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Inhibition of Prostaglandin Biosynthesis by 4-*O*-Methylcryptochlorophaeic Acid; Synthesis of Monomeric Arylcarboxylic Acids for Inhibitory Activity Testing and X-Ray Analysis of 4-*O*-Methylcryptochlorophaeic Acid

MASAAKI SHIBUYA, YUTAKA EBIZUKA, HIROSHI NOGUCHI, YOICHI IITAKA, and USHIO SANKAWA*

Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan

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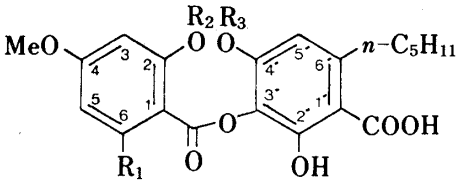
In order to clarify the structure-activity relationship of 4-*O*-methylcryptochlorophaeic acid (**1**), which is a lichen *meta*-depside and a potent inhibitor of prostaglandin (PG) biosynthesis found in our previous screening work, arylcarboxylic acids (**5**—**8**) corresponding to the monomeric moieties of 4-*O*-methylcryptochlorophaeic acid (**1**) were synthesized and tested for inhibitory effect against PG biosynthesis by an enzyme system prepared from rabbit renal medulla. They were a hundred times less active than 4-*O*-methylcryptochlorophaeic acid (**1**), indicating that the dimeric structure of the *meta*-depside is essential for inhibitory activity against PG biosynthesis. Kinetic studies on the mechanism of inhibition revealed that 4-*O*-methylcryptochlorophaeic acid (**1**) inhibits PG biosynthesis competitively with respect to the substrate, arachidonic acid. The three dimensional structure of 4-*O*-methylcryptochlorophaeic acid (**1**), which is expected to have a molecular structure able to fit into an active site that accommodates arachidonic acid, was determined by single crystal X-ray analysis with the direct approach. The obtained structure reveals that 4-*O*-methylcryptochlorophaeic acid (**1**) maintains a rigid conformation by forming a strong hydrogen bond between a hydroxy group and a methoxy group. Based on these findings, a new active site model of fatty acid cyclooxygenase is proposed in order to explain the inhibition by the *meta*-depside and acidic non-steroidal antiinflammatory drugs.

Keywords——prostaglandin; biosynthesis; inhibition; arylcarboxylic acid; synthesis; 4-*O*-methylcryptochlorophaeic acid; X-ray analysis; cyclooxygenase; active site

It is now firmly established that the inhibition of fatty acid cyclooxygenase, the first step of prostaglandin (PG) biosynthesis, is the underlying molecular mechanism of nonsteroidal antiinflammatory drugs such as aspirin and indomethacin (**16**).[1] A positive correlation between the potency of non-steroidal antiinflammatory drugs and their inhibitory activity against PG biosynthesis has been reported by many workers.[2] A bioassay method to test the inhibition of PG biosynthesis should thus be effective for screening biologically active compounds. Since PGs have a variety of biological activities, compounds which inhibit PGs biosynthesis are expected to have various pharmacological activities not limited to antiinflammatory action. In a previous paper,[3] we reported that [6]-gingerol, the main constituent of ginger root, was a strong inhibitor of PG biosynthesis. On the other hand, Aburada *et al.* reported that [6]-gingerol showed antipyretic and analgesic activities.[4] α-Cyperone, an inhibitor of PG biosynthesis contained in the rhizome of *Cyperus rhotundus,* was also shown to inhibit blood platlet aggregation.[5] In the course of their studies to find inhibitors of PG biosynthesis from microbial cultures, Endo and Kitahara isolated tridepsides, thielavins A and B, from a culture of *Thielavis terricola.*[6] Following this finding, lichen depsides and depsidones were tested for inhibitory activity against PG biosynthesis with an enzyme system prepared from rabbit renal medulla.[7] The most potent inhibitors among lichen depsides were 4-*O*-methylcryptochlorophaeic acid (**1**) and merochlorophaeic acid (**2**), which showed IC50 values of 0.34 and 0.43 μM, respectively, indicating that the *meta*-depsides are ten times more potent than indomethacin (**16**). This paper mainly reports the details of synthetic work and

X-ray analysis relevant to the study of the structure–activity relationship of the *meta*-depsides and acidic non-steroidal antiinflammatory drugs.



1: R_1=*n*-C_5H_{11}, R_2=Me, R_3=H
2: R_1=*n*-C_3H_7, R_2=Me, R_3=H
3: R_1=*n*-C_5H_{11}, R_2=Me, R_3=H
4: R_1=*n*-C_3H_7, R_2=H, R_3=Me

