The present series of examinations were always made with due considerations for the result of X-ray diffraction but urinary calculi are likely to be amorphous substances and a much better result can be obtained from infrared spectrophotometer than from X-ray diffraction apparatus, although the use of the latter in conjunction with the infrared photometer is desirable. In the case of a trace of metals, use should be made of flame analysis and other pertinent methods. Quantitative analysis by the KBr-pellet method is now being examined.

Sincere gratitude is expressed to Prof. S. Shibata and Prof. T. Ichikawa for their kind encouragement. The expenses for this work were defrayed by the Grant-in-Aid for Scientific Research from the Ministry of Education which is gratefully acknowledged.

Summary

Analytical method for various urinary calculi through infrared spectral measurement was established.

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116. **Atsuji Okano, Kazuhiko Hoji, Tōsaku Miki, and Akio Sakashita :**

Studies on the Constituents of *Digitalis purpurea* L. XIII.[*1]
On the Diacyl Derivatives of Digitalinum Verum.

(*Research Laboratory, Daiichi Seiyaku Co., Ltd.*[*2])

It has already been reported that deacetylation of the acetate of strospeside,[1] digitalinum verum[1] (I), gitostin,[2] and neogitostin[2] with potassium hydrogen carbonate results invariably in one residual acetyl group in the digitalose portion and a monoacetate is obtained. It had been observed by the present workers that in this deacetylation reaction there is also a reaction product which has not been hydrolyzed to the monoacetate but the formation of such a by-product has not been reported as yet. It has been found in the present series of work that deacylation of some of the digitalinum verum hexaacylates under a suitable condition effected fairly selective deacylation to form 16-acyldigitalinum verum monoacylate.

The objective substance could be obtained in a good yield by decreasing the amount of potassium hydrogen carbonate used in deacylation and by terminating the reaction period at a suitable time. Selection of the reaction time was examined by following the progress of reaction through paper chromatography (Fig. 1).

In the case of acetylated compound, the reaction product was submitted to column partition chromatography through Celite 535, with water-saturated methyl isobutyl ketone as the developing solvent, and needle crystals, m.p. 181~184°, $[\alpha]_D^{26}$ −24°(MeOH) (Fig. 2), were obtained. The product is easily soluble in methanol and ethanol, soluble in acetone and water, and insoluble in ether. Its analytical values and determination of acetyl group agreed with those for digitalinum verum diacetate.

[*1] Part XII : This Bulletin, **7**, 226(1959).

[*2] Hirakawabashi, Sumida-ku, Tokyo (岡野淳二, 傍士和彦, 三木藤作, 坂下昭夫).

1) W. Rittel, A. Hunger, T. Reichstein : Helv. Chim. Acta, **35**, 434(1952).

2) A. Okano : This Bulletin, **6**, 173(1958).

Molecular rotation of this diacetate and that of the monoacetate (II) are compared in Table I and the difference agrees well with the contribution of 16-acetyl group in molecular rotation of 16-acetylgitoxigenin glycosides noted in the same table.[1]

Reichstein and his co-workers[3] reported that treatment of compounds of 16-acetylgitoxigenin (oleandrigenin) series with alumina effected concurrent deacetylation and anhydrogenation to form the 16-anhydrogitoxigenin series compounds, with consequent conjugation with the double bond in unsaturated lactone, and a characteristic maximum newly appears at 270 mμ in the ultraviolet absorption spectrum.

The substance obtained by treatment of the foregoing diacetate with alumina also showed a newly formed absorption maximum at 270 mμ in its ultraviolet spectrum and a new spot, other than that of the diacetate, in its paper chromatogram (Fig. 6).

Treatment of digitalinum verum hexaacetate (IV) with alumina and the usual deacetylation with potassium hydrogen carbonate afforded anhydrodigitalinum verum monoacetate[3] and the anhydro compound obtained from the above-mentioned diacetate was identified with this anhydro-digitalinum verum monoacetate by paper chromatography. These results have proved that the said diacetate is 16-acetyldigitalinum verum monoacetate (VII).

The same deacylation reactions were carried out on the newly synthesized digitalinum verum hexapropionate (V) and digitalinum verum monoacetate pentapropionate (VI), derived from digitalinum verum monoacetate (II), and digitalinum verum monopropionate (III), m.p. 236~238°, 16-propionyldigitalinum verum monopropionate (VIII),[*3] m.p. 169~173° (Fig. 3), and 16-propionyldigitalinum verum monoacetate (IX),[*4] m.p. 175~180° (Fig. 4), were newly obtained. Table I also shows the contribution of 16-propionyl group in molecular rotation. The 16-propionyl compounds changed into the anhydro compounds by treatment with alumina (Fig. 6).

