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.

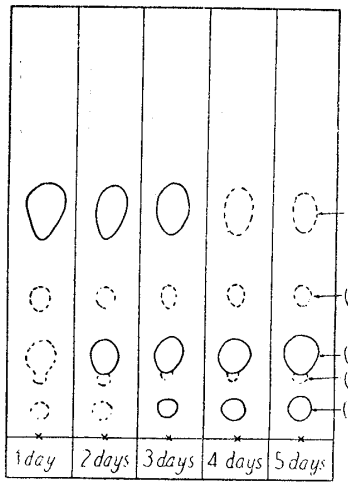

Fig. 1. Paper Partition Chromatography after Enzymatic Decomposition of 16-Acetyldigitalinum verum Monoacetate to 16-Acetyldigitalinum verum

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

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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).

This reaction product was isolated and purified through column partition chromatography but it failed to crystallize. This substance is easily soluble in water, methanol, ethanol, acetone, and ethyl acetate, soluble in chloroform, and almost insoluble in ether and benzene. $[\alpha]_D^{26}$ −21.1°(MeOH), UV : λ_{max}^{EtOH} 217 mμ (log ε 4.16). It is positive to the Frèrejacque reaction, its analytical values and determination of acetyl group indicate agreement with digitalinum verum monoacetate, and was assumed to be 16-acetyldigitalinum verum (IV). Its molecular optical rotation was compared with that of digitalinum verum (Table I) and its treatment with alumina converted it to anhydrodigitalinum verum

Table I. Comparison of Molecular Rotation (MeOH)

Substance	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$
Digitalinum verum	1.6°	11°	
16-Acetyldigitalinum verum	−21.1°	−159°	170°
Digitalinum verum	1.6°	11°	
16-Propionyldigitalinum verum	−23.4°	−180°	191°
Gitoxigenin	32.6°	127°	
16-Acetylgitoxigenin	− 8.4°	− 36°	163°
Gitoxigenin	32.6°	127°	
16-Propionylgitoxigenin	−11.1°	− 50°	177°

showing absorption maximum at 270 mμ in its ultraviolet spectrum. This anhydro compound was identified by paper chromatography with anhydrodigitalinum verum formed by enzymatic hydrolysis of anhydrodigitalinum verum monoacetate. This anhydro compound was also acetylated and derived to anhydrodigitalinum verum pentaacetate.[4] It is clear from these results that the new glycoside formed by hydrolysis with snail enzyme is 16-acetyldigitalinum verum. The presence of an acetyl group in the 16-position was further confirmed by the Mannich hydrolysis of the new glycoside in the cold, so as to prevent side-formation of gitoxigenin, to obtain 16-acetylgitoxigenin (oleandrigenin) (VI) (Fig. 2).

