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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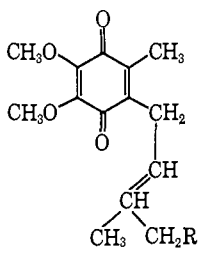
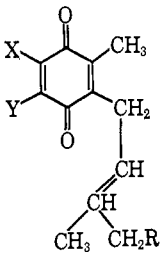
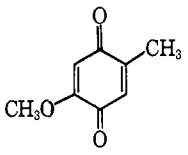




1a, R = —(CH$_2$CH=C(CH$_3$)CH$_2$)$_3$—H
b, R = —(CH$_2$CH$_2$C(CH$_3$)HCH$_2$)$_3$—H

2, X = NH$_2$; Y = OCH$_3$
3, X = OCH$_3$; Y = NH$_2$
4, X = OH; Y = OCH$_3$
5, X = OCH$_3$; Y = OH
6, X = H; Y = OCH$_3$
7, X = OCH$_3$; Y = H



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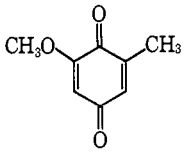

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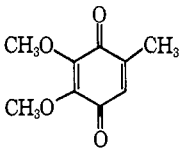

10

methylene substituents. The net effect is such that in benzene and pyridine solutions, substantial differences between isomers are observed in the separation (parts per million) of the ring methyl and methylene resonances (Table I).

Table I. Differentiation of Isomeric Multiprenyl Quinones by Separation of Ring Methylene and Ring Methyl Resonances

Compd	Separation, ppm		
	CCl_4	Benzene	Pyridine
2a	*a*	1.35	1.34
3a	*a*	1.17	*a*
4a	*a*	1.32	1.29
5a	*a*	1.20	*a*
6a	*a*	1.19	1.20
7a	*a*	1.31	1.26
2b	1.17	1.36	1.33
3b	1.10	1.17	1.16
4b	1.12	1.32	1.28
5b	1.13	1.20	1.20
6b	1.15	1.20	1.21
7b	1.12	1.32	1.27

[a] Ring methyl signal obscured by broad alkyl signal.

In the spectrum of rhodoquinone-10 (**2a**) in benzene, the ring methylene and methyl[12] resonances are separated by 1.35 ppm as compared with 1.17 ppm for isorhodoquinone-10 (**3a**). In pyridine solution, only **2a** exhibits an unobscured ring methyl signal (separated from the ring methylene signal by 1.34 ppm) and is readily differentiated from isomer **3a**.

Similar differences are observed for the isomeric hydroxyquinones **4** and **5**. The separation of the ring methylene and methyl resonances of **4a** in benzene is 1.32 ppm as compared with 1.20 ppm for **5a**. As for the isomeric aminoquinones, only one of the decaprenylhydroxyquinones (**4a**) exhibits an unobscured ring methyl signal in pyridine solution, which is separated from the methylene signal by 1.29 ppm.

Values for the separation of the ring methylene and methyl signals which allow for differentiation of the isomeric monomethoxyquinones are: **6a**, 1.19 (benzene) and 1.20 ppm (pyridine); **7a**, 1.31 (benzene) and 1.26 ppm (pyridine).

Included in Table I, in addition to data for quinones **2a–7a** which possess the decaprenyl side chain typical of natural quinones of the ubiquinone **1** group, are data for the analogous series of quinones (**2b–7b**) which possess the shorter, more nearly saturated phytyl side chain. The data for these two series closely correspond. The use of the phytylquinones **1b–7b** to locate ring methyl signals is of considerable advantage since in numerous spectra of decaprenylquinones (Tables II–V) the ring methyl signal is obscured by a broad signal due to allylic methylene protons of the decaprenyl side chain.[12] In several other spectra of decaprenylquinones, the ring methyl signal appears as a partially resolved band on the edge of the allylic methylene signals and could not be assigned with confidence without the benefit of comparison with spectra of the corresponding phytyl compounds in which the ring methyl signal is always clearly seen.

