The Structure of Amidoximes. II.[1] Oxamidoxime

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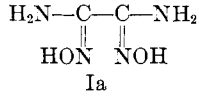
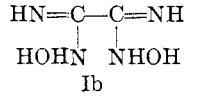
The two isomers of oxamidoxime described in the literature are shown to be identical. Infrared and proton n.m.r. evidence permits the assignment of the diaminoglyoxime structure to this compound and an analogous structure to ethyl aminoöximinoacetate.

Oxamidoxime (I) has been prepared from cyanogen and hydroxylamine,[2] by ammonolysis of dichloroglyoxime diacetate,[3] from dibromofuroxan and ammonia,[4] and by the reaction of dithioöxamide with hydroxylamine.[5] It is claimed[3] that the products from the first two methods are different compounds, yield different diacetates and should be regarded as isomers Ia and Ib.



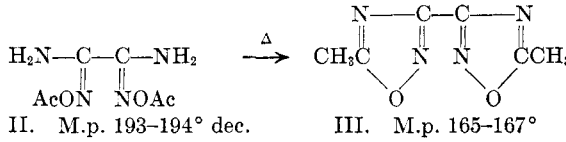
Ia



Ib

Since unsubstituted amidoximes usually exist in only one form,[6] the preparation of I has been reinvestigated.

The products from the two reactions[2,3] have been found to be identical as judged by infrared and ultraviolet absorption spectra and paper chromatography. Acetylation of I yields a diacetate II which decomposes on melting to the known 5,5′-dimethyl-3,3′-bi-1,2,4-oxadiazole (III).[2] The discrepancy in the decomposi-



II. M.p. 193–194° dec. III. M.p. 165–167°

tion points for different preparations of I[2,3] and the corresponding diacetates is probably due to different rates of heating or methods of determining melting points.

All modern structural evidence for unsubstituted amidoximes (RCN_2H_2OH) is based on infrared absorption spectra and the various investigators agree that a single structure is present rather than a mixture of isomers. The observed absorption bands have been assigned to O—H and N—H stretching and deformation and C=N and N—O stretching.[1,6–10] The differences which may be expected in the spectra of the two possible isomers are comparable with the differences between C=N stretching frequencies of oximes and imines, N—H stretching or deformation in amines, imines, and hydroxylamines, and O—H as well as N—O stretching in oximes and hydroxylamines. In some examples these differences have been observed to be small,[11–13] the frequencies are dependent on the substituents,[10–12] and few hydroxylamines with strictly comparable structures have been available. The structural assignments are therefore subject to some uncertainty.

In the case of oxamidoxime (I) and its derivatives a clear distinction between structures Ia and Ib on the basis of infrared absorption spectra is difficult for similar reasons. The diagnostic value of the C=N stretching frequencies has been impaired by later findings[10] and the C=N band shifts on salt formation[1] (Table I) may be due to electronic influences. The previously assigned structures[1] are therefore open to question.

In order to determine the structure for oxamidoxime (I), proton n.m.r. spectra have been determined for I, its diacetate (II), and for ethyl aminooximinoacetate (IV).[1] The only solvent which was suitable for this investigation was dimethylformamide (DMF). The assignment of the resonance at −10.00 p.p.m. to the hydroxyl proton in I is unambiguous. The observed chemical shift is identical to that of the C=N—OH proton resonance in propionaldoxime.[14] Additional evidence is provided by the disappearance of the resonance on addition of a few per cent of water. This disappearance is due to the expected rapid chemical exchange of the water protons with those of the amidoxime hydroxyls. A new resonance which reflects the averaged chemical shifts at the two positions occurs at −4.07 p.p.m. Only one additional resonance has been observed (−5.32 p.p.m.; −5.34 p.p.m. on addition of water). It is considerably broader than the hydroxyl resonance as is characteristic for protons attached to nitrogen (due to the shortened nuclear spin relaxation times induced by the large quadrupole moment of the nitrogen nucleus). The assignment of this line to N—H is strengthened by the similarity of the observed chemical shift to those usually found for amide protons. The ratio of NH to OH intensities is 2:1 giving evidence for structure Ia. For the diacetate (II) the NH_2 proton resonance is again observed (−6.50 p.p.m.). The OH line is missing, as expected, and instead the spectrum exhibits a CH_3 resonance (−2.09 p.p.m.) near the position of the DMF methyl proton resonances. The observed ratio of CH_3 to NH_2 intensities is 3:2. In the case of the ester (IV) the CH_2 (−4.2 p.p.m.) and CH_3 (−1.63 p.p.m.) assignments are unambiguous

