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Identification of new products of S-aminoethylcysteine ketimine autoxidation

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Summary. In continuation of a previous work (Pecci et al., 1993), dedicated to the detection of the autoxidation products of S-aminoethylcysteine ketimine (AECK), we give here data for the identification of 2,3,6,7-tetrahydro-4H-[1,4]thiazino[2,3-b]thiazine, thiomorpholine-3-one and 5,5′, 6,6′-tetrahydro-2,2′-dihydroxy-3,3′-bi-2H-thiazine among the products of AECK autoxidation. Identification has been done on the basis of mass spectrometry and NMR spectral analyses of the isolated products.

Keywords: Amino acids – S-Aminoethylcysteine ketimine – Thiomorpholine-3-one – Dithiazine derivative – Bithiazinyl derivative

Abbreviations: TLC: thin layer chromatography; HPLC: High performance liquid chromatography; AECK: S-aminoethylcysteine ketimine

Introduction

S-aminoethylcysteine ketimine (AECK), recently detected in bovine brain (Nardini et al., 1990), is the product of the enzymatic α-deamination and cyclization of S-aminoethylcysteine (Cini et al., 1978b; Costa et al., 1986). AECK and other sulfur-containing similar ketimines reduce molecular oxygen (Cini et al., 1978a; Pecci et al., 1991) various chemical reagents and some ferrihemoproteins (Solinas et al., 1992). The main product of AECK oxidation by molecular oxygen is the sulfoxide (Pecci et al., 1993; Solinas et al., 1993). Other oxidation products have been detected and other envisaged by the analytical procedures used but not identified. In continuation of this work we report here the isolation and the identification of three new products of the spontaneous oxidation of AECK to be added to those already communicated (Pecci et al., 1993).

Materials and methods

Products

AECK and its decarboxylated dimer (Pecci et al., 1991), thiomorpholine-3-one (Matarese et al., 1984) were prepared as indicated.

Analyses

UV spectra were recorded with a Kontron UVIKON 940 spectrophotometer. ^{1}H NMR and ^{13}C NMR were recorded on a Varian XL-300 spectrometer operating at 300 MHz and 75.43 Mhz, respectively. Mass spectra were determined with a Hewlett-Packard 5980 A operating at 70 eV. Positive Fast Atom Bombardment analysis was carried out on M-Scan's VG AUTOSPEC Mass Spectrometer operating at 8 kV for 4500 mass range at full sensitivity. High performance liquid chromatography (HPLC) was performed as previously described (Pecci et al., 1993). For preparative HPLC the column was a 7.8 × 300 mm Prep Nova-Pak HR C18, 6 micron. The mobile phases were: A, water; B, Acetonitrile-water (70:30, v/v). A linear gradient from A to 100% B was developed in 30 min. Flow rate: 2 ml/min at room temperature. Thin layer chromatography (TLC) was performed on silica gel plates (0.25 mm) using chloroform-methanol (93:7, v/v) as mobile phase. Detection was performed through exposure to I_2 vapours.

Isolation of AECK autoxidation products

A solution of AECK kept for 90 min at 38°C under O_2 bubbling as previously reported (Pecci et al., 1993) was extracted three times with the same volume of chloroform. The combined organic layers were washed with water, dried with anhydrous Na_2SO_4 and evaporated under vacuum. The resulting oily residue (0.4 g starting from 4 g of AECK) was chromatographed on a silica gel (12 g) column, using chloroform-methanol (97:3, v/v) as eluant. The eluted products, located by analytical TLC, were found in three separate fractions which were evaporated to dryness. The first fraction yielded a solid product indicated as P1 (0,05 g) which on TLC appears as a single spot with Rf value of 0.8. The second fraction gave a solid product indicated as P2 (0.035 g) with Rf value of 0.6. The third fraction provided an oily residue which was further purified by preparative HPLC. By the latter procedure two fractions were separated: the first one contained a product too unstable to be isolated, the second fraction, dried under vacuum at 40°C, yielded a solid residue which after crystallization from chloroform gave a product (Rf = 0.5 on TLC) indicated as P3 (0.15 g).

