Tlc (Eastman Chromatogram Sheets, $CHCl_3$–EtOAc–CH_3OH (2:2:1) developer) showed the gum was a complex mixture. One component had the same R_f value as natural (−)-mesembrine and was not separated from it on tlc. The gum was purified by tlc on Mallinckrodt SilicAR adsorbant using the solvent system last mentioned as the developer. In this system, natural mesembrine appeared in the 0.35–0.50-R_f range and, accordingly, in the purification, silica gel in this range was removed from the developed plates and extracted with 30 ml and again with 20 ml of 1 *N* HCl. The combined aqueous solutions were basified with 2 *N* NH_4OH and extracted with three 20-ml portions of $CHCl_3$. The combined organic layers were dried over Na_2SO_4. The drying agent and, by distillation *in vacuo*, the solvent were removed giving, as a pale yellow gum, 28 mg (7.5%) of *dl*-mesembrine. The ir and nmr solution ($CDCl_3$) spectra of this material were, except for the presence of minor contaminants such as stopcock grease, identical with that of the alkaloid. This synthetic material and 36.9 mg of that of equivalent purity from a previous synthesis were combined in 10 ml of HCl and the solution extracted with several small portions of CH_2Cl_2 (discarded), basified with 2 *N* NH_4OH, extracted with three 10-ml portions of CH_2Cl_2 and the combined organic layers dried over Na_2SO_4. Removal of the drying agent and, by distillation *in vacuo*, the solvent left 38.3 mg (4.6% after correction for dilution) of the synthetic alkaloid whose ir and nmr spectra were now identical with that of (−)-mesembrine. Tlc on Eastman Chromatogram Sheets of the natural alkaloid, the synthetic alkaloid, and a mixture of the two was carried out using six different developers. Each run showed only one spot and, with any one developer and within a few per cent, at the same R_f value: Et_2O–CH_3OH (4:1), R_f 0.43; $CHCl_3$–CH_3OH (3:1), R_f 0.73; $CHCl_3$, R_f 0.58; $CHCl_3$–EtOAc–CH_3OH (2:2:1), R_f 0.53; C_6H_6–CH_3OH (4:1), R_f 0.58; Et_2O, R_f 0.09.

dl-Mesembrine has also been prepared in the above manner (although reaction times appeared to be somewhat longer) and in comparable yield (*ca.* 3.0%) using the purified pyrroline as the starting material.

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Synthesis of Rhodoquinone and Other Multiprenyl-1,4-benzoquinones Biosynthetically Related to Ubiquinone[1a]

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Abstract: The knowledge of the biosynthetic sequence from *p*-hydroxybenzoic acid (HBA) to ubiquinone and then rhodoquinone has been confirmed and enlarged by the organic synthesis of three multiprenyl-1,4-benzoquinones, which correspond to biosynthetic precursors. They are 2-amino-3-hydroxy-5-decaprenyl-6-methyl-1,4-benzoquinone (rhodoquinone), 2-hydroxy-3-methoxy-5-decaprenyl-6-methyl-1,4-benzoquinone, and 2-methoxy-5-methyl-6-decaprenyl-1,4-benzoquinone. Boron trifluoride catalyzed condensation of decaprenol with 2-methoxy-5-methyl-1,4-benzohydroquinone followed by oxidation of the hydroquinone gave 2-methoxy-5-methyl-6-decaprenyl-1,4-benzoquinone which, in turn, reacted with ammonia by 1,4 addition to give rhodoquinone-10. Deamination of rhodoquinone-10 with cupric chloride in acetic acid yielded 2-hydroxy-3-methoxy-5-decaprenyl-6-methyl-1,4-benzoquinone. The three analogous and isomeric decaprenyl derivatives were synthesized similarly from 2-methoxy-6-methyl-1,4-benzoquinone, and other related compounds were prepared. In the past, complete structure elucidation, on a microscale, of newly isolated compounds of the ubiquinone group was often difficult or not feasible. The spectral and chromatographic data on the new synthetic 5- and 6-decaprenyl analogs of ubiquinone and related compounds now greatly facilitate structural elucidations in this field. New data on the relative rates of conversion of these benzoquinones to their corresponding chromenols also extend the feasibility of structural elucidation on a microscale.

The isolation of a series of multiprenylphenols[2] and multiprenyl-1,4-benzoquinones[3,4] from *Rhodospirillum rubrum* resulted in the formulation of a complete biosynthetic sequence[3] from *p*-hydroxybenzoic acid (HBA) to ubiquinone (Q, **1a**). Parson and Rudney[5] had earlier demonstrated that ubiquinone (**1a**) is a precursor to rhodoquinone (**2a**) in *R. rubrum*. The biosynthetic and structural relationships of the last four compounds in this sequence are given (Scheme I).

Compounds **2**, **3**, and **4** differ from ubiquinone (**1a**) in that one methoxy group is replaced by hydrogen (**3**) or by another substituent (**2**, **4**). This paper reports the syntheses of these products, 2-amino-3-methoxy-5-decaprenyl-6-methyl-1,4-benzoquinone (rhodoquinone-10,[6–8] **2a**, $n = 9$), 2-hydroxy-3-methoxy-5-decaprenyl-6-methyl-1,4-benzoquinone[4] (**4a**, $n = 9$), and 2-methoxy-5-methyl-6-decaprenyl-1,4-benzoquinone[3] (**3a**, $n = 9$) as well as their position isomers **12a**, **13a**, and **9a** and other related compounds. The availability of these synthetic compounds has made possible a study

(1) (a) Coenzyme Q. CII. (b) The Royal Veterinary and Agricultural College, Copenhagen, Denmark.

(2) (a) The nomenclature used in this paper is based on a recommendation of an IUPAC–IUB Commission of Biochemical Nomenclature, *Biochim. Biophys. Acta*, **107**, 5 (1965). (b) R. K. Olsen, G. D. Daves, Jr., H. W. Moore, K. Folkers, W. W. Parson, and H. Rudney, *J. Amer. Chem. Soc.*, **88**, 5915 (1966).

(3) P. Friis, G. D. Daves, Jr., and K. Folkers, *ibid.*, **88**, 4754 (1966).

(4) P. Friis, J. L. G. Nilsson, G. D. Daves, Jr., and K. Folkers, *Biochem. Biophys. Res. Commun.*, **28**, 324 (1967).

