Constituents of The Higher Fungi. Part XI.[1] Boviquinone-3, (2,5-Dihydroxy-3-farnesyl-1,4-benzoquinone), Diboviquinone-3,4, Methylenediboviquinone-3,3, and Xerocomic Acid from *Gomphidius rutilus* Fr.† and Diboviquinone-4,4 from *Boletus* (*Suillus*) *bovinus* (Linn ex Fr.) Kuntze

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Boviquinone-3, the second member of the boviquinone series, has been identified as a constituent of the basidiomycete *Gomphidius rutilus* Fr., in addition xerocomic acid and two new isoprenoid diquinones are described and identified. A new diquinone from *Boletus* (*Suillus*) *bovinus* (Linn ex Fr.) Kuntze is described and identified.

MODERN accounts of the higher fungi closely relate the *Gomphidiaceae* with the *Boletaceae*. Our attention was drawn to *G. rutilus* because of the bright pink colour which develops when the fruiting body is moistened with ethanol; this colouration is similar to that produced by *B. bovinus*.[2] No chemical investigation of this species has been previously carried out but an early report by Bachmann [3] described the presence of a red and a yellow colouring matter. Our investigations of this fungus have yielded three new quinones (II), (III), and (VI) and evidence for the existence of other homologues (IV) and (VII); in addition xerocomic acid (VIII) occurs as the blueing constituent. Unlike the crude extract from *B. bovinus*, which readily yielded crystalline bovinone,[2] the dark red gum from *G. rutilus* only gave the crystalline quinone (II) after an extensive purification procedure.

The occurrence of this multitude of quinones has necessitated a simple nomenclature for describing them. By analogy with the ubiquinones and other established series we propose the general name *boviquinones* for the 2,5-dihydroxyisoprenoid quinones; 2,5-dihydroxy-3-geranylgeranyl-1,4-benzoquinone (bovinone) (I) then becomes boviquinone-4 and the farnesyl analogue (II), boviquinone-3. The diquinones, directly linked at the 6,6-positions, are described as *diboviquinones* and the *compounds* (VI) and (VII) as diboviquinone-3,4 and -4,4 respectively. Amitenone (V), from *B. bovinus*, has been used to describe the diquinone formed by linking two molecules of boviquinone-4 at the 6,6-positions through a methylene group. It is now proposed to call this compound methylenediboviquinone-4,4 and compounds (III) and (IV) methylenediboviquinone-3,3 and -3,4 respectively.

† A voucher specimen of *G. rutillus* is deposited in the herbarium of the Royal Botanic Gardens, Edinburgh, under the collection number Wat 8191.

The crude lead salt, obtained from alcoholic extracts of the fresh fungus, was decomposed stepwise with methanolic hydrogen chloride to yield two fractions; both fractions contained considerable yellowish-red fatty material and small quantities of the farnesyl quinone (II). In addition, the first fraction gave the pure diboviquinone (VI) and the second the methylenediboviquinone (III). Boviquinone-3 (II) was separated from the large quantities of yellowish-red acidic material *via* the insoluble sodium salt or *via* the reduced acetate.

Boviquinone-3 (II) [m.p. 90—92°, $C_{21}H_{28}O_4$, *M* (mass spec.) 344] separates from acetic acid as yellow needles turning orange in air. The i.r., u.v., and ^1H n.m.r. spectra are all similar to those of boviquinone-4.[2] An alternative purification procedure, involving reductive acetylation of the crude gummy fractions gave the colourless leuco-tetra-acetate, $C_{29}H_{38}O_8$. This acetate showed weak signals in the mass spectrum at 582 and 513 in addition to the strong peaks at 514 and 445; the peak at 514 is an even number and is therefore a molecular ion since end of chain isoprene units show a loss of 69. These results indicate that this acetate is mainly the farnesyl compound mixed with a small quantity of the geranylgeranyl isoprenologue. Alkaline hydrolysis of the acetate with spontaneous aerial oxidation of the product yielded the pure boviquinone-3 (II).

