Determination of Nicotine N-Oxide by Gas Chromatography following Thermal Conversion to 2-Methyl-6-(3-pyridyl)tetrahydro-1,2-oxazine

Peyton Jacob, III,* Neal L. Benowitz, Lisa Yu, and Alexander T. Shulgin

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

Nicotine *N*-oxide is extracted from urine by use of silica gel columns and eluted with methanolic ammonia. The eluate is evaporated and then heated at 150–160 °C in anisole, which results in conversion to the ring-expansion product 2-methyl-6-(3-pyridyl)tetrahydro-1,2-oxazine. The oxazine derivative is readily extracted from aqueous solution with organic solvents and has good chromatographic properties. A method for determination of nicotine *N*-oxide in smokers' urine, using 5-methylnicotine *N*-oxide as an internal standard, is described.

The mammalian metabolism of tertiary amines generally involves α-carbon oxidation and N oxidation (*1*). The tobacco alkaloid nicotine is metabolized primarily by oxidation at the 5′ carbon, which leads to the lactam cotinine (*2, 3*). The other major route of metabolism appears to be oxidation of the pyrrolidine nitrogen to give the diasteriomeric 1′-*N*-oxides (Figure 1), although relatively little data on the quantitative importance of this pathway (*2, 4, 5*) has been published.

In order to evaluate the quantitative importance of the N-oxidation pathway in humans, we required a reliable method for the determination of nicotine *N*-oxide in biologic fluids. Determination of low concentrations of *N*-oxides in biologic samples presents a challenge to analytical chemists because the polarity of this functional group makes extraction difficult (*6*). Recently, a procedure was described for solid-phase extraction of radiolabeled nicotine *N*-oxide from urine, HPLC separation, and determination by liquid scintillation counting (*5*). We attempted to adapt this method for determination of nicotine *N*-oxide in urine using HPLC with UV detection but were unsuccessful owing to the presence of substances in the urine extract that gave peaks coeluting with nicotine *N*-oxide.

Most *N*-oxides are thermally labile (*7–9*) and, as a result, it is often not possible to analyze them directly with techniques that require vaporization, such as gas chromatography or mass spectrometry. Determination of *N*-oxides by these methods has generally involved reduction back to the parent tertiary amine using titanium trichloride (*2, 9, 10*). If the parent amine is present in relatively high concentrations, it must be removed prior to determination of the *N*-oxide. In smokers' urine, nicotine concentrations are generally higher than nicotine *N*-oxide concentrations (ref 2, Table III) and, consequently, several extractions are necessary to remove nicotine prior to the reduction step (*2*). Such procedures are prone to errors due to residual nicotine and/or losses of the *N*-oxide during nicotine extraction.

The determination of polar compounds by gas chromatography is often facilitated by conversion to derivatives that are less polar and more volatile. On thermal treatment, nicotine *N*-oxide undergoes a Meisenheimer rearrangement to the ring-expansion product 2-methyl-6-(3-pyridyl)tetrahydro-1,2-oxazine in high yield (*11*). We have found that the thermal rearrangement occurs smoothly with nanogram quantities of nicotine *N*-oxide in anisole at 150–160 °C. The oxazine derivative is easily extracted from aqueous solutions with organic solvents and has good chromatographic properties. This paper describes a new method for determination of nicotine *N*-oxide in urine based on its conversion to the oxazine derivative and gas chromatography with nitrogen–phosphorus detection.

EXPERIMENTAL SECTION

Instrumentation. Gas chromatographic analyses were carried out by use of a Hewlett-Packard Model 5880A chromatograph with a nitrogen–phosphorus detector, a Level IV computing integrator, Model 7672A automatic liquid sampler, a packed column injection port, and a split–splitless capillary injection port. A 2 m × 2 mm i.d. glass column packed with 2% Carbowax 20 M and 2% KOH on 100/120 mesh Chromosorb W (Alltech Associates), configured for on-column injection, was used for packed column determinations, at an oven temperature of 160 °C. A 25 m × 0.31 mm cross-linked 5% phenylmethylsilicone, 0.52-μm film thickness, fused silica column (Hewlett-Packard) was used for the capillary GC determinations. The carrier gas (nitrogen) flow rate was 30 mL/min for packed column analyses, and 2 mL/min (helium) for capillary column analyses; detector air and hydrogen flow rates were 50 mL/min and 5 mL/min, respectively. The N/P detector bead voltage was adjusted to give a background current of 12–20 pA. Mass spectral analyses were carried out with a Hewlett-Packard 5970B mass selective detector interfaced with a Hewlett-Packard 5890A gas chromatograph, or with a Kratos MS-25S mass spectrometer.

Chemicals and Reagents. Toluene and isopropyl alcohol were from Burdick and Jackson, distilled in glass. All other solvents and chemicals were reagent grade. Solid-phase extraction of urine was carried out by using 3-mL disposable columns packed with 40-μm silica gel (BondElute Si, Analytichem, or Baker-10 SPE). Nicotine *N*-oxide, 5-methylnicotine *N*-oxide, and 2-methyl-6-(3-pyridyl)tetrahydro-1,2-oxazine were synthesized as described below. Microanalyses of new compounds were carried out by Galbraith Laboratories, Knoxville, TN.

