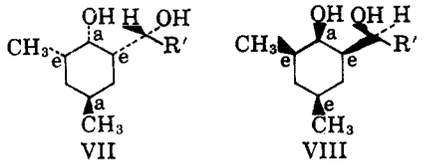
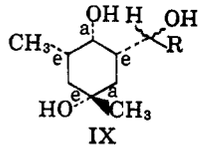
corresponding dihydrocycloheximide[6] VII [peaks at 246 (broad) and 233 cps (sharp)] and dihydro-α-epiisocycloheximide[7] VIII [peaks at 249 (broad) and 235



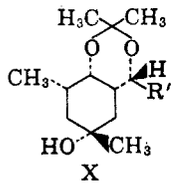
VII VIII

cps (sharp)]. Thus dihydrostreptovitacin A can be written as IX. The side chain hydroxyl orientation



IX

can now be determined by finding whether IX gives an acetonide or not, under mild conditions. Facile acetonide formation would indicate that the side chain hydroxyl group has the (*R*) configuration whereas no, or a very slow, reaction would point to the (*S*) configuration. Detailed arguments in support of this statement have been presented in earlier papers and will not be repeated here. It suffices to say that IX gave a noncrystalline product in almost quantitative yield, whose elemental analysis and spectral data left little doubt that it is the desired acetonide X. Its nmr



X

spectrum in deuteriochloroform for instance shows strong absorption at 83.0 and 84.5 cps characteristic of acetonide–methyl protons[7] whereas the characteristic broad and sharp peaks due to the CHO protons of the acetonide ring appear at 238 and 226 cps. These values again may be compared with those found for the acetonide of VIII[7] which are, respectively, 81.8, 83.9, 237 (broad), and 226 (sharp).

Attempts were also made to obtain the isomer of IX having an equatorial hydroxyl group at C-1 of the cyclohexane ring. If the stereochemistry assigned to X is correct, the isomeric triol should not easily form an acetonide. These efforts however were fruitless. Although this type of reduction could be accomplished successfully in the cycloheximide series using lithium tri-*t*-butoxyaluminum hydride, in the case of I only aluminum-containing complexes could be isolated. All attempts to remove the metal without destroying the molecule failed.

The new information then, while not unequivocal points to Ia as the complete structure for streptovitacin-A (R = H) and E-73 (R = Ac). Virtually the only glutarimide antibiotics whose stereochemistry is not known, are the isomeric streptovitacins-B, -C, and -D. Sufficient quantities of these have not been available for study as yet but it would not be surprising if they also had the same basic stereochemistry as cycloheximide.

Experimental Section

Nmr spectra were measured using a Varian A-60 instrument, and infrared spectra were obtained from a Baird Model 4-55 recording spectrophotometer. Melting points are corrected. ORD spectra were measured using a Bendix polarmatic recording spectropolarimeter Model 4600.

ORD determinations were (a) streptovitacin-A (chloroform, *c* 0.300) $[\alpha]_{400}$ −23°, $[\alpha]_{384.5}$ −32°, $[\alpha]_{370}$ −42°, $[\alpha]_{357}$ −55°, $[\alpha]_{345}$ −70°, $[\alpha]_{333}$ −95°, $[\alpha]_{322.5}$ −157°, $[\alpha]_{317.5}$ −200°, $[\alpha]_{361}$ −206°, $[\alpha]_{312.5}$ −202°, $[\alpha]_{303}$ −162°, $[\alpha]_{294}$ −107°, $[\alpha]_{289.5}$ 0°, $[\alpha]_{285.5}$ +88°, $[\alpha]_{278}$ +568°, $[\alpha]_{270}$ +674°, $[\alpha]_{263}$ +1015°; (b) cycloheximide (chloroform, *c* 0.300) $[\alpha]_{400}$ −20°, $[\alpha]_{384.5}$ −26°, $[\alpha]_{370}$ −37°, $[\alpha]_{357}$ −50°, $[\alpha]_{345}$ −75°, $[\alpha]_{333}$ −110°, $[\alpha]_{322.5}$ −168°, $[\alpha]_{312.5}$ −302°, $[\alpha]_{303}$ −250°, $[\alpha]_{294}$ −55°, $[\alpha]_{293}$ 0°, $[\alpha]_{285.5}$ +290°, $[\alpha]_{278}$ +750°, $[\alpha]_{263}$ +900°; (c) isocycloheximide (chloroform, *c* 0.304) $[\alpha]_{400}$ +53°, $[\alpha]_{384.5}$ +54°, $[\alpha]_{370}$ +56°, $[\alpha]_{357}$ +64°, $[\alpha]_{345}$ +73°, $[\alpha]_{333}$ +97°, $[\alpha]_{322.5}$ +140°, $[\alpha]_{312.5}$ +104°, $[\alpha]_{303}$ +72°, $[\alpha]_{294}$ +40°, $[\alpha]_{285.5}$ 0°, $[\alpha]_{278}$ −78°, $[\alpha]_{263}$ −134°.

