Ion-Pair Extraction of Thiocyanate from Plasma and Its Gas Chromatographic Determination Using On-Column Alkylation

Peyton Jacob, III,* Chin Savanapridi, Lisa Yu, Margaret Wilson, Alexander T. Shulgin, and Neal L. Benowitz

Division of Clinical Pharmacology of the Department of Medicine, University of California, Building 100, Room 235, San Francisco General Hospital Medical Center, San Francisco, California 94110

Barbara A. Elias-Baker, Sharon M. Hall, Ronald I. Herning, and Reese T. Jones

Drug Dependence Research Center, Langley Porter Psychiatric Institute, University of California, San Francisco, California 94143

David P. L. Sachs

Divisions of Critical Care Medicine and Pulmonary Medicine, Case Western Reserve University Medical School, Cleveland, Ohio 44106

Tributylsulfonium thiocyanate may be extracted from aqueous solutions with organic solvents. Injection into the heated injection port of a gas chromatograph results in conversion to the volatile derivative butyl thiocyanate. Tributylsulfonium selenocyanate undergoes analogous extraction and thermal conversion to the butyl derivative. A procedure has been developed for the extraction of thiocyanate from human plasma and gas chromatographic determination using selenocyanate as an internal standard. The method has good sensitivity and precision and has been applied to behavioral and pharmacologic studies of smokers.

Determination of thiocyanate in biologic fluids is frequently used as an index of exposure to tobacco smoke (*1*). A typical cigarette delivers 100–400 μg of hydrogen cyanide (*2*), which is rapidly metabolized to thiocyanate by the rhodanese enzyme system (*3*). Since thiocyanate has a long plasma half-life (*4*, *5*), its concentration is a fairly good index of long-term exposure to cigarette smoke. Human exposure to cyanide, and hence thiocyanate, also results from dietary sources and use of the vasodilator drug nitroprusside.

The most frequently cited methods for determination of thiocyanate in biologic samples are colorimetric procedures based on the König reaction (*5–10*). In these procedures, thiocyanate is converted to cyanogen bromide or cyanogen chloride by reaction with a suitable halogenating agent. The resulting cyanogen halide is reacted with pyridine to give glutaconic aldehyde, which in turn is condensed with a primary amine or active methylene compound yielding a colored product. An advantage of these methods is that sophisticated instrumentation is not required. On the other hand, most require rather extensive sample preparation, such as protein precipitation (*5–7*, *9*), column purification (*8*, *10*), distillation (*6*, *7*), and sequential addition of several reagents (*5–10*). In some cases, the chromophore is not stable, and spectrophotometric determination must be carried out within a short period of time (*8*). Thiocyanate in plasma has also been determined spectrophotometrically as the ferric (*11*) or cupric (*12*) complexes, but these methods appear to suffer from a lack of specificity (*9*). Other methods include electrochemical determination using an ion-specific electrode (*13*), HPLC using colorimetric detection (*14*), and gas chromatography following conversion of thiocyanate to cyanogen bromide (*15*) or methyl thiocyanate (*16*). The electrochemical method requires large samples (*8*) and is subject to interferences (*16*); the HPLC method has been applied only to urine. The gas chromatographic methods are either time-consuming (*15*) or have not been applied to plasma samples (*16*).

This paper describes a novel procedure for gas chromatographic determination of thiocyanate in plasma based on the formation of an ion pair with the tributylsulfonium ion. The ion pair is extracted into ethyl acetate and injected into a gas chromatograph, which results in formation of the volatile derivative butyl thiocyanate. The method is sensitive enough to measure thiocyanate in 100-μL plasma samples from nonsmokers, and the simplicity of the extraction procedure allows rapid processing of large numbers of samples.