(5) W. W. Parson and H. Rudney, *J. Biol. Chem.*, **240**, 1853 (1965).

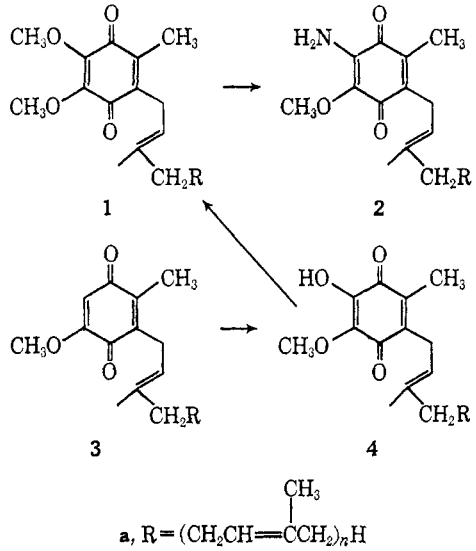
(6) J. Glover and D. R. Threlfall, *Biochem. J.*, **85**, 14p (1962).

(7) N. G. Carr, *ibid.*, **91**, 28p (1964).

(8) R. Powls and F. W. Hemming, *Phytochem.*, **5**, 1235 (1966).

of their chemical and physical properties which significantly resulted in a *micro and direct method*[9] for differentiation of the various isomer pairs. This method permits assignment of structure to certain natural and biosynthetic products in the ubiquinone group.

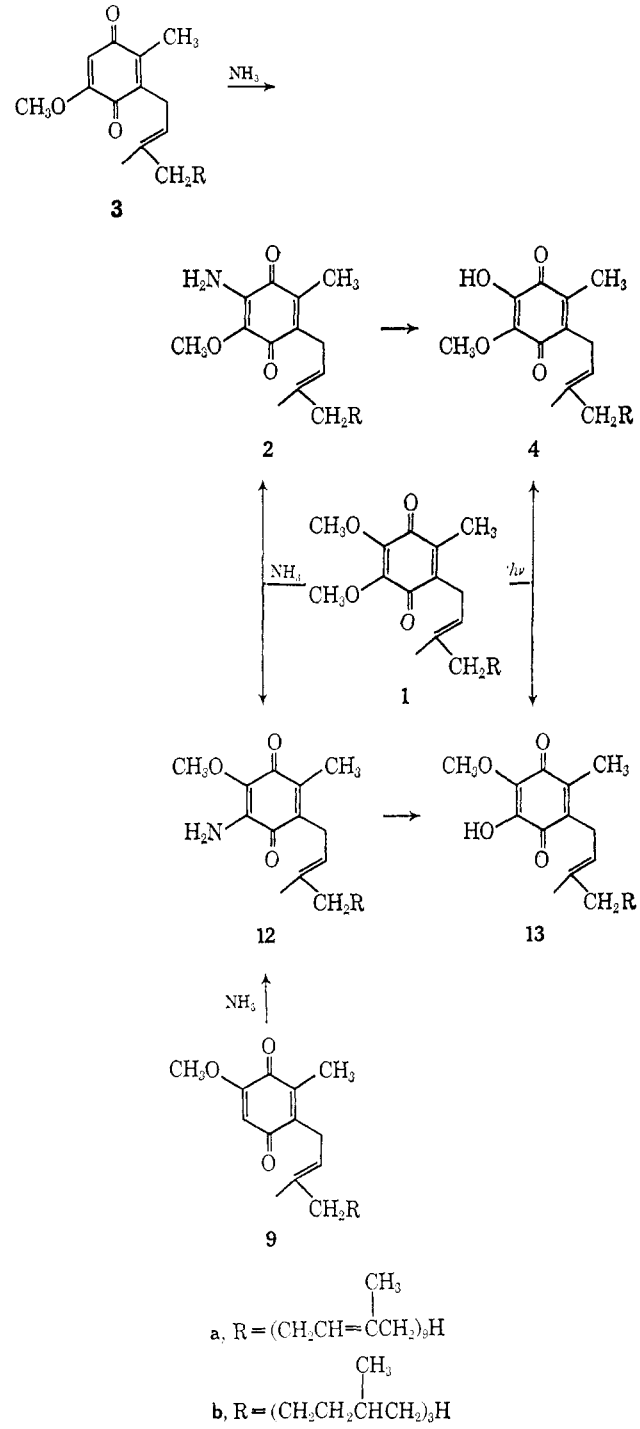
Scheme I. Biosynthetic Relationship of Multiprenyl-1,4-benzoquinones Isolated from *Rhodospirillum rubrum*



As shown in Schemes II and III, the synthetic sequence utilized for the preparation of multiprenylquinones **2**, **3**, and **4** consists of alkylation of 2-methoxy-5-methyl-1,4-benzohydroquinone[10] (**5**) followed by oxidation and separation of the desired 2-methoxy-5-methyl-6-multiprenyl-1,4-benzoquinone[3,11] (**3**); 1,4 addition of ammonia to **3** produced rhodoquinone (**2**) and treatment of rhodoquinone (**2**) with cupric chloride in acetic acid[12] yielded 2-hydroxy-3-methoxy-5-multiprenyl-6-methyl-1,4-benzoquinone[13] (**4**). The isomeric series (**9**, **12**, **13**)[14] was obtained in an analogous manner starting with 2-methoxy-6-methyl-1,4-benzohydroquinone[15] (**6**).

Boron trifluoride catalyzed condensation of 2-methoxy-5-methyl-1,4-benzohydroquinone[10] (**5**) with an appropriate isoprenoid alcohol followed by ferric chloride oxidation of the intermediate hydroquinones and thin layer chromatographic separation of the products yielded, in each case, three isoprenylated products (Scheme III). Two of these products were isomers resulting from monoalkylation at each of the two unsubstituted ring positions and the third product was the corresponding dialkylated product. Thus, condensation with phytol and subsequent oxidation of the intermediate hydroquinones yielded a readily separated mixture of 2-methoxy-5-methyl-6-phytyl-1,4-benzoquinone (**3b**), 2-methoxy-5-methyl-3-phytyl-1,4-benzoquinone (**7b**), and 2-methoxy-5-methyl-3,6-bisphytyl-1,4-benzoquinone (**8b**). Similarly, condensation of 2-methoxy-5-methyl-1,4-benzohydroquinone (**5**) with geraniol, farnesol, solanesol,[16] and decaprenol[17] yielded **3a** (n = 1, 2, 8, 9), **7a** (n = 1, 2, 8, 9), and **8a** (n = 1, 2). In the condensation of **5** with solanesol[16] and decaprenol[17] the bismultiprenyl products (**8a**, n = 8, 9) were formed in very low yield, as observed by tlc, and were not isolated.

