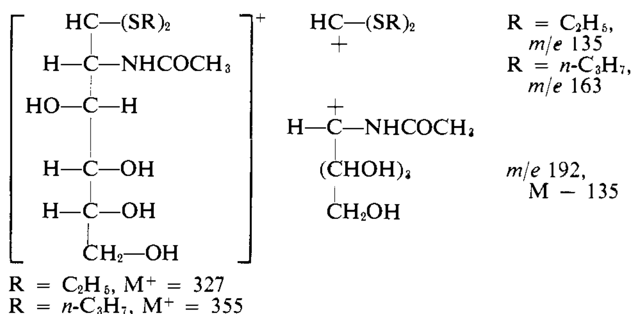
Assignment of the Position of Amino Groups in Amino Sugars by Mass Spectrometry

Sir:

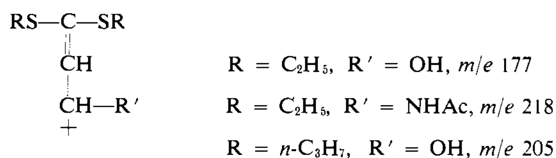
We wish to extend the study of carbohydrates by mass spectrometry[1] to one of the more important classes of derivatives, the amino sugars, in the form of their N-acetylated diethyl dithioacetals.[2]

Amino sugar dithioacetals are often products from the chemical degradation of antibiotics[3] and can be subjected directly to electron impact. Degradative procedures which allow the determination of stereochemistry at all carbon atoms except C-2[4] in 2-amino-,[5] 3-amino-,[6] and 2,6-diaminohexose[7] and 5-aminopentose[8] derivatives start with dithioacetals.[9] The classes investigated include dithioacetals[11] of 2-acetamido-, 3-acetamido-, 6-acetamido-, 2,6-diacetamido, and 3,6-diacetamidohexoses and 3-acetamido- and 5-acetamidopentoses. The 3,6-diacetamidohexose derivative is representative of a new class of diamino sugars[12] as yet not encountered in biological substances.

The mass spectra[13] of 2-acetamido-2-deoxy-D-glucose diethyl (I)[14] and di-*n*-propyl (II) dithioacetals contain molecular ion peaks at *m/e* 327 and 355, respectively, and peaks characteristic of cleavage of the C-1–C-2 bond.



A peak at *m/e* 268 in the mass spectrum of I and at *m/e* 296 in the spectrum of II, corresponding to elimination of acetamide from the molecular ion, is characteristic of the 2-acetamido substituent. A fragment retaining the substituent on C-3 is particularly useful for recognizing 3-acetamido-3-deoxyaldose dithioacetals



The mass spectra of 3-acetamido-3-deoxy-D-ribose diethyl dithioacetal (III) and of 3-acetamido-3-deoxy-D-mannose diethyl dithioacetal (IV) contain molecular ion peaks of such low intensity that they can be found only at high sample pressures. The dithioacetal portion of the 3-acetamido compounds is recognized by an intense peak at *m/e* 135 and the remainder of the molecule by a peak at M − 135. Compounds III and IV do not eliminate acetamide upon electron impact, as do the 2-acetamido analogs, but eliminate a molecule of water, indicating a preferential loss of the 2-substituent.

The mass spectra of 5-acetamido-5-deoxy-L-arabinose[10] (V) and 6-acetamido-6-deoxy-D-glucose (VI) diethyl dithioacetals can be differentiated from those of their respective 2-acetamido analogs by the elimination of one and two molecules of water from the molecular ion instead of elimination of acetamide, and from their 3-acetamido analogs by the peak at *m/e* 177. They give a molecular ion peak as well as peaks at *m/e* 135 and M − 135.

The mass spectra of compounds V and VI show fragmentation processes characteristic of the terminal acetamido function. Cleavage of successive carbon–carbon bonds in compound V with charge retention on the nitrogen-containing moiety gives fragments at *m/e* 162 for C-1–C-2 cleavage, 132 for C-2–C-3, 102 for C-3–C-4, and 72 for C-4–C-5, although *m/e* 72 may also result from the loss of ketene and water from *m/e* 132. These fragments are found 30 mass units higher for compound VI. Relative intensities indicate that C-1–C-2 and C-3–C-4 cleavages are preferred.

