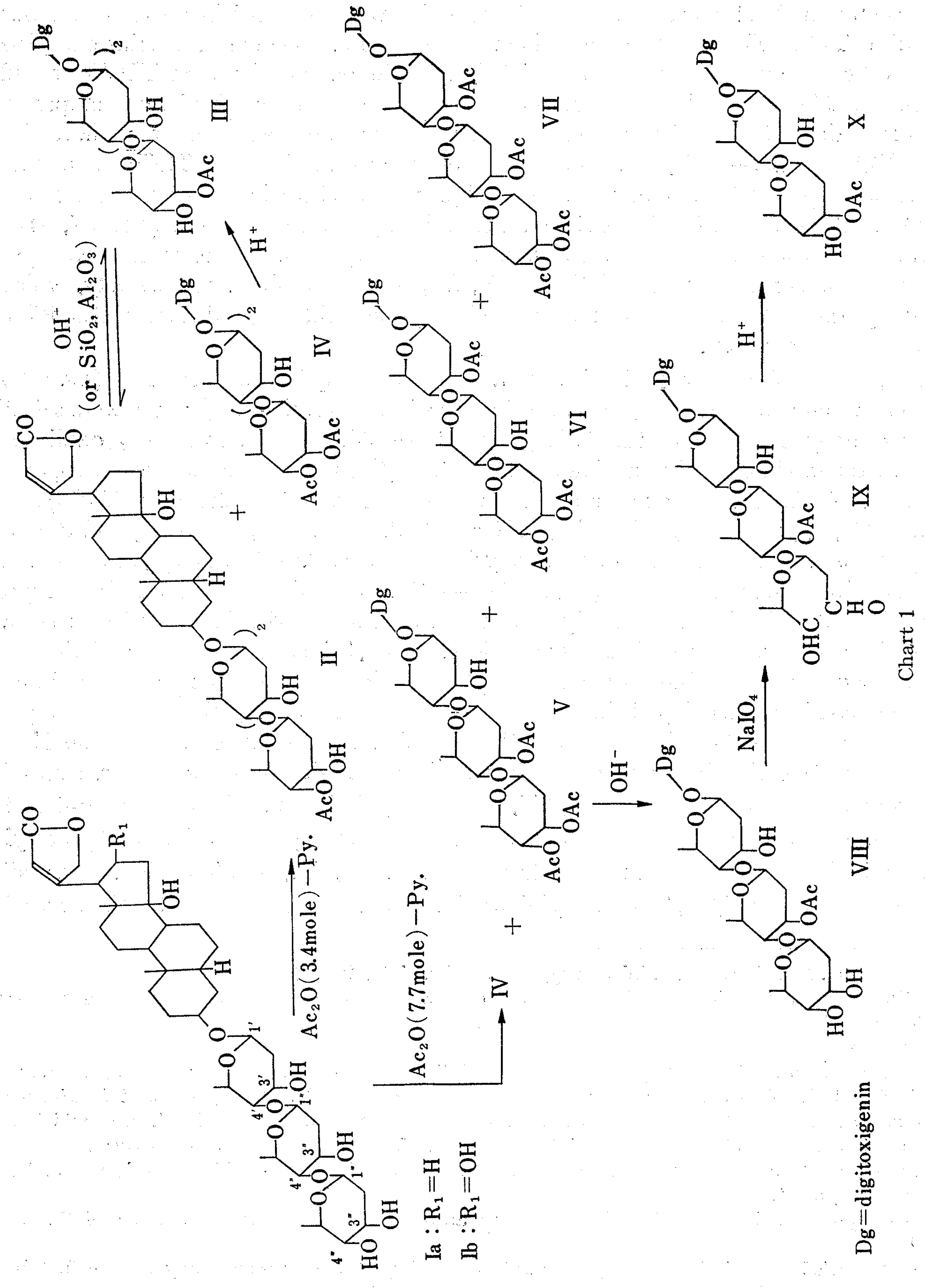

Chart 1

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[20] After the later experiments, 3′-monoacetyldigitoxin (acetyldigitoxin-γ), mp 170—172°, was obtained from VI by the partial hydrolysis with potassium hydrogen carbonate. The modified controlled degradation of this acetate gave digitoxigenin–bisdigitoxoside 3′-monoacetate, mp 140—145°, whose *cis*-glycol test was positive. This result proved the structure of VI.