EXPERIMENTAL SECTION

Equipment. Gas chromatography was carried out with a Hewlett-Packard Model 5880A chromatograph with a nitrogen–phosphorus detector, Level IV microprocessor, and Model 7672A autosampler. Extractions were carried out with a multitube vortex mixer (Kraft Apparatus, Inc.). Spectrophotometric determinations were performed with a Turner Model 330 or Beckman DB-G spectrophotometer.

Chemicals. Potassium thiocyanate (analytical reagent grade) was dried at 110 °C for 3–4 h and cooled in a desiccator prior to weighing and preparation of standard solutions. Potassium selenocyanate was obtained from Aldrich Chemical Co. Tributylsulfonium perchlorate was synthesized according to the method of Milligan and Minor (*17*). Butyl thiocyanate (IR 2154 cm^{-1}, sharp) was prepared by the reaction of butyl bromide and potassium thiocyanate (*18*), and butyl isothiocyanate (IR 2094 cm^{-1}, broad) was synthesized from *n*-butylamine and carbon disulfide (*19*). Ethyl acetate was from Burdick and Jackson Distilled-in-Glass. All other chemicals were reagent grade.

Preparation of Internal Standard/Ion-Pairing Reagent. The following solutions were prepared: (A) 0.2 N sodium hydroxide saturated with sodium chloride; (B) 200 μM aqueous potassium selenocyanate; and (C) 20 mg/mL tributylsulfonium perchlorate in methanol. Solutions A, B, and C were combined in the ratio of 10:1:1 just prior to carrying out the extractions. Aqueous solutions of the internal standard (B) were stable for several weeks under refrigeration. Solution C was made up fresh daily since the sulfonium salt appeared to decompose in methanol solutions left for long periods at room temperature.

Standards and Controls. Standards were prepared by spiking out-dated human plasma from a local blood bank with an appropriate amount of aqueous thiocyanate (99 parts plasma to 1 part aqueous thiocyanate). Several concentrations were prepared spanning the expected plasma range of 0–300 μM. A batch of controls, prepared by spiking a nonsmoker's plasma to 100 μM in thiocyanate, was used to determine the day-to-day and within-run precision.

Sample Preparation. Aliquots of the internal standard/ion-pairing reagent (1.2 mL) were pipetted into 13 × 100 mm culture tubes. Plasma samples (100 μL) were added, and the tubes were tapped to mix. Ethyl acetate (1.2 mL) was added and the tubes were capped and vortexed for 5 min and then centrifuged

0003-2700/84/0356-1692$01.50/0 © 1984 American Chemical Society

to break emulsions. If some tubes still had emulsions, they were tapped a few times and then centrifuged a second time. The aqueous phases were frozen by placing the tubes in a dry ice–acetone mixture, and the organic phases were poured into autosampler vials and capped for GC analysis.

Gas Chromatography. Analyses were carried out on a 2 m × 2 mm i.d. glass column, configured for on-column injection, and packed with 3% SP2401DA on 100/120 mesh Supelcoport (Supelco, Inc.). The column packing was extended about 2 cm into the heated injection zone. Carrier gas (nitrogen), detector air, and hydrogen flow rates were 30, 50, and 5 mL/min, respectively. The injection port and detector temperatures were 325 and 300 °C, respectively. The column oven temperature ranged from 90 to 105 °C depending on the age of the column. Autosampler parameters were as follows: three prewashes, three pumps, and 3-μL injection volume. Run times were 5 min per sample.

Calibration Procedure. By use of the internal standard method and peak area ratios, a best-fit linear regression line was calculated for spiked plasma standards. The concentration of thiocyanate prior to spiking was determined from the y intercept and was added to the spiked values to obtain the true concentrations. A standard in the middle of the expected concentration range was injected and its true concentration was used to calibrate the computing integrator.

