Communications to the Editor

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A Potent Mutagen isolated from a Pyrolysate of L-Ornithine

A potent mutagen was isolated from a pyrolysate of L-ornithine. The mutagenic activity of this compound on *Salmonella typhimurium* TA 98 was 28400 revertants/0.5 μg. This compound was deduced to be 4-amino-6-methyl-1*H*-2,5,10,10b-tetraazafluoranthene by X-ray crystallographic analysis.

Keywords——L-ornithine; bacterial mutagen; pyrolysate; azafluoranthene derivative; X-ray structural analysis

Recently many mutagenic compounds have been isolated from cooked foods and their structures have been determined.[1–3] These compounds are 3-amino-1-methyl- and 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indoles (Trp–P-1 and Trp–P-2, respectively) isolated from a pyrolysate of L-tryptophan,[4] 2-amino- and 2-amino-6-methyldipyrido[1,2-*a*: 3′,2′-*d*]imidazoles (Glu–P-1 and Glu–P-2) from a pyrolysate of L-glutamic acid,[5] 2-amino- and 2-amino-3-methylpyrido[2,3-*b*]indoles from a pyrolysate of soy bean globuline,[6] 3,4-cyclopentenopyrido[3,2-*a*]-carbazole (Lys–P-1) from a pyrolysate of L-lysine,[7] and 2-amino-3-methyl- and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinolines (IQ and MeIQ) from broiled sardines.[8] All these compounds except Lys–P-1 are heteroaromatic amines with three fused rings. The present paper reports the isolation of a mutagenic tetracyclic heteroaromatic amine from a pyrolysate of L-ornithine hydrochloride.

A tolal of 5 kg of L-ornithine hydrochloride was pyrolyzed in a flask over a direct flame and the products were collected as in preceeding studies.[4–9] The basic fraction of tar amounted to 600 g. During purification of the mutagen, the mutagenic activity of fractions was tested on *Salmonella typhimurium* TA 98 in the presence of a metabolic system, S-9 mix,[10] from the liver of rats treated with polychlorinated biphenyls. The basic mixture was separated by counter current distribution, and chromatographies on Sephadex LH 20, alumina and silica gel columns. Detailes of the chromatographic procedures will be described elsewhere. A chromatographically pure fraction was obtained in a yield of about 0.5 mg, and was crystallized as its hydrobromide from ethanol. In two tests on *Salmonella typhimurium* TA 98 the preparation induced 28400 and 28200 revertants/0.5 μg. The mutagenicity is as high as that of Trp–P-2. Though the recovery of total activity was low, this compound seems to be a major mutagen in the pyrolysate, judging from the activity distribution at each step of purification.

The mass spectrum of the hydrobromide showed a peak at *m/e* 237. The high resolution mass spectrum suggested that the molecular formula of the compound is $C_{13}H_{11}N_5$ (observed 237.009, calcd 237.1014).[11] X-Ray crystallographic analysis was performed using a crystal of $0.45\times0.12\times0.008$ mm. The space group of the crystal of the hydrobromide is orthorhombic $Pna2_1$ with four molecules per unit cell. The cell dimensions are $a=11.984$, $b=16.739$ and $c=6.695$Å. The independent structure factors obtained were 802. The structure of the compound was determined by the heavy atom method and refined by the method of block-diagonal least-squares. The final R value for all the structual factors, including anisotropic temperature factors for non-hydrogen atoms and isotropic ones for hydrogen atoms, was 0.072. Atomic species were assigned by isotropic temperature factors, bond lengths and the locations of hydrogen atoms, though some hydrogen atoms were difficult to find in a difference electron density map because insufficient numbers of structural factors were observed. The geometry of the molecule as well as its intra- and intermolecular atomic bond distances (Fig. 1) were of great help in the assignment. The structure was deduced to be 4-amino-6-methyl-1*H*-2,5,10,10b-tetraazafluoranthene (Fig. 2).[11] This compounds is tetracyclic, but it also con-