Scheme II. Organic Synthetic Conversions



(9) J. J. Wilczynski, G. D. Daves, Jr., and K. Folkers, *J. Amer. Chem. Soc.*, **90**, 5593 (1968).
(10) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler, and W. M. McLamore, *ibid.*, **74**, 4223 (1952).
(11) S. Imamoto and S. Senoh, *Tetrahedron Lett.*, 1237 (1967).
(12) H. W. Moore and K. Folkers, *J. Amer. Chem. Soc.*, **88**, 567 (1966).
(13) The nonaprenyl derivative **4a** (n = 8) and the phytyl derivative **4b** have been prepared by another method; see C. H. Shunk, J. F. McPherson, and K. Folkers, *J. Org. Chem.*, **31**, 1638 (1966).
(14) Compound **9b** has been prepared previously;[3,11] compound **13b** was prepared by a different method: I. Imada and H. Morimoto, *Chem. Pharm. Bull.* (Tokyo), **13**, 130 (1965).
(15) F. Henrich and G. Nachtigall, *Ber.*, **36**, 889 (1903).
(16) R. L. Rowland, P. H. Latimer, and J. A. Giles, *J. Amer. Chem. Soc.*, **78**, 4680 (1956).
(17) R. Rüegg, U. Gloor, R. N. Goel, G. Ryser, O. Wiss, and O. Isler, *Helv. Chim. Acta*, **42**, 2616 (1959).

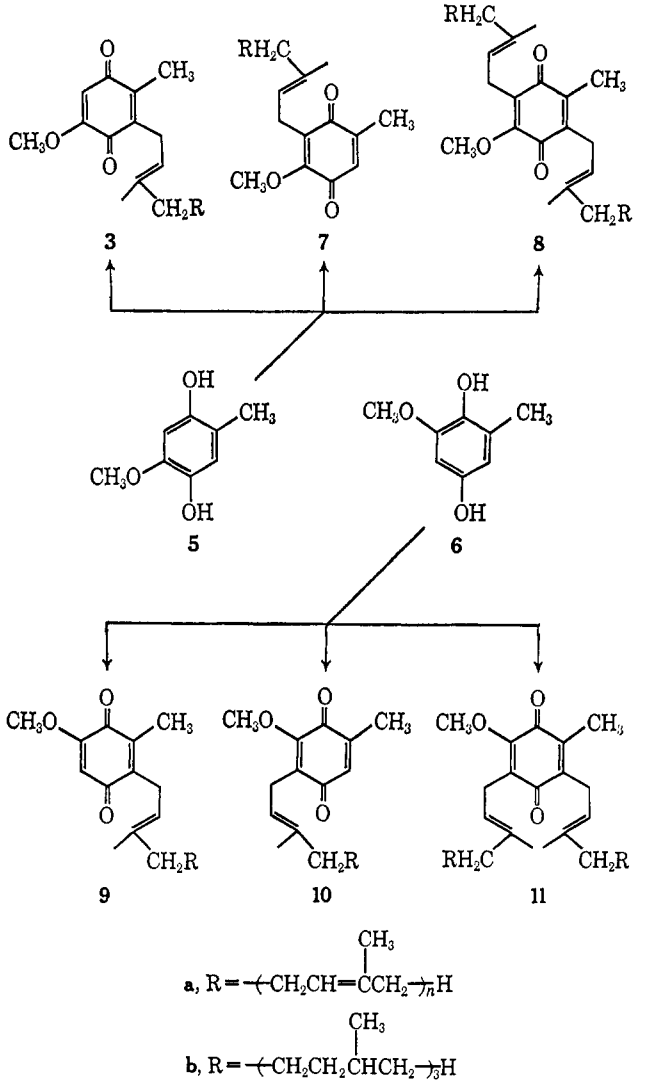
Table I. Spectral Data for Multiprenyl Derivatives of 2-Methoxy-5-methyl-1,4-benzoquinone

2-Methoxy-5-methyl-1,4-benzoquinone derivative	Uv λ_{max}^{hexane}, mμ	Nmr (CCl_4)[b] Ring proton	Olefinic	Methoxyl	Ring methylene	Ring methyl[c]	Alkyl
6-Geranyl (**3a**, n = 1)	265, 273	4.32 (s)	5.16 (m)	6.30 (s)	6.91 (d)		7.8–8.6
6-Farnesyl (**3a**, n = 2)	265, 273	4.28 (s)	5.04 (m)	6.27 (s)	6.87 (d)		7.8–8.6
6-Nonaprenyl (**3a**, n = 8)	265, 272	4.29 (s)	4.98 (m)	6.27 (s)	6.87 (d)		7.8–8.5
6-Decaprenyl (**3a**, n = 9)	265, 272	4.31 (s)	4.99 (m)	6.27 (s)	6.87 (d)		7.8–8.5
6-Phytyl (**3b**)	265, 272	4.31 (s)	5.14 (t)	6.29 (s)	6.89 (d)	8.04 (s)	7.8–9.3
3-Geranyl (**7a**, n = 1)	262	3.71 (q)	5.06 (m)	6.05 (s)	6.96 (d)		7.8–8.6
3-Farnesyl (**7a**, n = 2)	262	3.71 (q)	5.04 (m)	6.04 (s)	6.95 (d)		7.8–8.6
3-Nonaprenyl (**7a**, n = 8)	262	3.73 (q)	4.99 (m)	6.04 (s)	6.96 (d)		7.8–8.6
3-Decaprenyl (**7a**, n = 9)	260	3.71 (q)	4.98 (m)	6.03 (s)	6.95 (d)		7.8–8.6
3-Phytyl (**7b**)	262	3.71 (q)	5.05 (t)	6.02 (s)	6.95 (d)	8.02 (d)	7.8–9.3
3,6-Bisgeranyl (**8a**, n = 1)	269, 275[a]		5.08 (m)	6.07 (s)	6.94 (m)		7.8–8.6
3,6-Bisfarnesyl (**8a**, n = 2)	269, 275[a]		5.07 (m)	6.07 (s)	6.93 (m)		7.8–8.6
3,6-Bisphytyl (**8b**)	269, 275[a]		5.08 (m)	6.07 (s)	6.93 (m)	8.03 (s)	7.8–9.3

[a] Shoulder. [b] The letters in parentheses refer to peak shape: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. [c] Ring methyl signal is obscured by side-chain methylenes when unsaturated isoprenoid side chain is present.

