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156. Kazuo Miyatake, Atsuji Okano, Kazuhiko Hoji, Tōsaku Miki, and Akio Sakashita: Studies on the Constituents of *Digitalis purpurea* L. XVI.*1 On the Synthesis of 16-Acetyl-strospeside.

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In the previous papers,[1,2] it was reported that 16-acetyl-digitalinum verum was produced from digitalinum verum and the former has stronger activity than the latter. In the present investigation attempt was made to synthesize 16-acetyl-strospeside from strospeside to confirm the effect of acetylation of 16-position on its toxicity. Recently, Reichstein and his co-workers[3] reported that a new glycoside, neritaloside, was isolated from the seeds of *Nerium oleander* L. and it corresponds to 16-acetyl-strospeside, but the present authors obtained the same compound by synthesis from strospeside.

As the procedures for the synthesis of 16-acetyl-strospeside, three methods may be considered; a) enzymatic hydrolysis of 16-acetyl-digitalinum verum, b) selective deacetylation of the acetyl groups other than that in the 16-position of strospeside triacetate, and c) selective acetylation of the 16-position alone under a suitable condition. In the present work the last method was adopted.

The selection of a suitable condition of acetylation could be followed through paper chromatographic analysis, as the spot of 16-acetyl-strospeside,[2] which was formed from 16-acetyl-digitalinum verum by liberation of glucose with strophanthobiase, was detected on a paper chromatogram (Fig. 1). Pyridine and acetic anhydride were used as acetylation agents and reaction temperature was fixed at 30°. On the paper chromatogram, an unknown spot was recognized other than those of 16-acetyl-strospeside, strospeside monoacetate, and strospeside. The compound corresponding to this new spot was named substance-A (Fig. 2). A period of eight hours was suitable for this reaction and application of 2.5 moles of acetic anhydride per one mole of strospeside gave 16-acetyl-strospeside

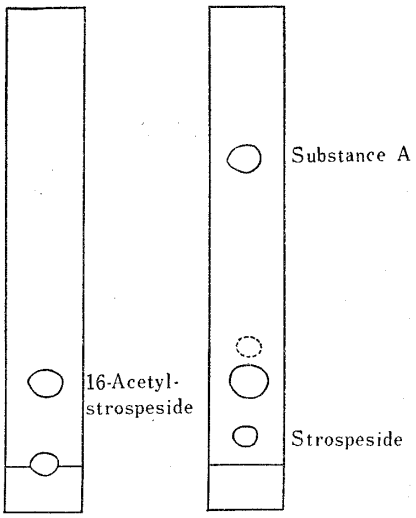

Fig. 1.

Fig. 2.

Fig. 1. Paper Partition Chromatography after Decomposition of 16-Acetyl-digitalinum verum by Strophanthobiase

Fig. 2. Paper Partition Chromatography of Reaction Mixture of Partial Acetylation of Strospeside

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

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1) Part XIII. A. Okano, *et al.*: This Bulletin, **7**, 627(1959).
2) Part XIV. A. Okano, *et al.*: *Ibid.*, **7**, 634(1959).
3) H. Jäger, O. Schindler, T. Reichstein: Helv. Chim. Acta, **42**, 977(1959).

in an excellent yield. When less than 2 moles of acetic anhydride was used, the greater part of strospeside remained unreacted, but when more than 3 moles were used the yield of 16-acetyl-strospeside rather decreased with the increased formation of substance-A.

Strospeside was therefore acetylated under the above fixed conditions. The reaction product was submitted to column partition chromatography through Celite 535, with water-formamide (1:2) as the stationary phase and benzene-chloroform mixture as the developing solvent, and 16-acetyl-strospeside and substance-A were isolated.

16-Acetyl-strospeside recrystallized from dioxane-ether as needle crystals, m.p. 135~143°, $[\alpha]_D^{30}$ −11.3°(MeOH), UV: λ_{max}^{EtOH} 217 mμ (log ε 4.18), soluble in water, methanol, ethanol, and acetone, and insoluble in ether and benzene. It is positive to the Frèrejacque reaction of the acetyl group. Its analytical values agreed well with the theoretical values for formula $C_{32}H_{48}O_{10}\cdot C_4H_8O_2$, calculated for monoacetyl-strospeside with one mole of dioxane. This dioxane would have been picked up from recrystallization solvent. The quantitative determination of acetyl group indicated agreement with the theoretical value.

