1977) to give dibenzofuran derivative **2a** and via radical pair **B** to form fluorophenyl derivative **2b**, both of which appear to be very stable photoproducts (Figure 3).

Only a few of the multitude of photoproducts of **3** and **4** are tentatively characterized (Figure 5). Reactivity is centered at the allylic position involving radical formation (**A**) followed by rearrangement (**B**). Either radical **A** or **B** can react with oxygen (through hydroperoxides **C** and **D**) to yield products tentatively identified as the α,β-unsaturated ketones **3a/b**. Additional photoproducts arise from isomerization and reaction with methanol based on their GC–MS characteristics. Although not shown in Figure 5, isomerization may proceed by hydrogen abstraction from **A** to give (*Z*)-**3** or (*Z*)-**4** and from **B** to give the corresponding positional isomers. Isomers may also arise from rupture of the cyclopropane ring and subsequent cyclization. Methanol addition to these radical intermediates can give rise to the observed (but unidentified) products, which incorporate either 30 or 32 amu. Oxidative cleavage of the double bond gives rise to a plethora of products (Figure 5), some of which are common to both **3** and **4**, i.e. those formed from the phenoxyfluorophenyl moiety.

In summary, a variety of central linkages, including ester, ether, alkane, and alkene, confer pyrethroid-like insecticidal activity. The nature of the central linkage also determines the degree of photostability and the type and variety of photoproducts formed.

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Registry No. **1**, 80844-07-1; **1a**, 117251-99-7; **1b**, 117252-00-3; **1c**, 50789-43-0; **1d**, 117252-01-4; **2e**, 3586-14-9; **1f**, 83493-63-4; **1g**, 39515-51-0; **1h**, 13826-35-2; **1i**, 50789-47-4; [2], 89764-44-3; **2a**, 117252-02-5; **2b**, 117252-03-6; **3**, 100846-02-4; **3a**, 117252-04-7; **3c**, 104-88-1; **3d**, 1126-46-1; **3e**, 7448-87-5; **3f**, 100845-90-7; **3g**, 117252-05-8; **3h**, 74483-53-7; **3i**, 117252-06-9; **3j**, 68359-57-9; **3k**, 117252-07-0; **3l**, 117252-08-1; **3m**, 81358-66-9; **3n**, 117252-09-2; **4**, 100846-04-6; **4c**, 10031-82-0; **4d**, 23676-08-6; **4e**, 117252-10-5.

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Hydroxycineole: Four New Metabolites of 1,8-Cineole in Rabbits

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The metabolites of 1,8-cineole in rabbit urine were isolated by preparative gas chromatography. With the aid of mass spectra, ^{1}H NMR spectra, ^{13}C NMR spectra, and infrared spectra, major metabolite **2** has been established as 2-*exo*-hydroxycineole, metabolite **3** as 2-*endo*-hydroxycineole, metabolite **4** as 3-*exo*-hydoxycineole, and metabolite **5** as 3-*endo*-hydroxycineole. Bioavailability was examined from blood levels of unchanged cineole and metabolites following oral dosing.

1,8-Cineole (**1**), eukalyptol, is a main constituent of essential oil from *Eucalyptus polybractea*, a component of a drug for urolithiasis. 1,8-Cineole and essential oils containing 1,8-cineole are used extensively in the food and cosmetic industries. Until recently, however, little was known about the metabolism of this terpene in mammals. It is known that 1,8-cineole is converted to oxidized products, 9-hydroxycineole and cineol-9-oic acid, by an enzyme of metabolism in brushtail possum (*Trichosurus vulpecula*) (Southwell et al., 1980; Flynn and Southwell, 1979). The aim of this study is to clarify natural smell substance biotransformation in animal biochemistry. The metabolism of α-pinene, β-pinene, pinane, 3-carene, carane,

