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Studies on Digitalis Glycosides. XXVII.[1] Alkoxy- and Cyclocarbonates of Gitoxin[2]

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Treatment of gitoxin (I) with alkyl chloroformate in pyridine gave rise to alkoxycarbonylation of hydroxyl groups in the order of 4‴-, 16- and 3‴-positions analogously to acetylation to afford gitoxin 4‴-mono-,·4‴, 16-di- and 3‴, 4‴, 16-trialkoxycarbonates (II, III, IV), but the further esterification encountered with more difficulties than the acetylation. Gitoxin 3‴,4‴-cyclocarbonate (V) was also prepared and the interconversion between 3‴-mono- and 4‴-monomethoxycarbonates (VIIa, IIa) and V was investigated. Alkyl pyrocarbonates showed a less selectivity in alkoxycarbonylation of I than alkyl chloroformates, resulting in the formation of V, 16-alkoxy- and 3‴,4‴-cyclocarbonyl-16-alkoxycarbonates (IX, VIII) besides II, III and IV. IX was also formed by partial hydrolysis of III and VIII with dilute alkali.

In the preceding papers, acetylation of gitoxin (I)[1,4] and deacetylation of its pentaacetate with diastase and potassium hydrogen carbonate[1] were reported as parts of a series of studies on the lipophylic derivatives of I. This paper concerns with preparation of alkoxy- and cyclocarbonates of I, thus constituting another part of the series.

When I was treated with an excess of methyl chloroformate in pyridine, gitoxin 4‴-monomethoxycarbonate (IIa)[5] was formed as a main product accompanied with a small quantity of gitoxin 4‴,16-dimethoxycarbonate (IIIa), but the formation of further substituted product was limited probably due to a insufficient solubility of methyl chloroformate-pyridine complex in pyridine. The position of methoxycarbonyl group in IIa was established by the following evidences. Since the *cis*-glycol test using periodate–benzidine reagent was positive with I but negative with IIa, the methoxycarbonyl group of IIa must be situated in the terminal sugar moiety, that is at 3‴- or 4‴-position. As the sugars of D-series form the chair conformation of Cl-type in general, the 3‴-hydroxyl group of the terminal D-digitoxose is axial and the 4‴-hydroxyl group is equatorial formulated as XI. Accordingly, the methoxycarbonyl group of IIa was considered to be introduced into the less hindered 4‴-hydroxyl group to give 4‴-monomethoxycarbonate (XIIb, partial formula of IIa) analogously to the acetylation of I, in which preferential formation of the 4‴-monoacetate (XIIa, partial formula) was proved by identification with the known acetylgitoxin-β.[1,4] Location of the further introduced methoxycarbonyl group of IIIa was established at 16-position, on the basis of the formation of 16,17-dehydrocardenolide (X) on treatment with alumina by the similar method applied to the 16-acetate.[6]

Treatment of IIa with anhydrous potassium hydrogen carbonate in acetone (suspended) at room temperature gave a mixture of intact IIa and its isomer, 3‴-monomethoxycarbonate

1) Part XXVI: J. Morita and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **16**, 1056 (1968).
2) This work was reported at the Meeting of Kinki Branch, Pharmaceutical Society of Japan, Nishinomiya, November 1967.
3) Loction: *Sagisu, Fukushima-ku, Osaka.*
4) D. Satoh, *Ann. Rept. Shionogi Res. Lab.*, **14**, 14 (1964).
5) Positions C-3 and C-4 of the terminal sugar moiety (D-digitoxose) of I are noted as 3‴ and 4‴, respectively.
6) K. Meyer, *Helv. Chim. Acta*, **29**, 718 (1946).

