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Studies on Digitalis Glycosides. XXXV.[1] Diels-Alder Type Reaction of 16,17-Dehydrodigitoxigenin 3-Acetate. (1). Dimerization[2]

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Dimerization of 16,17-dehydrodigitoxigenin 3-acetate (I) and its 21*R*- and 21*S*-ethoxy derivatives (II and III) was found to occur on heating the monomers in toluene. This dimerization appeared to take place between the 16,20(22)-diene system of one monomer and the 16,17-double bond of another monomer by a Diels-Alder type reaction, giving the dimers IV, V, and VI, respectively. Ultraviolet irradiation of a chloroform solution of II also gave the dimer V, but UV irradiation of I and III was ineffective. The structures of these dimers were established on the basis of their molecular weights, spectral data, and the properties of their dianhydro derivatives.

Keywords——16,17-dehydrodigitoxigenin 3-acetate; 16,17-dehydro-21*R*-ethoxydigitoxigenin 3-acetate; 16,17-dehydro-21*S*-ethoxydigitoxigenin 3-acetate; dimerization; Diels-Alder type reaction; ^{13}C-NMR

In the course of studies on the chemical transformation of gitoxigenin 3-acetate to digitoxigenin 3-acetate,[1] we observed the dimerization of 16,17-dehydrodigitoxigenin (16-anhydrogitoxigenin) derivatives on heating in less polar solvents. This dimerization might occur between the conjugated dienolactone systems of two monomer molecules by a Diels-Alder type reaction; such diene syntheses of cardenolide derivatives have not previously been reported in the literature. The present paper describes these results.

When 16,17-dehydrodigitoxigenin 3-acetate (I) was heated in toluene at 115—120° for 180 hr in a closed test tube under nitrogen, compound IV, mp 179—182°, $C_{50}H_{68}O_{10}\cdot 2H_2O$ was formed as a main product[4] and was isolated by multiple development TLC (SiO_2, benzene; ether; $CHCl_3$=2: 2: 1). The molecular weight and integration values of hydrogen in the proton nuclear magnetic resonance (^{1}H–NMR) spectrum proved that this product is a dimer of I. Spectral data are listed in Tables I, II, and III. In the ultraviolet (UV) spectrum of the dimer (IV), an absorption maximum at 217 nm was observed in place of that at 270 nm due to the 16,20(22)-conjugated dienolactone system of the monomer (I), and the infrared (IR) spectrum of IV showed absorptions at 1760 and 1610 cm^{-1} due to the cardenolide ring.[5] In the ^{1}H–NMR spectrum of IV, the vinyl proton signal due to 16-H of I, appearing as a triplet at δ 6.12, was absent. These data suggested that the 16,17-double bond rather than the 20(22)-double bond in the cardenolide ring had reacted as a dienophile. On the other hand, the proton signal (δ 5.95) due to 22-H in I appeared as separate signals at δ 4.06 and 6.04 in IV. The higher field shift of one 22-proton signal suggested that C-22 of one molecule of the monomer (I) also participates in the dimerization, with saturation

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[1] Part XXXIV: T. Hashimoto, H. Shibahara, K. Toyooka, and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **25**, 2468 (1977).

[2] A part of this work was reported at a Meeting of the Chugoku-Shikoku Branch, Pharmaceutical Society of Japan, October 29, 1978.

