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Studies on Digitalis Glycosides. XXVI.[1] Gitoxin Acetates. (1). Deacetylation of Pentaacetylgitoxin

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Hydrolysis of pentaacetylgitoxin (I) with diastase gave selectively 3′,3″,3‴,16–tetraacetylgitoxin (IIa) as a main product. When IIa was treated with potassium hydrogen carbonate, acetyl groups were successively eliminated in the order of those at C–3‴, –16, –3″ or –3′ positions to afford 3′,3″,16–triacetylgitoxin (IIc), 3′,3″–diacetylgitoxin (IId), 3′–monoacetylgitoxin (acetylgitoxin–γ, IIe) and gitoxin (IIf). These partial acetates did not agree with those obtained in acetylation of gitoxin (IIf), in which acetyl groups were introduced in the order at C–4‴, –16, –3‴, –3″ or –3′ positions. The partial acetates obtained by the former method (deacetylation) were thought to be more essential for the study of metabolism of I than those prepared by the latter method (acetylation).

Gitoxin (IIf),[3] one of the main cardiac glycosides of Digitalis species, has never been used in clinic due to its bad solubility. On the other hand, it was reported that pentaacetylgitoxin (I) showed a good intestinal abosprtion according to its lipophylic property and exhibited a sufficient cardiac activity,[4,5] and the revelation of activity was explained by deacetylation of I in body to regenerate IIf.[6,7] From this point of view, partial acetates of IIf were thought to be essential for the study on metabolism of I and synthesis of partial acetates by acetylation of IIf were performed by the several researchers.[8–11] Moreover, 16–acetylgitoxin (VIIb) was prepared by deacetylation of polyacetylgitoxin by the other workers.[12,13]

Recently, for the purpose of studying the metabolites of pentaacetylgitoxin (I), we investigated deacetylation of I in detail and could separate the several partial acetates of new type. This paper is concerned with these findings.

When I was hydrolyzed by treating with diastase in dilute alcohol, 3′,3″,3‴,16–tetraacetylgitoxin[14] (IIa), mp 140—144° was obtained as a main product, together with a trace of by–product which seemed to be 3′,3″,4‴,16–tetraacetate (IIb), an isomer of IIa. The structure of IIa and IIb were elucidated by the following data: Ultraviolet and infrared spectra,

