Quantitative Determination of Pentazocine in Plasma and of Pentazocine and Metabolites in Urine

K. A. PITTMAN▲ and C. DAVISON

Abstract □ GLC methods for the determination of plasma pentazocine and for the determination of urinary pentazocine and its known metabolites are described. The determination of plasma pentazocine involves the extraction of pentazocine and a suitable internal standard, the derivatization of these compounds with heptafluorobutyric acid anhydride, and the chromatography of the derivatives using tritium-foil, electron-capture detection. The determination of urinary pentazocine and two of its metabolites involves the extraction of these compounds and an internal standard, derivatization with a silylating reagent, and the chromatography of the derivatives using flame-ionization detection. The determination of a third urinary metabolite involves adsorption chromatography followed by the same derivatization and chromatographic analysis. The sensitivity and reliability of the techniques are discussed and compared to other methods in the literature.

Keyphrases □ Pentazocine in plasma, pentazocine and metabolites in urine—GLC analysis □ GLC—analysis, pentazocine in plasma, pentazocine and metabolites in urine

Pentazocine is a strong analgesic and weak narcotic antagonist possessing little dependence potential (1). Both parenteral and oral formulations are available and have found widespread clinical use. Although clinical studies of the efficacy of the compound and comparisons of it with other analgesics abound, only limited attempts have been made to study the behavior of the drug in man. Blood levels and the urinary excretion of free pentazocine in man after various routes of administration have been reported (2–5), as have some studies of its total human excretion and metabolism (6, 7) and of its metabolism in animals (8–13). With low and moderate doses of pentazocine, plasma levels in man rarely exceeded 0.2 mcg./ml. and were usually less than 0.1 mcg./ml.

Pentazocine is extensively metabolized in animals and in man. The dimethylallyl side chain is oxidized to two isomeric alcohols and the phenolic hydroxyl is conjugated. Both alcohols may be conjugated and one, the *trans*-isomer, is known to be further oxidized by man to a carboxylic acid. This acid is probably conjugated at the phenolic hydroxyl but to a lesser extent than are the other compounds. The urinary excretion of free pentazocine varies from less than 5 to more than 20% of the dose, depending upon the subject and the route of administration. The remainder of the 60–65% of the dose excreted in 24 hr. can be found in the form of the other known metabolites.

To compare various doses and routes of administration and to develop a pharmacokinetic model for the absorption, distribution, and excretion of pentazocine, adequate methods for the measurement of urinary metabolites, as well as of parent compound, and a sensitive method for the determination of plasma pentazocine were needed. This paper reports the development of methods that we have used in the analysis of a large number of plasma and urine samples from human subjects receiving pentazocine. Those data will be reported subsequently.

EXPERIMENTAL

Reagents—Technical grade ethylene dichloride was purified by fractional distillation in an all-glass apparatus. Nanograde benzene[1] and spectroquality hexane[2] were used without further treatment. Heptafluorobutyric acid anhydride[3] was distilled, 0.2-ml. amounts were sealed under nitrogen individually in small ampuls, and the ampuls were stored at −20°. *N,O*-Bis(trimethylsilyl)acetamide[4], *N,O*-bis(trimethylsilyl)trifluoroacetamide[4], β-glucuronidase[5], and the packings for GLC[6] were used as purchased. Amberlite XAD-2 resin[7] was cleaned by repeatedly and serially suspending it in water, methanol, acetone, methanol, and, finally, water. All other chemicals used were reagent grade.

Pentazocine [1,2,3,4,5,6-hexahydro-*cis*-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol], its "*trans*-alcohol" [1,2,-3,4,5,6-hexahydro-8-hydroxy-α,6(eq),11(ax)-trimethyl-2,6-methano-3-benzazocin-3-*trans*-2-buten-1-ol] and its "*trans*-acid" [1,2,3,4,5,6-hexahydro-8-hydroxy-α,6(eq),11(ax)-trimethyl-2,6-methano-3-benzazocin-3-crotonic acid] metabolites, and the internal standard (1,-2,3,4,5,6-hexahydro-*cis*-6,11-dimethyl-3-cyclopentyl-2,6-methano-3-benzazocin-8-ol) were prepared[8]. Solutions of these benzazocines were kept in the dark and refrigerated, and they are stable for at least 3 months under such conditions. Biological samples were stored in the frozen state (−20°), and they are stable for over 1 year.

