SYNTHESIS OF TWO ANALOGUES OF UBIQUINONES AND THEIR ACTION ON MITOCHONDRIAL RESPIRATION

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With the aim of obtaining ubiquinone analogues, a fraction of 5-n-alkyl(C_{19}–C_{25})resoreinols isolated from Azotobacter chroococcum 92 cells was converted by the action of diazomethane into mono-O-methyl derivatives, which were oxidized with m-chloroperbenzoic acid. The main oxidation products were characterized by spectrophotometric methods as 2-alkyl-6-methoxy-1,4-benzoquinones and 3-alkyl-5-methoxy-1,2-benzoquinomes. Both types of product accelerated the respiration of mitochondria on succinate in a similar way to natural CoQ_{10}, but, in contrast to it, inhibited respiration on an NAD-dependent substrate.

The majority of published studies devoted to the synthesis of analogues of natural ubiquinones (CoQs) had the further aim of using these compounds as tools for elucidating the mechanisms of the redox reactions taking place in mitochondrial and bacterial cells with the participation of CoQs, and also for determining the role of individual structural elements of the CoQ molecule in the functioning of the latter [1-3]. Furthermore, since some synthetic analogues, just like their natural prototypes, proved to be effective drugs in various pathologies [4, 5], the task was set of finding new drugs of this series with definite therapeutic properties.

The synthesis of the analogues under consideration is usually based on the condensation of two compounds, one of which has the structure of the aliphatic moiety, and the other that of the cyclic fragment of the molecule of the expected final product [1, 3, 6]. In the present work we have studied the possibility of using for this purpose the readily available 5-alkylresorcinols with long carbon chains, including those of natural origin. In particular, 5-n-alkyl(C_{19}–C_{25})resorcinols (I) are the dominating lipids of dormant cells (cysts) of bacteria of the genus Azotobactor [7] and their isolation in the form of individual fractions poses no difficulties. For the investigation discussed below, we selected fraction (I) isolated from A. chroococcum 92 with the following composition of the homologues: n = 18 (30%), n = 20 (64%), n = 22 (4%), n = 24 (2%) [8].

By the action of diazomethane, the alkylresorcinols (I) were converted into a mixture of mono- and di-O-methyl derivatives (II and III), which, under the conditions described below (see the Experimental part), were formed in a ratio of ~2:1. The mono-O-methyl derivatives (II) were isolated by chromatography on a silica gel column and were oxidized with m-chloroperbenzoic acid using the method of Asakawa et al. [9] modified for application to the task in hand. Oxidation led to a mixture of at least six products, among which compounds (IV) and (V), the most mobile in TLC, predominated. Filtering the mixture through alumina permitted the practically complete elimination of more polar components, after which the above-mentioned main reaction products were separated and purified to the state of individual fractions by chromatography on a column of silica gel.

The yields of the purified fractions (IV) and (V) were 47 and 29%, respectively. Their chemical natures were established by spectrometric methods. In the interpretation of the NMR and UV spectra use was made of published information on the spectroscopy of natural 2-(3',7'-dimethylocta-2',6'-dienyl)-6-methoxy-1,4-benzoquinone (VI) [10] and synthetic 2-decyl-6-methoxy-1,4-benzoquinone (VII) [3].

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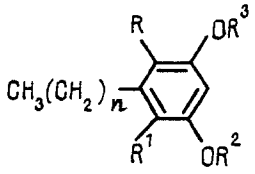
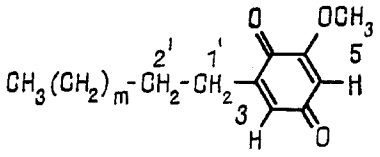
TABLE 1. PMR Spectra of Quinones (IV) and (V) and of the Previously Known Compounds (VI) [10] and (VII) [3]

Protons (see formula)	No. of H's	δ, ppm (structure of the signal; J, Hz) in the spectra of the quinones			
		IV	V	VI	VII
H-3	1	6,46 (m)		6,43 (dt; 2,8, 2,2)	6,50 (d)
H-4	1		6,54 (m)		
H-5	1	5,86(d; 2,8)		5,86 (d; 2,8)	5,83 (d)
H-6	1		5,68 (d; 2,7		
O—CH_3	3	3,80 (s)	3,81 (s)	3,80 (s)	3,81 (s)
H-1′	2	2,38 (t; 6,5)	2,36 (t; 6,5)		2,25 (t)
H-2′	2	1,48 (m)	1,46 (m)		
$(CH_2)_m$		1,26 (m)	1,26 (m)		1,26 (m)
C—CH_3	3	0,86 t; 6,5)	0,86 (t, 6,5)		0,88 (t)