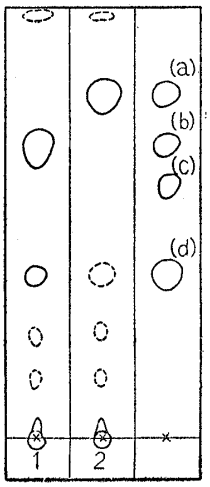
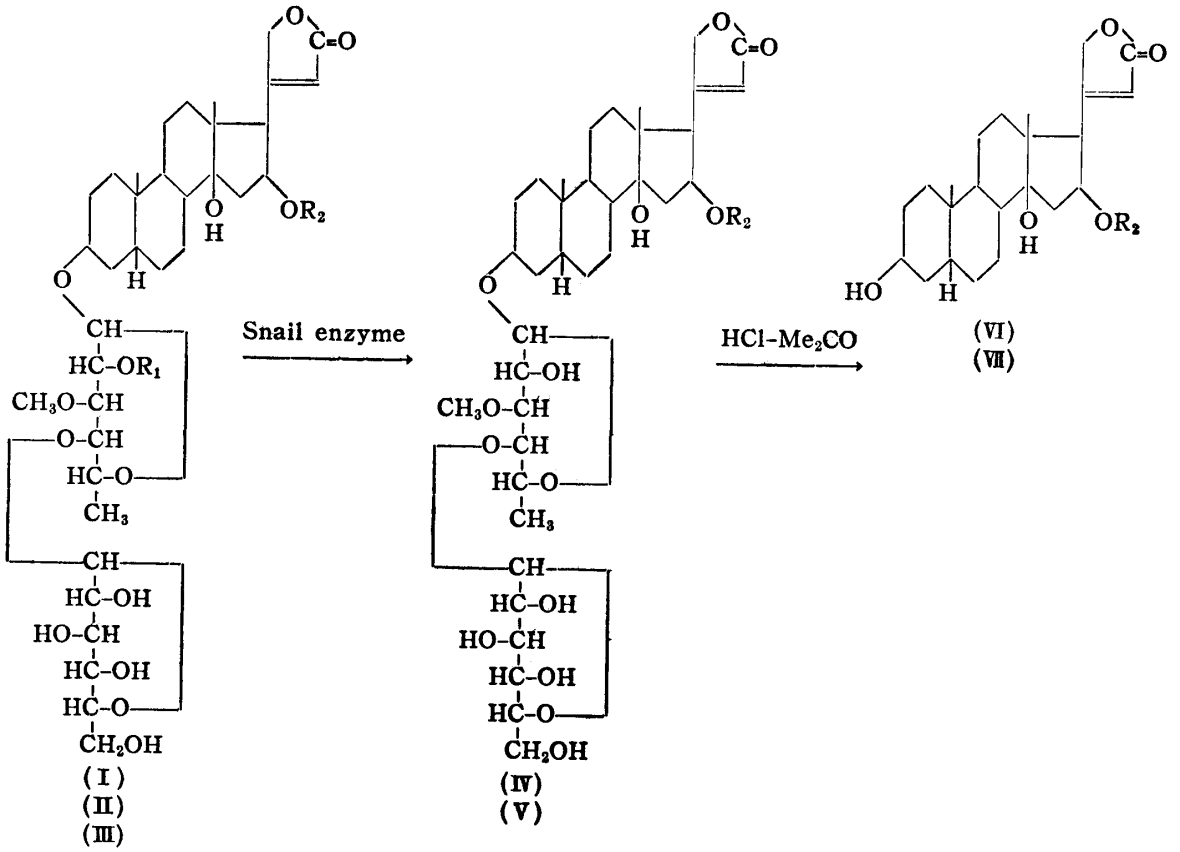


Fig. 2. Paper Partition Chromatography of Reaction Mixture of Mannich Hydrolysis

1. From 16-acetyldigitalinum verum
2. From 16-propionyldigitalinum verum
Toyo Roshi, No. 50; ascending method, at 18~22°.
Moving phase : Xylene-MeCOEt (1:1) saturated with formamide.
Stationary phase : Impregnated with formamide-Me_2CO (1:4).
Coloring agent : 20% $SbCl_3$-$CHCl_3$ solution.
(a) 16-Propionylgitoxigenin
(b) 16-Acetylgitoxigenin (oleandrigenin)
(c) 16-Anhydrogitoxigenin
(d) Gitoxigenin

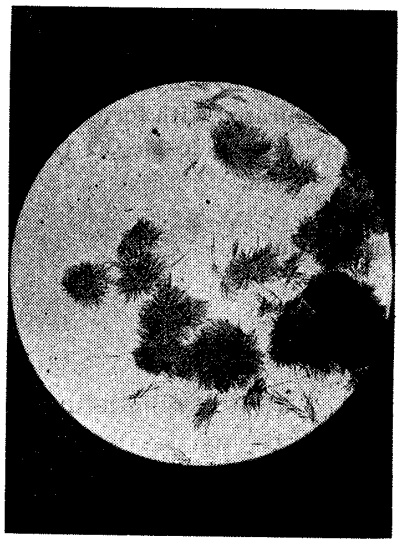
In accordance with the above-described formation of 16-acetyldigitalinum verum, 16-propionyldigitalinum verum monoacetate (III) was submitted to the same enzymatic hydrolysis and a new cardiotonic glycoside, 16-propionyldigitalinum verum (V) was obtained as needles, m.p. 166~172° (Fig. 3). Comparison of its molecular optical rotation (Table I) showed the same result as that of 16-acetyldigitalinum verum. The cold Man-