Treatment of Plasma Samples—To analyze plasma for unconjugated pentazocine, the following procedure was used. From 0.5- to 2-ml. plasma samples were placed in 150 × 16-mm. test tubes, and exactly 0.50 ml. of a 4×10^{-5}% solution of the internal standard in 0.001 *N* HCl, 0.2 ml. of a buffer consisting of a solution saturated with both sodium bicarbonate and sodium carbonate, and 5 ml. of nanograde benzene were added to each tube. The contents of the tubes were stirred in a vortex mixer and centrifuged, and the benzene layers were transferred to 175 × 20-mm. tubes. The aqueous phases were extracted with a second 5 ml. of benzene as before. The combined benzene phases were extracted as before with 2 ml. of 0.1 *N* HCl. The organic phases were removed and discarded and, after the addition of 0.2 ml. of the carbonate buffer, the aqueous phases were extracted as before with 5 ml. of benzene. The benzene extracts were transferred to 150 × 16-mm. tubes, and the aqueous phases were extracted again with 3 ml. of benzene.

The combined benzene extracts were centrifuged to remove the last traces of the aqueous phases, decanted into 100 × 16-mm. tubes, and dried in a heating block at 65° under a gentle stream of filtered air. The residues were taken up in 0.4 ml. of a 1.25% solution of heptafluorobutyric acid anhydride in ethyl acetate, and clean carborundum chips were added to each tube. The solutions were taken to dryness at 65° in a vacuum oven, the residues were taken up in approximately 0.25 ml. of ethyl acetate, and these solutions were dried in the vacuum oven again. At least 15 min. was allowed for each drying step. The residues were taken up in 0.1 ml. of ethyl acetate for chromatography; once dissolved, they

[1] Mallinckrodt Chemical Works.
[2] Matheson, Coleman and Bell.
[3] Pierce Chemical Co.
[4] Regisil, Regis Chemical Co.
[5] Glusulase, Endo Research Laboratories.
[6] Applied Science Laboratories.
[7] Rohm and Haas Co.
[8] By Dr. N. F. Albertson, Sterling-Winthrop Research Institute.

had to be analyzed on the same day. However, the dry heptafluorobutyryl esters could be kept in a desiccator for several days.

Treatment of Urine Samples—To analyze urine for unconjugated pentazocine and unconjugated *cis*-alcohol and *trans*-alcohol metabolites, the following procedure was used. Five-milliliter urine samples were placed in 50-ml. extraction tubes, and exactly 0.100 ml. of a 0.005% solution of the internal standard in 0.1 *N* HCl, 20 ml. of ethylene dichloride, and 0.5 g. of an equal mixture of sodium bicarbonate and sodium carbonate were added to each tube. The tubes were closed with polyethylene stoppers and shaken, horizontally, for 5 min. at 360 oscillations/min. All buffer had to be in solution at this point. The tubes were centrifuged and the aqueous phases were removed and discarded. The organic phases were washed in the same manner with 5 ml. of water and the washes were discarded. The organic phases were then extracted in the same manner with 5 ml. of 0.1 *N* HCl, and the acid phases were transferred to clean 50-ml. extraction tubes. The organic phases were discarded and, after adding 0.5 g. of the buffer used above to the aqueous phases, they were extracted with 15 ml. of ethyl acetate.

After centrifugation, approximately 10 ml. of the ethyl acetate phases was transferred to 15-ml. conical centrifuge tubes and dried in a heating block at 70–75° under a gentle stream of air. Fifty microliters of 10% *N,O*-bis(trimethylsilyl)acetamide in dimethylformamide was added to each tube, and the tubes were closed with disposable polyethylene stoppers. The desired trimethylsilyl derivatives formed almost immediately and had to be analyzed on the same day.