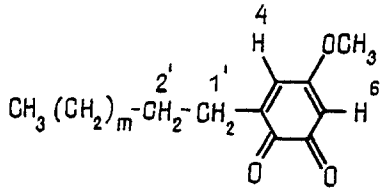


n = 18, 20, 22, 24
(see text)

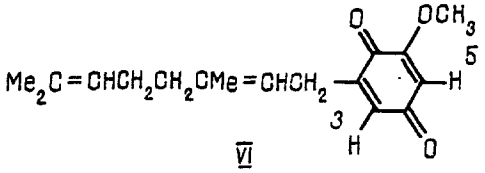
I. $R = R^1 = R^2 = R^3 = H$
II. $R = R^1 = R^2 = H$, $R^3 = CH_3$
III. $R = R^1 = H$, $R^2 = R^3 = CH_3$
VIII. $R = OH$, $R^1 = R^2 = H$, $R^3 = CH_3$
IX. $R = R^2 = H$, $R^1 = OH$, $R^3 = CH_3$



IV. m = 16, 18, 20, 22
VII. m = 7



V. m = 16, 18, 20, 22



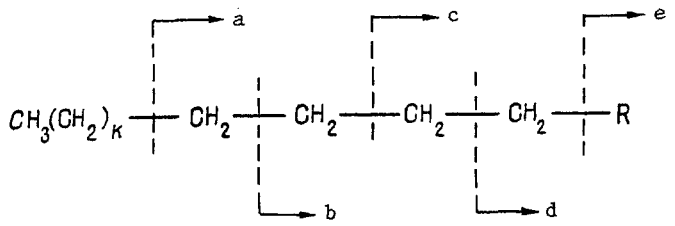
VI

In the UV spectrum of (IV) the selective absorption at 264 nm (ε 13000)* and 350 nm (ε 420) corresponding to the allowed and forbidden $\pi \rightarrow \pi^*$ transitions [11] that is characteristic for substituted 1,4-benzoquinones (see [3, 10]) was observed. For fraction (V) we recorded absorption maxima at 266 nm (ε 2900) and 385 nm (ε 170) with considerably lower molar extinctions, which is a distinguishing feature of 1,2-benzoquinones [11]. The UV spectra of the 1,4- and 1,2-hydroquinones (VIII) and (IX) formed as the result of the reduction of the quinone fractions under consideration each contained a single maximum in the 220-320 nm region, at 288 nm (ε 2800) (see [3]).

In the IR spectra of the synthesized substances (IV) and (V) there were no bands of hydroxy groups. The strongest bands of the spectra of the 1,4-quinones (IV) related to the stretching vibrations of the C—H bonds in the aliphatic chain (2955, 2916, and 2851 cm^{-1}), of the C=O bonds of keto groups conjugated with C=C bonds (1684 and 1653 cm^{-1},) of C=C bonds

*In the calculation of ε the "mean molecular mass" of the mixture of homologues was used, with the assumption that the homologous composition of the alkylresorcinol fraction (I) did not change during its transformations.

TABLE 2. Main Peaks in the Mass Spectra of the Fractions of Mono-O-methyl Derivatives of 5-Alkylresorcinols (II), the 1,4-Quinones (IV), the 1,2-Quinones (V), the 1,4-Hydroquinones (VIII), and the 1,2-Hydroquinones (IX)



Type of ion	m/z (I_{rel}, %) in the spectrum of the fractions				
	II	IV	V	VIII	IX
M^+	418 (39)	432 (52)	432 (14)	434 (32)	434 (32)
M^+	390 (21)	404 (23)	404 (8)	406 (17)	406 (17)
a+H	180 (17)	—	—	—	—
	179 (15)	193 (21)	193 (6)	195 (12)	195 (4)
a—H	—	192 (23)	—	194 (15)	194 (4)
b	165 (7)	179 (37)	179 (14)	181 (12)	181 (11)
c	151 (43)	165 (32)	165 (32)	167 (24)	167 (22)
d+H	138 (100)	152 (100)	152 (73)	154 (100)	154 (81)
d	137 (90)	151 (97)	151 (100)	154 (82)	153 (100)
e	123 (12)	137 (32)	137 (38)	139 (25)	139 (34)

"—" – intensity less than 4%.

conjugated with keto groups (1598 cm^{-1}), and of the C–O ether bond at an unsaturated carbon atom (1234 cm^{-1}). The IR spectra of the 1,2-quinones (V) differed only slightly: ν_{max} (cm^{-1}) 2955, 2917, 2850 (C–H); 1689, 1646 (C=O); 1585 (C=C); and 1221 (C–O). Details of the PMR spectra of quinones (IV) and (V) are given in Table 1, which also gives the signals from some of the protons in the spectra of compounds (VI) and (VII).

