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### Studies on the Metabolic Products of *Aspergillus terreus*. III. Metabolites of the Strain IFO 8835. (1)<sup>1)</sup>

NORIKI KIRIYAMA,<sup>2a)</sup> KEIICHI NITTA, YOSHIKI SAKAGUCHI,  
YASUHISA TAGUCHI, and YUZURU YAMAMOTO<sup>2)</sup>

*Faculty of Pharmaceutical Sciences, Kanazawa University<sup>2)</sup>*

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A new type metabolite (I) was isolated from *Aspergillus terreus* var. *africanus* IFO 8835 together with terrein, 3,6-dihydroxytoluquinone, emodin, questin, sulochrin, dihydroerdin, geodin, asterric acid, and aspulvinone D. The structure of I was determined as  $\alpha$ -oxo- $\beta$ -(*p*-hydroxyphenyl)- $\gamma$ -(*p*-hydroxy-*m*-3,3-dimethylallylbenzyl)- $\gamma$ -methoxycarbonyl- $\gamma$ -butyro-lactone by chemical and physical experimental data. Compound I was biosynthesized by intact condensation of two phenylpropanoids between carbons 2 and 3'.

**Keywords**—new metabolite; isolation; structure; biosynthesis; *Aspergillus terreus* IFO 8835

As reported in the previous papers, tryptophan-derived purple benzoquinone named asterriquinone was isolated from *Aspergillus terreus* IFO 6123,<sup>3)</sup> and its antitumor activity was demonstrated.<sup>4)</sup> In the course of the investigation of analogous quinones, many kinds of metabolites were isolated from *Aspergillus terreus* var. *africanus* IFO 8835. This paper deals with the metabolites except quinones which will be reported in the following papers.

This fungus was surface-cultured on a malt extract medium at 27° for 14 days. The culture filtrate was concentrated and extracted with ethyl acetate. The extract was dissolved in dichloromethane and then concentrated. The resulting asterric acid was filtered off and the mother liquor was applied on a column of oxalic acid-precoated silicagel. By elution with dichloromethane, 3,6-dihydroxytoluquinone, emodin, and geodin were isolated. Then, by elution with the mixture of dichloromethane and ethyl acetate, asterric acid, questin, sulochrin, dihydroerdin, and terrein were obtained. The yields of geodin and dihydroerdin were remarkably increased in the cultivation on a medium supplemented with sodium chloride. These metabolites were identified by comparison with the authentic samples in physical and chemical properties.

The dried mycelium was extracted with petroleum ether, and ether, successively. The petroleum ether extract gave ergosterol and purple quinones.

The ether extract was chromatographed on oxalic acid-precoated silicagel. By elution with benzene, deep purple metabolites were obtained together with yellow oil. Further elution with benzene containing ethyl acetate gave aspulvinone D as yellow prisms, mp 257—259°, Compound I as colorless prisms, mp 94—96° (dec.), and sulochrin, mp 253—256°, in this order. Aspulvinone D was identified with the authentic sample which had been isolated from *Aspergillus terreus* IAM 2054 by Ojima and Seto.<sup>5)</sup>

1) Part II: N, Kiriyaama, Y. Higuchi, and Y. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **25**, 1265 (1977).

2) Location: Takaramachi 13-1, Kanazawa, 920, Japan; a) Present address: Faculty of Pharmaceutical Sciences, Hokuriku University, Kanagawa-cho, Kanazawa, 920-11, Japan.

3) Y. Yamamoto, K. Nishimura, and N. Kiriyaama, *Chem. Pharm. Bull.* (Tokyo), **24**, 1853 (1976).

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5) a) N. Ojima, I. Takahashi, K. Ogura, and S. Seto, *Tetrahedron Lett.*, **1976**, 1013; b) N. Ojima, S. Takenaka, and S. Seto, *Phytochemistry*, **14**, 573 (1975); *idem, ibid.*, **12**, 2527 (1973); c) M.J. Begley, D.M. Knight, and G. Pattenden, *Tetrahedron Lett.*, **1976**, 131. Aspulvinone D was kindly supplied by Prof. S. Seto, Tohoku University.

Compound I was weakly acidic and soluble in sodium carbonate, but insoluble in sodium bicarbonate solution. The chemical formula was determined as  $C_{24}H_{24}O_7$  by elementary analysis and mass spectrum (MS) ( $M^+$   $m/e$ : 424). It had optical activity. It was fairly unstable and decomposed gradually to gummy compound even at room temperature, and molecular peak in MS was not recognized at elevated temperature above  $130^\circ$ . In the infrared (IR) spectrum in chloroform solution, several types of hydroxyl groups ( $3565$ ,  $3480$ , and  $3280\text{ cm}^{-1}$ ) and carbonyl groups ( $1755$  and  $1740\text{ cm}^{-1}$ ) were recognized. These carbonyl bands were not resolved in KBr method. Compound I afforded a trimethyl derivative (II) by methylation with diazomethane in methanol.

Proton nuclear magnetic resonance (NMR) spectrum of Compound I showed the presence of 3,3-dimethylallyl group, 1,4- and 1,2,4-substituted aromatic rings, O-methyl group, and methylene group which appeared as AB-type pattern.

When Compound I was treated with alcoholic hydrochloric acid at  $70^\circ$ , it afforded a cyclic ether (III), mp  $83\text{--}84^\circ$  ( $M^+$   $m/e$ : 424), which suggested the hydroxyl group and the dimethylallyl group were located at *ortho*-position in an aromatic ring. These properties resembled aspulvinones, but ultraviolet (UV) spectra were quite different (*cf.* Table I).

The cyclic ether (III) was hydrolyzed to an acid (IV),  $C_{23}H_{22}O_7$  ( $M^+$   $m/e$ : 410), mp  $169\text{--}170^\circ$  by mild treatment with dilute alkali. Therefore, one of the two carbonyl groups of I [ $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta_c$ :  $170.3$  and  $171.5$ ] could be assigned to methyl ester ( $1740\text{ cm}^{-1}$ ) and the other to a lactone ( $1755\text{ cm}^{-1}$ ).

