Zinniol: a major metabolite of *Alternaria zinniae*[1]

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Zinniol, a compound recently obtained from the phytopathogenic fungus *Alternaria zinniae*, is shown to be 1,2-bis(hydroxymethyl)-5-(3′,3′-dimethylallyloxy)-3-methoxy-4-methylbenzene (**1**) by chemical and spectroscopic studies. Attempts to isolate other metabolites are described briefly.

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A recent investigation (1) of filtrates from stationary liquid cultures of the phytopathogenic fungus *Alternaria zinniae* resulted in the isolation of a major metabolite, $C_{15}H_{22}O_4$, which has been named zinniol. This compound, which is an oil, was characterized by the preparation of crystalline diacetyl (**2**) and dibenzoyl (**3**) derivatives. Zinniol (**1**) is now shown by chemical and spectroscopic evidence to be a novel penta-substituted benzene closely related to quadrilineatin (**4**) (3).

The nuclear magnetic resonance (n.m.r.) spectrum of zinniol exhibited bands attributable to the presence of one aromatic proton (δ 6.66, singlet), a methoxyl (δ 3.72), and a methyl group on an aromatic nucleus (δ 2.14). In addition there were bands assignable to the methylene protons of two $-CH_2OH$ groupings at δ 4.69 and 4.61 (singlets, 2 protons each) which appeared at δ 5.21 and 5.15 in the spectrum of the diacetate. The spectrum also showed signals attributable to a 3,3-dimethylallyloxy grouping (2).

When zinniol diacetate (**2**) was warmed with acetic anhydride containing a small amount of sulfuric acid, a triacetate (**5**) was formed. The n.m.r. spectrum of this compound showed the absence of bands due to the dimethylallyloxy side chain and the presence of an additional acetyl grouping. This indicated that solvolysis of the allylic ether function followed by acetylation of the resulting phenol had occurred. Zinniol, similarly treated, gave the same product, though in lower yield.

Chromic acid oxidation of zinniol (**1**) in acetone afforded two isomeric phthalides, **6** and **10**, which could be reduced to zinniol with lithium aluminium hydride. Phthalide **6** was also obtained during the chromatographic isolation of zinniol but it was probably an artifact since aqueous alkali soluble substances had been removed from the original culture extract at an earlier stage. Phthalide **6**, when warmed with acetic anhydride – sulfuric acid, gave an acetate (**7**) lacking the dimethylallyl grouping. Similarly, phthalide **10** gave the isomeric acetate **11**.

Hydrolysis of acetate **7** gave a phenol, needles, m.p. 234–236°, corresponding to 6-hydroxy-4-methoxy-5-methyl phthalide (**8**), m.p. 234–235°, obtained previously from quadrilineatin, a metabolic product of *Aspergillus quadrilineatus* (3). Methylation of this phenol gave a substance, m.p. 158–159°, identical with 4,6-dimethoxy-5-methyl phthalide (**9**) which was obtained synthetically (3).

The presence of the dimethylallyloxy substituent on the aromatic nucleus of zinniol was confirmed by reacting **8** with 1-chloro-3-methyl-2-butene to give phthalide **6**.

Hydrolysis of acetate **11** yielded a phenol, m.p. 234–235° (change in crystal form approximately 190–200°) which differed from the m.p. of 198° reported (3) for 5-hydroxy-7-methoxy-6-methyl phthalide (**12**). This discrepancy is probably due to the occurrence of different crystalline forms as suggested by the phase change noted during determination of the melting point. Methylation with diazomethane yielded a substance, m.p. 172–173°, corresponding to 5,7-dimethoxy-6-methyl phthalide (**13**), m.p. 172–172.5°, synthesized by Birkinshaw *et al.* (4).