In order to clarify whether the dimeric structure of 4-*O*-methylcryptochlorophaeic acid (**1**) is essential for the inhibition of PG biosynthesis, arylcarboxylic acid derivatives (**5**—**8**) corresponding to the monomeric moieties of 4-*O*-methylcryptochlorophaeic acid (**1**) were synthesized by the methods of Korte *et al.*[8] and Elix,[9] starting from 2-octenoic acid and ethyl acetoacetate, as shown in Chart 1. Debromination of ethyl 2,4-dihydroxy-3,5-dibromo-6-pentylbenzoate (**9**) proceeded smoothly on adding sodium acetate. Selective and complete methylation of ethyl 2,4-dihydroxy-6-pentylbenzoate (**11**) with dimethyl sulfate gave the corresponding monomethyl and dimethyl ethers (**12, 13**). The ethyl esters were hydrolyzed with sulfuric acid or potassium hydroxide to give the corresponding arylcarboxylic acids (**6**—**8**). 2-Hydroxy-6-pentylbenzoic acid (**5**) was prepared *via* the 1-phenyl-5-oxytetrazole derivative (**14**). Synthesized arylcarboxylic acids (**5**—**8**) were tested for inhibitory activities against PG biosynthesis. The results, summarized in Table I, demonstrate that R_1 should be hydroxy for inhibitory activity, and the inhibitory activity was the highest when R_2 was hydrogen. Since the highest IC50 values of synthesized arylcarboxylic acids are a hundred times larger than that of 4-*O*-methylcryptochlorophaeic acid (**1**), it is quite clear that the dimeric structure of 4-*O*-methylcryptochlorophaeic acid (**1**) is essential for high inhibitory activity against PG biosynthesis. In order to clarify the inhibition mechanism of the *meta*-depside, a kinetic experiment was undertaken by measuring oxygen uptake in the cyclooxygenase reaction with an enzyme

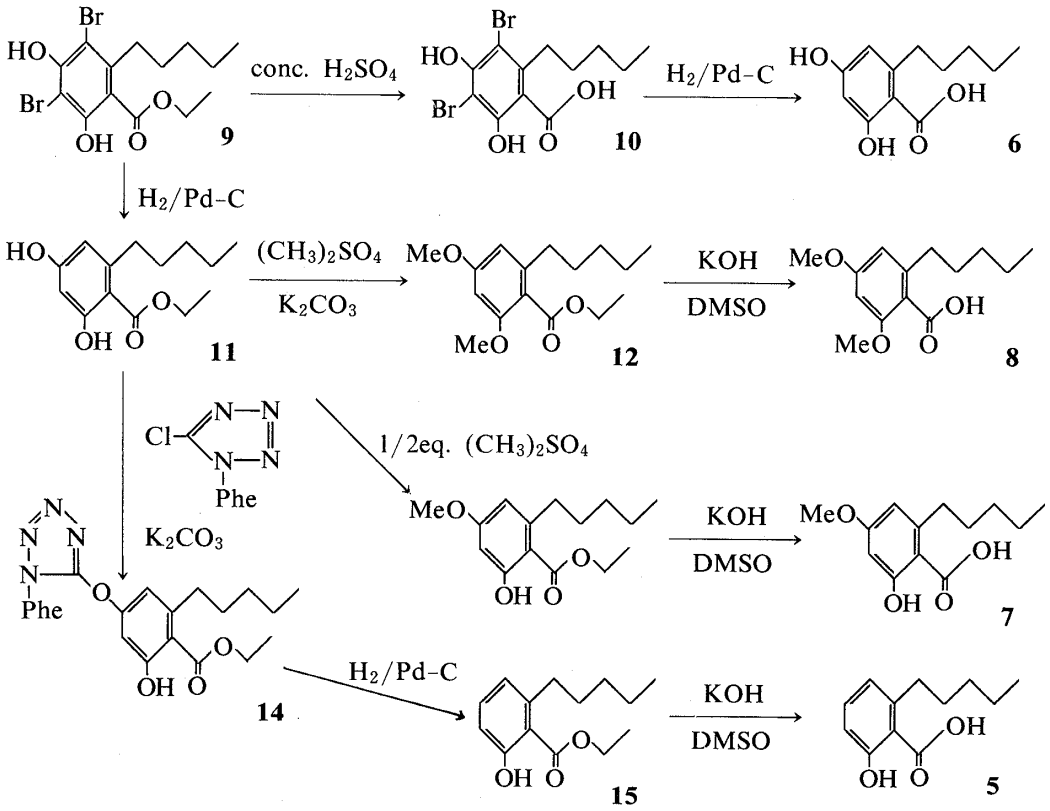
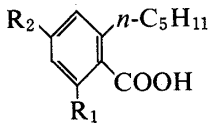


Chart 1

TABLE I. Inhibition of PG Biosynthesis by Monomeric Arylcarboxylic Acids



Compound	IC50 (μM)	R_1	R_2
2-Hydroxy-6-pentylbenzoic acid (**5**)	82	OH	H
2,4-Dihydroxy-6-pentylbenzoic acid (**6**)	160	OH	OH
2-Hydroxy-4-methoxy-6-pentylbenzoic acid (**7**)	310	OH	OCH_3
2,4-Dimethoxy-6-pentylbenzoic acid (**8**)	—[a]	OCH_3	OCH_3

a) 25% inhibition at 3.0 mM.

