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Decomposition of methoxamine in aqueous solution: identification of the decomposition products

B. J. MILLARD, D. J. PRIAULX AND E. SHOTTON

The School of Pharmacy, University of London, Brunswick Square, London, WC1N 1AX, U.K.

Combined gas-liquid chromatography-mass spectrometry is used to study the decomposition of methoxamine. The major breakdown product in aqueous solution under air is shown to be 2,5-dimethoxybenzaldehyde.

The mass spectrometer has been used to identify small quantities of medicinal substances, including sympathomimetics (Beckett, Tucker & Moffat, 1967; Reisch, Pagnucca & others, 1968; Reisch, Alfes & others, 1969), and the phenothiazines (Gilbert & Millard, 1969). Drugs and drug metabolites have also been identified using the mass spectrometer in combination with a gas chromatograph (Antony, Brooks & Middleditch, 1970; Capella & Horning, 1966; Gilbert, Millard & Powell, 1970).

We have used a gas chromatograph-mass spectrometer to identify the decomposition products of the sympathomimetic drug methoxamine hydrochloride B.P.C. [2-amino-1-(2,5-dimethoxyphenyl) propan-1-ol hydrochloride] which is unstable to light and heat (*Extra Pharmacopoeia*, 1967). The drug is often administered in aqueous solution and injections have to be sterilized by filtration or by autoclaving under nitrogen (B.P.C. 1968).

METHODS AND RESULTS

Apparatus

A Pye 104 gas chromatograph was used in conjunction with an A.E.I. MS 902 mass spectrometer. A Varian A-60A instrument was used for nmr spectrometry.

Degradation of sample. Methoxamine hydrochloride B.P.C. (5 mg) was dissolved in 0·1M borate buffer (2 ml) adjusted to pH 9, or 0·1M phosphate buffer (2 ml) adjusted to pH 6. These solutions were sealed in 10 ml clear glass ampoules to ensure excess oxygen was available and then stored at 80° in a constant temperature bath for 24 h and 1 week respectively.

Thin-layer chromatography. Thin-layer chromatograms were on Silica Gel G (Merck) layers, 300 μm thick. Developing solvent was the organic layer separated from n-butanol–acetic acid–water (5:1:4). Spots were detected by fluorescence under ultraviolet light (long wave), and by spraying with 0·2% ninhydrin in n-butanol, with colour development for 5 min at 110°.

Methoxamine (R_F 0·59) did not fluoresce but produced an intense red spot with the ninhydrin reagent. The degraded solution contained one main oxidation product (R_F 0·84), which fluoresced strongly under ultraviolet light; no colour developed after treatment with the ninhydrin reagent.

Gas-liquid chromatography–mass spectrometry. The column consisted of 2% SE-52 on Embacel (M & B Kieselguhr) packed in a 5 ft stainless steel column (¼ inch o.d.), column temperature 150°, inlet port temperature 250°, and a helium flow rate of 50 ml/min at 10 psi. The molecular separator, which worked on the opposed jet principle (Ryhage type) was contained within the g.l.c. oven. The inlet line to the mass spectrometer source was kept at 200°. The mass spectrometer had a resolving power of 1000 (10% valley definition) and was running at a source temperature of 220° and a beam energy of 70 eV. The total ion current monitor was used to record the presence of any material passing through the system.

The degraded solution was evaporated to dryness and then dissolved in 0·5 ml of methanol AR before injection into this system. An amount (5 μl) of this solution was injected into the heated inlet port and under the conditions described, one peak was recorded after 4·3 min. The spectrum of this compound was recorded (Fig. 1) as was the background spectrum. On raising the column temperature a second peak appeared; this was the remaining unchanged methoxamine. Both degraded samples of methoxamine produced this same peak, although less degradation product was formed at pH 6.

