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SYNTHESES AND BIOLOGICAL ACTIVITIES OF AZIDO UBIQUINONE DERIVATIVES

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SUMMARY--A general method for the synthesis of azido-ubiquinone derivatives has been developed directly by substituting one hydrogen atom on the benzoquinone ring with an azido group under weakly acidic conditions. The reaction takes several hours and the yield is generally low. The azido-ubiquinone was purified by preparative thin layer chromatography, and identified by NMR, IR and mass spectra. All the synthesized azido-ubiquinone derivatives show partial activity in mediating biological electron transfer in the dark, and show partial or complete inhibition upon photolysis.

The essential role of ubiquinone (Q) in mitochondrial and photosynthetic electron transfer systems has been well established (1-4). The reaction mechanism of ubiquinone-mediated redox reactions is, however, far from being understood (2,3). The low solubility of the naturally occurring ubiquinone (Q_6 or higher homologs) in aqueous solution and the low absorption of Q in the visible region have made the study of the reaction mechanism and the Q:protein interaction difficult. Low molecular weight synthetic Q analogs, especially those with a reporting group, have offered a promising approach to study the Q:protein interaction. Arylazido Q-derivatives have been successfully used for identification of the Q binding protein from the mitochondrial electron transfer chain (5). A more specific photoaffinity labeled Q-derivative, such as one with the azido group located on the benzoquinone ring, is needed in the study of Q:protein interaction and the identification of Q-binding proteins. Recently, we have developed a method to synthesize azido-Q derivatives by directly substituting one hydrogen atom on the benzoquinone ring with an azido group under weakly acidic conditions (6). It was found that if nonequivalent hydrogens are available on the benzoquinone ring for nucleophilic substitution, the azido group is preferentially substituted for the hydrogen with less steric hindrance. An adjacent electron-donating group was found to have a deactivating

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effect on the azidozation (7). For example, in the case of 2-methoxy-5-methyl-1,4-benzoquinone, which has two nonequivalent hydrogens available for substitution, the main product is 2-methoxy-5-methyl-6-azido-1,4- benzoquinone. NO monosubstitution reaction occurs if the 1,4-benzoquinone ring bears three hydrogens or two adjacent hydrogens. In this communication we will report the detailed synthetic procedures, the methods of identification and the relative biological activities of the synthesized azido ubiquninone derivatives.

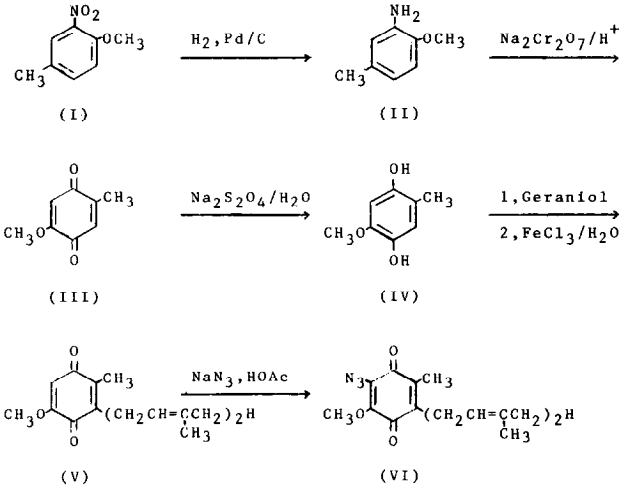
MATERIALS AND METHODS

The Q and phospholipid-depleted succinate-cytochrome c reductase used in determination of electron transfer activity of the synthesized azido ubiquinone derivatives was prepared according to the reported method (8). The enzyme was frozen at -80° in small aliquots in the presence of 20% glycerol, until use. The assay conditions were as described previously (9). All activities are expressed relative to that of 2,3-dimethoxy -5-methyl-6-(10-bromo-decyl)-1,4-benzoquinone ($Q_0C_{10}Br$), whose synthesis has been reported (10).

Cytochrome c, Type III, was obtained from Sigma Co. Starting materials for the syntheses of azido ubiquinone derivatives, 4-methoxy-3-nitrotoluene, 3-methoxy-2-nitrotoluene, 2,3-dimethoxyphenol and geraniol were products of Aldrich. Other chemicals used were obtained commercially at the highest purity available.

