# NEW BUTENOLIDES FROM ASPERGILLUS TERREUS

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Key Word Index—Aspergillus terreus; mould metabolites; 3-(p-hydroxyphenyl)-4-hydroxy-5-(p-hydroxy-benzylidene)-2(5H)-furanone.

Abstract—From the culture filtrate of Aspergillus terreus, seven related yellow substances were isolated and the simplest,  $C_{17}H_{12}O_5$ , was proved to be 3-(p-hydroxyphenyl)-4-hydroxy-5-(p-hydroxybenzylidene)-2(5H)-furanone.

### INTRODUCTION

ALTHOUGH the metabolites of Aspergillus terreus have been well studied,<sup>1</sup> no compound derived from a  $C_6$ - $C_3$  unit has yet been reported. While studying the biosyntheses of fungal products, we have recently found a series of butenolides which are presumably derived from a  $C_6$ - $C_3$  unit. In this paper the isolation of these metabolites and the determination of the fundamental structure are described.

## **RESULTS AND DISCUSSIONS**

Aspergillus terreus IAM 2054 was grown in surface culture for 3 weeks at  $27^{\circ}$  in a Czapek-Dox medium. Besides orsellinic and 3-methylorsellinic acids,<sup>2</sup> seven yellow acidic substances were isolated by chromatographic separation of the Et<sub>2</sub>O extract of the acidified culture filtrate, using a silica gel column and *n*-hexane-Et<sub>2</sub>O for elution; they were designated A-G, according to the order of their elution. Their yields and some properties are given Table 1.

	Formula*	UV (MeOH)					
Compound		m.p.	$\lambda_{\max} nm$	$(\log \epsilon)$	Yield†		
A	C27H28O5	243–245°	243 (4.34),	371 (4.49)	50		
В	$C_{27}H_{28}O_5$	187–189°	243 (4.33),	374 (4.49)	10		
С	$C_{27}H_{28}O_6$	257–259°	244 (4.23),	375 (4.50)	50		
D	C27H28O6	257–258°	245 (4.28),	377 (4.47)	160		
E	C17H12O5	282–284°	238 (4.32),	369 (4-46)	50		
F	C27H28O7	234–235°	242 (4.31),	375 (4.49)	60		
G	$C_{17}H_{12}O_6$	263–265°	237 (4-22)	369 (4.38)	30		

TABLE I, TILLES AND SOME TROTERTILS OF METABOLITE	FABLE 1.	Yields	AND SOME	PROPERTIES	OF	METABOLITE
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\* All the compounds gave acceptable elemental analyses.

† mg/l. of culture filtrate.

<sup>1</sup> SHIBATA, S., NATORI, S. and UDAGAWA, S. (1962) List of Fungal Products, University of Tokyo Press, Tokyo.

<sup>2</sup> TAKENAKA, S., OJIMA, N. and SETO, S. (1972) Chem. Commun. 391

Since the UV spectra suggest that they have a common skeleton and E is the simplest, the structural determination is described on this compound. The NMR spectrum of E showed signals due to eight aromatic protons as four pairs with *ortho* coupling (J 9 Hz) and one isolated olefinic proton. E (IR  $\nu_{max}^{KBT}$  1691 cm<sup>-1</sup>) is very stable to cold alkali, but on treatment with 6N NaOH at 100° for 13 hr gives oxalic acid, *p*-hydroxybenzaldehyde and *p*-hydroxyphenylacetic acid, and on methylation with CH<sub>2</sub>N<sub>2</sub> affords a trimethyl derivative (IR  $\nu_{max}^{KBT}$  1758 cm<sup>-1</sup>) which is converted to a carboxylic acid on mild treatment with weak alkali. These facts imply that E has structure (Ia) or (II). Some derivatives of I, e.g. vulpic acid (Ib)<sup>3</sup> and pulvic acid (Ic),<sup>4</sup> are known as lichen products and Id was synthesized by Claisen<sup>5</sup> via IIIa and IVa as shown below.



We followed Claisen's method: condensation of IIIb with ethyl oxalate yielded a triketone (IVb, as the enol), whose pyrolysis at  $210^{\circ}$  in an atmosphere of N<sub>2</sub> gave Ie, which was methylated with CH<sub>2</sub>N<sub>2</sub> to afford If identical with the trimethyl derivative of E described above. E itself was obtained by demethylation of Ic with pyridine hydrochloride. Therefore, the structure of E is 3-(p-hydroxyphenyl)-4-hydroxy-5-(p-hydroxybenzylidene)-2(5H)-furanone (Ia).

The other metabolites have structures substituted with one more hydroxy group and/or two 3,3-dimethylallyl groups (in some cases, bound to the hydroxy group on the benzene nucleus to form a cyclic ether or epoxide). These will be reported in detail elsewhere.