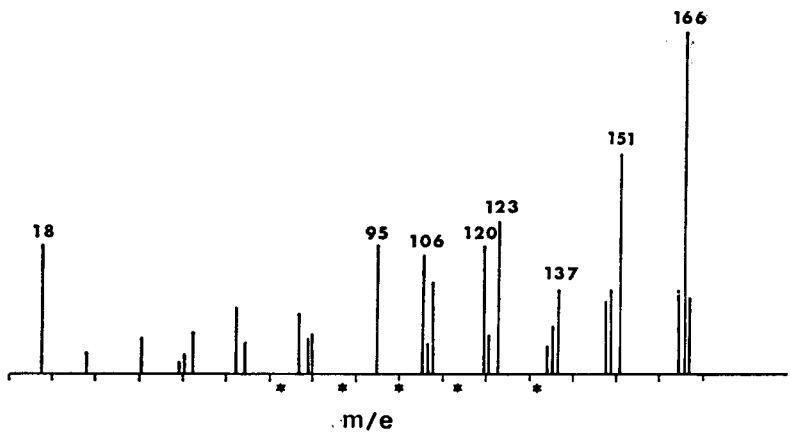


FIG. 1. Mass spectrum of the degradation product. Stars indicate position of metastable peaks.

Mass spectrum of the oxidation product. The molecular ion appearing at m/e of 166, was also the base peak in the spectrum, reflecting the relative stability of aromatic compounds, and corresponded to the aldehyde produced by oxidation of the alcoholic group with a subsequent loss of the amine side-chain. A fragmentation pattern (Fig. 2) for 2,5-dimethoxybenzaldehyde was derived which accounted for all the main peaks and metastable peaks observed. The spectrum was thus evidence for the formation of 2,5-dimethoxybenzaldehyde during the oxidation of aqueous solutions of methoxamine.

A commercial sample of this aldehyde was not available so a larger quantity was degraded, the aldehyde extracted and the nuclear magnetic resonance spectrum measured as conclusive evidence.

Preparation of sample for nmr determination. Methoxamine hydrochloride (100 mg) was degraded as described and the aldehyde separated by passing the solution through a cationic ion-exchange resin packed in a 2 ft chromatography

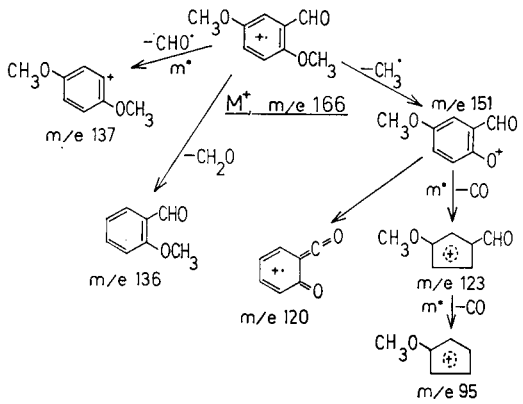


FIG. 2. Fragmentation pattern for 2,5-dimethoxybenzaldehyde.

column. The resin used was Zeokarb '225' 1% D.V.B. 50–100# (Permutit Ltd.). Intact methoxamine and other basic materials were retained on the resin, the aldehyde was then extracted from the eluent with diethyl ether in the presence of excess sodium hydroxide. The solvent was evaporated under reduced pressure, the sample was then dried at 50° for 2 h at 10 mm Hg. This sample produced one spot on the t.l.c. system at R_F 0·84. The melting point was 52° and that of the semicarbazone 208° (Dictionary of Organic Compounds, Vol. II, p. 1053, gives 53° and 208° respectively).

Nmr spectrum of the degradation product (Fig. 3). Chemical shifts were measured as τ values, using tetramethylsilane as an internal reference. The solvent was deuterochloroform, a peak, τ −0·43 (shown as one proton on the integrated curve)

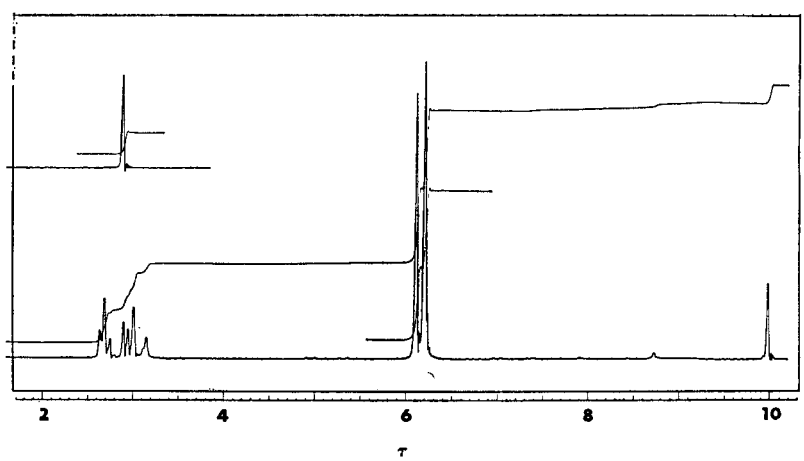


FIG. 3. Nmr spectrum of the degradation product.