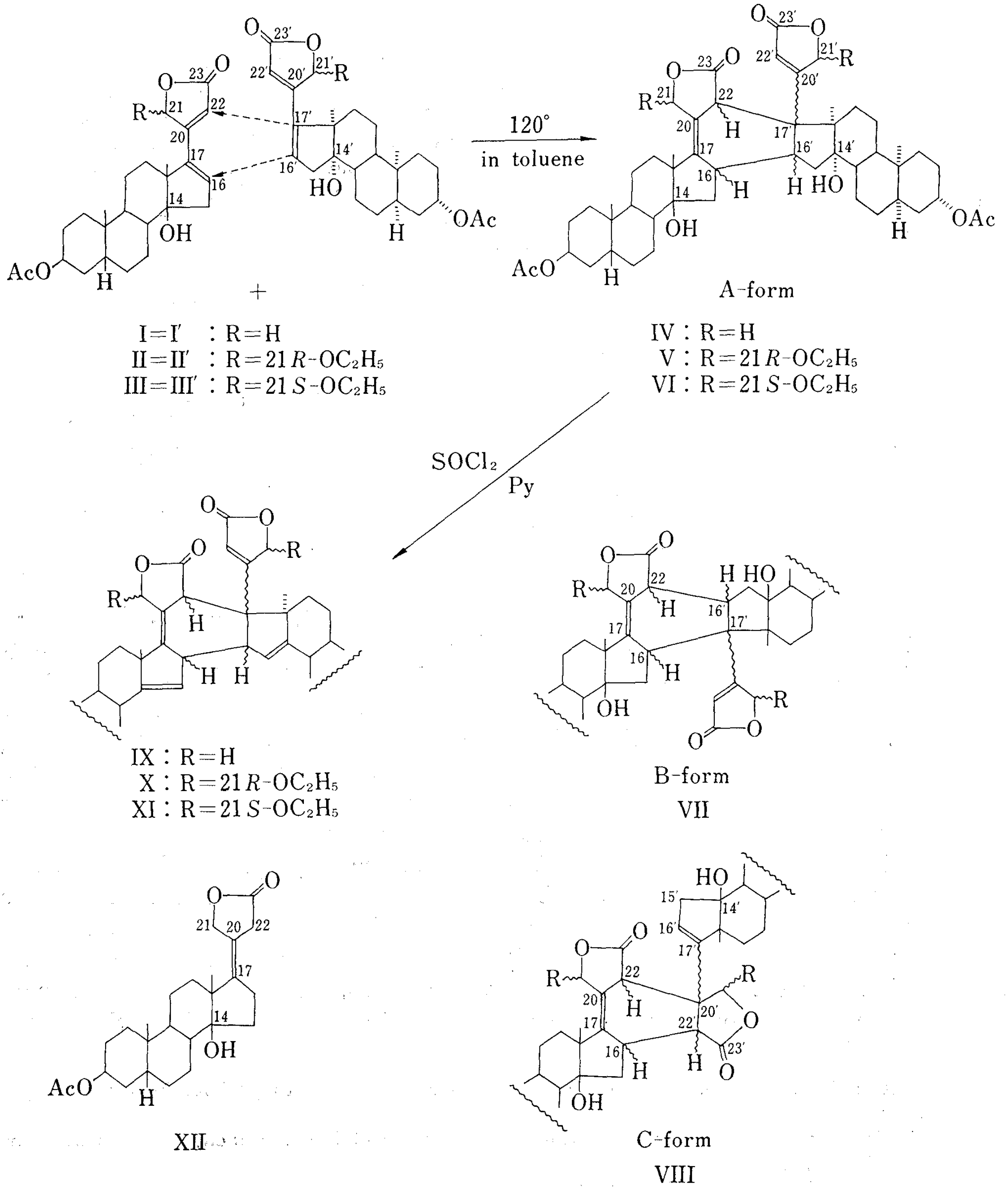
[3] Location: *Yamashiro-cho, Tokushima, 770, Japan.*

[4] An epimeric dimer was obtained as a minor by-product, as will be reported in a succeeding paper.

[5] R.N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

[6] Redrawn form of formula I, as shown in Chart 1.

of the 20(22)-double bond, while that of the other monomer (I′)[6] remains intact. Based on these spectral data, dimerization was considered to take place between the 16,20(22)-diene moiety of the monomer (I) and the 16,17-double bond of another monomer (I′) by a Diels-Alder type reaction to give a dimer, IV (A-form) or VII (B-form). Since the proton signal due to 22-H of the reaction product (IV) was a singlet, the B-form was excluded (this form should exhibit a doublet). Another type of dimer, VIII (C-form), which might be formed if the 20(22)-double bond of I′ reacts as a dienophile, was also excluded on the basis of the above spectral data and formation of the dianhydro derivative of IV. Thus, the dianhydro derivative (IX), mp 247—250°, $C_{50}H_{64}O_8$, did not show an absorption maximum (262 nm)



I=I′ : R=H
II=II′ : R=21*R*-OC_2H_5
III=III′ : R=21*S*-OC_2H_5

IV : R=H
V : R=21*R*-OC_2H_5
VI : R=21*S*-OC_2H_5

IX : R=H
X : R=21*R*-OC_2H_5
XI : R=21*S*-OC_2H_5

VII

XII

VIII

Chart 1

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7) R. Tschesche and R. Peterson, *Chem. Ber.*, **86**, 578 (1953).

due to a 14,16-diene system[7] which would be expected for the dianhydro derivative of the C-form dimer (VIII).

