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## Photodecomposition Products of Tetracycline in Aqueous Solution

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New photodecomposition products of tetracycline (TC) were found under photodecomposition conditions similar to a natural fish culture pond. Namely, using Extrelut and silica gel column chromatography, we were able to isolate seven photodecomposition products (I-VII) from a photodecomposition solution of TC under the following conditions: irradiation with wavelengths longer than 290 nm in air-saturated aqueous solution at pH 7.0 at 30 °C. The structures of compounds I, II, VI, and VII and partial structures of compounds III-V were clarified. Compounds I and II were lactones, and compounds VI and VII were hydroxy carboxylic acids. Compounds I and II were mainly converted from compounds VII and VI during the separation procedure, respectively, and so compounds VI and VII were mainly formed under the present photodecomposition conditions.

It is well-known that tetracycline (TC; Figure 1) photodecomposes easily and is converted to many photodecomposition products (Hlavka and Bitha, 1966; Wiebe and Moore, 1977; Davies et al., 1979; Sanniez and Pilpel, 1980). Further, the photodecomposition products may contribute to the photosensitization that appears frequently in patients undergoing treatment with TC (Wiebe and Moore, 1977; Davies et al., 1979; Sanniez and Pilpel, 1980; Hasan et al., 1984). In Japan, TC is widely applied to cultured fishes as a medicine and a feed additive because of its activity against a wide range of animal pathogens. Naturally, it is assumed that a part of TC applied to the fishes is photodecomposed, because TC is exposed to sunlight in a culture pond. So it is necessary for public health agencies to pay attention not only to residual TC but also to photodecomposition products of TC in the fish.

In previous studies on photodecomposition of TC, only three photodecomposition products have been reported. 4-Dedimethylaminotetracycline was identified after irradiation with UV light in methanol (Hlavka and Bitha, 1966). 5a,6-Anhydrotetracycline was isolated as a major photodecomposition product on photolysis of TC in the presence of  $\beta$ -mercaptoethanol (Hasan et al., 1985) and was also identified on photolysis in an oil-water mixture (Sanniez and Pilpel, 1980). Two groups suggested formation of a red product as a quinone form in aqueous air-saturated solution at pH 9.0 (Davies et al., 1979; Moore et al., 1983). However, we considered that photodecomposition of TC in a culture pond would produce compounds different from the above three, because photodecomposition conditions are quite different from the above

conditions; that is, a culture pond is usually aired compulsorily, the pH of the water is above 7.0, and the temperature is sometimes above 30 °C. Therefore, we planned to identify photodecomposition products of TC obtained on irradiation of an air-saturated solution at pH 7.0 and 30 °C and were able to isolate seven photodecomposition products, of which the structures for four were identified.

This paper describes the isolation and structural characterization of the seven photodecomposition products of TC.

### EXPERIMENTAL SECTION

**Instrumentation.** UV-visible absorption spectra were recorded on a Hitachi double-beam spectrophotometer, Model 200-10. IR spectra were obtained on a Hitachi IR spectrophotometer, Model 215. NMR spectra were obtained on either a Hitachi R-24B (60 MHz) or JEOL JNM-FX100 (100 MHz). Mass spectrometry was carried out by a Shimadzu GCMS-6020.

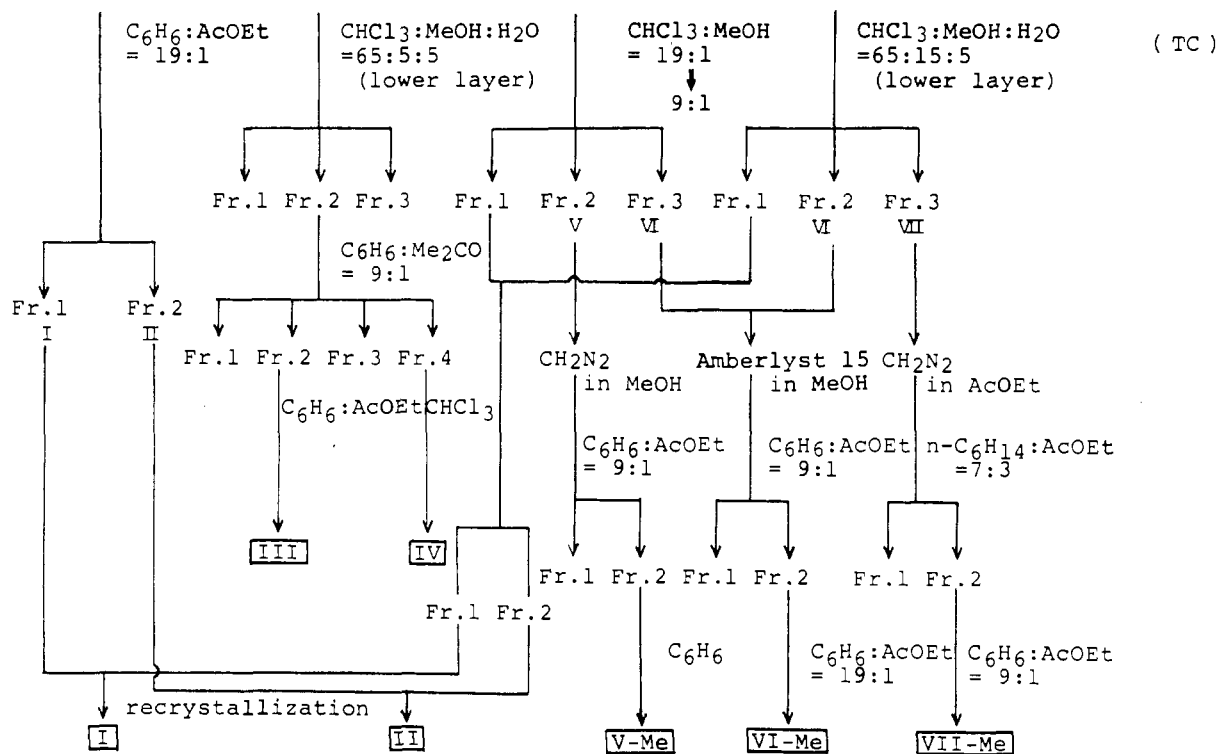
**Materials.** TC was purchased from PL-Biochemical Inc. (>95% pure) and was used without further purification. Column and thin-layer chromatographies were carried out with use of silica gel (E. Merck, No. 7729 and 7734) and silica gel plate (E. Merck, No. 5715), respectively. Extrelut was obtained from E. Merck. Other reagents were analytical grade.