RESULTS AND DISCUSSIONS

Scheme I shows the synthetic pathway of 2-azido-3-methoxy-6-methyl-5-geranyl-1,4-benzoquinone (VI). 2-Methoxyl-5-methyl-1,4-benzoquinone (III) was prepared from 4-methoxy-3-nitrotoluene (I) through catalytic hydrogenation and



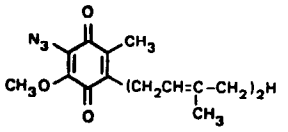
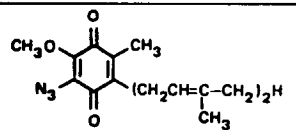
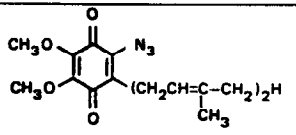
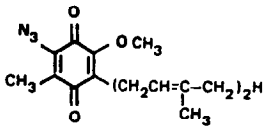
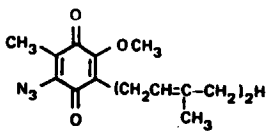
Scheme I. Synthesis of 2-azido-3-methoxy-6-methyl-5-geranyl-1,4-benzoquinone.

sodium dichromate oxidation under the conditions described previously (10-12). Compound III was purified from the oxidized mixture by methylene chloride extraction. The extract was stirred with an increasing amount of Florisil until the solution became yellow, and was then filtered. The filtrate was evaporated to dryness, yielding 3.3 g of yellowish needle crystals of III from 7.5 g of II. Compound III was identified by m.p 172-173°, ^{1}H NMR ($CDCl_3$) δ6.54 (q,1), 5.91 (s,1), 3.82 (s,3), 2.09 (d,3), and high-resolution mass spectra m/e 152.0421. Compound III (0.9 g) was reduced to 2-methoxy-5-methyl-1,4-hydrobenzoquinone (IV) by sodium hydrosulfite. The crystalline IV was alkylated to produce 0.31 g of 2-methoxy-5-methyl-6-geranyl- 1,4-benzoquinone (V) by the described method (13). Compound V has an R_f value of 0.21 in silica Gel-IB-F, developed by hexane and ether (3.5:1.0). Compound V, an orange oil, was identified by ^{1}H NMR ($CDCl_3$) δ5.87 (s,1), 4.96 (t,2), 3.78 (s,3), 3.20 (d,2), 2.02 (m,7), 1.65 (t,9), UV $\lambda_{max.(nm)}^{EtOH(95\%)}$ Oxid. 272, Red. 293, and high-resolution mass spectra, m/e 288.1742. The by-products of this alkylation reaction are 2-methyl-5-methoxy-6-geranyl-1,4-benzoquinone (15%) and 2-methyl-5-methoxy-3,6-digeranyl-1,4-benzoquinone (7%).

Compound V (25 mg) was dissolved in 1 ml of acetic acid (90%), and incubated at 75° with constant stirring. The subsequent operations were performed in the dark. Three additions of sodium azide (17 mg each in 0.05 ml of water) were made over a period of 3 hrs. The mixture was then stirred for 7 hrs, extracted with ether, concentrated, and chromatographed on silica Gel-H plates, developed with hexane and ether (3.5:1.0). 2-azido-3-methoxy-6-methyl-5-geranyl-1,4-benzoquinone (VI) has an R_f of 0.61 in this system and was eluted with ether. Upon removal of the ether, 0.5 mg of pure compound VI was obtained. The purity and structure of VI were confirmed by ^{1}H NMR ($CDCL_3$) δ 4.96 (t,2), 4.07 (s,3), 3.20 (d,2), 2.02 (m,7), 1.65 (t,9), UV $\lambda_{max.(nm)}^{EtOH(95\%)}$ Oxid. 290,252. Red. 304, and IR (KBr) 2900, 2120 (N_3), 1660, 1600, 1440, 1370, 1290, 1140 cm^{-1}.

Other azido-ubiquinone derivatives, such as 3-azido-2-methoxy-6-methyl-5-geranyl-1,4-benzoquinone (VII) and 6-azido-2,3-dimethoxy-5-geranyl-1,4-

Table 1. Structures and Properties of Azido-ubiqiunone derivatives.