The structures of the oxidation products (IV) and (V) were shown definitively with the aid of electron-impact ionization spectrometry (see scheme in Table 2). The mass spectra contained the peaks of the molecular ions of the dominating homologues of the quinone fractions, and in the spectra of the 1,2-quinones the intensities of these peaks were substantially lower. The main directions of fragmentation of the molecular ions of the 1,2- and 1,4-quinones coincided. They did not differ from the main directions of fragmentation of the 5-alkylresorcinols and their derivatives [8] [including the mono-O-methyl derivatives (II)] or from those of the reduction products – the 1,4- and 1,2-hydroquinones (VIII) and (IX) (see Scheme in Table 2). Thus, on the oxidation of 3-alkyl-5-methoxy phenols (II) with m-chloroperbenzoic acid the main products formed are 2-alkyl-6-methoxy-1,4-benzoquinones (IV) and 3-alkyl-5-methoxy-1,2-benzoquinones (V). No isomeric o-quinones – 4-alkyl-6-methoxy-1,2-benzoquinones – were detected among the reaction products.

A comparative study of the influence of the CoQ analogue synthesized and of natural CoQ_{10} on the rate of respiration of rat liver mitochondria on succinate and on an NAD-dependent substrate (equimolar mixture of pyruvate and malate) was studied. In the case of undecoupled respiration, as was to be expected, the rate of oxidation (V_4) of both substrates increased somewhat after the addition of CoQ_{10} to a suspension of mitochondria. The maximum increase (40-50%) was recorded on the addition of 110 μg of CoQ_{10} (here and below the amount of preparation per 1 mg of mitochondrial protein is given), and with a further rise in its concentration in the incubation medium V_4 scarcely changed.

The action of the analogues (IV) and (V) had a somewhat more complex nature. A successive increase in their amount in the suspension on the use of the NAD-dependent substrate first caused a rise in V_4 by 20-100% and then an inhibition of respiration. The maximum values of V_4 were reached with the addition of 100 μg of the 1,4-quinones (IV) or 200 μg of the 1,2-quinones (V). A fall in V_4 to 50% of the initial value took place in the presence of 250 and 350 μg of quinones (IV) and (V), respectively. When respiration was decoupled by

2,4-dinitrophenol, both synthetic analogs inhibited the oxidation of the substrate more actively, and in this case the rate fell smoothly with a rise in the concentration of the quinones in the medium. A twofold decrease in the rate was recorded on the addition of 75-125 μg of either analogue, the 1,4-quinones (IV) exhibiting a somewhat greater activity. The quinones synthesized accelerated mitochondrial respiration on succinate. The addition of 70 μg of the 1,4-quinones (IV) led to a twofold increase in V_4, i.e., in this respect they proved to be more active than CoQ_{10}, while the activity of the 1,2-quinones (V) was lower than that of the latter.

It must be mentioned that the effects of the synthetic preparations and of CoQ_{10} that have been described developed in time and reached their maximum values (those given above) 10-15 min after the addition of the quinones to the mitochondrial suspension. This period is probably determined by the rate of diffusion of the preparations through the mitochondrial membrane.

With respect to their type of action on mitochondrial respiration, quinones (IV) and (V) can be assigned to the series of inhibitors of the rotenone type [12]. The 1,4-quinones (IV) have a definite structural similarity to the natural CoQs and, most probably, are capable of competing with the endogenous CoQ of mitochondria for binding with the active center of NADH-dehydrogenase, like other known analogues [4, 5]. The 1,2-quinones (V) differ substantially from CoQ and therefore it is still difficult to propose any substantiated explanation whatever of their action. No appreciable influence of preparations (IV) and (V) on the respiratory control of mitochondria was detected when succinate was used, and in the presence of pyruvate with malate only a slight lowering of it was observed. Thus, these analogues, while modifying the flow of electrons along the transport chain, have practically no influence on the rate of synthesis of ATP.

EXPERIMENTAL

For column chromatography we used silica gel L 100/160 (Lachema, Czechoslovakia), which had first been washed with 10 volumes of MeOH, with MeOH–$CHCl_3$ (1:1; here and below the volume ratios of solvents in mixtures of them are given) and $CHCl_3$ by stirring with the solvent for 30 min, followed by filtration. The sorbent was dried at 20°C for 24 h and at 105°C for 12 h. TLC was conducted on plates (10 × 10 cm) coated with silica gel G60 (Merck) in ether–hexane (1:20) (system 1), (1:1) (2), and (2:1) (3). The substances were revealed on the chromatograms with a 7% solution of molybdophosphoric acid (100°C, 10-15 min), after which, for the specific detection of phenols we used Pauly's reagent and $FeCl_3$–$K_3[Fe(CN)_6]$ [13].

