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83. Kazuo Miyatake, Atsuji Okano, Kazuhiko Hoji, Tōsaku Miki, and Akio Sakashita : Studies on the Constituents of *Digitalis purpurea* L. XXIII.[*1] On the 16-Formyl Derivative of Digitalinum Verum.

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Studies on 16-acyl derivatives of cardiotonic glycosides of gitoxigenin series have been carried out and syntheses of 16-acetyldigitalinum verum,[1,2] 16-propionyldigitalinum verum,[1,2] and 16-acetylstrospeside[3] have already been reported. In the present series of work, 16-formyldigitalinum verum (VI) was obtained from digitalinum verum (I) by essentially the same method as in the preparation of 16-acetyldigitalinum verum.

Many cardiotonic glycosides having 16-formylgitoxigenin (gitaloxigenin) as the aglycone had been obtained from Digitalis species by Haack and his collaborators.[4] The presence of 16-formyldigitalinum verum (glucoverodoxin) had earlier been observed and it was isolated in recent years.[5]

Formylation of digitalinum verum (I) with 95~100% formic acid and acetic anhydride[6] afforded digitalinum verum hexaformate (II) as needle crystals, m.p. 145~150°/248~255°, $[\alpha]_D^{28}$ −10.0° (95% dioxane).

It was reported in Part XIII of this series[1] that partial deacetylation of digitalinum verum hexaacetate (III) with potassium hydrogencarbonate afforded 16-acetyldigitalinum verum monoacetate (V) and digitalinum verum monoacetate. Since the formyl group is more easily liberated than acetyl group, digitalinum verum hexaformate (II) was deformylated with a milder saponification agent than potassium hydrogencarbonate and the ion exchanger Amberlite IR-4B afforded 16-formyldigitalinum verum monoformate (IV) and digitalinum verum monoformate. Progress of this reaction was followed throughout by paper chromatographic examination, as shown in Fig. 1.

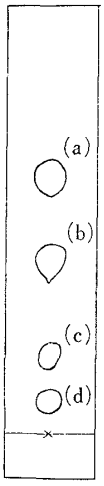

Fig. 1. Paper Partition Chromatography of Digitalinum verum and its Formyl Derivatives

(a) 16-Formyldigitalinum verum monoformate
(b) Digitalinum verum monoformate
(c) 16-Formyldigitalinum verum
(d) Digitalinum verum

Toyo Roshi No. 50; ascending method, at 18~22°
Moving phase : MeCOEt-iso-BuCOMe (1:1) saturated with H_2O
Stationary phase : Impregnated with H_2O-Me_2CO (1:4)
Coloring agent : 20% $SbCl_3$-$CHCl_3$ solution

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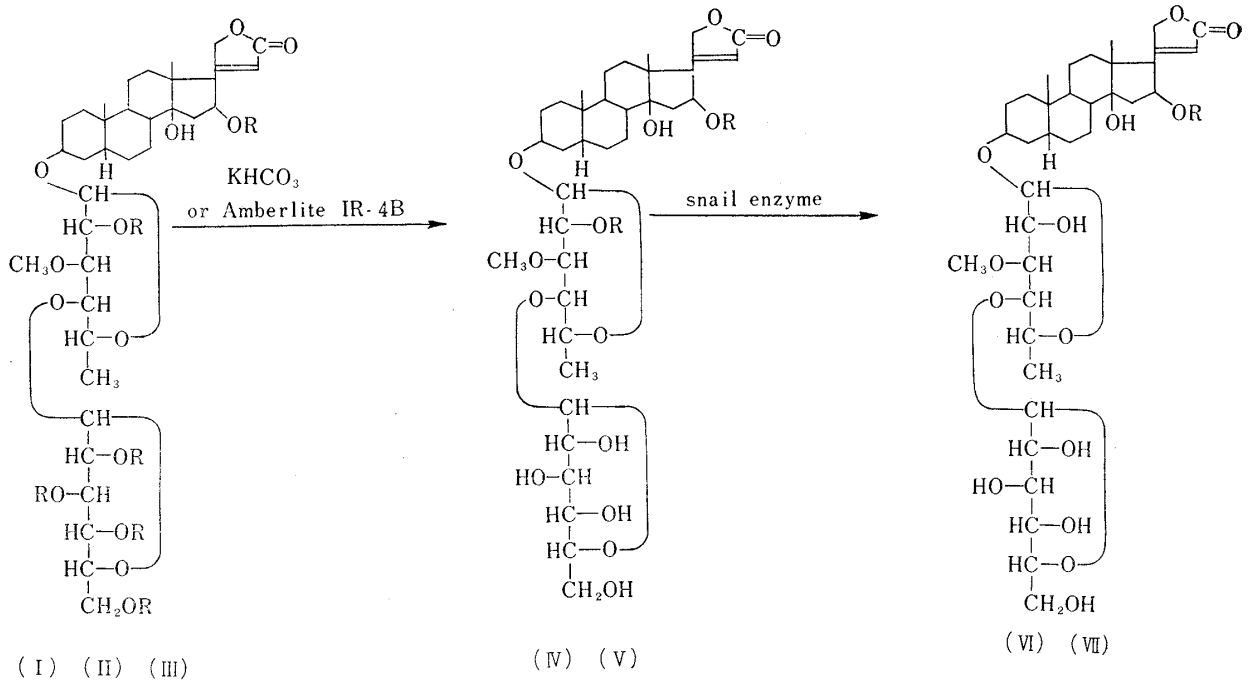
*1 Part XXII : This Bulletin, **9**, 519 (1961).
*2 Hirakawabashi, Sumida-ku, Tokyo (宮武一夫, 岡野淳二, 傍士和彦, 三木藤作, 坂下昭夫).
1) Part XIII. A. Okano, *et al.* : This Bulletin, **7**, 627 (1959).
2) Part XIV. K. Miyatake, *et al.* : *Ibid.*, **7**, 634 (1959).
3) Part XVI. *Idem* : *Ibid.*, 8, 945 (1960).
4) E. Haack, F. Kaiser, M. Gube, H. Spingler : Naturwiss., **43**, 301 (1956).
5) *Idem* : *Ibid.*, **45**, 338 (1958).
6) The method followed that of H.P. Sigg, Ch. Tamm, and T. Reichstein (Helv. Chim. Acta, **36**, 985 (1953)) with slight modifications.