The evidence so far adduced indicates that **1** represents the structure of zinniol. However, no direct proof other than melting point data has been introduced to exclude the possibility that the methoxy and dimethylallyloxy substituents may be interchanged. The following observations support the assigned structure. Phenol **12** did not give a color reaction with ferric chloride as would be expected for the alternative formulation,

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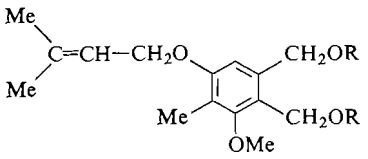

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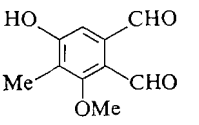
2 R = Ac
3 R = Bz



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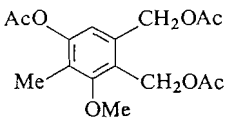

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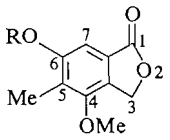
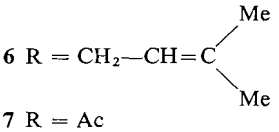
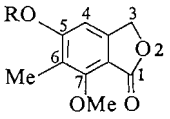
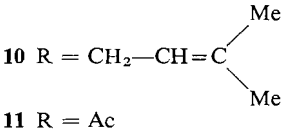




6 R = CH_2—CH=C(Me)(Me)

7 R = Ac
8 R = H
9 R = Me





10 R = CH_2—CH=C(Me)(Me)

11 R = Ac
12 R = H
13 R = Me

7-hydroxy-5-methoxy-6-methyl phthalide (3, 7), and the shift in the ultraviolet spectrum of phenol **12** when measured in alkali corresponded to that anticipated for a *p*-hydroxybenzoic acid (5). Since this phenol must therefore have structure **12** it follows that structure **1** represents zinniol.

Only acetate **7** could be isolated from the acetylated phenolic fraction of the culture medium extract. It is not known whether this compound is a natural product or an artifact although a late stage in the biosynthesis of zinniol probably involves the alkylation of a phenolic precursor with 3,3-dimethylallyl pyrophosphate (6).

Mannitol was isolated from the mycelium of *A. zinniae*.

Experimental

Melting points were measured on a Kofler hot stage and are uncorrected. Ultraviolet spectra of substances dissolved in 95% ethanol were recorded using a Beckman DK spectrophotometer and infrared spectra were obtained on a Perkin-Elmer model 21 spectrometer. Neutral Woelm alumina standardized according to Brockmann was used for column chromatography. Kieselgel (Camag) containing fluorescent indicator was used for thin-layer chromatography (t.l.c.). Nuclear magnetic resonance spectra were obtained with Varian A-60 and A-60A spectrometers using $CDCl_3$ as solvent. Chemical shifts are reported in p.p.m. (δ) downfield from an internal tetramethylsilane reference. Microanalyses were performed by Dr. C. Daesslé, Montreal. Petrol refers to a light petroleum fraction of b.p. 60–80°.

Zinniol (1)

The isolation of this compound has been described (1). Nuclear magnetic resonance (n.m.r.) spectrum: δ 1.73 (6 protons, broad singlet, $=CMe_2$), 2.14 (3 protons, singlet, aromatic Me), 3.22 (2 protons, broad band, hydroxyl protons), 3.72 (3 protons, singlet, —OMe), 4.50 (2 protons, doublet, J = 7 c.p.s., $=C(H)-CH_2-O$), 4.61 and 4.69 (singlets of 2 protons each, $-CH_2OH$), 5.46 (1 proton, triplet, J = 7 c.p.s., =CH—) and 6.66 (1 proton, singlet, aromatic).

Zinniol Diacetate (2)

The preparation of this compound has been described (1). Nuclear magnetic resonance spectrum: δ 1.77 (6 protons, broad singlet, $=CMe_2$), 2.05, and 2.08 (singlets of 3 protons each, —OAc), 2.17 (3 protons, singlet, aromatic Me), 3.73 (3 protons, singlet, —OMe), 4.53 (2 protons, doublet, J = 7 c.p.s., $=C(H)-CH_2-O$), 5.15 and 5.21 (singlets of 2 protons each, $-CH_2OAc$), 5.47 (1 proton, triplet, J = 7 c.p.s., =CH—) and 6.71 (1 proton, singlet, aromatic).