It is interesting that the acyl group in the 16-position is more difficult to be deacylated than that in the glucose portion and that 16-acyldigitalinum verum monoacylate is formed fairly selectively.

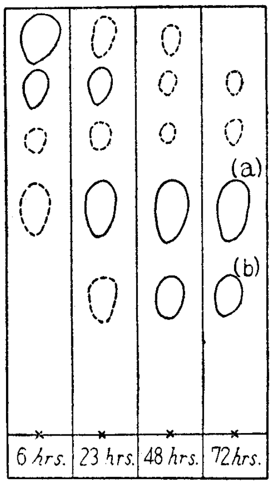


Fig. 1. Paper Partition Chromatography[*5] after Deacetylation of Digitalinum verum Hexaacetate to 16-Acetyldigitalinum verum Monoacetate

(a) 16-Acetyldigitalinum verum monoacetate
(b) Digitalinum verum monoacetate

[*3] The Mannich hydrolysis of (VIII) and examination of its reaction product by paper chromatography showed the presence of a spot of 16-propionylgitoxigenin, which will be described in the following paper.

[*4] The presence of two acyl groups was proved qualitatively by deriving this compound (IX) to the respective hydroxamic acids and submitting each to paper chromatography.

[*5] Procedures for all paper partition chromatography were as follows: Toyo Roshi No. 50; ascending method, at 18~22°; moving phase, MeCOEt—*iso*-BuCOMe (1:1) saturated with water; stationary phase, impregnated with H_2O-Me_2CO (1:1); coloring agent, 20% $SbCl_3$-$CHCl_3$ solution.

3) A. Hunger, T. Reichstein: Helv. Chim. Acta, **33**, 76(1950).

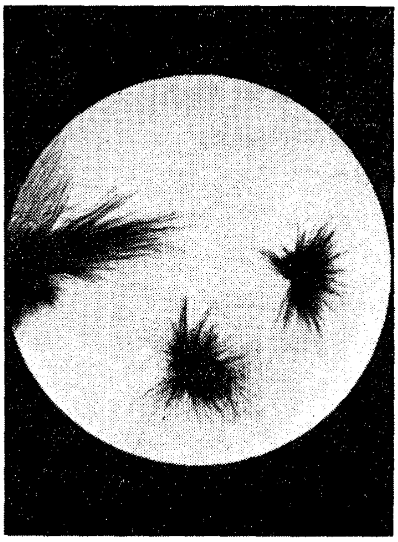


Fig. 2. 16-Acetyldigitalinum verum Monoacetate

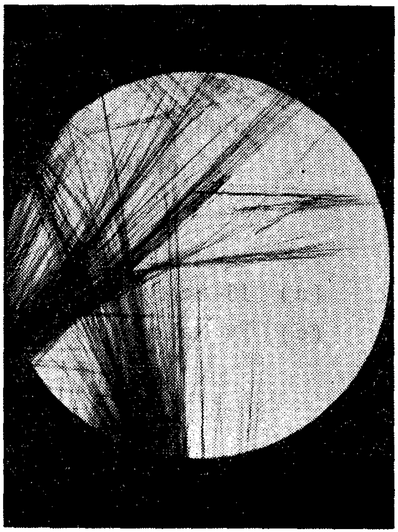


Fig. 3. 16-Propionyldigitalinum verum Monopropionate

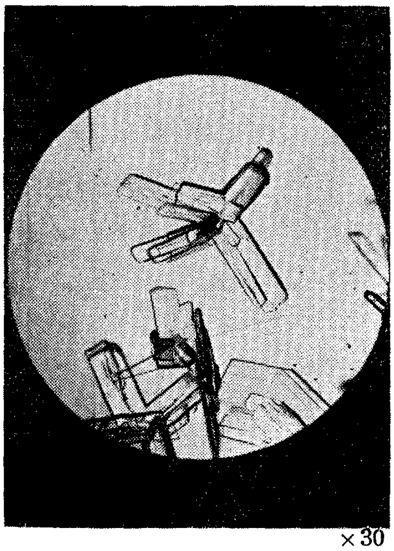


Fig. 4. 16-Propionyldigitalinum verum Monoacetate

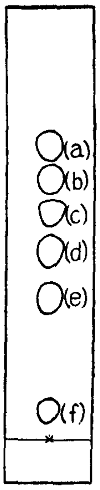


Fig. 5. Paper Partition Chromatography of Digitalinum verum and its Derivatives

(a) 16-Propionyldigitalinum verum monopropionate
(b) 16-Propionyldigitalinum verum monoacetate
(c) 16-Acetyldigitalinum verum monoacetate
(d) Digitalinum verum monopropionate
(e) Digitalinum verum monoacetate
(f) Digitalinum verum

