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Studies on Monoamine Oxidase Inhibitors

II.* A Note on the Reaction between 1,2-Diethylhydrazine and Acetaldehyde

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Some aspects of the reaction between acetaldehyde and 1,2-diethylhydrazine are discussed and it is concluded that this reaction is unsatisfactory as a model for monoamine oxidase inhibition. Specifically, it is shown that the spectral changes occurring at a pH of 7.2 and 8.0 in aqueous solutions of 1,2-diethylhydrazine and acetaldehyde are not due to the simple addition of 1,2-diethylhydrazine to the carbonyl group of acetaldehyde, but probably to a Mannich-type reaction giving rise to the Δ^3-pyrazoline ring system.

1,2-Di-*n*-alkylhydrazines are known to be potent inhibitors of monoamine oxidase (MAO).[1] Since the "carbonyl group theory" of irreversible MAO inhibition requires the interaction between a carbonyl group at the enzymic receptor and the hydrazine moiety, resulting in the formation of a stable covalent bond,[1] the potency of the 1,2-dialkylhydrazines is difficult to explain according to this theory. Using aliphatic aldehydes, *e.g.* acetaldehyde, as model compounds, Carbon, Burkhard, and Zeller [1] concluded that the reaction

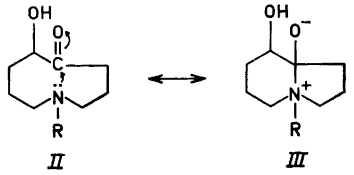
$$\text{Enzyme}{=}\text{C}{=}\text{O} + \text{RHN}{-}\text{NHR} \rightarrow \text{Enzyme}{=}\text{C(OH)}{-}\text{N(R)}{-}\text{NHR(I)} \quad (1)$$

would possibly account for the inhibition. Evidence for this reaction was obtained from a study of the spectral behaviour of aqueous solutions of 1,2-dialkylhydrazines and aldehydes at pH 7.2. Immediately upon mixing the solutions a shift of the absorption in the ultra-violet region towards longer wave-lengths was observed, a stable value of absorption being established within 15 sec. at 2° in the case of 1,2-dimethylhydrazine and acetaldehyde. The spectral changes were recorded as the increase in absorption at 230 mμ. It was found that aqueous solutions at pH 7.2 of 1,2-dimethyl-, 1,2-diethyl-,

* For the preceding paper in this series, see Eberson, L. and Persson, K. *J. Med. Pharm. Chem.* 5 (1962) 738.

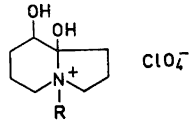
and 1,2-di-propylhydrazine and acetaldehyde exhibited small but significant increases in absorption, whereas 1,2-diisopropylhydrazine and acetaldehyde remained essentially unchanged. As the 1,2-di-*n*-alkylhydrazines were found to be potent MAO inhibitors and the diisopropyl compound a weak one the results were taken as evidence that reaction (1) is responsible for the enzyme inhibition.

However, the interpretation of the spectral shift was based on a false analogy with the behaviour of cyclic aminoketones [2] capable of transannular interaction between the keto and tertiary amine group (2).



II III (2)

Now (2) actually represents a resonance hybrid, whereas I or its model counterpart is an ordinary addition compound not possessing the structural features necessary for such an electronic transition. Consequently, there is no reason to expect any light absorption in the region around 230 mμ. This is further substantiated by the fact that the perchlorate of II, (IV), which is a closer structural analogue of I, has negligible ultraviolet absorption in the short wave-length region.



IV

As there is evidence that hydrazine derivatives may inhibit MAO by virtue of their reducing properties,[3] the reaction between acetaldehyde and 1,2-diethylhydrazine was investigated in some detail in order to seek some other explanation for the spectral shifts observed by Carbon *et al.*[1]

RESULTS

1. The titration curve of 1,2-diethylhydrazine dihydrochloride against standard sodium hydroxide or the free base against standard hydrochloric acid was unaffected by the presence of even a twenty-fold molar excess of acetaldehyde, indicating that any equilibrium analogous to (1) is either rapidly displaced to the left or has a very small equilibrium constant.