Effect of Multiprenyl Side Chain on Signals due to Ring Substituents. The obvious advantages of using phytyl analogs for the comparison of ring methyl and methylene signals (see above) made necessary an evaluation of the effect of changes in the isoprenoid side chain on the chemical shifts of signals due to ring substituents. In addition, the effect of the absence of an isoprenoid side chain on these signals was evaluated.

A. Decaprenyl and Phytyl Side Chains. Comparison of the chemical shifts of signals due to each ring substituent of decaprenylquinones **1a–7a** with the shifts due to corresponding ring substituents of phytylquinones **1b–7b** (*e.g.*, the methoxyl signal of **1a** as compared with the methoxyl signal of **1b** in a given solvent) shows that the average difference (52 comparisons) is 0.013 ppm. In two cases (see Tables IV and V), a difference of 0.04 ppm is noted. It seems likely that some of these observed differences may be attributed to concentration differences.

B. Nonmultiprenyl Quinones. Spectral data (Table VI) for three nonmultiprenyl quinones, 2-methoxy-5-methyl-1,4-benzoquinone (**8**), 2-methoxy-6-methyl-1,4-benzoquinone (**9**), and 2,3-dimethoxy-5-methyl-1,4-benzoquinone (**10**) were examined. The multiprenyl side chain has little effect on either the chemical shifts or solvent shifts of ring substituents (methoxyl or hydrogen) in position 2 or 3 (*i.e.*, *meta* or *para* to the position of the multiprenyl side chain) as shown by the near identity of the solvent shifts of these substituents for nonmultiprenyl quinones **8**, **9**, and **10** with those for the corresponding multiprenyl quinones **6**, **7**, and **1** (Table VII). In contrast, the effect on the behavior of the signal due to the *adjacent* ring methyl is substantial. For each of quinone pairs **8** and **6b**, **9** and **7b**, and **10** and **1b**, $\Delta_{CCl_4}^{benzene}$(methyl, nonmultiprenyl) − $\Delta_{CCl_4}^{benzene}$(methyl, multiprenyl) = +0.31 ppm. Similarly, $\Delta_{CCl_4}^{pyridine}$(methyl, nonmultiprenyl) − $\Delta_{CCl_4}^{pyridine}$(methyl, multiprenyl) yields values of +0.16, +0.16, and +0.15 ppm, respectively, for these three quinone pairs.

Solvent Shifts for Side-Chain Alkyl Proton Resonances. The protons of the isoprenoid side chains also exhibit selective solvent shifts in going from carbon tetrachloride to benzene[9d] or pyridine solutions. The

(12) The utility of benzene as a solvent for the purpose of observing the ring methyl signal has been reported: L. M. Jackman, I. B. O'Brien, G. B. Cox, and F. Gibson, *Biochim. Biophys. Acta*, **141**, 1 (1967).

Table II. Chemical Shifts (τ) for Rhodoquinone (2) and Isorhodoquinone (3) in Carbon Tetrachloride, Benzene, and Pyridine

Compd	Solvent	Type of proton: Olefinic	Amino	Methoxyl	Ring methylene[a]	Ring methyl	Alkyl
2a	CCl_4	5.00	5.52	6.18	6.92	*b*	8.05, 8.29, 8.36, 8.44
3a		5.00	5.56	6.18	6.95	*b*	8.05, 8.30, 8.37, 8.44
2b		5.16[c]	5.56	6.17	6.92	8.09	8.31, 8.82, 9.12, 9.18
3b		5.15[c]	5.48	6.18	6.96	8.06	8.32, 8.82, 9.12, 9.18
2a	Benzene	4.74	6.03	6.35	6.89	8.24	7.89, 8.34, 8.40, 8.46
3a		4.75	6.00	6.36	6.99	8.16	7.90, 8.35, 8.41, 8.46
2b		4.95[c]	6.05	6.36	6.89	8.25	8.33, 8.77, 9.08, 9.15
3b		4.98[c]	5.94	6.36	6.98	8.15	8.34, 8.76, 9.08, 9.14
2a	Pyridine	4.75	3.16	6.17	6.67	8.01	7.87, 8.21, 8.34, 8.40
3a		4.76	3.17	6.18	6.77	*b*	7.87, 8.22, 8.34, 8.40
2b		4.83[c]	3.18	6.19	6.69	8.02	8.23, 8.76, 9.11, 9.17
3b		4.85[c]	3.17	6.20	6.77	7.93	8.24, 8.76, 9.10, 9.17