is typical of an aldehyde proton resonance, while singlets at τ 6·12 and τ 6·22 represent the two methoxy groups (3 protons each), the aromatic resonance appears in the region τ 3·0 as expected (3 protons on integration). The position of these resonance peaks and the absence of any other proton resonance is confirmation that the major degradation product is a dimethoxybenzaldehyde.

Other oxidation products. A process resulting in the formation of this aldehyde would also be expected to produce acetaldehyde and ammonia, the former substance being itself oxidized to acetic acid. Using suitable t.l.c. and g.l.c. systems, acetaldehyde was not detected in the degraded sample.

A small quantity of acetic acid present in an aqueous solution containing several interfering substances is difficult to isolate, and the g.l.c. method of Emery & Keorner (1961), was used. This consisted of a 3 ft glass column containing 20% Tween 80 on acid-washed, silanized Chromosorb W 80–100♯, maintained at a column temperature of 108°, injection port temperature 200° and a carrier gas flow rate of 20 ml/min at 5 p.s.i. This system easily detected 1 μl of a 0·1% solution of acetic acid, the retention time being 6·1 min. A sample of the degraded solution was acidified (using hydrochloric acid) to liberate the free acid and then chromatographed (Fig. 4).

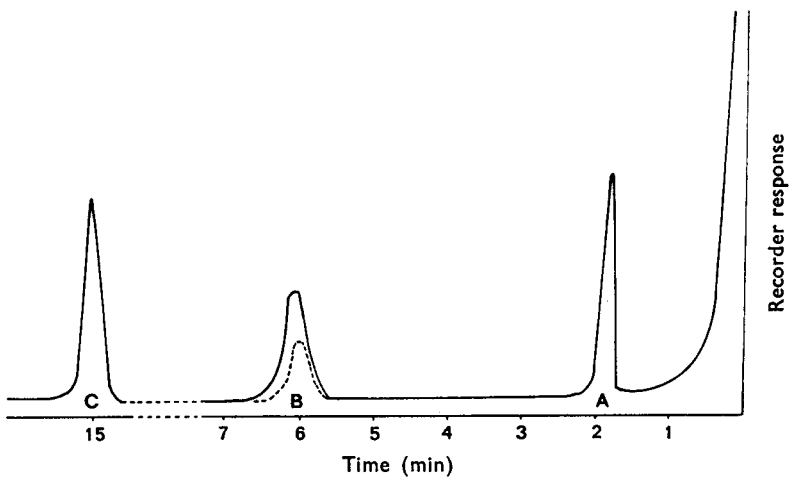
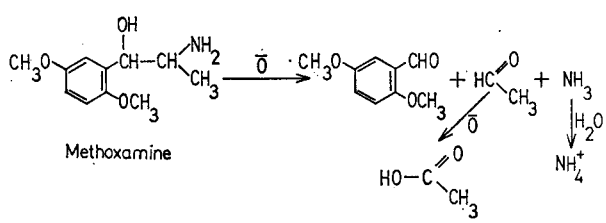


FIG. 4. Gas chromatographic separation of an aqueous sample of degraded methoxamine.

Peak B has the same retention time as acetic acid, a mixed sample showed only one peak. Peak A was due to 2,5-dimethoxybenzaldehyde and Peak C (programming at 4°/min) due to undegraded methoxamine. Due to high bleed, the column could not be used satisfactorily in the g.l.c.-m.s. system.

DISCUSSION

In aqueous solution, methoxamine hydrochloride is degraded by atmospheric oxygen to 2,5-dimethoxybenzaldehyde; this product was identified using a combination of the gas chromatograph and the mass spectrometer. The suggested oxidation pathway is shown below.



Proposed decomposition pathway.

The nmr spectrum of 2,5-dimethoxybenzaldehyde was recorded to confirm identification.

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