4) A. Hunger, T. Reichstein : Helv. Chim. Acta, 33, 96(1950).

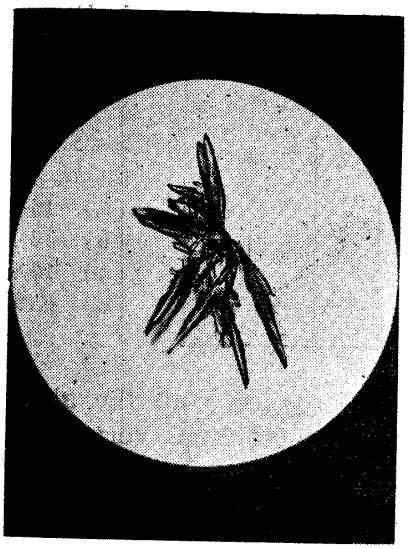


(I) (R_1=R_2=CH_3CO) 16-Acetyldigitalinum verum monoacetate
(II) (R_1=R_2=CH_3CH_2CO) 16-Propionyldigitalinum verum monopropionate
(III) (R_1=CH_3CO, R_2=CH_3CH_2CO) 16-Propionyldigitalinum verum monoacetate
(IV) (R_2=CH_3CO) 16-Acetyldigitalinum verum
(V) (R_2=CH_3CH_2CO) 16-Propionyldigitalinum verum
(VI) (R_2=CH_3CO) 16-Acetylgitoxigenin (oleandrigenin)
(VII) (R_2=CH_3CH_2CO) 16-Propionylgitoxigenin

Chart 1.



×80
Fig. 3. 16-Propionyldigitalinum verum



×30
Fig. 4. 16-Propionylgitoxigenin

nich hydrolysis of 16-propionyldigitalinum verum (V) afforded 16-propionylgitoxigenin (VII), m.p. 206~210° (Fig. 4) and comparison of its molecular rotation between (VII) and gitoxigenin was also made (Table I).

Further, 16-propionyldigitalinum verum monopropionate (II) was also submitted to enzymatic hydrolysis but the reaction hardly progressed and only a faint spot corresponding to that of 16-propionyldigitalinum verum (V) was detected by paper chromatography.

Liberation of glucose from 16-acetyl- (IV) and 16-propionyldigitalinum verum (V) was carried out with strophanthobiase*[3] isolated from the seeds of *Strophanthus kombé* and paper chromatographic examination revealed the presence of 16-acetyl- and 16-propionyl-strospeside formed by their conversion.

Tschesche[5] had pointed out the presence of 16-acetyldigitalinum verum in *Digitalis lanata* and reported that its separation from gitorin is difficult. Later, he denied its presence.[6] Reichstein had also anticipated the presence of 16-acetylstrospeside and its primary glycoside, 16-acetyldigitalinum verum, in *Adenium honghel* A. DC.[7] and *Ad. multiflorum*[8] but this was later denied.[9] Therefore, 16-acetylgitoxigenin digitalosides have not been obtained from natural source. A recent paper[10] reported the correction of the structure of rhodexin-B and -C to 16-acetylgitoxigenin rhamnoside and glucosido-rhamnoside, respectively, and another[11] reported the presence of acoschimperoside P, 16-acetylgitoxigenin L-acofrioside, and its primary glycoside, digluco-acoschimperoside P, in *Acokanthera Schimperi*. 16-Acetyldigitalinum verum obtained by the authors is the first example of the synthesis of 16-acetylgitoxigenin glycoside by partial acetylation of a glycoside possessing glucose, like digitalinum verum. Haack and others[12] obtained verodoxin and gitaloxin by respective partial formylation of strospeside and gitoxin.