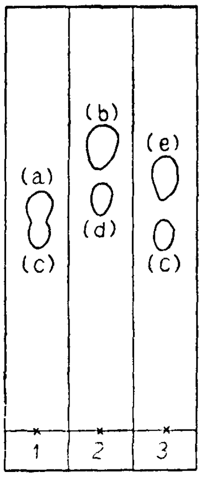


Fig. 6. Paper Partition Chromatography of Mixture after Alumina Deacylation

1. From 16-acetyldigitalinum verum monoacetate
2. From 16-propionyldigitalinum verum monopropionate
3. From 16-propionyldigitalinum verum monoacetate

(a) 16-Acetyldigitalinum verum monoacetate
(b) 16-Propionyldigitalinum verum monopropionate
(c) 16-Anhydrodigitalinum verum monoacetate
(d) 16-Anhydrodigitalinum verum monopropionate
(e) 16-Propionyldigitalinum verum monoacetate

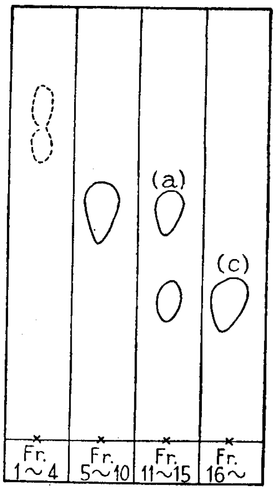
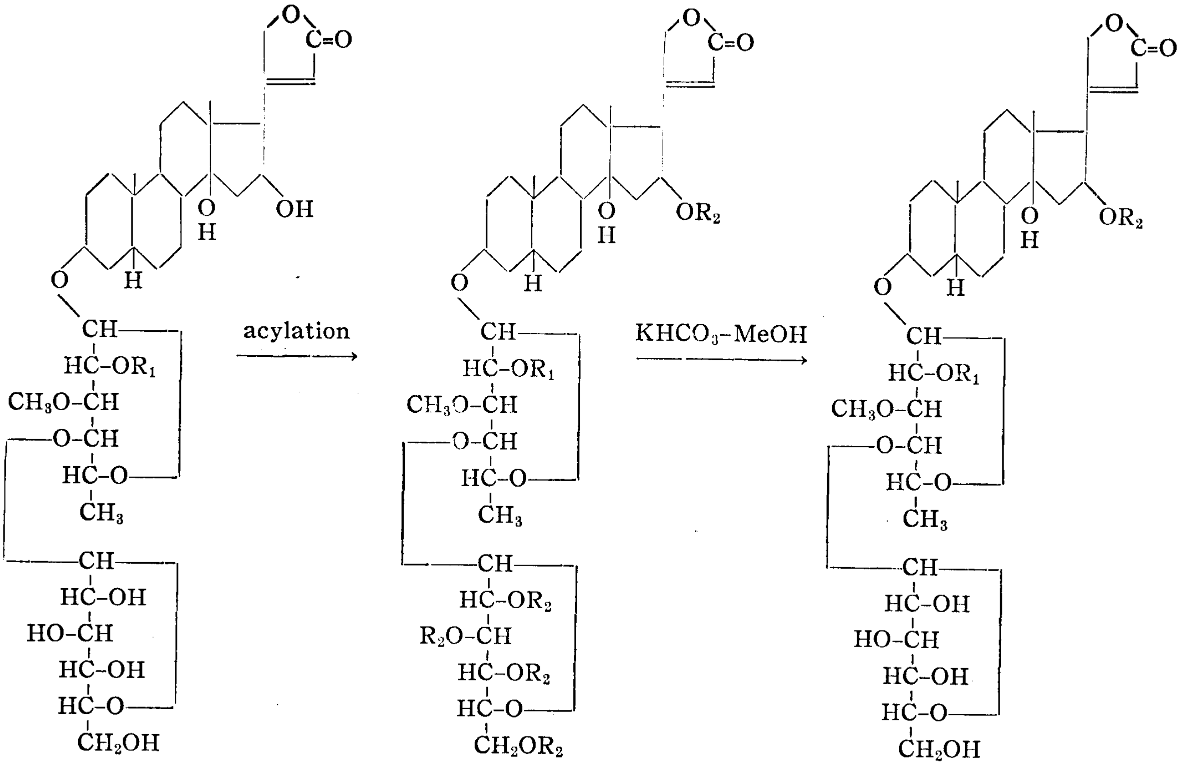