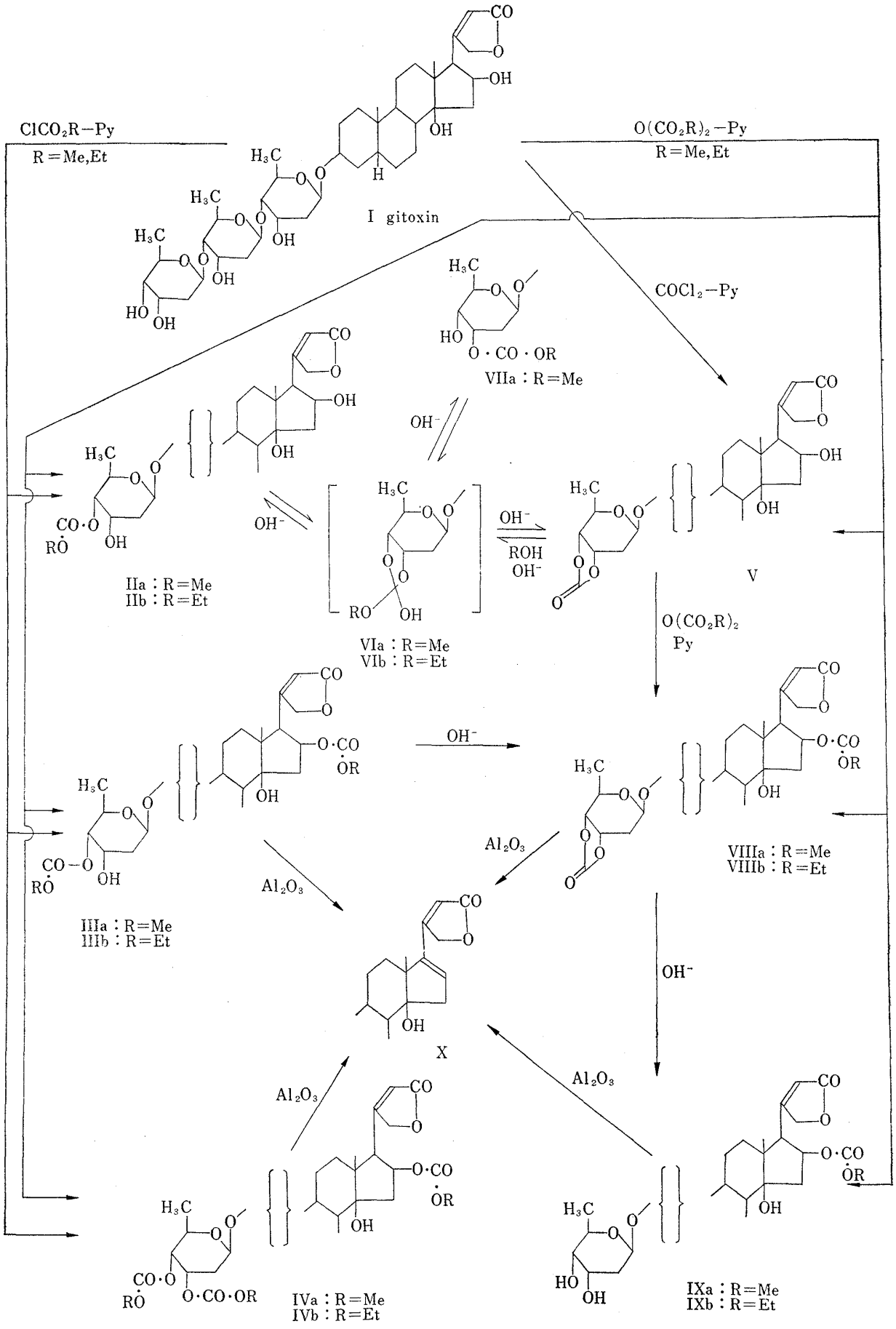

Chart 1

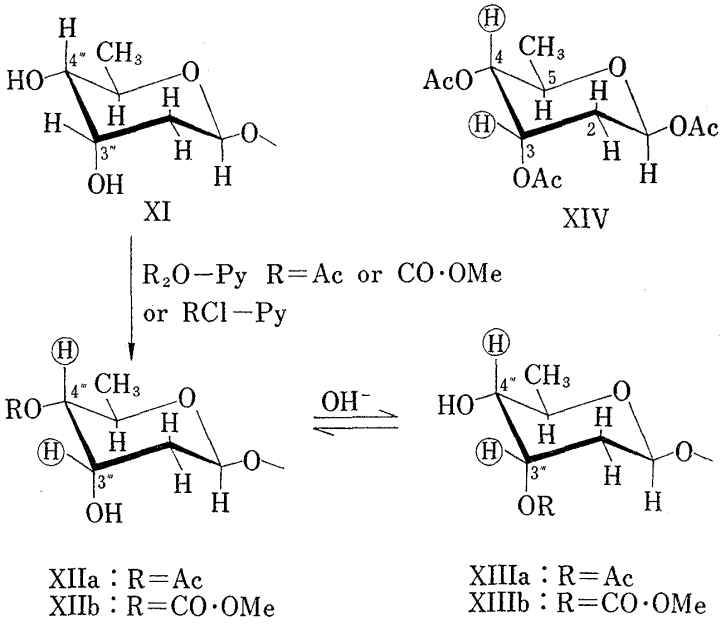

Chart 2

(VIIa) in almost equal quantity in which the methoxycarbonyl group connects with the axial hydroxyl group formulated as XIIIb (partial formula of VIIa). VIIa was thought to be formed by the migration of methoxycarbonyl group from 4‴ to 3‴ through a cyclic *ortho*-ester intermediate (VIa) analogously to the acetyl migration between gitoxin 4‴- and 3‴-monoacetate (XIIa, XIIIa). XIIIb was more polar than XIIb on thin-layer chromatography in analogy to the corresponding pair of acetate, in which XIIIa indicated a lower *Rf* value than XIIa. The migration of alkoxycarbonyl group was also observed in thiamine alkoxycarbonate, recently.[7]