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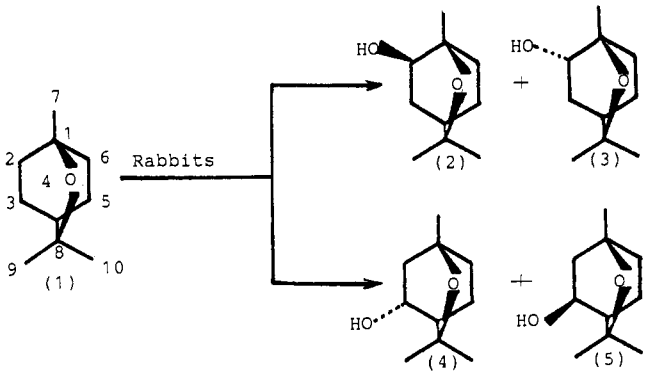
Scheme I. Metabolism of 1,8-Cineole in Rabbits



myrcene, *p*-cymene, *dl*-camphene, caryophyllene, and longifolene in rabbits was studied by Ishida and Asakawa (Ishida et al., 1977, 1978, 1981; 1982, Asakawa et al., 1981). In addition, terpenoid biotransformation of camphor terpenoids was studied in mammals (Robertson and Hussain, 1969). The purpose of this investigation was to isolate and identify the free and conjugated urinary metabolites of 1,8-cineole in rabbits.

MATERIALS AND METHODS

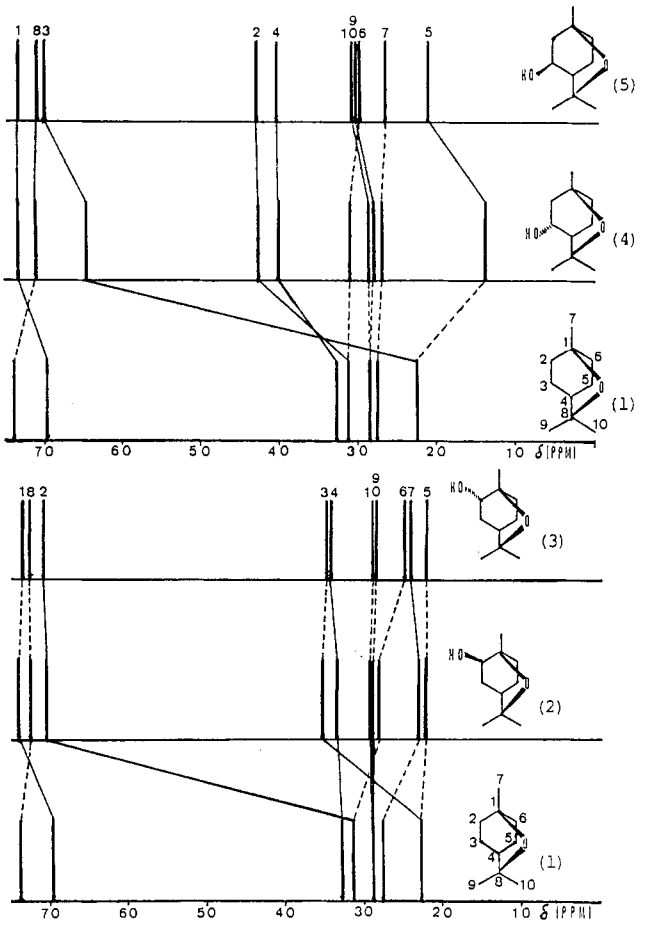
Materials. 1,8-Cineole (above 98%, Tokyo Kasei Kogyo), β-glucuronidase-arylsulfatase (Boehringer Manhein), tris(dipivaloyl metanate)europium (Eu(DPE)) (Wako Jiyunyaku Co. Ltd.), Wako gel 200, and reagent-grade solvents (Kanto Chemicals) were used. A Shimadzu GC-6A gas chromatograph was equipped with a hydrogen flame ionization detector and a thermal conductive detector. For neutral metabolite detection, the following were used: a 2 m × 2.6 mm (i.d.) glass column, 5% PEG-HT (60–80 mesh) and an injector at 250 °C; oven programmed at 100–210 °C at 10 °C/min; detector at 250 °C; nitrogen flow rate 40 mL/min. For preparative use, the same size glass column of 3% PEG-HT (60–80 mesh) was employed under the same conditions. The IR spectra were recorded on a Jasco IR-A2 spectrometer and taken in liquid films and KBr plates. ^{13}C and ^{1}H NMR spectra were measured on a JEOL FX200FT spectrometer (200 MHz) in $CDCl_3$ with $(CH_3)_4Si$ as an internal standard. Optical rotations were measured on a Jasco DIP-140 spectrometer. Mass spectra were recorded with a Shimadzu LKB-9000B.