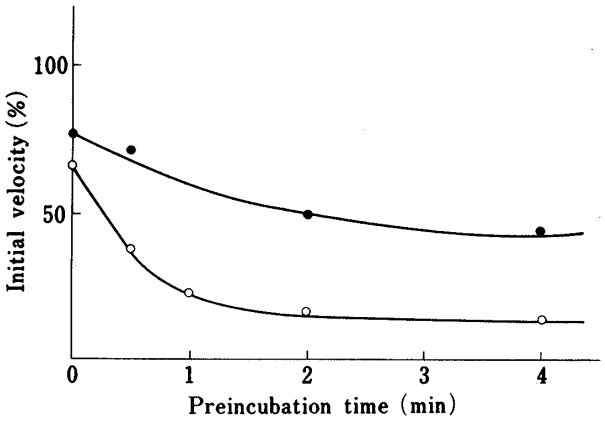


Fig. 1. Time-Dependent Inhibitory Effect

●, 4-*O*-methylcryptochlorophaeic acid 0.34 μM; ○, indomethacin 18 μM.

The enzyme reaction was initiated by adding 218 μM arachidonic acid after the enzyme and inhibitor had been preincubated. Details were given in a previous paper.[7]

system prepared from sheep seminal vesicles.[7] The results of kinetic investigation showed that 4-*O*-methylcryptochlorophaeic acid (**1**) acted competitively with respect to arachidonic acid, if the substrate and the inhibitor were added to the incubation mixture at the same time.[7] However, when the assay mixture was preincubated with 4-*O*-methylcryptochlorophaeic acid (**1**), the enzyme was inactivated in a time-dependent manner, as shown in Fig. 1. This indicates that the depside is an active-site-oriented irreversible inhibitor, like indomethacin.[10] The results of kinetic study suggest that 4-*O*-methylcryptochlorophaeic acid (**1**) acts on the active site of fatty acid cyclooxygenase to inhibit PG biosynthesis. The three dimensional structure of 4-*O*-methylcryptochlorophaeic acid (**1**) was expected to provide useful information for the formulation of a hypothetical complementary receptor site model of cyclooxygenase. We therefore carried out a single crystal X-ray analysis of 4-*O*-methylcryptochlorophaeic acid (**1**). 4-*O*-Methylcryptochlorophaeic acid (**1**) gave crystals suitable for X-ray analysis when it was slowly recrystallized from benzene. The structure was determined by the direct method and refined by the block-diagonal least-squares method to give a final *R* value of 0.107 without

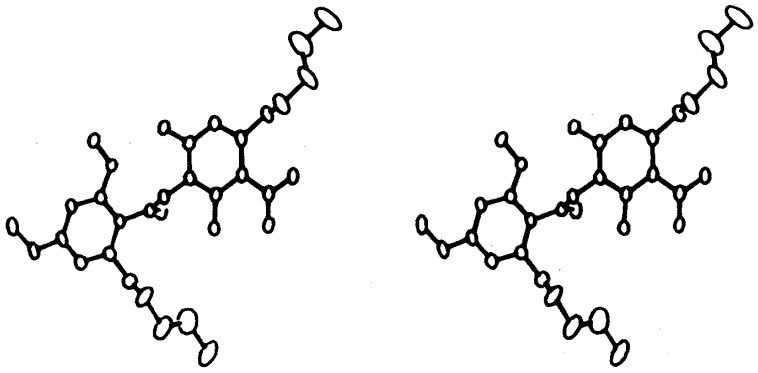


Fig. 2. ORTEP Drawing

hydrogen atoms. The structure (ORTEP drawing) is shown in Fig. 2. The 4′-hydroxy group and the carbonyl of the ester group cannot form a hydrogen bond, since the distance between the two oxygen atoms is 3.69 Å. However, the distance between the oxygen atoms of the 4′-hydroxy and 2-methoxy groups is 2.69 Å and it is evident that these two groups form a strong hydrogen bond to maintain a rigid conformation of 4-*O*-methylcryptochlorophaeic acid (**1**). The corresponding 4′-*O*-methyl derivative (**3**), which lacks a hydrogen bond between the two rings, was far less active than 4-*O*-methylcryptochlorophaeic acid (**1**). Moreover, the 4′-methoxy-2-hydroxy derivative (**4**), which lacks a hydrogen bond between the two rings and cannot maintain the same conformation as 4-*O*-methylcryptochlorophaeic acid (**1**) due to the presence of a hydrogen bond between the 2-hydroxy group and the carbonyl of the ester group, was thirty times less active than merochlorophaeic acid (**2**). Therefore the rigid conformation maintained by the hydrogen bond is very important for high inhibitory activity.