[21] A. Okano, K. Hoji, J. Miki, and K. Miyatake, *Chem. Pharm. Bull.* (Tokyo), 5, 171 (1957).

of the former researchers[3,4,6] are presumed to be due to acetyl migrations during chromatographic separations or acetyl group elimination in hydrolysis of the acetates.

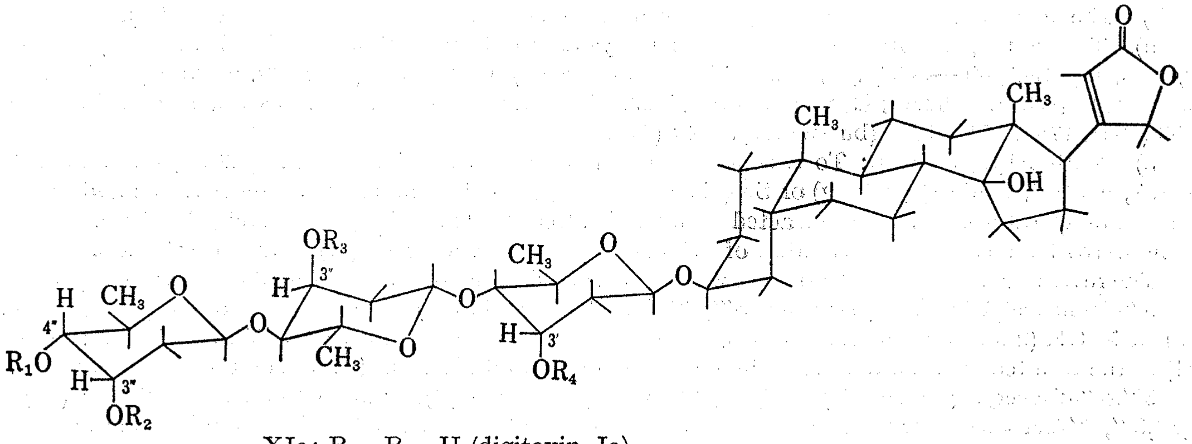
Voigtländer, *et al.*[6] studied the NMR spectra of acetates of cardiac glycosides and noticed their availability for the location of acetyl groups. The author and co-workers also used the NMR data for the assignment of positions of acyl groups in gitoxin acetates[7] and alkoxycarbonates.[22] In the present studies, NMR spectra of digitoxin acetates (Table I) contributed to the confirmation of the sugar moiety conformation of digitoxin (Ia).

The signal of 3‴-H of III appeared as narrow multiplet at 4.72 τ ascribable to be equatorial[23] as indicated in XIc, while that of 4‴-H in II overlapped that of 21-H (2H) at nearly 5.15 τ corresponding to axial one[23] as indicated in XIb. These data proved the C1-type conformation of the terminal digitoxose moiety of digitoxin as formula XIa. Preferential formation of II (XIb) in acetylation of Ia (XIa) described above was reasonably explained by the favorable situation of equatorial hydroxyl group (4‴-OH) to acetylation. The fact that numbers of equatorial protons were two in both V (XIf) and VI (XIg), and three in VII (XIh) (Table I) also proved C1-type conformations of the inner digitoxose moieties. As β-glycosidic linkages[9] and 1,4-linkages[9,17] of digitoxoses had been established, the whole con-

Table I. NMR Signals of Digitoxin Acetates (τ, in $CDCl_3$, 60 Mc)

