Short Communication

Quantitative Analysis of Emepronium Bromide by Demethylation and Selected Ion Monitoring GC/MS

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Emepronium bromide, *N,N*-dimethyl-*N*-ethyl-4,4-diphenyl-2-butylammonium bromide (Cetiprin®, **1**), is registered as a drug for the treatment of motor urge incontinence. In our studies on its bioavailability and biotransformation, a method was needed for converting the quaternary ammonium compound to GLC-suitable derivatives. Recently, a method for dealkylation of quaternary ammonium salts to tertiary amines has been developed[1] which could be used for demethylation of compounds isolated in small quantities from blood serum by ion-pair extraction.[2]

The demethylation of **1** using L-Selectride (lithium tri-*sec*-butylborohydride) in tetrahydrofuran was studied at 55 and 75 °C (Fig. 1). The reaction was performed on a deuterated analogue, *N,N*-dimethyl-*N*-ethyl-4,4-diphenyl-2-butyl-(1-d_3)-ammonium bromide (**2**) using demethylated **1**, i.e. *N*-ethyl-*N*-methyl-4,4-diphenyl-2-butylamine, as internal standard. The yield of the demethylation reaction was monitored by GC/MS using the selected ion monitoring (SIM) method. Maximum yield was obtained at 75 °C after only 10 min, and no loss of product seemed to occur on prolonged treatment; demethylation at 55 °C proceeded much slower. A neutral product, 4,4-diphenylbutan-2-ol, was detected in the diethyl ether extract of the reaction mixture after addition of sulfuric acid. According to liquid scintillation results using 1-^{14}C-labelled **1**, 10 % of the material was extracted into the diethyl ether phase. Formation of the alcohol could account for the yield of the demethylated product being only 90 %.

The total yield of product obtained through ion-pair extraction and demethylation including all purification steps was approx. 75 %, estimated by liquid scintillation analysis of serum containing 1-^{14}C-labelled **1**.

The method was tested using serum to which known quantities (5 to 20 ng ml^{-1}) of **1** and deuterated analogues as internal standards had been added. As no degradation seemed to occur on prolonged treatment, 20 min at 75 °C was chosen as the reaction conditions for serum samples. A linear correlation was obtained on plotting peak height ratios vs. weight ratios. In the analysis, a relative standard deviation of 9.5 % at a serum concentration of 10 ng ml^{-1} was obtained.

The choice of a standard deuterated at a position which does not influence the demethylation reaction, and which will give reaction by-products to the same extent as the parent compound, is a prerequisite for good accuracy of the method.

Good selectivity of the method is obtained using the SIM technique in combination with the ion-pair extraction step. No interference from emepronium metabolites in the serum will occur as these will be separated during the gas chromatography step and will also give different mass spectra. Furthermore, quantification of the different metabolites can be achieved with the described method by monitoring appropriate *m/z*-values.

The method has successfully been used in stud-

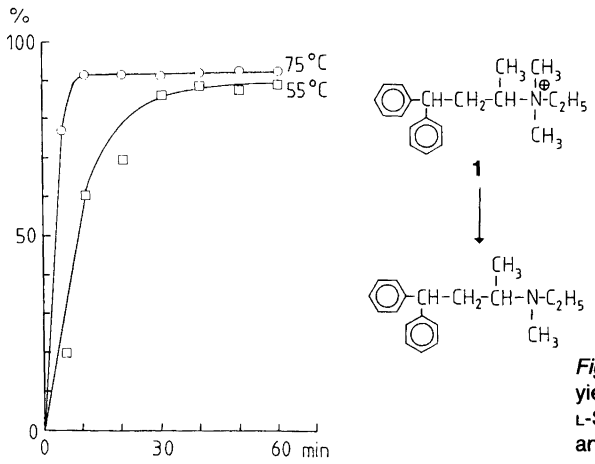

Fig. 1. Temperature and time dependence of the yield of demethylation of emepronium bromide (**1**) by L-Selectride in tetrahydrofuran using a 1-CD_3 analogue.

ies of the bioavailability of **1** in dogs, where serum concentrations of ≥3 ng ml^{-1} could be analysed. Various hydroxylated metabolites of **1** have also been identified by GC/MS using the described demethylation method and silylation of the hydroxy groups.