Dihydrostreptovitacin-A (IX).—Steptovitacin-A (0.5 g) in acetic acid (25 ml) was stirred at room temperature in the presence of a platinum catalyst (from 0.2 g of PtO_2) under hydrogen at atmospheric pressure. After 2.5 hr, gas absorption (86 ml, 95% of theory) ceased. The catalyst was removed and the solution evaporated to dryness under reduced pressure. The resulting glassy residue was then crystallized from ethyl acetate–petroleum ether (bp 30–60°) to give IX as white needles (0.41 g, 82%), mp 183–184.5°. A sample recrystallized from the same solvent pair had mp 187–187.5°. Its infrared spectrum taken as a Nujol mull showed significant peaks at 2.80, 2.95, 3.01, 3.04, 5.76, and 5.83 μ.

Anal. Calcd for $C_{15}H_{25}NO_5$: C, 60.18; H, 8.42; N, 4.68. Found: C, 60.15; H, 8.38; N, 4.63.

Acetonide of Dihydrostreptovitacin-A (X).—A solution of dihydrostreptovitacin-A (0.2 g) in acetone (20 ml) was refluxed in the presence of anhydrous copper sulfate (2.0 g) for 12 hr. Removal of the copper sulfate and excess acetone in the usual way afforded a colorless glassy residue which refused to crystallize. A sample prepared for analysis became mobile at 75–77°. Its infrared spectrum (KBr disk) showed peaks at 2.92 (OH), 3.12, 3.25 (NH), 5.88 (imide C=O), 7.25, 7.90, 8.30, 8.56, 8.70, 9.26, 9.75, 10.05, 10.25, 10.90, and 11.65 μ.

Anal. Calcd for $C_{18}H_{29}NO_5$: C, 63.69; H, 8.61; N, 4.13. Found: C, 63.45; H, 8.61; N, 4.10.

Registry No.—Ia (R = H), 523-86-4; Ia (R = Ac), 2885-39-4; *l*-II, 4630-76-6; III, 15314-11-1; IX, 15303-44-3; X, 15303-45-4.

Acknowledgment.—The authors wish to thank Dr. C. Smith of the Upjohn Co., who provided a generous sample of streptovitacin-A, and Dr. K. V. Rao of the John L. Smith Memorial for Cancer Research, for a sample of E-73. Special thanks are due to Professor J. Wolinsky and Mrs. H. Hauer of Purdue University who measured the ORD spectra.

Mass Spectrometry of Ubiquinones. Thermal Loss of a Methoxyl Group[1]

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In the study of the chemistry of the ubiquinones, Q-*n* (I), and ubiquinols H_2Q-*n* (II), we have critically examined the mass spectra of numerous samples from both natural and synthetic sources. In the mass spectra of samples of Q-10 (I, *n* = 10) and Q-9 (I, *n* = 9) of

