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Synthesis of four Ethyl-dimethylbenzenetriols in Relation to a new Phenolic Metabolite of *Penicillium baarnense*

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From the medium of *Penicillium baarnense* a new phenolic compound has been isolated and shown to be 5-ethyl-4,6-dimethylpyrogallol. In the course of the investigation four new isomers have been synthesized.

In the search for an intermediate of the series orsellinic acid → → → penicillic acid [1] a new phenolic compound was isolated, m.p. 145–146°. This substance, which we call barnol, appeared in the medium of a surface culture of *Penicillium baarnense* v. Beyma after about two weeks of incubation. In the medium of this mould, growing on Raulin-Thom, barnol was produced in great abundance, whereas this metabolite could not be isolated from the medium of a culture on Czapek-Dox. On the other hand, *Penicillium baarnense* is known to produce both orsellinic and penicillic acid on a medium of Czapek-Dox.[1]

The elementary analysis and molecular weight determination gave the formula $C_{10}H_{14}O_3$. Acetylation of barnol resulted in a triacetyl derivative, m.p. 130–131°, thus suggesting three free hydroxyl groups. Kuhn-Roth oxidation yielded 2.7 methyl groups bound to carbon.

For further analysis the nuclear magnetic resonance of the acetylated phenol (Fig. 1) was investigated. The spectrum of 15 mg of acetylated barnol in 0.5 ml of CCl_4 shows one ethyl group most likely attached to an aromatic ring. Three signals in the region of methyl groups, corresponding to those bound to an aromatic ring as well as to an acetyl group, show an integrated intensity equivalent to five methyl groupings. No signals could be detected from protons bound to aromatic ring systems.

Summarizing these results, barnol consists of one ethyl, two methyl and three hydroxy groups attached to a benzene nucleus. Six different isomeric compounds can be thought of (Fig. 2).

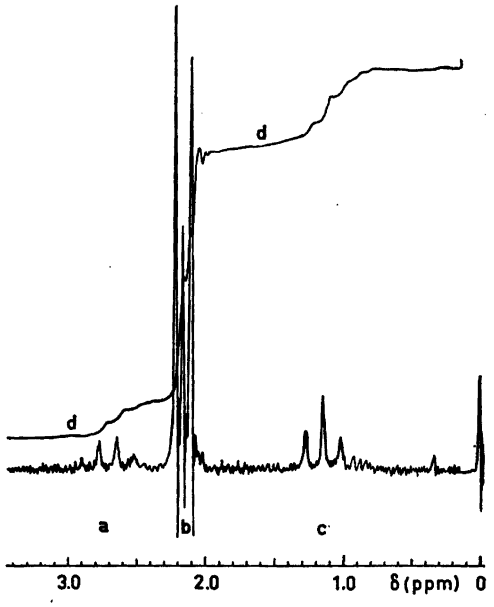

Fig. 1. NMR-spectrum of 15 mg of acetylated barnol in 0.5 ml of CCl_4. a.) CH_3 in ethyl group. b.) acetyl- and phenylmethylregion. c.) CH_3 in ethyl group. d.) integrated curve.

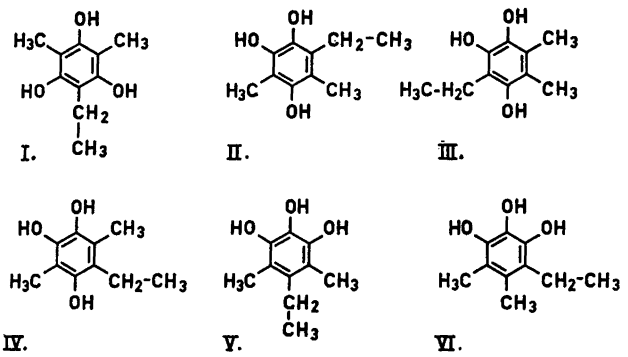

Fig. 2.