When Compound I was heated at  $190\text{--}200^\circ$  with pyridine hydrochloride under nitrogen, it afforded colorless prisms (V),  $C_{22}H_{22}O_5$ , mp  $229\text{--}231^\circ$  (dec.). This compound was also obtained from the cyclic ether (III) or the carboxylic acid (IV) by the same procedure. Compound V had no optical activity. It gave dimethyl ether (VI) with diazomethane in methanol. In NMR spectrum, Compound V showed the new signal of methine group in fairly low magnetic field ( $\delta$ ,  $5.60$ ), which was coupled with methylene group ( $\delta$ ,  $3.10$ ) as a typical ABX-pattern. In  $^{13}\text{C}$  NMR spectrum, the signal of quaternary carbon ( $\delta_c$ ,  $86.8$  in I,  $86.3$  in III) was not recognized, but a new signal of methine group at  $\delta_c$   $79.5$  (doublet) was observed. These results showed that Compound I had a quaternary carbon bound to a methylene, a methoxycarbonyl group, and an oxygen atom. The reaction from I to V involved the formation of an ether ring, demethoxycarbonylation, and racemization.

Compound I was hydrogenated with palladium to a dihydro-derivative (VII), mp  $87\text{--}88^\circ$ , by saturation of dimethylallyl group, but there was no change in UV absorption ( $310\text{ nm}$ ).

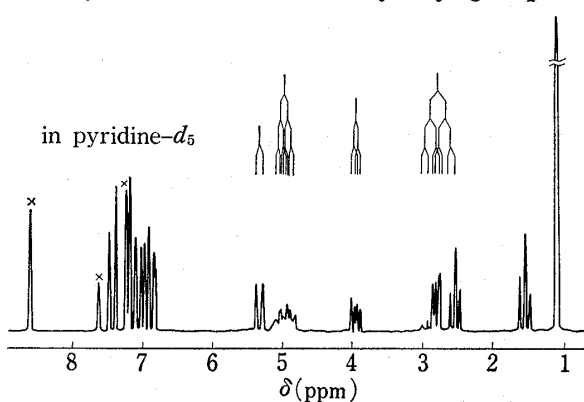


Fig. 1. NMR Spectrum of Dihydro-derivative of V (VIII)

Compound V and its dimethylether (VI) were hydrogenated with platinum oxide to the corresponding dihydro-derivatives, VIII, mp  $210\text{--}211^\circ$  and IX, mp  $131\text{--}132^\circ$ . The UV absorptions were shifted to shorter wave length (both to *ca.*  $290\text{ nm}$ ), and the carbonyl bands in IR spectrum (KBr) were shifted to higher wave number (to  $1775$  in VIII and to  $1770\text{ cm}^{-1}$  in IX). These results suggested the presence of an unsaturated five-membered lactone ring in Compound I, and the double bond was conjugating with an aromatic ring.

The NMR spectra of these hydro-derivatives gave important informations. By spin-spin decoupling among the methylene and methine groups, partial structure of  $\text{Ph}-\text{CH}_2-\text{CH}-\text{CH}-\text{CH}-\text{CO}-$  could be assigned to the hydro-derivatives, in which the centered methine group  $\text{ph O}$

TABLE I

Compound	Structure	UV $\lambda_{\max}$ nm (log $\epsilon$ ) (Solvent)	IR $\nu_{\max}$ cm <sup>-1</sup> (Method)	<sup>13</sup> C NMR $\delta_c$						
				C <sub>1</sub> /C <sub>6</sub>	C <sub>2</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>7</sub> (Solvent)		
Compound I		223 (4.18) 309 (4.31) (EtOH)	1755 1720 1740 (KBr) (CHCl <sub>3</sub> )	171.5 170.3	139.5	86.8	39.6	53.8 (CD <sub>3</sub> OD)		
Compound V		223 (4.22) 305 (4.31) (EtOH)	1775 1740 1725 1705 (THF) (KBr)	169.6	137.3	79.5	39.9	— (Acetone-d <sub>6</sub> )		
Compound X <sup>a)</sup>		290 (4.24) (EtOH)	1770 (sh) 1742 (KBr)	169.3 170.0	138.8	86.0	39.2	54.5 (CDCl <sub>3</sub> )		
Compound XI <sup>a)</sup>		288 (4.31) (EtOH)	1738 (KBr)	171.1	139.9	79.9	40.3	— (CD <sub>3</sub> OD)		
Vulpic acid <sup>b)</sup>		293 (4.41) 376 (3.96) (MeOH)	1770 1680 (KBr)							
Aspulvinone D <sup>a)</sup>		245 (4.28) 377 (4.47) (MeOH)	1720 (KBr)							
Dihydrovulpic acid <sup>b)</sup>		261 (MeOH)	1730 1710 (KBr)							
Isodihydrovulpic acid <sup>b)</sup>		262 (MeOH)	1755 1740 (KBr)							

Carbon No. for <sup>13</sup>C NMR:

R<sup>1</sup> = HO- R<sup>2</sup> = R<sup>3</sup> =   
R<sup>4</sup> = R<sup>5</sup> =

a) Compound X and the related compounds were the gift of Prof. T. Miwa, Osaka City University.

b) These samples were kindly supplied by Prof. Y. Arata, Kanazawa University.

might attach to an aromatic ring. From these results, it was suggested that Compound I was a derivative of  $\alpha$ -oxo- $\beta$ -phenyl- $\gamma$ -benzyl- $\gamma$ -methoxycarbonyl- $\gamma$ -butyrolactone. This type of the compounds had not been isolated from natural sources, but several compounds such as Compound X had been synthesized from phenylpyruvic acid or its derivatives.<sup>6)</sup> The spectral data of these compounds and pulvinone derivatives are listed in Table I. The properties of Compound I and V had similarity to X, but not to the corresponding pulvinones.

The methoxycarbonyl group of Compound X was removed with hydrochloric acid in acetic acid as reported by Sakan and Miwa.<sup>6b)</sup> The same reaction also proceeded smoothly by heating with pyridine hydrochloride.