16-Formyldigitalinum verum monoformate (IV) was recrystallized from hydrous dioxane to plates, m.p. 178~181°, $[\alpha]_D^{26}$ −13.2°(MeOH), $[\alpha]_D^{27}$ −12.5°(pyridine). It is soluble in methanol and ethanol, and slightly soluble in water. Digitalinum verum monoformate was recrystallized from a mixture of chloroform, methanol, and ether to a crystalline powder of m.p. 234~243°(decomp.), $[\alpha]_D^{27}$ −1.2°(pyridine). It is very sparingly soluble and only dissolves in a mixture of chloroform and methanol, and in pyridine. Further deformylation of (IV) gives digitalinum verum monoformate and this fact suggests that the formyl group in the sugar portion of these two compounds is present at the same position, which is assumed to be C-2 of digitalose (cf. Chart 1).



(I) (R=H) Digitalinum verum
(II) (R=HCO) Digitalinum verum hexaformate
(III) (R=CH_3CO) Digitalinum verum hexaacetate
(IV) (R=HCO) 16-Formyldigitalinum verum monoformate
(V) (R=CH_3CO) 16-Acetyldigitalinum verum monoacetate
(VI) (R=HCO) 16-Formyldigitalinum verum
(VII) (R=CH_3CO) 16-Acetyldigitalinum verum

Chart 1.

The preparation of 16-acetyldigitalinum verum (VII) by deacetylation with snail enzyme was described in Part XIV of this series.[2] Deformylation of 16-formyldigitalinum verum monoformate (IV) was carried out by the same method and it was found that 16-formyldigitalinum verum (VI) is formed as a main product with (I) as a by-product. Repeated purification through column partition chromatography failed to give (VI) in crystalline form. (VI), $[\alpha]_D^{28}$ −12.4°(MeOH), is easily soluble in water, methanol, ethanol, and acetone, and insoluble in ether and benzene.

