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# THE STRUCTURE OF THE BASIDIOMYCETE *ORTHO* QUINONE, PHLEBIARUBRONE, AND OF ITS NOVEL ACETYLATION PRODUCT

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**Abstract**—The red pigment produced by the basidiomycete *Phlebia strigosozonata* (Schw.) Lloyd is shown to be 3,6-diphenyl-4,5-methylenedioxy-1,2-benzoquinone by spectral evidence, and alkaline hydrolysis to polyporic acid and formaldehyde. Treatment of this quinone with acetic anhydride and hydrated sodium acetate gives in high yield the compound 2-acetoxy-3,6-diphenyl-4,5-methylenedioxybenzyl acetate.

TERPHENYL quinones, of which polyporic acid (I) is perhaps the best known example, are produced in nature mostly by wood rotting fungi (Basidiomycetes). These compounds possess a *para* quinone function in the central ring which, in nearly all cases, carries other oxygen functions as well.[1]

The basidiomycete *Phlebia strigosozonata* when grown in culture produces an insoluble red pigment which is an *ortho* quinone closely related to polyporic acid. We made a preliminary report some time ago on the structure of this compound, which was named Phlebiarubrone, and we present here a detailed account of the evidence.[2, 3]

The quinone crystallized from acetic acid as red needles, m.p. 248–250°, and had absorption maxima at 268, 332 and 465 mμ. Its IR spectrum showed $\nu_{max}$ 1653, 1640 $cm^{-1}$, expected for a quinone but there was no OH absorption. The insolubility prevented determination of the mol wt by cryoscopic methods. This was obtained indirectly by preparing the *leuco* acetate with acetic anhydride, zinc dust and pyridine. Analysis indicated a diacetate of molecular formula $C_{23}H_{18}O_6$. Hydrolysis of this compound with a mixture of acetic and dilute sulfuric acids gave the hydroquinone of phlebiarubrone which, though stable in the solid state, quickly reverted to the quinone in solution due to aerial oxidation.

Phlebiarubrone itself was quite stable in the solid state and in neutral and acid solution at room temperature. It dissolved readily in concentrated sulfuric acid giving the intense green colour of the oxonium salt. Dilution with water gave back the quinone unchanged.

The key reaction to elucidation of the structure was the action of alkali. The quinone was not soluble in alkali but when the acetic acid solution was added to

[1] R. H. Thomson, in *Naturally Occurring Quinones* p. 26. Academic Press, New York (1957). See also J. Gripenberg, *Tetrahedron* **10**, 135 (1960) and earlier papers in that series.

[2] T. C. McMorris and M. Anchel, *Tetrahedron Letters* No. 5, 335 (1963).

[3] The synthesis of Phlebiarubrone has been reported recently by J. Gripenberg in *Tetrahedron Letters* No. 7, 697 (1966).

excess dilute sodium hydroxide solution, an immediate purple solution resulted which on acidification gave a brown precipitate. This high melting product (m.p. ~305°) showed $\nu_{max}$ 3310, 1637, 1613, 1597 $cm^{-1}$ and had other properties corresponding to those of polyporic acid. Comparison with a synthetic sample of the latter compound established the identity.

The simple conversion to polyporic acid indicated that the four oxygen atoms present in phlebiarubrone were attached to the central ring. Two of these were required for the quinone, and the nature of the other two was revealed by the NMR spectrum (taken in DMSO). It showed a complex band at $\tau$ 2·55 attributable to 10 aromatic protons, and a sharp singlet at $\tau$ 3·75 suggesting the presence of a methylenedioxy ether. A corresponding singlet appeared at $\tau$ 4·03 (2H) in the spectrum of the *leuco* acetate ($CDCl_3$) which showed as well, signals at $\tau$ 2·57 (10H) and $\tau$ 7·98 (6H). Confirmation of the methylenedioxy ether came from the reaction with alkali which gave formaldehyde in high yield (isolated as the dimedone derivative).