UV spectra were recorded on a Hitachi model 220A spectrophotometer in hexane, UV spectra on a Perkin–Elmer model 1710 FTIR spectrograph using films of the substances, and PMR spectra on a Bruker CXP-200 instrument in $CDCl_3$ with HMDS as internal standard. Mass spectra were obtained on a Varian MAT 44 mass spectrometer at an energy of the ionizing electrons of 70 eV.

O-Methylation of the 5-Alkylresorcinols (I). At 20°C, 15 ml of a 0.7 M solution of CH_2N_2 in ether was added to 1.0 g of an individual fraction of 5-alkylresorcinols (I) isolated from A. chroococcum 92 cells by a method described previously [8]. The mixture was left to stand at the same temperature for 5 h, after which it was evaporated to dryness. The residue was dissolved in 15 ml of hexane–ether (50:1), and the solution was deposited on a column filled with 45 g of silica gel in hexane. Elution was conducted with hexane–ether mixtures having a linear increase in the concentration of the latter from 2 to 20% (a total of 620 ml of eluent). The eluate was collected in 10-ml fractions, which were analyzed with the aid of TLC in systems 1-3. The solvent mixtures containing from 2.5 to 3.5% of ether eluted 262 mg of the di-O-methyl derivatives (III); R_f 0.75 (system 1); these did not differ with their UV spectrum, mass spectrum, and TLC mobility from an authentic sample [8]. Mixtures containing from 4.5 to 6.0% of ether eluted 539 mg of the mono-O-methyl derivatives (II); R_f 0.7 (system 3); UV spectrum: λ_{max} nm: 274, 279 (ε 1180, 1210); IR spectrum: ν_{max} cm^{-1} 3328 (H–O), 1628 and 1600 (C=C arom.), 1204 (C–O of a phenol); for the mass spectrum, see Table 2. Mixtures containing from 16 to 20% of ether eluted 182 mg of the initial 5-alkylresorcinols (I); R_f 0.3 (system 3).

Oxidation of the Mono-O-methyl Derivatives (II). With stirring, at 20°C, a solution of 520 mg of m-chloroperbenzoic acid (70%; Aldrich Chemical Co.) in 20 ml of $CHCl_3$ was added over 20 min to a solution of 200 mg of the mono-O-methyl derivatives (II) in 5 ml of $CHCl_3$.

The mixture was left to stand at the same temperature for 4 h, after which it was filtered through a column containing 10 g of alumina (neutral, activity grade II, Reanal) in $CHCl_3$. The column was additionally washed with 50 ml of $CHCl_3$, and the combined filtrate was evaporated to dryness. The residue was dissolved in 5 ml of hexane–ether (15:1), and the solution was deposited on a column filled with 15 g of silica gel in hexane. Elution was carried out with hexane–ether in ratios of (15:1), (12:1), and (10:1) (250 ml each). The eluate was collected in 15-ml fractions, and these were analyzed with the aid of TLC in system 2.* The fractions collected on elution by the second solvent mixture yielded 94 mg of the 1,4-benzoquinones (IV); R_f 0.6 (system 2); and the fractions eluted by hexane–ether (10:1) yielded 58 mg of the 1,2-quinones (V); R_f 0.4 (system 2).

Reduction of the Quinones (IV) and (V). At 20°C, 2 mg of $NaBH_4$ was added to a vigorously stirred mixture of 1 ml of water and a solution of 5 mg of one of the quinones (IV) or (V) in 5 ml of ether. Stirring was continued for 30 min, after which 3% hydrochloric acid was added to the mixture until the aqueous phase had acquired a pH of ~1. The organic phase was separated off and was washed with water (2 × 1 ml) and evaporated to dryness. The boric acid was eliminated from the residue by distillation with MeOH. This gave about 5 mg of the hydroquinone (VIII) or (IX); R_f 0.55 and 0.45 (system 2), respectively.

Measurement of Mitochondrial Respiration. The methods for the isolation and characterization of mitochondria and for recording their respiration described previously [14] were used. CoQ_{10} (Sigma Chemical Co.) and the quinones (IV) and (V) that had been synthesized were introduced into the incubation medium in the form of aqueous suspensions that had been treated with ultrasound or of solutions in 99.5% EtOH (10 mg/ml of suspension or solution).

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*The first system of solvents eluted a mixture of substances (in different experiments, from 1.5 to 2.5% of the sum of the reaction products), which, judging from its UV and IR spectra, were not quinones but, presumably, arose in the process of filtering the reaction mixture through Al_2O_3.