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RAPID COMMUNICATIONS

PENTAFLUOROBENZENESULFONYL CHLORIDE AS A SENSITIVE REAGENT FOR THE RAPID GAS CHROMATOGRAPHIC ANALYSIS OF TRANYLCYPROMINE IN TISSUES AND BODY FLUIDS

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Gas chromatography with electron-capture detection (GC-ECD) has been widely used for the analysis of naturally-occurring amines and amine-containing drugs in tissues and body fluids (1-3). Often such analyses involve adjustment of the pH of the biological samples and extraction with an organic solvent, or extraction with, and elution from, ion-exchange columns. The phase containing the amines is then taken to dryness and reacted with a derivatizing reagent which imparts electron-capturing properties. In many cases, the derivatizing reagent is unstable in water, thus creating the necessity for reaction under anhydrous conditions. Reagents that would provide derivatization under aqueous conditions (i.e. the reagent or an organic solution of it is added to the aqueous solution containing the amine), and hence reduce analysis time, are important to researchers using GC-ECD. Examples of such reagents which have proven useful for the analysis of bioactive amines include nitro-substituted benzoyl chloride (4), 2,6-dinitro-4-trifluoromethylphenyl chloride (5) and pentafluorobenzoyl chloride (6). We describe here another such reagent, pentafluorobenzenesulfonyl chloride, which has the potential to be utilized for the analysis of a wide variety of compounds containing amine groups. Its application to the analysis of the antidepressant tranylcypromine (TCP), an amine structurally similar to amphetamine, is presented in this report.

MATERIALS AND METHODS

Pentafluorobenzenesulfonyl chloride (PFBSC), di-(2-ethylhexyl)phosphate (DEHPA), and TCP.HCl were obtained from the Sigma Chemical Co. (St. Louis, MO). Toluene (glass-distilled) and chloroform ACS were obtained from Fisher Scientific (Fair Lawn, NJ). The internal standard (I.S.) 2-(4-chlorophenyl)ethylamine (CPEA), obtained from the Aldrich Chemical Co. (Milwaukee, WI), was converted into its HCl salt by bubbling HCl gas through the ethereal solution of the free base. All other solvents were of the highest purity commercially available and were used without further purification. Water was double-distilled in an all-glass distillation apparatus.

Subjects. Male Sprague-Dawley rats weighing 150-220 g were obtained from Bio-Science Animal Services (Ellerslie, Alberta, Canada) and injected intraperitoneally with 0.1 mmole/kg of TCP.HCl dissolved in isotonic saline. The rats were killed by decapitation at predetermined time intervals. Brain, liver, heart, lungs, kidneys and spleen were removed as quickly as possible and frozen solid in isopentane on solid carbon dioxide. Blood was collected in vials containing 100 μl of saturated disodium EDTA solution, mixed thoroughly, and frozen. All samples were stored at -60° until the time of analysis.

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Derivatization. Frozen tissues were allowed to partially thaw and were cut into small pieces, weighed, and homogenized in 5 vol. of ice-cold 0.4 M perchloric acid containing EDTA (10 mg/100 ml). Homogenates were centrifuged at 12,000 g for 10 min at 4°. Aliquots (3 ml)of the resultant supernatant fractions were transferred to a set of clean tubes into which 2 μg of the I.S. was added. Blood samples were thawed completely and 1-g aliquots were weighed into polyethylene tubes to which 2.5 ml of ice-cold 0.4 M perchloric acid was added. The tubes were sonicated for 5 min in a sonicator bath and centrifuged at 3000 rpm for 5 min. The entire clear supernatant fraction was used in the assay after adding I.S.

Solid K_2CO_3 was added to all samples and the resultant precipitate was removed by centrifugation. To each clear supernatant a small excess of solid K_2CO_3 was added. Three milliliters of a mixture of toluene:acetonitrile:PFBSC (9:1:0.01, by vol.) was added to each tube, and the tubes were shaken vigorously for 2 min and centrifuged. The organic layer was transferred to another tube and taken to dryness under a stream of nitrogen. The residue was taken up in 300 μl of toluene; an aliquot (0.2-1.0 μl) was used for GC-ECD analysis. The above procedure works well for the tissue and blood samples described. However, if further "cleanup" is required (e.g. urine samples), an additional purification step may be added prior to derivatization. Briefly this involves adjustment of the pH of the sample to 7.8 and shaking with 4 ml of the liquid ion-pairing compound DEHPA in chloroform (2.5% v/v) for 5 min. Following brief centrifugation, the upper aqueous layer is aspirated off, and the TCP is back-extracted from the chloroform layer by shaking for 5 min with 0.5 M HCl (3 ml). The acid layer is transferred to another tube, and the TCP it contains is derivatized using the procedure described above.