The Mannich hydrolysis of the compound was carried out in cold storage and formation of oleandrigenin was shown by paper chromatography (Fig. 3). The difference of

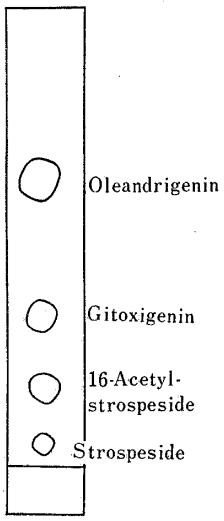

Fig. 3. Paper Partition Chromatography of Reaction Mixture of the Mannich Hydrolysis
(The same solvent system and coloring reagent as in Fig. 1)

molecular rotation between 16-acetyl-strospeside and strospeside was compared with that between 16-acetyl-digitalinum verum and digitalinum verum, and between oleandrigenin and gitoxigenin. This agreed with 16-acetyl contribution of molecular rotation (Table I).

Table I. Comparison of Molecular Rotation Difference

	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$
Strospeside	15.5°	85°	−162°
16-Acetyl-strospeside	−11.3°	− 77°	
Digitalinum verum	1.6°	11°	−170°
16-Acetyldigitalinum verum	−21.1°	−159°	
Gitoxigenin	32.6°	127°	−163°
Oleandrigenin	− 8.4°	− 36°	

Table II. Toxicity by Pigeon Method

	Mean lethal dose (mg./kg.)	Mole ($\times 10^{-6}$)
16-Acetyl-strospeside	0.4652	0.68
Strospeside	0.7785	1.4
16-Acetyldigitalinum verum	0.5008	0.66
Digitalinum verum	2.543	3.4

16-Acetyl-strospeside here obtained was identified with neritaloside*[3] by mixed fusion and paper chromatographic analysis.

The toxicity of 16-acetyl-strospeside was tested by the pigeon method J. P.*[4] and the result is presented in Table II. The anticipated fortification of physiological activity is obtained by acetylation of the 16-position.

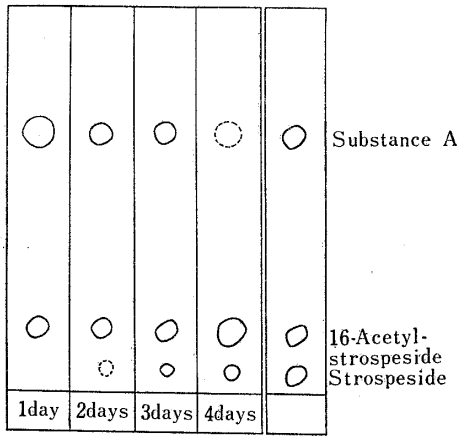

Fig. 4. Paper Partition Chromatography after Enzymatic Decomposition of Substance-A

(The same solvent system and coloring reagent as in Fig. 1)

Substance-A was recrystallized from acetone-ether as flat needles, m.p. 146~150°/213~218°, soluble in methanol, ethanol, and acetone, and insoluble in ether and benzene; $[\alpha]_D^{30}$ −12.0° (MeOH), UV: λ_{max}^{EtOH} 217 mμ (log ε 4.18). It is positive to the Frèrejacque reaction. Its analytical values and determination of acetyl groups agreed with theoretical values of strospeside diacetate.

Partial deacetylation of substance-A by snail enzyme was carried out and paper chromatographic analysis revealed the formation of 16-acetyl-strospeside (Fig. 5). On the basis of result of this partial deacetylation, it seems possible that 16-acetyl-strospeside is produced from substance-A.

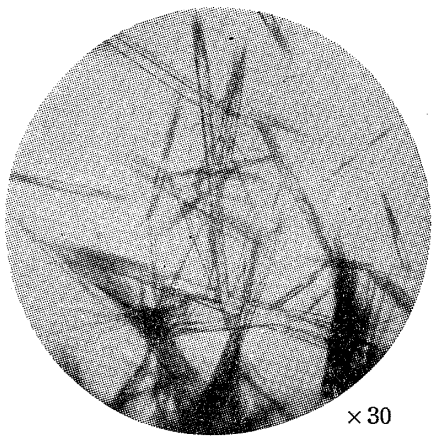

Fig. 5. 16-Acetyl-strospeside

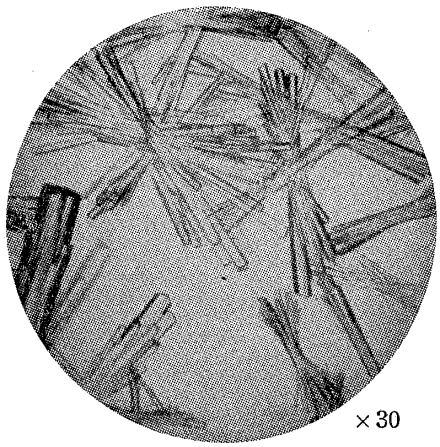

Fig. 6. Substance-A

From the result of paper chromatographic analysis and partial deacetylation by enzyme, it seems reasonable to assume that substance-A is a strospeside diacetate.

*3 Grateful acknowledgement is made to Dr. T. Reichstein for kind donation of a valuable sample of neritaloside.

*4 Japanese Pharmacopoeia, Ed. VI (Supplement).