Animals and Dosing. Five male albino rabbits, 2–3 kg, were starved for 3 days before the experiments. 1,8-Cineole (10.0 g) was dissolved in water (490 mL) containing CMC-Na (sodium carboxymethyl cellulose) (0.5 g) and the mixture homogenized for 10 min. This solution (20–30 mL) was administered to each rabbit through a stomach tube. This 1,8-cineole dose corresponds to 200 mg/kg, and the total amount of 1,8-cineole fed to all rabbits equaled 2.0 g. The animals were housed in individual stainless steel metabolism cages and were allowed rabbit food and water. The urine was collected daily for 2 days after drug administration and stored at −5 °C until use.

Extraction and Fractionation of Neutral Metabolites from Urine. The urine was treated according to the method of Bang Ourisson (1975). The urine was adjusted to pH 4.0 with phosphate buffer and then incubated with β-glucuronidase at 37 °C for 24 h followed by continuous ether extraction for 48 h. The ether extracts (1.9 g) were washed with 5% $NaHCO_3$ and 5% NaOH to remove the acidic and phenolic fractions, respectively, and dried over magnesium sulfate. Ether was evaporated under reduced pressure to give the free neutral metabolites (1.6 g).

Determination and Identification of Metabolites. The metabolites were chromatographed on a silica gel column and eluted by a gradient *n*-hexane–ethyl acetate system; each compound separated from the elute by preparative GLC. Four metabolites (**2–5**) were isolated by preparative GLC (Scheme I). Structure determination or identification was based on spectral data.

Table I. ^{13}C NMR Chemical Shift Assignments for 1–5



carbon	1	2	3	4	5
1	69.1 (s)	74.0 (s)	73.5 (s)	73.5 (s)	73.6 (s)
2	31.5 (t)	70.5 (d)	70.9 (d)	42.8 (t)	43.2 (t)
3	22.8 (t)	35.5 (t)	34.7 (t)	64.8 (d)	70.4 (d)
4	32.9 (d)	33.5 (d)	34.3 (d)	40.3 (d)	40.7 (d)
5	22.8 (t)	22.2 (t)	22.2 (t)	13.9 (t)	21.4 (t)
6	31.5 (t)	28.3 (t)	25.0 (t)	31.0 (t)	31.2 (t)
7	27.5 (q)	23.2 (q)	24.1 (q)	27.2 (q)	28.9 (q)
8	73.5 (s)	72.6 (s)	72.7 (s)	71.1 (s)	71.1 (s)
9	28.8 (q)	29.1 (q)	29.0 (q)	29.0 (q)	30.8 (q)
10	28.8 (q)	29.1 (q)	28.1 (q)	28.3 (q)	30.5 (q)

Measurement of the Blood Level. Rabbits, weighing about 2.5 kg, were administered 10 mL of 2% CMC-Na solution containing 500 mg of 1,8-cineole. An accurately measured 8 mL portion of blood was collected in a heparinized capillary tube from the ear vein prior to the administration and at an appropriate time after oral administration of the CMC-Na solution of 1,8-cineole (200 mg/kg). The concentrations of metabolites in plasma were determined 5, 15, 30, 60, 90, 120, 240, and 360 min after administration of 1,8-cineole. The free metabolite fractions were obtained from plasma by the ether extraction. For the quantitative analysis of free metabolites, the gas chromatography method was used, as an internal standard with 1,8-cineole. On the other hand, the conjugated metabolite fractions were obtained from the above treatment plasma by adjusting to pH 4.0 with phosphate buffer and then incubating with β-glucuronidase at 37 °C for 24 h followed by continuous ether extraction for 48 h. These