Gas chromatography. A Hewlett-Packard (HP) model 5890A gas chromatograph equipped with a linear ^{63}Ni-electron capture detector and a fused silica capillary column with a 0.25 μm film of 5% phenylmethylsilicone +1% vinyl stationary phase, dimensions 15 m x 0.246 mm (J. & W. Scientific, Inc., Rancho Cordova, CA) was used. Injection port and detector temperatures were 200° and 300° respectively. Helium was used as carrier gas at a flow rate of 2 ml/min and 5% methane in argon was used as makeup gas at the detector at a flow rate of 35 ml/min. The oven temperature was programmed to increase from 105° (maintained for 0.5 min) at a rate of 30°/min to 255° (maintained for 1 min) and then to increase at a rate of 25°/min to 280° (maintained for 10 min). Although GC-ECD was used for routine analysis, the structure of the final derivative was first confirmed by combined gas chromatography-mass spectrometry (GC-MS).

RESULTS AND DISCUSSION

The proposed structure and mass spectral fragmentation of the final derivative of TCP are shown in Fig. 1. The levels of TCP (in μg/g, means ± S.E.M. of 4-6 determinations) in various organs 0.5 hr after administration of TCP (0.1 mmole/kg) were: blood (2.49 ± 0.77), heart (3.26 ± 1.03), lung (11.82 ± 3.26), spleen (7.79 ± 1.52), kidney (24.16 ± 7.10), liver(8.60 ± 0.57) and brain (9.38 ± 0.91). Table 1 represents TCP concentrations in rat brain, liver and blood 1.5, 3.0 and 6.0 hr postinjection.

Described in this report is a simple, rapid and reproducible assay procedure for TCP, in which derivatization with PFBSC occurs in the aqueous medium containing the drug immediately prior to extraction. The PFBS derivative showed excellent chromatographic properties (see Fig. 2 for a typical GC trace) and provided good stability and high sensitivity. It was stable for at least 1 month at -10°. The "on column sensitivity" was < 5 pg, which could be further improved by reducing the volume of toluene (usually 300 μl) in which the derivative was finally taken up. The standard curve was linear over the range 2-2000 ng of

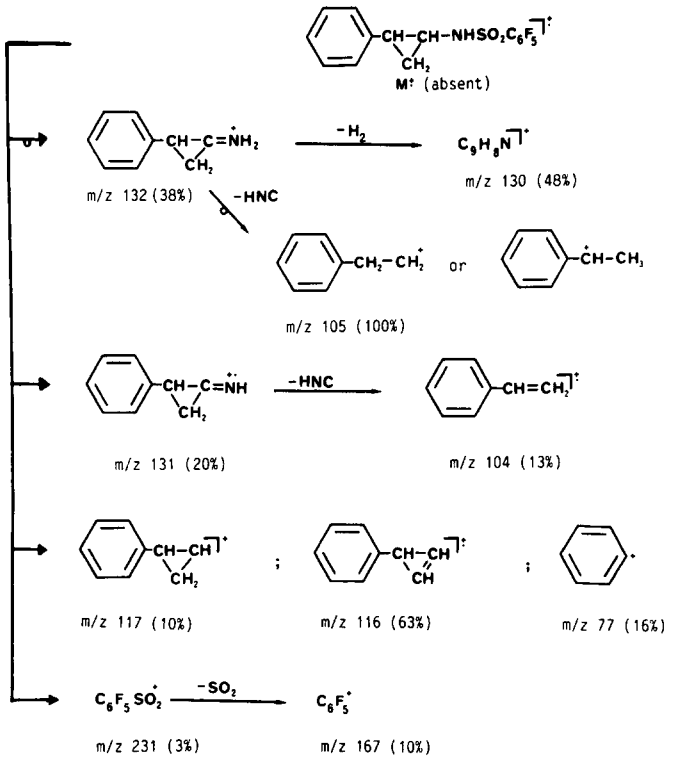

Fig. 1. Proposed electron-impact mass spectral fragmentation of derivatized tranylcypromine. The numbers in parentheses represent % relative abundance. Further confirmation of structure was obtained by conducting chemical ionization mass spectrometry (using methane), which yielded quasimolecular ions of masses m/z 364 $[(M+H)^+]$ and m/z 362 $[(M-H)^+]$ at relative abundances of 90.5% and 25.9% respectively.

