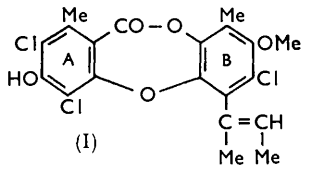
986. *Studies in Mycological Chemistry. Part XV.* Synthesis of 2,5-Dihydroxy-3-methyl-6-s-butyl-1,4-benzoquinone and its Bearing on the Structure of Nidulin.*

By B. W. Bycroft, J. A. Knight, and John C. Roberts.

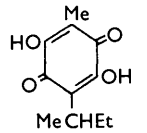
An unequivocal synthesis of 2,5-dihydroxy-3-methyl-6-s-butyl-1,4-benzoquinone is described. The identity of this substance with a degradation product of nidulin confirms the structure previously allocated to the metabolite and refutes an alternative claim.

Nidulin, a metabolite [1,2] of a non-ascosporic strain of *Aspergillus nidulans*, has been assigned [3] structure (I). Two of its degradation products have been formulated [3] as the chlorohydroxyquinone (II; R = Cl) and the dihydroxyquinone (II; R = OH). The nature of the unsaturated side-chain in these quinones was established [3] by oxidation of the chloroquinone (II; R = Cl) to tiglic acid. The final formulation of the dihydroxyquinone (II; R = OH) depended on evidence from its analysis, colour reactions,[3] and light-absorption properties.[3,4] Nevertheless, it was felt necessary to confirm its structure by synthesis, and the necessity of such action was emphasised when, recently, it was claimed [5] that nidulin should be represented by structure (IV).



(I)

(II)



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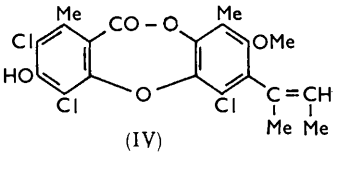
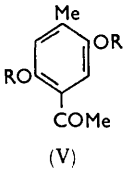


(IV)



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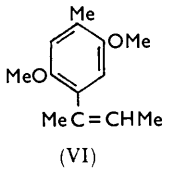

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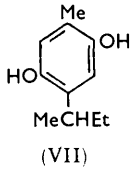

(VII)

(VIII)

We have made numerous unsuccessful attempts to synthesise a compound of structure (II; R = OH), the main difficulty encountered being the tendency of a substituted styrene [*e.g.*, (VI)] to polymerise under the strongly acidic conditions necessary for its subsequent demethylation. However, we have found that catalytic hydrogenation of the degradation compound (II; R = OH), followed by aerial oxidation of the product, readily yielded the dihydro-derivative (III), and we now describe an unequivocal synthesis of a quinone of this structure.

Submission of 2,5-diacetoxytoluene to the Fries reaction gave an acetophenone (V; R = H), the orientation of which [6] was confirmed thus. First, an examination of the proton magnetic resonance spectrum of the di-*O*-methyl compound (V; R = Me) revealed no splitting in those signals corresponding to the two aromatic protons, which are, therefore, placed 1,4 rather than 1,3 or 1,2. Secondly, vigorous oxidation of the ether (V; R = Me) gave an acid which was identified as 2,5-dimethoxyterephthalic acid by comparison with an authentic specimen prepared by an unambiguous method (see below).

* Part XIV, *J.*, 1963, 4868.

[1] Dean, Robertson, Roberts, and Raper, *Nature*, 1953, **172**, 344.
[2] Dean, Roberts, and Robertson, *J.*, 1954, 1432.
[3] Dean, Deorha, Erni, Hughes, and Roberts, *J.*, 1960, 4829.
[4] Bycroft and Roberts, *J. Org. Chem.*, 1963, **28**, 1429.
[5] Beach and Richards, *J. Org. Chem.*, 1961, **26**, 1339, 3011.
[6] Cf. Desai and Mavani, *Proc. Indian Acad. Sci.*, 1949, **29**, *A*, 269.