Determination of Derivatization Efficiency and Extraction Recovery. The derivatization efficiency was determined by comparing the peak area obtained from injection of synthetic butyl thiocyanate with the peak area obtained from injection of potassium thiocyanate and sulfonium salt. The internal standard/ion-pairing reagent was extracted with ethyl acetate; then the extract was spiked with methanolic potassium thiocyanate to a concentration of 10 μM and injected several times into the GC using the automatic sampler. The mean peak area was compared to the mean peak area obtained from several injections of 10 μM butyl thiocyanate in ethyl acetate.

Extraction recovery was determined by using equal volumes of 10 μM aqueous thiocyanate containing 1 mg/mL (3.3 mM) tributylsulfonium perchlorate and organic solvent. Peak areas were compared to the area obtained for 10 μM butyl thiocyanate multiplied by the derivatization efficiency previously determined.

RESULTS AND DISCUSSION

On-Column Alkylation. On-column, or "flash heater" alkylation is a commonly used method of derivatizing acidic substances for gas chromatographic analysis (*20–22*). The usual procedure is to inject a solution containing the analyte and a tetraalkylammonium hydroxide into the gas chromatograph, which results in formation of the alkylated derivative and a tertiary amine. The mechanism presumably involves formation of an ion pair, which undergoes the alkyl transfer reaction in the heated injection port. Recently, the use of trialkylsulfonium salts as on-column derivatizing agents has been reported (*23, 24*). The sulfonium salts are advantageous in certain cases, since the sulfides formed as byproducts are more volatile than the corresponding amine byproducts derived from tetraalkylammonium salts, and so are less likely to interfere with the analysis.

We found that injection of solutions containing potassium thiocyanate and tributylsulfonium perchlorate into a gas chromatograph resulted in a facile reaction producing butyl thiocyanate (eq 1). Selenocyanate was found to undergo an analogous transformation to the butyl derivative (eq 2). This suggested the possibility of gas chromatographic determination of thiocyanate using selenocyanate as an internal standard.

$$(n\text{-}C_4H_9)_3S^+SCN^- \xrightarrow{\Delta} n\text{-}C_4H_9SCN + (n\text{-}C_4H_9)_2S \quad (1)$$

$$(n\text{-}C_4H_9)_3S^+SeCN^- \xrightarrow{\Delta} n\text{-}C_4H_9SeCN + (n\text{-}C_4H_9)_2S \quad (2)$$

Gas Chromatography. The butyl derivatives of thiocyanate and selenocyanate were readily separated on a 2-m column packed with 3% SP2401DA. It was found necessary to fill the column such that the packing extended about 2 cm into the heated injection zone in order to achieve efficient alkylation. Apparently, the alkyl transfer reaction is facilitated by hot surfaces, such as provided by the segment of heated column packing. The identity of the thiocyanate derivative formed on-column was shown to be butyl thiocyanate, rather than butyl isothiocyanate, by comparison of retention times with synthetic materials.

Optimization of Extraction and Derivatization. Initial studies were aimed at finding optimum conditions for on-column alkylation. The efficiency of conversion increased with increasing injection port temperature up to 325 °C. Beyond this temperature the efficiency decreased slightly and appeared to be less reproducible. By comparing the detector response with that obtained from injection of synthetic butyl thiocyanate, we estimated the efficiency of on-column alkylation to be 96% at an injection port temperature of 325 °C.

Extraction of thiocyanate from aqueous solution was found to be markedly dependent upon ionic strength. With ethyl acetate as the extracting solvent, only about 1% was extracted from an aqueous solution containing thiocyanate and the ion-pairing agent tributylsulfonium perchlorate. Saturating the aqueous phase with ammonium sulfate increased the extraction efficiency to 20% but unfortunately led to emulsions when applied to plasma samples. Adding sodium hydroxide to the aqueous phase both increased extraction efficiency and minimized emulsions. With 0.2 N sodium hydroxide, the recovery was 5%, and by saturating 0.2 N sodium hydroxide with sodium chloride, the recovery was increased to 18%.