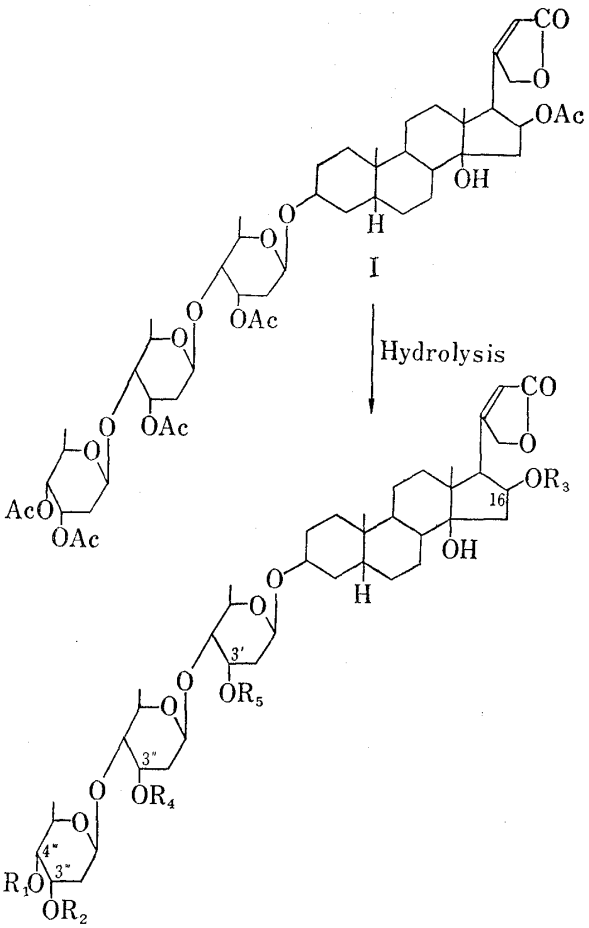
1) Part XXV: D. Satoh and M. Horie, *Chem. Pharm. Bull.* (Tokyo), **14**, 1133 (1966).
2) Location: *Sagisu, Fukushima–ku, Osaka.*
3) While the anomeric configurations and sugar linkages of gitoxin have never been established, they could be assumed all to be β–forms and 1,4–linkages analogously to digitoxin and digoxin as shown in formula (R. Tschesche, B. Niyomporn, and M. Machleidt, *Ber.*, **92**, 2258 (1959); H. Lichti, M. Kuhn, and A. von Wartburg, *Helv. Chim. Acta*, **45**, 868 (1962)).
4) A. Okano, K. Hoji, T. Miki, and K. Miyatake, *Chem. Pharm. Bull.* (Tokyo), **5**, 171 (1957).
5) D. Satoh, H. Ishii, Y. Oyama, and S. Takahashi, Japan. Petent Pub. 6982/60.
6) R. Megges and K. Repke, "Proc. First Intern. Meeting, Stockholm 1961," Vol. III, Pergamon Press, Oxford, 1962, p. 271.
7) T. Minesita and R. Hirota, Unpublished.
8) D. Satoh, Y. Oyama, and H. Ishiii, *Chem. Pharm. Bull.* (Tokyo), **5**, 493 (1957).
9) D. Satoh, *Ann. Rept. Shionogi Res. Lab.*, **14**, 14 (1964).
10) E. Haack, F. Kaiser, H.G. Kroneberg, and H. Spingler, Ger. Patent, 1063160 (1958).
11) R. Megges and K. Repke, *Monatsber. Deut. Akad. Wiss. Berlin*, **10/11**, 744 (1965).
12) K. Miyatake, A. Okano, K. Hoji, T. Miki, and A. Sakashita, *Chem. Pharm. Bull.* (Tokyo), **8**, 1144 (1960).
13) G. Baumgarten, *Arch. Pharm.*, **295**, 305 (1962).
14) Numbering of positions of C atoms of each sugar were described as follows: *e.g.* C–3 positions of three digitoxoses were noted as 3′,3″ and 3‴ in the order of connection with aglycone.

and color reactions (Legal test and Raymond test) proved IIa to belong to an acetylgitoxin, and the number of its acetyl groups was indicated to be four by the analysis. The intensities of chemical shifts of acetyl protons agreed with this finding as shown in Table I-a. From these facts IIa seemed to be produced from I by removing an acetyl group in treatment with diastase. When IIa was spotted on a silicagel thin-layer and developed after standing for 48 hr at room temperature, formation of a less polar product, whose *Rf* value corresponded to that of IIb, was detected besides the intact starting material. In the similar treatment with silicagel, the interconversion between well-known acetylgitoxin-α[15] (3'''-acetylgitoxin, VIIc, more polar) and acetylgitoxin-β[15] (4'''-acetylgitoxin, VIIa, less polar) was observed. Accordingly, these interconversions were thought to arise from the migration of acetyl group to the neighboring hydroxyl group. While the acetyl migration in the cardiac glycosides by heating in alcohol solution had been reported,[15,16] the procedure on silicagel thin-layer described here offered a convenient method for the acetyl migration test. The isomerization of IIa to IIb suggested that IIa has an acetyl group and a hydroxyl group at vicinal position which given only in the terminal digitoxose of sugar linkage of this glycoside, that is 3'''- and 4'''-positions. Considering that *Rf* value of acetylgitoxin-α (VIIc) is lower than that of acetylgitoxin-β (VIIa), it is reasonable to assign IIa of a low *Rf* value to 3',3'',3''',16-tetraacetylgitoxin and IIb of a high *Rf* value to 3',3'',4''',16-tetraacetylgitoxin. These assignments were verfied by hydrolysis of I to IIa with potassium hydrogen carbonate and by acetylation of 3',3'',16-triacetylgitoxin (IIc) to IIb as described below. It is interesting that diastase has an ability to eliminate selectively 4'''-acetyl group of I.