Treatment of the acetophenone (V; R = Me) with ethylmagnesium iodide gave a tertiary alcohol (not isolated) which was readily converted into the styrene (VI). Catalytic hydrogenation of this compound and demethylation of the product gave the quinol (VII). Bromination of this quinol, followed by gentle oxidation, yielded the quinone (VIII). Alkaline hydrolysis of the dibromoquinone (VIII) gave, in poor yield, a dihydroxyquinone of structure (III) which proved to be identical with the compound obtained from nidulin.

This result unambiguously fixes the relative positions of the methyl and 1'-methylpropenyl groups in the quinone degradation product (II; R = OH) and hence, together with other evidence previously presented,[2,3] confirms the orientation of the substituent groups in ring B of nidulin (I). The claim made by other workers [5] is thus refuted.

Experimental

M. p.s were determined, unless otherwise stated, on the Kofler block. Infrared absorption spectra were measured for compounds in potassium bromide discs with a Unicam (S.P. 200) spectrophotometer. Ultraviolet absorption spectra were determined on ethanolic solutions with a Unicam (S.P. 700) spectrophotometer.

2,5-*Dihydroxy-4-methylacetophenone* (V; R = H).—Toluquinol was acetylated, at room temperature for 5 min., with acetic anhydride containing a trace of concentrated sulphuric acid. The diacetate was isolated in the usual way and was obtained in 91% yield as needles (from ethanol), m. p. 49—50°. This material (30 g.) and anhydrous aluminium chloride (58·5 g.) were heated at 160° for 3 hr. The product (2,5-dihydroxy-4-methylacetophenone), isolated in the usual way, separated from benzene as golden-yellow plates (12 g., 53%), m. p. 145—146° (lit.,[6] 141°) (Found: C, 64·6; H, 5·9. Calc. for $C_9H_{10}O_3$: C, 65·0; H, 6·1%).

2,5-*Dimethoxy-4-methylacetophenone* (V; R = Me).—The foregoing substance (10 g.), methyl sulphate (17 ml.), anhydrous potassium carbonate (33 g.), and dry acetone (500 ml.) were heated under reflux for 18 hr. The product, isolated in the usual way, crystallised from ethanol to give the *acetophenone* as colourless needles (10·5 g.), m. p. 72—73° (Found: C, 67·8; H, 7·1. $C_{11}H_{14}O_3$ requires C, 68·0; H, 7·3%). The proton magnetic resonance spectrum of this compound in methylene dichloride solution showed, *inter alia*, two unsplit singlets (corresponding to the two aromatic protons) at 2·81 and 3·23 τ.

2,5-*Dimethoxyterephthalic Acid*.—(i) The foregoing substance (5 g.), together with pyridine (20 ml.) and aqueous 0·33N-sodium carbonate (90 ml.), was heated on the steam-bath. Potassium permanganate (24·8 g.) was added gradually during 1 hr. and the mixture was heated further for 1 hr. The mixture was filtered and the combined filtrate and washings, after evaporation to a volume of *ca.* 60 ml., were extracted with ether (2 × 30 ml.). Acidification, by 40% sulphuric acid, of the aqueous layer gave a yellow precipitate which was collected, washed, and crystallised from hot water to give the required acid as needles (1·6 g.), m. p. 266—268° unaltered on admixture with a sample prepared as below. The infrared absorption spectra of the two acids were identical.

(ii) A solution of 2,5-dimethylphenol (10 g.), m. p. 74—75°, in acetone (50 ml.) was added slowly, with stirring, to a solution of Frémy's salt (50 g.) and sodium acetate (1·6 g.) in water (500 ml.).[7] The mixture was stirred at room temperature for 1 hr. and the precipitated quinone was collected by filtration. (A further quantity was obtained by extraction of the filtrate with ether.) The combined crop was crystallised from light petroleum (b. p. 80—100°) to give 2,5-dimethyl-1,4-benzoquinone (8·0 g., 72%) as yellow needles, m. p. 124—125° (lit.,[8] 125°). A solution of this substance (7·5 g.) in ether (75 ml.) was shaken with a saturated aqueous solution (3 × 50 ml.) of sodium dithionite. The colourless ether layer was washed with water and was dried ($MgSO_4$). Removal of the ether and washing of the residue with light petroleum (b. p. 40—60°) gave 2,5-dihydroxy-1,4-dimethylbenzene (6·6 g., 88%), m. p. 209—212°. This substance (6·0 g.) was methylated (by the methyl sulphate–acetone–potassium carbonate method) to give 2,5-dimethoxy-1,4-dimethylbenzene (5·0 g., 69%) as needles (from aqueous ethanol), m. p. 107—108° (lit.,[9] 108°). Oxidation of this substance (4·8 g.) in aqueous