Acetates	Axial-OAc 3′,3″,3‴-Positions	Equatorial-OAc 4‴-Position	Equatorial-H[a] 3′,3″,3‴-Positions
4‴-Mono (II, XIb)		7.90 (3H)	
3‴-Mono (III, XIc)	7.87 (3H)		4.72 (1H)
3″-Mono (VIII, XId)	7.90 (3H)		4.62 (1H)
3‴,4‴-Di (IV, XIe)	7.90 (3H)	8.01 (3H)	4.56 (1H)
3″,3‴,4‴-Tri (V, XIf)	7.90 (6H)	8.01 (3H)	4.61 (2H)
3′,3‴,4‴-Tri (VI, XIg)	7.90 (6H)	8.00 (3H)	4.60 (2H)
3′,3″,3‴,4‴-Tetra (VII, XIh)	7.90 (9H)	8.01 (3H)	4.61 (3H)

a) narrow multiplet, respectively



XIa: $R_1-R_4=H$ (digitoxin, Ia)
XIb: $R_1=Ac$, $R_2-R_4=H$ (4‴-monoacetate, II)
XIc: $R_2=Ac$, $R_1=R_3=R_4=H$ (3‴-monoacetate, III)
XId: $R_3=Ac$, $R_1=R_2=R_4=H$ (3″-monoacetate, VIII)
XIe: $R_1=R_2=Ac$, $R_3=R_4=H$ (3‴,4‴-diacetate, IV)
XIf: $R_1-R_3=Ac$, $R_4=H$ (3″,3‴,4‴-triacetate, V)
XIg: $R_1=R_2=R_4=Ac$, $R_3=H$ (3′,3‴,4‴-triacetate, VI)
XIh: $R_1-R_4=Ac$ (tetraacetate, VII)

Chart 2

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22) D. Satoh, S. Kobayashi, and J. Morita, *Chem. Pharm. Bull.* (Tokyo), **17**, 682 (1969)

23) In NMR spectrum (in $CDCl_3$) of digitoxose 1,3,4-triacetate, the signal of 3-H (equatorial) appeared at 4.52 τ, and that of 4-H (axial) was observed at 5.36 τ.[22]

formation of sugar part of digitoxin (Ia) was confirmed to be XIa. Similarly to the NMR spectra of gitoxin acetate,[7] the signal of 4'''-acetyl group in IV (XIe), V (XIf), VI (XIg) and VII (XIh) shifted to higher field as compared with that of II (XIb) by the effect of the neibouring 3'''-acetoxyl group, respectively (Table I).

Experimental[24]

Acetyl Migration Test——A solution of sample (1 mg) in 0.1% $KHCO_3$ (0.2 ml) in 80% acetone was set aside at room temperature overnight, and the resulted solution was examined by TLC (SiO_2 Merck, $CHCl_3$: acetone=2:1). When acetyl group migrates from 4'''-position to 3'''-position, a new spot appeared under that of the intact sample, and, when acetyl migration occurred in the opposite direction, a new spot appeared upper that of the intact sample.

***cis*-Glycol Test**——To a solution of sample (1 mg) in 95% EtOH (0.2 ml) was added 10% $NaIO_4$ solution in H_2O (1 drop) and the mixture was set aside at room temperature. When the sample has *cis*-glycol grouping, the test solution became cloudy due to a deposition of $NaIO_3$ within 10 min.

4'''-Monoacetyldigitoxin (Acetyldigitoxin-*β*, II) and 3''',4'''-Diacetyldigitoxin (IV) from Digitoxin (Ia)——To a solution of Ia (500 mg) in pyridine (3 ml) was added acetic anhydride (0.2 ml, 3.4 moles) and the mixture was allowed to stand overnight at room temperature. After dilution with ice-water, the crude acetate (520 mg) there deposited was collected by filtration, washed with H_2O and dried *in vacuo*, which was separated into two fractions by preparative TLC (SiO_2 Merck, $CHCl_3$:acetone=1:1).

i) The less polar fraction (164 mg) was recrystallized from acetone to give IV (115 mg) as colorless crystals, mp 263—266°, $[\alpha]_D^{23}$ +23.4° (c=0.103, MeOH). *Anal.* Calcd. for $C_{45}H_{68}O_{15}$: C, 63.66; H, 8.07; $COCH_3$, 10.14. Found: C, 63.41; H, 8.07; $COCH_3$, 9.94. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3568 (OH), 1784, 1744, 1624 (butenolide), 1744 (Ac).

ii) The more polar fraction (236 mg) was recrystallized from acetone to give II (165 mg) as colorless crystals, mp 160°/264—267°, $[\alpha]_D^{23}$ +25.8° (c=0.244, MeOH), $[\alpha]_D^{23}$ +11.4° (c=0.527, pyridine). *Anal.* Calcd. for $C_{43}H_{66}O_{14}\cdot H_2O$: C, 62.60; H, 8.31; $COCH_3$, 5.22. Found: C, 62.64; H, 8.34; $COCH_3$, 4.98. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3615 (OH), 1790, 1751, 1630 (butenolide), 1751 (Ac).