Experimental

Solvents and chemicals were of analytical grade. L-Selectride (1M in tetrahydrofuran) was purchased from Aldrich Chemical Company (Milwaukee, Wisconsin).

Deuterated analogues of **1**, i.e. *N,N*-dimethyl-*N*-ethyl-4,4-diphenyl-2-butyl-(1-d_3)ammonium bromide (**2**), *N,N*-dimethyl-*N*-ethyl-(d_5)-4,4-diphenyl-2-butylammonium bromide (**3**), *N*-ethyl-*N*-methyl-4,4-diphenyl-2-butylamine and 1-^{14}C-labelled **1** were synthesized[3,4] in the Organic Chemistry Department, KabiVitrum, and used as standards in the quantitative analyses. Their identities were confirmed by ^{1}H NMR and mass spectrometry.

Gas chromatography-mass spectrometry. Mass spectra of demethylated **1, 2** and **3** were obtained on a Varian MAT 311A mass spectrometer (EI, 70 eV). A modified LKB 9000 gas chromatograph-mass spectrometer was used for selected ion monitoring (SIM). The gas chromatographic separations were achieved with an OV-1 fused-silica capillary column (25 m × 0,32 mm i.d., Orion Analytica, Finland) run isothermally at 215 °C with a helium flow of 2 ml min^{-1}.

Demethylation. The kinetics of the demethylation step were studied at 55 and 75 °C. An aqueous solution (2 ml) was prepared containing **2** (1.8 μg) and demethylated **1** (1.5 μg) as internal standard. Aqueous sodium perchlorate (1 ml, 1M) was added and the compounds were extracted with dichloromethane (3 ml).[2] After centrifugation, aliquots of the organic phase (100 μl) were transferred to each of eight 10 ml test tubes for subsequent demethylation. The dichloromethane was evaporated in a stream of nitrogen, and L-Selectride solution (50 μl) was added. The tubes were sealed and then heated to either 55 or 75 °C. The reaction was stopped at 0, 5, 10, 20, 30, 40, 50 and 60 min by addition of sulfuric acid (0.5 ml, 0.1M). The mixture was then washed with diethyl ether (2×3 ml) and the aqueous solution extracted with diethyl ether (3 ml) after alkalization with sodium hydroxide (0.2 ml, 2M). After centrifugation, the ether phase was dried over anhydrous sodium sulfate, transferred to another test tube and evaporated in a stream of nitrogen to dryness at 55 °C. The residue was dissolved in acetonitrile (50 μl) and injected (5 μl) into the GC/MS instrument for quantification.

Quantification. Quantitative data were obtained in the SIM mode by monitoring the immonium ions at *m/z* 86, 89 and 91. These ions, generated by α-cleavage at the nitrogen, constitute the base peaks in the mass spectra of **1, 2** and **3,** respectively. Quantitative calculations were made using calibration curves constructed for sample standards.

Sample preparation. Serum or plasma (1 ml) was diluted with water (3 ml) containing the internal standard **3** (10 ng), whereafter aqueous sodium perchlorate (0.5 ml, 1M) and dichloromethane (3 ml) were added. The mixture was shaken for 15 min, the organic phase separated by centrifugation, filtered through glass-wool and evaporated to dryness on a water-bath. L-Selectride solution (50 µl, 1M) was added, and the sample was then flushed with nitrogen and heated at 75 °C for 20 min in a sealed tube. The reaction mixture was cooled to room temperature and sulfuric acid (1 ml, 0.1M) was added. The mixture was washed with diethyl ether, and the amines were re-extracted after addition of sodium hydroxide (0.2 ml, 2M) and prepared for analysis by GC/MS as described above. The total yield was determined using 1-^{14}C-labelled **1** and analysis by liquid scintillation.

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