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Studies on the Metabolic Products of *Aspergillus terreus*. II.[1] Structure and Biosynthesis of the Metabolites of the Strain ATCC 12238

NORIKI KIRIYAMA,[2a] YOSHIHIRO HIGUCHI, and YUZURU YAMAMOTO[2]

Faculty of Pharmaceutical Sciences, Kanazawa University[2]

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New compounds, 6-hydroxytoluquinol-hydrate (3,4) (VI), terremutin-hydrate (VII), and 4-chloro-3,6-dihydroxytoluquinone (II) were isolated from *Aspergillus terreus* ATCC 12238 together with terreic acid, terremutin, 3,6-dihydroxytoluquinone, and terrein. The metabolic relationships among the metabolites were studied by administration experiments using ^{14}C-compounds and the metabolic scheme was proposed. The metabolic pattern of "epoxy compounds (terreic acid and terremutin)→quinone (3,6-dihydroxytoluquinone) or its quinol→hydroaromatic compound (VI)" was essentially the same as observed in *Aspergillus fumigatus* DH 413 and IFO 4399.

Keywords——*Aspergillus terreus*; biosynthesis; toluquinone; epoxy compound; hydroaromatic compound

As reported in the previous papers,[3] the biosynthetic pathway of toluquinones in *Aspergillus fumigatus* DH 413 and IFO 4399 had been clarified and it was found that an epoxy compound and several hydroaromatic compounds took important roles in the metabolic pathway.

Recently, many epoxy compounds, *i.e.* epoxydon[4] (phyllosinol),[5] phyllostine,[6] epoformin,[7] terreic acid,[8] and terremutin[9] had been isolated from several fungi. Although these epoxide compounds are also expected as the important intermediates in toluquinone biosynthesis, no investigations on such a point of view have been reported.

To demonstrate the universality of the participation of epoxy and hydroaromatic compounds in toluquinone metabolism in fungi, several strains of *Aspergillus terreus* which are known to produce epoxy compounds such as terreic acid and terremutin were investigated. Among the tested strains of *Aspergillus terreus* IFO 4100, 5445, 6123, 6346, 6365, 7078, 8835, and ATCC 12238, the last strain was found to produce considerable amounts of various metabolites relating to toluquinones.

This paper deals with the isolation procedure, the structure of new metabolites, and the metabolic relationships among the metabolites in ATCC 12238 strain.

This fungus was cultivated stationarily on a potato extract-glucose medium at 27°. The culture was harvested on the 10 th day of the cultivation when the most kinds of the

1) Part I: Y. Yamamoto, K. Nishimura, and N. Kiriyama, *Chem. Pharm. Bull.* (Tokyo), **24**, 1853 (1976).
2) Location: *Takara-machi 13-1, Kanazawa, 920, Japan*; *a*) Present adress: *Faculty of Pharmaceutical Sciences, Hokuriku University, Kanagawa-cho, Kanazawa, 920-11, Japan.*
3) *a*) Y. Yamamoto, K. Nitta, and A. Jinbo, *Chem. Pharm. Bull.* (Tokyo), **15**, 427 (1967); *b*) Y. Yamamoto, M. Shinya, and Y. Oohata, *ibid.*, **18**, 561 (1970); *c*) Y. Yamamoto, T. Hirai, K. Okada, and K. Saito, *ibid.*, **22**, 83 (1974).
4) A. Closse, R. Mauli, and H.P. Sigg, *Helv. Chim. Acta*, **49**, 204 (1966).
5) S. Sakamura, H. Niki, Y. Obata, R. Sakai, and T. Matsumoto, *Agr. Biol. Chem.*, **33**, 698 (1969).
6) S. Sakamura, J. Ito, and R. Sakai, *Agr. Biol. Chem.*, **34**, 153 (1970).
7) I. Yamamoto, E. Mizuta, T. Henmi, Y. Yamato, and S. Yamatodani, *Takeda Kenkyusho Nenpo*, **32**, 532 (1973).
8) J.C. Sheehan, W.B. Lawson, and R.J. Gaul, *J. Am. Chem. Soc.*, **80**, 5536 (1958).
9) M.W. Miller, *Tetrahedron*, **24**, 4839 (1968).