Compound I, 2-ethyl-4,6-dimethylphloroglucinol, differed from barnol in color reaction and melting point, m.p. 136°.[2]

Of the remaining five isomers, II—V were synthesized.

The melting point of barnol was not depressed on admixture with compound V, m.p. 145—146° and the IR spectra of both substances proved to be identical (Fig. 3).

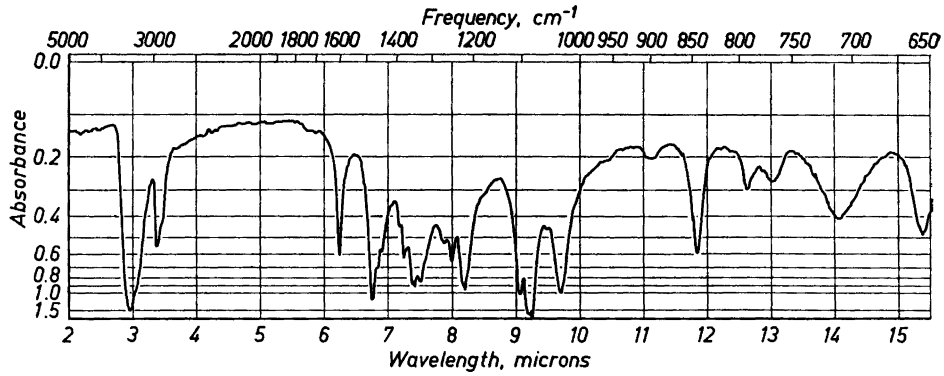

Fig. 3. IR-spectrum of 1.2 mg barnol in 303 mg KBr.

For studies of homogeneity and identification the two compounds were run on paper chromatogram in two different solvent systems. They were detected as blue spots by spraying with a 10 % solution of Folin and Ciocalteus phenol reagent; ferric chloride in ethanol gave a green color reaction. The R_F values were as follows: in solvent B [3] = 0.75 (chloroform:methanol:4 % formic acid, 10:1:1), in solvent D [3] = 0.40 (benzene:2 % formic acid, 10:1).

Compounds II—IV were synthesized from their corresponding ethyl-dimethyl-*p*-benzoquinones using Thieles oxidative acetylation with subsequent alkaline hydrolysis (Fig. 4). These substances showed a red color reaction with ferric chloride in ethanol contrary to the green color developed by barnol. The melting points of compounds II and III as well as their corresponding acetyl derivatives differed exceedingly from that of barnol. Compound IV had only a slightly higher melting point; however, its acetyl derivative deviated by about 10°.

Compound V was synthesized from 5-etyl-1,2,3-trimethoxybenzene by chloromethylation, reduction and subsequent demethylation.

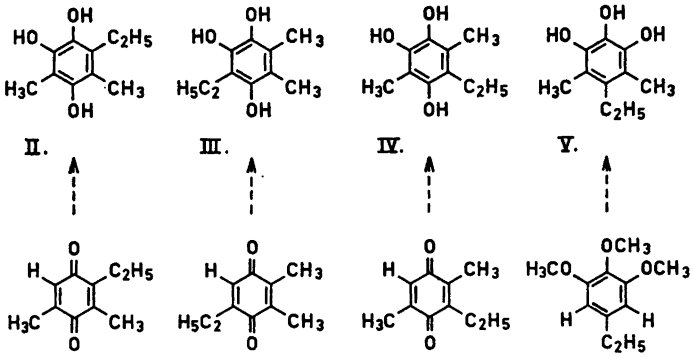

Fig. 4.

EXPERIMENTAL

Culture conditions. Penicillium baarnense v. Beyma was grown on Raulin-Thom medium* as a surface culture in 2 l Fernbach-flasks (0.5 l of medium) for two weeks at 27°.

