scribed by Blomquist for comparison, melted at 124.3–124.9°. The mixed m. p. was 121–124°.

When 3-nitro-4,4-bis-(nitroxymethyl)-oxazolidone was hydrolyzed as described below in the preparation of tris-(hydroxymethyl)-methylnitramine, no product could be isolated from the reaction mixture.

tris-(Hydroxymethyl)-methylnitramine (VII).—A 4.42-g. sample of the nitro derivative, VI, was stirred mechanically with 116.5 cc. of 0.9844 *N* sodium hydroxide for two hours, mild cooling being used at first to prevent the temperature from rising above 35°. The solid dissolved after about one hour. The solution was treated with an amount of standard hydrochloric acid exactly equivalent to the sodium hydroxide used, then concentrated to dryness *in vacuo* (bath, 50–65°). The crystalline residue was extracted at about 50° with 125 cc. of butanol in three portions (extraction at 100° with nitromethane caused considerable decomposition). After filtration of salt the butanol solution was concentrated to dryness *in vacuo* (bath, 53–65°), leaving 2.84 g. of crude nitramine containing some salt, m. p. 114–117° (gas). One crystallization from nitromethane gave 2.04 g. (77%) of material melting at 123–125°. After two additional crystallizations from nitromethane very large striated blades and plates were obtained, m. p. 124–126° (gas), varying somewhat with rate of heating. The analytical sample, Code No. IV-20, was ground and dried *in vacuo* at 80°.

Anal. Calcd. for $C_4H_{10}N_2O_5$: C, 28.92: H, 6.07; mol. wt., 166.1. Found: C, 29.00; H, 6.26; mol. wt., 165.3 (by titration).

This nitramine is very soluble in water, moderately soluble in alcohol and essentially insoluble in acetone and ether. It shows no evidence of decomposition during crystallization.

When tris-(hydroxymethyl)-methylnitramine was nitrated at −5°, using a slight excess of 98% nitric acid in acetic anhydride, there was evolution of gas during the reaction, and the water-insoluble product which was obtained crystallized very slowly. Attempted recrystallization caused further decomposition, and no pure product could be obtained.

Summary

There has been prepared tris-(hydroxymethyl)-methylnitramine and the isomeric, previously known tris-(hydroxymethyl)-methylnitrosohydroxylamine. The preparation of several related compounds from tris-(hydroxymethyl)-aminomethane is also described.

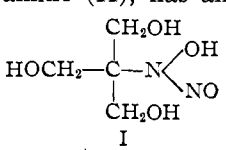
NASHVILLE, TENN. RECEIVED AUGUST 23, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING OF THE UNIVERSITY OF PENNSYLVANIA]

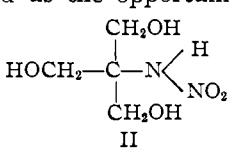
The Ultraviolet Absorption Spectra of tris-(Hydroxymethyl)-methylnitrosohydroxylamine, tris-(Hydroxymethyl)-methylnitramine, and their Salts[1,2]

BY MARVIN CARMACK AND J. J. LEAVITT[3]

The synthesis by Cason and Prout[4] of tris-(hydroxymethyl)-methylnitrosohydroxylamine (I) and its isomer, tris-(hydroxymethyl)-methylnitramine (II), has afforded us the opportunity



I



II

to investigate the ultraviolet absorption spectra of the primary nitramine and the isonitramine (nitrosohydroxylamine) chromophores in the rare, if not unique, situation in which both isomeric compounds are of the aliphatic series and isolable as reasonably stable crystalline compounds. In many of the more readily prepared nitrosohydroxylamine compounds, the presence of aromatic groups complicates the interpretation of the ultraviolet absorption spectra.

As was hoped, ultraviolet spectrometry proved useful in studying the nature of the functional groups in certain nitramines or isonitramines of ambiguous structure which have been reported in the literature, as for example, "methylenebisisonitramine," prepared by the Traube reaction.[2,5,6]

Kortüm and Finckh[7] have published the ultraviolet absorption spectra of nitramide and methylnitramine in dilute hydrochloric acid and of dimethylnitramine in water. They also reported the spectra of hyponitrous acid in dilute hydrochloric acid, and of sodium hyponitrite, nitrohydroxylamine, and methylnitrosohydroxylamine in dilute sodium hydroxide. The alkylnitramines were measured only in neutral or acidic solution, while methylnitrosohydroxylamine was, unfortunately, measured only in alkaline solution; hence, the spectra of the two types of isomeric compounds are not directly comparable, and it is difficult from the data of Kortüm and Finckh to evaluate the suitability of spectrometry as a means of distinguishing one functional group from the other.