Compound VIII was reduced with lithium aluminum hydride at room temperature to give an acetal (XII),  $C_{22}H_{26}O_5$ , mp 170—172° and a glycol (XIII),  $C_{22}H_{28}O_5$ , mp 213—215°. When this reaction was performed under reflux in tetrahydrofuran, only the glycol (XIII) was obtained. By oxidation with periodate, Compound XIII gave formaldehyde and an aldehyde (XIV),  $C_{21}H_{24}O_4$ .

Oxidative degradations gave various results according to the reaction conditions. When dimethylether (VI) was oxidized with potassium permanganate in the presence of magnesium sulfate at room temperature, *p*-anisic acid, mp 184—186°, and a ketol (XV),  $C_{21}H_{24}O_4$ , were obtained. The ketol (XV) was cleaved with periodate to *p*-anisic acid and 2,2-dimethylchroman-6-acetaldehyde (XVI) (colorless oil, 2,4-dinitrophenylhydrazone, mp 145°). Compound VI was oxidized with permanganate in acetone under reflux to *p*-anisic acid, 2,2-dimethylchromanone-6-carboxylic acid (XVII), mp 183—184°, and 4-(1-carboxy-isopropoxy)-isophthalic acid (XVIII), mp 290—291°. The structures of the above compounds were determined by their spectral properties and elementary analyses.

Trimethylether (II) was strongly oxidized with potassium permanganate in aqueous solution to give 4-methoxy-isophthalic acid, mp 261—263° (dec.) which was identified by comparison with the sample obtained from 5-methylsalicylic acid.

Ozonolysis of this series of compounds was not so fruitful, but Compound I gave *p*-hydroxybenzoic acid, and Compound V gave an  $\omega$ -benzylacetophenone derivative (XIX), mp 69—70° by reductive degradation of the ozonide with zinc in acetic acid.

From the experimental results above, the chemical structure of Compound I was determined as  $\alpha$ -oxo- $\beta$ -(*p*-hydroxyphenyl)- $\gamma$ -(*p*-hydroxy-*m*-3,3-dimethylallylbenzyl)- $\gamma$ -methoxycarbonyl- $\gamma$ -butyrolactone (I). The reactions are summarized in Chart 1.

The metabolites isolated from this fungus were interesting in the biogenetic points of view:

1) The co-existence of many types of metabolites which derived from different biosynthetic precursors, namely, isoprenoid from mevalonate, polyketide, and aromatic amino acids from shikimate.

2) There were a number of polyketide-derived metabolites, namely, anthraquinones such as emodin and questin; benzophenone derivatives such as sulochrin and dihydroerdin; spiran type compound such as geodin; and diphenylether derivative such as asteric acid were found in the same time. The biosynthetic pathway from benzophenone to diphenylether through spirocoumarin had been already confirmed.<sup>7)</sup> However, there are two types of speculations concerning sulochrin (benzophenone) biosynthesis.<sup>8)</sup> One is the condensation of two polyketide chains by Tatum,<sup>9)</sup> Aghoramurthy,<sup>10)</sup> and Curtis,<sup>11)</sup> and the other is one

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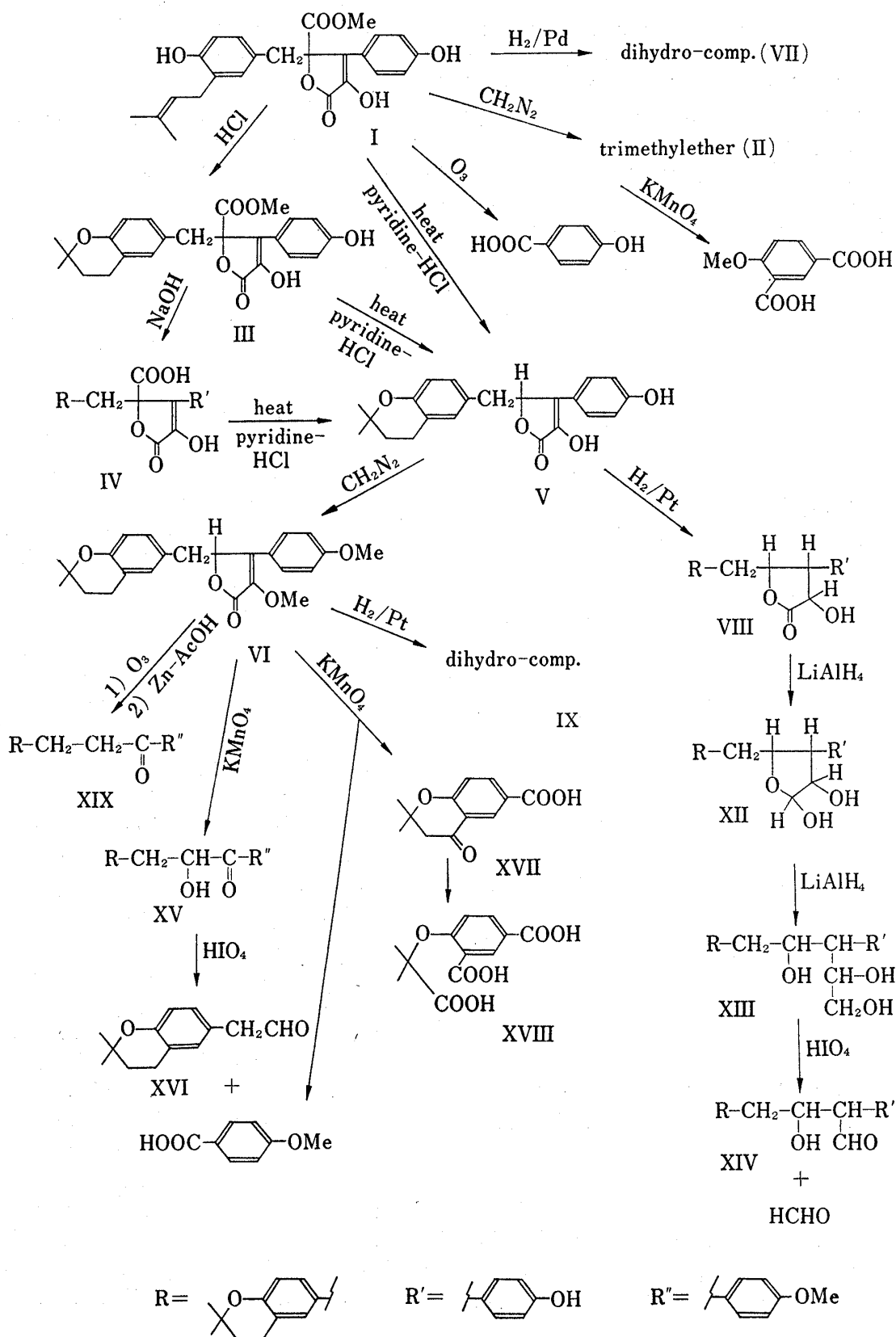


Chart 1

chain theory *via* anthraquinone by Gatenbeck,<sup>12)</sup> Mahmoodian,<sup>13)</sup> and Curtis.<sup>14)</sup> In our studies, anthraquinone such as emodin and questin were isolated together with other metabolites including sulochrin and dihydroerdin at the same time, so we support the single polyketide-chain theory *via* anthraquinone at least in this fungus.