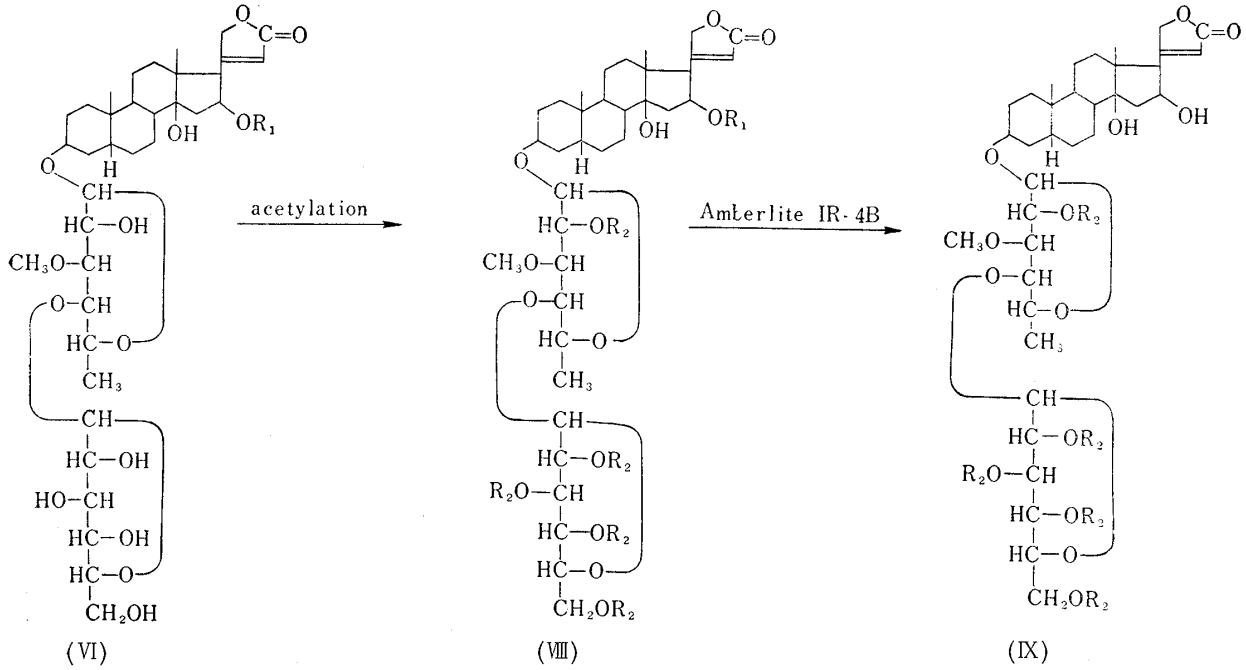
Comparison of molecular optical rotation of 16-formyldigitalinum verum (VI) and those of other 16-formyl derivatives (Table I) supports the structure of (VI).

In applying Mannich's acid hydrolysis[2] to (VI), 16-formylgitoxigenin was not obtained because of the facile liberation of its formyl group. In order to prove the presence of formyl group in 16-hydroxyl of (VI), (VI) was derived to digitalinum verum pentaacetate (IX) by the route shown in Chart 2. It had previously been shown[1,2] that compounds possessing 16-acetylgitoxigenin structure underwent deacetylation and anhydration by alu-

Table I. Comparison of Molecular Rotation

Substance	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$	Solvent
16-Formyldigitalinum verum	−12.4°	− 92°	−103°	MeOH
Digitalinum verum	1.6°	11°		
16-Formyldigitalinum verum monoformate	−12.5°	− 96°	− 87°	Pyridine
Digitalinum verum monoformate	− 1.2°	− 9°		
Digitalinum verum monoformate	− 1.2°	− 9°	− 18°	Pyridine
Digitalinum verum	1.3°	9°		
Gitaloxin (16-Formylgitoxin)[a]	− 7.0°	− 57°	− 84°	Pyridine
Gitoxin	3.5°	27°		
Lanatoside E (16-Formyllanatoside B)[b]	26.8°	272°	−102°	MeOH
Lanatoside B	38.0°	374°		

a) E. Haack, F. Kaiser, H. Spingler : Chem. Ber., **89**, 1353 (1956); *Idem* : Naturwiss., **42**, 441 (1955).
b) E. Angliker, F. Barfuss, J. Rinz : Helv. Chim. Acta, **41**, 479 (1958).



(VI) (VIII) (IX)

(VI) (R_1=HCO) 16-Formyldigitalinum verum
(VIII) (R_1=HCO, R_2=CH_3CO) 16-Formyldigitalinum verum pentaacetate
(IX) (R_2=CH_3CO) Digitalinum verum pentaacetate

Chart 2.

mina to form compounds with 16-anhydrogitoxigenin structure. Treatment of (IX) with alumina afforded a product which did not show the characteristic absorption for 16-anhydrogitoxigenin at 270 mμ in the ultraviolet region, indicating that the 16-anhydro derivative was not formed. Consequently, (IX) has a free hydroxyl in 16-position and the formyl group in (VI) is bonded to the hydroxyl in 16-position.

The toxicity test of the foregoing 16-formyldigitalinum verum was kindly carried out

Table II. Toxicity by Hatcher-Magnus Method (by Dr. K.K. Chen)

Substance	Mean lethal dose mg./kg. (cat)	Substance	Mean lethal dose mg./kg. (cat)
16-Formyldigitalinum verum	0.192	16-Propionyldigitalinum verum	0.819
16-Acetyldigitalinum verum	0.255	Digitalinum verum	1.332

by Dr. K.K. Chen of the Lilly Research Laboratories*3 and the results are shown in Table II. It is rather interesting that there is a great difference in physiological activity among the 16-formyl, 16-acetyl, and 16-propionyl derivatives of digitalinum verum.