To analyze urine for total (unconjugated plus conjugated) pentazocine and *cis*-alcohol, *trans*-alcohol, and *trans*-acid metabolites, the following procedure was used. Five-milliliter urine samples were placed in 50-ml. extraction tubes and 0.100 ml. of the 0.005% solution of internal standard, 15 ml. of 0.1 *N* acetate buffer (pH 5.5), 0.125 ml. of β-glucuronidase, and approximately 0.05 ml. of chloroform were added to each tube. The tubes were stoppered and incubated over 3 nights at 37°. Then the buffer salts were added as before and the tubes were stoppered and shaken by hand until all of the salts had dissolved. Twenty milliliters of ethylene dichloride was added to each tube and the tubes were mixed by inverting them by hand 100 times. The tubes were centrifuged, the aqueous phases were transferred to 100-ml. extraction tubes, the organic phases were washed in the same manner with 5 ml. of water, and the aqueous phases were combined.

At this point, the organic phases were carried through the rest of the procedure used earlier to obtain derivatives of pentazocine and its hydroxylated metabolites for GLC. The combined aqueous phases were adjusted to pH 4 by adding 3.0 ml. of glacial acetic acid gently down the side of each tube. The tubes were then agitated sufficiently to expel most of the carbon dioxide formed. The solutions were then passed through 1 × 10-cm. columns of Amberlite XAD-2 at a flow rate of 1 ml./min. The contents of each tube were rinsed into the columns with 20 ml. of water, and the columns were washed with one more 20-ml. portion of water. The columns were drained after the last rinse; all of the aqueous solutions were discarded. The columns were eluted with one 10-ml. and two 20-ml. portions of methanol. Flow was stopped halfway through the second elution, and the column bed was stirred to expel gas bubbles. The combined methanol eluates were dried on a steam bath, and the residues were transferred quantitatively with two 2.5-ml. washes of the upper phase of an equilibrated mixture of water–methanol–chloroform–hexane (1:3:4:4 v/v) to 20-ml., round-bottom centrifuge tubes. These solutions were extracted twice with 10-ml. portions of the lower phase of the same solvent mixture, the lower phases being discarded each time. To each tube was added 0.250 ml. of a 0.04% solution of the internal standard in 0.1 *N* HCl, and the contents were dried as in the other procedures. The residues were reacted for 15 min. at 60° with 100 μl. of *N,O*-bis(trimethylsilyl)trifluoroacetamide[4] before being chromatographed. The solutions had to be analyzed on the same day.

GLC—For the analysis of the heptafluorobutyryl derivatives of pentazocine, a gas chromatograph[9] equipped with a tritium-foil, electron-capture detector was used. Separation was performed isothermally on a 1.5-m. (5-ft.), 2-mm. i.d. glass column packed with 10% OV-1 on 100–120-mesh Gas Chrom Q. Injection volumes were 3–5 μl. The following conditions were used for all analyses: injection temperature, 250°; column oven temperature, 215°; detector temperature, 215°; and nitrogen carrier gas flow, 50 ml./min.

Table I—Relative Retention Times of Derivatives of Pentazocine and Its Metabolites[a,b]

Compound	Derivative: Heptafluorobutyryl	Derivative: Trimethylsilyl
Pentazocine	0.75	0.75
cis-Alcohol metabolite[c]	—	1.47
trans-Alcohol metabolite[c]	—	1.72
trans-Acid metabolite[c]	—	2.23

[a] Values were determined on 1.8-m. (6-ft.) columns packed with 10% OV-1 on Gas Chrom Q. [b] Retention times are relative to the appropriate derivative of the internal standard (1,2,3,4,5,6-hexahydro-*cis*-6,11-dimethyl-3-cyclopentyl-2,6-methano-3-benzazocin-8-ol). [c] These metabolites were characterized previously (8).

