The data obtained by GC corresponded well to those obtained by MC. Therefore, the present GC method appears to be suitable for the simultaneous determination of major pungent components in capsicum fruit; this method is simpler and more accurate than previous methods.

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

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Solvent effects on the dimerization of 16,17-dehydrodigitoxigenin 3-acetate (I) were investigated. Heating of I in less polar solvents, such as toluene, xylene, dioxane or tetrahydrofuran, gave the dimer III as the main product, in similar yields. The reaction in slightly basic media, such as toluene containing sodium acetate or dimethylformamide, afforded an isomeric dimer IV preferentially. The structure of IV was established on the basis of its spectral data and anhydration reaction in contrast to those of III. A mechanism is proposed for the formation of IV from I.

Keywords——16,17-dehydrodigitoxigenin 3-acetate; solvent effect in dimerization; Diels-Alder type reaction; enol-lactone; ^{13}C-NMR

In the previous paper,[1] we reported that a dimerization of 16,17-dehydrodigitoxigenin 3-acetate (I) took place on heating I in toluene to give a dimer III as the main product by a Diels–Alder type reaction. Later, we investigated the solvent effects in this reaction and observed the formation of an isomeric dimer IV as the main product in the reaction in slightly basic media. This paper deals with these results.

16,17-Dehydrodigitoxigenin 3-acetate (I) was heated in less polar solvents, such as toluene, xylene, dioxane, and tetrahydrofuran (THF), in a closed test tube under nitrogen, and the reaction products were separated by multiple development TLC (SiO_2, benzene: Et_2O: $CHCl_3$= 2: 2: 1). From the less polar fraction (main), the dimer III[1], mp 179—182°, $C_{50}H_{68}O_{10}\cdot 2H_2O$, was isolated, and unchanged material was recovered from the more polar fraction (minor). The yields of III were similar in all the solvents.

When the reaction was carried out in slightly basic media, such as toluene containing a small amount of anhydrous sodium acetate (toluene–AcONa) or dimethylformamide (DMF), compound IV, mp 296—298°, $C_{50}H_{68}O_{10}\cdot 2H_2O$, was formed as the main product.

The reaction conditions and the yields of products are summarized in Table I. The molecular weight and integration values of hydrogen in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed that this product is a dimer of I, *i.e.*, an isomer of III. Spectral

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[1] Part XXXV: T. Hashimoto, Y. Kato, H. Shibahara, K. Toyooka, T. Ohta, and D. Satoh, *Chem. Pharm. Bull.*, 27, 2975 (1979).

[2] A part of this work was reported at the meeting of the Chugoku-Shikoku Branch of the Pharmaceutical Society of Japan, October, 28, 1979.

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

data for both dimers[4] are listed in Tables II, III, and IV. The ultraviolet (UV) and infrared (IR) spectra of the dimers are similar (Table II). In the UV spectra, 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, while the IR spectra showed the characteristic absorption of the cardenolide ring.[5] In the ^{1}H-NMR spectra (Table III), the vinyl proton signal due to 16-H of I, appearing as a triplet at δ 6.12, was absent in both dimers. These results suggested that