Zinniol Dibenzoate (3)

A solution of zinniol (29 mg) in pyridine (10 drops) was treated for 18 h with freshly distilled benzoyl chloride (5 drops) at room temperature. After dilution with water, the product was extracted into ether. Removal of the ether afforded a gum which was separated by preparative t.l.c. (chloroform–benzene, 3:1). Isolation of material from the major band and recrystallization from ether–petrol gave zinniol dibenzoate (**3**), m.p. 79–80°; ν_{max} ($CHCl_3$) 1705, 1603, 1586 cm^{-1}.

Anal. Calcd. for $C_{29}H_{30}O_6$: C, 73.40; H, 6.37. Found: C, 73.75; H, 6.31.

Triacetate (5) from Zinniol Diacetate

Zinniol diacetate (50 mg) was heated on a steam bath

for 15 min in acetic anhydride (2 ml) containing 1 drop of concentrated sulfuric acid. The solution was diluted with water and extracted with chloroform. The product was chromatographed over alumina (Grade III; 5g). The material (34 mg) eluted with benzene was recrystallized from ether - isopropyl ether to give the triacetate m.p. 79–80°; ν_{max}(KBr) 1735 cm^{-1}; n.m.r. spectrum: δ 2.06 and 2.11 (singlets of 6 protons and 3 protons, —OAc) 2.31 (3 protons, singlet, aromatic Me), 3.74 (3 protons, singlet, —OMe), 5.13, and 5.23 (singlets of 2 protons each, —CH_2 OAc) and 6.88 (1 proton, singlet, aromatic).

Anal. Calcd. for $C_{16}H_{20}O_7$: C, 59.25; H, 6.22. Found: C, 59.15; H, 5.75.

Triacetate from Zinniol

Zinniol (77 mg) was treated with acetic anhydride containing a trace of sulfuric acid as described for zinniol diacetate. Chromatography of the product over alumina (Grade III; 5g) followed by separation by preparative t.l.c. (chloroform) yielded material (22 mg) which was recrystallized from ether - isopropyl ether to give the triacetate (**5**).

Oxidation of Zinniol

Zinniol (52 mg) in acetone (20 ml) was stirred at room temperature for 3 min with an excess of a stock chromic acid solution prepared from 11.0 g of chromium trioxide in 10 ml of concentrated sulfuric acid and diluted to 50 ml with water (8). The reaction solution was diluted with water and the product isolated by ether extraction. The product proved to be a mixture from which two isomeric crystalline compounds were obtained by preparative t.l.c. (chloroform). Recrystallization of the less polar component (13 mg) from ether–petrol gave phthalide **6**, m.p. 85–86°; ν_{max}($CHCl_3$) 1750 cm^{-1}; λ_{max} 253, 301 mμ (ε 7100, 3500); n.m.r. spectrum: δ 1.74 (6 protons, broad singlet, $=CMe_2$), 2.18 (3 protons, singlet, aromatic Me), 3.86 (3 protons, singlet, —OMe), 4.55 (2 protons, doublet, J = 7 c.p.s., $=\overset{|}{C}—CH_2—O$), 5.33 (2 protons, singlet, $—CH_2—O—C=O$), 5.46 (1 proton, triplet, J = 7 c.p.s., $=CH—$) and 7.03 (1 proton, singlet, aromatic).

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.60; H, 6.18.