2. The purity of the hydrazine compound appears to be a very critical point. In this work, 1,2-diethylhydrazine was prepared by lithium aluminium hydride reduction of acetaldehyde azine,[4] and it was found difficult to make this reduction go to completion. If there is an impurity of partly reduced azine in the 1,2-diethylhydrazine, this will lead to the formation of ethylhy-

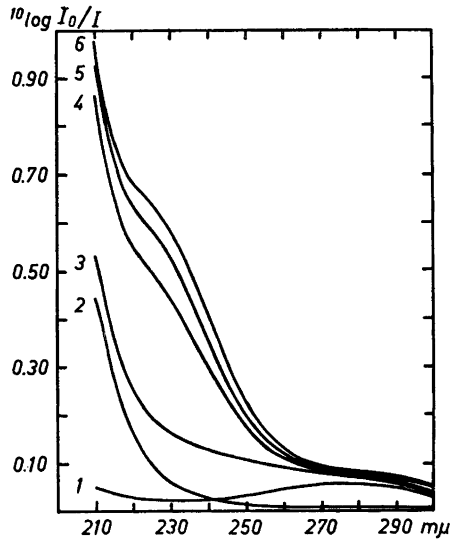


Fig. 1. Showing the spectral changes in a phosphate buffer of pH 8.0 of 1,2-diethylhydrazine and acetaldehyde at 20°. 1. 0.0070 M acetaldehyde. 2. 0.0035 M 1,2-diethylhydrazine. 3-6. 0.0070 M acetaldehyde and 0.0035 M 1,2-diethylhydrazine after 2, 103, 204, and 1980 min, respectively.

drazine during the preparation and purification of the dihydrochloride. In fact, a sample of 1,2-diethylhydraziane dihydrochloride which had been recrystallized only once showed a strong absorption around 230 mμ when mixed with acetaldehyde in equal concentrations in an aqueous buffer of pH 7.2. After three further recrystallizations the spectrum taken 2 min after mixing the solutions was essentially a superposition of the spectra of 1,2-diethylhydrazine and acetaldehyde taken alone, contrary to the findings of Carbon *et al.*[1] (curve 3, Fig, 1).

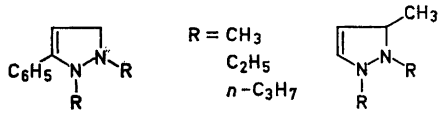
3. The spectrum of an aqueous solution of purified 1,2-diethylhydrazine and acetaldehyde slowly changed upon standing at pH 7.2 or 8.0 at 20°, the change being more rapid at pH 8.0 (curves 3-6, Fig. 1). After standing for 24 h an inflection point at 224 mμ had developed and the spectrum was then only very slowly altered upon further standing. During the first 24 h the spectra of the components measured separately under the same conditions did not change appreciably.

4. When acetaldehyde and 1,2-diethylhydrazine in a concentration ratio of 1:10 were allowed to stand under nitrogen at 100° at pH 8.0, a distinct maximum appeared at 225 mμ, which was fully developed after 72 h at this temperature (log ε_{max} 4.3 calculated from half the original aldehyde concentration and corrected for the spectral changes occurring in solutions of the components after the same treatment). This maximum was fairly well reproducible.

5. Mixing acetaldehyde and free 1,2-diethylhydrazine in a 2:1 molar ratio in petroleum ether produced an immediate separation of water, approximately one molecule/two molecules of acetaldehyde. From this mixture an easily oxidizable liquid was obtained, which according to VPC analysis consisted of a mixture of two compounds in about equal amounts (boiling points very

close to each other) with a contamination of a third compound (~5 %), none of which was identical with 1,2-diethylhydrazine. The compounds all had longer retention times than 1,2-diethylhydrazine. Elemental analyses roughly indicated that the composition of the mixture corresponded to the reaction of one molecule of the hydrazine with two molecules of acetaldehyde with the loss of one water molecule. The ultraviolet spectrum of the mixture in water at pH 7.2 or 8.0 showed a maximum at 226 mμ (log ε_{max} 2.5, calculated from the total concentration of the substance, which was assumed to have a molecular weight of 158, corresponding to the composition given above). The infra-red spectrum showed no significant absorption in the double bond stretching frequency region and no OH or NH stretching frequency band. The substance exhibited almost neutral properties upon titration with standard hydrochloric acid or sodium hydroxide, but on standing for half an hour in aqueous solution a basic substance was formed, which showed an apparent pK_a of 7.5 (The apparent pK_a of 1,2-diethylhydrazine under the same conditions was 7.6).[5] From the solution, acetaldehyde could be isolated in the form of its 2,4-dinitrophenylhydrazone.

It has been shown that 1,2-di-*n*-alkylhydrazines but not 1,2-diisopropylhydrazine can take part in a Mannich reaction with formaldehyde and acetophenone, with subsequent ring closure and elimination of water to a 1,2-di-*n*--alkyl-3-phenyl-Δ^3-pyrazoline (V).[6] In ethanolic solution, V, R = C_2H_5,



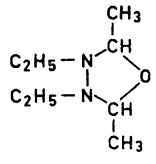
V VI

showed λ_{max} 229 and 288 mμ with logε_{max} 4.1 and 3.6, respectively, where the peak at longer wave-lengths was interpreted as due to conjugation of the phenyl group with the double bond.