(1) For a recent review on this subject see H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, Calif., 1964, p. 203.

(2) The advantage in using carbohydrates in the form of their dithioacetals has been discussed by D. C. DeJongh, *J. Am. Chem. Soc.*, **86**, 3149, 4027 (1964).

(3) For a recent comprehensive review on this subject see J. D. Dutcher, *Advan. Carbohydrate Chem.*, **18**, 259 (1963).

(4) The configuration at C-2 in 2-acetamido-2-deoxyhexose dithioacetals can also be determined by converting the dithioacetal to a 1-deoxy compound by reduction, oxidation of the latter, and isolation of D- or L-alanine resulting from C-1, C-2, and C-3, as described by M. L. Wolfrom, R. U. Lemieux, and S. M. Olin, *J. Am. Chem. Soc.*, **71**, 2870 (1949).

(5) L. Hough and M. Taha, *J. Chem. Soc.*, 3564 (1957).

(6) B. Coxon and L. Hough, *ibid.*, 1463, 1643 (1961).

(7) T. H. Haskell and S. Hanessian, *J. Org. Chem.*, **28**, 2598 (1963).

(8) S. Hanessian and T. H. Haskell, *J. Heterocyclic Chem.*, **1**, 57 (1964).

(9) It should also be possible to obtain the partial stereochemistry of terminal acetamidodeoxyhexose dithioacetals by application of the degradative procedure.[8] The products would be 5-acetamido-5-deoxypentoses.[10] The configuration at C-2 in these terminal acetamidodeoxyhexose dithioacetals could be secured independently from optical rotation rules established for the hydrazones of the parent sugars; see C. S. Hudson, "Scientific Papers of The Bureau of Standards," No. 533, The National Bureau of Standards, Washington, D. C., 1926, p. 297; E. Votocek, *Collection Czech. Chem. Commun.*, **3**, 250 (1931).

(10) S. Hanessian and T. H. Haskell, *J. Org. Chem.*, **28**, 2604 (1963).

(11) The general method of M. L. Wolfrom and Z. Yosizawa, *J. Am. Chem. Soc.*, **81**, 3474 (1959), was used for the free acetamidodeoxy sugars. The methyl glycosides (70 mg.) were stirred 24 hr. at 0° with 0.4 ml. of concentrated hydrochloric acid and 0.2 ml. of ethanethiol, followed by conventional processing. Depending on the behavior of the resulting product on paper or thin layer chromatograms, the dithioacetals were either isolated directly by crystallization from methanol–ether or purified by preparative paper (Whatman No. 1, BuOH–EtOH–H_2O 3:1:1) or thin layer chromatography (silica gel, benzene–ethanol 10:3). Mercaptolysis of methyl 3-amino-3-deoxy-D-mannoside hydrochloride was done essentially according to M. L. Wolfrom, D. Horton, and H. G. Garg, *J. Org. Chem.*, **28**, 1569 (1963). All the dithioacetals were chromatographically homogeneous solids which were characterized by their very close mobilities relative to 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal, by their specific color reactions on chromatograms with the bromine–methyl orange spray (F. Weygand, H. J. Bestmann, and H. Ziemann, *Ber.*, **91**, 1040 (1958)), alkaline silver nitrate spray (W. E. Trevelyan, D. P. Proctor, and J. S. Harrison, *Nature*, **166**, 444 (1950)), and whenever possible by infrared and n.m.r. data. The following new compounds were obtained crystalline: 2-acetamido-2-deoxy-D-glucose di-*n*-propyl dithioacetal, m.p. 120–121°, and 3-acetamido-3-deoxy-D-ribose diethyl dithioacetal, m.p. 91–92°. We thank Dr. D. Horton of Ohio State University for generous gifts of methyl 3-amino-3-deoxy-D-mannose hydrochloride and 3,6-diacetamido-3,6-dideoxy-D-altrose diethyl dithioacetal.