[7] Cf. Teuber and Rau, *Chem. Ber.*, 1953, **86**, 1036.
[8] Nietzki, *Ber.*, 1880, **13**, 472.
[9] Noelting and Werner, *Ber.*, 1890, **23**, 3251.

pyridine with potassium permanganate (27·8 g.), and isolation of the product [see method (i) above] gave a mixture (1·7 g.) of acids. Extraction of this mixture with cold chloroform removed one of the components (probably 2,5-dimethoxy-4-methylbenzoic acid), and the residue, when crystallised from hot water, yielded the required acid as needles (0·9 g.), m. p. 267—268° (lit.,[10] 265°).

2,5-*Dimethoxy*-1-*methyl*-4-*s*-*butylbenzene* (VII; OMe for each OH).—2,5-Dimethoxy-4-methylacetophenone (10 g.), in dry ether (100 ml.) was added dropwise, with stirring and cooling, to a solution of ethylmagnesium iodide prepared from ethyl iodide (6·7 ml.), magnesium (2·7 g.), and ether (70 ml.). The mixture was heated under reflux for 4 hr. and the ether was evaporated. The residue was heated on the steam-bath for 1 hr. and then under reflux for 15 min. with 2N-hydrochloric acid (250 ml.). The product, an oil (isolated by ether-extraction), was distilled over sodium [11] (*ca.* 0·2 g.), and the colourless oily fraction of b. p. 142—143°/14 mm. was collected. A solution of this oil (7 g.) in methanol (100 ml.) was shaken with 10% palladised charcoal (50 mg.) in an atmosphere of hydrogen. (In replicate experiments the uptake of hydrogen was 0·97 and 1·02 mol.) Filtration of the mixture and removal of the solvent from the filtrate left a residue which, on distillation, gave 2,5-*dimethoxy*-1-*methyl*-4-*s*-*butylbenzene* (5 g.), b. p. 130—133°/10 mm. (Found: C, 74·9; H, 9·8. $C_{13}H_{20}O_2$ requires C, 75·0; H, 9·7%).

2,5-*Dihydroxy*-1-*methyl*-4-*s*-*butylbenzene* (VII).—The foregoing ether (4·5 g.) was heated under reflux for 3 hr. with glacial acetic acid (45 ml.) and hydriodic acid (*d* 1·7; 15 ml.). The phenol (3·5 g.) was isolated as a pale brown oil and was used, without further purification, for the next stage in the synthesis (see below). A small portion was distilled at 150° (bath)/11 mm. to give a colourless oil, which, when triturated with light petroleum (b. p. 60—80°), gave the *phenol* as prisms, m. p. 102—103° [Found, on a sublimed (90°/0·1 mm.) sample: C, 73·4; H, 8·5. $C_{11}H_{16}O_2$ requires C, 73·3; H, 8·9%]. The *di*-p-*nitrobenzoate* formed needles (from ethanol), m. p. 201—202° (Found: C, 62·5; H, 4·2; N, 6·0. $C_{25}H_{22}N_2O_8$ requires C, 62·8; H, 4·6; N, 5·9%).