3) This fungus produced two different types of compounds such as aspulvinone D and Compound I. The biosynthesis of pulvinones had been studied by Mosbach<sup>15)</sup> and Maass,<sup>16)</sup> and was found to be synthesized from phenylpropanoid (phenylalanine or phenylpyruvate) *via* terphenyl benzoquinone. Since a symmetrical intermediate was involved in this course, the carbons originated from phenylalanine-1- and 2-C were randomized and distributed equally in four carbons as shown in Chart 2. Aspulvinones were shown to be biosynthesized from pulvinones by isoprenylation in the last step by Ojima and Seto.<sup>5a)</sup> Compound I is also supposed to be derived from phenylpropanoids and isoprenyl unit as well as aspulvinones. In

fact, mevalonate-2-<sup>14</sup>C was incorporated into Compound I in 7.9% yield, and phenylalanine-1- and 2-<sup>14</sup>C were much more efficiently incorporated (38% and 37%, respectively). The distribution of radioactivity in Compound I was determined by chemical degradation. In the case of phenylalanine-1-<sup>14</sup>C, Compound I was de-methoxycarbonylated with pyridine hydrochloride. The liberated carbon dioxide had 47% of the total radioactivity, and the remained moiety (Compound V) kept another 50% of the total activity. Compound V was further degraded into formaldehyde and Compound XIV. The formaldehyde had 44% of the total radioactivity, but Compound XIV had no activity. In the experiment using phenylalanine-2-<sup>14</sup>C, Compound XIV kept all the radioactivity. As shown in the experiments using phenylalanine-1-<sup>14</sup>C and 2-<sup>14</sup>C, the radioactivity in Compound I was located strictly in two carbons without randomization. These results showed Compound I was biosynthesized by intact condensation of two phenylpropanoids between position 2 and 3' followed by lactonization as shown in

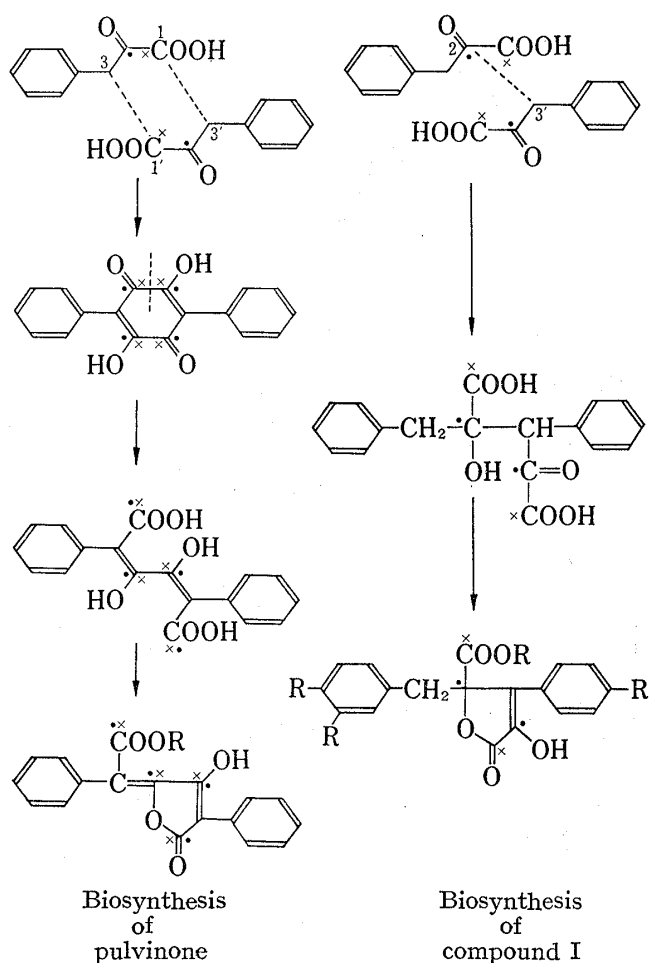


Chart 2

Chart 2. This mechanism is much different from the biosynthesis of pulvinones and aspulvinones, which involved condensation in two points (between 1 and 3', 1' and 3) to terphenyl benzoquinone. It is very interesting that different type-metabolites are biosynthesized simultaneously from common precursors by different condensation mechanisms.

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Experimental<sup>17)</sup>

**Cultural Conditions**—*Aspergillus terreus* var. *africanus* IFO 8835 was cultivated stationarily in 500 ml Roux flasks containing 200 ml of the culture medium: glucose, 20 g; malt extract (Difco), 20 g; polypeptone (Daigo-Eiyo), 1 g; tap water, 1 l. After cultivation at 27° for 14 days, the culture was harvested.