It has also been found during the present series of experiments that the esterase in the snail enzyme had a tendency to effect a more rapid deacetylation of the acetyl in the digitalose portion rather than that in the 16-position and that this enzyme hardly produced anhydro compound which is usually formed by deacetylation of the 16-position with other enzymes.

The toxicity of 16-acetyl- and 16-propionyldigitalinum verum obtained as described above was tested by the kind offices of Dr. K.K. Chen*[4] and the result is presented in

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

Substance	Mean lethal dose mg./kg. (cat)
16-Acetyldigitalinum verum	0.255
16-Propionyldigitalinum verum	0.819
Digitalinum verum	1.332
Oleandrin	0.197
16-Deacetyloleandrin	0.300
Honghelosid A	0.387
16-Deacetylhonghelosid A	0.669

*[3] The authors are deeply indebted to Prof. T. Reichstein for giving them a large amount of the valuable enzyme.

*[4] 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.

5) R. Tschesche, G. Grimmer, F. Neuwald: Chem. Ber., **85**, 1103(1952).
6) R. Tschesche, G. Grimmer: *Ibid.*, **88**, 1569(1955).
7) A. Hunger, T. Reichstein: Helv. Chim. Acta, **33**, 76(1950).
8) *Idem.*: *Ibid.*, **33**, 1993(1950).
9) W. Rittel, A. Hunger, T. Reichstein: *Ibid.*, **35**, 434(1952).
10) H. Nawa, M. Uchibayashi: This Bulletin, **5**, 508(1958).
11) F. Thudium, O. Schindler, T. Reichstein: Helv. Chim. Acta, **42**, 2(1959).
12) E. Haack, F. Kaiser, H. Spingler: Naturwiss., **42**, 41(1955); Chem. Ber., **89**, 1353(1956).

Table II. These facts have proved the desired fortification of physiological activity by acylation of the 16-position. Toxicity tests of some of the 16-acetylgitoxigenin glycosides described to date in the literature are also listed in Table II and indicated that these compounds have stronger activity than the corresponding 16-hydroxylated glycosides. It is interesting that there is a large difference in the physiological activity in acetyl and propionyl groups.

The authors express their deep gratitude to Dr. Junzo Shinoda, the President of this Company, Mr. Sakan Hashimoto, the Director of this Laboratory, and to Dr. Masao Shimizu, the Acting Director of the same, for their kind guidance and encouragement during the course of this work, and for permission to publish this work. The authors are indebted to Messrs. B. Kurihara and K. Abe for analytical data.

Experimental*5

Enzymatic Decomposition of 16-Acetyldigitalinum verum Monoacetate (I)—An enzyme solution obtained by extracting 0.65 g. of snail enzyme powder with two 100-cc. portions of water was added to the solution of 2.47 g. of (I) dissolved in 2500 cc. of water, the mixture was covered with 100 cc. of toluene, and allowed to stand at 32°. After 5 days, (I) was almost completely hydrolyzed by the enzyme and formed 16-acetyldgitalinum verum (IV), as evidenced by paper chromatography (Fig. 1). The solution was concentrated to 50 cc. under reduced pressure, 400 cc. of EtOH was added to the residue, the precipitate thereby formed was removed, and the solution was further concentrated, affording 2.4 g. of a reaction product.