A variety of solvents, including ether, chloroform, butyl acetate, butanol, and 10% isopropyl alcohol in ethyl acetate were compared to ethyl acetate, but either the extraction efficiency was less or endogenous substances extracted from plasma interfered with the analysis. Consequently, ethyl acetate in combination with 0.2 N sodium hydroxide saturated with sodium chloride was used for extraction of plasma, which gave an extraction efficiency of 14%. The effect of tributylsulfonium ion concentration on extraction efficiency was also investigated, but no significant differences were observed in the range of 100 μg/mL to 10 mg/mL.

Application to Plasma Samples. A simple, one-step extraction was developed for plasma samples. Aliquots of plasma were added to a saturated aqueous sodium chloride solution containing sodium hydroxide, the internal standard, and the derivatizing agent and then extracted with ethyl acetate. After the mixture was centrifuged to break emulsions, the extracts were transferred to autosampler vials for GC analysis. The chromatograms were clean, and coextracted endogenous substances did not lead to interferences (Figure 1). Quantitation was achieved by the internal standard method, using peak area ratios of thiocyanate/selenocyanate. Standard curves were linear over the entire range of concentrations studied, 0–300 μM. The standard curves were obtained from plasma specimens spiked with thiocyanate at several concentrations over the expected range. Since thiocyanate is a normal plasma constituent, the concentration prior to spiking had to be determined from the y intercept of the linear regression line. This value was added to the spiked value to obtain the "true" value, which was then used to calibrate the computing integrator.

The method has been used to analyze plasma samples as part of behavioral and pharmacologic studies of smokers. The mean plasma thiocyanate concentrations in 92 smokers was 158 μM as compared to a mean of 33 μM for 101 nonsmokers (Table I). These values agree well with values previously reported (144–157 μM for smokers, 42–57 μM for nonsmokers: ref 1, 11, and 25), although the mean plasma thiocyanate

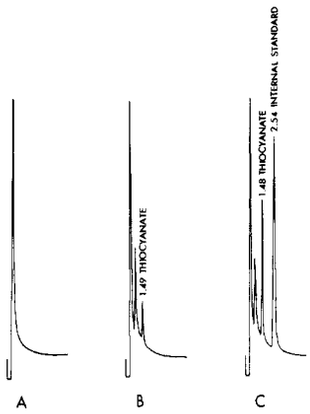

Figure 1. Chromatograms of plasma extracts: (A) extract of plasma without tributylsulfonium perchlorate; (B) nonsmoker's plasma containing 38 μM thiocyanate extracted with tributylsulfonium perchlorate but without internal standard; (C) nonsmoker's plasma spiked with thiocyanate (100 μM) and internal standard (200 μM). The numbers above each peak are the retention times in minutes. The peak eluting before thiocyanate is dibutyl sulfide, byproduct of the on-column alkylation reaction.

Table I. Plasma Thiocyanate Concentrations in Smokers and Nonsmokers Determined by the Gas Chromatographic Method Described in This Paper

	mean plasma thiocyanate concn, μM ± std dev (range)	no. of cigarettes smoked daily, mean ± std dev (range)
smokers (n = 92)	158 ± 51 (23–270)	32 ± 16 (4–82)
nonsmokers (n = 101)	33 ● 26 (5–146)	0

concentration for nonsmokers determined in our study was somewhat lower than those reported in most previous studies. Whether this was due to differences in analytical methodologies employed or due to the different populations of individuals studied is unclear. In our study, smokers and nonsmokers were distinguished by determination of the nicotine metabolite cotinine in plasma.

Precision and Sensitivity. Within-run precision was determined by analyzing 12 aliquots of the same plasma specimen and computing the relative standard deviation. At concentrations of 20 and 100 μM, the values were 5.9% and 1.9%, respectively. The between-run relative standard deviation (n = 8 over 5 months) was 7.2% at 100 μM. Since no difficulty was encountered in measuring thiocyanate at concentrations as low as 5 μM, the sensitivity of the assay is more than adequate for most applications.