When IIa was further hydrolyzed with potassium hydrogen carbonate in methanol at room temperature, acetyl groups were eliminated successively to afford 3',3'',16-triacetylgitoxin (IIc), mp 152—158°, 3',3''-diacetylgitoxin (IId), mp 246—249°, 3'-monoacetylgitoxin (IIe), mp 165—168°, and gitoxin (IIf).[17] The structures of these products were deduced from the following data respectively. While IIc bears three acetyl groups in its molecule as determined by analysis and nuclear magnetic resonance spectrum (Table I-a), it showed a positive *cis*-glycol test with periodate-benzidine reagent[18] which corresponded to 3''',4'''-glycol. These data assigned the structure 3',3'',16-triacetylgitoxin to IIc. Negative acetylmigration test of IIc by treatment with silicagel supported this structure. The product IId belongs to a diacetate and lacks an acetyl group at C-16 as indicated in nuclear magnetic resonance spectrum (Table I-a), and a failure of formation of 16-anhydro derivative on the treatment of IId with alumina[19] confirmed the absence of acetyl group at C-16. These results showed that hydrolysis of IIc eliminated the 16-acetyl group to give 3',3''-diacetylgitoxin (IId). Monoacetate (IIe), the deacetylated product of IId, was established to be 3'-monoacetylgitoxin by identification with an authentic sample of acetylgitoxin-γ,[20] mp 164—167°, $[\alpha]_D^{21}$ +35.7° (MeOH), by mixed fusion and comparisons of optical rotations, thin-layer chromatograms and infrared spectra. The velocity of hydrolysis of IId to IIe was inferior to that of I to IId due to steric hindrance. The final product of hydrolysis was gitoxin (IIf), and this fact proved that no other change than the fission of acetyl groups did not arise in the above mentioned hydrolysis sequence.

Hydrolysis of I with potassium hydrogen carbonate formed IIa more than IIb which proved by thin-layer chromatography,and acetylation of IIc under mild condition with acetic anhydride in pyridine gave IIb, mp 157—165°, as a main product. Since the sugars of D-

15) A. Stoll, A. von Wartburg, and W. Kreis, *Helv. Chim. Acta*, **35**, 1324 (1952).
16) A. Stoll and W. Kreis, *Helv. Chim. Acta*, **35**, 1318 (1952).
17) According to the later study of us, 3''-monoacetylgitoxin (mp 176—183°) was also isolated and it will be reported in the forthcoming paper.
18) D.H. Gregg and O. Gisvold, *J. Am. Pharm. Assoc.*, **43**, 106 (1954).
19) K. Meyer, *Helv. Chim. Acta*, **29**, 718 (1946).
20) K. Hoji, *Chem. Pharm. Bull.* (Tokyo), **9**, 296 (1961).



IIa: R_1=H, R_2—R_5=Ac } Tetraacetate
IIb: R_2=H, R_1,R_3—R_5=Ac }
IIc: R_1,R_2=H, R_3—R_5=Ac Triacetate
IId: R_1—R_3=H, R_4,R_5=Ac Diacetate
IIe: R_1—R_4=H, R_5=Ac Monoacetate
IIf: R_1—R_5=H Gitoxin

series build chair forms of Cl-type in general, hydroxyl and acetoxyl groups at 4'''-position are equatorial and those at 3'''-position are axial, respectively, as shown in formula III and VI. According to the general rule that equatorial substituents are more favorable for hydrolysis as well as acetylation than those of axial, it was considered that IIa has a partial structure of 3'''-acetate (IV) and IIb has that of 4'''-acetate (V), and acetyl migration between IIa and IIb is shown as IV⇄V.