The assignments of structures **3**, **7**, and **8** to the products of the isoprenylation reactions are made by examination of the uv and nmr spectra (Table I). The

Scheme III. Organic Synthetic Conversions



ultraviolet spectra of the synthetic multiprenylbenzoquinones compare closely with model compounds. The products of general structure **3** show λ_{max}^{hexane} at 265 and 272 (273) mμ; 2-methoxy-5,6-dimethylbenzoquinone exhibits corresponding maxima at 266 and 272 mμ.[18] The isomeric monoalkyl series (**7**) shows λ_{max}^{hexane} at 262 (260) mμ; the corresponding model compound, 2-methoxy-3,6-dimethylbenzoquinone, has a maximum of 262 mμ.[18] The bismultiprenyl products (**8**) exhibit λ_{max}^{hexane} at 269 and 275 mμ (shoulder); 2-methoxy-3,5,-6-trimethylbenzoquinone has λ_{max}^{hexane} at 268 mμ.[18]

The initial structure assignments made on the basis of the uv data were confirmed by examination of the nmr spectra (Table I). The bismultiprenylated series of compounds (**8**) is characterized by the absence of a ring proton absorption in the region τ 3.5–4.5 and by the appearance of a multiplet (4 H) due to ring methylene protons at τ 6.9. The ring proton signal at τ 4.3 (singlet) in one series and at τ 3.7 (quartet, J = 1.5 cps) in the second series of monoalkylated isomers allows unequivocal assignment of structure **3** to the former series and of structure **7** to the latter series. In the nmr spectrum of 2-methoxy-5-methyl-1,4-benzoquinone, the ring proton at C-3 (corresponding to the C-3 proton in structure **3**) appears as a singlet at τ 4.26; the ring proton at C-6 (corresponding to the C-6 proton in structure **7**) is coupled with the C-5 methyl group yielding a quartet at τ 3.60 and a doublet at τ 8.02 (J = 1.5 cps).[19]

By boron trifluoride catalyzed condensation of 2-methoxy-6-methyl-1,4-benzohydroquinone[15] (**6**) with phytol and with decaprenol,[17] compounds **9b**, **10b**, **11b**, and **9a** (n = 9) and **10a** (n = 9) were obtained (Scheme III). Unambiguous assignments of structures **9**, **10**, and **11** were made on the basis of their characteristic uv and nmr spectra (Table II), as was done in the assignment of structures **3**, **7**, and **8**.

Treatment of 2-methoxy-5-methyl-6-decaprenyl-1,4-benzoquinone (**3a**, n = 9) in methanol–ether (2:1) solution with ammonia under anhydrous conditions produced, in low yield, 2-amino-3-methoxy-5-decaprenyl-6-methyl-1,4-benzoquinone (**2a**, n = 9). This product is indistinguishable both spectrally and chro-

(18) W. Flaig, J. C. Salfeld, and E. Baume, *Ann.*, **618**, 117 (1958).
(19) For a discussion of long-range splitting in the nmr spectra of benzoquinones, see R. K. Norris and S. Sternhell, *Aust. J. Chem.*, **19**, 617 (1966).

Table II. Spectral Data for Multiprenyl Derivatives of 2-Methoxy-6-methyl-1,4-benzoquinone

Derivative	Uv λ_{max}^{hexane}, mμ	Nmr (CCl_4)[a] Ring proton	Olefinic	Methoxyl	Ring methylene	Ring methyl[b]	Alkyl
5-Decaprenyl (**9a**, n = 9)	265, 272	4.31 (s)	4.99 (m)	6.29 (s)	6.88 (d)		7.8–9.3
5-Phytyl (**9b**)	266, 273	4.31 (s)	5.16 (t)	6.28 (s)	6.91 (d)	8.03 (s)	7.8–9.3
3-Decaprenyl (**10a**, n = 9)	262	3.62 (q)	4.98 (m)	6.07 (s)	6.98 (d)		7.8–8.6
3-Phytyl (**10b**)	262	3.64 (q)	5.07 (t)	6.08 (s)	7.00 (d)	8.04 (d)	7.8–9.3
3,5-Diphytyl (**11b**)	269		5.08 (m)	6.08 (s)	6.94 (m)	8.06 (s)	7.8–9.3

[a] See Table I, footnote *b*. [b] See Table I, footnote *c*.

Table III. Spectral Data for Rhodoquinones (**2**) and Isorhodoquinones (**12**)

Compound	Uv λ_{max}^{hexane}, mμ	Nmr (CCl_4)[a] Olefinic	Amino	Methoxy	Ring methylene	Alkyl
2-Amino-3-methoxy-6-methyl-1,4-benzoquinone						
5-Decaprenyl (rhodoquinone-10, **2a**, n = 9)	227, 277, 495	5.00 (m)	5.58 (s)	6.18 (s)	6.92 (d)	7.8–8.6
5-Phytyl (**2b**)	227, 277, 495	5.16 (t)	5.56 (s)	6.17 (s)	6.92 (d)	7.8–9.3
2-Amino-3-methoxy-5-methyl-1,4-benzoquinone						
6-Decaprenyl (isorhodoquinone-10, **12a**, n = 9)	227, 277, 495	5.00 (m)	5.56 (s)	6.18 (s)	6.95 (d)	7.8–8.6
6-Phytyl (**12b**)	227, 277, 495	5.15 (t)	5.48 (s)	6.18 (s)	6.94 (d)	7.8–9.3

[a] See Table I, footnote *b*.

matographically from natural rhodoquinone-10 isolated from *R. rubrum*,[6] and differs chromatographically from the isomeric aminoquinone **12a** (n = 9) obtained by treatment of **9a** (n = 9) with ammonia under similar conditions. The comparison of isolated rhodoquinone-10 with these unambiguously synthesized aminoquinone isomers **2a** (n = 9) and **12a** (n = 9) provides confirmation of the structure assignment for rhodoquinone-10 made by Moore and Folkers.[12]