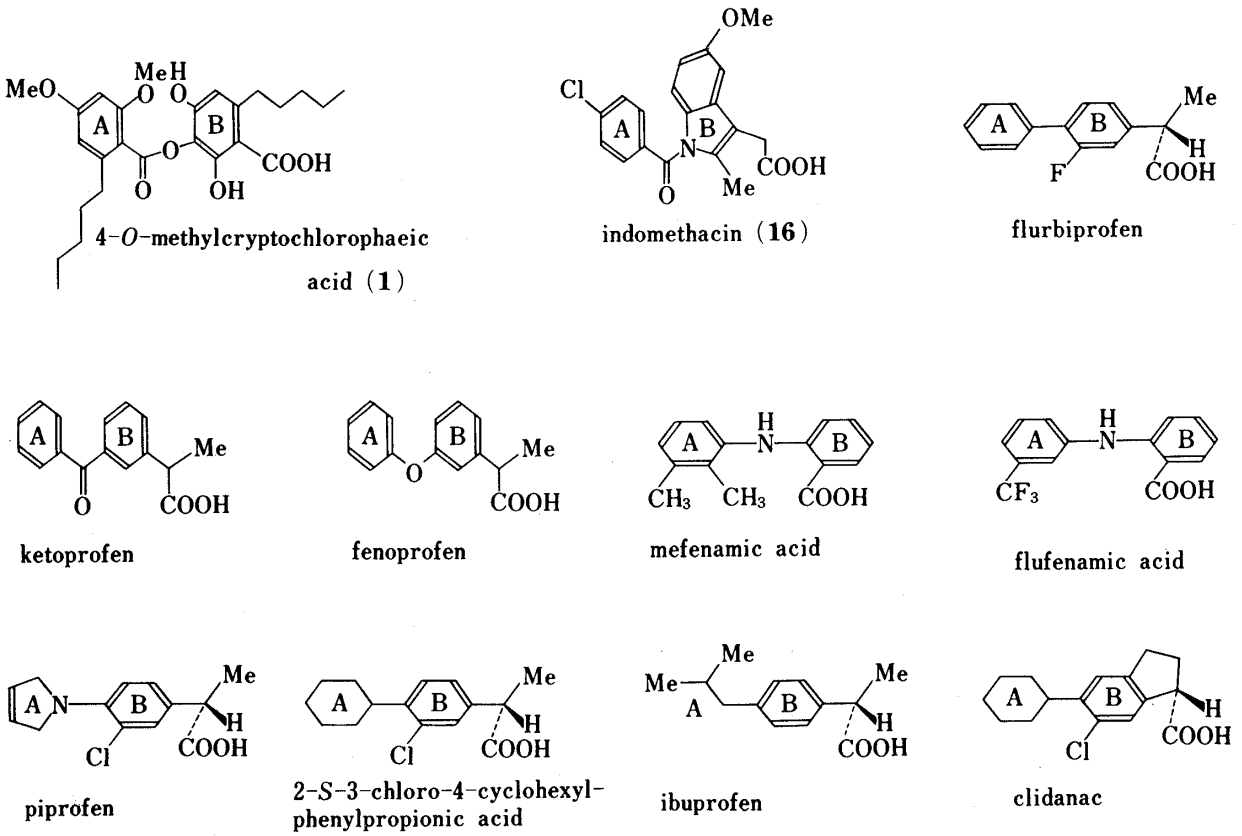


Chart 2

Based on these observations, we proposed a new active site model of fatty acid cyclooxygenase which consists of multiple regions accommodating the A and B rings, and two alkyl groups of 4-*O*-methylcryptochlorophaeic acid (**1**).[7] Acidic non-steroidal antiinflammatory drugs (Chart 2) have the common structural feature that they consist of two moieties, an aromatic ring with a carboxyl group corresponding to the B ring of 4-*O*-methylcryptochlorophaeic acid (**1**), and another aromatic ring or group corresponding to the A ring of 4-*O*-methylcryptochlorophaeic acid (**1**). The A and B groups presumably interact with the A and B regions where the A and B rings of 4-*O*-methylcryptochlorophaeic acid (**1**) would interact when the enzyme reaction is inhibited by 4-*O*-methylcryptochlorophaeic acid (**1**). The A and B regions of this active site model normally accommodate the double bond of arachidonic acid, so that they should have strong affinity for π electrons in aromatic rings or double bonds. In the previous paper,[7] we emphasized that the carboxyl group directly interacts with

an oxygenation site of the B region, resulting in irreversible deactivation of the enzyme. This hypothetical active site model well explains the inhibitory effect on PG biosynthesis of all the antiinflammatory drugs. Recently Humes *et al.* suggested a multiple site model for fatty acid cyclooxygenase, and designated the sites as catalytic and supplemntary sites.[11] They also indicated that the group interacting with the supplementary site is preferably hydrophobic. It is evident from the results so far obtained in our study that the A region corresponds to the supplementary site and the B region to the catalytic site. The multiple region model can well explain the inhibitory action of phenolic compounds,[12] [6]-gingerol,[3] diarylheptanoids,[13] flavonoids,[14] and a newly developped non-steroidal antiinfeammatory drug, 6,11-dihydro-11-oxodibenz[*b*, *e*]oxepin-3-acetic acid.[15] We will discuss this aspect in separate papers.