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TABLE I

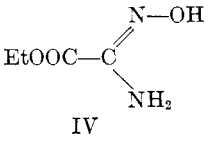
INFRARED ABSORPTION BANDS OF OXIMINO COMPOUNDS

Compound	Solvent	N—H[a] (N—D)		O—H[a] (O—D)	C=O	C=N	N—H	O—H (O—D)	C—O—C	N—O
Glyoxime	KBr			3.18 s		6.06 w		7.87 s		10.50 s
disodium salt	KBr					(6.16 m)[i]				9.95 s
diacetate	KBr				5.63 s[b]	6.17 m			8.47 s	10.85 s[c]
Oxamidoxime	KBr	2.91 s	3.01 s	3.13 s		6.06 s	6.33 s	6.98 m		10.71 s
	DMF	sh 2.88 m	3.02 s	3.12 m			6.39 m			10.70 s
disodium salt	KBr					6.33 m	6.49 m			10.35 s
diacetate	KBr	2.91 s	3.01 s		5.68 s	6.21 s	[d]		8.32 s	10.54 s[e]
	DMF	sh 2.88 m	3.01 s		5.66 s	6.17 m	[d]		8.30 s	10.62 m
	Py	2.88 m	3.02 s		5.65 s	6.13 s	[d]		8.35 s	(10.65 m)[i]
	Dioxan				5.64 s	6.14 s	[d]			
Et aminoöximinoacetate	KBr	2.89 m	2.97 m	3.18 m	5.80 s	5.99 s	6.37 m	6.92 m[f]	8.11 s	10.28 s
	DMF	2.88 m	3.02 m		(5.80 m)[i]		6.35 m		8.22 s	10.45 m
Glyoxime-d_2	KBr[g]			4.18 m		6.10 m		9.33 m		
Oxamidoxime-d_2[j]	KBr	2.92 m	3.85 m[h]	4.08 m		6.16 s	6.45 m[f]	9.29 m		10.99 s

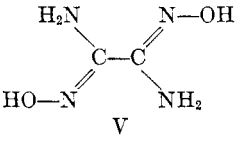
[a] Hydrogen-bonded stretching bands, *cf.* ref. 6. [b] Calcd. for acetone, *O*-acetyloxime, $\lambda_{C=O}$ 5.64 [M. Horak and O. Exner, *Chem. listy*, **52**, 1451 (1958)]. Found for purified acetone, *O*-acetyloxime ($n^{25}D$ 1.4340) $\lambda_{C=O}^{liq}$ 5.64, $\lambda_{C=N}$ 6.03, λ_{C-O-C} 8.27, λ_{N-O} 10.76, other bands at 9.98, and 11.40 μ. [c] Additional bands were found at 10.01 and 11.07 μ. [d] Fused band. [e] Additional bands occur at 9.93 and 10.95 μ. [f] Tentative assignment. [g] E. Borello and M. Colombo, *Gazz. chim. ital.*, **87**, 615 (1957). [h] The shift in the N—H bands indicates that some deuterium exchange occurred in the amino groups. [i] Values in parentheses occur where solvents or hydrates absorb. [j] Prepared according to ref. 16.

because these resonances exhibit characteristic spin-spin splittings. The NH and OH resonances occur at −5.6 p.p.m. and −10.0 p.p.m., respectively. The measured intensity ratios are: $CH_2:NH_2$ = 1:1 and $OH:NH_2$ = 1:2. The intensity ratios show that all N—H protons have been accounted for. Hence, structure Ib would be possible only if the chemical shifts of the protons associated with the two nitrogen atoms were accidentally the same in all three compounds. It has been pointed out[15] that rapid chemical exchange of non-equivalent protons in tautomeric compounds can also result in n.m.r. spectra in which these protons appear to be equivalent. This objection does not apply in the present case. The effect of adding water to I in DMF solution demonstrates the ease with which the hydroxyl protons exchange; no such effect is observed for the NH_2 resonance. The observation of separate NH_2 and OH proton resonances also argues strongly against rapid exchange of NH_2 protons. In view of the ease with which the OH protons participate in exchange it is certain that any exchange involving NH_2 protons would also involve the hydroxyls. If this were the case, only one resonance would be observed for all three protons. The possibility of accidental equivalence of the NH chemical shifts (in all three compounds investigated) if structure Ib were correct is felt to be extremely remote. Hence, only aminooximino structures corresponding to Ia can be assigned to compounds I, II, and IV in DMF solution. Furthermore, the absence of any resonances not assigned to structure Ia argues strongly that these compounds exist predominantly in one form. In view of the similarity of the infrared spectra for these compounds in DMF, other solvents, and in the solid state, it is believed that these structures are generally valid for unsubstituted amidoximes.