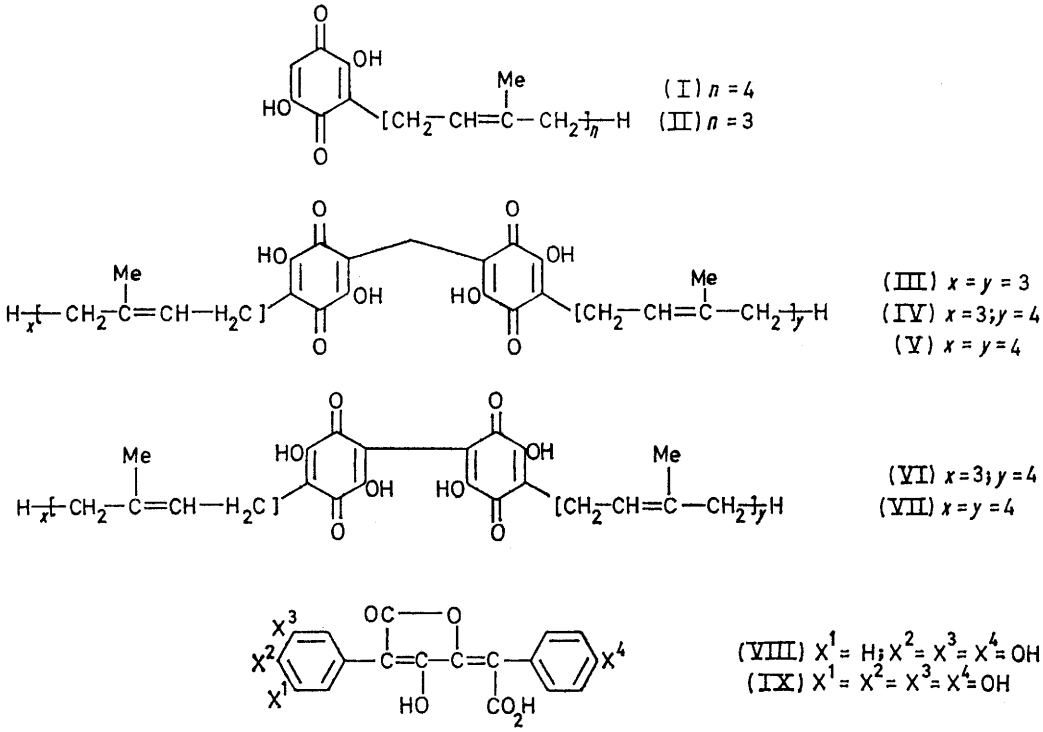
The methylenediboviquinone (III), $C_{43}H_{56}O_8$, separated from acetic acid as yellow fatty needles m.p. 197—201°. Like methylenediboviquinone-4,4 it is reversibly reduced and oxidised, forms an insoluble purple

[1] Part X, P. C. Beaumont and R. L. Edwards, *J. Chem. Soc.* (*C*), 1971, 1000.
[2] Part IX, P. C. Beaumont, and R. L. Edwards, *J. Chem. Soc.* (*C*), 1969, 2398.
[3] E. Bachmann, 'Programme des Gymnasiums und Realgymnasiums zu Plauen (i) V,' Ostern, 1886, p. 17.

sodium salt and forms gels in nonpolar solvents. In the ^{1}H n.m.r., the presence of a two proton peak at τ 4·41 and absence of a quinone nuclear proton at τ 4·26 identified this compound as a methylenediboviquinone. The u.v. and i.r. spectra are similar to those of methylenediboviquinone-4,4 and the mass spectrum shows a mass peak at 770 and peaks at 546 (methylenediboviquinone-3,3 — benzylium ion 154), 358 (boviquinone-3 + CH_2) and 334 (boviquinone-3); in addition much smaller peaks occur at 768, with ancillary peaks at 614, 426, and 412 showing the presence of the -3,4 analogue (IV) (1—2%).