**Thin-Layer Chromatography for Determination of TC.** A silica gel high-performance TLC plate (E. Merck, No. 5641) was predeveloped with saturated disodium ethylenediaminetetraacetate ( $\text{Na}_2\text{EDTA}$ ) solution and then dried in air at room temperature for 1 h and activated at 130 °C for 2 h. After a sample was applied the plate was developed with chloroform-methanol-5%  $\text{Na}_2\text{EDTA}$  solution (60:20:5) (lower layer). The developed TLC plate was placed under a chromatogram scanner (Shimadzu CS-910, Kyoto, Japan), and the components were determined by UV absorption spectrophotometry. The oper-

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Scheme I. Separation Procedure of Photodecomposition Products by Silica Gel Column Chromatography<sup>a</sup>

Hexane fraction Benzene fraction Chloroform fraction Ethyl acetate fraction Methanol fraction



<sup>a</sup> Abbreviations: C<sub>6</sub>H<sub>6</sub>, benzene; AcOEt, ethyl acetate; CHCl<sub>3</sub>, chloroform; MeOH, methanol; Me<sub>2</sub>CO, acetone; CH<sub>2</sub>N<sub>2</sub>, diazomethane.

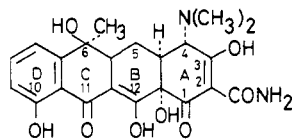


Figure 1. Structure of tetracycline.

ating conditions were as follows: dual-wavelength mode,  $R_{\text{sample}} = 360 \text{ nm}$  and  $R_{\text{ref}} = 600 \text{ nm}$ ; linear scanning in the reflection mode; size of beam,  $0.25 \times 9.0 \text{ mm}$ ; working linearizer, LINSX = 3 program; background correction, on.

**Photolysis.** The photolytic apparatus is shown in Figure 2. The UV light source was a 400-W high-pressure mercury lamp with Pyrex glassware. After dissolution of TC (0.5 g) in 150 mL of water, the solution was adjusted to pH 7.0 with 5 N sodium hydroxide solution and was placed in a glass vessel that was cooled with water to maintain the TC solution temperature at 30 °C and air was bubbled through the solution (300 mL/min) during irradiation. These procedures were repeated 107 times, and photolysis of 53.5 g of TC was finally carried out.

**Extraction of a Photodecomposition Mixture with Diaion HP-20.** A photodecomposition mixture was obtained by a batch method using Diaion HP-20; 500 mL of Diaion HP-20 was put into 3 L of photodecomposition solution obtained in the above experiment, and they were left overnight. The supernatant was discarded, and the Diaion HP-20 was rinsed with water until the supernatant was not colored. The photodecomposition mixture adsorbed on the Diaion HP-10 was eluted with 2 L of a mixture of methanol and acetone (1:1). The eluate was evaporated under reduced pressure to give 35.33 g of photodecomposition mixture.