For the analysis of trimethylsilyl derivatives of pentazocine and its metabolites, a gas chromatograph[10] equipped with flame-ionization detectors was used. Separation was performed isothermally on 1.8-m. (6-ft.), 2-mm. i.d. glass columns packed with 10% OV-1 on 100–120-mesh Gas Chrom Q. The injection volume was 2 μl. The following conditions were used for all analyses: injector and detector temperatures, 270°; nitrogen carrier gas flow, 55 ml./min.; hydrogen flow, 33 ml./min.; and air flow, 300 ml./min. The column oven temperature was varied between 245 and 265°, depending upon the particular analysis performed and the particular column used. Columns had to be replaced monthly under conditions of daily use.

Calculations—Standard curves were constructed by plotting the ratios of the peak heights of known amounts of authentic standards to the peak heights of the internal standard against the amounts of the standards added to human plasma or human urine and extracted by the methods outlined earlier in this paper. Since no authentic *cis*-alcohol metabolite was available, the standard curve established for the *trans*-alcohol metabolite was used, and all peak heights of the *cis*-alcohol were reduced by multiplying them by the ratio of the $^1/_3$ height width of the *cis*-alcohol to the $^1/_3$ height width of the *trans*-alcohol, on the assumption that the areas subtended by equal amounts of these two isomers would be equal.

RESULTS

Choice of Internal Standard—An analog of pentazocine was chosen because it could be expected to: (*a*) form the same derivatives as pentazocine and its metabolites, (*b*) behave very similarly to pentazocine and the hydroxylated metabolites in the extraction procedures involved, and (*c*) have a retention time near to that of the other compounds. Table I lists the relative retention times of pentazocine and its metabolites relative to the internal standard in the GLC systems used. The retention time of the internal standard conveniently falls just after that of pentazocine. Table II lists the absolute recovery of these various compounds from plasma and from urine after using the extraction procedures and indicates that the internal standard does behave very similarly to the compounds of interest.

Determination of Plasma Pentazocine—A chromatogram of the heptafluorobutyryl ester of the internal standard and of pentazocine extracted from human plasma shows the compounds to be well separated with symmetrical peaks, although a small contaminant often appears as a shoulder just prior to the peak representing the internal standard. Sample drying is a particularly important step in the procedure. If the temperature is too great, the derivatives are lost; if too slow, substances derived from the heptafluorobutyric anhydride are not removed and gross contamination occurs. Generally, further heating *in vacuo* of the latter type of samples will satisfactorily clean them.

The response of the electron-capture detector is linear with these benzazocine derivatives to about 150 ng. in the sample (about 5 ng. on the column). The 95% confidence interval of the slope of

[9] Packard series 7300.

[10] Varian Aerograph series 2100.

Table II—Absolute Recovery Obtained, by the Extraction Procedures Used, of Pentazocine, Its Hydroxylated Metabolite, and the Internal Standard from Plasma and Urine

Extracted Fluid	Compound Recovered, %: Pentazocine	*trans*-Alcohol	Internal Standard[a]
Urine[b]	66.4	64.2	61.5
Hydrolyzed urine[c]	68.8	60.0	56.5
Plasma[b]	95	—	97

[a] 1,2,3,4,5,6-Hexahydro-*cis*-6,11-dimethyl-3-cyclopentyl-2,6-methano-3-benzazocin-8-ol. [b] Values represent the mean recovery at 10 concentrations between 0.1 and 4.0 mcg./ml. of original sample. [c] Values represent the mean recovery at five concentrations between 0.1 and 4.0 mcg./ml. of original sample.

the line of the standard curve generally falls within ±10–15% of the value of the slope. If an unknown exceeds the range of the standard curve, it may be diluted and reinjected. A series of about 100 control plasma samples from 11 human volunteers was analyzed, giving an average blank value equivalent to 2.7 ± 2.5 (*SD*) ng. pentazocine/ml. While recovery of extracted standards relative to direct standards approaches 100%, extracted standards from plasma obtained before drug administration from subjects involved in experiments have been employed routinely. Duplicate series of 13 replicate plasma samples with 25 and 100 ng. pentazocine added/ml. were analyzed on consecutive days. At 100 ng./ml., the recoveries were 95 and 92 ng./ml. with percent standard deviations of 13 and 11, respectively. At 25 ng./ml., recoveries were 25 and 18 ng./ml. with percent standard deviations of 14 and 44, respectively.