3'''-Monoacetyldigitoxin (Acetyldigitoxin-*α*, III) from II——a) With Alkali: A solution of II (100 mg) in 0.1% $KHCO_3$ (25 ml) in aqueous acetone (4:1) was allowed to stand overnight at room temperature, neutralized with dil. HCl, 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 crude product (95 mg) which was separated into two fractions by preparative TLC (SiO_2 Merck, $CHCl_3$:acetone=2:1).

i) The less polar fraction (34 mg) was proved to be the intact starting material (II).

ii) The more polar fraction (45 mg) was recrystallized from acetone to give III (36 mg) as colorless crystals, mp 158°/245—249°, $[\alpha]_D^{23}$ +21.9° (c=0.434, MeOH), $[\alpha]_D^{23}$ +3.2° (c=0.539, pyridine). *Anal.* Calcd. for $C_{43}H_{66}O_{14}\cdot H_2O$: C, 62.60; H, 8.31; $COCH_3$, 5.22. Found: C, 62.89; H, 8.19: $COCH_3$, 5.19. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3563 (OH), 1783, 1747, 1624 (butenolide), 1747 (Ac).

b) With Al_2O_3 or SiO_2: To a solution of II (5 mg) in $CHCl_3$:MeOH=2:1 (0.2 ml) was added Al_2O_3 (Merck, neutral, grade I) (200 mg) or SiO_2 Merck (200 mg) and the mixture was allowed to stand overnight at room temperature, and then extracted with $CHCl_3$: MeOH=1:1. TLC (SiO_2 Merck, $CHCl_3$: acetone=2:1) of the extract indicated the formation of III beside the intact material (II) in about 1:1 ratio.

The reverse isomerization of III to II was also observed in the similar treatment of III with Al_2O_3 or SiO_2.

3'''-Monoacetyldigitoxin (III) from 3''',4'''-Diacetyldigitoxin (IV)——A solution of IV (5 mg) in 0.005 N HCl in MeOH (2 ml) was set aside overnight at room temperature. TLC (SiO_2 Merck, AcOEt: benzene=5:1) of the resulted solution indicated the formation of III and digitoxin (Ia) beside the intact material (IV).

3'''.4'''-Diacetyldigitoxin (IV), 3'',3''',4'''-Triacetyldigitoxin (V), 3',3''',4'''-Triacetyldigitoxin (VI) and 3',3'',3''',4'''-Tetraacetyldigitoxin (VII) from Ia——To a solution of Ia (500 mg) in pyridine (3 ml) was added acetic anhydride (0.45 ml, 7.7 moles) and the mixed solution was allowed to stand overnight at room temperature. After dilution with ice-water, the crude acetate (620 mg) there precipitated was collected by filtration, washed with H_2O and dried *in vacuo*, which was separated into the following three fractions (i, ii and iii) by preparative TLC (SiO_2 Merck, AcOEt:benzene=1:1).

i) The less polar fraction (101 mg) was recrystallized from aqueous MeOH to give VII (75 mg) as colorless crystals, mp 154—158°. $[\alpha]_D^{23}$ +63.9° (c=0.124, MeOH). *Anal.* Calcd. for $C_{49}H_{72}O_{17}\cdot H_2O$: C, 61.88; H, 7.84; $COCH_3$, 18.10. Found: C, 62.04; H, 7.73; $COCH_3$, 18.41. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3613 (OH), 1774, 1741, 1622 (butenolide), 1741 (Ac).

ii) The more polar fraction (280 mg) was recrystallized from aqueous MeOH to give V (112 mg) as colorless crystals, mp 232—235°, $[\alpha]_D^{23}$ +44.4° (c=0.812, MeOH). *Anal.* Calcd. for $C_{47}H_{70}O_{16}$: C, 63.35; H, 7.92; $COCH_3$, 14.49. Found: C, 63.24; H, 7.93; $COCH_3$, 14.25. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3533 (OH), 1781, 1742,

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[24] All melting points are uncorrected.