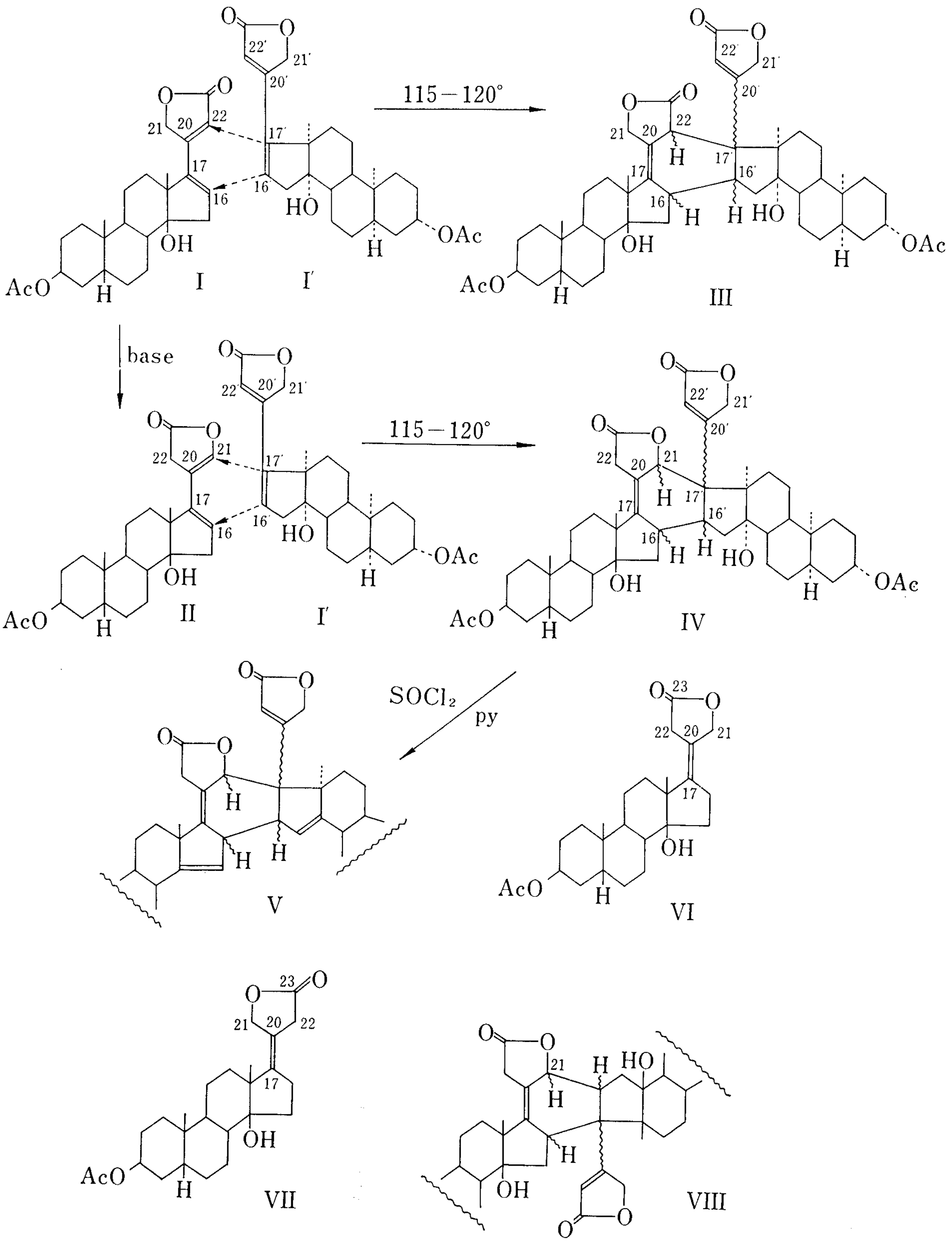

Chart 1

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[4] Spectral data for III were taken from the previous paper[1] for comparison with those of IV.
[5] R.N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

Table I. Reaction Conditions and Product Yields [a]

I mg	Solvents	Temp.	Time hr	Products Dimer III mg (%)	Dimer IV mg (%)
510	Toluene	115—120°	180	227(44.5)	
447	Xylene	115—120°	180	206(46.1)	
460	Dioxane	115—120°	180	220(47.6)	
480	THF	115—120°	180	236(49.2)	
300	Toluene–AcONa	115—120°	144	20(6.7)	206(68.7)
458	DMF	115—120°	180		283(61.8)

a) Theoretical yields.

Table II. UV and IR Spectral Data

Compds.	λ_{max}^{EtOH} nm (log ε)	ν_{max}^{KBr} cm^{-1} OH	C=O	C=C
I(I′)	270(4.27)	3520	1790, 1750, 1720	1620
III	217(4.20)	3500	1760, 1740	1610
IV	217(4.18)	3450	1780, 1735	1620

Table III. ^{1}H-NMR Spectral Data

Compds.	Chemical shifts (CDCl$_3$, δ) 22-H	22′-H	21-H	21′-H	16-H
I		5.95 (1H, s)		4.95 (2H, br. s)	6.12 (1H, t, J=2.5 Hz)
III	4.06 (1H, s)	6.04 (1H, s)	4.80 (2H, m)	4.80 (2H, m)	
IV	3.00 (2H, br. s)	6.06 (1H, s)	5.94 (1H, s)	4.63, 5.07 (2H, d. J=19 Hz)	
VI	3.24 (2H, m)		4.75 (2H, m)		
VII	3.05 (2H, m)		5.00 (2H, m)		