The structure of IV was further supported by the ^{13}C–NMR spectral data. Thus, the chemical shifts of C-17 and C-20 are comparable to those of card-17(20)-enolide[8] (XII) previously reported by our group, which confirms the presence of a 17(20)-double bond in the dimer. Furthermore, the chemical shifts due to C-14, C-14′, C-20′, C-21, C-21′, and C-22′[9] are also consistent with the structure of IV.[10] 21*R*-Ethoxy-16,17-dehydrodigitoxigenin 3-acetate[1] (II) and the 21*S*-epimer[1] (III) also gave the corresponding dimers (V), mp 162—163°,

Table I. UV and IR Spectral Data

Compds.	λ_{max}^{EtOH} nm (log ε)	ν_{max}^{KBr} cm^{-1} OH	C=O	C=C
IV	217(4.20)	3500	1760, 1740	1610
V	213(4.07)	3525	1770, 1740	1610
VI	212(4.10)	3525	1770, 1740	1620
IX	214(4.21)		1765, 1730	1620
X	211(4.28)		1780, 1740	1620
XI	210(4.22)		1770, 1740	1610

Table II. ^{1}H–NMR Spectral Data

Compounds		Chemical shifts (CDCl$_3$, δ) 22-H	22′-H	21-H	21′-H	16′-H
	IV	4.06(s)	6.04(s)	4.80(m)	4.80(m)	
Dimer	V	4.21(s)	6.26(s)	5.66(s)	5.66(s)	
	VI	3.91(s)	6.01(s)	5.71(s) or 5.67(s)	5.67(s) or 5.71(s)	
	I		5.95(s)		4.95(s)	6.12 (t, J=2.5 Hz)
Monomer	II		5.98(s)		5.95(s)	6.27 (t, J=2.5 Hz)
	III		5.97(s)		5.97(s)	6.48 (t, J=2.5 Hz)

Table III. ^{13}C–NMR Spectral Data

Compounds	Chemical shifts (CDCl$_3$, ppm) C-14	C-14′	C-17	C-20	C-20′	C-21	C-21′	C-22	C-22′
IV	87.5 or 87.0	87.0 or 87.5	123.9 or 117.7	149.0	173.8	67.1	76.7	65.3	117.7 or 123.9
V	87.7 or 87.0	87.0 or 87.7	125.0 or 125.6	155.3	170.1 or 169.8	100.4	104.1	64.4	125.6 or 125.0
VI	87.9 or 86.9	86.9 or 87.9	125.1 or 120.7	155.5	171.3 or 170.6	99.4	106.8	66.4	120.7 or 125.1
XII	86.2		119.0	145.9		70.3		33.7	

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[8] D. Satoh and T. Hashimoto, *Chem. Pharm. Bull.* (Tokyo), **24**, 1950 (1976).

[9] A tentative numbering of the carbon atoms of the dimer is given in Chart 1.

[10] K. Tori, H. Ishii, Z.W. Wolkowski, C. Chachaty, M. Sangare, F. Piriou, and G. Lukacs, *Tetrahedron Lett.*, **1973**, 1077.

$C_{54}H_{76}O_{12}\cdot H_2O$ and (VI), mp 298—300°, $C_{54}H_{76}O_{12}$, respectively, by reactions similar to that of I. The structures of these dimers were established to be as shown in Chart 1 on the basis of analytical and spectral data as well as the properties of the dianhydro derivatives (Tables I, II, and III), in the same way as for IV.[11] Thus, molecular weight determination showed that V and VI correspond to dimers of II and III, respectively, and spectral data proved that this dimerization proceeded in a similar way to that of I. Stereochemical analysis of these dimers is now in progress.