The above locations of the methoxycarbonyl groups in IIa and VIIa were further supported by nuclear magnetic resonance spectra (NMR). The 3‴-H (equatorial) signals[8] of the 3‴-monoacetate (XIIIa) and the 3‴-monomethoxycarbonate (XIIIb) were observed at τ 4.69 (multiplet) and τ 4.86 (multiplet), respectively, which were comparable to 3-H (equatorial) signal of digitoxose triacetate (XIV)[9] at τ 4.52 (quartet, $J_{2,3}=J_{3,4}=3.2$ cps). On the other hand, in the NMR of the 4‴-monoacetate (XIIa) and the 4‴-monomethoxycarbonate (XIIb), any significant signal was not observed in the range τ 4.2—5.0, and 4‴-H (axial) signals of XIIa and XIIb were thought to overlap 3-H signal of the steroid skeleton and 21-H_2 signals of the butenolide ring in the range τ 5.0—5.4, which corresponded to 4-H (axial) signal of XIV at τ 5.36 (doublet doublet, $J_{3,4}=3.2$, $J_{4,5}=9.1$ cps).

When IIa was refluxed with anhydrous potassium carbonate in acetone (suspended), a product was formed, in which methoxyl group was not detected by analysis and NMR, while the presence of a cyclocarbonyl group was shown by the appearance of an absorption band at 1809 cm^{-1}.[10] From these results, this product was presumed to be gitoxin 3‴,4‴-cyclocarbonate (V) which was formed from IIa by elimination of a molecule of methanol[11] probably through the cyclic orthoester intermediate (VIa). Actually, the structure V was confirmed by direct synthesis of this compound from I on treatment with phosgen in pyridine. It was also formed on treatment of IIa with alumina or silica gel. For example, when a solution

7) A. Takamizawa, K. Hirai, Y. Hamashima, and H. Ito, *Chem. Pharm. Bull.* (Tokyo), **15**, 816 (1967).
8) Chemical shifts were measured at 60 Mc in $CDCl_3$.
9) Chemical shifts of the methine protons of XIV were assigned by Dr. Y. Nozaki of this laboratory.
10) W.M. Doane, B.S. Shasha, E.I. Stout, C.R. Russel, and C.E. Rist, *Carbohyd. Res.*, **4**, 445 (1967); L. Hough, J.E. Priddle, R.S. Theobald, J. Douglas, and J.W. Spoors, *Chem. Ind.* (London), **1960**, 148.
11) L. Hough, J.E. Priddle, and R.S. Theobald, "Advances in Carbohydrate Chemistry," Vol. 15, Academic Press, New York and London, 1960, p. 91.

of IIa was spotted on a thin-layer plate made of silicagel and developed after standing overnitht at room temperature, formation of V was detected besides the intact starting material. This procedure was used for cyclocarbonylation test of alkoxycarbonate. Treatment of IIa, V or VIIa with a dilute (0.03%) solution of potassium hydrogen carbonate in aqueous methanol for a short time at room temperature afforded a similar mixture of IIa, V and VIIa, respectively. This interconversion was presumed to proceed through the common intermediate (VIa).

On refluxing with anhydrous potassium carbonate in acetone analogously to IIa, IIIa gave gitoxin 3‴,4‴-cyclocarbonyl-16-methoxycarbonate (VIIIa) which was also obtained by methoxycarbonylation of V with methyl pyrocarbonate, $O(CO_2CH_3)_2$, in pyridine.