Natural rhodoquinone-10 (**2a**, n = 9) has a melting point of 69–70°.[20] Rhodoquinone-10 and isorhodoquinone-10 (**12a**, n = 9), obtained by ammonia addition to the monomethoxyquinones **3a** (n = 9) and **9a** (n = 9), exhibit melting points of 47–49 and 45–50°, respectively. The low melting points of these synthetic products are explained by the fact that each is a mixture of *cis* and *trans* isomers at the double bond of the isoprenoid unit in the side chain which is nearest the quinone ring.[21] By treatment of Q-10 (**1a**, n = 9) with ammonia, Moore and Folkers[12,20] obtained a mixture of rhodoquinone-10 (**2a**, n = 9) and isorhodoquinone-10 (**12a**, n = 9) which melted at 39–45°. We have succeeded in separating this mixture by thin layer chromatography using the technique of multiple development.[22] Samples of synthetic rhodoquinone-10 (**2a**, n = 9) and isorhodoquinone-10 (**12a**, n = 9) obtained in this way melted at 69–70 and 61–62°, respectively.

The corresponding isomeric aminoquinones **2b** and **12b** were obtained by treatment of **3b** and **9b**, respectively, with ammonia. Treatment of phytylubiquinone[23] (**1b**) with ammonia yielded a mixture of **2b** and **12b** which was separated by thin layer chromatography using the multiple development technique.[22] The two isomer pairs **2a** (n = 9) and **12a** (n = 9) and **2b** and **12b** are readily separable by this method; in each case, the isomer which possesses the amino group *para* to the multiprenyl side chain, *i.e.*, **2a** and **2b**, exhibits the higher R_f value.

Deamination of rhodoquinone-10 (**2a**, n = 9), isorhodoquinone-10 (**12a**, n = 9), and the isomeric amino derivatives in the phytyl series **2b** and **12b** with cupric chloride dihydrate in hot glacial acetic acid as described by Moore and Folkers[12] yielded the two pairs of isomeric hydroxy compounds **4a** (n = 9) and **13a** (n = 9) and **4b** and **13b**. Attempts to separate mixtures of these isomer pairs obtained by photolysis[12,24,25] of Q-10 (**1a**, n = 9) and phytylubiquinone (**1b**) by chromatographic techniques similar to those used for the separation of the corresponding aminoquinone mixtures met with limited success.

It is evident that compounds **2**, **3**, and **4** cannot be distinguished from their respective isomers, **9**, **12**, and **13**, on the basis of spectral data as recorded in Tables I–IV. The great similarity in the chemical and physical properties of each of these compounds with those of its isomer, and the importance of definitive structural assignments of natural products of these types has led to a number of approaches[8,11,24,25] for the differentiation of the isomers. Moore and Folkers[25] described a method which involves several chemical transformations which they used for assignment of structure to rhodoquinone[12] (**2**), and for establishing that photodemethylation of ubiquinone[24] (**1**) produced a mixture of hydroxyquinones **4** and **13** in approximately equal amounts.[25] In principle, their method is adaptable for differentiation of the monomethoxymultiprenyl-1,4-benzoquinone isomers **3** and **9**. However, it was not suitable for assignment of structure to 2-methoxy-5-methyl-6-decaprenyl-1,4-benzoquinone (**3a**, n = 9), tediously isolated from *R. rubrum*,[3] since the limited

(20) H. W. Moore and K. Folkers, *J. Amer. Chem. Soc.*, **87**, 1409 (1965).

(21) In condensations of tertiary allylic alcohols with hydroquinones, as in these cases, as much as 30% of the *cis* isomer may be formed, see L. M. Jackman, R. Rüegg, G. Ryser, C. von Planta, U. Gloor, H. Mayer, P. Schudel, M. Kofler, and O. Isler, *Helv. Chim. Acta*, **48**, 1332 (1965).

(22) P. Friis, G. D. Daves, Jr., and K. Folkers, *Biochem. Biophys. Res. Commun.*, **24**, 252 (1966).

(23) C. H. Shunk, B. O. Linn, E. L. Wong, P. E. Wittreich, F. M. Robinson, and K. Folkers, *J. Amer. Chem. Soc.*, **80**, 4753 (1958).

(24) I. Imada, Y. Sanno, and H. Morimoto, *Chem. Pharm. Bull.* (Tokyo), **12**, 1056 (1964).

(25) H. W. Moore and K. Folkers, *J. Amer. Chem. Soc.*, **88**, 564 (1966).

Table IV. Spectral Data for 2-Hydroxy-3-methoxy-5-multiprenyl-6-methyl- (4) and 2-Hydroxy-3-methoxy-5-methyl-6-multiprenyl-1,4-benzoquinones (13)

Compound	Uv λ_{max}^{hexane}, mμ	Nmr (CCl_4)[a,b] Olefinic	Methoxy	Ring methylene	Alkyl
2-Hydroxy-3-methoxy-6-methyl-1,4-benzoquinones					
5-Decaprenyl (**4a**, n = 9)	271, 277	4.99 (m)	6.06 (s)	6.89 (d)	7.8–8.6
5-Phytyl (**4b**)	271, 278	5.18 (t)	6.06 (s)	6.90 (d)	7.8–9.3
2-Hydroxy-3-methoxy-5-methyl-1,4-benzoquinones					
6-Decaprenyl (**13a**), n = 9)	272, 278	4.99 (m)	6.08 (s)	6.90 (d)	7.8–8.6
6-Phytyl (**13b**)	271, 277	5.15 (t)	6.09 (s)	6.90 (d)	7.8–9.3

[a] See Table I, footnote *b*. [b] No discrete signals due to OH were observed.

Table V. Chromenol Formation from 2-Methoxy-5-methyl-6-phytyl-1,4-benzoquinone (**3b**) and 2-Methoxy-5-phytyl-6-methyl-1,4-benzoquinone (**9b**) in Pyridine Solution at 50°

Reaction time, hr	Chromenol formed, %: 2-Methoxy-5-methyl-6-phytyl-1,4-benzoquinone (**3b**)	2-Methoxy-5-phytyl-6-methyl-1,4-benzoquinone (**9b**)
1	0	Trace
34	Trace	60
58	10	80
87	15	

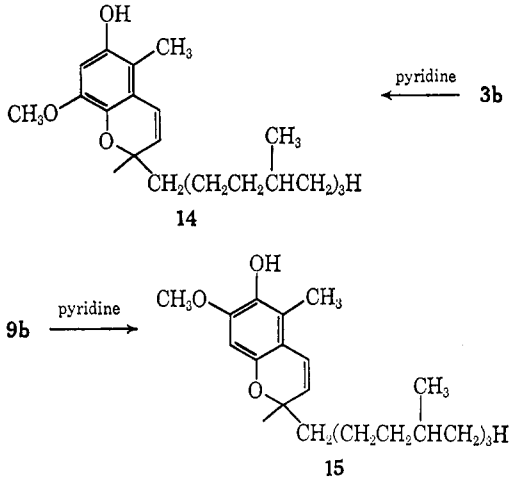
amount of product available (<3 mg) was insufficient for the number of chemical transformations required.