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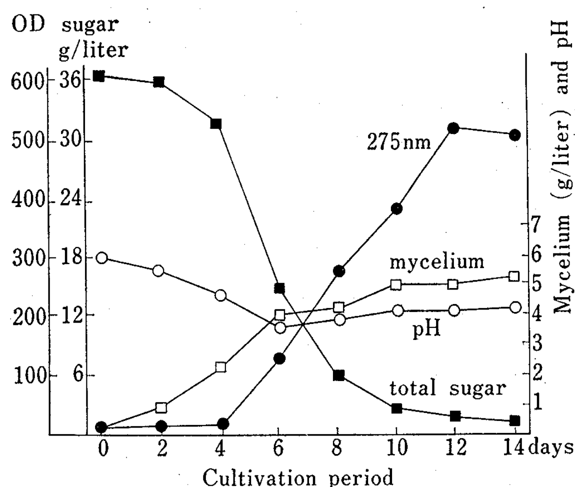

Fig. 1. Variation of pH, Optical Density, Mycelium Weight, and Sugar Content

OD: at pH 3.0
sugar: assayed by phenol-sulfuric acid method

metabolites were isolated. The growth of the mycelium, changes in pH, sugar content, and the optical density at 275 nm of the culture medium were shown in Fig. 1.

The culture medium (2 liters) was concentrated and extracted with ethyl acetate. The extract was concentrated and the resulting terremutin (I) was collected by filtration (yield, 1.0 g).

The filtrate was applied on a column of oxalic acid pre-treated silica gel and eluted with the mixture of benzene and ethyl acetate. The effluent was evaporated, and the residue was treated with benzene. From the less soluble part, compound II, mp 191° (yield, 1 mg), was isolated as red prisms.

The more soluble part in benzene was further separated by chromatography on silicagel into terreic acid (III) and a quinone (IV), mp 179°(yield, 30 mg and 3 mg, respectively). The quinone was identified with 3,6-dihydroxytoluquinone by comparison of the quinone and its tetraacetate, mp 198.5° (2,3,5,6-tetraacetoxytoluene) with the described data.[10]

The aqueous layer after extraction with ethyl acetate was adjusted to pH 5.0, and applied to a column of Dowex-1×8 (formate). The neutral fraction which passed through the column was concentrated and adsorbed on a column of charcoal and washed with water.

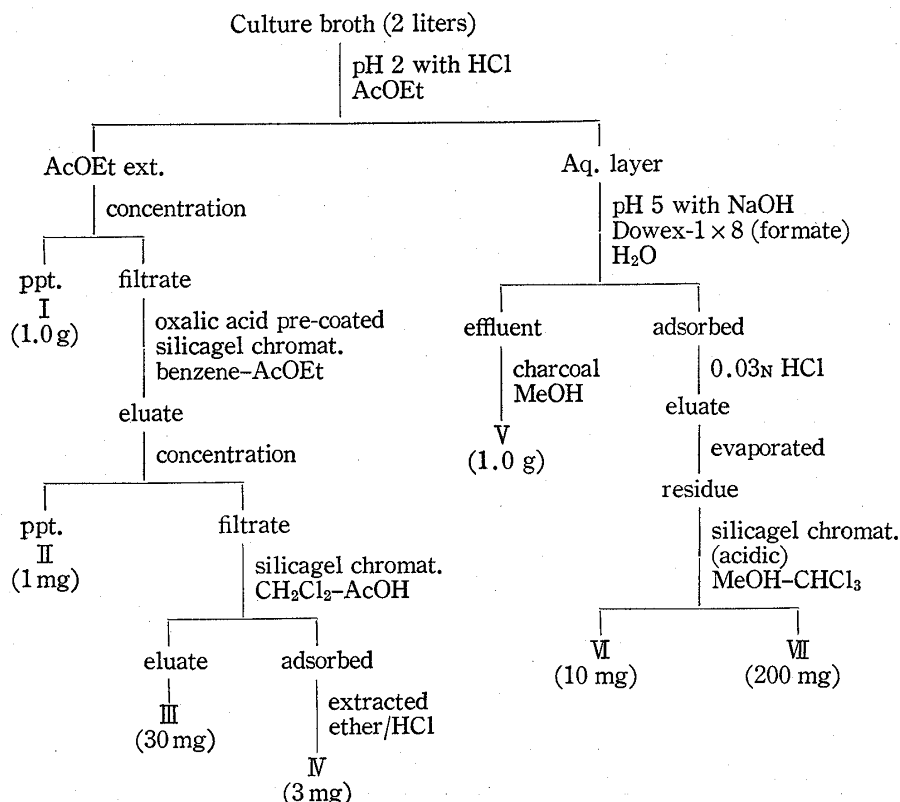

Fig. 2. Isolation Procedure of the Metabolites

10) D.S. Deorha and S.P. Sareen, *J. Indian Chem. Soc.*, **41**, 837 (1964); W.G. Hanger, W.C. Howell, and A.W. Johnson, *J. Chem. Soc.(C)*, **1958**, 496.