The second component (23 mg) was recrystallized from ether - isopropyl ether to give phthalide **10** as needles, m.p. 106–108°; ν_{max}($CHCl_3$) 1743 cm^{-1}; λ_{max} 259 mμ (ε 15 200); n.m.r. spectrum: δ 1.77 (6 protons, broad singlet, $=CMe_2$), 2.13 (3 protons, singlet, aromatic Me), 4.02 (3 protons, singlet, —OMe), 4.56 (2 protons, doublet, J = 6.5 c.p.s., $=\overset{|}{C}—CH_2—O$), 5.13 (2 protons, singlet, $—CH_2—O—C=O$), 5.46 (1 proton, triplet, J = 6.5 c.p.s., $=CH—$) and 6.58 (1 proton, singlet, aromatic).

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 69.31; H, 7.19.

Reduction of the crude oxidation product in anhydrous ether with lithium aluminium hydride yielded zinniol. No other product was detected by t.l.c.

Phthalide 6 from Extract of Culture Filtrate

Early fractions eluted with petrol–benzene (1:1) during the chromatographic isolation of zinniol (1) gave crystalline material (85 mg) which, after recrystallization from ether–petrol, was found to be identical (t.l.c., mixture m.p., infrared) with phthalide **6**, m.p. 84–85°, obtained by oxidation of zinniol.

Acetate 7

Phthalide **6** (22 mg) was heated on a steam bath for 15 min in acetic anhydride (1 ml) containing 1 drop of concentrated sulfuric acid. The reaction solution was diluted with water and the product isolated by chloroform extraction. Removal of the chloroform gave a crystalline residue which was chromatographed over alumina (Grade III; 3g). Elution with benzene afforded a substance which was recrystallized from chloroform - isopropyl ether to give acetate **7**, m.p. 134–135°; ν_{max}(KBr) 1753 cm^{-1}; n.m.r. spectrum: δ 2.17 (3 protons, singlet, aromatic Me), 2.36 (3 protons, singlet, —OAc), 3.94 (3 protons, singlet, —OMe), 5.45 (2 protons, singlet, $—CH_2—O—C=O$), and 7.35 (1 proton, singlet, aromatic).

Anal. Calcd. for $C_{12}H_{12}O_5$: C, 61.01; H, 5.12. Found: C, 60.98; H, 5.51.

Acetate 11

Phthalide **10** (109 mg) was warmed with acetic anhydride (3 ml) containing a drop of sulfuric acid under the conditions described above. Isolation and chromatography of the product over alumina gave crystalline material (46 mg) which was recrystallized from chloroform – isopropyl ether to yield acetate **11** as plates, m.p. 100–101°; ν_{max}(KBr) 1755, 1739 cm^{-1}; n.m.r. spectrum: δ 2.18 (3 protons, singlet, aromatic Me), 2.36 (3 protons, singlet, —OAc), 3.95 (3 protons, singlet, —OMe), 5.46 (2 protons, singlet, $—CH_2—O—C=O$) and 7.36 (1 proton, singlet, aromatic).

Anal. Calcd. for $C_{12}H_{12}O_5$: C, 61.01; H, 5.12. Found: C, 61.49; H, 5.26.

Hydrolysis of Acetate 7

Acetate **7** (37 mg) in methanol (3.5 ml) was refluxed under nitrogen for 15 min with potassium hydroxide solution (0.5 ml; 16%). After dilution with water, the reaction mixture was neutralized with dilute hydrochloric acid and extracted with ether. Removal of the ether and recrystallization of the product from methanol–chloroform gave phenol **8** as needles, m.p. 233–235°; ν_{max}(KBr) 3350, 1738 cm^{-1}; λ_{max} 254, 304 mμ (ε 5840, 3200) and, after addition of 1 drop of 0.1 *N* NaOH, λ_{max} 338 mμ (ε 3880). Sublimation in high vacuum at 125° gave needles, m.p. 234–236°.