identity of this compound is the presence of significant peaks of equal intensity at 344 (boviquinone-3) and 412 (boviquinone-4); there are no peaks at 426 and 360 typical of methylenediboviquinone-type compounds. In the ^{1}H n.m.r. spectrum the quinonoid proton of the boviquinones at τ 4·02 and the bridging methylene protons of the methylenediboviquinones at τ 6·41 are absent; the proton count is closer to the -3,4 than the -3,3 analogue. This data is indicative of a compound consisting of two directly linked quinone rings as in the natural products oosporein [4] and phoenicin [5].

A re-examination of *B. bovinus* has yielded a similar



The diboviquinone (VI), $C_{47}H_{64}O_8$, separated from acetic acid as clusters of small orange-yellow fatty needles m.p. 137—139°. The fatty crystalline form, the ability to form gels in nonpolar solvent, the i.r. peaks at 3305 and 1620 cm.$^{-1}$ and the $\lambda_{max.}$ at 288 nm. are similar to those of the methylenediboviquinones. The effect of caustic alkali on the u.v. absorption in ethanol solution is also similar to the methylenediboviquinones; diboviquinone-3,4, $\lambda_{max.}$ 288 nm. changes to 255 (infl.) and 326 nm. and methylenediboviquinone-4,4 from 285·5 to 255 (infl.) and 326 nm., whereas boviquinone-4, $\lambda_{max.}$ 287 nm. gives 240, 316 (infl.) and 324·5 nm. The mass spectrum of the new quinone shows a group of seven signals in the region of 686 suggestive of diboviquinone-3,3 but the most intense peak occurs at 684 and measurements at different probe temperatures indicated that substantial thermal decomposition of the molecule was taking place. The most significant indication of the orange-red quinone (VII), m.p. 132—134°. The i.r., u.v., and ^{1}H n.m.r. spectra are all identical with those of the above compound but the proton count and the combustion analysis indicated the presence of an extra isoprene unit. This is confirmed by the mass spectrum which is similar to the -3,4 analogue (apparent molecular ion peak at 684); the difference is the absence of the peak at 344 corresponding to a farnesyl fragment.

The large number of recrystallisations necessary to obtain a constant m.p., combined with the data from physical measurements, strongly suggested that the crude isolates comprised mixtures of closely related analogues. Other workers have successfully separated ubiquinone analogues on paraffin-impregnated silica-gel plates [6] but our attempts to separate the methyl ethers, prepared from the crude isolates, by this technique were unsuccessful. An inspection of the mass spectra fully revealed their composition; the crude boviquinone-3

[4] F. Kögl and van Wessem, *Rec. Trav. chim.*, 1944, **63**, 5.
[5] T. Posternak, *Helv. Chim. Acta*, 1938, **21**, 1326.
[6] E. Stahl, 'Thin-layer Chromatography,' Academic Press, New York and London, 1965, p. 234.

ether showed a strong molecular ion peak at 372 and a very weak peak at 440 corresponding to the presence of a small quantity of the tetraprenoid analogue (boviquinone-4). The crude diboviquinone ether from *G. rutilus* gave a strong peak at 810 and a very weak peak at 878 corresponding to the -3,4 and the -4,4 analogues respectively; the proportion of the latter is not greater than 1%. In contrast, the crude ether prepared from the diboviquinone from *B. bovinus* contains no other analogue and consists entirely of the symmetrical-4,4 compound. The crude methylenediboviquinone from *G. rutilus* gives a methylated product comprising the ethers of methylenediboviquinone-3,3 (III) 70%, methylenediboviquinone-3,4 (IV) 30%, and methylenediboviquinone-4,4 (V) (<1%).