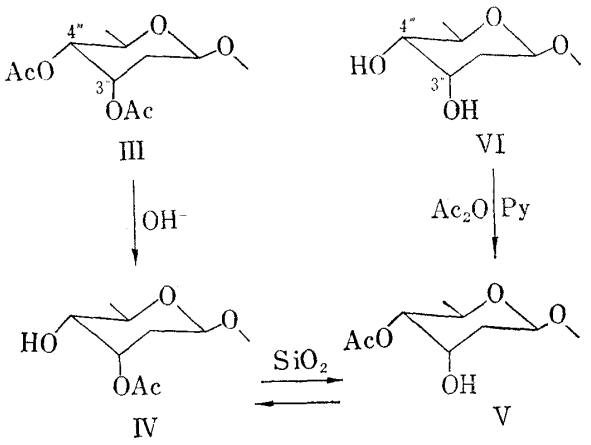


Chart 1

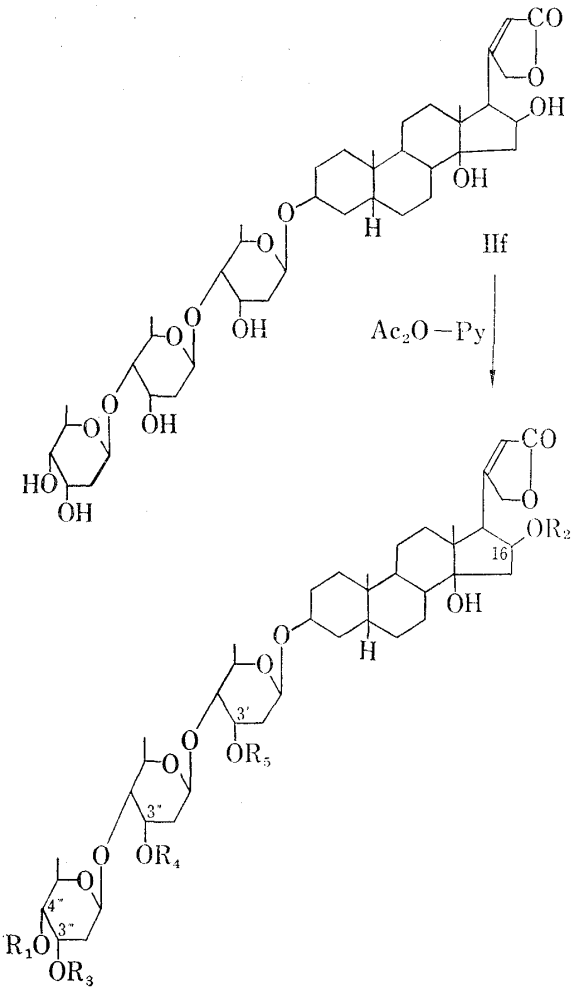
Table I. Chemical Shifts of Acetyl Protons (τ, in $CDCl_3$, 60 Mc)

Acetates	Axial-OAc[a] 3',3'',3'''-positions	Equatorial-OAc 4'''-position	16-OAc
a. Acetates prepared by Deacetylation of Pentaacetylgitoxin (I)			
Tetra (3',3'',3''',16-) (IIa)	7.88 (3H), 7.91 (6H)		8.04 (3H)
Tetra (3',3'',4''',16-) (IIb)	7.91 (6H)	7.91 (3H)	8.04 (3H)
Tri (3',3'',16-) (IIc)	7.90 (6H)		8.04 (3H)
Di (3',3''-) (IId)	7.91 (6H)		
Mono (3'-) (IIe)	7.91 (3H)		
b. Acetates prepared by Acetylation of Gitoxin (IIf)			
Penta (3',3'',3''',4''',16-) (I)	7.91(9H)	8.01[b] (3H)	8.04 (3H)
Tetra (3', or 3'',3''',4''',16-) (VIg)	7.87 (6H)	8.00 (3H)	8.03 (3H)
Tri (3''',4''',16-) (VIf)	7.88 (3H)	8.00 (3H)	8.03 (3H)
Di (4''',16-) (VId)		7.89 (3H)	8.04 (3H)
Di (3''',16-) (VIe)	7.87 (3H)		8.03 (3H)
Mono (3'''-) (VIc)	7.87 (3H)		
Mono (16-) (VIb)			8.04 (3H)
Mono (4'''-) (VIa)		7.89 (3H)	

a) The axial acetoxyl protons have slightly lower chemical shifts than those of equatorial groups. Ref. L.D. Hall, "Advances in Carbohydrate Chemistry," **19**, Academic Press, New York & London, 1964, p. 51.
b) The chemical shifts of 4'''-acetyl protons shifted to higher field by the effects of neighboring 3'''-acetyl groups.