Experimental

All melting points were determined on a Yanagimoto melting point apparatus (Serial No 345) and are uncorrected. Infrared (IR) spectra were recorded on a JASCO DS-701G spectrophotometer. Mass spectra (MS) were obtained at 15 eV on a JEOL JMS-DX300 mass spectrometer equipped with a JEOL JMA-2000 computer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken at 60 MHz on a Hitachi R-24 or at 100 MHz on a JEOL PS-100 spectrometer with tetramethylsilane as an internal standard. All reagents except for 1-phenyl-5-chlorotetrazole were purchased from Tokyo Kasei Co. 1-Phenyl-5-chlorotetrazole was synthesized according to the method described by Murphy and Kauer *et al.*[16] 2-Hydroxy-6-pentylbenzoic acid (**5**) was synthesized from ethyl 2,4-dihydroxy-6-pentylbenzoate (**11**) by followig the procedure of Inouye *et al.*[17] in which the phenolic hydroxy was removed *via* the 1-phenyl-5-oxytetrazole derivative (**14**). Enzyme assay to measure PG biosynthesis was performed as reported in the previous paper.[7]

2,4-Dihydroxy-3,5-dibromo-6-pentylbenzoic Acid (10)——Ethyl 2,4-dihydroxy-3,5-dibromo-6-pentylbenzoate (**9**)[8] (4 g) and conc. H_2SO_4 (10 ml), were stirred together at room temperature for 1 h. The mixture was poured into ice cold H_2O and extracted with ether. The extract was washed with H_2O and dried over $MgSO_4$. 2,4-Dihydroxy-3,5-dibromo-6-pentylbenzoic acid (**10**) (1.8 g, 48%) was obtained on evaporation of the solvent and was recrystallized from *n*-pentane to give colorless needles, mp 132—133 °C. IR ν_{max}^{KBr} cm^{-1}: 3440, 1640, 1590. ^{1}H-NMR (acetone-d_6) δ: 0.82 (3H, br t, CH_3), 1.2—1.6 (6H, $CH_2\times3$), 2.90 (2H, br t, $ArCH_2$). MS *m/z* (rel. int. %): 384 (M^++4, 7.0), 382 (M^++2, 16.2), 380 (M^+, 8.0), 366 (M^++4−18, 21.9), 364 (M^++2−18, 41.5), 362 (M^+−18, 20.7), 340 (M^++4−44, 23.6), 338 (M^++2−44, 49.4), 336 (M^+−44, 26.6), 284 (M^++4−100, 33.6), 282 (M^++2−100, 50.2), 280 (M^+−100, 25), 190 (M^+−190, 100). *Anal.* Calcd for $C_{12}H_{14}Br_2O_4$: C, 37.77; H, 3.69. Found: C, 38.01; H, 3.74.

2,4-Dihydroxy-6-pentylbenzoic Acid (6)——2,4-Dihydroxy-3,5-dibromo-6-pentylbenzoic acid (**10**) (2 g) was hydrogenated in saturated $NaHCO_3$ solution (10 ml) over 10% Pd-C (1.2 g) at room temperature until uptake of hydrogen gas ceased. The solution was then filtered, poured into cold dil. HCl and extracted with ether. The ethereal solution was washed with H_2O and saturated NaCl solution successively, and dried over $MgSO_4$. Evaporation of the solution gave 2,4-dihydroxy-6-pentylbenzoic acid (**6**) (830 mg, 71%), which was recrystallized from *n*-pentane to give colorless needles, mp 148—149 °C. IR ν_{max}^{KBr} cm^{-1}: 3380, 1625, 1585. ^{1}H-NMR (acetone-d_6) δ: 0.90 (3H, br t, CH_3), 1.1—1.8 (6H, m, $CH_2\times3$), 2.95 (2H, br t, $ArCH_2$), 6.20, 6.28 (each 1H, d, J=2.6 Hz, ArH, C-3, C-5). MS *m/z* (rel. int. %): 224 (M^+, 46.1), 206 (M^+−18, 45.3), 180 (M^+−44, 66.3), 168 (M^+−56, 27.3), 150 (M^+−74, 23.6), 124 (M^+−100, 100). *Anal.* Calcd for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found: C, 64.14; H, 7.26.

Ethyl 2,4-Dihydroxy-6-pentylbenzoate (11)——Ethyl 2,4-dihydroxy-3,5-dibromo-6-pentylbenzoate (**9**)[8] (105 mg) was hydrogenated in AcOH saturated with AcONa (5 ml) over 10% Pd-C (1.2 g) at room temperature until uptake of hydrogen gas ceased. The solution was then filtered, poured into H_2O and extracted with ether. The ethereal solution was washed with H_2O, saturated $NaHCO_3$ solution and saturated NaCl solution successively, and dried over $MgSO_4$. On evaporation of the solvent ethyl 2,4-dihydroxy-6-pentylbenzoate (**11**) was obtained as colorless needles (61 mg, 92%), mp 69 °C. ^{1}H-NMR (acetone-d_6) δ: 0.95 (3H, br t, CH_3), 1.1—1.7 (6H, m, $CH_2\times3$), 1.40 (3H, t, J=7 Hz, $OCH_2\underline{CH_3}$), 2.75—3.05 (2H, br t, $ArCH_2$), 4.5 (2H, q, J=7 Hz, $OC\underline{H_2}CH_3$), 6.20, 6.25 (each 1H, d, J=2 Hz, ArH, C-3, C-5).