This evidence established the structure II for phlebiarubrone. A dimeric structure V was ruled out by the mol wt found for the *leuco* acetate (III) by the Rast method, and by the mass spectrum ($M^+ = 390$). The mass spectrum of II was interesting in that it showed the molecular ion at *m/e* 304 (8% of base peak), but a stronger ion at *m/e* 306 (22% of base peak). At a lower sample pressure the intensity of the molecular ion peak relative to the base peak was the same, but the peak at *m/e* 306 was now only 9% of the base peak. This indicated that the ion of the hydroquinone was formed, by ion-molecule collisions, in the ionization chamber of the mass spectrometer.[4] The intense ion at *m/e* 276 (44% of base peak which occurred at *m/e* 129) was probably formed by loss of CO from the quinone.[5]

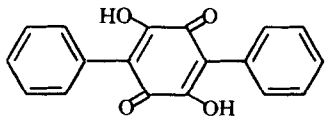
Phlebiarubrone reacted readily with *o*-phenylenediamine in boiling acetic acid to give a dark red crystalline phenazine (IV) which showed OH absorption at 3260 $cm^{-1}$. The compound formed a yellow diacetate ($\nu_{max}$ 1786, 1207 $cm^{-1}$) and it gave a yellow ether when refluxed with methylene iodide and potassium carbonate in acetone. Presumably, the phenazine was formed after base catalysed solvolysis of phlebiarubrone to polyporic acid which then reacted in the tautomeric *ortho* quinone form (Ia) with *o*-phenylenediamine. The derivative could be obtained equally well starting with polyporic acid itself.

In early work on phlebiarubrone a colourless diacetate, m.p. 115–117°, was obtained by refluxing the quinone with sodium acetate and acetic anhydride. It was later discovered that the sodium acetate used was not completely anhydrous and that a high yield of this compound could be obtained using hydrated sodium acetate and acetic anhydride. With anhydrous sodium acetate none of the compound was detected, but a number of other products were formed, including the normal *leuco* acetate in low yield.

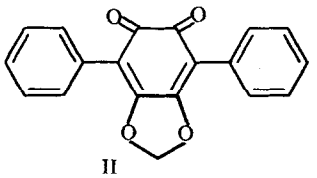
The compound, m.p. 115–117°, had an absorption spectrum $\lambda_{max}$ 241, 300 (inflexion) mμ (ε 41,000, 4000), similar to that of the *leuco* acetate (III), $\lambda_{max}$ 238, 262, 304 (infl) mμ (ε 28,300, 26,300, 3900). It analysed well for $C_{24}H_{20}O_6$ and this formula was confirmed by the mass spectrum ($M^+ = 404$). The spectrum showed strong peaks

[4] J. H. Beynon, *Mass Spectrometry and its Applications to Organic Chemistry* p. 275. Elsevier, Amsterdam (1960).

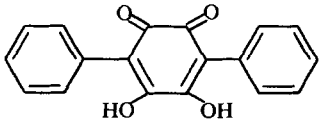
[5] Ref. 4, p. 360.



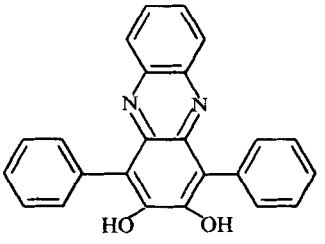

I



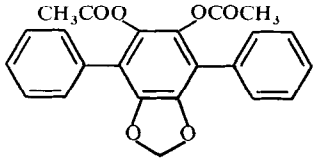

II



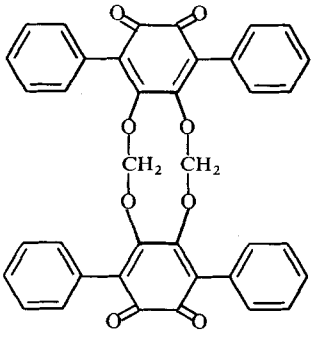

Ia




IV




III




V

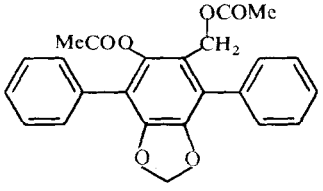
at *m/e* 362 (M-$CH_2CO$), 344 (M-$CH_3COOH$), 328 (M-$C_2H_4O_3$), 319 (M-$CH_2CO$—$CH_3CO$) and the base peak at *m/e* 302 (M-$CH_3CO$—$CH_3CO_2$). In contrast, the *leuco* acetate (III), $C_{23}H_{18}O_6$, showed a strong peak at *m/e* 348 (M-$CH_2CO$) and the base peak at *m/e* 306 (M-2 × $CH_2CO$) with metastable peaks at about 311 (390 → 348) and 269 (348 → 306).