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By elution with methanol, terrein (V) was isolated as colorless needles, mp 127° (yield,1.0g). The acidic part adsorbed on the ion exchange resin was eluted with 0.03 N hydrochloric acid. The fractions having absorption at 265 nm were combined, and evaporated under reduced pressure. The residue was chromatographed on a column of acidic silicagel to obtain colorless needles (VI), mp 152° and colorless prisms (VII), mp 189° (yield, 10 mg and 200 mg, respectively).

The isolation procedure of the metabolites is summarized in Fig. 2.

The molecular formula of the compound II was determined as $C_7H_5O_4Cl$ from elementary analysis and mass spectrum. The proton nuclear magnetic resonance (PMR) spectrum showed signals at δ 1.90 (singlet, CH_3) and 10.3 (broad singlet, 2 OH), but no olefinic protons were detected. In the infrared (IR) spectrum, II showed the absorptions at 3250 (OH), 1650 and 1620 (quinone), and 568 cm^{-1}(C-Cl). These results suggested II as a chlorinated dihydroxytoluquinone. The ultraviolet (UV) spectrum showed λ_{max} at 333 and 543 nm in aqueous solution at pH 10. It was corresponded to that of 3,6-dihydroxytoluquinone (IV) (328 and 536 nm)[11] and not to 3,4-dihydroxytoluquinone (440 nm)[3c] nor 4,6-dihydroxytoluquinone (337.5 and 583 nm).[12] The mass spectrum of II was well corresponded to that of 3,6-dihydroxytoluquinone. Compound II was derived from terreic acid (III) by treatment with dry hydrogen chloride in ether. From these results, compound II was determined to be 4-chloro-3,6-dihydroxytoluquinone.

Compound VI was assigned as $C_7H_{10}O_4$ by elementary analysis and mass spectrum. It was optically active mono-basic acid (p*K*a′, 5.7), and positive for ferric chloride test (purple). It gave a monomethylether with diazomethane. The UV spectrum showed λ_{max} at 264 nm in acidic aqueous solution which was reversibly shifted to 297 nm in basic medium. IR spectrum showed strong peaks at 3400 and 3260 (OH), 1650 (C=O), and 1620 cm^{-1} (C=C). These results showed VI had an α,β-unsaturated ketone and hydroxyl groups, one of which was enolic.

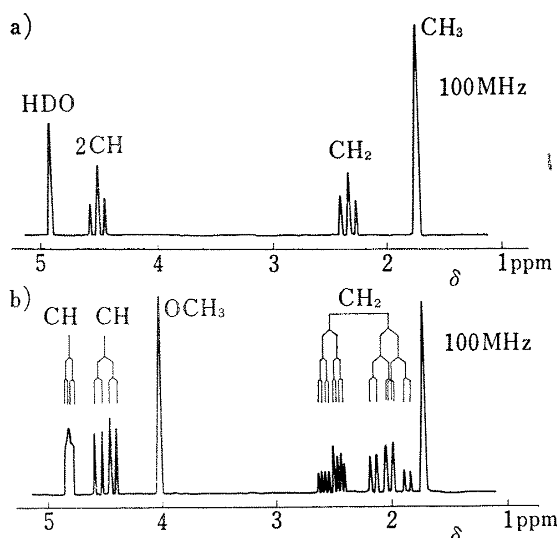

Fig. 3a. PMR Spectrum of Compound VI in D_2O

Fig. 3b. PMR Spectrum of Methyl Ether of VI in $CDCl_3$ (D_2O added)

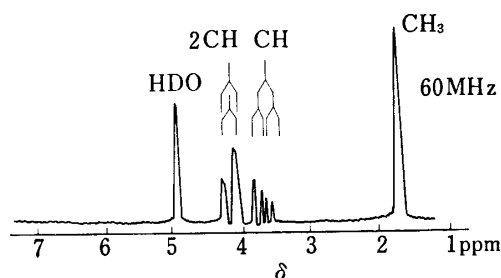

Fig. 4. PMR Spectrum of Compound VII in D_2O

Compound VI was dehydrated with ethanolic hydrochloric acid to give 6-hydroxytoluquinol, $C_7H_8O_3$, mp 122°, which suggested VI is a hydrate of 6-hydroxytoluquinol.

In PMR spectrum (Fig. 3a), VI had the signals of three hydroxyl groups, one methyl group attached to an olefinic carbon, one methylene group, and two methine protons locating on hydroxylated carbons. The signal of the methylene group was observed as a triplet (J=6 Hz), and the signals of the two methine groups were completely overlapped and also appeared as a triplet (J=6 Hz). In PMR spectrum of the methylether, as shown in Fig.

11) J.F. Corbett and A.G. Fooks, *J. Chem. Soc.* (*C*), **1967**, 1909.
12) J.F. Corbett, *J. Chem. Soc.* (*C*), **1967**, 2408.