**Separation of the Crude Photodecomposition Product with an Extrelut Column.** After dissolution

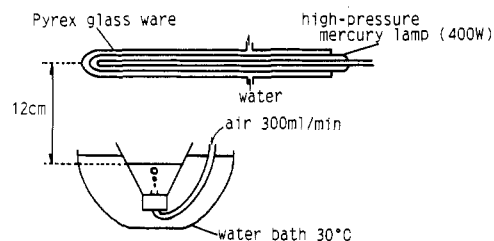


Figure 2. Photolytic apparatus.

of 35.33 g of the photodecomposition mixture in 450 mL of water, the solution was retained on an Extrelut column (Extrelut 350 g, 430 × 74 mm (i.d.)). Crude photodecomposition products were eluted with various organic solvents: *n*-hexane (7.6 L), benzene (6.6 L), chloroform (7.5 L), ethyl acetate (5.3 L), and methanol (4.0 L). With respect to elution volumes of each fraction, they were chosen according to the points that the spots of photodecomposition products were not detected on TLC plate any more. Each fraction was evaporated to dryness and 501.9 mg, 265.0 mg, 577.2 mg, 994.7 mg, and 20.2 g of crude photodecomposition products were obtained from *n*-hexane, benzene, chloroform, ethyl acetate, and methanol fractions, respectively.

**Photodecomposition Products from the Benzene Fraction.** Physical properties, mass spectral, and <sup>1</sup>H NMR data were shown in Tables I–III, respectively.

**Methylation of Compound V.** After dissolution of the fraction containing compound V in 0.5 mL of methanol, 3 mL of a diazomethane–ether solution was added to the solution and then the reaction mixture was allowed to stand for 2 days. The reaction mixture was evaporated under reduced pressure to give 42.0 mg. Further chromatographic procedures were shown in Scheme I.

**Methylation of Compound VI.** A fraction (109.6 mg) containing compound VI was dissolved in 4 mL of meth-

Table I. Physicochemical Properties of I-VII-Me

compd	mp, °C	appearance (recryst solvent)	anal. calcd (found)		EI high-res data for M <sup>+</sup> : calcd (found)	[α], deg (c)	IR, cm <sup>-1</sup>	UV, <sup>c</sup> nm (log ε)		
			C	H						
I	157-159	colorless prisms (AcOEt-MeOH)	67.23 (67.16)	5.21 (5.10)		-198.5 (0.2, CHCl <sub>3</sub> )	1785, 1770, 1632 <sup>a</sup>	335 (2.80)	258 (3.15)	218 (3.50)
II	159-162	colorless prisms (AcOEt-MeOH)	68.28 (68.23)	5.73 (5.77)		-91.5 (0.2, CHCl <sub>3</sub> )	1730, 1638 <sup>a</sup>	335 (3.46)	260 (3.77)	216 (4.03)
III	203-208	yellow needles (AcOEt)				-232.5 (0.13, CHCl <sub>3</sub> )	3550-3100, 1730 1640, 1260, 1100 <sup>b</sup>	372 (2.89)	295 (2.23)	262 (3.03)
IV		oily substance					3575, 3500-3150 1728, 1620 <sup>a</sup>	236 (3.34)	224 (3.35)	
V-Me		oily substance					<i>d</i>	364 (3.69)	269 (4.08)	251 (3.69)
VI-Me	65-68	colorless prisms (C <sub>6</sub> H <sub>6</sub> -C <sub>6</sub> H <sub>12</sub> )			278.1151 (278.1135)	-33.6 (0.2, CHCl <sub>3</sub> )	3600, 3550-3300 1730, 1640 <sup>a</sup>	228 (3.30)	279 (3.30)	223 (3.82)
VII-Me	78-80	colorless prisms (C <sub>6</sub> H <sub>6</sub> -C <sub>6</sub> H <sub>12</sub> )			264.0996 (264.0988)	-128.9 (0.2, CHCl <sub>3</sub> )	3550-3200, 1735 1635 <sup>a</sup>	205 (4.30)	261 (3.85)	216 (4.10)

<sup>a</sup> In chloroform solution. <sup>b</sup> In potassium bromide tablet. <sup>c</sup> In ethanol solution. <sup>d</sup> It was not able to be measured, because the amount obtained was very limited.

Table II. Mass Spectral Data for I-VII-Me

compd	m/z (relative intensity)								
	CI ( <i>i</i> -C <sub>4</sub> H <sub>10</sub> )			EI					
	MH <sup>+</sup>	MH <sup>+</sup> - 18	other ions	M <sup>+</sup>	M <sup>+</sup> - 15	M <sup>+</sup> - 18	other ions		
I	233 (100)			232 (100)	217 (78)		173 (78)	163 (25)	
II	247 (100)			246 (100)	231 (60)		203 (35)	174 (70)	163 (62)
III	249 (100)			248 (100)		230 (19)	202 (19)	189 (50)	188 (58)
IV	302 (100)	284 (16)		301 (82)			258 (36)	256 (36)	214 (100)
V-Me	347 (26)	329 (100)	311 (34)	346 (100)			163 (42)	287 (16)	215 (31)
VI-Me	279 (54)	261 (100)		278 (21)	263 (15)	260 (100)	288 (15)	185 (31)	163 (71)
VII-Me	265 (100)	247 (88)		264 (60)	249 (49)	246 (22)	213 (16)	204 (21)	192 (21)
							187 (37)	164 (80)	121 (66)
							217 (100)	189 (16)	187 (32)
							173 (19)	164 (88)	121 (44)