Perhaps for similar reasons, Imamoto and Senoh[11] chose different methods for the assignment of structure to 2-methoxy-5-methyl-6-nonaprenyl-1,4-benzoquinone (**3a**, n = 8), an apparent biosynthetic precursor to ubiquinone-9 (**1a**, n = 8) isolated from *Pseudomonas ovalis*. They reported that 2-methoxy-5-methyl-6-phytyl-1,4-benzoquinone (**3b**) and 2-methoxy-6-methyl-5-phytyl-1,4-benzoquinone (**9b**) exhibit sufficiently different uv spectra in carbon tetrachloride solution to allow unambiguous differentiation of the two compounds. Thus, they reported[11] that **4b** showed $\lambda_{max}^{CCl_4}$ at 269, 276 (sh), and 320 (sh) mμ, and that **7b** exhibited $\lambda_{max}^{CCl_4}$ at 269, 276 (sh), and 317 mμ. We have found that the uv absorption maxima of longest wavelength for **4b** and **7b** in carbon tetrachloride solutions appear at 321 mμ (distinct maximum; not a shoulder) and 319 mμ, respectively.

Imamoto and Senoh[11] have also noted that the conditions for isomerization of **3b** and **9b** to their corresponding chromenols were strikingly different; under conditions which readily converted **9b** to its corresponding chromenol **15b**, the isomeric quinone **3b** was unchanged. We obtained similar results (Table V); after 34 hr in pyridine solution at 50°, 60% of quinone **9b** was converted into its chromenol **15b**, while after 87 hr under similar conditions **3b** was isomerized to its chromenol **14b** to the extent of only 15%. When **3b** was heated in pyridine solution under reflux for 6 hr, conversion to its chromenol **14b** was essentially complete.

While quinones **3b** and **9b** possess virtually indistinguishable uv and nmr (CCl_4) spectral properties (Tables I and II), the respective chromenols **14** and **15** are readily differentiated by spectral methods (Table VI).

The obvious disadvantage, when dealing with milligram (or smaller) quantities of laboriously isolated natural products, of structural elucidations involving chemical conversions led us to seek a simpler, more direct method for differentiating isomeric compounds in the ubiquinone group. This search has led to the discovery[9] that the isomer pairs rhodoquinone (**2**) and isorhodoquinone (**12**), the corresponding hydroxy analogs **4** and **13**, and the monomethoxyquinones **3** and **9** exhibit characteristic differences in their nmr spectra in benzene and pyridine solutions which allow very facile and unequivocal differentiation of isomers. With the use of the synthetic compounds described herein as models, assignment of structure to related new natural products is easily possible.



Experimental Section

Materials. Geraniol, farnesol, and phytol were obtained commercially and used without purification. Solanesol[16] and the intermediate 3,7,11,15,19,23,27,31,35,39-decamethyltetraconta-6,10,14,18,22,26,30,34,38-nonaen-1-yn-3-ol[17] was generously provided by Dr. O. Isler and Professor Dr. Pl. A. Plattner of F. Hoffmann-LaRoche and Co. Ltd., Basel, Switzerland. 3-Decaprenol (3,7,11,15,19,23,27,31,35,39-decamethyltetraconta-1,6,10,14,18,22,26,30,34,38-decaen-3-ol) was prepared[17] by catalytic reduction of the intermediate acetylenic alcohol.

2-Methoxy-6-methyl-1,4-benzoquinone[15] was conveniently prepared by chromic acid oxidation of 2-methoxy-4-amino-6-methylphenol hydrochloride.[26] 2-Methoxy-4-nitrophenol was generously provided by the Maumee Chemical Co., Toledo, Ohio.

Dioxane was refluxed over sodium and distilled. Nuclear magnetic resonance (nmr) spectra were obtained using carbon tetrachloride solutions with a Varian HA-100 spectrometer; chemical shifts are expressed in τ units relative to tetramethylsilane as an internal standard. Ultraviolet spectra were obtained using a Cary 14 spectrophotometer. Thin layer chromatography was carried out using silica gel G plates having a 0.3-mm layer; for preparative thin layer chromatography, plates with 1.0-mm layer of adsorbent

(26) W. E. Solodar and M. Green, *J. Org. Chem.*, 27, 1077 (1962).

Table VI. Spectral Data for Chromenols

Chromenol	UV λ_{max}^{hexane}, mμ	Nmr (CCl_4)[b] Ring proton	Olefinic	Hydroxyl	Methoxyl	Ring methyl	Alkyl
2,5-Dimethyl-8-methoxy-2-(4,8,12-trimethyltridecyl)-6-chromenol (**14**)	227, 272, 282, 340	3.89 (s)	4.05[c]	5.02 (b)	6.39 (s)	7.95 (s)	8.2–9.3
2,5-Dimethyl-7-methoxy-2-(4,8,12-trimethyltridecyl)-6-chromenol (**15**)	236, 277, 283,[a] 328	3.91 (s)	4.18[d]	5.05 (b)	6.21 (s)	7.88 (s)	8.2–9.3

[a] Shoulder. [b] See Table I, footnote *b*. [c] AB (J = 10 cps, δ 96 cps). [d] AB (J = 10 cps, δ 106 cps).

were used. The plates were activated by heating at 130° for 1.5 hr and were stored in a dry cabinet after cooling.