The spectrometric curves for tris-(hydroxyl-

(1) This paper is based upon work carried out for the Office of Scientific Research and Development under Contract OEMsr-646, between the National Defense Research Committee and the University of Pennsylvania.

(2) R. Norman Jones and G. Denis Thorn have made a comprehensive study of the ultraviolet absorption spectra of nitramines and related compounds, including methylenebisisonitramine obtained by the Traube reaction. They will publish their results elsewhere (private communication).

(3) Present address: Calco Chemical Division, American Cyanamid Company, Bound Brook, N. J.

(4) Cason and Prout, THIS JOURNAL, **71**, 1218 (1949).

(5) Traube, *Ber.*, **27**, 1509, 3291 (1894); *cf.* also Wieland, *ibid.*, **61**, 2382 (1928).

(6) G. F. Wright and collaborators have studied the Traube product, methylenebisisonitramine, and will publish the results of their findings elsewhere (private communication).

(7) Kortüm and Finckh, *Z. physik. Chem.*, **43B**, 32 (1940).

methyl)-methylnitrosohydroxylamine (I) in water and in 0.25 *N* sodium hydroxide solutions are shown in Fig. 1. Curves for tris-(hydroxymethyl)-methylnitramine (II) in water, in *N* sodium hydroxide, and in *N* hydrochloric acid are shown in Fig. 2. For comparison, curves for methylnitramine in *N* hydrochloric acid and for potassium methylnitramine in 0.5 *N* potassium hydroxide are shown in Fig. 3. Data on the extinction values at the absorption peaks are summarized in Table I.

TABLE I

ULTRAVIOLET ABSORPTION MAXIMA OF COMPOUNDS

Compound and solvent	$\epsilon_{max.}$, mμ.	$\epsilon_{max.} \times 10^{-3}$
tris-(Hydroxymethyl)-methylnitroso-hydroxylamine (I)		
in water	229	6.0
in 0.25 *N* sodium hydroxide	249	8.73
tris-(Hydroxymethyl)-methylnitramine (II)		
in water	235	6.97
in *N* sodium hydroxide	236	8.07
in *N* hydrochloric acid	234	6.19
Methylnitramine (potassium salt)		
in *N* hydrochloric acid[8]	230	7.01
in *N* potassium hydroxide	228.5	7.60

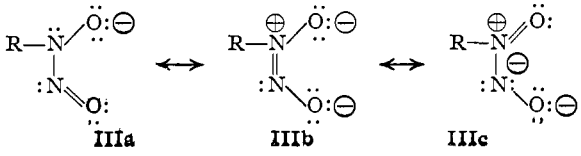
The spectra of the nitramines in water are very similar to the spectrum of tris-(hydroxymethyl)-methylnitrosohydroxylamine (I) in water. The differences in both the intensities and wave lengths of the absorption peaks are small and of the same order as those among homologous members of a series derived from one chromophore group.

A striking difference was observed between the nitramines and the isonitramine in alkaline solution. Upon the formation of the salt of the nitrosohydroxylamine (I) in alkali, it was noted that not only did the absorption peak increase in intensity, but the position of the maximum shifted 20 mμ toward the visible. In contrast, the nitramines showed only very slight shifts in the positions of their maxima although the extinction values at the maxima for the nitramines increased as the medium was varied from aqueous acid to water to dilute alkali.

The magnitude of the shift of the absorption peak for the isonitramine is sufficiently great to serve as a means of distinguishing the isonitramine (nitrosohydroxylamine) functional group from the nitramine.[9]

(8) Kortüm and Finckh (ref. 7) published a spectrum of methylnitramine in dilute hydrochloric acid, but the absorption maximum of their curve was apparently obtained by extrapolation.