Table III. <sup>1</sup>H NMR Spectral Data for I-VII-Me (δ)<sup>a</sup>

compd	C4 CH <sub>3</sub>	H6 ( <i>J</i> <sub>6,7</sub> )	H7 ( <i>J</i> <sub>7,8</sub> )	H8 ( <i>J</i> <sub>8,9</sub> )	C9 OH	OCH <sub>3</sub>	N(CH <sub>3</sub> ) <sub>2</sub>
I	1.86 s	7.09 dd (8)	7.56 t (8)	6.92 dd (2)	12.25 s		
II	1.79 s	7.16 dd (8)	7.54 t (8)	6.91 dd (2)	12.34 s		
III	1.90 s		7.18 d (10)	6.88 d	12.06 s		
IV	1.62 s	7.01 dd (8)	7.45 t (8)	6.90 dd (2)	12.66 s		3.06 s
V-Me	1.94 s	6.99 dd (8)	7.23 t (8)	6.89 dd (2)		3.68 s	2.96 s
VI-Me	1.64 s	7.16 dd (8)	7.50 t (8)	6.86 dd (2)	12.44 s	3.81 s	
VII-Me	1.37 s	7.20 dd (8)	7.50 t (8)	6.86 dd (2)	12.41 s	3.64 s	
						3.71 s	

<sup>a</sup> Measured in CDCl<sub>3</sub> at 100 MHz with TMS as an internal standard. Key: s, singlet; d, doublet; dd, double doublet; t, triplet. *J* values are expressed in hertz.

anol, 600 mg of Amberlyst 15 was added to the solution, and then the reaction mixture was left with stirring overnight. The mixture was evaporated under reduced pressure, and 112.3 mg of residue containing compound VI-Me was obtained. Further chromatographic procedures were shown in Scheme I. Compound VI-Me was recrystallized from benzene and cyclohexane.

**Methylation of Compound VII.** A fraction (32.0 mg) containing compound VII was dissolved in 2 mL of ethyl acetate, 3 mL of a diazomethane-ether solution was added to the solution, and then the reaction mixture was left overnight. The mixture was evaporated under reduced pressure, and 40.6 mg of the mixture containing compound VII-Me was obtained. Further chromatographic procedures were shown in Scheme I. Compound VII-Me was recrystallized from benzene and cyclohexane.

**Conversion of Compound VI to Compound II.** A fraction (26.7 mg) containing compound VI was dissolved in 3 mL of 5% *p*-toluenesulfonic acid-tetrahydrofuran solution, and the solution was left overnight. A 50-mL portion of water was added, and then the solution was neutralized with 5% sodium hydrogen carbonate solution. Crude compound II was extracted three times with 30 mL of ethyl acetate from the reaction mixture, and the ethyl acetate layer was dried with anhydrous sodium sulfate and evaporated under reduced pressure. Crude compound II (18.6 mg) was obtained. Compound II (6.6 mg) was obtained as colorless prisms after preparative TLC (benzene-ethyl acetate, 9:1) and recrystallization (ethyl acetate and methanol).

**X-ray Analysis of I.** Intensity measurements were performed with a Rigaku Denki automatic four-circle

Table IV. Crystal Data for I

molecular formula	C <sub>13</sub> H <sub>12</sub> O <sub>4</sub>
molecular weight	232
crystal system	monoclinic
space group	P2 <sub>1</sub>
cell dimensions	
<i>a</i> , Å	7.989 (1)
<i>b</i> , Å	10.504 (2)
<i>c</i> , Å	7.079 (1)
β, deg	112.07 (1)
<i>V</i> , Å <sup>3</sup>	550.4 (2)
<i>Z</i>	2
<i>D<sub>c</sub></i> , g·cm <sup>-3</sup>	1.401
final <i>R</i> value, %	3.8

Table V. Bond Lengths (Å)

C1-C2	1.39 (4)	C8-C9	1.53 (4)
C1-C10	1.38 (4)	C8-C11	1.52 (5)
C1-H1	1.00 (30)	C8-H8	0.98 (26)
C2-C3	1.37 (4)	C9-C10	1.52 (3)
C2-H2	1.01 (43)	C9-C13	1.52 (5)
C3-C4	1.40 (4)	C9-O3	1.48 (3)
C3-H3	0.96 (31)	C11-C12	1.51 (5)
C4-C5	1.41 (3)	C11-H111	0.98 (36)
C4-O1	1.35 (3)	C11-H112	0.91 (38)
C5-C6	1.47 (4)	C12-O3	1.36 (4)
C5-C10	1.41 (4)	C12-O4	1.19 (4)
C6-C7	1.50 (3)	C13-H131	0.93 (32)
C6-O2	1.23 (4)	C13-H132	0.97 (41)
C7-C8	1.52 (4)	C13-H133	1.00 (33)
C7-H71	0.99 (44)	O1-HO1	0.90 (39)
C7-H72	1.01 (44)		

diffractometer using monochromated Mo K $\alpha$  radiation. Colorless single crystals of I for X-ray study were obtained from ethyl acetate. The crystal data for I are listed in Table IV. The intramolecular bond distances (Å) and valence angles (deg) are given in Tables V and VI, respectively.