This type of dimerization was found to take place in sunlight as well as on UV irradiation of a chloroform solution of II to form V, but the dimerizations of I and III did not proceed in the same manner. The mechanism of this photodimerization has not yet been clarified.

Experimental[12]

16,17-Dehydrodigitoxigenin 3-Acetate (I)[13]——A mixture of gitoxigenin (20 g), anhydrous AcONa (7 g) and dry dimethylformamide (DMF, 500 ml) was heated at 95—100° under nitrogen with stirring for 5 hr in an oil bath. After cooling, AcONa was filtered off and the filtrate was concentrated to dryness *in vacuo*. The residue was digested with $CHCl_3$ (200 ml) and cooled in refrigerator overnight. Crystalline precipitates were collected by filtration, washed with ether and dried *in vacuo*. The crude product (10.3 g) thus obtained was acetylated with dry pyridine (50 ml) and Ac_2O (50 ml) in the usual manner and the crude acetate (10.2 g) was recrystallized from MeOH to give I (8.4 g) as colorless plates, mp 198—202°.

Dimer (IV) Formation from 16,17-Dehydrodigitoxigenin 3-Acetate (I)——A mixture of I (500 mg) and toluene (0.5 ml) was heated at 115—120° for 180 hr in a closed test tube under nitrogen in an oil bath. TLC (SiO_2, benzene: ether: $CHCl_3$=2: 2: 1) of the reaction solution showed the formation of a main product as well as a small amount of by-product and unchanged material. After triply developed preparative TLC using the same system, the main fraction (less polar, 291 mg) was collected and recrystallized from AcOEt to give IV (236 mg) as colorless prisms, mp 179—182°, $[\alpha]^{24}_{D}$ −24.0° (c=0.50, $CHCl_3$). *Anal.* Calcd. for $C_{50}H_{68}O_{10}\cdot 2H_2O$: C, 69.42; H, 8.39; Mol. Wt., 865.07. Found: C, 69.32; H, 8.34; Mol. Wt., 917.4. By-product (more polar, 25 mg) and unchanged material I (most polar, 96 mg) were also recovered.

Dimer (V) Formation from 21R-Ethoxy-16,17-dehydrodigitoxigenin 3-Acetate (II)——(a) Heating Method: A mixture of II (600 mg) and toluene (0.4 ml) was heated at 120—130° for 208 hr in a closed test tube under nitrogen. The reaction product was subjected to triply developed preparative TLC (SiO_2, benzene: ether: $CHCl_3$=4: 1: 1). The less polar fraction (217 mg) was collected and recrystallized from ether to give V (174 mg) as colorless needles, mp 162—163°, $[\alpha]^{24}_{D}$ −67.7° (c=0.50, $CHCl_3$), *Anal.* Calcd. for $C_{54}H_{76}O_{12}\cdot H_2O$: C, 69.35; H, 8.34; Mol. Wt., 935.2. Found: C, 69.41; H, 8.40; Mol. Wt., 904.0. The more polar fraction (330 mg) was collected and recrystallized from AcOEt-ligroin to give unchanged material (II, 264 mg) as colorless plates, mp 190—193.5°.

(b) Sunlight Method: A solution of II (800 mg) in $CHCl_3$ (1 ml) was exposed to sunlight at room temperature for 36 days in a closed test tube under nitrogen. TLC using the same system as in method (a) showed that a product was formed in about 1: 1 ratio with respect to unchanged material. The product was isolated by triple-deveopment preparative TLC (SiO_2, benzene: ether=2: 1) and the crude fraction (331 mg) was recrystallized from AcOEt to give V (143 mg) as colorless needles, mp 161—162°. Mixed melting point and comparison of IR spectra with the sample obtained by method (a) showed that the compounds were identical.

(c) Mercury Lamp Irradiation Method: A solution of II (200 mg) in $CHCl_3$ (0.5 ml) was irradiated with a high-pressure mercury lamp (12W) at room temperature in a closed test tube under nitrogen for 50 hr. The reaction solution was subjected to preparative TLC as in method (a) and the less polar fraction (126 mg) was recrystallized from AcOEt–ether to afford V (74 mg) as colorless needles, mp 162—163°. This product was identical with that obtained in method (a) by mixed melting point test and IR spectral comparison. From the more polar fraction (56 mg), unchanged material II (44 mg), mp 188—191°, was recovered as colorless plates after recrystallization.