The extra 14 units in the compound $C_{24}H_{20}O_6$ are accommodated very simply in the structure VI. The IR spectrum $\nu_{max}$ 1764, 1738 $cm^{-1}$ and the NMR spectrum ($CDCl_3$) $\tau$ 2·57 (10H) 4·03 (singlet, 2H), 5·07 (singlet, 2H), 8·03 (singlet, 6H) are in full agreement. The latter spectrum is remarkable because the aromatic proton signal is a sharp peak unlike the complex band given by the aromatic protons in the more symmetrical *leuco* acetate (III). The structure VI was confirmed by hydrogenolysis of the benzylic acetate group giving the monoacetate VII $\nu_{max}$ 1761 $cm^{-1}$, $\tau$ 2·53 (multiplet, 10H), 4·03 (singlet, 2H), 7·97 (singlet, 3H), 7·98 (singlet, 3H). When a solution of VII in acetic acid was refluxed with hydrobromic acid a crystalline

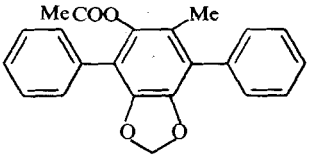
phenol was obtained which gave on oxidation with ferric chloride, a brown quinone identical with 2-hydroxy-5-methyl-3,6-diphenyl-1,4-benzoquinone.[6]

A plausible mechanism for the formation of VI assumes nucleophilic addition to one of the quinone carbonyls, of the anion formed from acetic anhydride and base ($CH_3COO^-$). The product is protonated ($CH_3COOH$) and elimination then occurs giving a quinone methide intermediate which yields VI on 1, 4 addition of acetic anhydride.

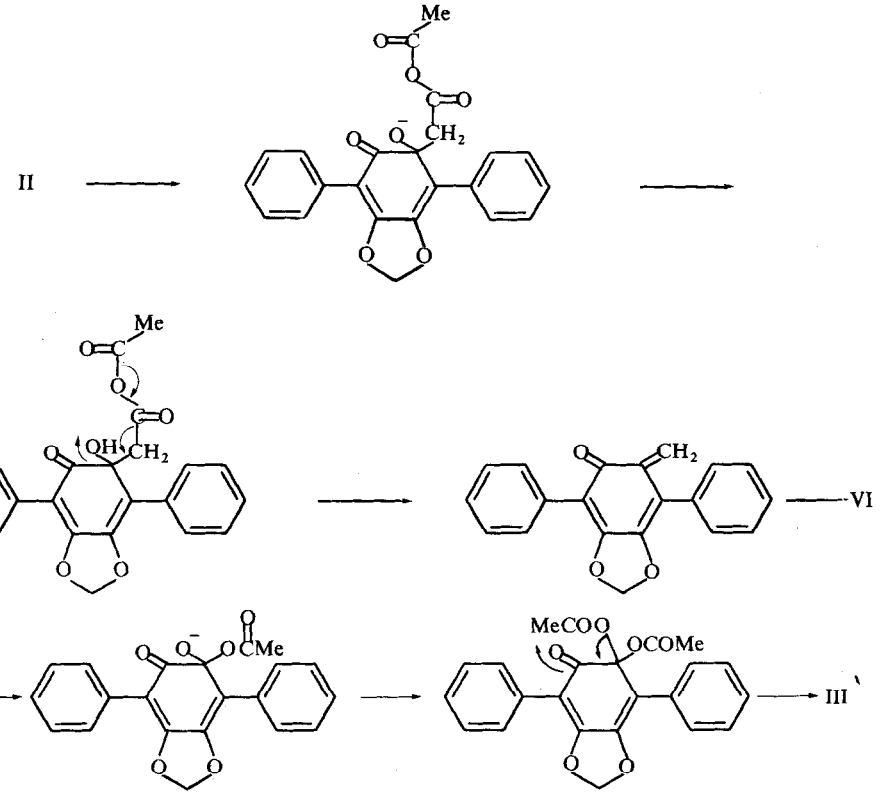
The reaction of phlebiarubrone with anhydrous sodium acetate and acetic anhydride is still being investigated, but it appears at this stage to be similar to that of 9,10-phenanthraquinone and sodium acetate–acetic anhydride. This latter reaction also involves nucleophilic attack of the anion from acetic anhydride.[7] The *leuco*



VI



VII



[6] B. F. Cain, *J. Chem. Soc.* 936 (1961). We are grateful to Dr. Cain for sending us a sample of this quinone.