This residue was submitted to partition chromatography using 400 g. of a mixture (1:1) of Celite and water, with water-saturated mixture of MeCOEt and *iso*-BuCOMe (1:1) as the developing solvent, collecting in 100-cc. fractions. The fraction Nos. 5~12 (0.35 g.) afforded (I) and a small amount of digitalinum verum monoacetate, the fraction Nos. 13 and 14 (0.15 g.) gave (IV) and a small amount of digitalinum verum monoacetate, fraction Nos. 15~25 (1.15 g.) gave (IV), fraction Nos. 26~28 (0.10 g.) gave (IV) and a small amount of 16-anhydrodigitalinum verum, and the fraction No. 29 and later portions (0.25 g.) contained (IV), the anhydro compound, and digitalinum verum.

16-Acetyldigitalinum verum (IV)—The combined fraction Nos. 15~25 afforded (IV) as amorphous substance, freely soluble in H_2O, MeOH, EtOH, Me_2CO, and AcOEt, soluble in $CHCl_3$, and insoluble in Et_2O, benzene, and petr. ether. Positive to the Frèrejacque reaction. $[\alpha]_D^{26}$ −21.1°(c=1.33, MeOH). UV λ_{max}^{EtOH} 217 mμ (log ε 4.16). *Anal.* Calcd. for $C_{38}H_{58}O_{15}$: C, 60.46; H, 7.73; CH_3CO, 5.70. Found: C, 60.38; H, 7.96; CH_3CO, 5.40.

Reacetylation of (IV): The usual acetylation of 50 mg. of (IV) with Ac_2O and pyridine and recrystallization of the crude acetylated compound from Me_2CO-Et_2O afforded 50 mg. of digitalinum verum hexaacetate as needles, m.p. 170~175°/217~224°.

Formation of 16-Anhydrodigitalinum verum from 16-Anhydrodigitalinum verum Monoacetate—16-Anhydrodigitalinum verum monoacetate (255 mg.) was dissolved in a small amount of EtOH, 500 cc. of water was added to it, and EtOH was distilled off under reduced pressure as much as possible. An enzyme solution, prepared by extraction of 130 mg. of snail enzyme powder with two 25-cc. portions of water, and 20 cc. of toluene were added to the former solution and the mixture was allowed to stand at 32° for 3 days. The reaction mixture was treated as in enzymatic decomposition of (I) and 250 mg. of product consisting of 16-anhydrodigitalinum verum and a small amount of the starting material was obtained.

This product was submitted to partition chromatography using 120 g. of a mixture (1:1) of Celite and water, with water-saturated MeCOEt as the developing solvent, collecting in 25-cc. fractions. The fraction Nos. 5 and 6 (40 mg.) was the starting material, the fraction Nos. 7 and 8 (30 mg.) contained the starting material and 16-anhydrodigitalinum verum, and the fraction Nos. 9~16 (150 mg.) gave 16-anhydrodigitalinum verum alone. The combined fraction Nos. 9~16 was recrystallized from EtOH and EtOH-Et_2O mixture to 16-anhydrodigitalinum verum as colorless crystalline powder. This substance is soluble in MeOH and EtOH, and insoluble in water, $CHCl_3$, and Et_2O. Frèrejacque reaction, negative. $[\alpha]_D^{27}$ +41.0°(c=1.17, MeOH). *Anal.* Calcd. for $C_{36}H_{54}O_{13}$: C, 62.23; H, 7,83. Found: C, 62.29; H, 8.02. UV λ_{max}^{EtOH} mμ (log ε): 225(3.60), 270(4.22).

Treatment of 16-Acetyldigitalinum verum (IV) with Alumina—A solution of 200 mg. of (IV) dissolved in 10 cc. of MeOH:$CHCl_3$ (1:9) or dioxane:$CHCl_3$ (1:1) mixture was adsorbed on 40 g. of activated alumina and this was covered with benzene. After allowing this mixture to stand at room temperature for 7 days, the mixture was treated as above and a product consisting of (IV), 16-an-

*5 All m.p.s were measured on a Kofler block and are uncorrected.

hydrodigitalinum verum, and a small amount of digitalinum verum was obtained. This product was dissolved in 10 cc. of Me_2CO and 100 mg. of insoluble substance, chiefly consisting of the 16-anhydro compound, was obtained. This was submitted to partition chromatography using 50 g. of a mixture (1:1) of Celite and water, and water-saturated solution of MeCOEt—*iso*-BuCOMe (1:1) as the developing solvent, collecting in 50-cc. fractions. The fraction Nos. 9~17 afforded 35 mg. of 16-anhydrodigitalinum verum as colorless powder, negative to the Frèrejacque reaction. UV λ_{max}^{EtOH} mμ (log ε): 222(3.82), 270(4.18).