Treatment of I with twenty moles of ethyl chloroformate in pyridine gave gitoxin 4‴-monoethoxycarbonate (IIb) and gitoxin 4‴,16-diethoxycarbonate (IIIb) as principal products. The structures of IIb and IIIb were elucidated on the basis of analogous experimental results to those of IIa and IIIa. After refluxing with anhydrous potassium carbonate in acetone, IIb and IIIb gave V and gitoxin 3‴,4‴-cyclocarbonyl-16-ethoxycarbonate (VIIIb), respectively, analogously to IIa and IIIa. When the ethoxycarbonylation was carried out using fifty moles of ethyl chloroformate, gitoxin 3‴,4‴,16-triethoxycarbonate (IVb) was formed mainly together with IIIb and an unidentified further substituted product in small quantities. The positions of ethoxycarbonyl groups in IVb were clarified by the following results. Thus, a *cis*-glycol test with periodate-benzidine reagent and cyclocarbonylation test on a silicagel thin-layer described above were both negative, two hydroxyl groups of IVb at 3‴- and 4‴-positions were both substituted, and moreover, ethoxycarbonylation of the 16-hydroxyl group was proved by the formation of 16,17-dehydro derivative (X) on treatment of IVb with alumina.

Treatment of VIIIa and VIIIb with dilute (0.4%) potassium hydrogen carbonate in aqueous acetone at room temperature resulted in the preferential hydrolysis of 3‴,4‴-cyclocarbonyl group to give gitoxin 16-methoxycarbonate (IXa) and 16-ethoxycarbonate (IXb), respectively. When IIIa and IIIb were submitted to the same reaction, IXa and IXb were also obtained by direct hydrolysis of 4‴-alkoxycarbonyl groups or through cyclocarbonates (VIIIa, VIIIb).

Alkoxycarbonylation of I with methyl pyrocarbonate,[12] $O(CO_2CH_3)_2$ or ethyl pyrocarbonate,[12] $O(CO_2C_2H_5)_2$ in pyridine gave mixtures comprising a number of carbonates of IIa or IIb, IIIa or IIIb, IVa or IVb, V, VIIIa or VIIIb, and IXa or IXb depending on the amounts of pyrocarbonate and the reaction conditions as shown in Table I. This alkoxycarbonylation differs from that with alkyl chloroformate described above in a direct formation of V, VIII and IX besides II, III and IV, the common products of the both methods.

Table I. Main Products in the Alkoxycarbonylation of Gitoxin (I) with Alkyl Chloroformate and Pyrocarbonates

Reagent		Reaction condition		Product
Formula	Amount (mole)	Temperature (°C)	Time (min)	
$ClCO_2Me$	100	R.T.	120	IIa, IIIa
$ClCO_2Et$	20	R.T.	80	IIb, IIIb
	50	R.T.	120	IVb
$O(CO_2Me)_2$	60	R.T.	30	IIa, IIIa, IVa, V, VIIIa, IXa
	60	70—75	30	IVa, V, VIIIa
$O(CO_2Et)_2$	20	70—75	10	V, VIIIb, IXb
	100	R.T.	60	IVb, VIIIb

12) Pyrocarbonates were supplied from Dr. S. Sumimoto of this laboratory.

The biological activities of gitoxin alkoxy- and cyclocarbonates described above are under examination.

Experimental[13]

Thin-Layer Chromatography (TLC)——TLC were performed by the following two systems:
A=SiO_2 (E. Merck), $CHCl_3$–MeOH (10:1, v/v)
B=Al_2O_3 (E. Merck), AcOEt–benzene (5:1, v/v)

Gitoxin 4'''-Monomethoxycarbonate (IIa) and Gitoxin 4''',16-Dimethoxycarbonate (IIIa) from Gitoxin (I) on Treatment with $ClCO_2Me$——To a solution of 580 mg of I in 70 ml of pyridine, 6.05 g of $ClCO_2Me$ (*ca.* 100 moles) was added dropwise under shaking at 0——5°, and the shaking was further continued at room temperature for 1 hr. The reaction mixture was concentrated *in vacuo* under 40° to *ca.* 20 ml, diluted with ice–water and extracted with $CHCl_3$. After the $CHCl_3$ solution was washed with 5% HCl, 3% $NaHCO_3$ and H_2O and dried over anhydrous Na_2SO_4, $CHCl_3$ was evaporated *in vacuo* to give 620 mg of a crude product which was shown to consist of two main substances by TLC (system A). The crude product was then separated by preparative TLC (system A) into the following two fractions.

i) The more polar fraction (310 mg) was recrystallized from acetone to give 255 mg of IIIa as colorless crystals, mp 224—227°, $[\alpha]_D^{21}$ +26.9° (c=1.054, MeOH). *Anal.* Calcd. for $C_{43}H_{66}O_{16}\cdot H_2O$: C, 60.26; H, 8.00; OCH_3, 3.62. Found: C, 60.47; H, 7.87; OCH_3, 3.37. UV λ_{max}^{EtOH} mμ (ε): 219 (15190). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (broad, OH), 1795, 1750, 1616 (butenolide), 1750, 1275 (ester).

ii) The less polar fraction (86 mg) was recrystallized from acetone to afford 46 mg of IIIa as colorless crystals, mp 155—159°, $[\alpha]_D^{22}$ +9.8° (c=0.633, pyridine). *Anal.* Calcd. for $C_{45}H_{68}O_{18}\cdot H_2O$: C, 59.06; H, 7.71; OCH_3, 6.78. Found: C, 59.54; H, 7.32; OCH_3, 6.33. UV λ_{max}^{EtOH} mμ (ε): 216 (15540). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480 (broad, OH), 1798, 1752, 1618 (butenolide), 1752, 1282 (ester).