[7] See S. M. Bloom, *J. Am. Chem. Soc.* **83**, 3808 (1961). We thank the referee for calling our attention to this paper.

acetate (III) may be formed by simple reduction with concurrent oxidation to one of the unknown products. Alternatively, it may result from nucleophilic addition, this time of acetate ion, leading to an *o*-quinol diacetate. Elimination and further acetylation would then give III.[8] Polyporic acid under similar conditions yields a *leuco* acetate and an analagous mechanism may be invoked for its formation.

Polyporic acid and certain of its derivatives have been reported by Cain to possess antitumour properties.[6] Dr. Cain kindly tested phlebiarubrone and phenazine (IV) but found them to be inactive.

## EXPERIMENTAL

*General.* M.ps were taken on a Köfler hot stage and are uncorrected. IR spectra were determined with a Perkin-Elmer Model 21 spectrophotometer in KBr disks. UV spectra were measured in EtOH with a Cary Model 11 spectrophotometer. We are indebted to Drs. A. K. Bose and P. Funke, Stevens Institute, New Jersey for the mass spectra, which were determined on a CEC 21-103 C Mass Spectrometer. NMR spectra were determined by Drs. D. P. Hollis and A. E. Pier, Varian Associates, California. All NMR and mass spectral data are given in the text. Microanalyses were carried out by Dr. F. Pascher, Bonn, Germany.

*Culture of* P. strigosozonata *and isolation of phlebiarubrone* (II). Batches of 40–60 Erlenmeyer flasks (500 ml) containing 130 ml each of 2% malt medium[9] were inoculated from malt slant cultures of *P. strigosozonata* (56454)[10] and incubated at 25° on a reciprocal shaker. Within 2 weeks the growing mycelium (pellets), originally white, became brown with bright red flecks. After 4 weeks all pellets had a red colour which deepened with time; they were harvested in 6–8 weeks. They were filtered off and extracted repeatedly with acetone in a Waring blendor until all the red colour was removed. The extract was concentrated, giving a crystalline red ppt mixed with a partly crystalline white ppt which could be removed by washing the mixture with MeOH, yield of washed red crystals, ca. 0·5 g per liter. Recrystallization of the red ppt from acetone or AcOH gave phlebiarubrone as red needles, m.p. 248–250°, $\lambda_{max}$ 268, 332, 465 mμ (ε 29,900, 4400, 3500); $\nu_{max}$ 1653, 1640 $cm^{-1}$. (Found: C, 74·70; H, 3·74; O, 21·65. $C_{19}H_{12}O_4$ requires: C, 74·99; H, 3·97; O, 21·03%.)

*Derivatives*

(a) Leuco *acetate* (III). The quinone (100 mg) suspended in $Ac_2O$ (2 ml) was treated with a few drops of pyridine and a little Zn dust. The red colour disappeared rapidly. After 15 min water was added and the mixture kept until the gummy ppt had hardened, then it was filtered. The ppt was washed with water, dried, then extracted with hot AcOEt and filtered away from the Zn. Removal of the solvent left the *leuco* acetate (120 mg), m.p. 224–225°, and after recrystallization from AcOEt, m.p. 227–229°, $\lambda_{max}$ 238, 262, 304 (infl) mμ (ε 28,300, 26,300, 3900), $\nu_{max}$ 1770, 1208, 1188, 749, 691 $cm^{-1}$. (Found: C, 71·15; H, 4·84; O, 24·05; Ac, 12·34; M.W. (Rast) 400. $C_{23}H_{18}O_6$ requires: C, 70·76; H, 4·65; O, 24·59; 2Ac, 22·03%; M.W. 390.)

The *leuco* acetate (100 mg) was hydrolyzed by gradual addition of 6N $H_2SO_4$ (1 ml) to its soln in 2 ml of boiling AcOH. A crystalline ppt soon formed and after 15 min the mixture was cooled and the crystals were filtered off, washed with water and dried (60 mg); m.p. 210–220°, $\nu_{max}$ 3500, 3400 $cm^{-1}$. The pale brown crystals of hydroquinone were stable in air, but in soln in EtOH or pyridine, oxidation to phlebiarubrone occurred rapidly.