[a] Doublet, $J \sim 7$ cps. [b] Ring methyl signal obscured by broad alkyl signal. [c] Triplet, $J \sim 7$ cps.

Table III. Chemical Shifts (τ) for 2-Hydroxy-3-methoxy-6-methyl-5-multiprenyl- (4) and 2-Hydroxy-3-methoxy-5-methyl-6-multiprenyl-1,4-benzoquinones (5) in Carbon Tetrachloride, Benzene, and Pyridine

Compd	Solvent	Type of proton: Olefinic	Methoxyl	Ring methylene[a]	Ring methyl	Alkyl
4a	CCl_4	4.99	6.06	6.89	*b*	8.05, 8.29, 8.37, 8.45
5a		4.99	6.08	6.90	*b*	8.06, 8.30, 8.38, 8.45
4b		5.18[c]	6.06	6.90	8.02	8.31, 8.82, 9.12, 9.18
5b		5.15[c]	6.09	6.90	8.03	8.32, 8.82, 9.12, 9.18
4a	Benzene	4.76	6.30	6.98	8.30	7.89, 8.34, 8.40, 8.46
5a		4.76	6.30	7.04	8.24	7.90, 8.35, 8.40, 8.46
4b		5.03[c]	6.32	6.98	8.30	8.36, 8.76, 9.08, 9.14
5b		5.03[c]	6.32	7.04	8.24	8.36, 8.74, 9.08, 9.14
4a	Pyridine	4.75	6.04	6.69	7.98	7.89, 8.22, 8.36, 8.41
5a		4.74	6.02	6.76	*b*	7.88, 8.22, 8.35, 8.41
4b		4.83[c]	6.04	6.69	7.97	8.22, 8.78, 9.11, 9.17
5b		4.83[c,d]	6.05	6.76	7.96	8.24, 8.77, 9.11, 9.17

[a] Doublet, $J \sim 7$ cps. [b] Ring methyl signal obscured by broad alkyl signal. [c] Triplet, $J \sim 7$ cps. [d] This spectrum was run at 80° (dia[l] temperature). No shifts were observed between spectra run at 34 and 80°.

Table IV. Chemical Shifts (τ) for 2-Methoxy-5-methyl-6-multiprenyl- (6) and 2-Methoxy-6-methyl-5-multiprenyl-1,4-benzoquinones (7) in Carbon Tetrachloride, Benzene, and Pyridine

Compd	Solvent	Type of proton: Ring proton	Olefinic	Methoxyl	Ring methylene[a]	Ring methyl	Alkyl
6a	CCl_4	4.31	4.99	6.29	6.90	*b*	8.05, 8.29, 8.36, 8.44
7a		4.31	4.99	6.29	6.89	*b*	8.05, 8.29, 8.37, 8.44
6b		4.31	5.16[c]	6.28	6.89	8.04	8.31, 8.82, 9.12, 9.18
7b		4.30	5.16[c]	6.29	6.91	8.03	8.31, 8.81, 9.12, 9.18
6a	Benzene	4.58	4.76	7.18	6.95	8.14	7.91, 8.36, 8.42, 8.45
7a		4.58	4.76	7.16	6.89	8.20	7.91, 8.36, 8.42, 8.46
6b		4.57	4.96[c]	7.16	6.92	8.12	8.36, 8.76, 9.08, 9.14
7b		4.56	4.93[c]	7.12	6.87	8.19	8.32, 8.76, 9.08, 9.16
6a	Pyridine	4.02	4.76	6.43	6.76	7.96	7.89, 8.24, 8.36, 8.42
7a		4.03	4.77	6.44	6.74	8.00	7.90, 8.23, 8.37, 8.42
6b		4.01	4.87[c]	6.42	6.73	7.94	8.23, 8.77, 9.10, 9.17
7b		4.01	4.85[c]	6.42	6.71	7.98	8.23, 8.78, 9.11, 9.17