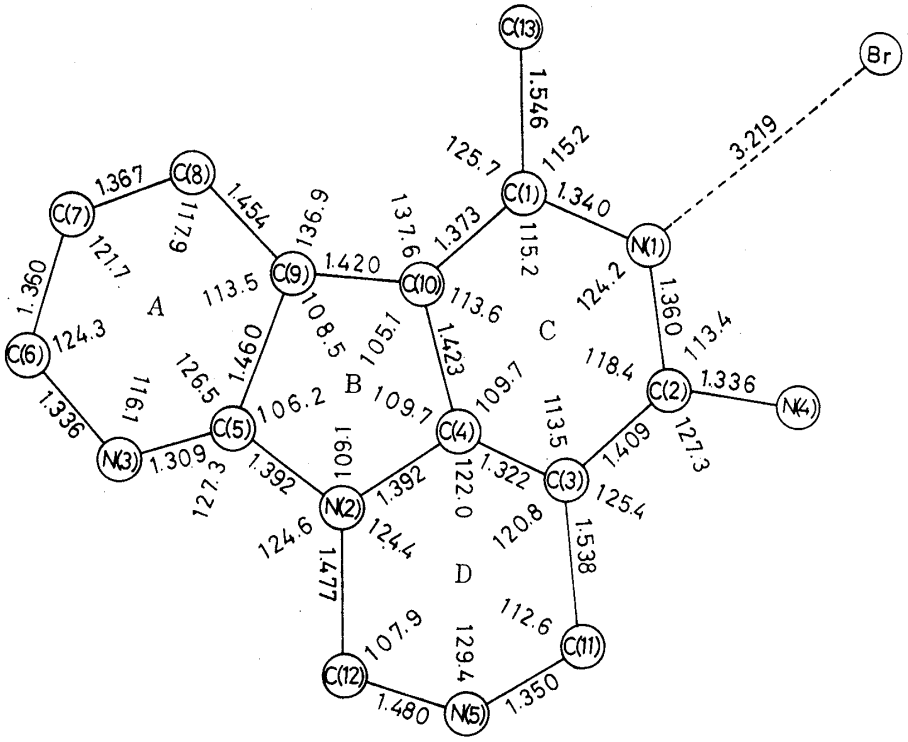

Fig. 1. Bond Lengths (Å) and Angles (°) of the Mutagenic Principle in a Pyrolytic Product of L-Ornithine

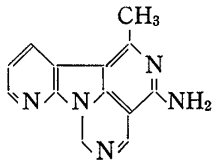

Fig. 2. The Chemical Structure of the Mutagenic Principle in a Pyrolytic Product of L-Ornithine

tains an aminopyridine moiety which has been shown to be the most important group of this type of mutagen.[12] The final determination of the structure and its exact biological activity will be possible after total synthesis of the compound.

References and Notes

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11) An unidentified peak at *m/e* 238 was observed, which is consistent with the formula $C_{14}H_{14}N_4$. This suggested that the D ring has a trimethylene bridge instead of C–N=C. However, data on bond lengths obtained by X-ray crystallography are incompatible with the assignment.
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New Methods and Reagents in Organic Synthesis. 14.[1] A Simple Efficient Preparation of Methyl Esters with Trimethylsilyldiazomethane ($TMSCHN_2$) and Its Application to Gas Chromatographic Analysis of Fatty Acids

Trimethylsilyldiazomethane ($TMSCHN_2$), known as a stable and safe substitute for highly toxic and explosive diazomethane in the Arndt–Eistert synthesis and homologation of carbonyl compounds, has smoothly reacted with various carboxylic acids in methanolic benzene solution to give the corresponding methyl esters in excellent yields.

Keywords——trimethylsilyldiazomethane; carboxylic acid; methyl ester; esterification; fatty acid; gas chromatographic analysis

Preparation of methyl esters of carboxylic acids, especially for analytical purposes, has been generally carried out[2] by the reaction of carboxylic acids with (1) methanol in the presence of acidic catalysts such as sulfuric acid, hydrogen chloride, boron trifluoride *etc.* which are mostly corrosive, (2) diazomethane which presents an explosive hazard in addition to its high toxicity, or (3) dimethylformamide dimethylacetal or trimethylphenylammonium hydroxide which requires heating for esterification.

We have reported recently that trimethylsilyldiazomethane ($TMSCHN_2$),[3] which is a stable and safe substitute for hazardous diazomethane, can be efficiently used for the Arndt–Eistert synthesis[4] and homologation of ketones[5] and aldehydes.[6] We now wish to report that the reaction of $TMSCHN_2$ with carboxylic acids in the presence of methanol quickly gives methyl esters in excellent yields at room temperature and the method can be efficiently applied to analytical works such as determination of carboxylic acids by gas chromatography:

$$RCO_2H \xrightarrow[CH_3OH \text{ at room temp.}]{(CH_3)_3SiCHN_2} RCO_2CH_3$$