(b) *Phenazine derivative* (IV). The quinone (100 mg) dissolved in hot AcOH (10 ml) was treated with a soln of *o*-phenylenediamine (100 mg) in AcOH (1 ml). The resulting soln was refluxed for 30 min, during which deep red spangles separated. The mixture was cooled and the ppt collected (60 mg). It showed partial melting at 305° but did not melt completely even up to 350°. The phenazine was slightly soluble in ACOH or $CHCl_3$ but insoluble in other common solvents, $\nu_{max}$ 3270 $cm^{-1}$. (Found: C, 78·19; H, 4·34; O, 9·63; N, 7·81. $C_{24}H_{16}O_2N_2$ requires: C, 79·10; H, 4·43; O, 8·78; N, 7·69%.)

[8] For examples of the reaction of quinol diacetates with acetic anhydride under acidic and basic conditions, see S. Goodwin and B. Witkop, *J. Am. Chem. Soc.* **79**, 179 (1957).

[9] This consists of a 2% soln of Bacto-Malt extract (Difco Laboratories, Detroit).

[10] This culture was obtained from Dr. R. W. Davidson, U.S.D.A., Beltsville.

Polyporic acid (I) readily gave the same phenazine when it was refluxed with excess of a solution of *o*-phenylenediamine in AcOH. A dark red colour quickly appeared and the phenazine crystallized out from the boiling soln. The yield was quantitative.

Acetylation of the phenazine with excess $Ac_2O$ and pyridine overnight gave pale yellow needles, m.p. 260–265° (from AcOEt–pet ether), $\nu_{max}^{Nujol}$ 1785 $cm^{-1}$. (Found: C, 75·62; H, 5·09; O, 13·96; N, 5·64; Ac, 17·58. $C_{28}H_{20}O_4N_2$ requires: C, 74·99; H, 4·50; O, 14·27; N, 6·25; 2Ac, 19·2%.)

The methylene ether of the phenazine was made by refluxing the latter with excess $CH_2I_2$ in acetone together with $K_2CO_3$ for 48 hr. The acetone soln was then filtered off and the solvent removed, leaving a brown-red residue. This was dissolved in benzene and filtered through a column of alumina (Brockmann, Grade 1) giving a yellow eluate which on evaporation yielded yellow crystals. Recrystallization from benzene–pet ether gave yellow needles, m.p. 300–306°.

The phenazine ether was prepared also by another method.[11] Dimethylformamide (3 ml) was added to NaH (50%, 200 mg) in a flask with a $N_2$ atm. A soln of the phenazine (100 mg) in the same solvent (5 ml) was then added and the resulting orange suspension heated to about 80°. A soln of $CH_2I_2$ (400 mg) in dimethylformamide was next added. The mixture, now red, was kept for 1 hr, then cooled and diluted carefully with water giving a brown ppt. This was purified by filtration of its benzene soln through alumina as above, giving yellow needles (80 mg) of the ether.

*Alkaline hydrolysis of phlebiarubrone.* The quinone (100 mg) was dissolved in hot AcOH (10 ml) and the soln added gradually to 30% NaOH aq (35 ml) cooled in ice water. A purple soln resulted; this was kept for 5 min, then acidified with HCl giving a tan ppt which was removed by centrifugation. The ppt (76 mg) melted at 305° (sublimation). It was identical (mixture m.p., IR spectrum) with I synthesized from benzoquinone.[12] The lemon yellow diacetate, m.p. 214–215°, was identical with polyporic acid diacetate, and the *leuco* acetate, m.p. 267–269°, with polyporic acid *leuco* acetate.[13]

The colorless filtrate after separation from the polyporic acid was treated with dimedone (100 mg) in AcOH (2 ml). The soln soon clouded and, after standing overnight, the formaldehyde dimethone (72 mg) was filtered off, m.p. 195°. This represents a 75% yield of formaldehyde from phlebiarubrone.