Experimental*4

Formylation of Digitalinum verum (I)——After a mixture of 180 cc. of 95~100% HCOOH and 80 cc. of Ac_2O was allowed to cool for 4 hr., a solution of 9 g. of (I) dissolved in 110 cc. of pyridine was added to it, chilling to 0°. The mixture was allowed to stand for 24 hr. at a room temperature and poured into ice water. The precipitate was collected by filtration and recrystallized from Me_2CO-Et_2O to 9.5 g. of digitalinum verum hexaformate (II) as needles, m.p. 145~150°/248~255°; $[\alpha]_D^{28}$ −10.0°(c=1.23, 95% dioxane); soluble in pyridine and hydr. dioxane, and insoluble in $CHCl_3$ and MeOH. *Anal.* Calcd. for $C_{42}H_{56}O_{20}$: C, 57.26; H, 6.41. Found : C, 56.98; H, 6.50. UV : λ_{max}^{EtOH} 217 mμ (log ε 4.17).

Hydrolysis of Digitalinum verum Hexaformate (II) with Amberlite IR-4B——To a solution of 9.5 g. of (II) dissolved in a mixture of 1500 cc. of MeOH and 150 cc. of water, 95 cc. of Amberlite IR-4B was added and the mixture was stirred for 3 hr. at a room temperature. After Amberlite IR-4B was removed by filtration, the filtrate was concentrated at below 40° in a reduced pressure, and the residue was submitted to partition chromatography using 1600 g. of a mixture (1:1) of Celite and water, water-saturated iso-BuCOMe as the developing solvent, and 1000-cc. fractions were collected. The fraction Nos. 2~20 (3.8 g.) gave (IV) and the fraction later than No. 21 (3.9 g.) gave (I) and digitalinum verum monoformate.

16-Formyldigitalinum verum Monoformate (IV)——The foregoing fraction Nos. 2~20 (3.8 g.) was recrystallized from hydr. dioxane to 1.9 g. of (IV) as plates, m.p. 178~181°; $[\alpha]_D^{26}$ −13.2°(c=1.15, MeOH), $[\alpha]_D^{27}$ −12.5°(c=1.52, pyridine), easily soluble in MeOH and EtOH, soluble in water, and almost insoluble in Et_2O. *Anal.* Calcd. for $C_{38}H_{56}O_{16}$: C, 59.36; H, 7.34; CHO, 7.55. Calcd. for $C_{38}H_{56}O_{16}\cdot 2H_2O$: C, 56.70; H, 7.51; CHO, 7.21. Found : C, 56.41; H, 7.35; CHO, 7.18. UV : λ_{max}^{EtOH} 217 mμ (log ε 4.16).

Digitalinum verum Monoformate——The portion eluted after the fraction No. 21 (3.9 g.) was again submitted to partition chromatography on a mixture (1:1) of Celite and water, with water-saturated MeCOEt-$CHCl_3$ (4:1) mixture as the developing solvent. The fraction of digitalinum verum monoformate was recrystallized from $CHCl_3$-MeOH-Et_2O mixture to 0.95 g. of a crystalline powder, m.p. 234~243°(decomp.); $[\alpha]^{27}$ −1.2°(c=1.31, pyridine), soluble in pyridine and $CHCl_3$-MeOH mixture, and insoluble in water, $CHCl_3$, and MeOH. *Anal.* Calcd. for $C_{37}H_{56}O_{15}$: C, 59.98; H, 7.62; CHO, 3.92. Found : C, 59.65; H, 7.94; CHO, 4.15. UV : λ_{max}^{EtOH} 219 mμ (log ε 4.15).

Enzymatic Decomposition of 16-Formyldigitalinum verum Monoformate (IV)——(IV) (2.4 g.) was dissolved in a small amount of dioxane, 2400 cc. of water was added to it, and dioxane was distilled off as much as possible. An enzyme solution, prepared by extraction of 0.8 g. of snail enzyme with two 50-cc. portions of water, was added to the solution of (IV), this mixture was covered with 30 cc. of toluene, and allowed to stand at 32° for 6 days. This reaction mixture was concentrated to 30 cc. at below 50° in a reduced pressure, 100 cc. of dioxane was added to the residue, the precipitate thereby formed was removed, and the solution was further concentrated, affording 2.35 g. of a reaction product.