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3b, the signal of the methylene group appeared as a couple of AB type pattern, each of which was further splitted into double-doublet by adjacent two methine groups. The signals of methine groups were separatedly observed as double-doublet.

These PMR data suggested the location of a methylene group between two methine groups and the existence of keto-enol tautomerism.

From these results, the chemical structure of VI was proposed as keto-form of 6-hydroxytoluquinol-hydrate (3,4). Rapid exchange between two keto-enol tautomers and the change of the conformation caused the equivalency of the two methine protons and the two hydrogens of the methylene group in PMR spectrum of compound VI. Since VI had optical activity, one of the two alcoholic hydroxyl groups must be axial and the other must be equatrial.

Compound VII was optically inactive and a molecular formula, $C_7H_{10}O_5$ was assigned from elementary analysis and mass spectrum. It was mono-basic acid (pK_a', 4.6) and positive for ferric chloride test (red-brown). The UV spectrum shownd λ_{max} at 263 nm in acidic aqueous solution and 292 nm in basic medium. Compound VII was methylated with etherial diazomethane to give monomethylether, mp 128—129°. In the IR spectrum, VII showed the presence of two types of hydroxyl groups (3400 and 3275 cm^{-1}) and α,β-unsaturated ketone group (1650 and 1620 cm^{-1}).

PMR spectrum (Fig. 4) showed the presence of one methyl group and a series of three hydroxylated methine groups. The coupling constants between the methine groups were 11 and 8 Hz, showing all of the three methine hydrogens were in axial conformation.

Compound VII gave 2,3,5,6-tetraacetoxytoluene, mp 198.5° on acetylation with acetic anhydride. In this process, dehydration and enolization of the ketone group must be involved. By treating with ethanolic hydrochloric acid, VII was dehydrated to the mixture of 3,6-dihydroxytoluquinone (IV) and its quinol. Furthermore, the formation of VII from terremutin (I) by treatment with hot water showed the close structural relation of them. Terreic acid (III) did not give compound VII under the same condition.

From these results above, the structure of VII was assigned as terremutin-hydrate. The alcoholic hydroxyl group of terremutin (I) had been determined as quasi-equatrial by

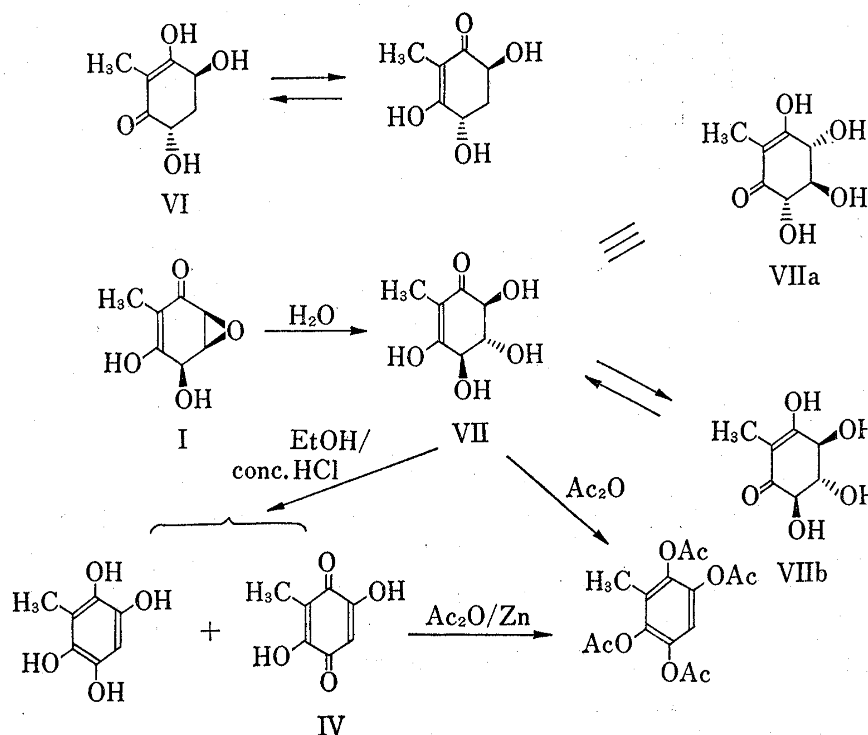

Chart 1

Miller.[9] Compound VII was optically inactive in spite of the presence of three asymmetric carbons. But this is reasonably elucidated by tautomerism between VIIa and VIIb, which gave a mixture of equal amounts of antipodes as shown in Chart 1.