## RESULTS AND DISCUSSION

(a) **Photolysis of Tetracycline.** As mentioned in the introduction, a culture pond is usually aired compulsorily, the pH of the water is above 7.0, and the temperature is above 30 °C, especially in the summer. The wavelength of sunlight is longer than 286 nm (Nakagawa, 1979). In this study, since we want to clarify the photodecomposition of TC under conditions similar to those in a natural culture pond, the photodecomposition was carried out under the conditions shown in the Experimental Section and the reaction course was monitored by high-performance thin-layer chromatography (HP TLC). As shown in Figure 3, TC in the solution decreased with prolonged irradiation and 50% of the TC was photodecomposed when the solution was irradiated for 3 h. Therefore, it was assumed that TC in a culture pond was also photodecomposed by sunlight. So we tried to identify photodecomposition products after irradiation for 3 h in the subsequent work.

(b) **Effective Extraction of the Photodecomposition Mixture.** As the total volume of the photodecomposition solution was more than 15 L, it was hard to extract directly the photodecomposition mixture from the solution by conventional liquid-liquid partitioning. We considered the use of adsorbing resins such as Amberlite XAD-2, XAD-7, and Diaion HP-20, which were often used for extraction of antibiotics from their culture brothes (Kurita et al., 1976; Yamamoto et al., 1978). In order to choose the most suitable resin for the extraction of a photodecomposition mixture, their adsorbing powers were compared. After each resin was packed into a column (100 × 50 mm (i.d.)), 150 mL of the photodecomposition solution was passed through. The photodecomposition mixture was eluted with 200 mL of methanol-acetone (1:1), and then the yields

Table VI. Bond Angles (deg)

C2-C1-C10	120.3 (3)	C2-C1-H1	121.7 (17)
C10-C1-H1	117.9 (17)	C1-C2-C3	121.3 (3)
C1-C2-H2	116.3 (18)	C3-C2-H2	122.4 (18)
C2-C3-C4	119.9 (2)	C2-C3-H3	124.1 (26)
C4-C3-H3	116.1 (26)	C3-C4-C5	119.6 (2)
C3-C4-O1	117.7 (2)	C5-C4-O1	122.6 (3)
C4-C5-C6	119.5 (2)	C4-C5-C10	119.3 (2)
C5-C6-C7	118.3 (2)	C6-C5-C10	121.2 (2)
C7-C6-O2	120.0 (3)	C5-C6-O2	121.7 (2)
C6-C7-H71	109.7 (18)	C6-C7-C8	113.7 (2)
C8-C7-H71	107.8 (22)	C6-C7-H72	108.9 (19)
H71-C7-H72	101.5 (35)	C8-C7-H72	114.4 (2)
C7-C8-C11	116.5 (3)	C7-C8-C9	112.6 (20)
C9-C8-C11	101.8 (2)	C7-C8-H8	109.3 (17)
C11-C8-H8	107.3 (19)	C9-C8-H8	108.6 (20)
C8-C9-C13	114.0 (2)	C8-C9-C10	113.1 (2)
C10-C9-C13	111.6 (2)	C8-C9-O3	103.0 (2)
C13-C9-O3	107.1 (2)	C10-C9-O3	107.2 (2)
C1-C10-C9	118.8 (2)	C1-C10-C5	119.7 (2)
C8-C11-C12	103.1 (3)	C5-C10-C9	121.6 (2)
C8-C11-H112	115.0 (27)	C8-C11-H111	111.8 (23)
C12-C11-H112	110.8 (24)	C12-C11-H111	109.3 (23)
C11-C12-O3	109.1 (2)	H111-C11-H112	106.8 (35)
O3-C12-O4	120.7 (3)	C11-C12-O4	130.2 (3)
C9-C13-H132	108.2 (25)	C9-C13-H131	114.3 (28)
H131-C13-H132	104.3 (34)	C9-C13-H133	112.6 (22)
H132-C13-H133	114.9 (31)	H131-C13-H133	102.5 (32)
C9-O3-C12	110.5 (2)	C4-O1-HO1	107.2 (22)

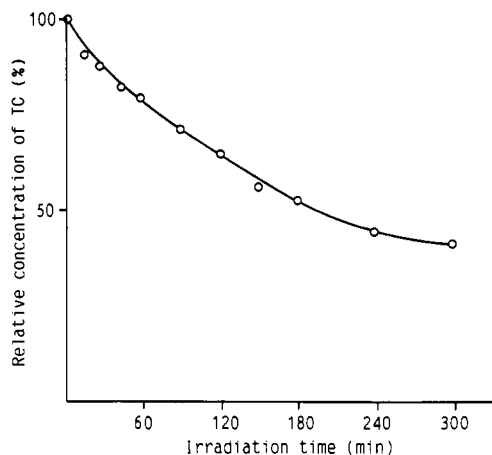


Figure 3. Decrease of tetracyclines during photolysis.