Compound	Structural Formula	Spectrum data			Yield of final step (%)	Biological Activity % (Relative to $Q_0C_{10}Br$)
		H' NMR ($CDCl_3$)	UV λ^{EtOH}_{max}, (nm)	IR (cm^{-1})		
VI		δ 1.65(t,9), 2.02(m,7) 3.20(d,2), 4.07(s,3) 4.96(t,2)	Oxid. 290, 252 Red. 304	2900,2120($-N_3$), 1660,1600,1440, 1370,1290,1140	1.8	22
VII		δ 1.65(t,9), 2.04(m,7) 3.20(d,2), 4.08(s,3) 4.95(t,2)	Oxid. 291, 252 Red. 303	2900,2120($-N_3$), 1660,1600,1440, 1370,1290,1140	3.0	18
VIII		δ 1.70(t,9), 2.0(m,4) 3.14(d,2), 3.98(s,3) 4.14(s,3), 5.04(t,2)	Oxid. 315 Red. 305	2900,2120($-N_3$), 1640,1590,1440, 1340,1270,1130	13.0	39
IX		δ 1.66(t,9), 1.98(m,7) 3.16(d,2), 3.98(s,3) 5.04(t,2)	Oxid. 306 Red. 297	2900,2120($-N_3$), 1660,1590,1440, 1380,1290,1130, 920	10.8	12
X		δ 1.62(q,9), 1.96(s,3) 2.00(s,4), 3.16(d,2) 4.05(s,3), 5.08(t,2)	Oxid. 307 Red. 298	2900,2120($-N_3$), 1640,1590,1450, 1370,1290,1140, 920	11.0	9

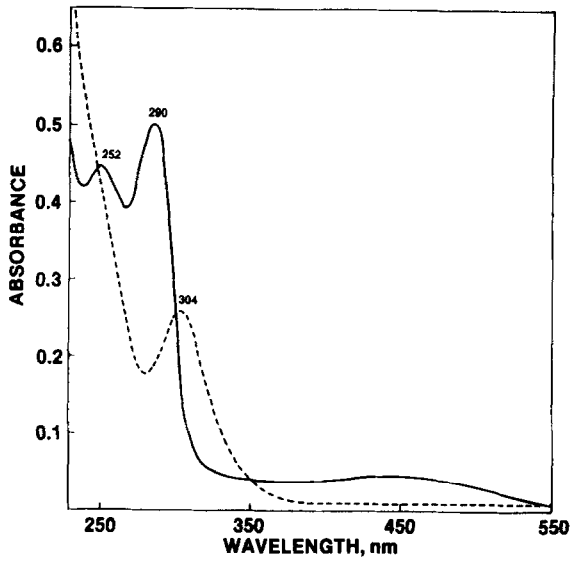


Figure 1. Absorption spectra of 2-azido-3-methoxy-6-methyl-5-geranyl-1,4-benzoquinone in ethanol. The solid (——) and the dotted (----) curves represent oxidized and reduced forms, respectively. The reduction was effected by the addition of a few grains of sodium borohydride.

benzoquinone (VIII) were also synthesized by a similar procedure, with 3-methoxy-2-nitrotoluene and 2,3-dimethoxyphenol as starting materials, respectively. The yield of VIII in the final step (10%) is much higher than that of VI or VII. Two isomers of azido-ubiquinone, 2-azido-3-methyl-6-methoxy-5- geranyl-1,4-benzoquinone (IX) and 3-azido-2-methyl-6- methoxy-5-geranyl-1,4-benzoquinone (X), have been synthesized by the same methods as VI and VII, respectively. The spectral characteristics, synthetic yields, and relative electron transferring activities of these azido ubiquinone derivatives are summarized in Table I.

Introduction of the azido group to the benzoquinone ring caused a red shift in the UV absorption peak. The absorption spectra of VI is shown in Figure 1. The oxidized form shows aborption peaks at 252 and 290 nm. Upon reduction by sodium borohydride, the 290 nm peak shifts to 304 nm with a concurrent decrease in intensity. Upon photolysis, the absorption peaks at 290 nm and 252nm decreased in proportion to the degree of photodecomposition (data not shown). All the synthetic azido-Q derivatives show partial biological electron transfer

activity. The order of effectiveness, as compared to $Q_0C_{10}Br$ (10), is as follows: VIII (39%), VI (22%), VII (18%), IX (12%), and X (9%). Upon photolysis, compounds VI and IX totally inactivated succinate-cytochrome c reductase activity. This inhibition is apparently due to the formation of a covalent bond between azido-Q and the protein at the active site (9).

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