Table IV. ^{13}C-NMR Spectral Data

Compds.	Chemical shifts (CDCl$_3$, ppm) C-17	C-20	C-20′	C-21	C-21′	C-22′
III	123.9 or 117.7	149.0	173.8	67.1	76.7	117.7 or 123.9
IV	121.1 or 118.4	148.6	171.5	85.1	77.6	118.5 or 121.1
VI	118.3	145.2		72.3		
VII	119.0	145.9		70.3		

both the 16,17-double bonds of two monomer units (I and I′)[6] participate in the dimerization. Thus, the 16,17-double bond of one monomer (I′) was thought to add as a dienophile to the diene system including the 16,17-double bond of the other monomer (I) by a Diels–Alder type reaction to form the dimer. For the formation of dimer III, the 16,20(22)-diene system of I reacts as a diene moiety, as shown in Chart 1.[1] Therefore, another mode of Diels–Alder reaction should take place at an alternative diene system to form the new dimer (IV). On comparison of the ^{1}H-NMR spectra of the dimers, a remarkable difference was observed in the signals of 21-H and 22-H, as shown in Table III. A characteristic singlet (1H, δ 4.06) of III shifts to lower field (δ 5.94) in IV and a two-proton signal (δ 4.80) in III shifts to higher field (δ 3.00) in IV. These results indicate that the dimers are structural isomers rather than stereoisomers. Judging from the above-mentioned chemical shifts, the signal at δ 5.94 was assigned to a methine located between an oxygen atom and a carbon-carbon double bond, and the signal at δ 3.00 was assigned to a methylene located between a carbonyl group and a carbon-carbon double bond. These assignments were supported by the chemical shifts of 21-H and 22-H of 17(20)-cardenolides[7] (VI and VII), as shown in Table III.

On the basis of the above UV, IR and ^{1}H-NMR spectra, the structure IV can be assigned to the new product. UV absorption, $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 216 (4.17). The dianhydro derivative, mp 266—270°, $C_{50}H_{64}O_8 \cdot 2H_2O$ was assigned the structure V. Another type of dimer (VIII) which might be formed was excluded, because the 21-H signal of this form should be a doublet.

Furthermore, the structure of the dimer (IV) was also supported by the ^{13}C-NMR spectra (Table IV). Thus, the chemical shifts of C-17 and C-20 of IV are comparable with those of III and 17 (20)-cardenolides (VI and VII), and the chemical shifts of C-20′, C-21′, and C-22′[8] of IV also agree with those of III. On the other hand, the chemical shift of C-21 in IV is at lower field than in III, VI and VII.

The mechanism of formation of the dimer IV in basic media can be explained as follows. The shift of the double bond from the α,β-position to the β,γ-position in the cardenolide ring caused by a base is well known.[9] Thus, one molecule of the monomer (I) was presumably isomerized to the enol-lactone (II) by base, and the 16,17-double bond of another molecule of monomer (I′) added to 16,20-diene system of II by a Diels–Alder type reaction to form IV, as shown in Chart 1. Stereochemical analysis of III and IV is now in progress.

Experimental[10]

I. Formation of Dimer III from 16,17-Dehydrodigitoxigenin 3-Acetate (I) in less Polar Solvents. (a) **Dimerization in Toluene**——A mixture of I (510 mg) and toluene (0.6 ml) was heated at 115—120° for 180 hr in a closed test tube under nitrogen in an oil bath. TLC (SiO_2, benzene: Et_2O: $CHCl_3$ = 2: 2: 1) of the reaction solution showed the formation of a main product in addition to the presence of unchanged material (I). After triply developed preparative TLC using the same system, the less polar fraction (262 mg) was collected and recrystallized from AcOEt to give III (227 mg) as colorless prisms, mp 179—182°. This material was identical with the dimer reported in the previous paper.[1] The more polar fraction (123 mg) was recrystallized from AcOEt to recover the unchanged material (I, 98 mg).