Determination of Urinary Pentazocine and Its Metabolites—A chromatogram of the trimethylsilyl derivatives of the internal standard and of pentazocine and its metabolites run under the conditions described in the *Experimental* section shows the compounds to be well separated and their peaks to be symmetrical. The following basic drugs were checked and found not to interfere with peaks due to the internal standard, pentazocine, or its metabolites: amphetamine, atropine, caffeine, cocaine, codeine, meperidine, methadone, methamphetamine, morphine, nalorphine, nicotine, and normeperidine. Quinine interferes with the *trans*-alcohol metabolite. Acidic and neutral drugs would not appear in the final extract. The response of the gas chromatograph to all of the pertinent compounds was linear over a wide range of concentrations, extending above and below the concentrations that were expected to be measured in urine (Fig. 1). Each point on the graph is the result of the measurement of a single injection. Since no crystalline *cis*-alcohol metabolite was available, an amount sufficient to obtain the results shown in Fig. 1 was isolated from a human urine sample by extraction and TLC; the approximate amount present was determined by reference to known amounts of the *trans*-alcohol metabolite.

Whenever a column became unsuitable for use, the first compound to be affected was the *trans*-acid metabolite. Pronounced tailing of this compound was noted, and the linearity of response disappeared at lower concentrations. The primary reason for the deterioration of the quality of the chromatograms seemed to be the accumulation of salts and charred organic material in the glass-wool plug at the head of the columns. The source of the contamination probably was the prepared samples of the *trans*-acid metabolite, which remain very impure preparations. Often, chromatograms could be improved or restored to acceptable appearance simply by changing the glass-wool plug.

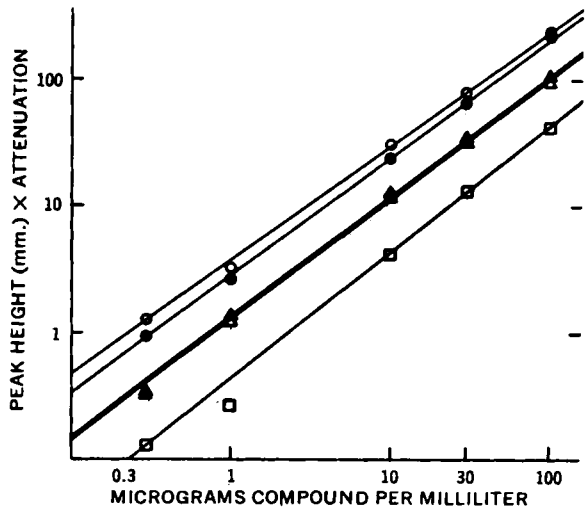

Figure 1—*Response of the flame-ionization detector to various amounts of trimethylsilyl derivatives of pentazocine and related compounds. A single measurement of pentazocine* (○), *the internal standard* (●), *the* cis*-alcohol metabolite* (△), *the* trans*-alcohol metabolite* (▲), *and the* trans*-acid metabolite* (□) *was made at each concentration. (A Varian model 2100 gas chromatograph was used.)*

Preliminary experiments using urine from human subjects given pentazocine were carried out to establish satisfactory conditions for the complete enzymatic hydrolysis of conjugates of pentazocine and its metabolites. Incubation periods less than 2 days or a β-glucuronidase concentration one-tenth that recommended resulted in less than maximum release of pentazocine or its metabolites from their conjugated forms. No degradation of unconjugated pentazocine or of its unconjugated metabolites occurs under the conditions used for enzymatic hydrolysis.

Typical standard curves for extracted standards of pentazocine and the *trans*-alcohol and *trans*-acid metabolites show low blank values and good linearity. The 95% confidence intervals of the slopes of the lines of the standard curves for pentazocine and the *trans*-alcohol and *trans*-acid metabolites generally fall within ±5–10% of the value of the slope. More variation in the *trans*-acid metabolite curve might be expected, since the internal standard could not be carried through the entire operation with this compound.