This product was acetylated with Ac_2O and pyridine and the crude acetylated compound was recrystallized from benzene-Et_2O mixture to 25 mg. of 16-anhydrodigitalinum verum pentaacetate as needles, m.p. 244~248°(decomp.). UV λ_{max}^{EtOH} mμ (log ε): 222(3.73), 270(4.26).

Formation of 16-Acetylgitoxigenin (Oleandrigenin) (VI) from 16-Acetyldigitalinum verum (IV)—A solution of 660 mg. of (IV) dissolved in 66 cc. of Me_2CO, added with 0.66 cc. of conc. HCl, was allowed to stand at 5° for 3 weeks. To this solution, 66 cc. of water was added, neutralized with $NaHCO_3$, and Me_2CO was evaporated under reduced pressure. The residual solution was extracted with two 50-cc. portions of $CHCl_3$, the extract was concentrated, and a product containing (VI), gitoxigenin, and unreacted starting material was obtained (Fig. 2).

In order to isolate (VI), this product was submitted to partition chromatography using 150 g. of Celite and 75 cc. of a mixture (2:1) of formamide and water as the stationary phase. The column was first eluted with 600 cc. of benzene and later elution with a mixture (9:1) of benzene and $CHCl_3$ afforded (VI). (VI) was collected and recrystallized from a mixture of Me_2CO, Et_2O, and petr. ether to 110 mg. of 16-acetylgitoxigenin (VI) as prisms, m.p. 226~229°. $[\alpha]_D^{20}$ −8.4°(c=1.23, MeOH). UV λ_{max}^{EtOH} 216 mμ (log ε 4.18). *Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 69.41; H, 8.39. Found: C, 69.68; H, 8.21.

Enzymatic Decomposition of 16-Propionyldigitalinum verum Monoacetate (III)—(III) (3.09 g.) was dissolved in a small amount of EtOH, 3000 cc. of water was added to it, and EtOH was distilled off as much as possible. An enzyme solution, prepared by extraction of 1.2 g. of snail enzyme powder with two 100-cc. portions of water, was added to the solution of (III), this was covered with 100 cc. of toluene, and the mixture was allowed to stand at 32° for 4 days. This reaction mixture was treated as in enzymatic decomposition of (I) and 3.0 g. of a reaction product, almost solely consisting of 16-propionyldigitalinum verum (V) was obtained. This product was submitted to partition chromatography using 1000 g. of a mixture (1:1) of Celite and water, and water-saturated MeCOEt as the developing solvent, collecting in 100-cc. fractions. The fraction Nos. 10~14 (2.16 g.) contained (V) alone.

In the paper chromatography used to date with water-saturated solution of MeCOEt—*iso*-BuCOMe (1:1), the spots of (V) and digitalinum verum monoacetate had approximately the same Rf values. In the paper chromatography carried out for confirmation of (V), a mixture of toluene-BuOH (1:1) saturated with formamide was used (Toyo Roshi No. 50, 18~22°) and the Rf values were 0.40 for (V) and 0.27 for digitalinum verum monoacetate.