Comparison with Colorimetric Methods. Thiocyanate in plasma has most frequently been determined colorimetrically by using the König reaction. Consequently, we felt that a comparison with such methods would be informative. Plasma specimens from both smokers and nonsmokers were split and analyzed by GC and by either of two published procedures (Figure 2). Correlation coefficients were r = 0.85 (n = 17) comparing GC with the method of Lundquist et al. (8) and r = 0.97 (n = 15) comparing GC with the method of Grgurinovich (9).

CONCLUSIONS

The method described in this paper appears to have certain advantages over previously reported methods. The simplicity of the extraction procedure, short chromatographic run time, and the use of an automatic sampler have made the method convenient for the analysis of large numbers of samples. Good sensitivity and precision for small plasma samples were obtained. This may be in part due to the use of an analogue of thiocyanate, selenocyanate, as an internal standard to correct for variations in recovery during extraction and chromatography.

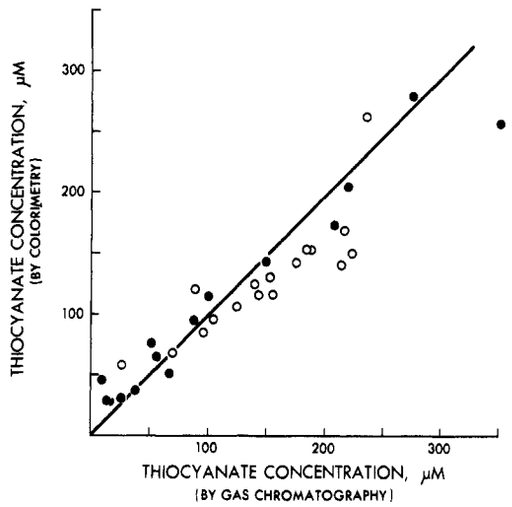

Figure 2. Plasma thiocyanate concentrations determined by the gas chromatographic method described in this paper (x axis) compared to concentrations determined by colorimetric methods (y axis). Open circles (O) represent samples analyzed by gas chromatography and by the method of Lundquist et al. (ref 8); closed circles (●) represent samples analyzed by gas chromatography and by the method of Grgurinovich (ref 9).

Preliminary experiments have indicated that the method is applicable to the analysis of urine and saliva as well as plasma. This successful extraction and derivatization of thiocyanate and selenocyanate suggests that analogous procedures may be feasible for the determination of other inorganic anions in aqueous matrices.

ACKNOWLEDGMENT

The authors are grateful to Kaye Welch, Beverly Busa, and Mitzi Speer for their assistance in this research.

Registry No. Thiocyanate, 302-04-5; tributylsulfonium, 39895-78-8.