In order to elucidate the metabolic relationships among the above metabolites, incorporation experiments using ^{14}C-labeled compounds were carried out. ^{14}C-Labeled metabolites were prepared by administration of sodium acetate-1-^{14}C to the fungus. The radioactivity of acetate-1-^{14}C was distributed partially in the mycelium and respiratory carbon dioxide (24 and 7%, respectively), and mainly in the culture medium (47%). And about half in the medium could be isolated as labeled metabolites (23%). Each labeled metabolite was purified to constant specific radioactivity. Since the yield of 3,6-dihydroxytoluquinone (IV) was not sufficient for tracer experiments, the labeled sample was prepared chemically from labeled terremutin (I). Each labeled compound was administered to the medium on the 4 th day and further incubated for 7 days. Compound VI could not be studied because of poor yield.

TABLE I

Compound added	Incorporation ratio (%)						
	I	III	IV	VI	VII	VIII	V
Terremutin (I) 42.0 mg, 5.10×10^6 dpm	41.1	1.1	0.8	1.3	15.0	*a*)	0
Terreic acid (III) 30.0 mg, 5.13×10^6 dpm	40.1	0.5	0.5	3.0	14.3	*a*)	0
3,6-Di-OH-toluquinone (IV) 50.0 mg, 2.21×10^6 dpm	0	0	~0	20.7	0	53.4	0
Compound VII 38.7 mg, 4.56×10^6 dpm	0	0	0	0	71.0	*a*)	0
Terrein (V) 70.2 mg, 5.48×10^6 dpm	0	0	0	0	0	*a*)	86.6

a) No metabolite was detected.

The results of administration experiments are summarized in Table I. In every experiments, no radioactivity was detected in respiratory carbon dioxide or mycelium, which showed further degradation of the metabolites in the fungus was negligible.

Terremutin (I) was incorporated into terreic acid (III) (1.1%), 3,6-dihydroxytoluquinone (IV) (0.8 %), compound VI (1.3 %), and into compound VII (15.0 %) with unchanged terremutin (41.1%), but it was not incorporated into terrein (V).

Terreic acid (III) was incorporated into terremutin (I) (40.1 %), 3,6-dihydroxytoluquinone (IV) (0.5 %), compound VI (3.0 %), and into compound VII (14.3 %) with 0.5% recovery, but not to terrein (V).

The incorporation pattern of terremutin (I) and terreic acid (III) into other metabolites was quite similar. The interconversion between I and III was recognized inspite of the predominant formation of the former. Read, *et al.*[13] had proposed terremutin is the precursor of terreic acid (III), but the above results and time course studies by the yields of both metabolites did not give clear conclusion which is the precursor of the other.

3,6-Dihydroxytoluquinone (IV) was not incorporated into terremutin (I), terreic acid (III), or compound VII, but it was well incorporated into compound VI (20.7%). And much radioactivity was found in an unidentified compound (VIII) which had not isolated in usual culture. Compound VIII had the molecular formula $C_{14}H_{14}O_8$, and presumed as the condensation product of two C–7 compounds. In fact, VIII was formed by condensation of

13) G. Read, D.W.S. Westlake, and L.C. Vining, *Can. J. Biochem.*, **47**, 1071 (1969).

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3,6-dihydroxytoluquinol and terreic acid (III). To minimize the formation of VIII, the incubation time after administration of labeled 3,6-dihydroxytoluquinone (IV) was shortened until 6 hr, but the results were almost the same.

Compound VII was not incorporated into any other metabolites, and showed to be an end product in the metabolism. Terremutin (I) could be assigned as the immediate precursor of VII, because VII was easily obtained chemically from terremutin (I), but not from terreic acid (III).

Terrein (V) had no metabolic relation to other metabolites.

From these results above, the metabolism around 3,6-dihydroxytoluquinone in *Aspergillus terreus* ATCC 12238 proceeds as follows: epoxy compounds (I and III)→quinone (IV) or its quinol→hydroaromatic compound (VI). This is quite the same scheme as the biosynthesis of toluquinones by *Aspergillus fumigatus* DH 413 (fumigatin) and IFO 4399 (spinulosin). However, in *Aspergillus terreus* ATCC 12238, the pathway to quinone from epoxy compounds was rather narrow and resulted in considerable accumulation of epoxy compounds which were mainly changed to another hydroaromatic compound (VII) *via* terremutin (I).

Read, *et al.*[13] proved 6-methylsalicylic acid was the precursor of terreic acid (III). Terrein (V) was not incorporated into any other metabolites, nor any of them incorporated into terrein (V). Therefore, terrein (V) is biosynthesized through an independent course as suggested by Birch, *et al.*[14]

The metabolic pathway in *Aspergillus terreus* ATCC 12238 is summarized in Chart 2.