Gitoxin 4'''-Monoethoxycarbonate (IIb) and Gitoxin 4''',16-Diethoxycarbonate (IIIb) from I on Treatment with $ClCO_2Et$——To a solution of 500 mg of I in 40 ml of pyridine, 1.39 g of $ClCO_2Et$ (*ca.* 20 moles) was added dropwisely at 0——5° under shaking and the shaking was further continued at room temperature for 80 min. The reaction mixture was treated in the similar manner to the above described experiment to give 530 mg of a crude product which showed to contain two main substances on TLC (system A). The crude product was then separated by TLC (system A) into the following two fractions.

i) The morpolar fraction (220 mg) was recrystallized from acetone to afford 153 mg of IIb as colorless crystals, mp 230—233°, $[\alpha]_D^{21}$ +22.4° (c=0.738, pyridine). *Anal.* Calcd. for $C_{44}H_{68}O_{16}$: C, 61.95; H, 8.04; OC_2H_5, 5.29. Found: C, 62.35; H, 8.20; OC_2H_5, 5.26. UV λ_{max}^{EtOH} mμ (ε): 219 (14250). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (broad, OH), 1789, 1748, 1617 (butenolide), 1748, 1257 (ester).

ii) The less polar fraction (165 mg) was recrystallized from aqueous MeOH to afford 126 mg of IIIb as colorless crystals, mp 145—149°, $[\alpha]_D^{23}$ +11.9° (c=0.662, pyridine). *Anal.* Calcd. for $C_{47}H_{72}O_{18}$: C, 61.02; H, 7.85; OC_2H_5, 9.74. Found: C, 60.87; H, 7.94; OC_2H_5, 10.13. UV λ_{max}^{EtOH} mμ (ε): 216 (14580). IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3540 (broad, OH), 1795, 1749, 1616 (butenolide), 1749, 1264 (ester).

Gitoxin 3''',4''',16-Triethoxycarbonate (IVb) from I on Treatment with $ClCO_2Et$——To a solution of 500 mg of I in 40 ml of pyridine, 3.48 g of $ClCO_2Et$ (*ca.* 50 moles) was added to 0——5° dropwise under shaking and the mixture was shaked further for 2 hr at room temperature. The reaction mixture was treated in the usual manner to give 540 mg of a crude product which was shown to contain a main product together with several by-products. The main product (165 mg) was separated by preparative TLC (system B) and recrystallized from aqueous MeOH to give 113 mg of IVb as colorless crystals, mp 144—149°, $[\alpha]_D^{23}$ +15.1°(c=0.973, pyridine). *Anal.* Calcd. for $C_{50}H_{76}O_{20}$: C, 60.22; H, 7.68; OC_2H_5, 13.56. Found: C, 60.51; H, 7.71; OC_2H_5, 13.17. UV λ_{max}^{EtOH} mμ (ε): 216 (14130). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470 (broad, OH), 1792, 1749, 1618 (butenolide), 1749, 1270 (ester).

Gitoxin 3''',4'''-Cyclocarbonate (V)—i) From IIa: A mixture of a solution of 80 mg of IIa in 16 ml of acetone and 20 mg of powdered anhydrous K_2CO_3 was refluxed for 2 hr. K_2CO_3 was filtered off and the filtrate was concentrated *in vacuo* and the residue was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give 72 mg of a crude product which was submitted to preparative TLC (system A) to separate the main product. The main fraction (56 mg) was recrystallized from acetone to give 45 mg of V as colorless crystals, mp 238—240° (decomp.), $[\alpha]_D^{22}$ +21.6° (c=0.518, $CHCl_3$). *Anal.* Calcd. for $C_{42}H_{62}O_{15}$: C, 62.51; H, 7.75. Found: C, 62.39; H, 7.87. UV λ_{max}^{EtOH} mμ (ε): 218 (14590). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3458 (broad, OH), 1808 (cyclocarbonyl), 1746, 1616 (butenolide).