2,5-*Dibromo*-3-*methyl*-6-*s*-*butyl*-1,4-*benzoquinone* (VIII).—A solution of bromine (7·5 g.) in chloroform (25 ml.) was added dropwise and with stirring to a solution of the foregoing phenol (3 g.) in chloroform (75 ml.). The solution, having been left overnight at room temperature, was washed with aqueous sodium hydrogen sulphite and then with water. Evaporation of the chloroform from the dried ($MgSO_4$) solution gave a yellow oil (4·9 g.) which was heated under reflux for 4 hr. with a mixture of ethanol (250 ml.) and concentrated nitric acid (3 ml.).[12] The excess of acid in the cooled mixture was neutralised with sodium hydrogen carbonate, and *ca.* 200 ml. of ethanol were removed by distillation. The concentrated solution, when kept overnight at 0°, gave a precipitate which was collected and crystallised from ethanol to give the *quinone* (*ca.* 3 g.) as yellow plates, m. p. 55—56° (Found: C, 39·0; H, 3·8. $C_{11}H_{12}Br_2O_2$ requires C, 39·3; H, 3·6%), $\nu_{max.}$ included a strong band at 1670 cm.$^{-1}$ (>C=O stretch).

2,5-*Dihydroxy*-3-*methyl*-6-*s*-*butyl*-1,4-*benzoquinone* (III).—(i) Aqueous N-sodium hydroxide (90 ml.) was added, during 30 min., to a warm (60°) solution of the foregoing quinone (2·5 g.) in methanol (100 ml.), and the solution was then heated under reflux for 30 min. Acidification by 2N-hydrochloric acid of the cooled solution gave an orange-brown solid which was collected, dried, and extracted (2 × 150 ml.) with boiling light petroleum (b. p. 40—60°). The light petroleum solution, having been filtered, reduced in volume to 25 ml., and then cooled, gave an orange solid (200 mg.), which, at 115°/0·1 mm., yielded a semi-crystalline reddish-brown sublimate. The sublimate crystallised from light petroleum (b. p. 60—80°) to give the *quinone* as sheaves of slender, orange needles (70 mg.), m. p. 189—190° (sealed capillary tube) [Found (on a resublimed sample): C, 62·9; H, 6·8. $C_{11}H_{14}O_4$ requires C, 62·8; H, 6·7%], $\lambda_{max.}$ 293 and 432 (broad peak) mμ (ε 21,850 and 219, respectively), $\nu_{max.}$ included peaks at 3320 (OH) and 1620 cm.$^{-1}$ (hydrogen bonded >C=O).

(ii) The chloroquinone (II; R = Cl) was obtained by periodate oxidation [3] of methyl *O*-methylnidulinate. A solution of this quinone (100 mg.) in 5% aqueous sodium hydroxide (10 ml.) was kept at 50—60° for 2 hr. Acidification of the cooled solution gave an orange precipitate which was collected in ether. The ethereal solution was dried ($MgSO_4$) and the ether was removed. Crystallisation of the residue from light petroleum (b. p. 40—60°) gave

[10] Nef, *Annalen*, 1890, **258**, 298.
[11] Cf. Johnson, Robertson, and Whalley, *J.*, 1950, 2971.
[12] Cf. Smith and Nichols, *J. Amer. Chem. Soc.*, 1943, **65**, 1742.

the dihydroxyquinone (II; R = OH) as orange needles (70 mg.), m. p. (sealed capillary) 197—198°, unaltered by admixture with a sample, m. p. 197°, prepared by the alternative method (see ref. 3). This dihydroxyquinone (50 mg.) was hydrogenated in methanol (20 ml.) in presence of 10% palladised charcoal (20 mg.), absorption of hydrogen ceasing after 2 mol. had been taken up. The mixture was filtered and the filtrate, initially colourless, became red on exposure to air. Evaporation of the methanol and crystallisation of the residue from light petroleum (b. p. 40—60°) gave the quinone (III) as slender, orange needles (30 mg.), m. p. (sealed capillary) 190°, unaltered on admixture with the sample prepared as above [method (i)]. It had $\lambda_{max.}$ 293 and 427 (broad peak) mμ (ε 21,200 and 208, respectively). Its infrared absorption spectrum was identical with that of the quinone prepared by method (i) above.

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