(9) Kortüm and Finckh (ref. 7) proposed for the anion of methylnitrosohydroxylamine a resonating structure, of which IIIa and IIIb would represent the most important contributing forms



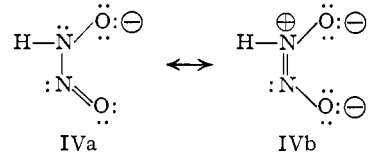
IIIa IIIb IIIc

Experimental

Materials.—tris-(Hydroxymethyl)-methylnitrosohydroxylamine (I) was kindly supplied to us by Dr. James Cason[4] (his Sample No. NB-I-50B which had been recrystallized four times). When received in this Laboratory the sample had a well-defined crystalline appearance. The spectrum was determined immediately after receipt in order to minimize the possibility of deterioration upon storage. Solutions were freshly made up to appropriate concentrations immediately before being measured. Another sample of I (NB-I-51) gave similar results except that slightly lower extinction values were obtained at the maximum. tris-(Hydroxymethyl)-methylnitramine was also furnished to us by Dr. Cason (his Sample No. IV-20, m. p. 124–126°). The sample of potassium methylnitramine was provided through the courtesy of Dr. A. T. Blomquist, Cornell University.

Ultraviolet Absorption Spectral Measurements.—All spectrometric measurements were made on dilute aqueous solutions in 1-cm. quartz cells in the Beckman Quartz Spectrophotometer, Model DU.[10] Appropriate dilution, kept the readings within the range of 10–80% transmission. In the case of potassium methylnitramine, curves were determined in several concentrations of potassium hydroxide as well as in water alone. Although the curves were similar in appearance and location of the peak on the wave length scale, the value of $\epsilon_{max.}$ varied from 7.21 ×

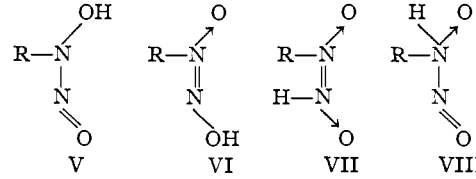
These are analogous to the important resonance forms of the tautomeric structure favored by Kortüm and Finckh for the hyponitrite ion ($HN_2O_2^-$)



IVa IVb

They pointed out that the spectra of the hyponitrite ion and of methylnitrosohydroxylamine in alkaline solutions are very similar. It would be interesting to know whether hyponitrous acid and free methylnitrosohydroxylamine show similar spectra in neutral and acidic solutions and whether the magnitude of the shift on passing from neutral to basic medium is similar for hyponitrous acid and for the alkylnitrosohydroxylamines. Kortüm and Finckh did not extend their measurements of the solution of hyponitrous acid sufficiently far into the ultraviolet region to reach the absorption maximum, and they did not report the spectrum of the methylnitrosohydroxylamine in neutral or acidic solution.

For the free isonitramines, the general structural formula V is preferred because it does not involve a separation of charges; it is used to represent the crystalline compound prepared by Cason and Prout (I). It may be pointed out, however, that three other structures might be written (VI, VII, and VIII). Thus, the hydrogen atom could conceivably be attached to either of the oxygen atoms or either of the nitrogen atoms. Structure VII would be least probable, since it involves two positive charges on the adjacent nitrogen atoms. Kortüm and Finckh considered only the structure V. The possibility that a tautomeric equilibrium between two or more forms exists in solution must also be considered. In any case, only one resonating anion would be expected. The shift of the absorption peak upon formation of the salt from the isonitramine suggests that the distribution of electrical charges is different in the free parent isonitramine and in the salt anion.



V VI VII VIII

(10) We gratefully acknowledge the technical assistance of Mr. P. W. Wilcox and Mr. Joseph L. Ciminera of the Research Laboratory, Sharp and Dohme, Glenolden, Pennsylvania, in the determination of the absorption spectra.