ii) From IIb: A mixture of a solution of 30 mg of IIb in 3 ml of acetone and 10 mg of powdered anhydrous K_2CO_3 was refluxed for 2 hr and the reaction mixture was treated as in IIa to give 27 mg of a crude product. The main product was separated by preparative TLC (system A) and recrystallized to afford 14 mg of V, mp 237—240° (decomp.).

iii) From I: To a solution of 500 mg of I in 25 ml of pyridine, 6 ml of 10% solution of $COCl_2$ in toluene was added dropwise for 30 min at 0——5° under shaking and the shaking was further continued for 60 min

13) All melting points are uncorrected.

at about 0°. Excess of $COCl_2$ was decomposed by addition of ice-water and the reaction product was extracted with $CHCl_3$. The $CHCl_3$ solution was washed successively with 5% HCl, 3% $NaHCO_3$ and H_2O to neutral, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give 530 mg of a crude product, which was proved to consist of a main product together with small quantities of two by-products by TLC (system A). The crude product was submitted to preparative TLC (system A) to separate the main fraction (340 mg) which was recrystallized from acetone to afford 310 mg of V, mp 238—240° (decomp.).

Gitoxin 3‴-Monomethoxycarbonate (VIIa) and V from IIa——To a solution of 180 mg of IIa in 60 ml of acetone was added 50 mg of powdered anhydrous $KHCO_3$, and the mixture was stirred for 4 days at room temperature. After filtration of $KHCO_3$, the acetone solution was evaporated *in vacuo* and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to afford 178 mg of a crude product which showed to comprise the intact starting material and a more polar product in almost equal quantities, accompanying a small quantity of a less polar product on TLC (system A). The crude product was purified by repeated recrystallization from acetone to separate into the following two components.

i) 46 mg of VIIa (more polar and more soluble), mp 227—229°, $[\alpha]_D^{23}$+28.1° (c=0.533, $CHCl_3$). *Anal.* Calcd. for $C_{43}H_{66}O_{16}$: C, 61.56; H, 7.93; OCH_3, 3.70. Found: C, 61.45; H, 8.04; OCH_3, 3.69. UV λ_{max}^{EtOH} mμ (ε): 218 (15050). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3510 (broad, OH), 1788, 1746, 1618 (butenolide), 1746, 1287 (ester).

ii) 42 mg of IIa (intact starting material, less soluble), mp 223—227°.

Preparative TLC (system A) of the mother liquor of VIIa gave 12 mg of V, mp 237—240°.

IIa and V from VIIa——To a solution of 10 mg of VIIa in 3 ml of acetone, 4 mg of powdered anhydrous $KHCO_3$ was added and the mixture was stirred for 4 days at room temperature. TLC (system A) indicated the formation of IIa and V beside the intact starting material in the resulting solution.

IIa and VIIa from V——A solution of 150 mg of V in 25 ml of 0.03% $KHCO_3$ in MeOH was allowed to stand at room temperature for 3 hr and then was neutralized with 5% HCl, concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to afford 140 mg of a crude product which showed to contain two products with more polarity besides the intact starting material (V) on TLC (system A). The crude product was separated into the following three fractions in the order of polarity by preparative TLC (system A).

i) The first fraction (27 mg) was recrystallized from acetone to give 19 mg of VIIa, mp 226—228°.

ii) The second fraction (24 mg) was recrystallized from acetone to give 17 mg of IIa, mp 223—227°.

iii) The third fraction (45 mg) was recrystallized from acetone to give 35 mg of V (intact starting material), mp 237—240°.

Gitoxin 3‴,4‴-Cyclocarbonyl-16-ethoxycarbonate (VIIIb)——i) From IIIb: To a solution of 100 mg of IIIb in 20 ml of acetone, 20 mg of powdered anhydrous K_2CO_3 was added and the mixture was refluxed for 2 hr. K_2CO_3 was filtered off and the filtrate was concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give 94 mg of a crude product which was purified by preparative TLC (system A) and recrystallized from acetone to afford 57 mg of VIIIb as a main product, mp 152—156° (decomp.), $[\alpha]_D^{23}$+17.7°(c=0.942, $CHCl_3$). *Anal.* Calcd. for $C_{45}H_{66}O_{17}$: C, 61.49; H, 7.59; OC_2H_5, 5.13. Found: C, 61.94; H, 7.53; OC_2H_5, 5.66. UV λ_{max}^{EtOH} mμ(ε): 218 (15300). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3510 (broad, OH), 1810 (cyclocarbonyl), 1748, 1618 (butenolide), 1748, 1268 (ester).

ii) From V: To a solution of 120 mg of V in 3 ml of pyridine, 0.65 ml (*ca.* 30 moles) of $O(CO_2Et)_2$ was added dropwisely at 5—10° under stirring. After the mixture was allowed to stand at room temperature for 30 min, it was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 5% HCl, 3% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. Evaporation of $CHCl_3$ *in vacuo* gave 125 mg of a crude product which was submitted to preparative TLC (system A) to afford 85 mg of the main product which was recrystallized from acetone to give 62 mg of VIIIb, mp 151—156° (decomp.).