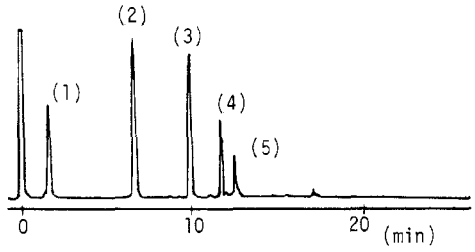


Figure 1. Gas chromatograms of an extract of urine from rabbits dosed with 1,8-cineole [5%-PEG-HT (2.5 mm × 2.25 m), CT 100–200 °C (5 °C/min); N_2 flow 40 mL/min]. See Scheme I.

ether extracts were analyzed by the same method used for the free metabolite fractions.

RESULTS AND DISCUSSION

A gas chromatogram of the urinary extract was shown in Figure 1. Compound **2** was isolated as an unracemate, $[\alpha]^{20}_D$ +13.9° (*c* 0.5, EtOH). Sublimation [80 °C (1 mm)] gave crystals of alcohol **2**, mp 90 °C (Gandini et al., 1972), for a compound called 2-*exo*-cineolylol: mp 94 °C; $[\alpha]^{20}$ −30 to −14.5° (*c* 0.2, EtOH); IR(ν, neat, cm^{-1}) 3410, 1100, 1085, 1050 and 990 (C–OH); MS, *m/z* (%) 170 (M^+, $C_{10}H_{18}O_2$, 12.0), 155 ($M^+ - CH_3$, 1.0), 152 ($M^+ - H_2O$, 3.5), 126 ($M^+ - C_2H_4O$, 96.8), 111 ($M^+ - C_3H_7O$, 45.0), 108 (100.0), 93 (35.2), 82 (23.7); ^{1}H NMR ($CDCl_3$) δ 1.11 (3 H, s, 7-Me), 1.29 (6 H, s, 9,10-Me), 1.40–1.60 (2 H, br s), 1.70–2.20 (5 H, m), 1.89 (1 H, br s, –OH), 3.50 (1 H, br m, 16 Hz, collapses to a triplet on adding D_2O, H6, J(6,5exo), J(6,5endo) = 6.5 hz). The presence of one hydroxy group was suggested from these spectra. The position of the hydroxy group was determined to be C-2 (endo) on the basis of the lanthanide-induced shift experiment (Figure 2). According to the increase in the europium reagent, a methine proton (exo H-2) and methylene proton (endo H-3) on the cyclohexane ring and the C-7 methyl group slanted more sharply than the C-9 and C-10 methyl groups. This result suggests that the metabolized hydroxy group is in exo configuration with respect to the pyrane ring. Hence, this hydroxycineole was determined to be (+)-2-*endo*-hydroxy-1,8-cineole (**2**).