*Action of sodium acetate–acetic anhydride on phlebiarubrone.* The quinone (500 mg), $AcONa.3H_2O$ (1 g) and $Ac_2O$ (10 ml) were refluxed for 1 hr giving a green-brown soln. It was diluted with water and a crystalline ppt soon formed. This was dried (633 mg) then dissolved in benzene and filtered through activated alumina (Brockmann, Grade 1) to remove the coloured impurity. About 550 mg of colourless crystals (VI), m.p. 115–117°, were obtained. These were recrystallized from AcOEt–acetate/pet ether for analysis; m.p. 115–117°. $\lambda_{max}$ 241, 300 (infl) mμ (ε 41,000; 4000), $\nu_{max}$ 1764, 1738 $cm^{-1}$. (Found: C, 71·02, 71·24; H, 4·72, 5·09; O, 23·99, 23·73; Ac, 20·66; M. W. 424. $C_{24}H_{20}O_6$ requires: C, 71·28; H, 4·98; O, 23·74; 2Ac, 21·77%; M. W. 404.)

When the quinone (50 mg) was refluxed with anhyd AcONa (100 mg) and $Ac_2O$ (2 ml) a vigorous reaction occurred and solid material separated. The dark coloured reaction product was diluted with water giving a dark green partly crystalline solid (65 mg). On preparative TLC with silica gel F254 (Merck, Darmstadt, Germany) and $CHCl_3$–MeOH 10:1, 5 coloured bands were obtained: brown, brown, pale green, yellow and pale brown in order of increasing $R_f$ value. The pale green band gave 20 mg of crystalline solid, m.p. 250–255° (dec), $\nu_{max}$ 1815, 1757 $cm^{-1}$. The yellow band gave 5 mg of the *leuco* acetate III. The pale brown band gave 5 mg crystals, m.p. 283–285° (dec), $\nu_{max}$ 1818 $cm^{-1}$.

*Action of sodium acetate–acetic anhydride on polyporic acid.* A mixture of polyporic acid (100 mg), anhyd AcONa (500 mg) and $Ac_2O$ (2·5 ml) were refluxed for 3 hr then cooled and diluted with water. A brown insoluble oil was obtained which solidified on standing overnight. It was dissolved in benzene and the dark brown soln filtered through activated alumina. The initial yellow eluate was collected separately, then the colourless eluate was collected and the solvent evaporated from it giving about 40 mg of crystalline solid. After recrystallization from MeOH it had m.p. 260–265° and the IR spectrum was identical with that of polyporic acid *leuco* acetate.[13]

*Hydrogenolysis of* VI. The diacetate VI (300 mg) dissolved in AcOEt–MeOH was stirred with 30% Pd–C, (500 mg) in the presence of $H_2$ at room temp and atm press. After 3 hr the uptake of $H_2$ was complete (approx 1 mole). The catalyst was filtered off and the filtrate evaporated leaving a gum which soon crystallized, yield of VII 290 mg, m.p. 157–160°. A sample for analysis was recrystallized from

[11] cf. J. S. Brimacombe, A. B. Foster, B. D. Jones and J. J. Willard, *Chem. Commun.* 174 (1965).

[12] R. L. Frank, G. R. Clark and J. N. Coker, *J. Am. Chem. Soc.* **72**, 1824 (1950).

[13] P. R. Shildneck and R. Adams, *J. Am. Chem. Soc.* **53**, 2373 (1931).

pet ether, m.p. 165–167°. (Found: C, 75·70; H, 5·18; O, 18·05; Ac, 12·73. $C_{22}H_{18}O_4$ requires: C, 76·28; H, 5·24; O, 18·48; 1Ac, 12·43%.)

*Conversion of* VII *to 2-hydroxy-5-methyl-3,6-diphenyl-1,4-benzoquinone.* A soln of VII (30 mg), in AcOH (1 ml) was refluxed with 0·3 ml 48% HBr for 30 min. The soln was cooled and diluted with water giving a crystalline orange ppt (20 mg). This product was dissolved in AcOH (0·5 ml) and the soln added to one of $FeCl_3 \cdot 6H_2O$ (35 mg) in 0·25 ml AcOH. Dilution with water gave a dark brown ppt which crystallized readily from dioxan–water as red-brown plates, m.p. 145–150°. It was identical (mixture m.p., IR spectrum) with an authentic sample of 2-hydroxy-5-methyl-3,6-diphenyl-1,4-benzoquinone.[6]

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