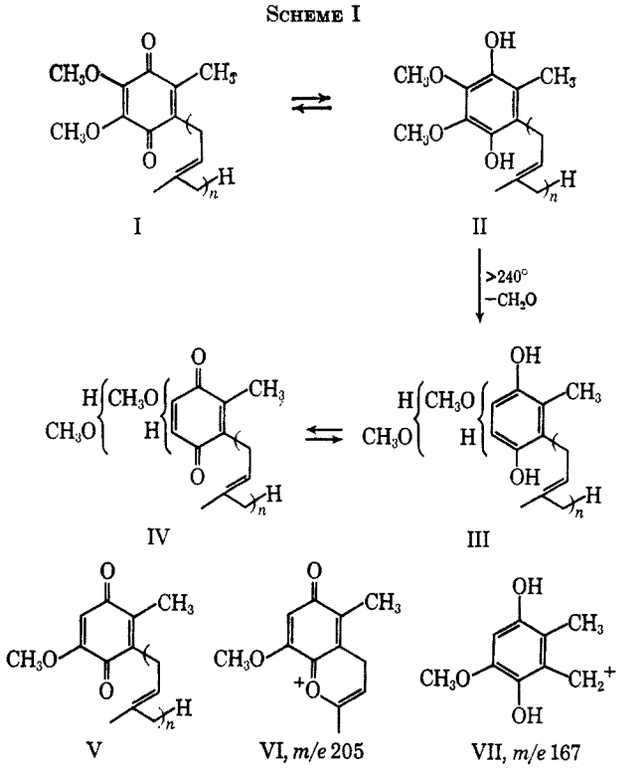
(1) Coenzyme Q. XCIV.

natural origin, prominent peaks were observed at *m/e* values which are 30 mass units lower than peaks due to the parent ions. These peaks (M − 30), which appear as typical quinone and hydroquinone parent ions,[2] are coupled with intense peaks at *m/e* 205 (ion VI) and 167 (ion VII) and correspond to a monomethoxymethylmultiprenylquinone (IV), *i.e.*, a demethoxyubiquinone. While many methoxy-containing compounds readily lose formaldehyde upon electron bombardment,[3] the absence of prominent M − 30 peaks in the mass spectra of Q-1, -2, and -3 (synthetic) and in the spectra of Q-5, -7, and -8 (isolated)[2] indicates that formaldehyde expulsion is not an important process in the mass spectrometric breakdown of the ubiquinones.

Two possible explanations for the peaks at M − 30 in the mass spectra of Q-10 and Q-9 seemed plausible: (a) these peaks are due to the presence of a monomethoxyquinone in the isolated samples implying the presence in the source material of this biosynthetic precursor[4] (V) to ubiquinone or (b) the monomethoxy species are formed in the mass spectrometer prior to electron impact.

To elucidate the origin of the peaks at M − 30, the mass spectrum of highly purified synthetic Q-9 (I, n = 9) was obtained. Prominent peaks were observed at *m/e* 764 (M − 30) and at 205 and 167 corresponding to the presence of monomethoxyquinones. This result ruled out the possibility that monomethoxyquinone (V) was present in the samples and established that monomethoxyquinones (IV) are formed during the process of obtaining the mass spectrum (Scheme I).

SCHEME I



The absence of M − 30 peaks in the spectra of the lower molecular weight Q's indicated that methoxyl loss probably was brought about thermally[5] in the inlet system at the elevated temperatures required for the higher molecular weight homologs. Spectra of Q's-1 to -8 were obtained using probe temperatures of less than 200°, while normally spectra of Q-9 and Q-10 are obtained using probe temperatures higher than 275°.[2] In several instances, where very limited samples of the higher Q's were available, probe temperatures from 350–400° have been used. Synthetic Q-9 was placed in the mass spectrometer probe at a temperature of 125° and the region from mass 750 to 800 was scanned at intervals as the temperature was increased. The data in Table I show that at 223°, parent ion peaks at *m/e* 766

TABLE I

RELATIVE INTENSITIES OF PARENT IONS OF UBIQUINONE-9 AND DEMETHOXYUBIQUINONE-9 AS A FUNCTION OF PROBE TEMPERATURES

Probe temp, °C	M⁺ peak intensities[a]: I (*m/e* 794) + II (*m/e* 796)	III (*m/e* 766) + IV (*m/e* 764)	(III + IV)/(I + II)
	Q-9 (I, n = 9)		
223	3.6	0	0
240	9.2	5.6	0.61
250	7.7	4.4	0.59
268	26.6	14.6	0.55
280	31.6	14.0	0.45
300[b]	37.6	15.8	0.42
	H_2Q-9 (II, n = 9)		
300	6.4	11.5	1.80
320	11.0	17.5	1.59
330	36.0	57.0	1.58
350	47.2	75.2	1.59

[a] Arbitrary units. [b] In another series of Q-9 spectra the ratio (III + IV)/(I + II) remained 0.41–0.42 over a temperature range of 300–380°.

and 764 due to the monomethoxy species, III and IV, were not observed. However, spectra obtained at temperatures higher than 240° contained prominent peaks at *m/e* 764 and 766, establishing that the process I (II) → III (IV) is brought about thermally.