Methylation of Phenol 8

Phenol **8** (25 mg) in ether was treated overnight at room temperature with diazomethane in ether. The reaction mixture was evaporated to dryness and chromatographed over alumina (Grade III; 2g). Elution with benzene yielded material (21 mg) which was recrystallized from chloroform - isopropyl ether to give needles, m.p. 158–159°; ν_{max}($CHCl_3$) 1753 cm^{-1}; ν_{max}(KBr) 1755, 1735 cm^{-1}; n.m.r. spectrum: δ 2.22 (3 protons, singlet, aromatic Me), 3.90 (6 protons, singlet, —OMe), 5.38 (2 protons, singlet, $—CH_2—O—C=O$) and 7.11 (1 proton, singlet, aromatic). This substance was identical (m.p., mixture m.p., t.l.c., infrared) with 4,6-dimethoxy-5-methyl phthalide (***9***) obtained synthetically (3).

Phthalide 6 from Phenol 8

Phenol **8** (3.2 mg) in anhydrous acetone (3 ml) containing potassium carbonate (200 mg) was refluxed 6 h with 1-chloro-3-methyl-2-butene (0.05 ml). The potassium carbonate was removed and washed with acetone and the solvent evaporated *in vacuo*. The product was purified by t.l.c. (chloroform) and recrystallization from ether-petrol gave phthalide **6**, m.p. 85–86°, undepressed by mixture with phthalide **6** obtained by oxidation of zinniol. Thin-layer chromatography behavior and the infrared spectra further demonstrated these compounds to be identical.

Hydrolysis of Acetate 11

Acetate **11** (29 mg) was hydrolyzed in the same manner as described for acetate **7**. The product (23 mg) was recrystallized from methanol–chloroform to yield phenol **12**, m.p. 233–234° (change in crystal form approximately 190–200°); ν_{max}(KBr) 3280, 1717 cm^{-1}; λ_{max} 260 mμ (ε 11 700) and after addition of 1 drop of 0.1 *N* NaOH, $\lambda_{inflection}$ 234, λ_{max} 302 mμ, (ε 17 500, 26 800). Phenol **12** showed no color reaction with ferric chloride.

Anal. Calcd. for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 61.80; H, 5.19.

Methylation of Phenol 12

Phenol **12** (17 mg) in ether (4 ml) and methanol (0.5 ml) was treated overnight at room temperature with diazomethane in ether. The solvent was removed and the residue chromatographed over alumina (Grade III; 2 g). Elution with benzene yielded material which, after recrystallization from chloroform – isopropyl ether, gave **13** as needles, m.p. 172–173°; ν_{max}(KBr) 1738, 1608 cm^{-1}; n.m.r. spectrum: δ 2.15 (3 protons, singlet, aromatic Me), 3.92, 4.06 (singlets of 3 protons each, —OMe), 5.18 (2 protons, singlet, $—CH_2—O—C=O$), and 6.64 (1 proton, singlet, aromatic). Birkinshaw *et al.* (4) report m.p. 172–172.5° for 5,7-dimethoxy-6-methyl phthalide.

Anal. Calcd. for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.51; H, 5.88.

Phenol 8 from Alkaline Extract

Sodium hydroxide soluble material (900 mg) from the extraction of two batches of culture medium (1) was chromatographed over silica gel (British Drug Houses; 100 g). Benzene–ether (3:1) eluted a crystalline fraction (47 mg) which was acetylated (acetic anhydride – pyridine, overnight at room temperature). The product was purified by preparative t.l.c. (chloroform–methanol, 49:1) and recrystallized from chloroform – isopropyl ether to give a substance (12 mg) identified (m.p., mixture m.p., infrared) as the acetate of phenol **8**, m.p. 136–137°. No other compound could be isolated from the complex mixture.

Isolation of Mannitol from the Mycelium

Mycelium of *Alternaria zinniae* was continuously extracted in a Soxhlet apparatus with petrol (1 day), chloroform (4 days), and methanol (3 days). The methanol extract yielded material which was recrystallized from methanol to give needles, m.p. 165–166°, identical (m.p., mixture m.p., infrared) with authentic mannitol. Acetylation yielded mannitol hexaacetate, m.p. 123–124° (chloroform–methanol). No other crystalline substance could be obtained from the mycelial extracts.

Acknowledgments

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