[a] Doublet, $J \sim 7$ cps. [b] Ring methyl signal obscured by broad alkyl signal. [c] Triplet, $J \sim 7$ cps.

Table V. Chemical Shifts (τ) for Ubiquinone (1) in Carbon Tetrachloride, Benzene, and Pyridine

Compd	Solvent	Type of proton: Olefinic	Methoxyl	Ring methylene[a]	Ring methyl	Alkyl
1a	CCl_4	5.00	6.12	6.92	*b*	8.06, 8.29, 8.37, 8.45
1b		5.18[c]	6.16	6.94	8.08	8.32, 8.83, 9.12, 9.19
1a	Benzene	4.75	6.42	6.96	8.20	7.90, 8.34, 8.40, 8.46
1b		4.98[c]	6.43	6.94	8.20	8.34, 8.77, 9.08, 9.15
1a	Pyridine	4.77	6.16	6.80	8.03	7.90, 8.25, 8.37, 8.42
1b		4.91[c]	6.13	6.78	8.01	8.25, 8.77, 9.11, 9.17

[a] Doublet, $J \sim 7$ cps. [b] Ring methyl signal obscured by broad alkyl signal. [c] Triplet, $J \sim 7$ cps.

Table VI. Chemical Shifts (τ) for 2-Methoxy-5-methyl- (**8**), 2-Methoxy-6-methyl- (**9**), and 2,3-Dimethoxy-5-methyl-1,4-benzoquinone (**10**) in Carbon Tetrachloride, Benzene, and Pyridine

		Type of proton			
Compd	Solvent	5(6) proton	3 proton	Methoxyl	Methyl[a]
8	CCl_4	3.60[b]	4.26	6.26	8.02
9		3.61[c]	4.30[d]	6.26	8.00
10		3.74[b]		6.10, 6.12	8.05
8	Benzene	4.04[b]	4.63	7.19	8.41
9		3.95[c]	4.62[d]	7.17	8.47
10		4.14[b]		6.42, 6.47	8.48
8	Pyridine	3.51[b]	4.00	6.41	8.08
9		3.49[c]	4.02[d]	6.42	8.11
10		3.62[b]		6.10, 6.13	8.13

[a] Doublet, $J \sim 1.5$ cps. [b] Quartet, $J \sim 1.5$ cps. [c] Unresolved multiplet. [d] Doublet, $J \sim 2.5$ cps.

changes in the concentration of rhodoquinone-10 (**2a**) produced changes in the chemical shifts of ring substituents of <0.03 ppm. For this experiment, both carbon tetrachloride and benzene were used, and the concentrations chosen were in the range used throughout this study.

Correlation of Selective Solvent Effects with Structure. The concept is well developed that the differences often observed between nmr spectra that are obtained using "complexing" solvents such as benzene or pyridine from spectra obtained using "noncomplexing" or "inert" solvents such as carbon tetrachloride or cyclohexane are due to specific solvent–solute interactions in the case of complexing solvents which are absent when inert solvents are used.[13-15] Several empirical rules correlating such "nmr solvent shift" phenomena have

Table VII. Solvent Shifts ($\tau_{solvent} - \tau_{CCl_4}$) for Quinone Ring Substituents