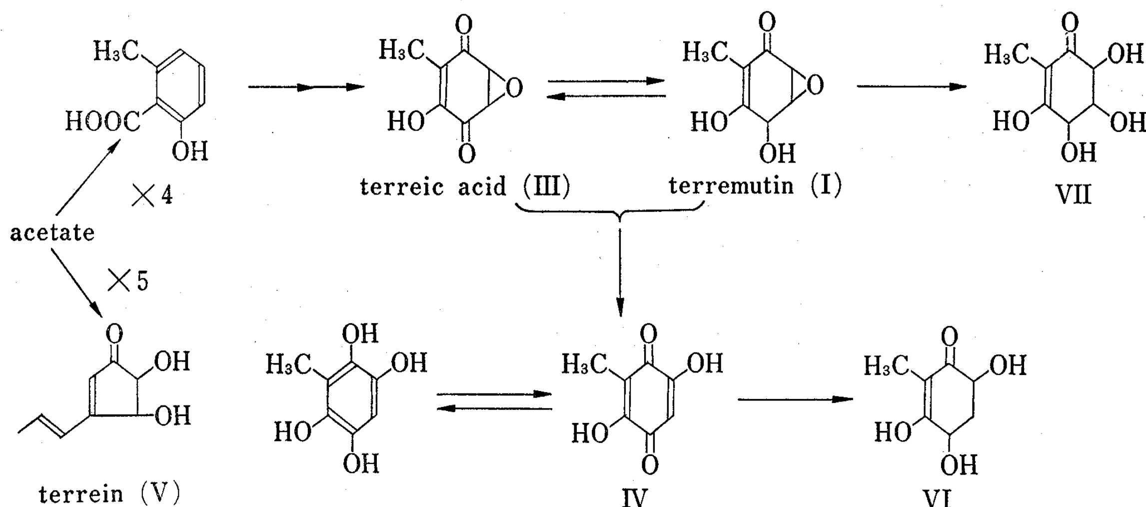

Chart 2. Biosynthetic Pathway of Terreic Acid by *Asp. terreus*

It shows many epoxy and hydroaromatic compounds are participating in toluquinone metabolism, and this metabolic pathway is essentially the same as in *Aspergillus fumigatus* DH 413 and IFO 4399 reported previously.

Experimental[15]

Cultivation of the Fungus——*Aspergillus terreus* ATCC 12238 was cultivated stationarily at 27° for 10 days in 500 ml Roux flasks containing 200 ml of potato extract-glucose medium [diced potato (300 g) was cooked with 1 liter of water, filtered, and 30 g of glucose was added].

Isolation of Metabolites——The culture broth (2 liters) was concentrated to 1/5 volume at 40° under reduced pressure, and pH was adjusted to 2.0 with HCl. It was extracted with AcOEt at least 6 times

14) A.J. Birch, A. Cassera, and A.R. Jones, *Chem. Commun.*, **1965**, 167.
15) All melting points are not corrected.

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(total *ca.* 1.2 liters), and the extract was concentrated under reduced pressure to *ca.* 50 ml. The precipitates were filtered and recrystallized from AcOEt as colorless prisms, mp 145—146°. It was identified with terremutin (I).

The filtrate was chromatographed on a column of oxalic acid pre-treated silica gel (100—200 mesh, 3×40 cm)[16] with benzene–AcOEt (100: 5, v/v) as the elution solvent. The orange-red effluent was evaporated to dryness and the residue was recrystallized from benzene as red prisms (II), mp 191°. *Anal.* Calcd. for $C_7H_5O_4Cl$: C, 44.59; H, 2.67. Found: C, 45.09; H, 2.59. Mass Spectrum *m/e*: 188 (M^+), 160, 125. UV $\lambda_{max}^{H_2O}$ nm (log ε): 298 (4.30), 488 (2.48) at pH 2.0; 333 (4.40), 543 (2.29) at pH 10.0. IR ν_{max}^{KBr} cm^{-1}: 3250, 1650, 1620, 568. PMR (acetone-d_6) δ: 1.90 (s, CH_3), 10.3 (bs, 2 OH).

After filtration of II, the mother liquor was adsorbed on a column of silica gel (100—200 mesh, 2×20 cm, for chromatography, Kanto Chemical Co., Ltd.) and eluted with CH_2Cl_2–AcOH (100: 1, v/v). The effluent was evaporated and recrystallized from CH_2Cl_2–*n*-hexane to give terreic acid (III) as colorless leaflets, mp 127°.