Hydrolysis of I with potassium hydrogen carbonate was not so selective as with diastase described above, and the further hydrolysis products (IIc, IId, IIe and IIf) were produced. From the above mentioned results of hydrolysis sequence it was clarified that the order of fission of acetyl groups in I was at C-4‴→ -3‴→ -16→ -3″ or -3′ positions.

On the other hand, we had briefly reported[9,21] that acetyl groups were introduced to gitoxin (IIf) in the order of C-4‴→-16→-3‴ →-3″ or -3′ positions as decided by the following data: Acetylation of IIf with acetic anhydride in pyridine gave two monoacetate (VIIa), mp 268—271°; (VIIb), mp 227—235°, a diacetate (VIId), mp 158—161°, a triacetate (VIIf), mp 270—275°, a tetraacetate (VIIg), mp 157—162° and pentaacetate (I), mp 173—176°, in proportion to the amount of acetic anhydride. The two monoacetates agreed with the known acetylgitoxin-β(4‴-acetylgitoxin, VIIa) and 16-acetylgitoxin (VIIb) respectively. As mentioned above, acetylgitoxin-β (VIIa) converted to the more polar acetylgitoxin-α(3‴-acetylgitoxin, VIIc), mp 199—203°, in the treatment with silicagel. When diacetate was treated with silicagel, a more polar isomer, mp 155—157°, was formed, and hydrolysis of diacetate gave 16-acetylgitoxigenin, and so this diacetate was considered to be 4‴,16-diacetylgitoxin (VIId) and the isomer corresponded to 3‴,16-diacetylgitoxin (VIIe). As the triacetate, a further acetylated product of VIId, did not isomerize in the contact with silicagel, the newly introduced



VIIa: R_1=Ac, R_2—R_5=H
VIIb: R_2=Ac, R_1,R_3—R_5=H } Monoacetate
VIIc: R_3=Ac, R_1,R_2,R_4,R_5=H
VIId: R_1R_2=Ac, R_3—R_5=H } Diacetate
VIIe: R_2,R_3=Ac, R_1,R_4,R_5=H
VIIf: R_1—R_3=Ac, R_4,R_5=H Triacetate
VIIg: R_1—R_3,R_4 or R_5=Ac, R_5 or R_4=H Tetraacetate
I: R_1—R_5=Ac Pentaacetate

Chart 2

Table II. Comparison of the Positions of Acetyl Groups in Gitoxin Acetates prepared by the Both Methods

Gitoxin acetates	Prepared by deacetylation of pentaacetylgitoxin (I)						Prepared by acetylation of Gitoxin (IIf)					
	Position of Ac / Formula	3′	3″	3‴	4‴	16	Position of Ac / Formula	3′	3″	3‴	4‴	16
Penta							I	+	+	+	+	+
Tetra	IIa	+	+	+		+	VIIg	+		+	+	+
Tetra	IIb	+	+		+	+	or		+	+	+	+
Tri	IIc	+	+			+	VIIf			+	+	+
Di	IId	+	+				VIIe			+		+
							VIId				+	+
Mono	IIe	+					VIIc			+		
							VIIb					+
							VIIa				+	

21) This work was collaborated with T. Okumura of this laboratory.

acetyl group should locate at C-3‴ position as formula VIIf. An acetyl group furthermore introduced in tetraacetate (VIIg) has not been established by us yet.[22] The signals of acetyl groups in the synthesized acetates were shown in Table I-b.

While, as mentioned above, the order of fission of acetyl groups from pentaacetylgitoxin (I) and that of introduction of acetyl groups into gitoxin (IIf) were similar as such as at C-4‴→-3‴→-16→-3″ or -3′ and at C-4‴→-16→-3‴→-3″ or -3′, the partial acetates by the both methods were different respectively as shown in Table II.

Deacetylation of 3′ or 3″,3‴,4‴,16-tetraacetylgitoxin (VIIg) also gave mono-, di- and triacetate which did not agree with those described here, and the details of these results will be reported in the forthcoming paper.