Gitoxin 3‴,4‴-Cyclocarbonyl-16-methoxycarbonate (VIIIa)——One hundred milligrams of IIIa was treated with K_2CO_3 in the analogous manner as described above with IIIb to give 54 mg of VIIIa, mp 158—162° (decomp.), $[\alpha]_D^{23}$+6.3° (c=0.937, $CHCl_3$). *Anal.* Calcd. for $C_{44}H_{64}O_{17}\cdot H_2O$: C, 59.85; H, 7.53; OCH_3, 3.51. Found: C, 59.53; H, 7.64; OCH_3, 3.97. UV λ_{max}^{EtOH} mμ (ε): 217 (14950). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3510 (broad, OH), 1809 (cyclocarbonyl), 1750, 1617 (butenolide), 1750, 1281 (ester).

Gitoxin 16-Methoxycarbonate (IXa)——After a solution of 100 mg of VIIIa in 30 ml of aqueous acetone (acetone:H_2O=3:1, v/v) containing 0.4% $KHCO_3$ was allowed to stand at room temperature for 4 days, the resulting solution was neutralized, concentrated *in vacuo* and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give 95 mg of a crude product which was separated into the following three fractions in the order of polarity by preparative TLC (systemA).

i) The first fraction (3 mg) was proved to be I by TLC (system A and B).

ii) The second fraction (54 mg) was recrystallized from acetone to give 42 mg of IXa as colorless crystals, mp 219—223°, $[\alpha]_D^{22}$+1.8 (c=0.541, MeOH). *Anal.* Calcd. for $C_{43}H_{66}O_{16}$: C, 61.56; H, 7.93; OCH_3, 3.70. Found: C, 61.34; H, 7.99; OCH_3, 3.80. UV λ_{max}^{EtOH} mμ (ε): 215 (14950). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3537 (broad, OH), 1783, 1746, 1621 (butenolide), 1746, 1283 (ester).

iii) The third fraction (21 mg) was proved to be VIIIa (intact starting material) by TLC (system A and B).

Gitoxin 16-Ethoxycarbonate (IXb)——i) From VIIIb: A solution of 100 mg of VIIIb in 30 ml of aqueous acetone containing 0.4% $KHCO_3$ was treated in the similar manner to VIIIa as described above to afford 94 mg of a crude product which was separated into the following three fractions by preparative TLC (system A).

i) The first fraction (2 mg) was shown to be I by TLC (system A).

ii) The second fraction (52 mg) was recrystallized from acetone to give 39 mg of IXb as colorless crystals, mp 208—210°, $[\alpha]_D^{22}$+0.9 (c=0.546, MeOH). *Anal.* Calcd. for $C_{44}H_{68}O_{16}$: C, 61.95; H, 8.04; OC_2H_5, 5.29. Found: C, 61.72; H, 8.18; OC_2H_5, 4.92. UV λ_{max}^{EtOH} mμ (ε): 216 (15050). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3541 (broad, OH), 1788, 1747, 1620 (butenolide), 1747, 1273 (ester).

iii) The third fraction (23 mg) was shown to be VIIIb (intact starting material) by TLC (system A and B).

ii) From IIIb: A solution of 60 mg of IIIb in 18 ml of aqueous acetone containing 0.4% $KHCO_3$ was treated analogously to the partial hydrolysis of VIIIb described above to give 57 mg of a crude product which afforded 23 mg of IXb, mp 207—209°, after fractionation by preparative TLC (system A) and recrystallization from acetone.

Formation of 16,17-Dehydro Derivatives (X) from 16-Acylates——Each 5 mg of samples was dissolved in 0.5 ml of $CHCl_3$, and 0.3 ml of benzene and 1 g of Al_2O_3 (activated at 120° for 3 hr) was added. After the mixture was set aside at room temperature overnight, Al_2O_3 was filtered and washed with a mixture of $CHCl_3$–MeOH (1:1, v/v). The filtrate was evaporated *in vacuo* and the residue was dissolved in EtOH and UV absorption spectrum was determined. The test solutions with IIIa, IIIb, IVa, IVb, VIIIa, VIIIb, IXa and IXb exhibited the absorption band at about 272 mμ due to 16,17-dehydrocardenolide (X), respectively.