from each column were compared after concentration to dryness. The results showed that 154.6, 76.9, and 35.1 mg of the mixtures were obtained from Diaion HP-20, Amberlite XAD-2, and XAD-7, respectively. Therefore, we decided to use Diaion HP-20 for the extraction. Finally, 35.3 g of photodecomposition mixture was obtained from photolysis of 53.5 g of TC using Diaion HP-20 extraction.

Next, in order to separate the desired crude photodecomposition product from the photodecomposition mixture obtained in the Diaion HP-20 extraction step, we first used conventional liquid-liquid partition using ethyl acetate after dissolution of the photodecomposition mixture in water. However, it formed an emulsion and the further silica gel column chromatography for isolation was complicated. Therefore, Extrelut column chromatography, which can extract chemical compounds by a simple procedure without formation of emulsion, was used instead of conventional liquid-liquid partition. The column is filled with a large-pore Kieselguhr of granular structure and high-pore volume. It is based on the principle of liquid-liquid extraction, and no emulsion is formed (Breiter et al., 1976).

As described in the Experimental Section, the crude photodecomposition products were eluted with five organic

Table VII.  $^{13}\text{C}$  NMR Spectral Data for I, II, VI-Me, and VII-Me ( $\delta$ )<sup>a</sup>

compd	C1	C2	C3	C4	C4 CH <sub>3</sub>	D ring				C11	C12	COOR	OCH <sub>3</sub>	
I	200.4 s	34.8 <sup>b</sup> t	41.2 d	82.0 s	27.6 q	161.8 s	143.5 s	138.0 d	118.9 d	117.8 d	114.4 s	36.7 <sup>b</sup> t	173.9 s	
II	200.8 s	29.5 <sup>c</sup> t	38.8 d	82.5 s	30.8 q	162.6 s	145.8 s	138.0 d	117.8 d	116.6 d	113.6 s	23.8 t	169.7 s	
VI-Me	202.9 s	31.9 <sup>d</sup> t	44.9 d	72.4 s	30.8 q	162.4 s	149.1 s	137.4 d	118.9 d	115.8 d	114.8 s	24.1 t	174.5 s	51.7 q
VII-Me	202.4 s	34.6 <sup>e</sup> t	42.1 d	72.8 s	23.8 q	162.6 s	152.3 s	137.4 d	117.0 d	115.2 d	114.8 s	41.8 <sup>e</sup> t	173.3 s	52.1 q

<sup>a</sup> Measured in CDCl<sub>3</sub> at 25 MHz with TMS as an internal standard. <sup>b-e</sup> Assignments may be reversed.

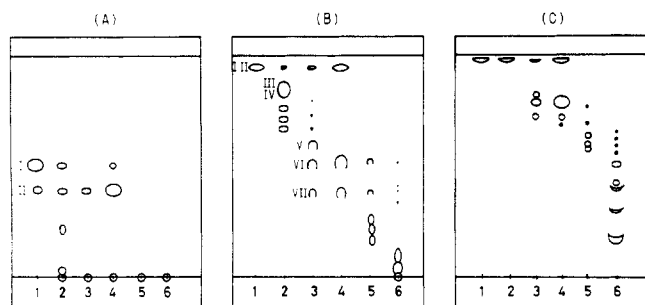


Figure 4. Thin-layer chromatograms of each fraction from Extrelut column (plate, silica gel TLC). Solvent systems: (A) benzene-ethyl acetate, 8:2; (B) chloroform-methanol-water, 65:20:5 (lower layer); (C) chloroform-methanol-water, 30:20:4.

solvents (*n*-hexane, benzene, chloroform, ethyl acetate, methanol) in order of their polarities. The thin-layer chromatograms of each fraction are shown in Figure 4. It was clarified that the crude photodecomposition products were separated according to their polarities and that this Extrelut step made ready further silica gel column chromatography. For example, when conventional liquid-liquid partition was used at this step, we had to repeat silica gel column chromatography four times to isolate I and II, whereas we were able to achieve the purpose by further silica gel column chromatography only once in the case of the Extrelut column.

Silica gel column chromatography was repeated as summarized in Scheme I to isolate photodecomposition products from each fraction obtained at the Extrelut column step. As a result, we were able to isolate seven photodecomposition products (I-VII) in order of *R<sub>f</sub>* values on TLC plate in Figure 4. Compounds I-IV were directly isolated, and compounds V-VII were isolated after methylation. Undecomposed tetracycline was in the methanol fraction.