Similarly, mass spectra of H_2Q-9 were obtained at various temperatures and a comparison was made of the relative intensities of the parent ion peaks of the monomethoxy species III and IV and the parent ion peaks of Q-9 and H_2Q-9 from each of the spectra obtained (Table I). The fourfold differences in these ratios (III + IV/I + II) observed in H_2Q-9 spectra (1.80–1.58) from the same ratio calculated using Q-9 spectra (0.61–0.42) strongly suggests that the hydroquinone (II) rather than the quinone (I) is the species from which a methoxyl is lost.[5] Additional support for this interpretation was provided by experiments in which Q-9 and H_2Q-9 were heated at 285–320° under reduced pressure (<1 mm) or in sealed tubes for periods of 10–20 min. Thin layer chromatographic separation of the products obtained by pyrolysis of H_2Q-9 under these conditions yielded a monomethoxy "product" (IV) which was characterized by comparison with authentic material.[4] When the products obtained from a

(2) R. F. Muraca, J. S. Whittick, G. D. Daves, Jr., P. Friis, and K. Folkers, *J. Am. Chem. Soc.*, **89**, 1505 (1967).

(3) See, for example, D. M. Clugston and D. B. MacLean, *Can. J. Chem.*, **44**, 781 (1966); C. S. Barnes and J. L. Occolowitz, *Australian J. Chem.*, **16**, 219 (1963); G. Spiteller-Friedman, *Monatsh.*, **93**, 1395 (1962).

(4) P. Friis, G. D. Daves, Jr., and K. Folkers, *J. Am. Chem. Soc.*, **88**, 4754 (1966).

(5) Pyrolytic demethoxylation (loss of formaldehyde) of methoxy-containing aromatic compounds has been observed previously. See, for example, L. K. Freidin, A. A. Balandin, and N. M. Nazarova, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk., No. 1*, 102 (1949); K. U. Ingold and F. P. Lossing, *Can. J. Chem.*, **31**, 30 (1953).

similar treatment of Q-9 were separated, no monomethoxyquinone was observed. Heating of H_2Q-9 at 170° for 12 hr failed to yield a monomethoxy product.

The process which takes place in the mass spectrometer inlet system is visualized as occurring as depicted in I → IV. Peaks due to hydroquinone species have been observed in all spectra of ubiquinones[2] and the related multiprenylquinones.[6] The suggestion has been made[7] that the reduction of quinone to hydroquinone is brought about by residual material on the surface of the spectrometer inlet system. While the presence of peaks due to quinone, observed in the spectra of hydroquinone samples, may, in some instances, be due to air oxidation occurring during sample introduction, the present data suggest that in this case the process hydroquinone → quinone occurs in the inlet system.[7]

The monomethoxyquinone hydroquinones obtained thermally in the mass spectrometer inlet system are undoubtedly mixtures of the two possible isomers (III and IV). It has been previously demonstrated that ultraviolet irradiation of ubiquinone produced a "monomethoxyhydroxybenzoquinone"[8-10] which has more recently been shown to be a 50:50 mixture of the two possible isomers.[11] Nucleophilic displacement of methoxy groups of ubiquinone with ethanol in base[12,13] and with ammonia[14] have been shown to yield mixtures of both possible monomethoxy isomers.

Recently, 2-decaprenyl-3-methyl-6-methoxy-1,4-benzoquinone (V, n = 10) isolated[4] from *Rhodospirillum rubrum* has been implicated as a biosynthetic precursor to ubiquinone-10 in this organism.[4] Similarly, 2-nonaprenyl-3-methyl-6-methoxy-1,4-benzoquinone (V, n = 9) has since been isolated from *Pseudomonas ovalis*.[15] Prominent peaks in the mass spectrum of 2-decaprenyl-3-methyl-6-methoxy-1,4-benzoquinone (V, n = 10) at m/e 205 and 167 are characteristic[2,4] of the pyrilium (VI) and benzylium (VII) ions, respectively. It is important to recognize that the appearance of these prominent peaks coupled with corresponding parent ion peaks on mass spectra of ubiquinones-9 and -10 is artifactual and should not be mistaken for the presence of a naturally occurring monomethoxyquinone.