Experimental[23]

Thin-layer Chromatography (TLC)——Analytical and preparative TLC were performed by the following three systems;

A: SiO_2, pyridine–$CHCl_3$=1:4
B: SiO_2, AcOEt
C: SiO_2, $CHCl_3$–acetone=1:1

Test of Acetyl Migration by TLC——After 50—100 μg of sample was spotted on a silicagel thin-layer and left aside for 48 hr at room temperature, the intact sample was newly spotted in a line on the same thin-layer and developed with $CHCl_3$–acetone=1:1. When an acetyl group migrated from 4‴-position to 3‴-position, a new spot of slightly lower *Rf* value was detected and when migration arose in the opposite direction, a slightly less polar product was formed.

Hydrolysis of Pentaacetylgitoxin (I) to 3′,3″,3‴,16-Tetraacetylgitoxin (IIa) with Diastase——A solution of 500 mg of I in 250 ml of 95% EtOH was added to a solution of 25 g of diastase (JP VII) in 1250 ml of H_2O and the mixture was agitated at room temperature. After 66 hr the mixture was extracted with $CHCl_3$, and $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated under reduced pressure to give 448 mg of a crude hydrolysis product which was shown to consist of a main product together with some admixture (TLC=system C).

The main product (320 mg) was separated by preparative TLC (system C) and recrystallized from acetone–ether–petroleum ether to afford 210 mg of IIa as colorless crystalline powder, mp 140—144°, $[\alpha]_D^{22}$ +16.9±0.5° (*c*=1.083, pyridine). *Anal.* Calcd. for $C_{49}H_{72}O_{18}\cdot H_2O$: C, 60.85; H, 7.71; $COCH_3$, 17.80. Found: C, 60.63; H, 7.85; $COCH_3$, 18.65. UV λ_{max}^{EtOH} mμ (ε): 216 (15650). IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3675, 3550, 1743, 1621. *cis*-Glycol test of IIa with benzidine–periodate reagent was negative.

Beside the main product, a small amount of less polar fraction was obtained which corresponded to IIb (TLC=system C).

Hydrolysis of IIa to 3′,3″,16-Triacetylgitoxin (IIc) and 3′,3″-Diacetylgitoxin (IId) by $KHCO_3$——A solution of 100 mg of IIa in 20 ml of 0.06% $KHCO_3$ (MeOH–H_2O=9:1) was allowed to stand for 88 hr at room temperature and then the solution was neutralized with 0.1N HCl and MeOH was distilled off under reduced pressure at room temperature 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 98 mg of crude hydrolysis product which was separated into two fractions by preparative TLC (system C).

i) Less polar fraction (72 mg) was recrystallized from MeOH–ether–petroleum ether to give 52 mg of IIc as colorless crystals, mp 152—158°, $[\alpha]_D^{21}$ +10.1±0.4° (*c*=1.015, pyridine). *Anal.* Calcd. for $C_{47}H_{70}O_{17}\cdot H_2O$: C, 61.02; H, 7.85; $COCH_3$, 13.96; H_2O, 1.90. Found: C, 60.78; H, 7.83; $COCH_3$, 14.15; H_2O, 2.09. UV λ_{max}^{EtOH} mμ (ε): 216 (13850), IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3700, 3615, 1743, 1630, 1622.

ii) More polar fraction (20 mg) was recrystallized from acetone and MeOH–petroleum–ether to give 17 mg of IId as colorless crystals, mp 246—249°, $[\alpha]_D^{22}$ +25.2±0.6° (*c*=1.026, pyridine). *Anal.* Calcd. for $C_{45}H_{68}O_{16}$: C, 62.48; H, 7.92; $COCH_3$, 9.95. Found: C, 62.42; H, 7.97; $COCH_3$, 9.68. UV λ_{max}^{EtOH} mμ (ε): 219 (14800), IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3665, 3465, 3530, 1743, 1633, 1616.

Acetylation of 3′,3″,16-Triacetylgitoxin (IIc) to 3′,3″,4‴,16-Tetraacetylgitoxin (IIb)——To a solution of 33 mg of IIc in 1.2 ml of pyridine, 0.7 ml of acetic anhydride was added at about 0°, and the mixture was allowed to stand for 40 min at the same temperature. After dilution with ice-water, crude acetate (31 mg) was collected by filtration, washed with water and dried *in vacuo*, which was separated into three fractions by preparative TLC (system C).

i) First fraction (4 mg) was proved to be I by TLC (system C).