Results

The HPLC elution profile of the solution of AECK at the end of autoxidation reported in the previous paper (Pecci et al., 1993), indicates, beside the main peak running fast in the chromatogram and identified as the AECK sulfoxide, the presence of a group of compounds absorbing at 250 nm with relatively high retention times. We have now paid attention to this group of compounds. As first step of isolation of these products, the final autoxidation solution of AECK has been extracted with chloroform. Figure 1 shows the HPLC elution pattern of the organic extract. With the addition of authentic samples only the peak of the decarboxylated dimer (D) of AECK could be identified. Purification of the organic extract by the procedure reported in Materials and

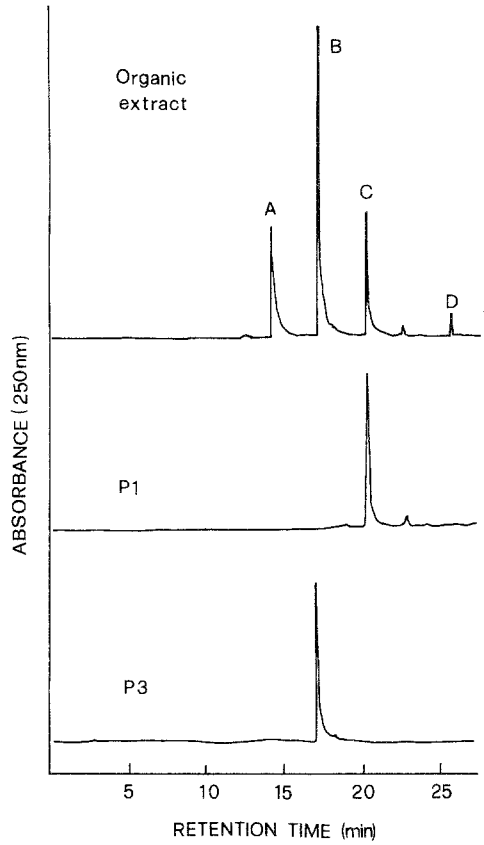


Fig. 1. HPLC elution profile of the organic extract of the oxidized AECK solution and of the isolated products *P1* and *P3*. See text for details

methods afforded three products indicated as P1, P2 and P3. HPLC analysis shows that P1 correspond to peak C and P3 to peak B of the chromatogram of the chloroform extract (Fig. 1). P2 was not detectable in the chromatogram because not absorbing at the wavelength used. Isolation of the component corresponding to peak A of the chromatogram was unsuccessful due to the chemical lability of this component. The absorption spectra of the isolated compounds are reported in Fig. 2. Thus the formation of P1 and P3 contribute to the absorbance at 250 nm appearing in the course of AECK autoxidation (Pecci et al., 1993).

Spectral analysis of P1

The spectral data of compound P1 are in agreement with the structure of the dithiazine derivative reported in Fig. 3, **I**. In particular the mass spectrum shows the molecular ion at m/z 174 (Fig. 4A); ^{13}C NMR spectrum in $CDCl_3$ (Fig. 5A), supported by attached proton test (APT) experiments (Patt et al., 1982), reveals the presence of five proton-bearing sp^3 carbon atoms, one of them consistent with the CH group ($\delta = 68.63$ ppm) and four of them attributable to the CH_2 groups ($\delta = 32.71, 35.93, 52.85, 64.22$ ppm). Furthermore

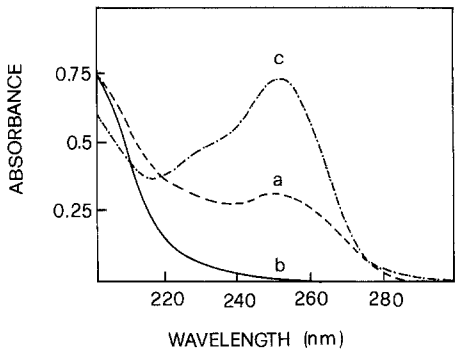
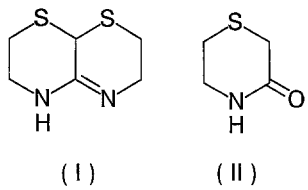
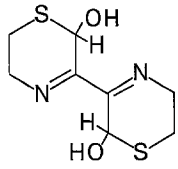