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[11] The chemical shifts of 21-H, 21′-H, C-21, C-21′ of V and VI are shifted compared to those of IV due to the effect of the ethoxyl substituents.

[12] All melting points are uncorrected. Specific rotations were measured with a Jasco DIP-180 digital polarimeter. UV spectra were recorded on a Shimadzu UV-300 spectrophotometer, IR spectra on a Shimadzu IR-27G spectrometer, ^1H-NMR spectra on a Varian HA-100 spectrometer at 100 MHz and ^{13}C-NMR on a Varian NV-14FT NMR spectrometer at 15.087 MHz. Merck Silica Gel PF_{254} was used for preparative TLC.

[13] J.H. Russel, O. Schindler, and T. Reichstein, *Helv. Chim. Acta*, 43, 167 (1960).

Dimer (VI) Formation from 21S-Ethoxy-16,17-dehydrodigitoxigenin 3-Acetate (III)——A mixture of III (600 mg) and toluene (0.4 ml) was heated at 120—130° for 208 hr in a closed test tube under nitrogen. The reaction product was recrystallized from acetone to afford VI (281 mg) as a colorless crystalline powder, mp 297—300°, $[\alpha]_D^{24}$ +38.1° (c=0.50, $CHCl_3$), *Anal.* Calcd. for $C_{54}H_{76}O_{12}$: C, 70.71; H, 8.35; Mol. Wt., 917.2. Found: C, 70.49; H, 8.52; Mol. Wt., 930.0. The mother liquor was subjected to triple-development preparative TLC (SiO_2, benzene: ether: $CHCl_3$=1: 1: 1), yielding two fractions. The less polar fraction (69 mg) was recrystallized from acetone to give a second crop of VI (36 mg), mp 297—300°, and the more polar fraction (126 mg) gave unchanged III (83 mg), mp 206—208°, after recrystallization from AcOEt–*n*-hexane.

Dianhydro Derivative (IX) from the Dimer (IV)——A solution of IV (100 mg) in dry pyridine (2 ml) was chilled at −15° with ice-NaCl mixture in a desiccator and a solution of $SOCl_2$ (0.6 ml) in dry pyridine (1 ml) was added dropwise with stirring. After stirring for 1 hr at the same temperature, excess $SOCl_2$ was decomposed with ice and the precipitate was collected by filtration and dried *in vacuo*. This reaction was repeated four more times and the total crude product (444 mg) was subjected to preparative TLC (SiO_2, benzene :ether: $CHCl_3$=10: 1: 2). The main fraction was recrystallized from $CHCl_3$–MeOH to give IX (240 mg) as colorless crystals, mp 247—250°, *Anal.* Calcd. for $C_{50}H_{64}O_8$: C, 75.73; H, 8.13. Found: C, 75.59; H, 8.28.

Dianhydro Derivative (X) from the Dimer (V)——A solution of V (50 mg) in dry pyridine (1 ml) was chilled at −16° with ice-NaCl mixture in a desiccator and a solution of $SOCl_2$ (0.1 ml) in dry pyridine (0.5 ml) was added dropwise with stirring. After further stirring for 1 hr at the same temperature, excess $SOCl_2$ was decomposed by adding ice and the precipitate thus formed was collected by filtration and dried *in vacuo*. The crude product (50 mg) was recrystallized from MeOH–ether to afford X (16 mg) as a colorless crystalline powder, mp 190—195°, *Anal.* Calcd. for $C_{54}H_{72}O_{10}$: C, 73.60; H, 8.24. Found: C, 73.10; H, 8.28.

Dianhydro Derivative (XI) from the Dimer (VI)——VI (50 mg) was treated with $SOCl_2$ by a method analogous to that used for V to give the crude dianhydro derivative (47 mg), which was recrystallized from MeOH to afford XI (25 mg) as colorless crystals, mp 198—202°. *Anal.* Calcd. for $C_{54}H_{72}O_{10}\cdot 3\frac{1}{2}H_2O$: C, 68.69; H, 8.43. Found: C, 68.32; H, 8.10.

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