Isolation The mycelium was filtered off and the medium was extracted with ether after acidification. The ether was evaporated and the residue was submitted to sublimation at 12 mm Hg and 100°. The sublimed white substance was recrystallized three times from CCl_4. The colorless needle-shaped crystals gave a melting point of 145–146°. (Found: C 65.8; H 7.8; O 26.5).

Synthesis of II. 2-Ethyl-3,5-dimethyl-*p*-benzoquinone was synthesized as described by Smith and Opie.[4]

272 mg of this quinone were dissolved in 2.5 ml of Ac_2O (containing 3 % (v/v) conc. H_2SO_4) and kept in a closed vessel for about four days at room temperature. After dilution with 25 ml of water, the triacetate precipitated and was recrystallized from 2 M AcOH. Yield 315 mg, m.p. 95°. (Found C 62.0; H 6.5; O 31.1. Calc. for $C_{16}H_{20}O_6$ C 62.3; H 6.5; O 31.2).

Hydrolysis of the triacetate: 40 mg of the triacetate were dissolved in 40 ml of 50 % aqueous ethanol in a three-necked flask. After flushing with N_2 gas for 10 min, 6 ml of 1 N NaOH were added through a dropping funnel. The reaction mixture was then kept for 48 h at room temperature and thereafter acidified with diluted HCl under N_2-atmosphere and the triphenol extracted with ether. The ether was evaporated in vacuum and the residue sublimed at 12 mm Hg and 100°. The white crystals were recrystallized from CCl_4. Color reactions: with ferric chloride red, with Folin-Ciocalteus reagent blue and with alkali red-violet. Yield 8 mg, m.p. 135°. (Found C 65.7; H 7.4; O 26.6. Calc. for $C_{10}H_{14}O_3$ C 65.9; H 7.7; O 26.4).

Synthesis of III. 2-Ethyl-5,6-dimethyl-*p*-benzoquinone was synthesized as desribed by Smith and Opie.[4] The triacetate was prepared from 650 mg of the benzoquinone analogous to the procedure above. Yield 515 mg, m.p. 120–121°. (Found C 62.3; H 6.5; O 31.0. Calc. for $C_{16}H_{20}O_6$ C 62.3; H 6.5; O 31.2).

Hydrolysis of the triacetate: 40 mg of the triacetate was hydrolysed and the triphenol purified in the same way as described above. The color reactions were as for compound II. Yield 6.5 mg, m.p. 124°. (Found C 65.5; H 7.5; O 26.5. Calc. for $C_{10}H_{14}O_3$ C 65.9; H 7.7; O 26.4.).

Synthesis of IV. 2-Ethyl-3,6-dimethyl-*p*-benzoquinone was synthesized as described by Smith and Opie.[4] The triacetate was prepared from 250 mg of the benzoquinone analogous to the procedure above. Yield 135 mg, m.p. 139–140°. (Found C 62.6; H 6.3; O 31.0. Calc. for $C_{16}H_{20}O_6$ C 62.3; H 6.5; O 31.2.).

Hydrolysis of the triacetate: 40 mg of the triacetate were hydrolysed and the triphenol purified in the same way as described for compound II. The color reactions were as for II and III. Yield 6 mg, m.p. 147–148°. (Found C 65.9; H 7.7; O 26.3. Calc. for $C_{10}H_{14}O_3$ C 65.9; H 7.7; O 26.4.).

Synthesis of V. 4-Hydroxy-3,5-dimethoxyacetophenone = acetosyringon. Acetosyringon was prepared from 2-hydroxy-1,3-dimethoxybenzene as described by Mauthner.[5] The latter was acetylated with acetyl chloride and distilled in vacuum. 20 g acetyldimethylpyrogallol were rearranged in 100 g of nitrobenzene after addition of 12 g of aluminium chloride. It was found, that leaving the reaction mixture at room temperature for 48 h instead of 24 as stated by Mauthner resulted in a higher yield of acetosyringon, (5 g instead of 1.5 g). After addition of ice, acidification with HCl and warming on the water-bath, the mixture was extracted with ether. The ether phase was then shaken with 1 N NaOH and after acidification of the alkaline solution, acetosyringon was isolated by extraction with ether. After recrystallization from water it showed a melting point of 120–123°. Yield 5 g.