**Isolation of Metabolites from the Culture Filtrate**—Six liters of the culture filtrate was concentrated to one tenth under reduced pressure, and extracted with AcOEt at pH 3. The solvent was evaporated and the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub>. The solution was concentrated, and precipitated asterric acid, mp 211° was filtered off. The mother liquor was chromatographed on a column of oxalic acid-precoated silica gel<sup>18)</sup> (2.5 × 65 cm). By elution with CH<sub>2</sub>Cl<sub>2</sub>, 3,6-dihydroxytoluquinone, mp 179° (13 mg), emodin, mp 257° (4 mg), and geodin, mp 236° (trace amount) were isolated. AcOEt was then added stepwisely to the elution solvent (3, 5, 7, and 10–20% AcOEt in CH<sub>2</sub>Cl<sub>2</sub>) to elute the following compounds: From the 3% fraction, asterric acid (340 mg); from the 5% fraction, questin as orange needles, mp 300° (12 mg) and sulochrin, mp 256° (60–120 mg); from the 7% fraction, dihydroerdin, mp 232° (10 mg); and from the 10–20% fraction, terrein, mp 127° (430 mg). When the fungus was grown on the malt extract medium supplemented with NaCl (1%), the yield of geodin and dihydroerdin increased remarkably (260 mg and 300 mg, respectively).

**Isolation of Metabolites from Mycelium**—The harvested mycelium was dried (50 g from 6 l culture medium), powdered and extracted in Soxhlet apparatus with petr. ether, and then ether. The ether extract was chromatographed on a column of oxalic acid-precoated silica gel (3.5 × 60 cm). Purple quinones were obtained by benzene elution together with yellow oil. AcOEt was added stepwisely (5%, 10%, and then 15%) to the elution solvent. The first fraction gave yellow prisms, C<sub>27</sub>H<sub>28</sub>O<sub>6</sub>, mp 257–259° (aspulvinone D) by crystallization from MeOH (36 mg). The second brownish-yellow fraction (eluted with 10% AcOEt) was evaporated, and the oily residue was crystallized from CH<sub>2</sub>Cl<sub>2</sub> to colorless prisms (Compound I), mp 94–96° (dec.) (2.24 g). From the mother liquor, sulochrin, mp 256° was isolated by rechromatography (17 mg).

**Aspulvinone D**—*Anal.* Calcd. for C<sub>27</sub>H<sub>28</sub>O<sub>6</sub>: C, 72.30; H, 6.29. Found: C, 72.15; H, 6.31. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 246 (4.34), 378 (4.55). IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 1720, 1600.

**Compound I**—The melting point of this compound was not clear. It began to melt at 74° and decomposed at 94–96°.  $[\alpha]_{\text{D}}^{25} = +100^{\circ}$  ( $c=1$ , EtOH). *Anal.* Calcd. for C<sub>24</sub>H<sub>24</sub>O<sub>7</sub>: C, 67.91; H, 5.70; M<sup>+</sup>,  $m/e$  424.144. Found: C, 67.38; H, 5.76; M<sup>+</sup>,  $m/e$  424.150. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 223 (4.18), 309 (4.31). NMR<sup>19)</sup> (CDCl<sub>3</sub>)  $\delta$ : 1.62, 1.67 (each s, CH<sub>3</sub>), 3.13 (d,  $J=7$  Hz, CH<sub>2</sub>), 3.47 (center of AB-pattern,  $J=14$  Hz, CH<sub>2</sub>), 3.82 (s, OCH<sub>3</sub>), 5.06 (t,  $J=7$  Hz, -CH=), 5.34 (2 OH), 6.12 (OH), 6.5 (m, 3H, aromatic H), 6.78, 7.50 (each d,  $J=8$  Hz, 2H aromatic H). MS  $m/e$ : 424 (M<sup>+</sup>), 380, 348, 320, 291, 175, 131, 91, 69, etc.

**Trimethylether of I (II)**—To the solution of I (2.0 g) in MeOH (20 ml), large excess of CH<sub>2</sub>N<sub>2</sub> in ether was added and kept overnight. II was purified by silica gel chromatography (elution with CHCl<sub>3</sub>) to colorless oil (1.4 g). IR  $\nu_{\text{max}}^{\text{film}}$  cm<sup>-1</sup>: 1765, 1745. NMR (CDCl<sub>3</sub>)  $\delta$ : 1.56, 1.65 (each s, CH<sub>3</sub>), 3.10 (d,  $J=7.5$  Hz, CH<sub>2</sub>), 3.45 (center of AB-pattern,  $J=14.5$  Hz, CH<sub>2</sub>), 3.73 (s, OCH<sub>3</sub>), 3.78 (s, 2 OCH<sub>3</sub>), 3.84 (s, OCH<sub>3</sub>), 5.0 (t,  $J=7.5$  Hz, -CH=), 6.6 (m, 3H, aromatic H), 6.91, 7.55 (each d,  $J=8$  Hz, 2H, aromatic H).

**Formation of Cyclic Ether (III)**—I (500 mg) in EtOH (20 ml) and conc. HCl (5 ml) was warmed at 70° for 30 min. After evaporation of the solvent, the reaction mixture was extracted with ether to obtain colorless plates (from benzene), mp 83–84°. *Anal.* Calcd. for C<sub>24</sub>H<sub>24</sub>O<sub>7</sub> · 1.5 C<sub>6</sub>H<sub>6</sub>: C, 73.18; H, 6.15. Found: C, 73.66, 72.60; H, 6.15, 6.13.  $[\alpha]_{\text{D}}^{25} = +72^{\circ}$  ( $c=1$ , MeOH). UV  $\lambda_{\text{max}}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 227 (4.16), 290 (4.13), 309 (4.26). IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  cm<sup>-1</sup>: 1757, 1740. NMR (CDCl<sub>3</sub>)  $\delta$ : 1.24 (s, 2 CH<sub>3</sub>), 1.68, 2.57 (each t,  $J=7$  Hz, CH<sub>2</sub>), 3.47 (center of AB-pattern,  $J=15$  Hz, CH<sub>2</sub>), 3.72 (s, OCH<sub>3</sub>), 6.45 (m, 3H, aromatic H), 6.82, 7.53 (each d,  $J=9$  Hz, 2H, aromatic H).

**Hydrolysis of III**—III (424 mg) was dissolved in 1.5 N NaOH (2.0 ml, 3 equivalents) and kept at room temperature under N<sub>2</sub> gas for 3 hr. The reaction mixture was acidified and extracted with ether. The product was crystallized from CHCl<sub>3</sub> to give colorless prisms (IV), mp 169–170° (380 mg). This acid did not give correct analysis owing to hygroscopic property. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 224 (4.25), 291 (4.25), 305 (4.31). IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 1738, 1703.  $[\alpha]_{\text{D}}^{25} = +88^{\circ}$  ( $c=1$ , EtOH). NMR (acetone-*d*<sub>6</sub>)  $\delta$ : 1.24 (s, 2 CH<sub>3</sub>), 1.72, 2.60 (each t,  $J=7$  Hz, CH<sub>2</sub>), 3.49 (s, CH<sub>3</sub>), 6.55 (m, 3H, aromatic H), 7.00, 7.76 (each d,  $J=9$  Hz, 2H, aromatic H), 9.0 (b, 3 OH).