Attempted reductive acetylation of the diboviquinones and methylenediboviquinones gave mixtures of compounds for which no consistent analyses could be obtained. The abnormally low molecular weights as indicated by mass spectrometry is indicative of other reactions taking place (*e.g.* dehydration as occurs in vilangin [7]) during reductive acetylation.

The dimerisation of benzoquinones is known to take place in the presence of acid [8] and the dimerisation of boviquinone-4 and boviquinone-3 to yield the diboviquinones during the isolation procedure could not be ruled out. This was tested by treating boviquinone-4 with methanolic hydrogen chloride; the quinone was unchanged during 12 hr. but was converted into a red polymeric oil during several days at room temperature; no diquinone could be isolated.

The water-soluble fraction of this fungus contains one major yellow component. Acetylation of the crude gummy material gave crystalline xerocomic acid dilactone acetate, identified by its m.p., u.v., i.r., and mass spectrum and by the identification of protocatechuic acid and *para*-hydroxybenzoic acid as the products of chromium trioxide oxidation. Xerocomic acid (VIII) has recently been identified as the major colouring matter of the related species *G. glutinosus* (Schaeff. per Fr.) Fr.,[9] gomphidic acid (IX), which also occurs in the latter species, does not occur in *G. rutilus*. The occurrence of boviquinone-3 and related quinones in *G. rutilus* establishes a further chemotaxonomic link between the *Gomphidiaceae* and *Boleteceae* and especially with the sub-group *Suillus*; it has been stated that both families must have ancestors which are closely related to each other.[10]

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus, i.r. spectra on a Perkin-Elmer model 237 spectrophotometer, u.v. spectra on a Unicam SP spectrophotometer, ^{1}H n.m.r. spectra on a Varian A-60 spectrometer (the chemical shift values being expressed in τ units relative to tetramethylsilane as an internal standard), and the mass spectra on an M.S. 9 mass spectrometer.

Extraction of Gomphidius rutilus.—Freshly collected fruiting bodies of *G. rutilus* (14 kg.) were sliced and steeped in cold ethanol (14 l.) for 2 hr. The bright red solution was decanted and the mushrooms were extracted overnight with fresh alcohol (7 l.). Treatment of the combined extracts with an excess of aqueous lead acetate solution yielded the blue-green lead salt which was filtered off and washed twice with water and then twice with methanol. Methanolic hydrogen chloride (550 ml.; 7%) was added dropwise to the stirred suspension of the lead salt in methanol. After addition of the first 350 ml. of reagent, the mixture was filtered and the filtrate was evaporated at 35° to yield a red gum [fraction (i)]. The remaining lead salt was decomposed as above by the addition of the remaining methanolic hydrogen chloride (200 ml.); the filtrate yielded a red gum on evaporation [fraction (ii)]. The fractions were each extracted separately with boiling water (5 × 100 ml.) and the aqueous extracts combined [fraction (iii)]. The water-insoluble residues were each dissolved in ether, the ether solutions were dried and evaporated, and the residual red gums were examined separately.

Diboviquinone-3,4 (VI) *from Fraction* (i).—The gum dissolved in acetic acid (40 ml.) was set aside for three days. Filtration and recrystallisation of the orange residue (220 mg.), five times from acetic acid, gave *diboviquinone-3,4* as clusters of small orange-yellow fatty needles, m.p. 137—139° (Found: C, 74·5; H, 8·15. $C_{47}H_{62}O_8$ requires C, 74·8; H, 8·2%); $\lambda_{max.}$ (EtOH) 288 nm. (log ε 4·46), (EtOH + NaOH, 2 drops 2N) 326 nm. (log ε 4·63); $\nu_{max.}$ (KBr) 3305, 2965, 2923, 2850, 1620, 1050 cm.$^{-1}$ (w); τ ($CDCl_3$) 2·03—2·40 (4H), 4·67—5·13 (7H), 6·88 (d, *J* 7 Hz, 4H), 8·02 (20H), 8·27 (6H), 8·32 (6H), 8·41 (15H); mass spec. 684, 616, 548, 529, 461, 412 (M^+ boviquinone-4), and 344 (M^+ boviquinone-3).