Fig. 7. Paper Partition Chromatography of Each Fraction from Partition Chromatography of Reaction Mixtures

(a) 16-Acetyldigitalinum verum monoacetate
(c) Digitalinum verum monoacetate



(I) (II) (III) (IV) (V) (VI) (VII) (VIII) (IX)

(I) (R_1=H) Digitalinum verum
(II) (R_1=CH_3CO) Digitalinum verum monoacetate
(III) (R_1=CH_3CH_2CO) Digitalinum verum monopropionate
(IV) (R_1=R_2=CH_3CO) Digitalinum verum hexaacetate
(V) (R_1=R_2=CH_3CH_2CO) Digitalinum verum hexapropionate
(VI) (R_1=CH_3CO, R_2=CH_3CH_2CO) Digitalinum verum monoacetate pentapropionate
(VII) (R_1=R_2=CH_3CO) 16-Acetyldigitalinum verum monoacetate
(VIII) (R_1=R_2=CH_3CH_2CO) 16-Propionyldigitalinum verum monopropionate
(IX) (R_1=CH_3CO, R_2=CH_3CH_2CO) 16-Propionyldigitalinum verum monoacetate

Chart 1.

Table I. Comparison of Molecular Rotation (in MeOH)

Substance	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$
Digitalinum verum monoacetate	− 2.9°	− 22°	
16-Acetyldigitalinum verum monoacetate	−24.0°	−191°	169°
Digitalinum verum monopropionate	− 1.8°	− 14°	
16-Propionyldigitalinum verum monopropionate	−24.9°	−205°	191°
Digitalinum verum monoacetate	− 2.9°	− 22°	
16-Propionyldigitalinum verum monoacetate	−25.0°	−203°	181°
16-Deacetylhongheloside A	13.6°	73°	
Hongheloside A	−14.0°	− 81°	154°
16-Deacetyloleandrin	−24.9°	−133°	
Oleandrin	−52.1°	−300°	167°
16-Deacetylcryptograndoside A	− 3.4°	− 18°	
Cryptograndoside A	−32.9°	−190°	172°
Gitoxigenin	32.6°	127°	
Oleandrigenin (16-Acetylgitoxigenin)	− 9.8°	− 42°	169°
Digitalinum verum	1.6°	11°	
Digitalinum verum monoacetate	− 2.9°	− 22°	33°
Digitalinum verum	1.6°	11°	
Digitalinum verum monopropionate	− 1.8°	− 14°	25°
Strospeside	15.5°	85°	
Strospeside monoacetate	18.8°	112°	− 27°

The authors are deeply grateful to Dr. Junzo Shinoda, the President of this Company, Mr. Sakan Hashimoto, the Director of this Laboratory, Dr. Masao Shimizu, the Acting Director of the Laboratory, and to Dr. Kazuo Miyatake, the Director of Yanagishima Factory, for their kind and unfailing guidance throughout the course of the present work and for giving permission for publication of this work. The authors are also indebted to Messrs. B. Kurihara and K. Abe for analytical data.

Experimental[*6]

Hydrolysis of Digitalinum verum Hexaacetate (IV) with $KHCO_3$—A solution of 1.2 g. of $KHCO_3$ dissolved in 200 cc. of water was added to a solution of 10 g. of (IV) dissolved in 1800 cc. of MeOH and the mixture was allowed to stand at room temperature. After 72 hr., (IV) was almost completely hydrolyzed to 16-acetyldigitalinum verum monoacetate (VII) and digitalinum verum monoacetate (II), as evidenced by the result of paper chromatography (Fig. 1). The reaction mixture was diluted with 200 cc. of water, neutralized with dil. HCl, and MeOH was evaporated under reduced pressure at below 50°. The residual solution was extracted with four 400-cc. portions of a mixture (2:1) of $CHCl_3$ and EtOH, the extract was concentrated, and dried, affording 8 g. of a residue.

This residue was submitted to partition chromatography using 800 g. of a mixture (1:1) of Celite and water, and water-saturated *iso*-BuCOMe as the developing solvent, collecting in 300-cc. fractions. The fraction Nos. 1~4 (0.12 g.) was the intermediate acetylated compound, the fraction Nos. 5~10 (3.25 g.) gave (VII), the fraction Nos. 11~15 (0.50 g.) gave (VII) and (II), and the fractions later than No. 16 gave (II) (3.9 g.) (Fig. 7).