Metabolite **3** was isolated as an unracemate: $[\alpha]^{20}_D$ + 15.0° (*c* 0.2, EtOH); mp 97 °C; MS, *m/z* (%) 170 (M^+, 12.0), 155 (1.0), 152 ($M^+ - H_2O$, 3.2), 126 (96.8), 111 (45.0), 108 (98.9), 93 (26.6), 83 (22.5), 71 (86.0), 43 (100.0); IR (ν_{max}, KBr, cm^{-1}) 3390 (C–OH), 1100, 1140 (C–O–C), 1075 (alcoholic C–O); ^{1}H NMR ($CDCl_3$) δ 1.10 (3 H, s, CH_3 at C-7), 1.19 (3 H, s, CH_3 at C-9), 1.28 (3 H, s CH_3 at C-7), 1.28 (3 H, s, CH_3 at C-10), 2.52 (1 H, m, J(3endo2) = 10 Hz, J(*gem*) = 13 Hz, J(3endo4) = 3 Hz, J(3endo,5endo) = 3 Hz attributed to a *W* conformation (Silverstein et al., 1981), C_3-H endo), 3.75 (1 H, ddd, J(2,3endo) = 10 Hz, J(2,3exo) = 4 Hz, J(2,6endo) = 2 Hz, HCOH); ^{13}C NMR ($CDCl_3$, TMS) δ 71.1 (d), 34.7 (t) (indicating that an OH group is attached to C-2 of 1,8-cineole) (Johnson and Jankowski, 1972). The position of the hydroxy group was determined to be C-2 (exo) on the basis of the lanthanide-induced shift experiment (Figure 2). According to the increase in europium reagent, a methine proton (endo H-2), methylene proton (exo H-3), and methylene proton (exo H-6) on the cyclohexane ring and the C-7 methyl group slanted more sharply than the C-9 and C-10 methyl groups. These results suggest that the metabolized hydroxycineole was determined to be (+)-2-*exo*-hydroxy-1,8-cineole (**3**). The discrimination of structures **2** (endo alcohol) and **3** (exo alcohol) had previously been reported in connection with studies on the stereochemistry of the 1,8-cineole derivative (Gandini et al., 1972). From a comparison between present and previously reported data (Gandini et al., 1972; Macrae et al., 1979), compounds **2** and **3** were identified as unracemic 2-hydroxycineole: i.e., (1*S*,4*R*,6*R*)-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-ol (**2**) and (1*S*,4*R*,6*S*)-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-ol (**3**). Metabolite **4** was isolated as colorless crystals: mp 55.0–56.0 °C; $[\alpha]^{20}_D$ +5.4° (*c* 0.4, EtOH); IR (ν_{max}, KBr, cm^{-1}) 3420 (–OH), 1130, 1100 (C–O–C), 1060 (alcoholic –C–O). The ^{1}H NMR ($CDCl_3$) signals at δ 4.46 (1 H, dd, HCOH) and 1.98–2.20 (2 H, m) [endo proton signal at ca. δ 2.17 collapsed to a doublet with J(*gem*) = 13 Hz on irradiation at δ 4.46 (CH_2–COH)] indicate that the OH group giving ^{13}C NMR signals at δ 64.8 (d) and 42.8 (t) is attached to C-3 of 1,8-cineole. On the other hand, metabolite **5** isolated as a colorless oil was determined to be a stereoisomer of 3-hydroxycineole (**4**) from the following spectral data: $[\alpha]^{20}_D$ +2.8°; IR (ν_{max}, neat, cm^{-1}) 3410 (OH); MS, *m/z* (%) 170 (M^+, 2.5), 155 (46.1), 137 (6.3), 127 (6.1), 126 (2.8), 108 (8.5), 93 (30.0), 87 (8.3), 85 (12.3), 43 (100); ^{13}C NMR δ 70.4