	Benzene					Pyridine				
Compd	Ring proton	Methoxyl	Ring methylene Obsd	Ring methylene Cor[a]	Ring methyl	Ring proton	Methoxyl	Ring methylene Obsd	Ring methylene Cor[b]	Ring methyl
1a		+0.30	+0.04				+0.04	−0.12		
2a		+0.17	−0.03				−0.01	−0.25		
3a		+0.18	+0.04				0	−0.18		
4a		+0.24	+0.09				−0.02	−0.20		
5a		+0.22	+0.14				−0.06	−0.14		
6a	+0.27	+0.89	+0.05			−0.29	+0.14	−0.14		
7a	+0.27	+0.87	0			−0.28	+0.15	−0.15		
1b		+0.27	0	+0.12[a]	+0.12		−0.03	−0.16	−0.07[b]	−0.07
2b		+0.19	−0.03	+0.09	+0.16		+0.02	−0.23	−0.14	−0.07
3b		+0.18	+0.02	+0.14	+0.09		+0.02	−0.19	−0.10	−0.13
4b		+0.26	+0.08	+0.20	+0.28		−0.02	−0.21	−0.12	−0.05
5b		+0.23	+0.14	+0.26	+0.21		−0.04	−0.14	−0.05	−0.07
6b	+0.26	+0.88	+0.03	+0.15	+0.08	−0.30	+0.14	−0.16	−0.07	−0.10
7b	+0.26	+0.83	−0.04	+0.08	+0.16	−0.29	+0.13	−0.20	−0.11	−0.05
8	+0.37[c] / +0.44[d]	+0.93			+0.39	0.26[c] / −0.09[d]	+0.15			+0.06
9	+0.32[c] / +0.34[e]	+0.91			+0.47	−0.28[c] / −0.12[e]	+0.16			+0.11
10	+0.40	+0.32 / +0.35			+0.43	−0.12	0 / +0.01			+0.08

[a] $\Delta^{benzene}_{CCl_4}(cor) = \Delta^{benzene}_{CCl_4}(obsd) - (-0.12$ ppm); -0.12 ppm is the approximate value for the benzene solvent shifts for the ring methylene groups due to interaction of benzene with the side chain estimated using method B (see text). [b] $\Delta^{pyridine}_{CCl_4}(cor) = \Delta^{pyridine}_{CCl_4}(obsd) - (-0.09$ ppm); -0.09 ppm is the approximate value for the pyridine solvent shifts for the ring methylene groups due to interaction of pyridine with the side chain estimated using method B (see text). [c] 3 proton. [d] 6 proton. [e] 5 proton.

allylic methylene proton resonances of decaprenylquinones **1a–7a** occur 0.14–0.16 ppm toward lower field in benzene, and 0.15–0.18 ppm toward lower field in pyridine (as compared with carbon tetrachloride). The allylic methyl proton signals show positive benzene solvent shifts of 0–0.05 ppm, and negative pyridine solvent shifts of 0.01–0.07 ppm. Protons of the saturated portions of the phytyl side chains of compounds **1b–7b** show lesser shifts. The olefinic protons exhibit $\Delta^{benzene}_{CCl_4} = -0.12$ to -0.26 ppm and $\Delta^{pyridine}_{CCl_4} = -0.22$ to -0.35 ppm. Since the majority of the protons of the isoprenoid side chain are at considerable distance from the quinone ring, it seems clear that the solvent shifts observed for these groups must be due to interactions with solvent which are independent of those occurring with the quinone ring.