Ethyl 2-Hydroxy-6-pentyl-4-(1-phenyl-5-tetrazolyl)-oxybenzoate (14)——Ethyl 2,4-dihydroxy-6-pentylbenzoate (**11**) (7.3 g), 1-phenyl-5-chlorotetrazole[16] (3 g) and anhydrous K_2CO_3 (3.5 g) were stirred together and heated under reflux in acetone (130 ml) for 15 h. The mixture was then cooled, poured into cold dil. HCl and extracted with ether. The extract was washed with water and dried over $MgSO_4$. The oily product left on removal of the solvent was passed several times through a silica gel column with benzene containing 0—2% acetone as an eluent. Ethyl 2-hydroxy-6-pentyl-4-(1-phenyl-5-tetrazolyl)-oxybenzoate (**14**) (3.2 g) was recrystallized from *n*-pentane to give a colorless powder, mp 81—82 °C. IR ν_{max}^{KBr} cm^{-1}: 2920, 1650,

1530, 1260. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, br t, CH_3), 1.1—1.7 (6H, m, $CH_2\times3$), 1.40 (3H, t, J=5.5 Hz, $OCH_2\underline{CH_3}$), 2.84 (2H, br t, $ArCH_2$), 4.38 (2H, q, J=5.5 Hz, $OC\underline{H_2}CH_3$), 6.78, 6.90 (each 1H, d, J=2.5 Hz, ArH, C-3, C-5), 7.4—7.8 (5H, m, ArH), 10.9 (1H, br s, ArOH). MS m/z (rel. int. %): 396 (M^+, 49.2), 322 (M^+-74, 22.6), 236 (M^+-160, 53.8), 190 (M^+-206, 100), 149 (M^+-247, 50.5). *Anal.* Calcd for $C_{21}H_{24}N_4O_4$: C, 63.62; H, 6.10; N, 14.13. Found: C, 63.62; H, 6.00; N, 14.41.

Ethyl 2-Hydroxy-6-pentylbenzoate (15)——Ethyl 2-hydroxy-6-pentyl-4-(1-phenyl-5-tetrazolyl)-oxybenzoate (**14**) (3.2 g) was hydrogenated in AcONa saturated AcOH (10 ml) over 10% Pd-C (1.5 g) at room temperature until uptake of hydrogen gas ceased. The solution was then filtered, poured into water and extracted with ether. The ethereal solution was washed with H_2O, saturated NaCl solution and saturated NaCl solution successively, and dried over $MgSO_4$. Evaporation of this solution gave ethyl 2-hydroxy-6-pentylbenzoate (**15**) as a colorless oil. IR ν_{max}^{cap} cm^{-1}: 2960, 2920, 1660, 1605, 1575. ^{1}H-NMR (acetone-d_6) δ: 0.96 (3H, br t, CH_3), 1.1—1.7 (6H, m, $CH_2\times3$), 1.40 (3H, t, J=7.6 Hz, $OCH_2\underline{CH_3}$), 4.40 (2H, q, J=7.6 Hz, $OC\underline{H_2}CH_3$), 6.70 (2H, d, J=7.6 Hz, ArH, C-3, C-5), 7.22 (1H, t, J=7.6 Hz, ArH, C-4), 10.64 (1H, s, ArOH). MS m/z (rel. int. %): 208 (M^+, 56.8), 190 (M^+-18, 100), 162 (M^+-46, 13.7), 134 (M^+-74, 39.5). High resolution MS: $C_{14}H_{30}O_3$ (M^+: m/z 236.1420, Calcd: 236.1413).

2-Hydroxy-6-pentylbenzoic Acid (5)——A mixture of ethyl 2-hydroxy-6-pentylbenzoate (**15**) (50 mg), KOH (50 mg), H_2O (0.2 ml) and dimethylsulfoxide (0.6 ml) was stirred and heated at 90 °C for 20 h. The solution was then cooled and poured into cold dil. HCl and extracted with ether. The ethereal solution was washed with H_2O and saturated NaCl solution successively, and dried over $MgSO_4$. 2-Hydroxy-6-pentylbenzoic acid (**5**) (35 mg, 80%) was recrystallized from *n*-pentane to give colorless needles, mp 88—89 °C. IR ν_{max}^{KBr} cm^{-1}: 3420, 1645, 1600. ^{1}H-NMR ($CDCl_3$) δ: 0.80 (3H, br t, CH_3), 1.1—1.7 (6H, $CH_2\times3$), 2.88 (2H, br t, $ArCH_2$), 6.68 (2H, d, J=8 Hz, ArH, C-3, C-5), 7.22 (1H, t, J=8 Hz, ArH, C-4). MS m/z

TABLE II. Final Atomic Parameters and Their Standard Deviation ($\times10^4$)