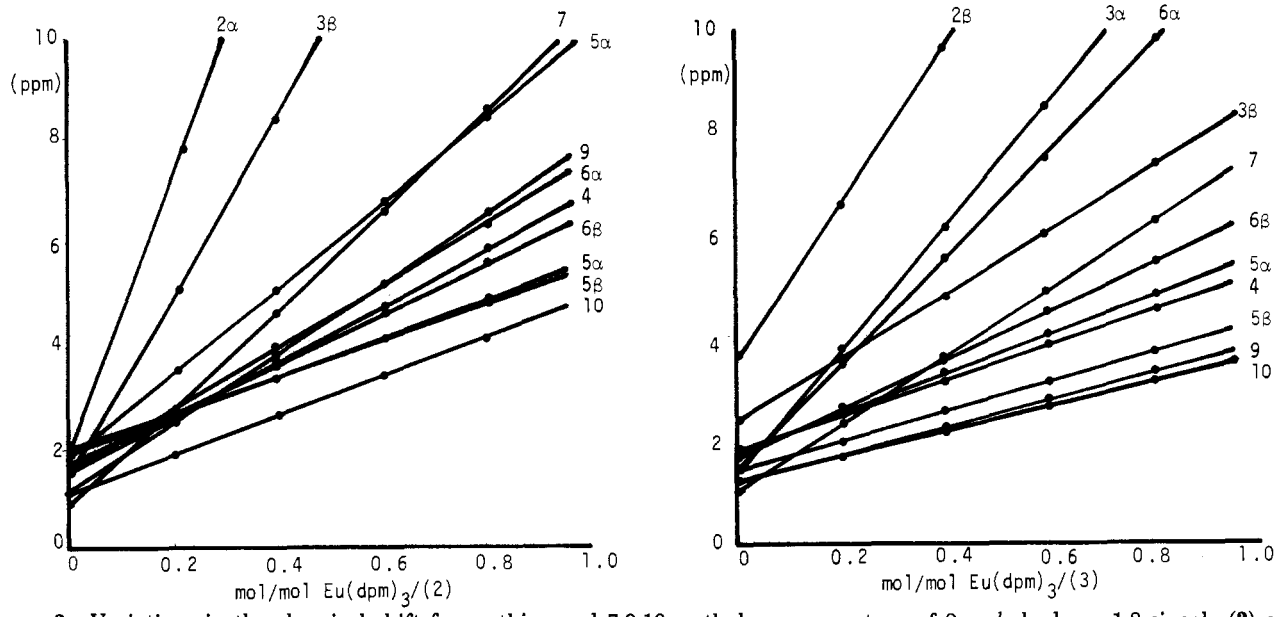


Figure 2. Variations in the chemical shift for methine and 7,9,10-methyl groups protons of 2-*endo*-hydroxy-1,8-cineole (**2**) and 2-*exo*-hydroxy-1,8-cineole (**3**) with increasing concentration of $Eu(DPM)_3$.