Concentration Effects. Concentrations of the samples used for spectral determinations varied from 2 to 5% w/v (see Experimental Section). A control experiment was performed to assess the importance of concentration effects on the shifts observed. Fivefold

been suggested which are of potential use in structural, stereochemical, and conformational problems.[13,15-18]

The solvent–solute interactions which give rise to these specific and highly selective effects on solute proton resonance signals are of the dipole-induced dipole type in which the "complexing" solvent (*e.g.*, benzene) is weakly ordered[14] about the positive end of the solute dipole. In general, the magnitude of the solvent shifts for benzene ($\tau_{benzene} - \tau_{CCl_4}$, $\Delta^{benzene}_{CCl_4}$, ppm) are greatest for protons spatially near (*i.e.*, shielded by) the transiently oriented benzene molecule(s) about the positive end of the solute dipole.[13] That is, in general, the magnitude of the solvent shift de-

(13) J. Ronayne and D. H. Williams, *J. Chem. Soc., B*, 540 (1967), and references cited there.
(14) R. C. Fort, Jr., and T. R. Lindstrom, *Tetrahedron*, **23**, 3227 (1967).
(15) D. H. Williams, J. Ronayne, H. W. Moore, and H. R. Shelden, *J. Org. Chem.*, **33**, 998 (1968).
(16) J. Seyden-Penne, P. Arnaud, J. L. Pierre, and M. Piat, *Tetrahedron Letters*, 3719 (1967).
(17) J. Ronayne and D. H. Williams, *J. Chem. Soc., B*, 805 (1967).
(18) D. H. Williams, *Tetrahedron Letters*, 2305 (1965).

creases with increasing distance of the proton from the positive end of the dipole.[13] The situation for pyridine solvent effects is less clear but appears to be similar.[18]

The quinone systems studied here are relatively complex; however, the observed differences in solvent shifts (Tables I and VII), which allow the differentiation of isomers, correlate well with the direction of solvent-induced polarization of the quinone systems which is predictable from resonance considerations (Figure 1). The relative magnitudes of the solvent shifts in benzene and pyridine for the ring methylene and methyl substituents of each of quinones **2**–**7** as compared with similar shifts for the corresponding isomers are in accord with predictions. For example, the methyl group of **2** is closer to the positive end of the induced dipole, and exhibits solvent shifts ($\Delta_{CCl_4}^{benzene} = +0.16$ and $\Delta_{CCl_4}^{pyridine} = -0.07$ for **2b**, Table VII) which are of greater positive value than those exhibited by the methyl proton resonance of **3** ($\Delta_{CCl_4}^{benzene} = +0.09$ and $\Delta_{CCl_4}^{pyridine} = -0.13$ for **3b**) in which the methyl group is nearer the negative end of the dipole. The situation for the ring methylene resonances of **2** and **3** is reversed. In this case, the ring methylene group of **2** is nearer the negative end of the dipole and the ring methylene group of **3** is nearer the center of positive charge. The solvent shifts reflect these relationships; solvent shifts for the ring methylene of **2b** are $\Delta_{CCl_4}^{benzene} = -0.03$ and $\Delta_{CCl_4}^{pyridine} = -0.23$ and for **3b** are $\Delta_{CCl_4}^{benzene} = +0.02$ and $\Delta_{CCl_4}^{pyridine} = -0.19$. The solvent shifts observed for the ring methyl and methylene substituents of isomers **4** and **5**, and **6** and **7** correlate equally well.

The concept of solvent-induced polarization of the quinones (Figure 1) also accounts for the absence of any significant difference in the methoxyl signals of isomeric quinones. The electronic environments of the methoxy groups of both isomers are apparently the same (compare polarized structures **2** and **3**, Figure 1). Similarly, the absence of separate methoxyl signals in the benzene and pyridine spectra of ubiquinone (**1**, **a** or **b**, Table V) is undoubtedly due to the nearly equal probability for polarization involving each of the carbonyls.