(b) **Dimerization in Xylene**——A mixture of I (447 mg) and xylene (0.5 ml) was heated under the conditions described in Table I, and the reaction mixture was separated by a method similar to that described for dimerization (a). From the less polar fraction (250 mg) and the more polar fraction (153 mg), III (206 mg,

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

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

[8] A tentative numbering of the carbon atoms of the dimers is given in Chart 1.

[9] W.D. Paist, E.R. Brout, F.C. Uhle, and R.C. Elderfield, *J. Org. Chem.*, 6, 273 (1941); M. Kimura, *Chem. Pharm. Bull.*, 3, 81 (1955).

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

mp 179—182°) and unchanged material I (122 mg) were obtained after recrystallization from AcOEt, respectively.

(c) **Dimerization in Dioxane**——A mixture of I (460 mg) and dioxane (0.5 ml) was heated under the conditions described in Table I. The reaction mixture was separated as described for dimerization (a). The less polar fraction (246 mg) and the more polar fraction (97 mg) were recrystallized from AcOEt to afford III (220 mg, mp 178—181°) and unchanged material I (77 mg), respectively.

(d) **Dimerization in THF**——A mixture of I (480 mg) and THF (0.5 mg) was heated under the conditions described in Table I. The reaction product was separated as described above. The less polar fraction (247 mg) and the more polar fraction (73 mg) were recrystallized from AcOEt to give III (236 mg, mp 179—181°) and unchanged material I (58 mg), respectively.

II. Formation of Dimer IV from 16,17-Dehydrodigitoxigenin 3-Acetate (I) in Slightly Basic Medium. (a) **Dimerization in Toluene-AcONa**——A mixture of I (300 mg), anhydrous sodium acetate (10 mg), and toluene (0.5 ml) was heated at 115—120° for 144 hr in a closed test tube under nitrogen in an oil bath, and the reaction mixture was separated into three fractions by quintuply developed preparative TLC (SiO_2, benzene: Et_2O: $CHCl_3$=2: 2: 1). The less polar fraction (27 mg) was recrystallized from AcOEt to give III (20 mg) mp 178—181°. The more polar fraction (234 mg) was recrystallized from AcOEt to give IV (206 mg) as colorless needles, mp 296—298°, $[\alpha]_D^{24}$ −40.1° (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, 69.36; H, 8.48; Mol. Wt., 914.0. The most polar fraction (17 mg) was identified as unchanged material (I) by TLC.

(b) **Dimerization in DMF**——A mixture of I (458 mg) and redistilled DMF (0.5 ml) was heated under the conditions described in Table I, and the reaction mixture was separated by the method used in the case of dimerization (a). The more polar fraction (345 mg) was recrystallized from AcOEt to afford IV (283 mg) as colorless needles, mp 296—298°. The most polar fraction (23 mg) was identified as unchanged I by TLC.

Dianhydro derivative (V) from IV——A solution of IV (50 mg) in dry pyridine (1 ml) was cooled at −15° with ice-NaCl mixture 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 additng ice and the precipitate thus formed was collected by filtration and dried *in vacuo*. The crude product (43 mg) was recrystallized from $CHCl_3$–MeOH to give V (25 mg), mp 266—270°. *Anal.* Calcd for $C_{50}H_{64}O_8\cdot 2H_2O$: C, 72.44; H, 8.27. Found: C, 72.18; H, 8.12.

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Chemical Modification of Tryptophan and Histidine Residues in Lipoprotein Lipase from *Pseudomonas fluorenscens*[1]

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Lipoprotein lipase (glycerol ester hydrolase; EC 3.1.1.3) from *Pseudomonas fluorescens* was oxidized with N-bromosuccinimide at 37°. One mol of tryptophan residue per mol of enzyme was oxidized, but the enzymatic activity was unaffected.

The enzyme was inactivated by photooxidation in the presence of methylene blue, and the inactivation was pH-dependent. In addition, the decrease in the enzymatic activity was accompanied by the loss of a histidine residue. It appears that the histidine residue in involved in the catalytic activity of the enzyme.

Keywords——*Pseudomonas fluorescens*; chemical modification; tryptophan; histidine; lipoprotein lipase; methylene blue; photooxidation

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