Since the ultraviolet spectrum for I is quite similar to those of glyoxime and dimethylglyoxime, an *anti*-structure and s-*trans* configuration is indicated for the compound.[16] This is further confirmed by the absence of a doublet in the C=N stretching region. It is felt therefore that on the basis of the present evidence the properties of I are best explained by the structure V, in which both amino and oximino groups are further involved in hydrogen bonds.



IV



V

Experimental[17]

Oxamidoxime (I).—Hydroxylamine hydrochloride (27.80 g., 0.4 mole) in 30 ml. of water was added to a solution of sodium hydroxide (16.00 g., 0.4 mole) in 64 ml. of water. The solution was cooled to 0° and treated with a slow stream of cyanogen gas. Successive precipitates were filtered with suction and dried until the amounts became quite small. During this time the originally neutral solution became acid. The average yield of crude product from several runs was 20.2 g. (85%). Aqueous solutions of oxamidoxime were chromatographed on Whatman no. 3 filter paper with water and the dried strips were sprayed with alcoholic *p*-benzoquinone. R_f values of crude and crystallized product were 0.69 and 0.70. A small spot with R_f 0.89 disappeared after crystallization. Oxamidoxime was soluble in dioxane, DMF, pyridine, and water and could be crystallized from the latter two solvents. The crystallized product was analytically pure and chromatographically uniform and gave capillary decomposition points of 192–197°. On the Dennis bar all crystallized preparations melted at 210° dec., lit. m.p. 196°,[2,4] 198°,[5b,18] 202°,[19] and 203°.[5a] The ultraviolet absorption spectrum, $\lambda_{max}^{H_2O}$ 233 mμ (log ϵ 4.03), was in good agreement with the literature value.[5a] Potentiometric titration of the compound with 0.1 *N* sodium hydroxide at 26.5° under nitrogen gave a pK_a of 10.62.[20]

Anal. Calcd. for $C_2H_6N_4O_2$: C, 20.34; H, 5.12; N, 47.44. Found: C, 20.11, 20.30; H, 5.51, 5.30; N, 47.05, 47.67.

Oxamidoxime was recovered unchanged when the aqueous solution of the disodium salt was carefully acidified with aqueous acetic acid.

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(19) J. Houben and E. Schmidt, *ibid.*, **46**, 3616 (1913).
(20) Potentiometric titrations by J. F. Baytos of this laboratory. For pK_a values of other glyoximes see ref. 16.

A sample of I, prepared by ammonolysis of dichloroglyoxime diacetate (m.p. 161–162°),[3] was identical in melting behavior and infrared spectra. It had $\lambda_{max}^{H_2O}$ 233 mμ (log ε 4.03) and R_f 0.69.

Anal. Calcd. for $C_2H_6N_4O_2$: C, 20.34; H, 5.12; N, 47.44. Found: C, 20.64, 20.29; H, 5.35, 5.24; N, 46.89.

Oxamidoxime gave blue-green solutions with aqueous nickel salts from which the yellow-orange nickel derivative was precipitated by addition of little dilute ammonia or pyridine.[21] Aqueous I was oxidized by aqueous or alcoholic solutions of *p*-benzoquinone with formation of dark-colored products. The reaction with molar equivalents of *p*-benzoquinone leads to destruction of a small portion of I; the remainder was unchanged. Both nickel salts and quinone solutions were used as spray reagents for the paper chromatography of I.

Oxamidoxime Diacetate (II).—An exothermic reaction occurred when I was added to excess boiling acetic anhydride. The mixture was shaken and allowed to cool. Filtering the colorless solid with suction, washing and drying gave 99% of pure diacetate, m.p. 193–194° dec., remelt 165–167°. Its solubility in pyridine at 25° was 2 g. per 100 ml. From dioxan the diacetate crystallized in colorless leaflets, m.p. 193–194°. The corresponding melting points on the Dennis bar were 229° dec., lit. m.p. 184–187°,[2] 206°,[3] 212°.[3]

Anal. Calcd. for $C_6H_{10}N_4O_4$: C, 35.65; H, 4.98; N, 27.72. Found: C, 35.62, 35.40; H, 5.22, 4.96; N, 27.52, 27.55.