5-Ethyl-1,2,3-trimethoxybenzene.[6] Acetosyringon was submitted to a Clemmensen reduction as follows. 5 g of acetosyringon were dissolved in a mixture of 100 ml of water and 20 ml of conc. HCl. After addition of 10 g of amalgamated Zn dust and refluxing for 3 h, during which time 20 ml of 50 % HCl were added every hour, the 5-ethyl-2-hydroxy-1,3-dimethoxybenzene was extracted with ether. The ether was evaporated and the oily residue methylated with a total amount of 25 ml of dimethyl sulphate. In doing so, the

* No $CuSO_4$ present.

compound was dissolved in 50 ml of methanol, the dimethyl sulphate was added in small portions during one hour together with small amounts of 30 % KOH to keep the reaction mixture alkaline. It was heated for another 2 h at 60—70°, thereafter diluted with water, acidified and extracted with ether. After evaporation of the solvent, an oily residue was obtained which was distilled, b.p. 132—135°/12 mm. Yield 3.4 g, $_D^{20}$ = 1.5175. (Found C 67.4; H 8.2. Calc. for $C_{11}H_{16}O_3$ C 67.3; H 8.1.)

5-Ethyl-1,2,3-trimethoxy-4,6-dimethylbenzene. 3 g of the 5-ethyl-1,2,3-trimethoxybenzene were dissolved in 40 ml of CCl_4 in a three-necked flask equipped with condenser and stirrer. 1.2 g of paraformaldehyde and 3 g of anhydrous $ZnCl_2$ were added and dry HCl bubbled through the solution, which was refluxed for 3 h. After dilution with 200 ml of water, the chloromethylated compound was extracted with ether. The ether solution was then washed several times with water and the solvent driven off in vacuum. The oily residue obtained weighed 4 g. It was reduced by boiling in 50 ml of AcOH together with 5 g of Zn dust for 3 h. The mixture was then diluted with 200 ml of water and extracted with ether. The ether solution was evaporated in vacuum and gave 2.7 g of a thick oil.

5-Ethyl-4,6-dimethylpyrogallol = compound V. Demethylation of 1.5 g of the 5-ethyl-1,2,3-trimethoxy-4,6-dimethylbenzene was accomplished by refluxing with a mixture of 20 ml of AcOH, 500 mg of red phosphorus and 4 ml of freshly distilled HI (b.p. 127°) for 3—4 h. After dilution with 100 ml of water and extraction several times with 50 ml portions of ether, the solution was evaporated in vacuum and the residue sublimed at 12 mm Hg and 100°. The sublimed product was recrystallized from CCl_4 yielding colorless, needleshaped crystals. The purity was further checked by paper chromatography as mentioned above. Yield 150 mg, m.p. 145—146°. (Found C 65.5; H 7.6; O 26.6. Calc. for $C_{10}H_{14}O_3$ C 65.9; H 7.7; O 26.4.).

Triacetyl derivative of compound V. 2 ml of Ac_2O and 25 µl of conc. H_2SO_4 were added to 100 mg of compound V and the reaction mixture kept boiling for 1 min. After cooling and diluting with water, the white triacetyl derivative precipitated. Recrystallization from 2 M AcOH gave 135 mg of this compound, m.p. 130—131°. (Found C 62.4; H 6.4; O 31.2. Calc. for $C_{16}H_{20}O_6$ C 62.3; H 6.5; O 31.2.).

Added in proof: The *Penicillium* species responsible for the production of barnol has been characterized as a special type of *Penicllium baarnense* v. Beyma carrying two different forms of conidia spores.

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