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Note

Derivatization of 2-amino-2-oxazolines with trimethylanilinium hydroxide

Determination of reaction products by gas chromatography–mass spectrometry

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The five-membered heterocyclic 2-amino-2-oxazolines have been widely tested for pharmaceutical use[1,2]. Derivatization of these polar compounds is necessary in order to analyse them by gas chromatography (GC)[3,4]. Methylation with a methanolic solution of trimethylanilinium hydroxide (TMAH) is a common derivatization method for the analysis of drugs in biological samples[5,6]; because of the well known tautomerism of 2-amino-2-oxazolines[7–9], derivatization with TMAH gives a mixture of methylated compounds. A secondary solvolysis reaction also occurs, yielding the corresponding 2-oxazolidones. All the products were detected by combined gas chromatography–mass spectrometry (GC–MS) using high-resolution fragmentography.

The stability of 2-amino-2-oxazolines in alcoholic solutions was investigated. When aged or heated solutions are used, N-alkyl-2-oxazolidones are always detected by GC. Their structure was confirmed by MS.

EXPERIMENTAL

Materials and sample preparation

5-Phenoxymethyl-2-amino-2-oxazoline (oxazoline1) was treated with a methanolic solution of TMAH. Oxazoline 1 was synthesized in our laboratory by condensation of monosodium cyanamide with 1-phenoxymethyl2,3-epoxypropane. The structure of oxazoline 1 was supported by elemental analysis, IR and NMR spectral data[10].

TMAH (0.2 *M* trimethylanilinium hydroxide in methanol solution) was obtained from Pierce (Rockford, IL, U.S.A.). Methanol, ethanol, 1-propanol and 2-propanol were of analytical-reagent grade (Merck, Darmstadt, F.R.G.).

Three solutions of the oxazoline 1 were prepared in methanol: A (1 g/l), just

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before analysis; B (0.1 g/l), just before analysis; and C (0.1 g/l), 1 week before analysis.

The methylation procedure was adapted from that of Brochmann-Hanssen and Oke[11]: to 50 μl of solution A, B of C were added 10 μl of the 0.2 *M* TMAH solution, the mixture was vortexed for 30 s and 2 μl were injected into the chromatograph.

For the alcoholysis study, working solutions were prepared by dissolving 100 mg of oxazoline 1 into 100 ml of methanol, ethanol, 1-propanol or 2-propanol, then 10 ml of these solutions were refluxed under nitrogen for 24 h, the alcohol was evaporated under a stream of nitrogen and the residue was dissolved in 50 μl of methanol for injection.

Gas chromatography and mass spectrometry

GC analysis were performed on a Pye 204 gas chromatograph (Pye Unicam, Cambridge, U.K.) equipped with a 25 m × 0.22 mm I.D. CPSil 8 CB (Chrompack, Middelburg, The Netherlands) fused-silica WCOT capillary column. Samples were introduced by split injection at 250°C. The column temperature was programmed from 120°C (maintained for 5 min) to 250°C at 4°C/min. High-purity N55 helium (Dinal, Toulouse, France) was used as the carrier gas.

Electron-impact (EI) mass spectra were recorded on a VG Micromass 70/70 F double-focussing spectrometer (VG Analytical, Manchester, U.K.) with electron energy 70 eV, ionization current 200 μA and source temperature 180°C. The mass spectral recording conditions were as follows: full spectra recorded at low resolution (1000), high-resolution fragmentography (10 000) of molecular ions for oxazoline 1 derivatization.

RESULTS AND DISCUSSION

TMAH methylation

2-Amino-2-oxazolines are tautomeric with 2-iminooxazolines (Fig. 1), and this tautomeric equilibrium leads to structural imprecision[12,13]. The chemical properties of 2-amino-2-oxazolines indicate many reactions in both forms.

Depending on the experimental conditions, methylation of oxazoline 1 gives one monomethylated compound (2) and three dimethylated compounds ($C_{12}H_{16}N_2O_2$) (3, 4 and 5), detected by high-resolution mass fragmentography of their molecular ions (Figs. 2 and 3). The proportions of the methylated products depends on the TMAH concentration. As the TMAH concentration increases the proportions of the dimethylated compounds 3, 4 and 5 increase (Fig. 2b). The tautomeric equilibrium of 2-amino-2-oxazolines explains why both a mono- and two dimethylated compounds (4 and 5) are obtained[7–9]. Similar results were observed when structurally related heterocyclic compounds were methylated with diazomethane or methyl iodide[9,14]. The other dimethylated compound (3) could be assigned to an unsaturated urea caused by thermal opening of the heterocyclic ring. The EI mass spectrum of 3 is characterized by two principal peaks; the first (m/z = 72; 100%) is assigned to the $CON(CH_3)_2$ terminal group and the other (m/z = 127; 75%) might result from elimination of the phenoxy group after allylic scission. The formation of a corresponding unsaturated urea was observed by Tanaka *et al.*[4] on the trifluoroacetylation of 5-phenyl-2-amino-2-oxazoline.