The purple band was cut out from the column and extracted with ether containing HCl. The ether extract was evaporated and recrystallized from benzene to obtain 3,6-dihydroxytoluquinone (IV) as orange leaflets, mp 179°. *Anal.* Calcd. for $C_7H_6O_4$: C, 54.55; H, 3.92. Found: C, 54.50; H, 3.88. Mass Spectrum *m/e*: 154 (M^+). UV $\lambda_{max}^{H_2O}$ nm (log ε): 287 (4.37), 428 (2.44) at pH 2.0; 328 (4.47), 536 (2.36) at pH 8.5. IR ν_{max}^{KBr} cm^{-1}: 3300, 1640, 1618.

The broth after extraction with AcOEt was adjusted to pH 5.0 with aqueous NaOH and passed through a column of Dowex-1×8 (formate, 3×50 cm). The column was washed with 5 liters of H_2O and the washing was concentrated to about 2 liters, and adsorbed on a column of charcoal (3×40 cm, for chromatography, Wako Pure Chemical Ind. Ltd.). After washing with H_2O, the adsorbed part was eluted with MeOH and the eluate was evaporated to dryness, and recrystallized from AcOEt to give terrein (V) as colorless needles, mp 127°.

The column of Dowex was then eluted with 0.03N HCl, and the effluent having λ_{max} at 265 nm was collected. The combined effluent was evaporated at 40° under reduced pressure to dryness, and the MeOH solution of the residue was applied on a column of silica gel (Mallinckrodt, Silic AR CC-4). Elution with $CHCl_3$–MeOH (9: 1, v/v) gave two fractions. The first fraction was evaporated and the residue was recrystallized from AcOEt as colorless needles (VI), mp 152°. *Anal.* Calcd. for $C_7H_{10}O_4$: C, 53.16; H, 6.37. Found: C, 53.16; H, 6.31. Mass Spectrum *m/e*: 158 (M^+). $[\alpha]_D^{20°}=-185.3°$ (c=0.68 in MeOH). p$K_{a'}$=5.7. UV $\lambda_{max}^{H_2O}$ nm (log ε): 264 (4.14) at pH 2.0; 297 (4.36) at pH 8.0. IR ν_{max}^{KBr} cm^{-1}: 3260, 1650, 1620. PMR (D_2O) δ: 1.75 (s, CH_3), 2.30 (t, J=6 Hz, CH_2), 4.55 (t, J=6 Hz, 2 CH); (acetone-d_6): 5.8 (bs, 3 OH).

The second fraction was crystallized from MeOH–acetone as colorless prisms (VII), mp 189°. *Anal.* Calcd. for $C_7H_{10}O_5$: C, 48.27; H, 5.79. Found: C, 48.39; H, 5.74. Mass Spectrum *m/e*: 174 (M^+). p$K_{a'}$=4.6. UV $\lambda_{max}^{H_2O}$ nm (log ε): 263 (4.23) at pH 2.0; 292 (4.43) at pH 7.0. IR ν_{max}^{KBr} cm^{-1}: 3400, 3275, 1650, 1620. PMR (D_2O) δ: 1.67 (s, CH_3), 3.64 (dd, J=8 and 11 Hz, CH), 4.24 (d, J=8 Hz, CH), 4.24 (d, J=11 Hz, CH); (DMSO-d_6): 4.5 (bs, 4 OH).

Formation of 4-Chloro-3,6-dihydroxytoluquinone (II) and 3,6-Dihydroxytoluquinone (IV) from Terreic Acid (III)——Terreic acid (220 mg) was dissolved in 30 ml of dry ether, and dry HCl gas was saturated. After standing for 30 min, the solvent and HCl was evaporated. The residue was chromatographed on oxalic acid pre-treated silica gel (2.5×20 cm). By elution with benzene–AcOEt (100: 5, v/v), a mixture of red compounds was obtained. The mixture was purified by fractional crystallization from benzene to obtain compound II, mp 191° and compound IV, mp 179° (yield, 40 mg and 30 mg, respectively).

Methylation of VI——Etherial solution of diazomethane was added to 41 mg of VI and the solution was evaporated after 30 min. The residue was purified by preparative thin-layer chromatography on silica gel (Merck, PF_{254}) with $CHCl_3$–AcOEt (3: 2, v/v) as the developing solvent. The band having UV absorption was extracted with AcOEt and colorless oil was obtained (yield, 41.5 mg). Mass Spectrum *m/e*: 172 (M^+). $[\alpha]_D^{20°}=-202.4°$ (c=4.15 in MeOH). UV λ_{max}^{EtOH} nm: 267. IR ν_{max}^{Nujol} cm^{-1}: 3380, 1650, 1620. PMR ($CDCl_3$+D_2O) δ: 1.70 (s, CH_3), 2.00 (ddd, J=13, 12.5, and 4 Hz, CH), 2.50 (ddd, J=13, 5.5, and 2.5 Hz, CH), 4.04 (s, OCH_3), 4.52 (dd, J=12.5 and 5.5 Hz, CH), and 4.82 (dd, J=4 and 2.5 Hz, CH).