**Formation of V from I**—Freshly prepared pyridine-HCl (17.5 g) and I (500 mg) was heated at 190–200° for 1 hr under N<sub>2</sub> atmosphere. Water was added to the mixture and extracted with ether. The solvent was evaporated and the residue was crystallized from benzene to obtain colorless prisms (V), mp 229–231° (370 mg). In the degradation experiments using <sup>14</sup>C-compounds, the liberated CO<sub>2</sub> was collected as BaCO<sub>3</sub>.

17) All melting points are not corrected.

18) Silica gel (for chromatography, Kanto Chemical Co., Ltd.) was suspended in 0.1 M oxalic acid overnight, filtered, washed with H<sub>2</sub>O, and dried in an oven at 100°.

19) Abbreviation in the description of NMR data: s, singlet; d, doublet; t, triplet; m, multiplet; dd, double-doublet; b, broad, etc.

*Anal.* Calcd. for  $C_{22}H_{22}O_5$ : C, 72.11; H, 6.05. Found: C, 72.40, 71.69; H, 6.18, 5.96. UV  $\lambda_{\max}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 223 (4.22), 291 (4.27), 305 (4.31).  $[\alpha]_D^{25} = 0^\circ$  ( $c=5$ , EtOH). NMR (acetone- $d_6$ )  $\delta$ : 1.27 (s, 2  $\text{CH}_3$ ), 1.75, 2.68 (each t,  $J=7$  Hz,  $\text{CH}_2$ ), 3.10 (center of AB-part of ABX-pattern,  $J=11.5$ , 6.5, and 3.5 Hz,  $\text{CH}_2$ ), 5.60 (X-part of ABX-pattern,  $J=6.5$  and 3.5 Hz, CH), 6.48–6.90 (m, 3H, aromatic H), 7.00, 7.70 (each d,  $J=12$  Hz, 2H, aromatic H), 3.04 (b, OH), 8.80 (b, OH).

V was also obtained from III or IV by the same procedure.

**Dimethylether of V (VI)**—V (600 mg) was dissolved in MeOH (30 ml) and large excess of  $\text{CH}_2\text{N}_2$  in ether was added. After standing overnight, the solvent was evaporated and the residue was crystallized from MeOH as colorless prisms (VI), mp 136–138°. *Anal.* Calcd. for  $C_{24}H_{26}O_5$ : C, 73.07; H, 6.64;  $M^+$ ,  $m/e$  394.178. Found: C, 72.79; H, 6.64;  $M^+$ ,  $m/e$  394.180. UV  $\lambda_{\max}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 223 (4.25), 292 (shoulder, 4.29), 303 (4.32). NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.29 (s, 2  $\text{CH}_3$ ), 1.75, 2.68 (each t,  $J=7$  Hz,  $\text{CH}_2$ ), 2.95 (center of AB-part of ABX-pattern,  $J=14$ , 6.5, and 3 Hz,  $\text{CH}_2$ ), 3.85, 3.90 (each s,  $\text{OCH}_3$ ), 5.42 (X-part of ABX-pattern,  $J=6.5$  and 3 Hz, CH), 6.5–6.88 (m, 3H, aromatic H), 6.95, 7.52 (each d,  $J=9$  Hz, 2H, aromatic H).

When the methylation was performed in ether solution, monomethyl derivative, mp 142–144° was obtained as colorless needles (from  $\text{CH}_2\text{Cl}_2$ - $n$ -hexane).

**Dihydro-derivative of I (VII)**—I was hydrogenated with Pd/C in EtOH by usual method. The product was crystallized from AcOEt to colorless prisms (VII), mp 87–88° (dec.).  $[\alpha]_D^{25} = +90^\circ$  ( $c=1$ , EtOH). *Anal.* Calcd. for  $C_{24}H_{26}O_7$ : C, 67.59; H, 6.15. Found: C, 67.29; H, 5.88. UV  $\lambda_{\max}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 310 (3.84). MS  $m/e$ : 426 ( $M^+$ ).

**Catalytic Hydrogenation of V and VI**—Each compound was hydrogenated with  $\text{PtO}_2$  in EtOH (V) or in AcOEt (VI) by usual method. The products were Compounds VIII and IX, respectively.

VIII, colorless prisms from AcOEt, mp 210–211°. *Anal.* Calcd. for  $C_{22}H_{24}O_5$ : C, 71.72; H, 6.57. Found: C, 71.79, 71.45; H, 6.93, 6.55. UV  $\lambda_{\max}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 229 (4.21), 279 (3.59), 289 (3.49). IR  $\nu_{\max}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3450, 3350, 1775. NMR (pyridine- $d_5$ )  $\delta$ : 1.24 (s, 2  $\text{CH}_3$ ), 1.62, 2.62 (each t,  $J=7$  Hz,  $\text{CH}_2$ ), 2.83 (center of AB-part of ABX-pattern,  $J=14$ , 8, and 5.5 Hz,  $\text{CH}_2$ ), 3.92 (dd,  $J=8$  and 5 Hz, CH), 5.01 (ddd,  $J=8$ , 5.5, and 5 Hz, CH), 5.26 (d,  $J=8$  Hz, CH), 6.88 (m, 3H, aromatic H), 7.08, 7.36 (each d,  $J=8.5$  Hz, 2H, aromatic H), ca. 8.3 (b, OH).