16-Acetyldigitalinum verum Monoacetate (VII)—The residue from fraction Nos. 5~10 was recrystallized from water-saturated *iso*-BuCOMe and 2.62 g. of needles, m.p. 181~184°, was obtained. This substance is easily soluble in MeOH and EtOH, soluble in Me_2CO and H_2O, and insoluble in Et_2O. *Anal.* Calcd. for $C_{40}H_{60}O_{16}$: C, 60.29; H, 7.59; CH_3CO, 10.80. Calcd. for $C_{40}H_{60}O_{16}\cdot H_2O$: C, 58.95; H, 7.67; CH_3CO, 10.57. Found : C, 59.20; H, 7.31; CH_3CO, 10.19. UV λ_{max}^{EtOH} 217 mμ (log ε 4.18). $[\alpha]_D^{26}$ −24.0°(c=1.25, MeOH).

Digitalinum verum Monoacetate (II)—The portion eluted after the fraction No. 16 was recrystallized from hydrous MeOH to 2.76 g. of needles, m.p. 225~243°(decomp.), identical with an authentic sample of (II).

[*6] All m.p.s were measured on Kofler block and are uncorrected.

Alumina Treatment of 16-Acetyldigitalinum verum Monoacetate (VII)—A solution of 5 mg. of (VII) dissolved in 0.5 cc. of a mixture (9:1) of $CHCl_3$ and MeOH was adsorbed on 1 g. of Al_2O_3, this was covered with benzene, and allowed to stand at room temperature. After 5 days, Al_2O_3 was extracted with water-saturated BuOH, the extract was concentrated under reduced pressure, and dried. This residue was submitted to paper chromatography and a spot with Rf value same as that of 16-anhydrodigitalinum verum monoacetate was observed besides that of (VII) (Fig. 6). Ultraviolet spectrum of this residue exhibited absorption maxima at 217 and 270 mμ.

16-Anhydrodigitalinum verum Monoacetate from Digitalinum verum Hexaacetate—A solution of 0.4 g. of (IV) dissolved in 20 cc. of $CHCl_3$ was adsorbed on 60 g. of activated Al_2O_3, this was covered with benzene, and allowed to stand at room temperature. After 7 days, Al_2O_3 was extracted with water-saturated BuOH, the extract was concentrated under reduced pressure, and dried, affording 0.3 g. of an extract. This extract was dissolved in 30 cc. of MeOH, a solution of 0.2 g. of $KHCO_3$ dissolved in 6 cc. of water was added to it, and the mixture was allowed to stand for 3 days at room temperature. This mixture was diluted with 6 cc. of water, neutralized with dil. HCl, and MeOH was evaporated under reduced pressure. The residual solution was extracted with a mixture (2:1) of $CHCl_3$ and EtOH, the extract was concentrated and dried, and 0.25 g. of 16-anhydrodigitalinum verum monoacetate was obtained as granules. In order to effect purification, this substance was submitted to partition chromatography with Celite and water-saturated MeCOEt or water-saturated *iso*-BuCOMe as the developing solvent, and fractions containing 16-anhydrodigitalinum verum monoacetate were collected. This fraction was recrystallized from MeOH-Et_2O and $CHCl_3$-MeOH-Et_2O mixture to 170 mg. of needles, m.p. 276~280°(decomp.). UV λ_{max}^{EtOH} mμ(log ε): 225(3.66), 270(4.24). $[\alpha]_D^{26}$ −8.2°(c=1.10, pyridine). *Anal.* Calcd. for $C_{38}H_{56}O_{14}$: C, 61.94; H, 7.66. Found: C, 61.75; H, 7.58.

Acetate: Acetylation of 50 mg. of 16-anhydrodigitalinum verum monoacetate with pyridine and Ac_2O by the usual method and recrystallization of the crude acetylated compound from benzene-Et_2O and Me_2CO-benzene mixture afforded 50 mg. of 16-anhydrodigitalinum verum pentaacetate as needles, m.p. 246~249°(decomp.). UV λ_{max}^{EtOH} mμ(log ε): 222(3.71), 270(4.25). $[\alpha]_D^{26}$ +23.4°(c=1.28, $CHCl_3$). *Anal.* Calcd. for $C_{46}H_{64}O_{18}$: C, 61.05; H, 7.13. Found: C, 61.02; H, 6.91.