16-Propionyldigitalinum verum (V)—The fraction Nos. 10~14 in the above chromatography was recrystallized from Me_2CO-petr. ether mixture and 1.75 g. of (V) was obtained as needles, m.p. 166~172; $[\alpha]_D^{25}$ −23.4°(c=1.42, MeOH). Frèrejacque reaction, positive. UV λ_{max}^{EtOH} 217 mμ (log ε 4.16). This substance is freely soluble in H_2O, MeOH, EtOH, and Me_2CO, soluble in $CHCl_3$, and insoluble in Et_2O and petr. ether. *Anal.* Calcd. for $C_{39}H_{60}O_{15}$: C, 60.92; H, 7.87. Found: C, 60.83; H, 8.02.

Repropionylation of (V): The usual propionylation of 50 mg. of (V) with propionic anhydride and pyridine, and recrystallization of a crude propionate from hydr. EtOH gave 40 mg. of digitalinum verum hexapropionate as needles, m.p. 137~142°.

Qualitative Estimation of the Propionyl in (V): The propionyl group in (V) was derived to hydroxamic acid by the method described in the preceding paper and the acid was submitted to paper chromatography with BuOH:AcOH:H_2O (4:1:5) for confirmation.

Formation of 16-Propionylgitoxigenin (VII) from 16-Propionyldigitalinum verum (V)—A solution of 870 mg. of (V) dissolved in 87 cc. of Me_2CO and added with 0.87 cc. of conc. HCl was allowed to stand at 5° for 4 weeks. This reaction mixture was treated as in the case of (IV) and a reaction product consisting of (VII), gitoxigenin, and unreacted starting material was obtained (Fig. 2). This product was submitted to partition chromatography as in the isolation of (VI) and the fraction eluted with a mixture (9:1) of benzene and $CHCl_3$ was collected. The residue from this fraction was recrystallized from a mixture of Me_2CO, Et_2O, and petr. ether affording 180 mg. of 16-propionylgitoxigenin (VII) as prisms, m.p. 206~210°. $[\alpha]_D^{21}$ −11.1°(c=1.35, MeOH). UV λ_{max}^{EtOH} 216 mμ (log ε 4.17). *Anal.* Calcd. for $C_{26}H_{38}O_6$: C, 69.93; H, 8.58. Found: C, 69.65; H, 8.74.

Enzymatic Decomposition of 16-Propionyldigitalinum verum Monopropionate (II)—(II) (50 mg.) was dissolved in a small amount of EtOH, 50 cc. of water was added, and EtOH was distilled off as much as possible. An enzyme solution, prepared by extraction of 30 mg. of snail enzyme powder with two 4-cc. portions of water, was added to the foregoing solution of (II), 2 cc. of toluene was added, and the mixture was allowed to stand at 32° for 5 days. This was treated as in the case of

(I) and a reaction product consisting of unreacted (II) and a small amount of (V) was obtained.

This product was submitted to partition chromatography with a mixture (1:1) of Celite and water, and water-saturated *iso*-BuCOMe as the developing solvent. The fractions containing (II) were collected and the residue was recrystallized from MeOH-Et_2O to 35 mg. of (II) as needles, m.p. 166~171°.

Enzymatic Decomposition of (IV) and (V) with Strophanthobiase—Enzymatic decomposition of (IV) and (V) was carried out by adding 20 mg. of strophanthobiase to each solution of 7 mg. of (IV) or 10 mg. of (V) dissolved in 10 cc. of distilled water, 2 drops of toluene, and the mixture allowed to stand at 32° for 5 days. The solutions were treated as in the case of (I) and the product was examined by paper chromatography. The decomposition product from (IV) and (V) respectively gave spots at Rf 0.19 and 0.32, against Rf 0.05 for the control strospeside. It was assumed that the spot of Rf 0.19 is that of 16-acetylstrospeside and that of 0.32, 16-propionylstrospeside.

Summary

Deacetylation with snail enzyme was carried out on 16-acetyl- and 16-propionyldigitalinum verum monoacetates and the corresponding 16-acetyl- and 16-propionyldigitalinum verum were obtained. Characteristics of these compounds were examined and it was found that the toxicity of 16-acetyldigitalinum verum was far higher than that of the original monoacetate.

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