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Note

Quantitative micro-determination of 2,6-pipecoloxylidide by gas–liquid chromatography

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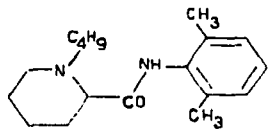
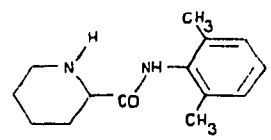
Recently, 2,6-pipecoloxylidide (PPX) was detected by gas–liquid chromatography (GLC)[1] in blood and urine samples of patients anaesthetized with bupivacaine (Marcaine®). However, the use of a flame ionization detector limited the determination of this metabolite to a level of about 50 ng. With the aim of lowering the detection limit to the sub-nanogram level, an attempt was made to prepare derivatives with high electron affinity, suitable for electron capture detection. Acylation with heptafluorobutyric anhydride (HFBA), as described by various workers[2,3], was found to be inapplicable, as inconsistent and irreproducible results were observed throughout all the experiments.



Bupivacaine



PPX

The purpose of this paper is to explain this unusual behaviour and to propose a modification of the general method which allows the quantitative micro-determination of PPX.

A primary acylation product was synthesized on a preparative scale and its structure elucidated by means of elemental and spectroscopic analyses. It could not be quantitatively determined by GLC. However, when treated with an aliphatic alcohol it affords a new acylated compound, which was found to be suitable for GLC determination. The structure of this second product was also elucidated.

EXPERIMENTAL*

Preparation and analysis of acylation products

Primary acylation. A 1-ml volume of heptafluorobutyric anhydride (Macherey, Nagel & Co., Düren, G.F.R.) was added to a solution of 50 mg of 2,6-pipecoloxylidide (AB Bofors, Mölndal, Sweden) in 1 ml of ethyl acetate. The mixture was heated in a water-bath at 70° for 30 min, cooled, washed with saturated sodium

* Elemental analysis performed by Janssen Pharmaceutica, Beerse, Belgium.

hydrogen carbonate solution in order to eliminate the excess of reagent, then extracted with diethyl ether. The ethereal solution was washed with water, dried over sodium sulphate and evaporated to dryness. The residue was purified by preparative thin-layer chromatography on silica gel with a solvent mixture of diethyl ether and *n*-hexane (1:3). An oil was obtained ($R_f = 0.52$), which crystallized very slowly. The composition based on the formula $C_{22}H_{18}F_{14}N_2O_3$ was: C 42.32% (experimental 42.09%); H 2.91% (experimental 3.25%); F 42.60% (experimental 43.14%).

Alcoholysis of the primary acylation product. A 1-ml volume of methanol was added to 50 mg of the purified primary acylation product and the mixture evaporated to dryness at 50° to yield white crystals (corrected m.p. 178°). The composition based on the formula $C_{18}H_{19}F_7N_2O_2$ was: C 50.47% (experimental 48.84%); H 4.47% (experimental 4.40%); F 31.05% (experimental 29.31%). The same compound was obtained by treating the primary product with ethanol, *n*-propanol and *n*-butanol.

Infrared and mass spectrometry

A Perkin-Elmer 237 grating infrared (IR) spectrometer was used and mulls of isolated material were prepared with Nujol. An AEI-MS12 mass spectrometer was used with a ionization energy of 70 eV and a chamber temperature of 100°.

GLC determination

Equipment. The apparatus used for this work was a Hewlett-Packard Model 5750 gas chromatograph equiped with an electron capture detector (^{63}Ni) and a 1-m spiral glass column (3.5 mm I.D.). The column was packed with 3% OV-17 on Gas-Chrom Q, 100–120 mesh (Applied Science Labs., State College, Pa., U.S.A.). The detector was maintained at 300° while the column temperature was 200° and the injection port temperature 250°. The pressure and flow-rate of nitrogen carrier gas were 2.5 kg/cm^2 and 35 ml/min, respectively.

Analytical procedure. The extraction of urine and blood samples was carried out by the method described by Reynolds[1]. The residues were treated with a solution of 0.2 ml of HFBA in 1 ml of ethyl acetate in a water-bath at 70° for 30 min. The reaction mixture was cooled and treated with 2 ml of water saturated with sodium hydrogen carbonate. The aqueous solution, which had a pH between 7.5 and 8.0, was extracted three times with 5 ml of peroxide-free diethyl ether, and the organic phases were combined, washed with 3 ml of water, dried by freezing the water in a cooling bath of solid carbon dioxide and acetone, followed by centrifugation. The ethereal solution was then poured into a ground-glass stoppered Pyrex conical tube, treated with 0.5 ml of methanol and evaporated to dryness at a temperature not exceeding 40°. The final residue was dissolved in a benzene solution of 800 ng/ml of the internal standard (heptafluorobutyrylbenzoctamine). The injected amounts of acylated PPX ranged from 0.5 to 3 ng, corresponding to 0.26 to 1.56 ng of PPX. The calibration curve for the determination was constructed by plotting the ratio of peak heights of PPX and internal standard against the weight ratio of these two compounds, whereby good linearity was observed. The accuracy of the determination was ±3% for amounts of PPX ranging from 0.5

to 1.5 ng and ±5% for 0.25 ng of PPX. Careful siliconization of the tubes reduced the adsorption losses to a considerable extent.