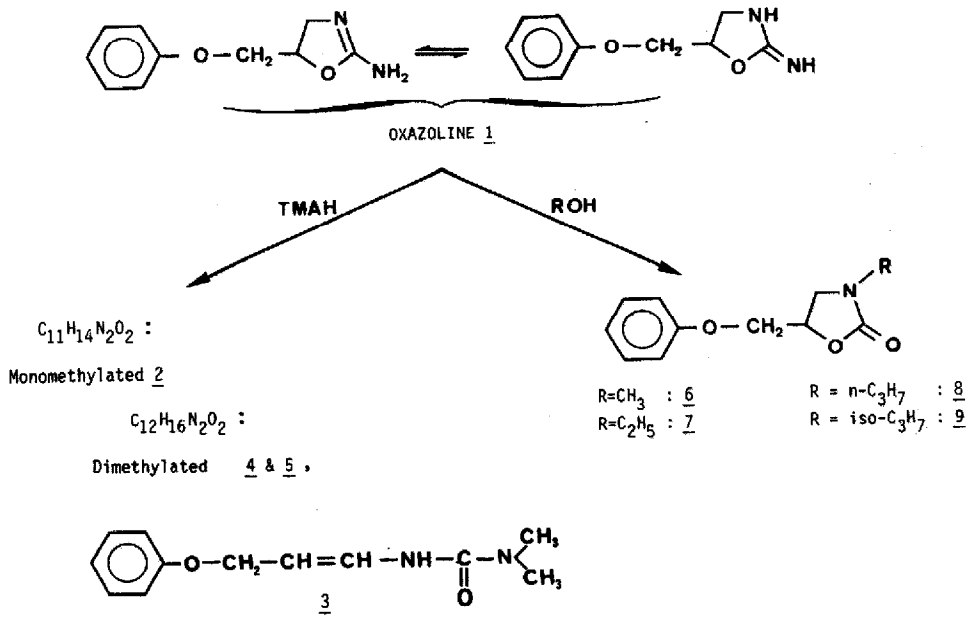
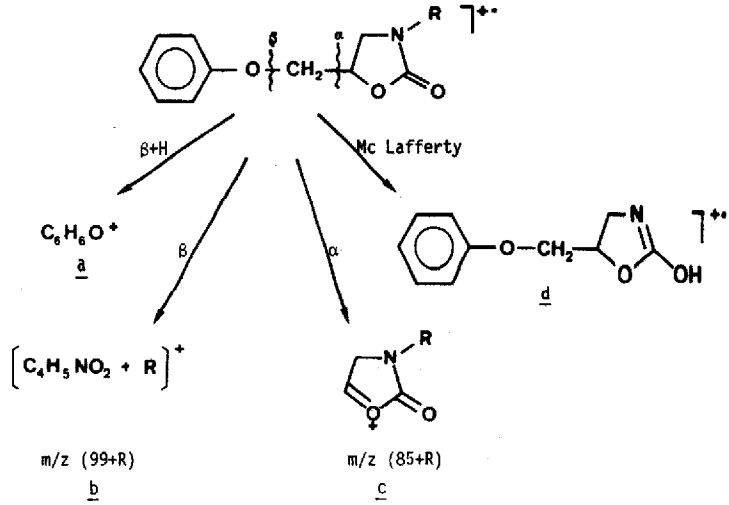


Fig. 1. 5-Phenoxymethyl-2-amino-2-oxazoline: TMAH methylation and solvolysis reaction products.

TABLE I

FRAGMENTATION PATTERN OF 5-PHENOXYMETHYL-3-ALKYL-2-OXAZOLIDONES



Compound	*R*	M^+	*m/z (relative intensity, %)*			
			a	*b*	*c*	*d*
6	CH_3	207 (49)	94 (15)	114 (100)	100 (52)	–
7	C_2H_5	221 (42)	94 (100)	128 (63)	114 (7)	193 (26)
8	*n*-C_3H_7	235 (24)	94 (100)	142 (6)	128 (7)	193 (73)
9	*iso*-C_3H_7	235 (6)	94 (100)	142 (3)	128 (3)	193 (47)

When an aged methanolic solution of oxazoline 1 is derivatized with TMAH, compound 6 is always found (Fig. 2c). This compound was identified as 5-phenoxymethyl-3-methyl-2-oxazolidone. It is found only with aged methanolic solutions and its formation occurs before methylation of oxazoline 1. A chromatogram of a heated methanolic solution of oxazoline 1 without derivatization is shown in Fig. 2d; about 10% of 2-oxazalidone appears after heating for 24 h.

Stability of 2-amino-2-oxazolines

The previous solvolysis reaction is observed when oxazoline 1 is refluxed in ethanol, 1-propanol or 2-propanol; N-substitued 2-oxazolidones (7, 8 and 9) are isolated (Fig. 1). The formation of compounds 6–9 can be explained on the basis of tautomeric 2-aminooxazolidines, with oxidation of the imino group and the introduction of an alkyl group on the endocyclic nitrogen. Comparable behaviour of 2-amino-2-oxazolines was found when they were hydrolysed[1,9].