Dehydration of VI with Ethanolic HCl——VI (56 mg) was refluxed with ethanolic HCl solution (10 drops of conc. HCl in 5 ml of EtOH) for 3.5 hr. The pale yellowish solution was diluted with H_2O and extracted with ether. The ether extract was chromatographed on a column of silica gel (Mallinckrodt, Silic AR CC-4, 2×30 cm) and eluted with $CHCl_3$–AcOEt–AcOH (100: 30: 1, v/v). The pale yellowish effluent was evaporated and recrystallized from $CHCl_3$–*n*-hexane as colorless needles, mp 122° (yield, 9 mg). It was identified with 6-hydroxytoluquinol by IR and mixed mp.

Acetylation of VII——The mixture of VII (113 mg) and fused AcONa (100 mg) in Ac_2O (5 ml) was refluxed for 1 hr, and poured into H_2O. The resulting precipitates were recrystallized from MeOH as colorless needles, mp 198.5° (yield, 181 mg). It was identified with 2,3,5,6-tetraacetoxytoluene by IR and mixed mp. This compound was also obtained from IV by reductive acetylation with Ac_2O and Zn powder.

16) Silica gel (for chromatography, Kanto Chemical Co., Ltd.) was suspended in 0.1M oxalic acid overnight, filtered, washed with H_2O, and dried in an oven at 100°.

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Methylation of VII——VII (370 mg) was treated with etherial diazomethane for 1 hr. The methylether was recrystallized from AcOEt–*n*-hexane as colorless prisms, mp 128—129° (yield, 163 mg). *Anal.* Calcd. for $C_8H_{12}O_5$: C, 51.06; H, 6.43. Found: C, 51.23; H, 6.40. Mass Spectrum *m/e*: 188 (M^+). UV λ_{max}^{EtOH} nm: 264. IR ν_{max}^{KBr} cm^{-1}: 3380, 1640, 1600. PMR (DMSO-d_6) δ: 1.60 (s, CH_3), 3.48 (dd, J=10 and 7 Hz, CH), 3.80 (d, J=10 Hz, CH), 3.96 (s, OCH_3), 4.40 (d, J=7 Hz, CH), and 5.20 (bs, 3 OH).

Formation of VII from Terremutin (I)——Terremutin (166 mg) was dissolved in H_2O and heated on a water bath for 2 hr. The solvent was evaporated and the residue was recrystallized from MeOH-acetone as colorless prisms, mp 189° (yield, 121 mg). It was identified with VII by IR and mixed mp.

Formation of 3,6-Dihydroxytoluquinone (IV) and Its Quinol from VII——VII (121 mg) was refluxed with EtOH (5 ml) containing 5 drops of conc. HCl for 5 hr. The reaction mixture was diluted with H_2O and extracted with AcOEt. The solvent was evaporated under reduced pressure and the residue was recrystallized from benzene–AcOEt to obtain 3,6-dihydroxytoluquinol, mp 171°, as colorless prisms (yield, 42 mg). From the mother liquor, 3,6-dihydroxytoluquinone (IV) was also obtained (yield, 19 mg).

Compound VIII——This compound was detected in a trace amount in the MeOH elution from the column of oxalic acid pre-treated silica gel after removing compound (II, III, and IV).

It was also obtained chemically as follows: Terreic acid (93 mg) was added to the solution of 3,6-dihydroxytoluquinol (104 mg) in H_2O (50 ml) and kept for 3 hr at room temperature. The reaction mixture was evaporated under reduced pressure to dryness and crystallized from AcOEt–EtOH as colorless prisms (VIII), mp 218° (yield, 135 mg). Mass Spectrum *m/e*: Calcd. for $C_{14}H_{14}O_8$: 310.069. Found: 310.063. UV $\lambda_{max}^{H_2O}$ nm: 267 at pH 2.0. IR ν_{max}^{KBr} cm^{-1}: 3375, 1660, 1645, 1625. PMR (acetone-d_6) δ: 1.74, 1.84 (each s, CH_3), 4.36, 5.28 (each d, J=4 Hz, CH).

Administration Experiments and Determination of Radioactivity——Sodium acetate-1-^{14}C (0.95 mCi) was administered to the fungus and each labeled metabolite was obtained by the methods described above. The respiratory CO_2 evolved was collected in NaOH and the radioactivity of mycelium was assayed after combustion. Several millions dpm of each labeled metabolites was administered on the 4th day of the cultivation and harvested on the 10th day. Metabolites were isolated and the radioactivity was assayed by liquid scintilation spectrometer (Packard, Tri-Carb, model 3320) using dioxane scintilator.

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