The values presented in Table III were obtained in separate analyses of nine sets of samples containing 10 premedication samples of human urine each. They indicate the magnitudes and variabilities of the experimental blanks that would be expected to be included in the values obtained for pentazocine and its metabolites if a sample of human urine were analyzed as described earlier. Much of the variability in the overall means, as expressed by their percent standard deviations, is due to day-to-day variation in the slope–*y* intercept of the standard curves. As indicated by the mean percent standard deviations (Table III) calculated from the standard deviations of each set of samples, the variability within samples analyzed on a given day is less. Therefore, the reliability of a deter-

Table III—Analysis for Apparent Pentazocine and Metabolites in Urine Taken from Human Subjects Prior to Medication

Compound	Microgram Equivalents[b] per Milliliter: Urine: Mean	Urine: % *SD*[c]	Urine: % $\overline{SD}$[d]	Hydrolyzed Urine: Mean	Hydrolyzed Urine: % *SD*[c]	Hydrolyzed Urine: % $\overline{SD}$[d]
Pentazocine	0.048	133	56	0.026	234	101
cis-Alcohol metabolite	0.058	236	64	0.018	728	124
trans-Alcohol metabolite	0.012	842	156	−0.033	215	90
trans-Acid metabolite	—	—	—	0.66	100	84

[a] A total of 90 samples of human urine was analyzed, by the methods presented in the text, in nine sets of 10 samples each. [b] Microgram equivalents = micrograms compound (mol. wt. pentazocine per mol. wt. compound). [c] The percent standard deviation (% *SD*) was calculated by comparing each sample to the overall mean. [d] The average percent standard deviation (% $\overline{SD}$) is the mean of the nine relative standard deviations, each calculated by comparing the samples in a set to the mean for that set.

Table IV—Accuracy and Precision of the Measurement of Pentazocine and Its Known Metabolites in Human Urine[a]

Treatment of Urine	Compound Added	Microgram Equivalents of Compound Added per Milliliter[b]	Microgram Equivalents of Compound Recovered per Milliliter[b] Day 1	% SD[c]	Day 2	% SD[c]	Pooled SE[d]
None	Pentazocine	1.00	0.91	5	1.04	6	0.03
	trans-Alcohol metabolite	1.00	0.92	9	0.96	7	0.02
Hydrolysis	Pentazocine	1.00	0.97	4	1.00	3	0.01
	trans-Alcohol metabolite	1.00	1.12	5	0.94	5	0.03
	trans-Acid metabolite	3.00	3.31	12	2.76	10	0.13

[a] Seventeen replicate samples, prepared by adding known amounts of the compounds to premedication human urine, were analyzed on each of 2 consecutive days, against extracted standards, by the methods described in the text. [b] Microgram equivalents = micrograms compound (mol. wt. pentazocine per mol. wt. compound). [c] The percent standard deviations were calculated by comparing each value obtained on a given day to the mean value for that day. [d] The pooled standard error includes the error due to the "day of analysis" effect, which was significant in every case ($p < 0.05$ to $p < 0.001$).

mination of urinary pentazocine and its metabolites would be increased if a sample of urine known to be free of those compounds would be analyzed at the same time and used as an experimental blank.

An estimate of the accuracy and precision of the measurement of pentazocine and its metabolites in urine and in hydrolyzed urine was obtained by adding known amounts of the compounds to human urine and subjecting replicate samples to analysis either directly or after hydrolysis. Replicate sets were analyzed in this manner on 2 consecutive days (Table IV).

DISCUSSION

The advantages of the internal standard technique in multistep procedures are obvious. The fact that the internal standard used is so close in its physicochemical properties to that of pentazocine and its hydroxylated metabolites is insurance against variations in temperature and salt concentrations changing significantly the relative amounts of these compounds recovered in the final step of the assay. Also, this internal standard can be expected to behave similarly to pentazocine in derivatization procedures. However, any such compound with the proper retention time and polarity should be satisfactory. For example, Beckett *et al.* (4) used α-3-hydroxy-6-dimethylamino-4,4-diphenylheptane (α-methadol) as an internal standard in the GC assay of pentazocine in blood and urine, and Ahmad and Medzihradsky (15) used cyclazocine as an internal standard.