1623 (butenolide), 1742 (Ac). The residue (140 mg) from the mother liquor of V was separated into the following two fractions (a and b) by preparative continuous development TLC (SiO_2 Merck, $CHCl_3$:AcOEt =5:1, developed for 24 hr continuously).

a) The less polar fraction (56 mg) was recrystallized from aqueous MeOH to give VI (42 mg) as colorless amorphous powder, mp 142—145°, $[\alpha]_D^{23}$ +42.7° (*c*=0.455, MeOH). *Anal.* Calcd. for $C_{47}H_{70}O_{16} \cdot H_2O$: C, 62.09; H, 7.98; $COCH_3$, 14.21. Found: C, 61.93; H, 7.91; $COCH_3$, 13.95. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3564 (OH), 1782, 1743, 1623 (butenolide), 1743 (Ac).

b) The more polar fraction (75 mg) was recrystallized from aqueous MeOH to give V (58 mg) as colorless crystals, mp 231—234°.

iii) The most polar fraction (165 mg) was recrystallized from acetone to give IV (118 mg) as colorless crystals, mp 263—265°.

3′′-Acetyldigitoxin (VIII) from V——A solution of V (50 mg) in 0.3% $KHCO_3$ in 90% MeOH (20 ml) was set aside overnight at room temperature, and the resulted solution was neutralized with dil. HCl, 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 dryness to give residue (42 mg), which was shown to be almost homogenous by TLC (SiO_2 Merck, $CHCl_3$:acetone=2:1). The crude product was recrystallized from aqueous MeOH to give VIII (28 mg) as colorless amorphous powder, mp 158—163°, $[\alpha]_D^{23}$ +29.9° (*c*=0.646, MeOH). *Anal.* Calcd. for $C_{43}H_{66}O_{14}$: C, 64.00; H, 8.24; $COCH_3$, 5.33. Found: C, 63.96; H, 8.47; $COCH_3$, 5.15. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1784, 1743, 1623 (butenolide), 1743 (Ac).

Digitoxigenin-bisdigitoxoside 3′′-Monoacetate (X) from VIII through Dialdehyde Acetate (IX)——To a solution of VIII (139 mg) in 95% EtOH (7.7 ml) was added a solution of $NaIO_4$ (139 mg) in H_2O (1.4 ml) dropwise under stirring at room temperature, and the mixed solution was allowed to stand at the same temperature for 3 hr. $NaIO_3$ there deposited was filtered off and the filtrate, after diluted with H_2O (1 ml), was 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 IX (146 mg) as a colorless amorphous powder which was proved to be almost homogenous by TLC (SiO_2 Merck, $CHCl_3$:acetone=2:1).

IX (140 mg) was dissolved in a mixture of MeOH (24 ml) and 0.05N HCl (3.6 ml) and the solution was set aside overnight at room temperature. The resulted 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 dryness to yield a crude product (116 mg) which was separated into the following fractions by preparative TLC (SiO_2 Merck, $CHCl_3$:acetone=2:1).

i) The less polar fraction (4 mg) was unidentified by-product.

ii) The more polar fraction (62 mg) was recrystallized from aqueous MeOH to give X (47 mg) as colorless crystals, mp 143—147°, $[\alpha]_D^{23}$ +17.5° (*c*=0.258, MeOH). *Anal.* Calcd. for $C_{37}H_{56}O_{11} \cdot \frac{1}{2}H_2O$: C, 64.80; H, 8.38; $COCH_3$, 6.28. Found: C, 64.74; H, 8.25; $COCH_3$, 6.01. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3604 (OH), 1787, 1749, 1622 (butenolide), 1749 (Ac).

iii) The most polar fraction (18 mg) was unidentified by-product.

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