An estimation of the solvent shifts of the resonances for the ring methylene, which are due solely to interactions of solvent with the quinone ring, may be obtained by subtraction from the observed shift (which consists of a component due to solvent–quinone ring interactions and a component due to solvent–isoprenoid side-chain interactions) of the average solvent effect for all side-chain methylene protons (−0.15 ppm for benzene and −0.17 ppm for pyridine, Tables II–V).[19] This estimation (method A) of the separate components of the solvent shifts for the ring methylene is based on the assumption that solvation of the double bond adjacent to the quinone ring is as effective as that occurring at double bonds further removed from the ring moiety. If the steric nature of the solvated quinone ring interferes with effective solvent interaction with the adjacent double bond, the actual side-chain component of the solvent shift for the ring methylene would be smaller than estimated by method A. That this is likely to be the case is supported by the fact that estimation (method B) of the side-chain components of the solvent

(19) Ronayne and Williams[13] cite evidence that benzene interacts separately with isolated dipolar sites in a molecule producing additive effects on the chemical shift of a proton resonance

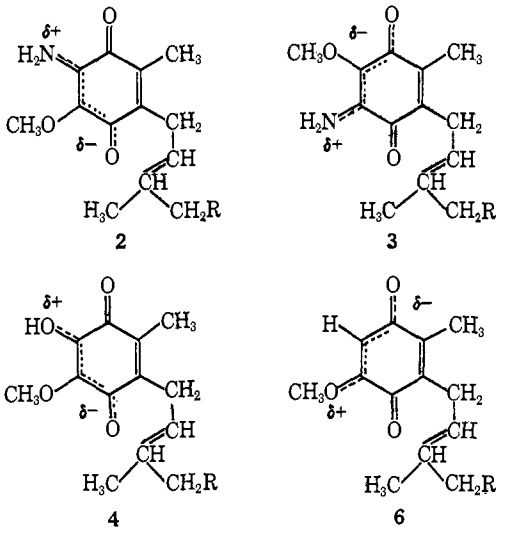

Figure 1. Direction of solvent-induced polarization of functionally substituted multiprenyl quinones.

shifts for the ring methylene using 2,3-dimethoxy-5-methyl-6-phytyl-1,4-benzoquinone (**1b**) as a model produces lower values. Since the contributions of dipolar structures involving each carbonyl are approximately equal for **1b**, the solvent shifts for the ring methyl and methylene resonances should be approximately equal. The differences in the observed benzene and pyridine solvent shifts for these resonances are measures of the effect on the ring methylene resonance of solvent interaction with the side chain. Method B gives values of −0.12 ppm for benzene and −0.09 for pyridine compared with −0.15 (benzene) and −0.17 (pyridine) obtained by method A (see above).

Using approximate values for the solvent shifts of the ring methylene resonances due only to solvent interaction with the quinone ring[19] it is possible to make two additional correlations. If the solvent shifts for the ring methylene and methyl substituents are due only to interaction of benzene with the quinone ring, the most effectively shielded group (methylene or methyl), *i.e.*, the group closest to the positive end of the dipole (Figure 1), would be expected to exhibit the greatest benzene solvent shift.[13] Similarly, one might expect that the magnitude of the solvent shift for a substituent (ring methylene or methyl) of a quinone (*e.g.*, the methyl of **2b**) would closely approximate the magnitude of the shift for the substituent of the isomeric quinone which is similarly located with respect to the direction of the dipole[13] (in this case, the ring methylene of **3b**). These correlations are observed for each of compounds **2b**–**7b** using values for benzene solvent shifts corrected by either of methods A or B. When the pyridine solvent shifts for ring methylene are corrected by subtraction of the average shift (−0.17 ppm) for the side-chain methylenes (method A) no useful correlations result. When values based on corrections for compound **1b** are used (method B), the correlations are very nearly as good as those observed for the benzene shifts. For this reason, the corrected values listed in Table VII were obtained by method B.

Implicit in the correlations which compare the magnitude of a solvent shift for a methylene group with

that of a methyl group, is the assumption that differences in substitution of a rotationally free methylene group, *i.e.*, $-CH_2H$ or $-CH_2R$, are not important in determining the magnitude of an nmr solvent shift. The excellent correlations obtained for this series of closely related quinones **2–7** suggest that the assumption is valid; however, the assumption is not necessarily valid for other systems.[20]

Experimental Section

Nmr Measurements. All measurements were carried out using a Varian Associates HA-100 nmr spectrometer with 2–5% w/v solutions at ambient temperatures unless otherwise indicated. Chemical shifts are expressed in τ units relative to tetramethylsilane as an internal standard.