(12) S. Hanessian and T. H. Haskell, *J. Org. Chem.*, in press.

(13) The mass spectra were determined with an Atlas CH_4 mass spectrometer at an ionizing potential of 70 e.v. and an ionizing current of 18 μa. The solid samples were ionized by electron bombardment after sublimation directly into the electron beam from a small furnace heated by a tungsten coil. The mass spectrometer was purchased by Wayne State University under Grant CP-1474 from the National Science Foundation.

(14) M. L. Wolfrom and K. Anno, *J. Am. Chem. Soc.*, **74**, 6150 (1952).

The mass spectrum of 2,6-diacetamido-2,6-dideoxy-L-idose diethyl dithioacetal[7] (VII) can be recognized as that of a 2-acetamido compound by the fragments involving the loss of a molecule of acetamide. A peak at m/e 177 shows it is not a 3-acetamido compound. Peaks at m/e 132, 102, and 72 are characteristic of the terminal acetamido function. A molecular ion peak and peaks at m/e 135 and M − 135 are present.

The mass spectrum of 3,6-diacetamido-3,6-dideoxy-D-altrose diethyl dithioacetal (VIII)[15] is recognized as that of a diethyl dithioacetal by the peak at m/e 135, and as that of a 3-acetamido compound by the peak at m/e 218, the small molecular ion peak of intensity 0.02% of the base peak, and the peak at M − H_2O. Fragments 132, 102, and 72 are characteristic of the 6-acetamido-6-deoxy function.

A detailed study of these amino sugars as well as 4-amino-, 5-amino-, and diaminoaldoses, by mass spectrometry, will be the subject of a forthcoming publication.

(15) M. L. Wolfrom, D. Horton, and Y.-L. Hung, Abstracts, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964, p. 3D. The stereochemistry was communicated privately by Dr. D. Horton.

(16) The support of Grant GM 12328-01 from the National Institutes of Health, U. S. Public Health Service, is acknowledged.

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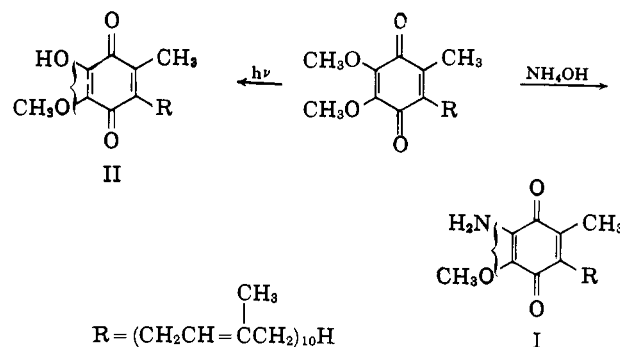
Coenzyme Q. LXII. Structure and Synthesis of Rhodoquinone, a Natural Aminoquinone of the Coenzyme Q Group[1]

Sir:

Rhodoquinone (I), a naturally occurring quinone from *Rhodospirillum rubrum*, and *Athiorhodaceae*, has now been shown by structural and synthetic studies to be an aminoquinone belonging to the coenzyme Q group. The apparent enzymic formation of rhodoquinone from coenzyme Q_{10} may indicate that the aminoquinone has a photosynthetic function.

It was previously reported[2] that the structure of rhodoquinone is a hydroxyquinone (II), based on C–H analysis, molecular weight measurement, micro hydrogenation data, and ultraviolet and infrared spectra, with particular significance attached to absorptions at 3495 and 3370 cm.$^{-1}$ in the infrared spectrum. The assignment of the hydroxyquinone function was based essentially upon the above two bands in the infrared spectrum; however, primary amines also show two bands in this region.[3]