Atom	x	y	z	β_{11} or B	β_{22}	β_{33}	β_{13}	β_{13}	β_{23}
1 C1	1429(3)	4525(4)	4757(5)	5(0)	9(0)	14(1)	−2(0)	2(0)	−2(0)
2 C2	858(3)	3820(4)	5394(5)	5(0)	10(0)	15(1)	−2(0)	2(0)	−1(0)
3 C3	477(3)	2699(4)	4415(5)	5(0)	10(0)	17(1)	−3(0)	1(0)	−3(0)
4 C4	715(3)	2287(4)	2829(6)	6(0)	9(0)	16(1)	−2(0)	1(0)	−3(0)
5 C5	1285(3)	2943(5)	2195(6)	7(0)	11(1)	17(1)	−3(0)	3(0)	−1(0)
6 C6	1644(3)	4091(4)	3158(5)	5(0)	10(0)	16(1)	−2(0)	3(0)	0(0)
7 C7	1748(3)	5792(4)	5784(5)	4(0)	10(0)	16(1)	−2(0)	2(0)	−1(0)
8 O8	1656(2)	6878(3)	5377(4)	7(0)	11(0)	20(1)	−3(0)	0(0)	2(0)
9 O9	2147(2)	5568(3)	7330(4)	5(0)	9(0)	16(1)	−2(0)	1(0)	−2(0)
10 O10	656(2)	4322(3)	6957(4)	6(0)	13(0)	17(1)	−4(0)	4(0)	−4(0)
11 O11	402(3)	1179(3)	1759(4)	8(0)	12(0)	19(1)	−5(0)	2(0)	−6(0)
12 C12	2311(4)	4741(5)	2473(7)	7(0)	15(1)	20(1)	−3(0)	6(0)	1(1)
13 C13	3178(4)	4224(9)	3137(10)	6(0)	34(1)	37(2)	−7(1)	4(1)	7(1)
14 C14	3913(8)	4671(15)	2400(17)	15(1)	56(3)	59(3)	−20(1)	16(1)	−11(3)
15 C15	4149(9)	5865(12)	2966(15)	21(1)	33(2)	49(3)	−13(1)	13(2)	3(2)
16 C16	4974(6)	6297(11)	2334(15)	10(1)	31(2)	68(3)	−9(1)	8(1)	11(2)
17 C17	158(3)	3546(5)	7728(6)	6(0)	16(1)	17(1)	−5(0)	4(0)	−1(1)
18 C18	−229(5)	474(6)	2361(8)	10(0)	16(1)	24(1)	−8(0)	4(1)	−4(1)
19 C1′	3204(3)	8452(4)	10004(5)	4(0)	10(0)	17(1)	−2(0)	1(0)	0(0)
20 C2′	3041(3)	7362(4)	8668(6)	4(0)	10(0)	17(1)	−2(0)	1(0)	0(0)
21 C3′	2302(3)	6710(4)	8542(5)	4(0)	10(0)	16(1)	−2(0)	2(0)	−2(0)
22 C4′	1732(3)	7105(5)	9634(6)	4(0)	12(1)	19(1)	−2(0)	3(0)	−1(1)
23 C5′	1884(3)	8176(5)	10950(6)	5(0)	13(1)	20(1)	−3(0)	3(0)	−3(1)
24 C6′	2611(3)	8857(4)	11146(6)	5(0)	10(0)	17(1)	−2(0)	1(0)	−2(0)
25 C7′	3982(3)	9118(4)	10038(6)	4(0)	11(0)	19(1)	−3(0)	1(0)	−1(1)
26 O8′	4478(2)	8734(4)	9028(5)	5(0)	16(0)	27(1)	−5(0)	3(0)	−6(0)
27 O9′	4138(2)	10165(4)	11216(5)	6(0)	17(0)	29(1)	−6(0)	4(0)	−8(1)
28 O10′	3545(2)	6901(3)	7539(4)	5(0)	13(0)	22(1)	−3(0)	4(0)	−5(0)
29 O11′	1009(2)	6473(4)	9467(5)	5(0)	18(0)	24(1)	−5(0)	4(0)	−5(0)
30 C12′	2720(4)	9977(5)	12663(6)	7(0)	12(1)	19(1)	−3(0)	1(0)	−3(1)
31 C13′	3219(5)	9498(7)	14187(8)	12(1)	17(1)	19(1)	−2(0)	0(1)	−2(1)
32 C14′	3290(8)	10641(9)	15740(9)	19(1)	23(1)	20(1)	−4(1)	4(1)	−8(1)
33 C15′	2554(7)	11015(12)	16538(13)	13(1)	36(2)	41(2)	7(1)	0(1)	−14(2)
34 C16′	2696(8)	12091(11)	18148(11)	19(1)	34(2)	27(2)	8(1)	4(1)	−9(1)

The anisotropic temperature factors are of the form; $T=\exp[-(\beta_{11}h+\beta_{22}k+\beta_{33}l+2\beta_{12}hk+2\beta_{13}hl+2\beta_{33}hl)]$.
The estimated standard deviations are given in parentheses.

(rel. int.%): 208 (M^+, 56.9), 190 (M^+-18, 100), 162 (M^+-44, 13.7), 134 (M^+-74, 39.6). *Anal.* Calcd for $C_{12}H_{16}O_3$: C, 69.21; H,7.74. Found: C, 68.91; H, 7.85.

X-Ray Analysis——Crystal Data for 4-*O*-Methylcryptochlorophaeic Acid (1): $P\bar{1}$ (triclinic); a= 16.2479(8), b=10.1150(5), c=8.1060(4)Å, α=99.26(5), β=98.29(5), γ=94.24(5); V=1261 Å^3; Z=2; Dx= 1.22 cm^{-3}. The diffraction intensities were measurued on a Phillips PW 1100 four-circle diffractometer using Cu$K\alpha$ radiation. The intensities of 3222 reflections with 2θ less than 65° were collected and used in the structural analysis.