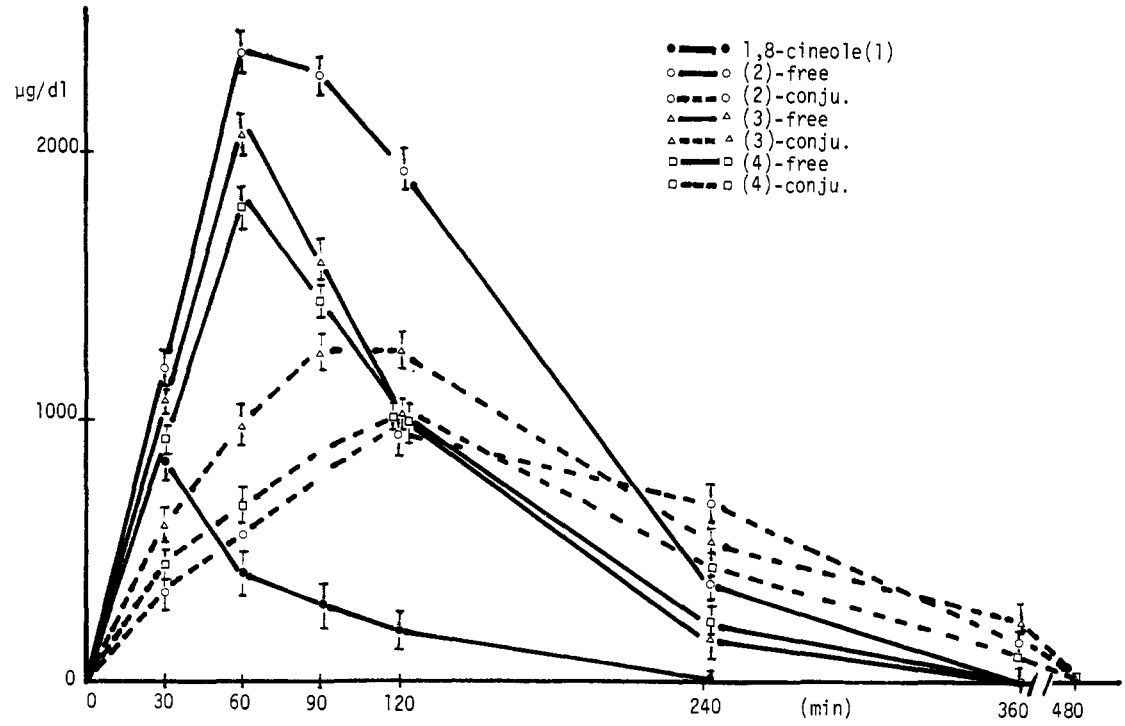


Figure 3. Plasma concentration of 1,8-cineole and metabolites after oral administration. See Scheme I.

(d, CHOH), 43.2 (t, CH_2COH); ^1H NMR δ 4.15 (1 H, ddd, J(3,2exo) = 10 Hz, J(3,2endo) = 6 Hz, J(3,4) = 2. Hz, HCOH), 1.98–2.15 (2 H, m, exo proton signal at ca. δ 2.07 collapses to a doublet with $J(gem)$ = 13 Hz on irradiation at δ 4.15, CH_2-COH). The chemical shift of a proton (H-3) attached to the carbinol carbon of metabolite **4** shows deshielding by the anisotropic effect of an ethereal oxygen as in 2-hydroxycineoles (**2**) while intramolecular hdyrogen bonding of metabolite **5** gives rise to deshielding of C-3 of 1,8-cineole in the ^{13}C NMR. On the basis of those spectral interpretations, compounds **4** and **5** were identified as 3-*exo*-hydroxy-1,8-cineole [(1*S*,4*R*,5*R*)-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-5-ol] and 3-*endo*-hydroxy-1,8-cineole [(1*S*,4*R*,5*S*)-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-5-ol], respectively.

The metabolites in plasma were analyzed by GC and GC–MS. Four metabolites with retention times of 9.0, 13.5, 16.0, and 16.5 min corresponding to those of (+)-2-*endo*-hydroxy-1,8-cineole (**2**), (+)-2-*exo*-hydroxy-1,8-cineole (**3**), 3-*exo*-hydroxy-1,8-cineole, and 3-*endo*-hydroxy-1,8-cineole, respectively, were observed. These metabolites were confirmed further by GC–MS.

The time courses of changes in the mean plasma 1,8-cineole free **2**, free **3**, free **4**, conjugate **2**, conjugate **3**, and conjugate **4** concentrations after administration of 1,8-cineole are shown in Figure 3. Open circles in Figure 3 show the time course of plasma concentration of **3**. The plasma 2-*exo*-hydroxy-1,8-cineole (**3**) concentration reached a maximum of 2400 μg/dL as soon as 1 h after administration of 1,8-cineole and then decreased slowly between 2 and 6 h. Free metabolite **3** and **4** also reached a maximum as soon as 1 h after administration of 1,8-cineole and then decreased. On the other hand, conjugated **3** concentration was higher than that of other conjugated metabolites after adminstration of 1,8-cineole and reached a maximum of 1250 μg/dL at 1.5–2.0 h after administration of 1,8-cineole. Conjugated metabolites **2** and **4** also reached a maximum at 1.5–2.0 h and then decreased slowly between 4 and 8 h. The closed circles in Figure 3 show the time course of the plasma concentration of 1,8-cineole after oral administration. The concentrations of 1,8-cineole 30 min after administration reached a maximum of 840 μg/dL and then decreased slowly between 1 and 4 h.

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Registry No. 1, 470-82-6; 2, 38223-63-1; 3, 38223-62-0; 4, 118013-28-8; 5, 118013-29-9.

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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 β-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 (Na_2EDTA) 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% Na_2EDTA 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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