Propionylation of Digitalinum verum (I)—A mixture of 10 cc. of propionic anhydride added to a solution of 3 g. of (I) dissolved in 45 cc. of pyridine was allowed to stand for 3 days at room temperature. The mixture was concentrated at below 50° under reduced pressure, the residue was dissolved in 100 cc. of $CHCl_3$, and the $CHCl_3$ solution was washed consecutively with water, 5% HCl, water, 5% NaOH, and water. After evaporation of $CHCl_3$, the residue was recrystallized twice from hydrous EtOH to 3.96 g. of digitalinum verum hexapropionate (V) as needles, m.p. 138~142°; $[\alpha]_D^{24}$ −20.7° (c=1.21, $CHCl_3$); soluble in MeOH, EtOH, and $CHCl_3$, insoluble in water. UV λ_{max}^{EtOH} 217 mμ (log ε 4.20). *Anal.* Calcd. for $C_{54}H_{80}O_{20}$: C, 61.82; H, 7.69. Found: C, 62.06; H, 7.66.

Hydrolysis of Digitalinum verum Hexapropionate (V) with $KHCO_3$—A solution of 0.42 g. of $KHCO_3$ dissolved in 70 cc. of water was added to a solution of 3.51 g. of (V) dissolved in 700 cc. of MeOH and the mixture was allowed to stand tor 1 week at room temperature. This mixture was treated as in the hydrolysis of (IV) and 2.7 g. of a mixture of reaction products was obtained, chiefly consisting of 16-propionyldigitalinum verum monopropionate (VIII) and digitalinum verum monopropionate (III). This mixture was submitted to partition chromatography on 600 g. of a mixture (1:1) of Celite and water, with water-saturated *iso*-BuCOMe as the developing solvent, collecting in 100-cc. fractions. The fraction Nos. 1 and 2 gave the intermediate reaction product, the fraction Nos. 3~8 (2.0 g.) gave (VIII) and a small amount of the intermediate, the fraction Nos. 9~14 (0.1 g.) gave (VIII) and (III), and fraction No. 15 and subsequent fractions (0.6 g.) gave only (III).

The combined fraction Nos. 3~8 was again submitted to partition chromatography on 700 g. of a mixture (4:3) of Celite and water, with water-saturated MeCOEt-benzene (1:2) mixture as the developing solvent, collecting in 250-cc. fractions. The fraction Nos. 1~4 (0.7 g.) gave a small amount of the intermediate and (VIII), and the fraction Nos. 5~24 (1.2 g.) only (VIII).

16-Propionyldigitalinum verum Monopropionate (VIII)—The foregoing fraction Nos. 5~24 (1.2 g.) was recrystallized from MeOH-Et_2O mixture to 0.63 g. of (VIII) as needles, m.p. 169~173°; $[\alpha]_D^{24}$ −24.9° (c=1.22, MeOH), easily soluble in MeOH and EtOH, soluble in $CHCl_3$ and H_2O, and insoluble in Et_2O. *Anal.* Calcd. for $C_{42}H_{64}O_{16}$: C, 61.15; H, 7.82. Calcd. for $C_{42}H_{64}O_{16}\cdot H_2O$: C, 59.84; H, 7.89. Found: C, 59.99; H, 7.73. UV λ_{max}^{EtOH} 217 mμ (log ε 4.15).

Digitalinum verum Monopropionate (III)—The fraction No. 15 and later portion (0.6 g.) was recrystallized from hydrous MeOH and MeOH-Et_2O mixture to 0.31 g. of (III) as plates, m.p. 236~238°; $[\alpha]_D^{25}$ −1.8°(c=1.28, MeOH); soluble in MeOH and EtOH, and insoluble in $CHCl_3$ and Et_2O. UV λ_{max}^{EtOH} 219 mμ (log ε 4.20). *Anal.* Calcd. for $C_{39}H_{60}O_{15}$: C, 60.92; H, 7.87. Calcd. for $C_{39}H_{60}O_{15}\cdot H_2O$: C, 59.53; H, 7.94. Found: C, 59.73; H, 7.56.

Hydrolysis of 16-Propionyldigitalinum verum Monopropionate (VIII) by the Mannich Method—A solution of 10 mg. of (VIII) dissolved in 1.2 cc. of Me_2CO and added with 0.012 cc. of conc. HCl was allowed to stand at 5° for 15 days. After neutralization, this mixture was extracted with $CHCl_3$ and the $CHCl_3$ solution was concentrated. The reaction product so obtained was examined by paper chromatography using formamide-saturated mixture (1:1) of xylene and MeCOEt, and Toyo Roshi No. 50, at 18~22°. The chromatogram revealed spots of the unreacted starting compound and gitoxigenin (Rf 0.39), as well as a spot at Rf 0.80, a value slightly greater than that (Rf 0.68) of 16-acetylgitoxigenin. This latter spot was assumed to be that of 16-propionylgitoxigenin.