IX, colorless needles from  $n$ -hexane, mp 131–132°. *Anal.* Calcd. for  $C_{24}H_{28}O_5$ : C, 72.70; H, 7.12. Found: C, 72.35; H, 7.11. UV  $\lambda_{\max}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 229 (4.30), 278 (3.56), 289 (3.42). IR  $\nu_{\max}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3450, 1770. NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.32 (s, 2  $\text{CH}_3$ ), 1.76, 2.68 (each t,  $J=7$  Hz,  $\text{CH}_2$ ), 2.52 (center of AB-part of ABX-pattern,  $J=15$ , 8, and 6.5 Hz,  $\text{CH}_2$ ), 3.40, 3.84 (each s,  $\text{OCH}_3$ ), 3.80 (dd,  $J=7.5$  and 5 Hz, CH), 4.38 (d,  $J=7.5$  Hz, CH), 4.72 (ddd,  $J=8$ , 6.5, and 5 Hz, CH), 6.72 (m, 3H, aromatic H), 6.89, 7.07 (each d,  $J=9$  Hz, 2H, aromatic H).

**LiAlH<sub>4</sub> Reduction of VIII**—VIII (600 mg) was dissolved in tetrahydrofuran and reduced with  $\text{LiAlH}_4$  (400 mg) under stirring at room temperature for 8 hr. The reaction mixture was treated with dilute HCl and extracted with ether. The solvent was evaporated and the residue was chromatographed on silica gel by eluting with  $\text{CHCl}_3$ -AcOEt (3:1). From the first fraction, acetal (XII) was obtained as colorless needles, mp 170–172° (from benzene-AcOEt, 180 mg). *Anal.* Calcd. for  $C_{22}H_{26}O_5$ : C, 71.33; H, 7.08. Found: C, 71.44, 71.15; H, 6.97, 7.12. UV  $\lambda_{\max}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 228 (4.23), 280 (3.59), 289 (shoulder, 3.46). From the second fraction, glycol (XIII) was obtained as colorless prisms, mp 213–215° (from AcOEt, 190 mg). *Anal.* Calcd. for  $C_{22}H_{28}O_5$ : C, 70.94; H, 7.58;  $M^+$ ,  $m/e$  372.194. Found: C, 71.09; H, 7.84;  $M^+$ ,  $m/e$  372.196. UV  $\lambda_{\max}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 228 (4.21), 280 (3.57), 290 (shoulder, 3.49).

When this reaction was carried out under reflux for 12 hr, only XIII was obtained in 54% yield.

**HIO<sub>4</sub> Oxidation of XIII**—To the solution of XIII (100 mg) in EtOH (10 ml), 60 mg of  $\text{HIO}_4 \cdot 2\text{H}_2\text{O}$  was added, and kept at room temperature for 2–3 min. The product was extracted with ether and separated on preparative thin-layer chromatography (TLC) (silica gel PF<sub>254</sub>, Merck,  $\text{CHCl}_3$ -AcOEt (4:1) as the solvent). From the main spot, colorless amorphous solid (XIV) was isolated. MS  $m/e$ : Calcd. for  $\text{C}_{21}\text{H}_{24}\text{O}_4$ : 340.167. Found: 340.170. NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.31 (s, 2  $\text{CH}_3$ ), 1.76 (t,  $J=7$  Hz,  $\text{CH}_2$ ), 2.72, 2.64 (overlapped 2  $\text{CH}_2$ ,  $J$  was not clear), 3.57 (d,  $J=5$  Hz, CH), 4.56 (m, CH), 6.7–7.3 (7H, aromatic H), 9.70 (observed as singlet, CHO), 3.0 (b, OH). To the aliquoted reaction mixture corresponding to 50 mg of XIII, aqueous solution of dimedone (50 mg) was added. After standing for 1 hr, the product was extracted with ether and purified from  $n$ -hexane to obtain colorless needles, mp 191° (12.4 mg). It was identified with formaldehyde-dimedone by IR and mixed melting point.

**KMnO<sub>4</sub> Oxidation of VI (1)**—The aqueous solution of  $\text{KMnO}_4$  (500 mg in 15 ml) and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (600 mg) was added to the solution of VI (500 mg) in acetone (25 ml), and stirred vigorously at room temperature. After decoloring, another 100 mg portion of  $\text{KMnO}_4$  was added and stirred until decolorized. The reaction mixture was treated with  $\text{NaHSO}_3$  and  $\text{H}_2\text{SO}_4$  and extracted with ether. The ether solution was washed with  $\text{NaHCO}_3$  solution and evaporated. The residue was purified by preparative TLC [silica gel PF<sub>254</sub>, benzene-AcOEt (7:1) as the solvent] to obtain the ketol (XV) as colorless oil (134 mg). IR  $\nu_{\max}^{\text{film}}$   $\text{cm}^{-1}$ : 3450, 1670. NMR ( $\text{CDCl}_3 + \text{D}_2\text{O}$ )  $\delta$ : 1.33 (s, 2  $\text{CH}_3$ ), 1.77, 2.76 (each t,  $J=7$  Hz,  $\text{CH}_2$ ), 2.98 (center of AB-part of ABX-pattern,  $J=14$ , 7, and 4.5 Hz,  $\text{CH}_2$ ), 3.95 (s,  $\text{OCH}_3$ ), 5.26 (X-part of ABX-pattern,  $J=7$  and 4.5 Hz, CH), 6.7–6.9 (m, 3H, aromatic H), 6.92, 7.96 (each d,  $J=9.5$  Hz, 2H, aromatic H), 3.8 (b, in  $\text{CDCl}_3$ , OH).



The  $\text{NaHCO}_3$  washing was acidified and extracted with ether. The ether extract was crystallized from  $\text{H}_2\text{O}$  to obtain colorless needles, mp  $184\text{--}186^\circ$ , which was identified with *p*-anisic acid by IR and mixed melting point.