**(c) Structures of the Photodecomposition Products.** Spectral data of the isolated photodecomposition products are summarized in Tables I-III and VII. As a result, they are divided into the following three classes according to their characteristics: (i) lactones (I and II); (ii) photodecomposition products from the benzene fraction (III and IV); (iii) carboxylic acids (V-VII). The processes of purification and structural confirmation of each photodecomposition product are detailed below.

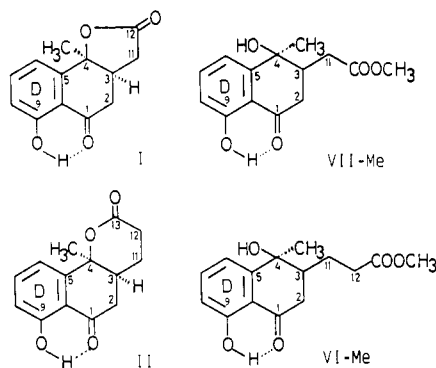
*(i) Lactones (I and II).* As shown in Scheme I, we isolated 137.4 mg of I and 33.4 mg of II from 501.9 of the *n*-hexane fraction. Compound I was obtained as colorless prisms (mp 157-159 °C), and its composition corresponded to C<sub>13</sub>H<sub>12</sub>O<sub>4</sub> (Table I). A protonated molecule (MH<sup>+</sup>) in the chemical ionization (CI) mass spectrum with isobutane as a reagent gas was observed at *m/z* 233 as a base peak, so the molecular weight was estimated to be 232 (Table II). In the <sup>1</sup>H nuclear magnetic resonance (NMR) spectrum, a singlet signal (1 H), originated by an associated hydroxyl group, was observed at  $\delta$  12.25. Appearance of three signals of  $\delta$  7.09 (1 H, dd, *J* = 8, 2 Hz), 7.56 (1 H, t, *J* = 8 Hz), and 6.92 (1 H, dd, *J* = 8, 2 Hz) showed the presence of consecutive C6 H, C7 H, and C8 H, respec-

tively, corresponding to those on the D ring of TC (Table III). Additionally an angular methyl signal was found at  $\delta$  1.86. Strong bands at 1785 and 1770 cm<sup>-1</sup> and at 1632 cm<sup>-1</sup> in the infrared (IR) absorption spectrum indicated the presence of *r*-lactone and conjugated ketone with hydrogen bonding (Table I). All signals in the <sup>13</sup>C NMR spectrum were assigned as shown in Table VII. Therefore, the structure of compound I was determined as shown in Figure 5, and the structure including its absolute stereochemistry was steadily confirmed by X-ray crystallographic analysis.

Compound II was obtained as colorless needles, mp 159-162 °C, and its formula, C<sub>14</sub>H<sub>14</sub>O<sub>4</sub>, was confirmed by elemental analysis (Table I). In the CI mass spectrum, MH<sup>+</sup> was observed at *m/z* 247 as a base peak, so that the molecular weight was estimated to be 246 (Table II). Although the chromophores of I and II were found to be very similar by their <sup>1</sup>H NMR and UV spectra, a detailed comparison of the <sup>13</sup>C NMR spectra of I and II indicated that II possesses one more methylene group (Table VII). The IR spectrum also demonstrated the presence of *d*-lactone (1730 cm<sup>-1</sup>) in II. These results clearly showed that II has the structure shown in Figure 5 where the *r*-lactone is replaced by the *d*-lactone in I. The results of minimum inhibitory concentration for compounds I and II showed that compounds I and II do not have antibacterial activity.

*(ii) Photodecomposition Products from the Benzene Fraction (III and IV).* Compounds III and IV were isolated from the benzene fraction (265.0 mg) by repeated silica gel column chromatographies (Scheme I). Compound III (3.5 mg) was obtained as yellowish needles. The molecular weight was determined to be 248 by CIMS (Table II). The presence of an associated hydroxyl group [ $\delta$  12.06, 1 H, s) and a tetrasubstituted benzene ring [ $\delta$  7.18 (1 H, d, *J* = 10 Hz) 6.88 (1 H, d, *J* = 10 Hz)] with an ortho coupling was suggested in the <sup>1</sup>H NMR spectrum (Table III). The IR spectrum showed ester (1730-cm<sup>-1</sup>) and ketone (1640-cm<sup>-1</sup>) absorptions (Table I) as in the case of II. Therefore, it was considered that compound III has the D ring of TC as a partial structure in itself. However, a further detailed structural study was not performed, because the obtained amount was very limited.