Preparation of Multiprenyl Derivatives of 2-Methoxy-5-methyl-1,4-benzoquinone.[10] General Procedure. An ether solution of 1.52 g (0.01 mol) of 2-methoxy-5-methyl-1,4-benzoquinone[10] was shaken in a separatory funnel with an aqueous solution of 10 g of sodium hydrosulfite until the organic phase was essentially colorless. The organic phase was separated and carefully dried, and the solvent was removed. To the residue, was added 0.003 mol of the appropriate isoprenoid alcohol in 75 ml of dioxane. To the resulting well-stirred solution was added dropwise 1.5 ml of boron trifluoride etherate. After 3 hr, the reaction mixture was poured into 3 volumes of ether and 3 volumes of water. The organic phase was removed and shaken in a separatory funnel with 150 ml of water–methanol (2:1) containing an excess (10–15 g) of ferric chloride hexahydrate. The organic phase was separated and dried over magnesium sulfate. The residue obtained upon removal of the solvent was triturated with 15–25 ml of hexane. The insoluble material (recovered 2-methoxy-5-methyl-1,4-benzoquinone) was removed and the hexane-soluble residue was subjected to preparative thin layer chromatography using chloroform–benzene (1:1) as developing solvent system. After development the plates showed four distinct yellow-to-orange bands. Elution of these bands yielded (in order from the origin) recovered 2-methoxy-5-methyl-1,4-benzoquinone, 2-methoxy-5-methyl-6-multiprenyl-1,4-benzoquinone (**3**), 2-methoxy-3-multiprenyl-5-methyl-1,4-benzoquinone (**7**), and 2-methoxy-3,6-bismultiprenyl-1,4-benzoquinone (**8**). A second purification by the same procedure was necessary to produce a pure sample of 2-methoxy-5-methyl-6-multiprenyl-1,4-benzoquinone (**3**). In the alkylations using solanesol[16] and 3-decaprenol,[17] the dialkylated products were present in only minor amounts and were not isolated. Spectral data (uv and nmr) which establish the structures of the various products appear in Table I.

Preparation of Decaprenyl and Phytyl Derivatives of 2-Methoxy-6-methyl-1,4-benzoquinone.[15] 2-Methoxy-6-methyl-1,4-benzoquinone[15] (see Materials) was reduced as described above and the resulting hydroquinone was treated with 3-decaprenol[17] or phytol as described for the multiprenylation of 2-methoxy-5-methyl-1,4-benzoquinone. Following thin layer chromatographic separation as described above, 2-methoxy-5-decaprenyl-6-methyl-1,4-benzoquinone (**9a**, n = 9), 2-methoxy-3-decaprenyl-6-methyl-1,4-benzoquinone (**10a**, n = 9), 2-methoxy-5-phytyl-6-methyl-1,4-benzoquinone (**9b**), 2-methoxy-3-phytyl-6-methyl-1,4-benzoquinone (**10b**), and 2-methoxy-3,6-bisphytyl-1,4-benzoquinone (**11b**) were obtained. Characteristic spectral data (uv and nmr) for these compounds are recorded in Table II.

Addition of Ammonia to 2-Methoxy-5-methyl-6-multiprenyl- (3) and 2-Methoxy-6-methyl-5-multiprenyl-1,4-benzoquinones (9). General Procedure. A solution of 10–15 mg of the appropriate monomethoxymultiprenylbenzoquinone (**3a,b** or **9a,b**) in 30 ml of ether–methanol (1:2) was cooled to 0°, and ammonia was passed continuously through the solution for 1.5 hr. Ammonia addition was stopped and the reaction mixture was allowed to stand for 4 hr at room temperature. The solvent was then removed *in vacuo* and the residue was subjected to preparative thin layer chromatography on silica gel G plates developed in chloroform. An upper yellow band (unreacted starting material) and a purple aminoquinone band immediately below it were separately eluted using ether. Approximately 50% of unchanged starting material was recovered. By this procedure, 10–30% yields (based on consumed starting material) of 2-amino-3-methoxy-5-decaprenyl-6-methyl-1,4-benzoquinone (rhodoquinone-10 **2a**, n = 9), 2-amino-3-methoxy-5-methyl-6-decaprenyl-1,4-benzoquinone (isorhodoquinone-10, **12a**, n = 9), 2-amino-3-methoxy-5-phytyl-6-methyl-1,4-benzoquinone (**2b**), and 2-amino-3-methoxy-5-methyl-6-phytyl-1,4-benzoquinone (**12b**) were obtained. Characteristic nmr and uv spectral data for these products are recorded in Table III.

Separation of Mixtures of Rhodoquinone-10 (2a, n = 9) and Isorhodoquinone-10 (12a, n = 9). A mixture of rhodoquinone-10 (**2a**, n = 9) and isorhodoquinone-10 (**12a**, n = 9) was prepared from ubiquinone-10 (**1a**, n = 9) by the procedure of Moore and Folkers.[12] The crude reaction mixture was subjected to preparative thin layer chromatography on silica gel G plates developed in chloroform; the mixture of aminoquinones appeared as a purple band and was isolated by elution with ether. This mixture was then rechromatographed in chloroform; the plate was dried and redeveloped. By this procedure, two distinct purple bands became evident. These bands were eluted separately and individually rechromatographed as described above. Finally, each product was chromatographed by the multiple development procedure[22] using hexane–ether (9:1) as the developing solvent system to yield pure rhodoquinone-10 (**2a**, n = 9), mp 69–70°, and isorhodoquinone-10 (**12a**, n = 9), mp 61–62°. Rhodoquinone-10 exhibits a greater mobility than isorhodoquinone-10 in each of the solvent systems used.

By similar techniques, the mixture of aminoquinones obtained by treatment of 2,3-dimethoxy-5-methyl-6-phytyl-1,4-benzoquinone[23] (phytylubiquinone, **1b**) with ammonia was separated to yield pure samples of 2-amino-3-methoxy-5-phytyl-6-methyl-1,4-benzoquinone (**2b**) and 2-amino-3-methoxy-5-methyl-6-phytyl-1,4-benzoquinone (**12b**).