#### EXPERIMENTAL

Isolation of metabolites. The culture filtrate (201.) was concentrated to 0·1 vol. under a reduced pressure at 40°, and after acidification to pH 2 with HCl continuous extraction with  $Et_2O$  in a Soxhlet gave the crude material (16 g), which was chromatographed on a column of silica gel (1·5 kg). Graded elution with *n*-hexane- $Et_2O$  (starting with a 7:3 mixture) afforded the fractions shown in Table 1.

Treatment of E with hot NaOH solution. E (150 mg) with 6N NaOH (3 ml) was heated on a water bath for 13 hr. After cooling, colorless crystals (ca. 10 mg) separated, which were identified as Na oxalate by comparison of IR spectra. The filtrate was neutralized to pH 7 and extracted with  $Et_2O$  to give colorless crystals (ca. 10 mg) identical with p-hydroxybenzaldehyde in IR spectrum. Then the aqueous layer acidified to pH 1 was extracted with  $Et_2O$  to afford colorless crystals (7 mg), which were identified as p-hydroxybenylacetic acid by IR spectral comparison.

*Trimethyl derivative* (If) of E. E (300 mg) was methylated with CH<sub>2</sub>N<sub>2</sub> in Et<sub>2</sub>O to give If (210 mg), m.p. 137–139° (Found: C, 71·23; H 5·03. C<sub>20</sub>H<sub>18</sub>O<sub>5</sub> required: C, 70·99; H, 5·36%) UV  $\lambda_{max}$  (MeOH) 237 and 353 nm (log  $\epsilon$  4·26 and 4·52).

<sup>3</sup> Möller, F. and Strecker, A. (1860) Annalen 113, 56.

<sup>4</sup> SPIEGEL, A. (1883) Annalen 219, 1.

<sup>5</sup> CLAISEN, L. and EWAN, TH. (1895) Annalen 284, 245.

Cleavage of the lactone ring of If. To a solution of If (140 mg) in a mixture of Et<sub>2</sub>O (10 ml), EtOH (15 ml) and dioxan (1 ml) was added 2N NaOH (5 ml) with stirring. The reaction mixture was stirred for 30 min at room temperature. After acidification with 1N HCl and evaporation of the solvent, extraction with Et<sub>2</sub>O followed by chromatographic purification with silica gel and Et<sub>2</sub>O-*n*-hexane (1: 1) gave an acid (45 mg), m.p. 95-96° (Found: C, 67·51; H, 5·71.  $C_{20}H_{20}O_6$  required: C, 67·40; H, 5·66%). UV:  $\lambda_{max}$  (MeOH) 225 and 275 nm (log  $\epsilon$  4·38 and 3·78).

Triketone (IVb). In N<sub>2</sub>, a solution of IIIb (500 mg) and diethyl oxalate (450 mg) in EtOH (5 ml) was added to NaOEt (Na 200 mg in EtOH 6 ml). The mixture was warmed at 40° for 10 min and then HOAc added until development of a yellow color. Evaporation of EtOH from the mixture under a reduced pressure followed by addition of H<sub>2</sub>O and 6N HCl to pH 2 and extraction with Et<sub>2</sub>O gave IVb (400 mg), m.p. 187–189° (Found: C, 70.67; H, 4.97. C<sub>19</sub>H<sub>16</sub>O<sub>5</sub> required: C, 70.36; H, 4.98%). UV:  $\lambda_{max}$  (MeOH) 251 and 349 nm (log  $\epsilon$  4.31 and 4.20).

Ie. In N<sub>2</sub>, IVb (100 mg) was heated at 215° for 15 min. The recrystallization from MeOH gave Ie (70 mg), m.p. 214–215° (Found: C, 70·26; H, 4·83, C<sub>19</sub>H<sub>16</sub>O<sub>5</sub> required: C, 70·36; H, 4·98%). UV:  $\lambda_{max}$  (MeOH) 236 and 365 nm (log  $\epsilon$  4·26 and 4·34).

Methylation of Ie. Methylation of Ie with  $CH_2N_2$  gave If which was identical with the trimethyl derivative of E.

Demethylation of Ie. Ie (30 mg) was heated with a large excess of pyridine hydrochloride in N<sub>2</sub> at 190° for 1 hr. After addition of H<sub>2</sub>O, the mixture was extracted with  $Et_2O$ . From the  $Et_2O$  solution, acidic material was extracted with 1N NaOH. The alkaline extract was acidified and shaken with  $Et_2O$ . The  $Et_2O$  layer was purified through a silica gel column to afford Ia (13 mg) which was identical with E.

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