Multiprenylquinones. The multiprenylquinones used for this study have been described.[10]

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(20) Note (ref 15) the significant difference in the benzene solvent shift for the methyl group of epoxypropene (+0.30) as compared with that for the exocyclic methylene of 1,2-epoxybutene (+0.22).

Synthesis of a Cyclic Disulfide-Linked Octapeptide Corresponding to Residues 65 to 72 of Bovine Pancreatic Ribonuclease A[1a]

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Abstract: This report describes the synthesis of the cyclic disulfide-linked octapeptide, cysteinyllysylasparaginylglycylglutaminylthreonylasparaginylcysteine, which corresponds to residues 65 to 72 in bovine pancreatic ribonuclease A. The protected tetrapeptides, tosyl-S-benzylcysteinyl-ε-*t*-BOC-lysylasparaginylglycine and glutaminylthreonylasparaginyl-S-benzylcysteine benzyl ester, were prepared by stepwise condensation of protected amino acids. These tetrapeptides were then coupled to give tosyl-S-benzylcysteinyl-ε-*t*-BOC-lysylasparaginylglycylglutaminylthreonylasparaginyl-S-benzylcysteine benzyl ester. The *t*-BOC group was removed by treatment with trifluoroacetic acid, and the remaining protecting groups were removed by sodium in liquid ammonia to give the linear octapeptide. The disulfide bridge was then allowed to form in dilute solution at pH 6.5.

The optical rotatory dispersion of cyclic peptides composed of ten amino acid residues has been found to be similar to that of peptides with the α-helix structure.[2,3] Since many proteins of known structure contain cyclic peptide moieties of approximately this size in the form of disulfide "loops," it is possible that such loops contribute to the over-all rotatory dispersion of the protein in such a way that the result is an overestimate of the per cent α helix in the molecule. For the purpose of estimating such a contribution in a protein of known structure, it seemed worthwhile to synthesize the loop peptide of bovine pancreatic ribonuclease A corresponding to residues 65 to 72.[4,5] This paper describes that synthesis.

The over-all scheme for the synthesis is outlined in Figures 1, 2, and 3. Carbobenzoxyasparaginylglycine ethyl ester (I)[6,7] was obtained in 78% yield from carbobenzoxyasparagine and glycine ethyl ester using the coupling reagent NEPIS[8a] according to the procedure of Woodward, *et al.*[7] No dehydration of the asparagine residue to β-cyanoalanine[8b,c] was detected by infrared spectroscopy. The carbobenzoxy group was removed by hydrogenolysis in the presence of palladium catalyst. The resultant dipeptide (II) was not isolated because it was found to be extremely hygroscopic but was treated directly with α-carbobenzoxy-ε-*t*-BOC-lysine N-hydroxysuccinimide ester. Schwyzer and Rittel[9] have prepared α-carbobenzoxy-ε-*t*-BOC-lysine in 25% over-all yield by acylation of the ε-amino group of lysine with *t*-BOC azide while protecting the α-amino group by means of a complex with copper. The copper was then removed and a carbobenzoxy group added to the α-amino group. In the present work, 48% over-all yield was obtained in a less tedious procedure by reversing the sequence of acylations. Thus, α-carbobenzoxylysine was prepared by the method of Bezas and Zervas[10] and was allowed

(1) (a) This work was supported by U. S. Public Health Service Research Grant 1-F2-GM-29,584-01. A portion of this material was presented by this author at the Biochemical Section of the 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966, Abstract C215. (b) Postdoctoral Research Fellow. Address correspondence to Mount Sinai School of Medicine, New York, N. Y. 10029.

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