LITERATURE CITED

(1) Haley, N. J.; Axelrad, C. M.; Tilton, K. A. *Am. J. Public Health* **1983**, *73*, 1204–1207.
(2) Schmeltz, I.; Hoffmann, D. *Chem. Rev.* **1977**, *77*, 295–311.
(3) Bodansky, M.; Levy, M. *Arch. Intern. Med.* **1923**, *31*, 373.
(4) Schulz, V.; Bonn, R.; Kindler, J. *Klin. Wochenschr.* **1979**, *57*, 243–247.
(5) Pettigrew, A. R.; Fell, G. S. *Clin. Chem. (Winston-Salem, N.C.)* **1972**, *18*, 996–1000.
(6) Boxer, G. E.; Rickards, J. C. *Arch. Biochem. Biophys.* **1952**, *39*, 292–300.
(7) Nyström, C.; Sörbo, B. *Scand. J. Clin. Lab. Invest.* **1957**, *9*, 223–225.
(8) Lundquist, P.; Mårtensson, J.; Sörbo, B.; Öhman, S. *Clin. Chem. (Winston-Salem, N.C.)* **1979**, *25*, 678–681.
(9) Grgurinovich, N. *J. Anal. Toxicol.* **1982**, *6* 53–55.
(10) Lundquist, P.; Mårtensson, J.; Sörbo, B.; Öhman, S. *Clin. Chem. (Winston-Salem, N.C.)* **1983**, *29*, 403.
(11) Butts, W. C.; Kuehneman, M.; Widdowson, G. M. *Clin. Chem. (Winston-Salem, N.C.)* **1974**, *20*, 1344–1348.
(12) Danchik, R. S.; Boltz, D. F. *Anal. Chem.* **1968**, *40*, 2215–2216.
(13) DuCailar, J.; Mathieu-Daude', J. U.; Deschodt, J.; Griffe, O. *Ann. Anesthesiol. Fr.* **1976**, *17*, 519.
(14) Imanari, T.; Tanabe, S.; Tolda, T. *Chem. Pharm. Bull.* **1982**, *30*, 3800–3802.
(15) Brabander, H. F.; Verbeke, R. *J. Chromatogr.* **1977**, *138*, 131–142.
(16) Funazo, K.; Tanaka, M.; Shono, T. *Anal. Chem.* **1981**, *53*, 1377–1380.
(17) Milligan, T. W.; Minor, B. C. *J. Org. Chem.* **1963**, *28*, 235–236.
(18) Knoke, D.; Kottke, K.; Pohloudek-Fabini, R. *Pharmazie* **1973**, *28*, 574–584.
(19) Dains, F. B.; Brewster, R. Q.; Olander, C. P. "Organic Synthesis"; Gilman, H., Ed.; Wiley: New York, 1932; Vol. 1, pp 447–449.
(20) Knapp, D. R. "Handbook of Analytical Derivatization Reactions"; Wiley: New York, 1979.
(21) Perchalski, R. J.; Wilder, B. J. In "Antiepileptic Drugs: Quantitative Analysis and Interpretation"; Pippenger, C. E., Penry, J. K., Kutt, H., Eds.; Raven Press: New York, 1978; Chapter 8.
(22) Gambario, V.; Mariani, R.; Marozzi, E. *J. Anal. Toxicol.* **1982**, *6*, 321–323.
(23) Butte, W.; Eilers, J.; Kirsch, M. *Anal. Lett.* **1982**, *15*, 841–850.

(24) Butte, W. J. Chromatogr. 1983, 261, 142–145.
(25) Kornitzer, M.; Vanhemeldonck, A.; Boudoux, P.; DeBacker, G. J. Epidemiol. Commun. Health 1983, 37, 132–136.

RECEIVED for review February 21, 1984. Accepted April 19, 1984. This research was supported in part by Grants DA02277, CA32389, HL29476, DA01696, DA02088, DA02538, DA00053, and DA00065 from the National Institutes of Health.

Quantitative Measurement of Octopamines and Synephrines in Urine Using Capillary Column Gas Chromatography Negative Ion Chemical Ionization Mass Spectrometry

Kamal E. Ibrahim, Margaret W. Couch, and Clyde M. Williams*

Veterans Administration Medical Center and Department of Radiology, University of Florida, Gainesville, Florida 32610

Mary Beth Budd and Richard A. Yost

Chemistry Department, University of Florida, Gainesville, Florida 32611

John M. Midgley

Department of Pharmacy, University of Strathclyde, Glasgow, Scotland G1 1XW, United Kingdom

The isomeric octopamines and synephrines were measured in urine by a new assay which combines ion-exchange chromatography, capillary column gas chromatography, and electron capture negative ion chemical ionization mass spectrometry. Deuterium labeled analogues of each compound were added to urine containing 1 mg of creatinine and the acid hydrolysate was subjected twice to cation exchange chromatography. The resultant amine fraction was derivatized with pentafluoropropionic anhydride and, under electron capture negative chemical ionization conditions, the PFP derivatives gave M^- and $(M - HF)^-$ ions which were sufficiently abundant to be suitable for selected ion monitoring. The limit of detection was approximately 100 pg mg^{-1} creatinine. In 10 normal adults, the concentrations of *o*-, *m*-, and *p*-octopamine and *o*-, *m*- and *p*-synephrine were, respectively, 0.6 (±0.2), 2.1 (±0.8), 25 (±19), ND (<0.1), 1.8 (±0.7), and 16 (±10) ng mg^{-1} creatinine. The occurrence of *o*- and *m*-octopamine and *m*-synephrine in human tissue or fluid has not been reported previously.