Fig. 2. Absorption spectra of P1 (*a*), P2 (*b*) and P3 (*c*)



(I) (II)



(III)

Fig. 3. Structures of P1 (2,3,6,7-tetrahydro-4H-[1,4]-thiazinol[2,3-b]thiazine) (**I**), P2 (thiomorpholine-3-one) (**II**) and P3 (5,5′,6,6′-tetrahydro-2,2′-dihydroxy-3,3′-bi-2H-thiazine) (**III**)

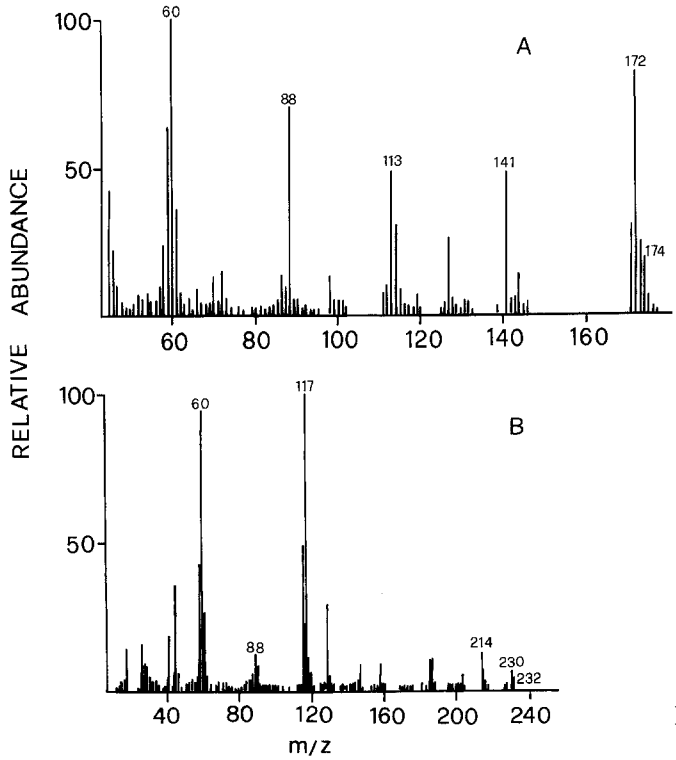


Fig. 4. Mass spectra of P1 (**A**) and P3 (**B**)