Alumina Treatment of 16-Propionyldigitalinum verum Monopropionate (VIII)—A solution of 5 mg. of (VIII) dissolved in 0.5 cc. of $CHCl_3$ was adsorbed on 1 g. of activated Al_2O_3, covered with benzene, and this was allowed to stand at room temperature for 4 days. This was treated as in the case of (VII), the reaction product was submitted to paper chromatography, and a spot of anhydro compound was detected (Fig. 6). The ultraviolet spectrum of the reaction product showed absorption maxima at 217 and 270 mμ.

Propionylation of Digitalinum verum Monoacetate (II)—A solution of 6 g. of (II) dissolved in 90 cc. of pyridine and added with 25 cc. of propionic anhydride was allowed to stand at room temperature for 3 days and treated as in the case of (V). Two recrystallizations from hydrous EtOH afforded 7.8 g. of digitalinum verum monoacetate pentapropionate (VI) as needles, m.p. 138~143°, whose properties resembled those of (V). $[\alpha]^{24}_{D}$ −20.5°(c=1.50, $CHCl_3$). UV λ^{EtOH}_{max} 217 mμ (log ε 4.20). *Anal.* Calcd. for $C_{53}H_{78}O_{20}$: C, 61.49; H, 7.60. Found : C, 61.25; H, 7.70.

Hydrolysis of Digitalinum verum Monoacetate Pentapropionate (VI) with $KHCO_3$—A solution of 1.8 g. of $KHCO_3$ dissolved in 200 cc. of water was added to the solution of 10.2 g. of (VI) dissolved in 1800 cc. of MeOH and the mixture was allowed to stand for 1 week at room temperature. This was treated as in hydrolysis of (IV) and a reaction mixture chiefly containing 16-propionyldigitalinum verum monoacetate (IX) and digitalinum verum monoacetate (II) was obtained. This reaction product was submitted to partition chromatography using 1400 g. of a mixture (1:1) of Celite and water, and water-saturated *iso*-BuCOMe as the developing solvent, collecting in 300-cc. fractions. The fraction Nos. 3~4 (1.8 g.) gave the intermediate product, the fraction Nos. 5~19 (4.7 g.) gave (IX), and the fraction No. 20 and later (1.5 g.) gave (II). The fractions later than No. 20 were collected and recrystallized from hydrous MeOH affording 1.15 g. of (II) as needles, m.p. 243~252°(decomp.).

16-Propionyldigitalinum verum Monoacetate (IX)—The foregoing fraction Nos. 5~19 (4.7 g.) was repeatedly recrystallized from MeOH-Et_2O mixture and 3.93 g. of 16-propionyldigitalinum verum monoacetate (IX) was obtained as plates, m.p. 175~180°; $[\alpha]^{25}_{D}$ −25.0°(c=1.20, MeOH); easily soluble in MeOH and EtOH, soluble in $CHCl_3$ and water, insoluble in Et_2O. UV λ^{EtOH}_{max} 217 mμ (log ε 4.16). *Anal.* Calcd. for $C_{41}H_{62}O_{16}$: C, 60.72; H, 7.71. Calcd. for $C_{41}H_{62}O_{16} \cdot H_2O$: C, 59.41; H, 7.78. Found: C, 59.63; H, 7.46.

Alumina Treatment of 16-Propionyldigitalinum verum Monoacetate (IX)—A solution of 180 mg. of (IX) dissolved in 10 cc. of $CHCl_3$ was adsorbed on 23 g. of activated Al_2O_3, this was covered with benzene, and allowed to stand for 1 week at room temperature. This was treated as in the case of (VII) and the reaction product was examined by paper chromatography (Fig. 6). A spot showing Rf value same as that of 16-anhydrodigitalinum verum monoacetate was detected besides the spot of (IX). The reaction product was then submitted to partition chromatography using 50 g. of a mixture (1:1) of Celite and water, with water-saturated *iso*-BuCOMe as the developing solvent, collecting in 25-cc. fractions. The fraction Nos. 3~6 afforded (IX), the fraction No. 7 gave (IX) and 16-anhydrodigitalinum verum monoacetate, and the fraction Nos. 8~10 (20 mg.) gave only the anhydro compound. The collected fraction Nos. 8~10 was recrystallized from MeOH-Et_2O mixture to 10 mg. of 16-anhydrodigitalinum verum monoacetate as needles, m.p. 275~279°(decomp.). UV λ^{EtOH}_{max} mμ (log ε) : 222 (3.65), 270 (4.21).