Experimental*5

Formation of 16-Acetyl-strospeside (II) and Substance-A (III) from Strospeside (I)—To a solution of 1.266 g. of (I) dissolved in 30 cc. of pyridine, 0.55 cc. of Ac_2O, at the rate of 2.5 moles per 1 mole of (I), was added and the mixture was allowed to stand at 28~30° for 8 hr. The reaction mixture was diluted with 250 cc. of water and extracted with five 150-cc. portions of $CHCl_3$. The combined $CHCl_3$ extract was washed twice with water and evaporated at below 55°, affording 1.75 g. of a residue. The residue was submitted to partition chromatography through 200 g. of Celite 535, with 200 cc. of water-formamide (2:1) mixture as the stationary phase, and benzene, benzene-$CHCl_3$ mixture (97:3) 300 cc., (9:1) 500 cc., (7:3) 500 cc., (1:1) 500 cc., (1:3) 500 cc., and $CHCl_3$ as the developing solvent, collecting in 50-cc. fractions. The fraction Nos. 23~33, developed with benzene-$CHCl_3$ (7:3) mixture, gave (III) and the fraction Nos. 48~51, developed with benzene-$CHCl_3$ (1:3) mixture gave (II).

16-Acetyl-strospeside (II)—To the residue from fraction Nos. 48~51, 30 cc. of water was added and extracted with five 15-cc. portions of $CHCl_3$-EtOH (19:1) mixture to remove formamide. The extract was washed with water and evaporated to 0.67 g. of a residue, which was recrystallized from dioxane-Et_2O to 0.473 g. of needles, m.p. 135~143°. This substance is soluble in water, MeOH, EtOH, and Me_2CO, and insoluble in Et_2O and benzene. $[\alpha]_D^{30}$ −11.3° (c=1.50, MeOH), UV: λ_{max}^{EtOH} 217 mμ (log ε 4.18). *Anal.* Calcd. for $C_{32}H_{48}O_{10}$: C, 64.82; H, 8.19; CH_3CO, 7.50. Calcd. for $C_{32}H_{48}O_{10}\cdot C_4H_8O_2$: C, 63.51; H, 8.29; CH_3CO, 6.32 Found : C, 63.72; H, 8.27; CH_3CO, 6.00.

It showed no depression of m.p. on admixture with neritaloside, m.p. 135~142°, and further identified with neritaloside by paper chromatography (Rf. 0.16).

Substance-A (III)—To the residue from fraction Nos. 28~33, 30 cc. of water was added and extracted with five portions of $CHCl_3$ to remove formamide. The extract was washed with water and evaporated to 0.33 g. of a residue. It was recrystallized from Me_2CO-EtOH and 0.164 g. of flat needles, m.p. 146~150°/213~218°, was obtained. This substance is soluble in MeOH, EtOH, and Me_2CO, and insoluble in Et_2O and benzene. $[\alpha]_D^{30}$ −12.0 (c=1.50, MeOH), UV: λ_{max}^{EtOH} 217 mμ (log ε 4.18). *Anal.* Calcd. for $C_{34}H_{50}O_{11}$: C, 64.33; H, 7.94; CH_3CO, 13.57. Calcd. for $C_{34}H_{50}O_{11}\cdot 1\frac{1}{2}H_2O$: C, 61.74; H, 8.08; CH_3CO, 13.02. Found : C, 61.75; H, 8.34; CH_3CO, 13.34.

Mannich Hydrolysis of (II)—A solution of 10 mg. of (II) dissolved in a mixture of 12 cc. of Me_2CO and 0.12 cc. of conc. HCl was allowed to stand at 5° for 16 days. The reaction mixture was submitted to paper partition chromatography, using a mixture of MeCOEt-xylene (1:1) saturated with formamide on a formamide-impregnated paper, and spots corresponding to oleandrigenin, gitoxigenin, (II), and (I) were detected.

Enzymatic Decomposition of Substance-A (III)—To a solution of 10 mg. of (III) dissolved in 1 cc. of MeOH, 25 cc. of water was added and MeOH was removed under a reduced pressure. An enzyme solution prepared from 5 mg. of snail enzyme was added to this solution, together with 1 cc. of toluene, and the mixture was allowed to stand at 32°. After 1, 2, 3, and 4 days, a portion of the reaction mixture was extracted with $CHCl_3$-EtOH (19:1) mixture. Each extract was evaporated and each residue was submitted to paper chromatography, using the solvent system described above (Fig. 4).

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Summary

16-Acetyl-strospeside and substance-A were produced by partial acetylation of strospeside. 16-Acetyl-strospeside was identified with neritaloside which had been isolated from the seeds of *Nerium oleander* L. by Dr. T. Reichstein. Substance-A was proved to be diacetyl derivative of strospeside possessing one acetyl group in the 16-position of gitoxigenin.

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