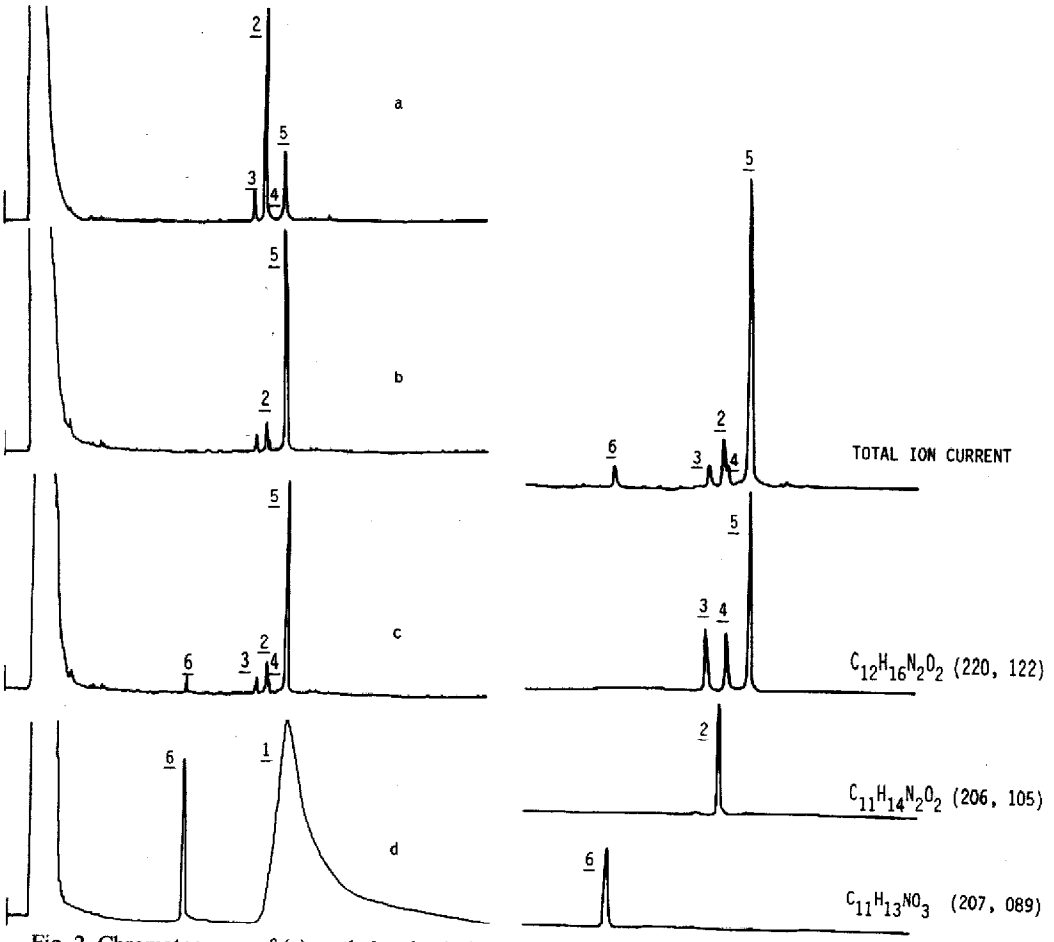


Fig. 2. Chromatograms of (a) methylated solution A; (b) methylated solution B; (c) methylated solution C; (d) heated methanolic solution.

Fig. 3. Methylation products detection by high-resolution mass fragmentography.

Characteristic fragmentation pathways occur in the EI mass spectra of the 2-oxazolidones (Table I), as follows:

(a) phenoxy group-induced fragmentation with an H-rearrangement leading to ion a (m/z = 94), as previously observed with similar compounds[15];

(b) α-side chain scission leading to the conjugated oxonium ion c (m/z = 85 + R);

(c) β-side chain scission leading to ion b (m/z = 99 + R); its structure is certainly homologous with one observed for α-substitued tetrahydrofuran ring extension[16]; and

(d) carbonyl-induced McLafferty rearrangement with elimination of the nitrogen substituent as an alkene (ethylene for 7 and propene for 8 and 9), leading to the same ion d (m/z = 193).

CONCLUSION

The GC analysis of 2-amino-2-oxazolines requires their derivatization. Because 2-amino-2-oxazolines are tautomeric with 2-iminooxazolidines, methylation with TMAH solution leads to a mixture of methylated compounds. The proportions of the derivatization products depends on the experimental conditions. When aged alcoholic solutions are used, a secondary reaction occurs, yielding N-alkyl-2-oxazolidones. The structure of these alcoholysis compounds was established by mass spectrometry. This original side reaction involving solvent should be considered for the quantitative analysis of 2-amino-2-oxazolines.

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