Methylenediboviquinone-3,3 (III) *from Fraction* (ii).—The gum dissolved in acetic acid (40 ml.) was set aside for three days. Filtration and recrystallisation of the yellow residue (190 mg.) six times from acetic acid gave methylenediboviquinone-3,3 (III) as yellow fatty needles m.p. 197—201° (Found: C, 73·6; H, 8·25. $C_{43}H_{56}O_8$ requires C, 73·7; H, 8·0%); $\lambda_{max.}$ (EtOH) 288 nm. (log ε 4·40), (EtOH + NaOH, 2 drops 2N) 327 nm. (log ε 4·56); $\lambda_{max.}$ (KBr) 3305, 2965, 2923, 2850, 1620, and 1048 cm.$^{-1}$ (s); τ ($CDCl_3$) 2·25—2·48 (4H), 4·68—5·05 (6H), 6·41 (2H), 6·86 (d, *J* 7 Hz, 4H), 7·99 (16H), 8·27 (6H), 8·30 (6H), 8·40 (12H); mass spec. 700 (M^+), 546, 358, and 344 (M^+ boviquinone-3).

Boviquinone-3 (II) *from Fractions* (i) *and* (ii).—The combined mother liquors from fractions (i) and (ii) above were evaporated under reduced pressure and the residual dark brown gum was added to sodium hydroxide solution (500 ml.; 2N). The mixture was set aside overnight and then the alkaline solution was filtered off. The dark brown gummy residue was washed with ethanol (3 × 350 ml.) and the red washings were set aside overnight; they were then filtered and evaporated, and the residue was dissolved in water (600 ml.). Acidification of the solution, ether extraction, and evaporation of the ether gave a brown gum which was applied in 1-g. fractions to plates (20 × 1000 cm.)

[7] C. B. Rao and V. Venkateswarlu, *J. Org. Chem.*, 1961, **26**, 4529.

[8] F. M. Dean, A. M. Osman, and A. Robertson, *J. Chem. Soc.*, 1955, 11.

[9] W. Steglich, W. Furtner, and A. Prox, *Z. Naturforsch.*, 1969, **24***b*, 941.

[10] R. Singer, 'The Agaricales in Modern Taxonomy,' J. Cramer, Weinheim, 1962, p. 703.

coated with silica gel (80 g.). After development with a mixture of light petroleum (b.p. 60—80°), ether, and acetic acid (70 : 30 : 3), the bright pink band was eluted with chloroform; evaporation of the chloroform and crystallisation of the residue from acetic acid gave *boviquinone*-3 (18 mg.), m.p. 90—92° (Found: C, 73·4; H, 8·4. $C_{21}H_{28}O_4$ requires C, 73·3; H, 8·1%); *M* (mass spec.) 344; $\nu_{max.}$ (KBr) 3290, 2960, 2915, 2850, 1615, and 1335, ($CHCl_3$) 3360, 2920, 1645, and 1370 cm.$^{-1}$; $\lambda_{max.}$ (EtOH) 288 nm. (log ε 4·27); τ (CCl_4) 2·37 (2H), 4·06 (1H), 4·72—5·15 (3H), 6·88 (d, *J* 7 Hz, 2H), 8·03 (8H), 8·28 (3H), 8·44 (6H), mass spectrum 344, 275, 207, 193, 155, 154, 153, 81, and 69 (base).