Compound IV (6.6 mg) was obtained as oily substance. The molecular weight was estimated to be 301 by CIMS (Table II), indicating that IV possesses a nitrogen atom. Although 18 signals were observed in the <sup>13</sup>C NMR spectrum, a detailed assignment cannot be done at present. As shown in Table III, the <sup>1</sup>H NMR spectrum showed the presence of characteristic signals derived from the D ring of TC [ $\delta$  12.66 (1 H, s), 7.45 (1 H, t, *J* = 8 Hz), 7.01 (1 H, dd, *J* = 8, 2 Hz), 6.09 (1 H, dd, *J* = 8, 2 Hz)], a singlet methyl (s,  $\delta$  1.62, 3 H), and a dimethylamino group [ $\delta$  3.06, (3 H, s), 2.96 (3 H, s)]. Therefore, it was supposed that compound IV have a dimethylamino group and C- and D-ring systems of TC. However, the decomposition of compound IV was found after measurement of <sup>13</sup>C NMR spectra, so we were compelled to abandon further study for compound IV. For these reasons the structures of the degradation products from the benzene fraction were unfortunately not made clear.



**Figure 5.** Structures of photodecomposition products of tetracycline.

(iii) *Carboxylic Acids (V-VII)*. We attempted to isolate compound V directly from the chloroform fraction; however, we were not able to obtain good results. We considered that compounds V-VII have a carboxyl group, because they always gave tailing spots on TLC plate but they showed no tailing spots with a solvent system containing acetic acid. Therefore, the isolation of compound V after methylation with diazomethane was performed, so that compound V was isolated as a methyl ester (V-Me) in a pure state from the chloroform fraction by silica gel chromatography (Scheme I). Compound V-Me was an oily substance, and its molecular weight was elucidated firmly to be 346 by CIMS (Table II). Although three aromatic and an angular methyl signals similarly appeared as compounds I and II in the  $^1\text{H}$  NMR spectrum, a signal due to the hydroxyl group at C-9 was not recognized around  $\delta$  12 and conversely two methoxy signals were observed at  $\delta$  3.68 and 3.81. These results suggested the hydroxy group was methylated under the present conditions and no keto group was present at C-1. In addition, the UV spectrum supported our suggestion. However, we were compelled to abandon further study of compound V-Me, because the obtained amount was very limited.

As described above, it was assumed that compound VI also contained a carboxyl group, so we planned to isolate it after methylation of compound VI (Scheme I). Methylation of crude VI was carried out with Amberlyst 15 in methanol. Methylated VI (VI-Me, 10 mg) was obtained as colorless prisms by silica gel column chromatography (Scheme I), whose molecular weight was estimated to be 278 by CIMS (Table II). In addition to three aromatic signals and an angular methyl and a hydroxy with hydrogen-bonding signals corresponding to the D-ring system of TC, a methoxy signal was newly observed at  $\delta$  3.64 in the  $^1\text{H}$  NMR spectrum (Table III). The IR spectrum showed a characteristic ester band at  $1730\text{ cm}^{-1}$ . These spectra data strongly indicated that VI-Me corresponds to the methanolysis product of II as shown in Figure 5. The  $^{13}\text{C}$  NMR spectrum also supported the conclusion because a new methoxy carbon signal appeared at  $\delta$  51.7.

Methylation of VII was performed with diazomethane to give the corresponding methyl ester (VII-Me). VII-Me was obtained as colorless prisms, and its molecular weight was determined to be 264 by CIMS (Table II). Comparison of  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of I with those of VII-Me suggested that VII-Me corresponds to the linearized methyl ester. This assumption was strongly confirmed by comparison of both IR spectra where the *r*-lactone ( $1785, 1770\text{ cm}^{-1}$ ) was converted to the ester with normal value ( $1735\text{ cm}^{-1}$ ).

For the relation between lactones (I and II) and carboxylic acids (VI and VII), when the direct isolation of carboxylic acid from the ethyl acetate fraction was at-

tempted, it was found that the lactones always coexisted with carboxylic acids. This fact strongly suggested that compounds VI and VII, which are hydroxy carboxylic acids in the crude photodecomposition product, were mainly converted to lactones (I and II) during separation. So we planned an experiment converting compound VI to II. As a result, it was clarified that compound VI is converted readily to compound II in 5% *p*-toluenesulfonic acid-tetrahydrofuran. Therefore, we conclude that compounds VI and VII are the main photodecomposition products under the present photodecomposition conditions.

## CONCLUSION

As mentioned above, the photodecomposition of TC was carried out under conditions similar to those of a natural culture pond. A combination of Diaion HP-20, Extrelut, and silica gel chromatography made possible effective isolation of seven photodecomposition products (I-VII). Structures of compounds I, II, VI, and VII and partial structures of compounds III-V were clarified; however, the previously reported three photodecomposition products were not found under the conditions similar to the natural culture pond. Compounds I and II were lactones, and compounds VI and VII were hydroxy carboxylic acids. We consider that compounds I and II were mainly converted from compounds VII and VI, respectively, during the separation procedure. Therefore compounds VI and VII were mainly formed under the present photodecomposition conditions. In the near future, it will be necessary to check biological properties including toxicity of these compounds in a culture pond.

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**Registry No.** I, 117687-07-7; II, 117687-08-8; VI, 117687-09-9; VII, 117687-10-2; TC, 60-54-8.

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