IIa, IIIa, Gitoxin 3‴,4‴,16-Trimethoxycarbonate (IVa), V, VIIIa and IXa from I on Treatment with $O(CO_2Me)_2$——To a solution of 500 mg of I in 15 ml of pyridine, 5.16 g (*ca.* 60 moles) of $O(CO_2Me)_2$ was added at 5—10° dropwise under shaking and the mixture was further shaked for 30 min at room temperature. The resulting solution was concentrated *in vacuo* under 35° and H_2O was added to decompose the excess of $O(CO_2Me)_2$ and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 5% HCl, 3% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give 551 mg of a crude product which was separate by preparative TLC (system A) into the following fractions in the order of polarity.

i) The first fraction (52 mg) was proved to be I recovered intact.

ii) The second fraction (57 mg) was recrystallized from acetone to give 29 mg of IXa, mp 219—224°.

iii) The third fraction (75 mg) was recrystallized from acetone to give 37 mg of IIa, mp 224—227°.

iv) The fourth fraction (69 mg) was recrystallized from acetone to give 40 mg of V, mp 238—240° (decomp.).

v) The fifth fraction (65 mg) was recrystallized from acetone to afford 34 mg of IIIa, mp 154—158°.

vi) The sixth fraction (68 mg) was recrystallized from aqueous MeOH to afford 35 mg of VIIIa, mp 158—162°.

vii) The seventh fraction (69 mg) was recrystallized from aqueous MeOH to afford 38 mg of IVa as colorless crystals, mp 162—165°. $[\alpha]_D^{23}$+23.4° (c=0.974, $CHCl_3$). *Anal.* Calcd. for $C_{47}H_{70}O_{20}$: C, 59.10; H, 7.29; OCH_3, 9.75. Found: C, 58.56; H, 7.33; OCH_3, 9.28. UV λ_{max}^{EtOH} mμ (ε): 215 (13170). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520 (broad, OH), 1790, 1754, 1621 (butenolide), 1754, 1284 (ester).

IVa, V and VIIIa from I on Treatment with $O(CO_2Me)_2$——To a solution of 600 mg of I in 13 ml of pyridine, 6.2 g (*ca.* 60 moles) of $O(CO_2Me)_2$ was added dropwise under shaking at 70—75° and the mixture was further shaked at the same temperature for 30 min. The resulting solution was treated in the analogous manner described above to give 630 mg of a crude product which was submitted to preparative TLC (system A) to separate in the following three fractions.

i) The first fraction (127 mg) was recrystallized from acetone to give 72 mg of V, mp 237—239° (decomp.).

ii) The second fraction (170 mg) was recrystallized from aqueous MeOH to afford 120 mg of VIIIa, mp 158—160°.

iii) The third fraction (58 mg) was recrystallized from aqueous MeOH to give 30 mg of IVa, mp 160—163°.

V, VIIIb and IXb from I on Treatment with $O(CO_2Et)_2$——To a solution of 600 mg of I in 18 ml of pyridine, 2.5 g (*ca.* 20 moles) of $O(CO_2Et)_2$ was added dropwise at 70—75° under shaking. After the mixture was further shaked at the same temperature for 30 min, the reaction mixture was treated analogously to the above reaction with $O(CO_2Me)_2$ to obtain 608 mg of a crude product which was separated into the following three fractions in the order of polarity.

i) The first fraction (303 mg) was recrystallized from acetone to afford 210 mg of V, mp 238—240°.

ii) The second fraction (171 mg) was recrystallized from acetone to give 110 mg of VIIIb, mp 151—156°.

iii) The third fraction (32 mg) was recrystallized from acetone to give 17 mg of IXb, mp 207—209°.

IVb and VIIIb from I on Treatment with $O(CO_2Et)_2$——To a solution of 500 mg of I in 20 ml of pyridine, 12.5 g (*ca.* 100 moles) of $O(CO_2Et)_2$ was added dropwise at room temperature under shaking and the mixture was further shaked at the same temperature for 1 hr. The resulting solution was treated in the usual manner to obtain 540 mg of a crude product which was separated into the following two fractions by preparative TLC (system A).

i) The first fraction (160 mg) was recrystallized from acetone to give 77 mg of VIIIb, mp 150—155°.

ii) The second fraction (110 mg) was recrystallized from aqueous MeOH to give 54 mg of IVb, mp 143—148°.

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