RESULTS AND DISCUSSION

The IR spectrum of the primary product shows CO vibrational absorption bands at 1725 and 1680 cm^{-1}. The NH deformation band that appears at 1540 cm^{-1} in PPX is missing. This observation suggests an acyclic imide structure, CO–NR–CO[4,5]. Moreover, the elemental analysis and the mass spectrum indicate the presence of two heptafluorobutyryl groups in one molecule (Fig. 1). The ions at *m/e* 308 and 280 correspond to the acylated pipecolyl moiety and its product of decarbonylation, whereas the peak at *m/e* 344 is thought to arise as a result of the breaking of the bond between the imide group and the heterocyclic ring. Those results provide strong evidence of the structure of a diacylated PPX, as shown in Fig. 1.

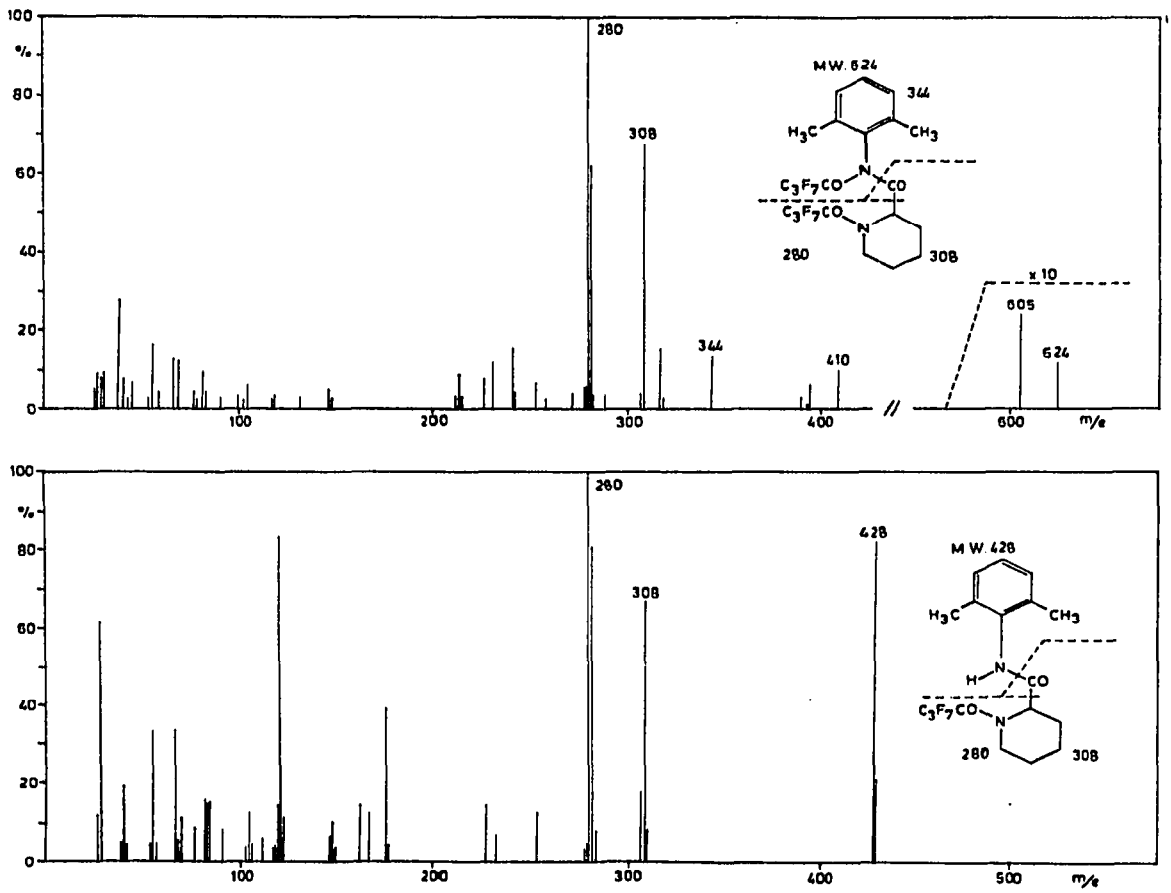


Fig. 1. Mass spectrum of di- and monoacylated products of PPX.

The diacylated PPX, when treated with an alcohol, undergoes rapid and quantitative solvolysis to yield the monoacylated PPX. Its IR spectrum does not show the imide stretching at 1725 cm^{-1} but shows an absorption band at 1530 cm^{-1}, which has been assigned to the NH bending of a secondary amide structure. The elemental analysis and the mass spectrum agree with the presence of a single heptafluorobutyryl group. The ion-fragments at *m/e* 308 and 280 are present but the peak at *m/e* 344 is missing. The product is thus a monoacylated PPX with the structure shown in Fig. 1.

In order to interpret the unusual one-step acylation of the very dissimilar amine and amide groups of PPX, the reaction conditions should be considered. Owing to the fact that HFBA was used without any base catalyst, the presence of a small amount of free acid is inevitable. The amine function of PPX is partially transformed into its conjugated acid, with the result of a decrease in its reactivity towards the anhydride. On the other hand, the trace amount of acid is likely to increase the reactivity of the anhydride towards the amide function, as observed previously[6], so that both the amine and amide are acylated simultaneously. The diacylated PPX contains an imide structure, which is liable to react with an alcohol to yield one amide molecule and one ester molecule.

CONCLUSION

The transformation of PPX into a derivative with high electron affinity by means of HFBA is a useful method for increasing the selectivity and the sensitivity of the chemical determination of PPX by GLC to the sub-nanogram level. However, in order to obtain successful results, the preparation of the derivative requires the treatment of the primary acylation product with an alcohol. This procedure permits the determination of PPX and possibly other aminoacylamine drugs and metabolites in small samples of biological fluid, such as foetal blood or the urine of new-borns.

ACKNOWLEDGEMENTS

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