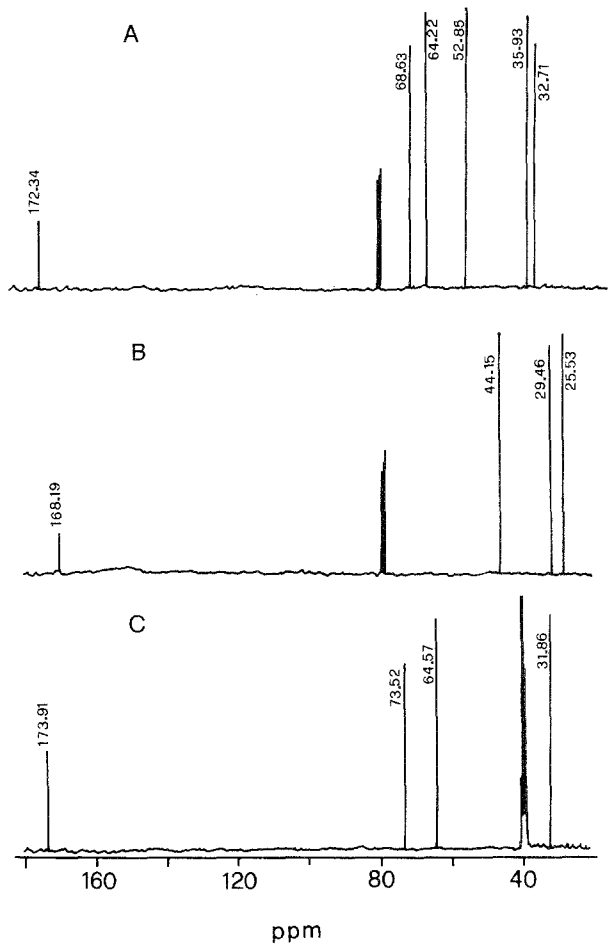


Fig. 5. ^{13}C NMR spectra of P1 (**A**), P2 (**B**) and P3 (**C**). Chemical shifts are given in δ ppm from TMS

the ^1H NMR spectrum ($CDCl_3$) shows the CH group as a singlet resonating at low field ($\delta = 5.2$ ppm) and an exchangeable proton consistent with the NH group is present as broad triplet ($\delta = 2.4$ ppm). Other signals present in the ^1H NMR spectrum: $\delta = 2.9$ (m, 2H), 3.15–3.4 (m, 4H), 4.2 ppm (m, 2H).

Spectral analysis of P2

The spectral data of compound P2 are in accordance with the structure of thiomorpholine-3-one (Fig. 3, **II**). ^{1}NMR ($CDCl_3$) $\delta = 2.7$ (m, 2H), 3.2 (s, 2H), 3.5 (m, 2H), 7.2 ppm (broad, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 25.53$, 29.46, 44.15, 168.19 ppm (Fig. 5B). Moreover these data were identical with those of an authentic specimen prepared as previously reported (Matarese et al., 1984). The presence of thiomorpholine-3-one among the products of AECK autoxidation was already reported (Pecci et al, 1993) on the basis of detection

of carboxymethylcysteamine on the HCl hydrolysate of the final oxidation solution of AECK.

Spectral analysis of P3

The spectral data of compound P3 are in accordance with the dimeric structure (bithiazinyl derivative) reported in Fig. 3, **III**. The mass spectrum (Fig. 4B) reveals a molecular ion at m/z 232 and other fragmentation ions including m/z ion at 117 indicating the monomer derived from fragmentation of the dimer. Particularly informative is the fast atom bombardment (FAB) mass spectrum which shows the molecular ion as base peak ($MH^+ = 233$). ^{13}C- and ^1H-NMR spectra taken in $(CD_3)_2SO$ and in $CDCl_3$ reveal the presence of only one set of resonances for the two heterocyclic six-membered rings, indicating that this compound adopts in solution a symmetric conformation. In particular in the ^{13}C-NMR spectrum [$(CD_3)_2SO$] (Fig. 5C) a characteristic signal attributable to the hemithioacetal structure is present at $\delta = 73.52$ ppm and a non-protonated sp^2 carbon atom is centered at $\delta = 173.91$ ppm. Furthermore in the ^1H NMR spectrum [$(CD_3)_2SO$] coupling is observed between the exchangeable proton at $\delta = 5.8$ (d, J = 6.0 Hz) and the CH group at $\delta = 4.5$ (d, J = 6.0 Hz) as well as between the two CH_2 groups resonating at $\delta = 3.15$ and 4.15 ppm respectively.

Discussion

The analytical data obtained for the three compounds isolated are consistent with the structures reported in Fig. 3. The known tendency of AECK to dimerize is overemphasized by the detection of the P1 and P3 products, to be added to the carboxylated and decarboxylated dimers previously described (Hermann, 1961; Pecci et al., 1991). Since AECK is expected to be a normal biological product (Nardini et al., 1990) and autoxidation, eventually speeded up by biological catalysts (Solinas et al., 1992), is assumed to occur also in vivo it is likely that the products identified in this work could have some biological relevance. It is of interest at this regard that the decarboxylated AECK dimer has been recently found to be a reversible inhibitor of the mitochondrial respiration (Pecci et al., 1994). Comparison of the new products described here with the effect of the decarboxylated dimer is therefore a demanding question to answer.

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