Estimation of the Acyl Group in 16-Propionyldigitalinum verum Monoacetate (IX) (Hydroxamic Acid Method[4])—To a solution of 15 mg. of (IX) dissolved in 0.5 cc. of EtOH, 0.5 cc. of a mixture (1:1) of 5% $NH_2OH \cdot HCl$ and 12.5% NaOH solution was added, and the mixture was allowed to stand at 25° for 20 min. This reaction mixture was neutralized with dil. HCl, a precipitate thereby formed was removed, and the solution was evaporated to one-half the original volume. The residual solution was submitted to paper chromatography using Toyo Roshi No. 50 filter paper and BuOH-AcOH-H_2O (4:1:5) as the developing solvent, at 18~22°, with 16% aq. solution of $FeCl_3 \cdot 6H_2O$ as the coloring agent. A spot (Rf 0.49) of hydroxamic acid due to the acetyl group and that (Rf 0.63) of hydroxamic acid due to the propionyl group in (IX) were detected. Digitalinum verum hexaacetate (IV) and digitalinum verum hexapropionate (V) were respectively run as the standard substance.

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4) E. Bayer, K. H. Reuther : Angew. Chem., **68**, 698(1956); E. Angliker, F. Barfuss, J. Renz : Helv. Chim. Acta, **41**, 479(1958).

Summary

Digitalinum verum hexaacetate, hexapropionate, and monoacetyl pentapropionate were submitted to deacylation with potassium hydrogen carbonate under suitable conditions and these acyl derivatives were converted to diacyl derivatives possessing one acyl group each in the 16-position of gitoxigenin and in the digitalose portion. Some examinations were made on the properties of the newly formed derivatives.

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117. **Kazuo Miyatake, Atsuji Okano, Kazuhiko Hoji, Tōsaku Miki, and Akio Sakashita**: Studies on the Constituents of *Digitalis purpurea* L. XIV.[*1] 16-Acetyl and 16-Propionyl Derivatives of Digitalinum Verum.

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Partial deacetylation with snail enzyme was carried out on 16-acetyl- and 16-propionyl-digitalinum verum monoacetate, whose formation was described in the preceding paper,[*1] and 16-acetyl- and 16-propionyl-digitalinum verum were obtained successfully.

Various degrees of hydrolysis effected by snail enzyme has already been described in previous papers of this series.[1~3] This snail enzyme was applied to 16-acetyldigitalinum verum monoacetate (I) and the state of hydrolysis was followed through paper chromatography. It was thereby found, as indicated in Fig. 1, that there was no formation of digitalinum verum monoacetate by liberation of the acetyl group in 16-position and the majority turned into a new glycoside having different Rf value from that of anhydrodigitalinum verum, besides partial formation of digitalinum verum by complete removal of the two acetyl groups.

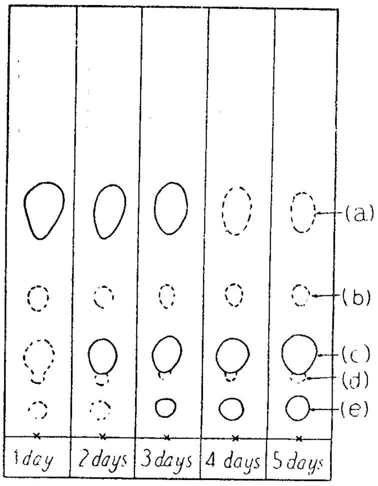

Fig. 1. Paper Partition Chromatography after Enzymatic Decomposition of 16-Acetyldigitalinum verum Monoacetate to 16-Acetyldigitalinum verum

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

(a)	16-Acetyldigitalinum verum monoacetate
(b)	Digitalinum verum monoacetate
(c)	16-Acetyldigitalinum verum
(d)	16-Anhydrodigitalinum verum
(e)	Digitalinum verum

[*1] Part XIII: This Bulletin, 7, 627 (1959).

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1) Part IV. A. Okano, *et al.* : This Bulletin, **5**, 167(1957).

2) Part VII. A. Okano : *Ibid.*, **5**, 279(1957).

3) Part IX. *Idem.* : *Ibid.*, **6**, 178(1958).