22) According to the later study of R. Megges and K. Repke,[11] tetraacetate consisted of nearly the same amounts of 3′,3‴,4‴,16- and 3″,3‴,4‴,16-tetraacetylgitoxin.
23) All melting points are uncorrected.

ii) Second fraction (19 mg) was recrystallized from acetone–petroleum ether to give 15 mg of IIb as colorless crystalline powder, mp 157—165°. *Anal.* Calcd. for $C_{49}H_{72}O_{18}$: C, 62.01; H, 7.65; $COCH_3$, 18.14. Found: C, 61.76; H, 7.72; $COCH_3$, 17.56. UV λ_{max}^{EtOH} mμ (ε): 215 (18850), IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3584, 1742, 1622. In the acetyl migration test, formation of IIa from this product was observed.

iii) Third fraction (4 mg) was shown to be IIc by TLC (system C).

Hydrolysis of IIc to IId by $KHCO_3$——Ten milligrams of IIc was dissolved in 2 ml of 0.06% $KHCO_3$ (MeOH–H_2O=9:1) and the solution was set aside for 7 days at room temperature. The hydrolyzed solution was neutralized with 0.1 N HCl and MeOH was distilled off under reduced pressure and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by preparative TLC (system C) and recrystallized to colorless crystals, mp 240—246°. TLC (system A, B and C) and mixed fusion showed the identity of this crystals with IId.

Hydrolysis of IId to 3′-Monoacetylgitoxin (IIe) with $KHCO_3$——When 13 mg of IId was treated with 3 ml of 0.06% $KHCO_3$ by the analogous procedure as described above, the formation of hydrolized product was observed after 20 days. When the product reached to the maximum (after 69 days), the hydrolysis was stopped and treated as described above. The crude product was found to consist of two components which were proved to be identical with 3′–monoacetylgitoxin (IIe) and gitoxin (IIf) by the three systems of TLC (system A, B and C), respectively.

Hydrolysis of IIe to Gitoxin (IIf)——After 5 mg of IIe was treated with 0.06% $KHCO_3$ analogously as described above for 80 days, hydrolysis was almost finished to give gitoxin (IIf) which was identified by TLC (system A, B and C).

Hydrolysis of I with $KHCO_3$——Five-hundred milligrams of I was dissolved in 100 ml of 0.06% $KHCO_3$ (MeOH–H_2O=9:1) and the solution was allowed to stand at room temperature. The progress of hydrolysis was checked by TLC (system C) as follows:

Duration of hydrolysis	Products
2 days	I⋙IIb<IIa≪IIc≫IId
16 days	IIc<IId>IIe
25 days	IId>IIe≫IIf

After 25 days the hydrolysis solution was neutralized with d. HCl, diluted with 100 ml of H_2O and MeOH was distilled off under reduced pressure. The precipitates were extracted with $CHCl_3$ and $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to give 412 mg of crude product which was separated into following fractions by preparative TLC (system B).

i) First fraction (185 mg) was recrystallized to colorless crystals, mp 242—248° which were proved to be identical with IId by mixed fusion, NMR and TLC (system A, B and C).

ii) Second fraction (79 mg) was recrystallized from acetone–petroleum ether to afford colorless crystals of IIe, mp 165—168°, $[\alpha]_D^{24}$ +42.2±0.8° (c=1.001, MeOH). *Anal.* Calcd. for $C_{43}H_{66}O_{15}\cdot H_2O$: C, 61.41; H, 8.15; $COCH_3$, 5.12. Found: C, 61.04; H, 7.98; $COCH_3$, 5.31. UV λ_{max}^{EtOH} mμ (ε): 219 (14900), IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3490, 1784, 1744, 1633, 1618.

iii) Third fraction (11 mg) was recrystallized from MeOH–$CHCl_3$ to afford colorless crystalline powder of IIf, mp 258—268°.

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