It was not possible to find an internal standard for use in the analysis of the *trans*-acid metabolite of pentazocine more suitable than the one used for pentazocine analysis. Since this internal standard cannot be added at the beginning of the procedure for the analysis of the *trans*-acid metabolite, the power of the internal standard technique is correspondingly diminished.

The technique used here for the determination of pentazocine in plasma is more sensitive and/or specific than other methods developed to date. The fluorometric technique developed by Berkowitz *et al.* (2) is less specific, since a metabolite of pentazocine interferes (2), and has a practical limit of determination of about 30–50 ng./ml. of plasma (2)[11]. The procedures used by Borg and Mikaelsson (14) and by Ahmad and Medzihradsky (15) are more specific but not more sensitive than the fluorometric one. The procedure of Beckett *et al.* (4), although specific, is not likely to be as sensitive as one involving the electron-capture detection of a highly quenching derivative. They reported a recovery of pentazocine of 98 ± 5% from plasma containing 0.2 mcg. of drug/ml., but no information concerning the lower limit of detection was provided. In addition, the Beckett *et al.* procedure requires large volumes of blood, up to 15 ml., for the determination of small amounts of pentazocine. In the present study, the use of benzene as the extracting solvent and of a carbonate buffer system conforms to the general experience that benzene results in a clean extract and, at the pH of 10 or so obtained, a quantitative extraction. Borg and Mikaelsson (14) discussed the pKa's and extraction of pentazocine. Benzene is not a suitable extraction solvent for the hydroxylated, metabolites of pentazocine, because only about 10% can be extracted by use of the same procedure used for pentazocine, nor for the acid metabolite, because none is extracted. The plasma technique, as described, is useful only for pentazocine.

The fluorometric method, with some modification, has been applied to the analysis of urinary pentazocine (3). With a lower limit of detection of about 0.2 mcg. pentazocine/ml., it should possess adequate sensitivity. Hydrolyzed urine has also been used for pentazocine and pentazocine conjugates. The direct GC method as used for the analysis of blood was used by Beckett *et al.* (4) in the analysis of urinary pentazocine. An average recovery of pentazocine of 93 ± 3% from urine containing 1 mcg. of drug/ml. was reported, but no indication of the lower limit of detection was mentioned, nor was any attempt made to apply this method to other metabolites. This method also should be adequate for determining urinary pentazocine. The technique described herein for the analysis of urine is not more sensitive for pentazocine than are the other techniques already available. The use of benzene as the extracting solvent in the other GLC procedures probably results in a cleaner final extract. The advantage of the procenures reported here is that they allow the determinationof pentazocine, its major metabolites, and most of their conjugates by a unified approach. Based on the values in Tables III and IV, the practical lower limit for the quantitative determination of pentazocine and of the hydroxylated metabolites is about 0.1–0.2 mcg./ml. and that for the acid metabolite is about 1–2 mcg./ml.

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▲ To whom inquiries should be directed.

Mass Spectra of Nine Medicinal Carbamates

R. T. COUTTS

Abstract □ The mass spectra of nine medicinal carbamates (meprobamate, mebutamate, carisoprodol, emylcamate, bethanechol chloride, styramate, hydroxyphenamate, mephenesin carbamate, and methocarbamol) were recorded and examined. Proposed fragmentation pathways were deduced, either by deuterium labeling or by means of accurate mass measurements. The carbamate grouping was expelled at various stages during fragmentation and was lost in a variety of ways.

Keyphrases □ Carbamates—mass spectral characterization of nine drugs □ Meprobamate—mass spectral characterization, fragmentation pathways □ Mebutamate—mass spectral characterization, fragmentation pathways □ Carisoprodol—mass spectral characterization, fragmentation pathways □ Emylcamate—mass spectral characterization, fragmentation pathways □ Bethanechol chloride—mass spectral characterization, fragmentation pathways □ Styramate—mass spectral characterization, fragmentation pathways □ Hydroxyphenamate—mass spectral characterization, fragmentation pathways □ Mephenesin carbamate—mass spectral characterization, fragmentation pathways □ Methocarbamol—mass spectral characterization, fragmentation pathways □ Mass spectroscopy—characterization, nine medicinal carbamates

There are several reports in the literature on the mass spectral behavior of carbamates (1–5), but all of these studies are concerned with *N*-substituted and *N*,*N*-disubstituted carbamates, including many of the carbamate pesticides. Unsubstituted carbamates are employed in medicine as minor tranquilizers and muscle relaxants and for other purposes. Their mass spectra have not been examined in detail.