Determination of the Structure of 4-*O*-Methylcryptochlorophaeic Acid (1): The structure was solved by the direct method and refined by the block-diagonal least squares method. The final R index without hydrogen atoms was 0.107. The final atomic parameters and their standard deviations are shown in Table II, and bond angles and distances in Fig. 3.

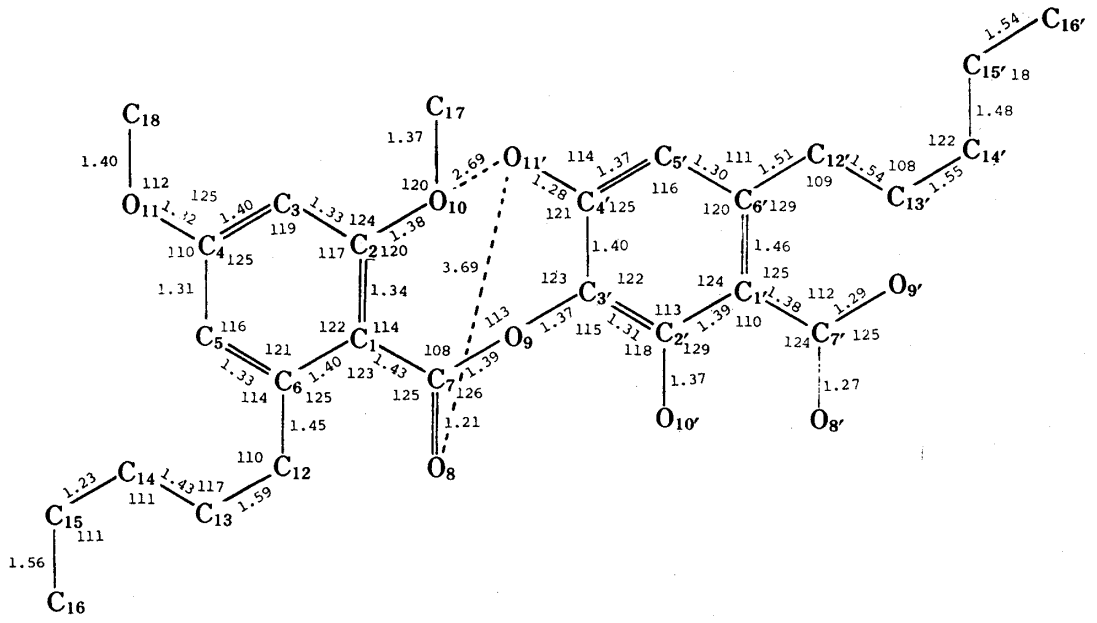


Fig. 3. Bond Angles and Distances

References

1) T. Miyamoto, S. Yamamoto and O. Hayaishi, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3645 (1974).
2) R.J. Flower and J.R. Vane, *Biochem. Pharmacol.*, **23**, 1439 (1974); J. Garcia-Rafanell and J. Forn, *Arzneim.-Forsch.*, **29**, 630 (1979).
3) F. Kiuchi, M. Shibuya and U. Sankawa, *Chem. Pharm. Bull.*, **30**, 754 (1982).
4) M. Aburada, K. Sudo, K. Yuasa, M. Morita and Y. Iketani, Proceedings of the Annual Meeting of the Japanese Society of Pharmacognosy, Nagoya, 1980, p. 42.
5) F. Kiuchi, M. Shibuya and U. Sankawa, Proceedings of the Annual Meeting of the Pharmaceutical Society of Japan, Osaka, 1982, p. 520.
6) N. Kitahara, A. Endo, K. Furuya and S. Takahashi, *J. Antibiot.* (Tokyo), Ser. A, **34**, 1562 (1981).
7) U. Sankawa, M. Shibuya, Y. Ebizuka, H. Noguchi, T. Kinoshita, Y. Iitaka, A. Endo and N. Kitahara, *Prostaglandins*, **24**, 21 (1982).
8) F. Korte and H, Shieper, *Justus Liebigs Ann. Chem.*, **630**, 71 (1960).
9) J.A. Elix, *Aust. J. Chem.*, **27**, 1767 (1974).
10) R.J. Gryglewski, *Adv. Lipid Res.*, **16**, 327 (1978).
11) J.L. Humes, C.A. Winter, S.J. Sadowski and F.A. Kuehl, Jr., *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 2053 (1981).
12) F.E. Dewhirst, *Prostaglandins*, **20**, 209 (1980).
13) F. Kiuchi, M. Shibuya and U. Sankawa, *Chem. Pharm. Bull.*, **30**, 2279 (1982).
14) J.Baumann, F.V. Bruchhausen and G. Wurm, *Prostaglandins*, **20**, 627 (1980).
15) T. Yoshioka, M. Kitagawa, M. Oki, S. Kubo, H. Tagawa, K. Ueno, W. Tsukada, M. Tsubokawa, and A. Kasahara, *J. Med. Chem.*, **21**, 633 (1978).
16) B.B. Murphy, *J. Org. Chem.*, **29**, 1613, (1964); J.C. Kauer and W.A. Sheppard, *J. Org. Chem.*, **32**, 3580 (1967).
17) H. Inouye, S. Ueda, K. Inoue and H. Matsumura, *Phytochemistry*, **18**, 1301 (1979).