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A NOVEL TRICYCLIC PYRAZINE FROM LYSINE HYDROCHLORIDE PYROLYSIS

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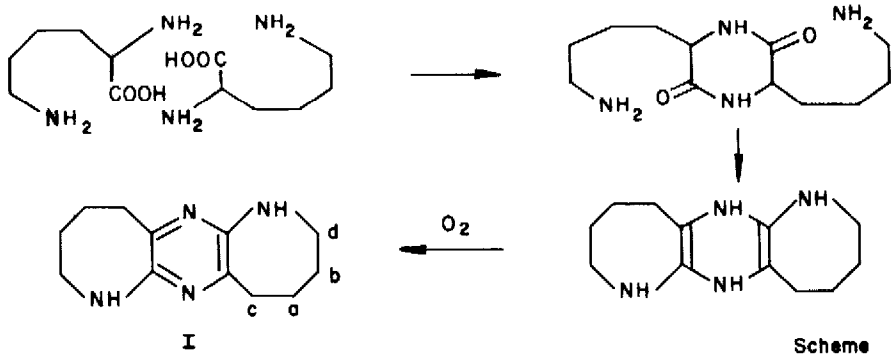
Summary: The pyrolysis of lysine hydrochloride at 600° yields a novel ditetrahydroazepinopyrazine (I).

Investigations in several laboratories have confirmed the formation of chemical mutagens on pyrolysis of amino acids, peptides and proteins[1]. Some of these mutagens are nitrogen heterocycles[2]. Our own studies on lysine hydrochloride pyrolysis have now revealed the formation of a novel tricyclic pyrazine (I) which, although non-mutagenic, is capable of inhibiting microsomal aryl hydrocarbon hydroxylase activity[3].



I Scheme

Lysine hydrochloride (200 g, in 10-15 g portions) was pyrolyzed for 8-10 minutes at 600° under a stream of air. The volatile products were collected in a Dry Ice-acetone trap and extracted with chloroform-methanol (1:1). The basic (nitrogenous) mutagenic and nonmutagenic products were isolated from this extract as chloroform soluble dark oily residue by standard acid base extractions[4]. The product (I) was isolated as ether soluble fraction from this oily residue. It was further purified by recrystallization from methanol and silica gel chromatography (3% methanol in dichloromethane as eluent). The product (2 mg) is a pale yellow solid which sublimes at 175-180° with decomposition and gives a single peak on GC analysis.

The GC-MS of this product shows a strong M^+ peak at 218 m/e, and the GC-CI/MS, a strong $M+1^+$ peak at 219 m/e[5]. Elemental analysis (Galbraith Lab., Knoxville, Tenn.) gave C: 66.11%; H: 8.02%;

N: 25.63%, consistent with the molecular formula $C_{12}H_{18}N_4$ (Calc. C: 66.02%; H: 8.31%; N: 25.66%). The UV spectra in chloroform: λ_{352} (ε = 6526), λ_{260} (ε = 8437) and in methanolic HCl: λ_{413} (ε = 2717), λ_{336} (ε = 3729), λ_{260} (ε = 8657) indicate lack of extended conjugation and incorporation of the nitrogen atoms in the chromophoric system.

The proton decoupled FT-^{13}C nmr spectrum (in D_2O, pH 2) demonstrates the presence of six types of carbon atoms, while the proton coupled spectrum shows that four of the carbons are present as CH_2 groups (a: δ=21.0ppm, J_{CH}=131Hz; b: δ=25.8ppm, J_{CH}=125Hz; c: δ=30.5ppm, J_{CH}=129Hz; d: δ=44.2ppm, J_{CH}=142Hz). The other two carbon atoms are olefinic with no attached protons (e: δ=140.7ppm; f: δ=141.8ppm). All six signals are of equal intensities. The 360MHz FT-^{1}H nmr spectrum (in $CDCl_3$) shows the presence of a secondary amino group (δ=4.28ppm) in addition to four methylene signals, which are present as complex multiplets (chemicals shifts: a: δ=1.59ppm; b: δ=1.80ppm; c: δ=2.78ppm; d: δ=3.05ppm). The relative intensities are NH:a:b:c:d = 1:2:2:2:2. Assignments of these signals to individual CH_2 groups were based on the following homonuclear proton-decoupling experiments (^{1}H nmr): When the amine resonance was irradiated, only signal d was affected, resulting in the simplification of its splitting pattern. Irradiating signals a or b caused signals c or d, respectively, to be reduced to singlets. Irradiation of signal a has no effect on signal d, and irradiation of signal b does not affect signal c. These results are in accord with the linkages of NH-CH_2(d)-CH_2(b) and also CH_2(a)-CH_2(c). Both the ^{1}H and ^{13}C nmr spectra indicate a highly symmetrical structure.

As shown in the scheme, we propose that I is formed by initial conversion of lysine to a diketopiperazine which, under the conditions of acid catalysis and thermolysis, undergoes intramolecular cyclization. The intermediate tricyclic condensation product is then aromatized by air oxidation.

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4) The mutagenicity was tested by Ames test on Salmonella strain TA98. Studies on mutagenic products are in progress.

5) The GC-MS and GC-CI/MS were performed on an HP5985 instrument.

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