The study now reported is a continuation of previous investigations (6, 7) on the characterization of medicinal compounds by means of their mass spectra. For this study, nine medicinal carbamates were available. The mass spectrum of each was recorded, and fragmentation pathways were proposed either as a result of deuterium labeling or accurate mass measurements. At the outset, each spectrum was inspected to see whether the chosen carbamates lost the carbamate grouping early in the fragmentation sequence and, if so, whether it was consistently lost as an identifiable molecule or radical such as HNCO or $NH_2COO^{\cdot}$. It soon became clear that the carbamate grouping was expelled at various stages during fragmentation and that it was lost in a variety of ways. However, one diagnostic peak was observed in five of the carbamates. An ion of mass 62 was formed from meprobamate, mebutamate, carisoprodol, mephenesin carbamate, and methocarbamol. An accurate mass measurement in each instance revealed that this ion was $NH_2COOH_2^+$. The elimination of NH_2COOH (or simultaneous expulsion of NH_3 and CO_2) at some stage during the fragmentation sequence was also common to the same five compounds and to emylcamate. The formation of the NH_2COOH molecule is considered to be the result of a hydrogen-transfer mechanism such as that illustrated in the fragmentation of emylcamate.

RESULTS[1] AND DISCUSSION

In the nine carbamates investigated (I–IX), the molecular ion generally either was present in very low abundance or was absent from each spectrum.

The spectrum of meprobamate (I) (Fig. 1) displayed six fragment ions at *m/e* 144 [146], 114 [115], 101 [102], 96 [96], 84 [84], and 83 [83], which could be useful for characterization purposes. Tetradeuterated meprobamate (I, NH_2, groups replaced with ND_2) gave a spectrum with corresponding deuterated fragment ions. This information, together with the appearance of appropriate metastable ions, permits a rationalization of fragmentation pathways as illustrated in Scheme I. (In all schemes, the mass of a deuterated ion is enclosed in a square bracket, and an asterisk indicates the presence of a supporting metastable ion.)

The chemical structures of mebutamate (II) and carisoprodol (III) are closely related to that of meprobamate. As expected, some of the fragment ions that appear in the spectra of II and III arise in ways similar to those just described for meprobamate. Thus, the spectrum of mebutamate (Fig. 1) possesses abundant ions of *m/e* 158 [160], 128 [129], 110 [110], 115 [116], 97 [97], and 69 [69], which arise in the same manner as the ions *a* and *c–g*, respectively, illustrated in Scheme I.

Other abundant ions in the spectrum of mebutamate were located at *m/e* 97, 72, 71, 62, and 55. A rational explanation of their formation is given in Scheme II. In addition, accurate mass measurements were made of all ions and, in this way, the elemental composition of each ion was confirmed as shown. The peak at *m/e* 71 was a doublet,

[1] Mass spectra were recorded by Dr. A. M. Hogg and his associates using an AEI MS-9 mass spectrometer at an ionizing potential of 70 ev. Samples were introduced using the direct probe method. The source temperature was between 75 and 155°, depending on the compound being examined. Accurate mass measurements were carried out by the peak matching method.

All of the compounds examined were gifts from pharmaceutical firms. All had literature melting points and gave IR spectra consistent with their structures. Each deuterated compound was prepared by boiling, under reflux for 4 hr., a solution of the compound in dioxane containing deuterium oxide and then repeated recrystallization from the same solvent until the O—H and N—H stretching bands in the IR spectra of the unlabeled carbamate were replaced by O—D and N—D stretching bands at longer wavelengths.