Experimental Section

Mass Spectrometric Determinations.—The mass spectra were determined using a modified CEC-103 spectrometer[2] at an ionizing voltage of 70 ev and probe temperatures as listed in Table I. It is pertinent to note that this instrument is equipped with a stainless steel inlet system, since thermal reactions at high temperatures are known to be promoted by metal surfaces.[16]

Pyrolysis of Ubiquinol-9 (II, n = 9). A.—Highly purified synthetic ubiquinone-9 (I, n = 9; 50 mg) was reduced with sodium hydrosulfite as previously described.[2] The resulting colorless ubiquinol-9 (II, n = 9) was heated at 285° under reduced pressure (<1 mm) for 20 min. The sample was then separated by thin-layer chromatography using silica gel G plates (1.0 mm) developed in hexane–ether (3:2). During this procedure the hydroquinones present were air-oxidized to the corresponding quinones which appeared as yellow-to-orange bands on the thin layer plates. The adsorbent was removed from the area of the plate below the Q-9 band (identified by use of a reference). This material was eluted and subjected to a second thin layer chromatographic separation using silica gel G plates (1.0 mm) developed five times using hexane–ether (9:1). Three distinct bands were apparent. The two more polar bands (R_{Q-9} 0.0, 0.4) showed uv absorption, λ_{max}^{hexane} 272 mμ, similar to Q-9.[17] The third quinone band (R_{Q-9} 0.65) was at λ_{max}^{hexane} 265 and 272 mμ (sh), characteristic of a monomethoxymethylmultiprenyl-1,4-benzoquinone;[4,15] V (n = 9) showed λ_{max}^{hexane} 265 and 272 mμ; and 2-phytyl-3-methyl-5-methoxy-1,4-benzoquinone exhibited λ_{max}^{hexane} 266 and 273 mμ.[18]

B.—When 40 mg of ubiquinol-9 was heated at 170° for 12 hr at 1-mm pressure, no monomethoxymethylmultiprenylbenzoquinone was detected.

C.—A sample (30 mg) of ubiquinol-9 in a nitrogen-flushed sealed ampoule was pyrolized by heating to 320° in a Wood's metal bath during ~30 min and then allowed to cool. Separation of the reaction mixture as described above yielded a purified product with λ_{max}^{hexane} 266 and 273 mμ (sh) identifying it as a monomethoxymethylmultiprenylbenzoquinone (IV).[4,15,18]

Pyrolysis of Ubiquinone-9 (I, n = 9).—Samples of ubiquinone-9 (I, n = 9) were pyrolized using each of the procedures (A, B, and C) described above for ubiquinol-9. Under none of these conditions was a product corresponding to the monomethoxymethylmultiprenylbenzoquinone detected.

Registry No.—I (n = 9), 15393-57-4; II (n = 9), 5677-54-3; III (n = 9),[19a] 15393-55-2; III (n = 9),[19b] 15393-56-3; IV (n = 9),[19a] 15350-50-2; IV (n = 9),[19b] 7200-28-4.

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(15) S. Imamoto and S. Senoh, *Tetrahedron Letters*, 1237 (1967).
(16) See K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 21.
(17) C. H. Shunk, R. E. Erickson, E. L. Wong, and K. Folkers, *J. Am. Chem. Soc.*, **81**, 5000 (1959).
(18) G. D. Daves, Jr., J. J. Wilczynski, P. Friis, and K. Folkers, unpublished data.
(19) (a) CH_3O *para* to chain; (b) CH_3O *meta* to chain.

Synthesis of O,O-Dialkyl S-Aryl Phosphorothiolates

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The dialkyl S-aryl phosphorothiolates are isomeric with important phosphorothionate insecticides but only the dimethyl and diethyl S-(4-nitrophenyl) derivatives have been examined for toxicological and other bio-

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