(b) *Boviquinone*-3 (II) via *the Reduced Acetate*.—Zinc dust (5·5 g.) was added during 3·5 hr. to a refluxing mixture of the brown gum (18 g.), acetic anhydride (90 ml.), and fused sodium acetate (1 g.). The mixture was filtered, poured into water (750 ml.), and set aside overnight. The resulting dark brown oil was washed with water (2 × 750 ml.), taken up in ether (750 ml.), dried (anhydrous Na_2SO_4), and the ether evaporated to yield a residue which was extracted with boiling, light petroleum (b.p. 60—80°) (2 × 200 ml.). The petroleum was decanted from the brown oils which separated from the cool mixture and was then set aside in open conical flasks for three weeks. The colourless precipitates which slowly deposited in each flask were filtered, combined, and recrystallised from methanol to yield solvated needles of *boviquinone*-3-*leuco-tetra-acetate* (140 mg.); recrystallisation from light petroleum (b.p. 60—80°) gave the pure product as small needles m.p. 99—100° (Found: C, 67·9; H, 7·7. $C_{29}H_{38}O_8$ requires C, 67·7; H, 7·4%); *M* (mass spec.) 514, $\nu_{max.}$ (KBr) 1767, 1470, and 1367 cm.$^{-1}$; $\lambda_{max.}$ (EtOH) 269 nm. (log ε 2·87); τ ($CDCl_3$) 2·90 (1H), 4·77—5·18 (3H), 6·79 (d, *J* 7 Hz, 2H), 7·74 (6H), 7·77 (6H), 8·02 (8H), 8·33 (6H), and 8·42 (6H); mass spectrum 514, 472, 430, 388, 346, 320, 294, 277, 252, 239, 154, 81, and 69 (base).

A suspension of the reduced acetate (90 mg.), in a mixture of ethanol (4 ml.) and sodium hydroxide solution (0·75 ml., 2N), was set aside for 1·5 hr. The suspension of purple-grey sodium salt was acidified and the mixture was shaken with ether. The yellow, ether solution was washed with water (2 × 25 ml.), dried, evaporated, and the orange solid (62 mg.) which resulted was recrystallised from acetic acid to give orange needles of *boviquinone*-3, m.p. 90—92° identical in all respects with the product described above.

Diboviquinone-4,4 (VII) *from* Boletus bovinus.—The lead salt from the alcoholic extracts of *B. bovinus* (25 kg.) was decomposed with methanolic hydrogen chloride (450 ml., 5%) in three equal stages. The filtrate from the third stage was evaporated until an orange solid was deposited. Crystallisation from acetic acid gave *diboviquinone*-4,4 (1·49 g.) as clusters of small orange-red needles m.p. 132·5—134° (Found: C, 76·4; H, 8·3. $C_{52}H_{70}O_8$ requires C, 76·0; H, 8·5%); $\nu_{max.}$ (KBr) 3320, 2970, 2920, 2860, 1620, and 1320, ($CHCl_3$) 3340, 2960, 2920, 2850, 1640, and 1355 cm.$^{-1}$; $\lambda_{max.}$ (EtOH) 287 nm. (log ε 4·53), (EtOH + 2 drops 2N-NaOH) 253 (inf.), 325 nm.; τ ($CDCl_3$) 4·69—5·12 (8H), 6·86 (d, *J* 7 Hz, 4H), 7·98 (24H), 8·28 (6H), 8·38 (6H), 8·68 (18H); mass spectrum 684, 616, 529, 412 (M^+ boviquinone-4).

Xerocomic Acid from Fraction (iii).—The yellow aqueous solution was continuously extracted with ether and the ether extract was evaporated until precipitation occurred; the precipitated fumaric acid was filtered off. Evaporation of the filtrate to dryness and treatment of the residual gum (2·87 g.) with acetic anhydride (6 ml.) and sulphuric acid (2 drops) yielded a yellow solid which after recrystallisation from acetic acid yielded yellow needles of xerocomic acid dilactone triacetate (320 mg.), m.p. 221—223° (Found: C, 62·3; H, 3·5. Calc. for $C_{24}H_{16}O_{10}$: C, 62·1; H, 3·5%); $\nu_{max.}$ (KBr) 1830, 1770, 1665, 1605, 1505, 1370, and 1200 cm.$^{-1}$; $\lambda_{max.}$ ($CHCl_3$) 389 nm. (log ε 4·48); mass spectrum 464, 422, 380, 338, 282, 259, 226, 185, 177, 161, 149, 133, 121, and 105. Oxidation of this product with chromium trioxide and hydrolysis of the oxidation products produced a mixture of protocatechuic and *p*-hydroxybenzoic acids.