This product was submitted to partition chromatography using 600 g. of a mixture (1:1) of Celite and water, water-saturated mixture of MeCOEt and iso-BuCOMe (1:1) as the developing solvent, and 200-cc. fractions were collected. The fraction Nos. 1~10 (0.85 g.) gave (VI) and a small amount of digitalinum verum monoformate, the fraction Nos. 11~18 (1.04 g.) gave (VI), and the fraction No. 19 and later portions (0.32 g.) afforded (I).

16-Formyldigitalinum verum (VI)——The fraction Nos. 11~18 afforded (VI) as an amorphous substance, freely soluble in water, MeOH, EtOH, and Me_2CO, and insoluble in Et_2O and benzene. $[\alpha]_D^{28}$ −12.4°(c=1.31, MeOH), UV : λ_{max}^{EtOH} 217 mμ (log ε 4.14). *Anal.* Calcd. for $C_{37}H_{56}O_{15}$: C, 59.98; H, 7.62; CHO, 3.92. Found : C, 59.74; H, 7.51; CHO, 4.60.

Hydrolysis of 16-Formyldigitalinum verum (VI) by the Mannich Method——A solution of 10 mg. of (VI) dissolved in 1.2 cc. of Me_2CO and added with 0.012 cc. of conc. HCl was allowed to stand at 5° for 9 days. The reaction product was submitted to paper chromatography using formamide-saturated mixture of xylene and MeCOEt (1:1) as the developing solvent; a spot was observed and identified as gitoxigenin, but that of 16-formylgitoxigenin was not detected.

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*3 Deep gratitude is expressed to Dr. K.K. Chen (The Lilly Research Laboratories, Indianapolis, U.S.A.) for kindly undertaking the toxicity tests and offering detailed data.

*4 All melting points were measured on a Kofler block and are uncorrected.

Acetylation of 16-Formyldigitalinum verum (VI)——A mixture of 0.8 cc. of Ac_2O added to a solution of 150 mg. of (VI) dissolved in 2.4 cc. of pyridine was allowed to stand for 2.5 hr. at a room temperature and poured into ice water. The precipitate was collected by filtration and recrystallized from Me_2CO-Et_2O to 110 mg. of 16-formyldigitalinum verum pentaacetate (VIII) as needles, m.p. 151~156°/232~238°; $[\alpha]_D^{25}$ −12.3°(c=1.22, $CHCl_3$). UV : λ_{max}^{EtOH} 217 mμ(log ε 4.19). *Anal.* Calcd. for $C_{47}H_{66}O_{20}$: C, 59.36; H, 7.00. Found : C, 59.62; H, 6.94. (VIII) was tested for acetyl and formyl groups by the method[7] of hydroxamic acid and gave positive results.

Hydrolysis of 16-Formyldigitalinum verum Pentaacetate (VIII) with Amberlite IR-4B——30 cc. of Amberlite IR-4B was added to a solution of 110 mg. of (VIII) dissolved in 36 cc. of MeOH and the mixture was stirred for 4 hr. at a room temperature. After removing Amberlite IR-4B, the reaction mixture was concentrated and the residue was recrystallized from hydr. MeOH to 80 mg. of digitalinum verum pentaacetate (IX) as needles, m.p. 247~251°. *Anal.* Calcd. for $C_{46}H_{66}O_{19}$: C, 59.86; H, 7.21; CH_3CO, 23.32. Found : C, 59.84; H, 7.38; CH_3CO, 23.82. $[\alpha]_D^{23}$ +0.8°(c=1.21, $CHCl_3$). UV : λ_{max}^{EtOH} 219 mμ(log ε 4.18). (IX) was tested for formyl and acetyl groups by the method[7] of hydroxamic acid; formyl group was negative but acetyl group was positive.

Treatment of Digitalinum verum Pentaacetate (IX) with Alumina——A solution of 5 mg. of (IX) dissolved in 0.5 cc. of $CHCl_3$ was adsorbed on 1 g. of activated alumina, the mixture was covered with benzene, and allowed to stand for 4 days at a room temperature. The reaction product was extracted from alumina with hydr. BuOH, the extract was concentrated, and dried. The ultraviolet spectrum of this product did not show an absorption maximum at 270 mμ.

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Summary

Digitalinum verum hexaformate was submitted to deformylation with Amberlite IR-4B and was converted to a new formyl derivative, 16-formyldigitalinum verum monoformate. Deformylation with snail enzyme was carried out on this derivative and the corresponding 16-formyldigitalinum verum was obtained.

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7) This method was described in the preceding paper (Part XIII : This Bulletin, **7**, 627 (1959)).