That rhodoquinone is not a hydroxyquinone was apparent from its ultraviolet and visible spectra in neutral and basic ethanol solutions. Acidic hydroxyl groups of hydroxyquinones react with base to give resonance-stabilized anions which absorb at longer wave length, but rho-



doquinone shows no change in the ultraviolet or visible regions of the absorption spectrum in ethanolic potassium hydroxide as compared to ethanol (λ_{max} 283 mμ ($E^{1\%}_{1cm}$ 121), 512 mμ ($E^{1\%}_{1cm}$ 14)). For comparison, the hydroxyquinone from coenzyme Q_{10}(II) was newly prepared by the technique used by Imada[4] for converting coenzyme Q_7 to its O-demethylated analog. The spectral and chromatographic properties of II are quite different from those of rhodoquinone; a marked change in the visible region of the ultraviolet absorption spectrum of II was observed when the spectrum of an ethanolic solution was compared to that of an ethanolic potassium hydroxide solution (λ_{max} 277 mμ ($E^{1\%}_{1cm}$ 44), 428 mμ ($E^{1\%}_{1cm}$ 5.7), as compared to λ_{max} 281 mμ ($E^{1\%}_{1cm}$ 28), 536 mμ ($E^{1\%}_{1cm}$ 15.4)). The infrared spectrum of a carbon tetrachloride solution of II showed only one peak in the O–H stretching region at 3350 cm.$^{-1}$, and the nuclear magnetic resonance spectrum is definitive for the hydroxyquinone structure II; τ 4.96 (10) m, CH=; 6.04 (3) s, $-OCH_3$; 6.88 (2) d, ring $-CH_2-$; 8.07 (38) m, $-CH_2CH=C(CH_3)CH_2-$ and ring $-CH_3$; 8.45 (32) m, $-CH_2CH=C(CH_3)CH_2-$.

The R_f values (t.l.c.) of the hydroxyquinone II and rhodoquinone on silica gel G plates in 40% ether in n-hexane are 0.1 and 0.33, respectively. Nitrogen determinations in natural and synthetic rhodoquinone gave high values averaging about 2.7%; all spectral data were consistent with the presence of one methoxyl and one amino group.

The nuclear magnetic resonance spectrum of a carbon tetrachloride solution of rhodoquinone is in agreement with structure I; τ 4.94 (10) m, CH=; 5.50 (2) b, $-NH_2$; 6.13 (3) s, $-OCH_3$; 6.88 (2), ring $-CH_2-$; 8.04 (38) m, $-CH_2CH=C(CH_3)CH_2-$ and ring $-CH_3$; 8.42 (32) m, $-CH_2CH=C(CH_3)CH_2-$. The assignment of the peak at τ 5.50 to the two amino protons was substantiated by taking the spectrum of a carbon tetrachloride solution of rhodoquinone containing a catalytic amount of formic acid. Under these conditions the only change in the spectrum was the disappearance of the peak due to the exchangeable amino protons at τ 5.50.

Rhodoquinone gives a monoacetate (amide) derivative when treated with acetic anhydride. The amide functional group was confirmed by spectral methods; infrared (smear) 3250 cm.$^{-1}$ (N–H stretching), 1655 and 1618 cm.$^{-1}$ (amide I band and quinone carbonyl); ultraviolet (C_2H_5OH) λ_{max} 275 mμ ($E^{1\%}_{1cm}$ 178), and 400 mμ (sh) ($E^{1\%}_{1cm}$ 8); n.m.r. (CCl_4) τ, 3.15 (1) s, $-NH$; 4.96 (10) m, CH=; 5.96 (3) s, $-OCH_3$; 6.88 (2) d, ring $-CH_2-$; 7.94 (3) s, CH_3CO-; 8.08 (38) m, $-CH_2$-

(1) This research was partially supported by funds from the Merck Sharp and Dohme Research Laboratories and we express our appreciation to Dr. Max Tishler. We are grateful to Dr. Harry Rudney of Western Reserve University for a sample of rhodoquinone.

(2) J. Glover and D. R. Threlfall, *Biochem. J.*, **85**, 14P (1962).

(3) L. J. Bellamy in "The Infra-red Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1959, p. 248.

(4) I. Imada, *Chem. Pharm. Bull.* (Tokyo), **11** (6), 815 (1963).