Boviquinone-3 *Dimethyl Ether*.—To a solution of bovinone-3 (50 mg.) in ether (10 ml.) was added an ethereal solution of diazomethane. After 30 min. the excess diazomethane was destroyed by the addition of acetic acid and the solution was evaporated. The orange-yellow gum was purified by t.l.c. on silica gel (Merck $P.f._{254+366}$) in the system benzene–ether (5 : 1). Elution of the leading yellow band (R_F 0·8) with chloroform gave *boviquinone*-3 *dimethyl ether* as a bright yellow oil (19 mg.) (Found: C, 73·9; H, 8·6. $C_{23}H_{32}O_4$ requires C, 74·2; H, 8·6%); $\nu_{max.}$ ($CHCl_3$) 1655 and 1600 cm.$^{-1}$; $\lambda_{max.}$ (EtOH) 283 nm. (log ε 4·15); τ ($CDCl_3$) 4·27 (1H), 4·72—5·10 (3H), 5·96 (3H), 6·22 (3H), 6·85 (d, *J* 7 Hz, 2H), 8·01 (8H), 8·24 (3H), 8·31 (3H), and 8·41 (6H); *M* (mass spec.) 372.

Diboviquinone-3,4 *tetramethyl ether* was similarly prepared from dibovinone-3,4 as a yellow viscous oil (56 mg.) (Found: C, 75·3; H, 8·8. $C_{51}H_{70}O_8$ requires C, 75·6; H, 8·6%); $\nu_{max.}$ ($CHCl_3$) 1655, 1605 cm.$^{-1}$: $\lambda_{max.}$ (EtOH) 285·5 nm.; τ (CCl_4) 4·70—5·10 (7H), 6·00 (6H), 6·02 (6H), 6·89 (d, *J* 7 Hz, 4H), 8·00 (20H), 8·28 (6H), 8·34 (6H), and 8·42 (15H); *M* (mass spectrum 810).

Similarly, diboviquinone-4,4 gave *diboviquinone*-4,4 *tetramethyl ether* as a viscous yellow oil (42 mg.) (Found: C, 76·6; H, 9·0. $C_{56}H_{78}O_8$ requires C, 76·5; H, 8·9%); *M* (mass spec.) 878; $\nu_{max.}$ ($CHCl_3$) 1645 and 1605 cm.$^{-1}$; $\lambda_{max.}$ (EtOH) 285·5 nm.; τ ($CDCl_3$) 4·76—5·12 (8H), 6·02 (6H), 6·07 (6H), 6·92 (d, *J* 7·5 Hz, 4H), 8·03 (24H), 8·28 (6H), 8·33 (6H), and 8·42 (18H). Methylenediboviquinone-3,3 gave *methylenediboviquinone*-3,3 *tetramethyl ether* as a viscous yellow oil (49 mg.) (Found: C, 75·0; H, 8·7. $C_{47}H_{64}O_8$ requires C, 74·6; H, 8·5%); *M* (mass spec.) 756; $\nu_{max.}$ ($CHCl_3$) 1645 and 1605 cm.$^{-1}$; $\lambda_{max.}$ (EtOH) 288·5 nm.; τ ($CDCl_3$) 4·70—5·07 (6H), 5·95 (6H), 6·01 (6H), 6·43 (2H), 6·86 (d, *J* 7 Hz, 4H), 8·00 (16H), 8·29 (6H), 8·32 (6H), and 8·40 (12H).

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