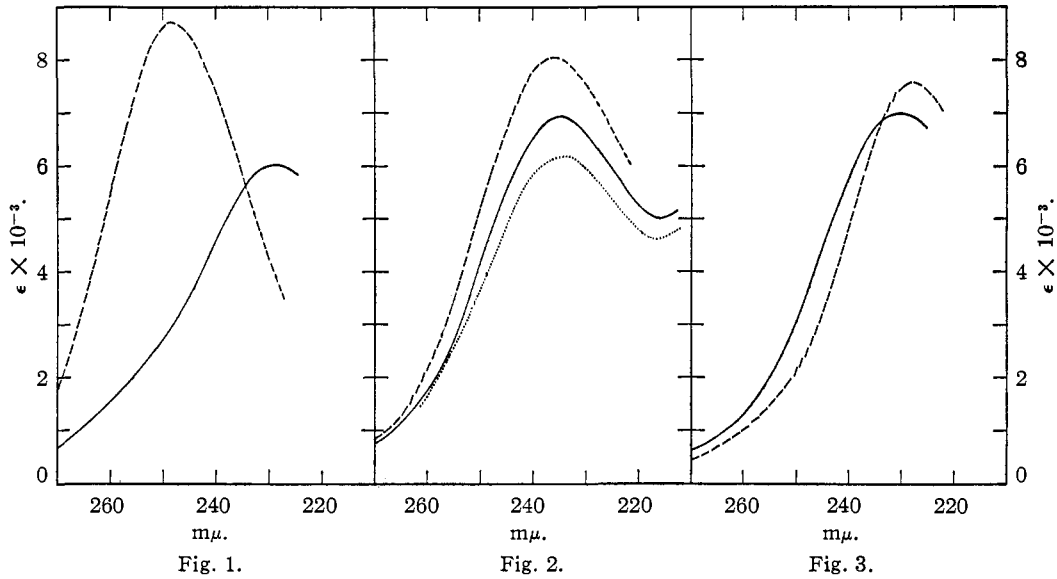


Fig. 1. Fig. 2. Fig. 3.

Fig. 1.—Solid curve: tris-(hydroxymethyl)-methylnitrosohydroxylamine in water; dash curve, same in 0.25 *N* NaOH.

Fig. 2.—Solid curve: tris-(hydroxymethyl)-methylnitramine in water; dash curve, same in *N* NaOH; dotted curve: same in *N* HCl.

Fig. 3.—Solid curve: methylnitramine in *N* HCl; dash curve, same in 0.5 *N* KOH.

10^3 in water to 7.60 × 10^3 in 0.5 *N* potassium hydroxide. No further increase of $\epsilon_{max.}$ was noted in *N* potassium hydroxide. The spectrum of free methylnitramine was obtained by dissolving potassium methylnitramine in a large excess of dilute hydrochloric acid.

Summary

The ultraviolet absorption spectrum of tris-(hydroxymethyl) - methylnitrosohydroxylamine in neutral aqueous solution is similar in location and intensity to that of tris-(hydroxymethyl)-methylnitramine and methylnitramine in neutral, aqueous solution. In aqueous alkaline solution the absorption of the nitrosohydroxylamine is greatly intensified and the maximum shifts 20 mμ toward the visible region. The spectra of the alkylnitramines are intensified by conversion into the salts but the location of the maximum remains almost unchanged. This differing behavior in alkaline medium affords a means of distinguishing nitramines from isonitramines (nitrosohydroxylamines).

PHILADELPHIA 4, PENNSYLVANIA

RECEIVED AUGUST 23, 1948

[CONTRIBUTION FROM DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

The Separation of Choline Esterase, Mucoprotein, and Metal-Combining Protein into Subfractions of Human Plasma[1a,h,c]

BY D. M. SURGENOR, L. E. STRONG,[1d] H. L. TAYLOR,[1e] R. S. GORDON, JR., AND D. M. GIBSON[1f]

A system for the separation of the protein components of human plasma has been described in an earlier paper in this series.[2] The fractions so obtained contain groups of proteins of somewhat similar solubility characteristics, each of which is susceptible of further purification. Many of the components so isolated, either in

(1a) This work was originally supported by grants from the Rockefeller Foundation and from funds of Harvard University. It was aided early in 1941 by grants from the Committee on Medicine of the National Research Council, which included a grant from the American College of Physicians. From August, 1941, to July, 1946 it was carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University. Since then it has been aided by a grant recommended by the Panel on Hematology of the National Institute of Health.

(1b) This paper is Number XX in the series "Preparation and Properties of Serum and Plasma Proteins" from the Department of Physical Chemistry, Harvard Medical School.

(1c) This paper is Number 75 in the series "Studies on the Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

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(2) E. J. Cohn, L. E. Strong, W. L. Hughes, Jr., D. J. Mulford, J. N. Ashworth, M. Melin and H. L. Taylor, THIS JOURNAL, 68, 459 (1946).