Table 1. TCP levels in rat brain, liver and blood 1.5, 3.0 and 6.0 hr following 0.1 mmole/kg TCP

	TCP levels (μg/g) (mean ± S.E.M., n = 6)		
Time (hr)	Brain	Liver	Blood
1.5	6.09 ± 0.99	5.99 ± 0.83	2.23 ± 0.37
3.0	4.07 ± 0.56	5.48 ± 0.90	0.65 ± 0.039
6.0	0.39 ± 0.037	0.55 ± 0.065	0.113 ± 0.034

TCP (in 3-ml volumes) with a correlation coefficient > 0.99. Recovery of TCP from aqueous solution was virtually quantitative. The addition of the DEHPA purification step provides cleaner traces and still affords good recoveries; the overall recovery of 100 ng TCP standards using this longer procedure was shown to be 89.2%.

In summary, the use of PFBSC provides for the rapid extractive derivatization of TCP. This reagent has the potential for use in the analysis of many other important drugs and endogenous substances. It has been employed for derivatization of standards of tyrosyl peptides (7) and nucleic acid pyrimidine bases (8), and preliminary experiments in our laboratories have demonstrated that the reagent reacts readily with β-phenylethylamine (PEA) and amphetamine and several of their analogues and with tricyclic antidepressants containing a secondary amine group. A number of the bioactive amines we have investigated can be derivatized with either PFBSC or pentafluorobenzoyl chloride (PFBC) under the conditions described in this report. However, with analyses of TCP and PEA we have found cleaner GC traces are obtained with extracts of urine, blood and brain homogenates when PFBSC is used, thus reducing interference problems in samples containing low concentrations of these

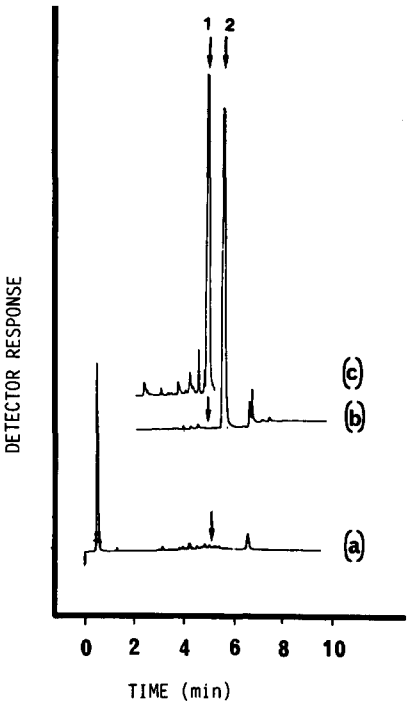

Fig. 2. Typical gas chromatographs of extracts carried through the procedure described in the text: (a) perchloric acid blank; (b) brain from a rat treated with saline; and (c) brain from a rat treated with tranylcypromine (0.1 mmole/kg, i.p., 1.5 hr). 1 = derivatized tranylcypromine, 2 = derivatized internal standard. Peak 2 was also present in trace (c). The attenuation values at the position corresponding to peak 1 are 2^{4}6 in traces (a) and (b) and 2^{4}9 in trace (c).

amines. Similarly, we have noted that some secondary amines, such as bioactive N-alkylated analogues of PEA and TCP which we are testing as potential "prodrugs" of these amines, give more sensitive derivatives with PFBSC than with PFBC and/or are easier to separate from the parent amine when they are derivatized with the former reagent. In addition, we have observed, in preliminary studies on plasma levels of tricyclic antidepressants containing secondary amine groups, that the PFBS derivatives sometimes separate from interfering peaks in the plasma extract more readily than do the corresponding derivatives formed with PFBC. It thus appears that PFBSC represents an important addition to the armamentarium of the analytical neurochemist.

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