Deamination of Rhodoquinones[12] (2a,b) and Isorhodoquinones (12a,b) to the Corresponding Hydroxy Analogs 4a,b and 13a,b. General Procedure. A mixture of approximately 25 mg of the appropriate aminomultiprenylquinone (**2a,b** or **12a,b**) and 25 mg of cupric chloride dihydrate in 2 ml of glacial acetic acid was heated to the boiling point, or until the deep purple color of the reaction mixture changed to yellow. The reaction mixture was then poured onto crushed ice which was covered with a layer of hexane. The organic layer was removed, dried, and evaporated. The crude product was purified by column chromatography on florisil. The hydroxyquinone product was strongly adsorbed and appeared as a purple band on the column. After 100–200 ml of ether had passed through the column, the product was eluted using 1% acetic acid in ether. By this procedure, 30–50% yields of 2-hydroxy-3-methoxy-5-decaprenyl-6-methyl-1,4-benzoquinone (**4a**, n = 9), 2-hydroxy-3-methoxy-5-methyl-6-decaprenyl-1,4-benzoquinone (**13a**, n = 9), 2-hydroxy-3-methoxy-5-phytyl-6-methyl-1,4-benzoquinone (**4b**), and 2-hydroxy-3-methyl-5-methyl-6-phytyl-1,4-benzoquinone (**13b**) were obtained. Characteristic nmr and uv spectral data for these products are recorded in Table IV.

2,5-Dimethyl-8-methoxy-2-(4,8,12-trimethyltridecyl)-6-chromenol (14). A solution of 25 mg of 2-methoxy-5-methyl-6-phytyl-1,4-benzoquinone (**3b**) in 5 ml of pyridine was heated under reflux for 6 hr. The solution was cooled and poured into a mixture of ice and dilute hydrochloric acid covered with a layer of hexane. After shaking, the hexane layer was separated, washed with aqueous sodium bicarbonate, and dried. The residue obtained upon removal of the solvent was subjected to preparative thin layer chromatography using benzene–chloroform (1:1) as a developing system. The chromenol (detected as an Emmerie–Engel[27] sensitive band) was removed and eluted. Spectral data which characterize the product as 2,5-dimethyl-8-methoxy-2-(4,8,12-trimethyltridecyl)-6-chromenol (**14**) are recorded in Table VI.

2,5-Dimethyl-7-methoxy-2-(4,8,12-trimethyltridecyl)-6-chromenol (15). A solution of 25 mg of 2-methoxy-5-phytyl-6-methyl-1,4-benzoquinone (**9b**) in 5 ml of pyridine was heated on a steam bath for 12 hr. The reaction mixture was worked up as described above for the preparation of **14**. 2,5-Dimethyl-7-methoxy-2-(4,8,12-trimethyltridecyl)-6-chromenol (**15**) is characterized by spectral data recorded in Table VI.

(27) F. L. Crane and R. A. Dilley, *Methods Biochem. Analysis*, **11**, 279 (1963).

Rate of Chromenol Formation from 3b and 9b in Pyridine at 50°. Samples (10 mg) of quinones **3b** and **9b** were dissolved in 0.2 ml of pyridine-d_5 and sealed in nmr tubes. These sealed tubes were placed in a constant-temperature bath maintained at 50 ± 5°. Periodically, the tubes were removed and the nmr spectra were recorded. The per cent chromenol formed was estimated by comparison of the area due to the signal of the methoxyl of the quinone (τ 6.41 for **3b** or τ 6.40 for **9b**) with the area due to the signal of the methoxyl peak due to chromenol (τ 6.31 for **14** or τ 6.36 for **15**). The data are in Table V.

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Structure Determination of Ubiquinone Analogs by Solvent Shifts in Nuclear Magnetic Resonance Spectra[1]

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Abstract: A facile and micro method for structural assignments of compounds related to ubiquinone which takes advantage of characteristic features of nuclear magnetic resonance (nmr) spectra in benzene and pyridine solutions has been found. The naturally occurring rhodoquinone-10 (2-amino-3-methoxy-5-decaprenyl-6-methyl-1,4-benzoquinone) and the two biosynthetic precursors, 2-hydroxy-3-methoxy-5-decaprenyl-6-methyl-1,4-benzoquinone and 2-methoxy-5-methyl-6-decaprenyl-1,4-benzoquinone, exhibit nmr spectra in carbon tetrachloride that are indistinguishable from those of their 2-amino-6-decaprenyl, 2-hydroxy-6-decaprenyl, and 2-methoxy-5-decaprenyl analogs. However, these three isomer pairs are readily distinguishable by their nmr spectra in either benzene or pyridine. The key differences in the benzene and pyridine spectra of such isomers occur in the chemical shifts of signals due to ring methylene and ring methyl resonances. These two signals in the spectra of rhodoquinone-10 are separated by 1.35 (benzene) and 1.34 ppm (pyridine) as compared with a separation of 1.17 ppm (benzene) for isorhodoquinone and no ring methyl signal in pyridine. The other two isomer pairs exhibit similar differences. The observed benzene and pyridine solvent shifts correlate with the direction of solvent-induced polarization of the quinone systems.

A facile and micro method for assignment of structure to compounds related to ubiquinone has been found. This method takes advantage of characteristic features of nuclear magnetic resonance spectra of compounds of this class in benzene and pyridine solutions.

The unambiguous assignment of structures to compounds of the ubiquinone (**1**) group in which one of the two methoxy groups has been replaced by another substituent (*e.g.*, amino in rhodoquinone (**2a**)) is of considerable importance due to the continuing discoveries of such new compounds in nature.[2-7] Several approaches have been used to determine the structures of such compounds;[5-8] however, no generally suitable method has been available for assigning such structures using the very small quantities (often <5 mg) of laboriously isolated new natural products which are initially accessible.

Numerous examples are now available of the effective use of nmr spectra of benzene and pyridine solutions for structural studies.[9] In the hope of developing a method for assignment of structure which does not involve chemical transformations, we have studied the nmr spectra of ubiquinone (**1**) and a series of related compounds[10] (**2**–**7**) in carbon tetrachloride, benzene, and pyridine solutions.

In carbon tetrachloride, the position isomer pairs **2** and **3**,[8,10] **4** and **5**,[8,10,11] and **6** and **7**[5,6,10] exhibit sufficiently similar nmr spectra (Tables II–IV) that a spectrum of one pure compound of a pair (*e.g.*, **2a**) cannot be distinguished from a spectrum of a mixture of the isomers (in this example, **2a** and **3a**). However, each of the compounds of a pair **2**–**7** (**a** or **b**) exhibits an nmr spectrum in either benzene or pyridine solution which is sufficiently different from that of its isomer to allow identification. Presumably, new isomer pairs in this series also could be differentiated by their nmr spectra in benzene and pyridine solutions.

Differentiation of Isomers. The differences in the spectra of isomeric multiprenylquinones in benzene and pyridine, which allow structural assignment, occur in the chemical shifts of signals due to the ring methyl and

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