The development of a radiochemical enzyme assay for the quantitative determination of *p*-octopamine (*1*, *2*) led to its discovery in several invertebrate nerve systems and in sympathetically innervated mammalian organs. However, it was subsequently discovered that the method was not specific for *p*-octopamine because the meta and para isomers were not resolved. The use of a modified method showed that both *m*- and *p*-octopamine were present in rat salivary gland (*3*) and brain (*4*). The radiochemical enzyme assay depends upon norepinephrine *N*-methyltransferase, which can accept all three positional isomers of octopamine as substrates with varying efficacy. The resultant products, the corresponding synephrines, are also substrates for the enzyme (*5*, *6*), and consequently, any one or more of these amines could have been detected and quantified as *p*-octopamine by the unmodified assay.

Gas chromatography/mass spectrometry (GC/MS) techniques permit the unequivocal identification and quantitative determination of the three isomeric octopamines and three isomeric synephrines and there is now conclusive evidence for the natural occurrence in mammalian tissue of *o*-octopamine (*7*), *m*-octopamine (*3*, *4*, *8*), *m*-synephrine (*9*, *10*), and *p*-synephrine (*10*). Our initial attempts to determine naturally occurring isomeric octopamines and synephrines in mammalian urine by electron impact GC (packed column) MS methods were unsuccessful because the concentrations of these amines in most of the samples were below the lower limits of detection (50–100 ng mg^{-1} creatinine) of the method. For this reason, we turned to electron capture negative chemical ionization (NCI) GC/MS because of its reported 10–100-fold increase in sensitivity over electron impact methods (*11*, *12*). The use of an ion-exchange resin (to separate and concentrate very small amounts of amines for analysis by NCI GC/MS) together with an "ultrabond" capillary column has enabled us to demonstrate the natural occurrence of *o*-, *m*-, and *p*-octopamine and *m*- and *p*-synephrine in normal human urine. *m*-Synephrine and *o*- and *m*-octopamine have not been identified previously in any human tissue or fluid.

EXPERIMENTAL SECTION

Reagents. These were obtained from the following sources: pentafluoropropionic anhydride (PFPA), Pierce Chemical Co. (Rockford, IL); *m*-octopamine hydrochloride, Interchim (Montlucon, France); *m*-synephrine, Sterling Chemical Co. (New York, NY); phenylethanolamine hydrochloride, *p*-synephrine, epinine hydrochloride, *p*-octopamine hydrochloride, and epinephrine bitartrate, Regis Chemical Co. (Morton Grove, IL); dopamine hydrochloride and metanephrine hydrochloride, Sigma Chemical Co. (St. Louis, MO); norepinephrine hydrochloride, 3-methoxytyramine hydrochloride, and normetanephrine hydrochloride, Calbiochem (Los Angeles, CA); *m*-tyramine hydrochloride, Vega Fox Biochemicals (Tucson, AZ); *p*-tyramine, Aldrich Chemical Co. (Milwaukee, WI); strong cation-exchange resin (AG 50W-X2, 100/200 mesh, H^+ form), Bio-Rad (Richmond, CA); *o*-octopamine-α,α'-d_2-β-d_1 hydrochloride, Merck, Sharp & Dohme (Montreal, Canada).

0003-2700/84/0356-1695$01.50/0 © 1984 American Chemical Society