A similar Mannich-type reaction is conceivable with 1,2-di-*n*-alkylhydrazines and acetaldehyde, in which case two molecules of acetaldehyde would replace formaldehyde and acetophenone. This reaction would lead to the formation of a 1,2-di-*n*-alkyl-5-methyl-Δ^3-pyrazoline (VI), for which we would predict a maximum in the neighbourhood of 230 mμ, but not at longer wavelengths as there is no aryl group conjugated with the double bond. (Compounds containing the C=C—N chromophore have all been found to have a maximum around 230 mμ [7]).

We interpret the above findings in the following way: In an aqueous solution of acetaldehyde and 1,2-diethylhydrazine there exists a series of rapidly displaceable equilibria, similar to the interaction between aldehydes and water, alcohols, amines, monosubstituted hydrazines, semicarbazides, and hydroxylamine.[8] Upon standing, the equilibrium is slowly and irreversibly shifted towards the formation of a pyrazoline (VI, R=ethyl) which accounts for the strong absorption at 225 mμ. Due to the short reaction period in the preparative experiment, only a small amount of pyrazoline (~5 %) is formed and, instead, compounds resulting from the reaction between one molecule

of 1,2-di-ethylhydrazine and two molecules of acetaldehyde with loss of only one molecule of water are formed. The absence of O—H and N—H absorption bands in the infra-red spectrum of the mixture is compatible with the structure VII which can exist in several stereoisomeric forms. Also, the absence



VII

of any significant absorption in the double bond stretching frequency region can be explained by assuming that the proportion of VI, R=C_2H_5, is small, combined with the fact that the C=C stretching frequency band generally is of low intensity.[9] VII would also be expected to possess almost neutral properties and to be hydrolysed into the original components in aqueous solution, as judged from the behaviour of 1,3-oxazolidines.[10]

In conclusion, model experiments of the kind described here are probably not representative of the reaction responsible for the inhibition of MAO by 1,2-dialkylhydrazines. Instead, the reducing properties of these compounds together with the possibility of radical formation during the oxidation of substituted hydrazines[3] may well account for the irreversible inhibition of MAO by hydrazine derivatives.

EXPERIMENTAL

Materials. 1,2-Diethylhydrazine was prepared by lithium aluminium hydride reduction of ethylideneazine according to Renaud and Leitch.[4] The dihydrochloride was recrystallized four times from isopropanol, neutr. equiv. 161, calc. 161. The acetaldehyde was of commercial quality and was distilled immediately before use.

Methods. Ultra-violet spectra were recorded by a Beckman DK-2 spetrophotometer. All solutions were prepared under nitrogen and in order to further minimize the risk of autoxidation they were made 10^{-5} M in EDTA, which has been shown to inhibit the autoxidation of hydrazines.[3] The long-time experiments were made in sealed, nitrogen-filled ampoules. Titration curves were recorded by an automatic titration apparatus from Radiometer, Copenhagen.VPC analyses were carried out on a column with Carbowax 1500 as a stationary phase. Analyses were carried out at the Department of Analytical Chemistry, University of Lund, Sweden. Analyses for N and O are uncertain due to the difficulty in handling the substance.

Reaction between 1,2-diethylhydrazine and acetaldehyde in light petroleum. Free 1,2-diethylhydrazine (purified *via* the dihydrochloride), 0.05 mole, was mixed with 0.10 mole of redistilled acetaldehyde in 200 ml of light petroleum (b.p. 30—50°). The mixture immediately boiled up for a few seconds and deposited droplets of water. After 3 h, the organic layer was decanted through a filter, leaving behind about 0.05 mole of water. The solvent was distilled off at a pressure of about 100 mm Hg and the residue was distilled *in vacuo*, b.p. 44—5°/10 mm (4.6 g), n_D^{20} 1.4355, d_4^{20} 0.8972, r_D 0.2911. (Found: C 60.8; H 11.4; N about 20; O about 8. Calc. for VII ($C_8H_{18}H_2O$): C 60.7; H 11.5; N17.7; O 10.1; r_D 0.2942. Calc. for VI ($C_8H_{16}N_2$): C 68.5; H 11.5; N 20.0; r_D 0.3170.)

The liquid was very sensitive towards oxygen and air moisture, which made the handling very difficult.

In aqueous solution, the substance, immediately after dissolution, exhibited almost neutral properties against standard hydrochloric acid and sodium hydroxide. After

standing for 30 min, a basic compound had formed with an apparent pK_a of 7.5*, as determined from the pH value at the half neutralization point. The equivalent weight calculated from this titration was 175, whereas VII requires the value 158. In the titrated solution acetaldehyde could be identified as its 2,4-dinitrophenylhydrazone.

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* Under the same conditions, purified 1,2-diethylhydrazine had an apparent pK_a of 7.6.