Oxamidoxime Disodium Salt.—Oxamidoxime (0.118 g., 0.001 mole) was only partially soluble in 10 ml. of ethanol containing sodium ethoxide (0.002 mole). It was dissolved by adding water (5 ml.). Evaporation of the solution at 0.3 mm. gave 0.22 g. of solvated salt. The anhydrous salt obtained by heating at 100° proved to be very hygroscopic and was not obtained analytically pure.

Anal. Calcd. for $C_2H_4N_4Na_2O_2$: Na, 28.38. Found: Na, 29.69.

Glyoxime diacetate, prepared by acetylation of glyoxime with acetic anhydride, melted at 120–121°, lit. m.p. 120°,[22] 126°.[23]

Anal. Calcd. for $C_6H_8N_2O_4$: C, 41.87; H, 4.68; N, 16.28. Found: C, 42.11; H, 4.96; N, 16.25.

Glyoxime, Disodium Salt.—Glyoxime (0.088 g., 0.001 mole) was dissolved in a solution of sodium (0.0460 g., 0.002 mole) in 10 ml. of ethanol. Evaporation at 0.3 mm. gave 0.11 g. of hygroscopic sodium salt which was analyzed as the hydrate.

Anal. Calcd. for $C_2H_2N_2Na_2O_2 \cdot 1.5H_2O$: C, 15.10; H, 3.16; N, 17.62. Found: C, 14.83; H, 2.61; N, 18.07.

N.m.r. Spectra. All proton n.m.r. spectra were obtained at 56.4 Mc./sec. and 28°. Because of the low solubilities, the most prominent resonances in all cases are those of DMF.

Other solvents, including acetone, water, carbon tetrachloride, dioxane, and pyridine, were tried. In all cases, however, the solubilities were insufficient or the solvent spectrum interfered with that of the oximes. Chemical shifts are given in parts per million. The internal reference substance in this study was tetramethylsilane, the chemical shift of which was taken as 0.00 p.p.m. Relative concentrations of protons were determined by electronic integration techniques. All concentration ratios quoted in the discussion have been rounded to the nearest simple ratio of integral numbers. In all cases the measured values were within ±5% of the reported values.

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Halogenation of Estrone and Derivatives[1]

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The halogenation of estrone and certain of its analogs has been investigated. Bromination, even with excess bromine, resulted only in substitution of the aromatic ring. Chlorination using sulfuryl chloride, chlorine, or N-chlorosuccinimide gave 10-chlorodien-3-one derivatives with chlorine substitution in rings A and D. Mixtures of different products, separable by chromatography, were obtained in all experiments. While substitution of the 2-position in estrone by halogen or nitro groups precluded chlorination in the 4-position, a similar substitution at carbon 4 did not interfere with the entrance of chlorine into the 2-position.

An observation made in this laboratory[2] that tumor growth in animals was inhibited by certain pentacyclic terpenoid methylene quinones such as pristimerin[3] led us to attempt the preparation of such structures from steroidal estrogens. Oxidation of estrone and similar estrogens with lead tetraacetate resulted in dienone–quinols with a hydroxyl group in the 10-position.[4] However, attempts to convert these substances into $\Delta^{9,10}$-methylene quinones by eliminating the 10-hydroxyl group as water were unsuccessful. Consequently, we decided to study the elimination of hydrogen halide from 10-halogenated estrogens. It was thought that the latter compounds would be formed by a reaction comparable to the action of bromine on *para*-substituted phenols.[5] While such work was in progress in this laboratory, Mukawa[6] reported that isocyanuric chloride converted estradiol 17-acetate (A-III) into 2,4,10β-trichloro-17-acetoxy-$\Delta^{1,4}$-estradien-3-one (B-I) and Mills, *et al.*,[7] obtained a number of 10β-chloro-$\Delta^{1,4}$-estradien-3-one derivatives by the action of N-chlorosuccinimide on aromatic steroids. These authors assigned the beta configuration to the halogen in the 10-position.

We began the present investigation by repeating Woodward's work[8] on the bromination of estradiol (A-II) with N-bromoacetamide. Even with a large excess of the same or other brominating agents only 2,4-dibromoestradiol (A-VI) was isolated by direct crystallization, though a small amount of 2,4-dibromoestrone (A-V) which was not found by Woodward, was separated from the crude reaction product using Girard's reagent. In agreement with Woodward, no dienone formation was observed. Similarly, estrone gave only 4-bromoestrone (A-IV) and 2,4-dibromoestrone (A-V), depending upon the amount of bro-

(1) Supported by Grant No. 4550 from the U. S. Public Health Service, National Cancer Institute, Institutional Grant No. EIN-56 from the American Cancer Society and a grant from the Massachusetts Division of the American Cancer Society.

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