**$\text{HIO}_4$  Oxidation of Ketol XV**—To the solution of XV (75 mg) in 50% EtOH, 77 mg of  $\text{HIO}_4 \cdot 2\text{H}_2\text{O}$  was added. After standing at room temperature for 1.5 hr, the reaction mixture was extracted with ether. The ether solution was washed with  $\text{NaHCO}_3$  solution and evaporated. The residue was purified by preparative TLC (silica gel PF<sub>254</sub>, ligroin–AcOEt (5:1) as the solvent) to obtain colorless oil (XVI) (27 mg). IR  $\nu_{\text{max}}^{\text{film}}$   $\text{cm}^{-1}$ : 1720. NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.33 (s, 2  $\text{CH}_3$ ), 1.80, 2.76 (each t,  $J=6.5$  Hz,  $\text{CH}_2$ ), 3.56 (d,  $J=2.5$  Hz,  $\text{CH}_2$ ), 6.7–7.0 (m, 3H, aromatic H), 9.70 (t,  $J=2.5$  Hz, CHO). XVI gave 2,4-dinitrophenylhydrazone, orange plates, mp  $145^\circ$ . Anal. Calcd. for  $\text{C}_{15}\text{H}_{20}\text{N}_4\text{O}_5$ : C, 59.37; H, 5.24; N, 14.58. Found: C, 59.44; H, 5.07; N, 14.87.

From the  $\text{NaHCO}_3$  layer above, *p*-anisic acid was obtained (25 mg).

**$\text{KMnO}_4$  Oxidation of VI (2)**—VI (500 mg) was dissolved in acetone (20 ml), and 2.5 g of  $\text{KMnO}_4$  was added gradually under refluxing and stirring. After 2.5 hr, another portion of  $\text{KMnO}_4$  (2.5 g) was added and refluxed for further 2.5 hr. The excess of  $\text{KMnO}_4$  and  $\text{MnO}_2$  were treated with  $\text{NaHSO}_3$  and  $\text{H}_2\text{SO}_4$ , and the mixture was extracted with ether. The ether extract was separated into three fractions by chromatography on silica gel (Mallinckrodt, Silic AR CC-4) with  $\text{CHCl}_3$  containing increased amount of AcOEt (0–20%). From the first fraction, *p*-anisic acid was obtained (110 mg). The second fraction was crystallized from benzene to obtain 2,2-dimethyl-chromanone-6-carboxylic acid (XVII) as colorless prisms, mp  $183\text{--}184^\circ$  (60 mg). Anal. Calcd. for  $\text{C}_{12}\text{H}_{12}\text{O}_4$ : C, 65.44; H, 5.49. Found: C, 65.32; H, 5.65. IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 2500–2800, 1700, 1670, 1615, 930. NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.49 (s, 2  $\text{CH}_3$ ), 2.77 (s,  $\text{CH}_2$ ), 6.99 (d,  $J=9$  Hz, 1H), 8.13 (dd,  $J=9$  and 2.5 Hz, 1H), 8.60 (d,  $J=2.5$  Hz, 1H), 10.8 (b, OH).

The third fraction was crystallized from benzene–*n*-hexane to colorless prisms (XVIII), mp  $290\text{--}291^\circ$  (75 mg). It was determined as 4-(1-carboxy-isopropoxy)-isophthalic acid. Anal. Calcd. for  $\text{C}_{12}\text{H}_{12}\text{O}_7$ : C, 53.73; H, 4.51. Found: C, 53.28; H, 4.24. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 254 (4.14). IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 2500–2800, 1740, 1700, 1690. NMR (acetone- $d_6$ )  $\delta$ : 1.80 (s, 2  $\text{CH}_3$ ), 7.28 (d,  $J=9$  Hz, 1H), 8.18 (dd,  $J=9$  and 2 Hz, 1H), 8.62 (d,  $J=2$  Hz, 1H), 10.7 (b, 3 COOH).

**$\text{KMnO}_4$  Oxidation of II**—The trimethylether (II) (395 mg) was suspended in  $\text{H}_2\text{O}$  (40 ml), and 2.0 g of  $\text{KMnO}_4$  was added gradually under heating on a water bath. After treatment with  $\text{NaHSO}_3$  and  $\text{H}_2\text{SO}_4$ , the mixture was extracted with ether. The ether solution was treated with  $\text{NaHCO}_3$  solution and the acidic fraction was separated on preparative TLC (silica gel, acid washed, Nakarai Chemical,  $\text{CHCl}_3$ –acetone (3:1) as the solvent). From the upper spot, *p*-anisic acid was isolated (19 mg). The lower spot gave colorless needles, mp  $261\text{--}263^\circ$ . Anal. Calcd. for  $\text{C}_9\text{H}_8\text{O}_5$ : C, 55.10; H, 4.11. Found: C, 54.99; H, 4.18. It was identified with 4-methoxy-isophthalic acid prepared from 5-methylsalicylic acid by  $\text{KMnO}_4$  oxidation in IR and mixed melting point.

**Ozonolysis of VI**—VI (500 mg) in  $\text{CHCl}_3$  (50 ml) was treated with  $\text{O}_3$  at room temperature for 1 hr. The solvent was evaporated under reduced pressure, and the residue was treated with Zn-powder (2 g) and AcOH (10 ml) at room temperature for 12 hr. After filtration and evaporation of the solvent, the product was purified by chromatography on silica gel with  $\text{CHCl}_3$  as the solvent. The isolated oil (123 mg) was crystallized from isopropyl ether–*n*-hexane as colorless plates (XIX), mp  $69\text{--}70^\circ$  (83 mg). Anal. Calcd. for  $\text{C}_{21}\text{H}_{24}\text{O}_3$ : C, 77.75; H, 7.46. Found: C, 77.86; H, 7.56. IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 1660, 1600. NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.32 (s, 2  $\text{CH}_3$ ), 1.76, 2.72 (each t,  $J=6.5$  Hz,  $\text{CH}_2$ ), 3.07 (center of  $\text{A}_2\text{B}_2$ -pattern, 2  $\text{CH}_2$ ), 3.84 (s,  $\text{OCH}_3$ ), 6.6–7.0 (m, 5H), 6.90, 7.90 (each d,  $J=8.5$  Hz, 2H). When the ozonide was decomposed by heating with  $\text{H}_2\text{O}$ , *p*-anisic acid was obtained in a small yield.

**Administration Experiments with  $^{14}\text{C}$ -Labeled Compounds**—DL-Mevalonic acid-2- $^{14}\text{C}$ , DL-phenylalanine-1- $^{14}\text{C}$ , and -2- $^{14}\text{C}$  were used. Each labeled compound was administered on the 4th day of the cultivation. After further 11 days' cultivation, metabolites were isolated. Radioactivity was assayed as previously described.<sup>3)</sup>

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