Synthesis of 5-Methylnicotine *N*-Oxide. (*A*) *Synthesis of 5-Methylmyosmine.* Condensation of ethyl 5-methylnicotinate (15 g, 91 mmol) (*12*) with *N*-vinylpyrrolidinone (11 g, 100 mmol) was carried out in a manner analogous to the reported method for the synthesis of 5-bromomyosmine (*13*). The crude product was purified by a bulb-to-bulb distillation (Kugelrohr oven) at 110–120 °C (0.1 mmHg), giving 10.4 g (71% yield) of white solid. The analytical sample was recrystallized from methylcyclohexane, mp 69.5–71 °C. Anal. Calcd for $C_{10}H_{12}N_2$: C, 74.96; H, 7.55; N, 17.49. Found: C, 74.79; H, 7.59; N, 17.47.

(*B*) *Synthesis of 5-Methylnicotine.* To a suspension of 3 g of 5-methylmyosmine in 150 mL of H_2O was added 1 g of sodium cyanoborohydride. Concentrated hydrochloric acid was added dropwise with stirring over 30 min, during which the pH of the solution was reduced to 1.7. The solution was stirred for an additional 15 min, and then enough solid sodium acetate was added to bring the pH to 6. Formaldehyde (2 mL of 40% aqueous) was added, followed by 1 g of sodium cyanoborohydride. After being stirred for 15 min, the solution was made basic with sodium hydroxide and was extracted twice with 100-mL portions of methylene chloride. The organic phase was extracted with two

0003-2700/86/0358-2218$01.50/0

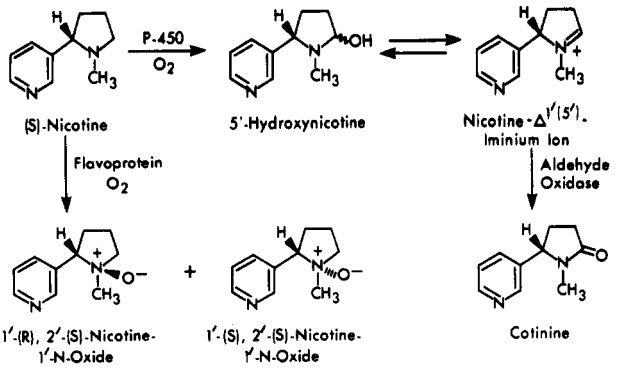


Figure 1. Oxidative metabolism of nicotine.

50-mL portions 1 N H_2SO_4, which were combined, and the methylene chloride layer was discarded. The acidic aqueous phase containing the product was made basic with sodium hydroxide and then was extracted with methylene chloride (2 × 50 mL). Evaporation of the methylene chloride using a rotary evaporator followed by bulb-to-bulb distillation (Kugelrohr oven, 110–120 °C, 0.1 mmHg) provided 2.1 g (70% yield) of light yellow mobile liquid. The bis(oxalate) was prepared by combining 0.29 g of the base with 0.42 g of oxalic acid dihydrate in methanol. Evaporation of the methanol (rotary evaporator) followed by recrystallization from methanol yielded 0.28 g of white crystalline powder, mp 131.5–133 °C. Anal. Calcd for $C_{15}H_{20}N_2O_8$: C, 50.56; H, 5.66; N, 7.86. Found: C, 50.87, H, 5.72; N, 7.89.

(*C*) *Preparation of 5-Methylnicotine N-Oxide Bis(picrate).* Hydrogen peroxide (0.1 mL of 35%) was added to 0.16 g of 5-methylnicotine in 10 mL of water. The solution was allowed to stand at room temperature for 3 days, after which it was extracted twice with 50-mL portions of ether to remove unreacted 5-methylnicotine. The ether extracts were discarded, and the aqueous phase was evaporated on a rotary evaporator to give an oil, which was combined with a solution of 0.4 g of picric acid in methanol. A gum separated which, on scratching with a glass rod, crystallized. Recrystallization from 30 mL of boiling 75% aqueous ethanol provided 0.25 g of bright yellow crystalline solid, mp 200–201 °C dec. Anal. Calcd for $C_{23}H_{22}N_8O_{15}$: C, 42.46; H, 3.41; N, 17.23. Found: C, 42.41; H, 3.36; N, 17.00.

Synthesis of Nicotine *N*-Oxide Bis(picrate). The sample of nicotine *N*-oxide used as the analytical standard was prepared from commercial nicotine by the method of Taylor and Boyer (*14*) and converted to the bis(picrate) in a manner analogous to the preparation of the 5-methyl analogue described above. The product was obtained as yellow needles from 65% ethanol, mp 170–171 °C (lit. mp 168–169 °C). Anal. Calcd for $C_{22}H_{20}N_8O_{15}$: C, 41.51; H, 3.17; N, 17.61. Found: C, 41.21; H, 3.20; N, 17.58.

Synthesis of 2-Methyl-6-(3-pyridyl)tetrahydro-1,2-oxazine (11). Nicotine *N*-oxide, prepared from 8.1 g (50 mmol) of nicotine, was heated at 150–160 °C under 25 mmHg using a Kugelrohr oven, and 6.4 g of light yellow liquid distilled. The electron impact mass spectrum (Kratos MS-25S) displayed prominent peaks at *m/z* 178 (9%, molecular ion), 119 (78%), 118 (44%), and 60 (100%), consistent with reported (*15*) mass spectral data for this compound. A small portion was converted to the bis(perchlorate) salt, which was recrystallized from ethanol as white needles, mp 155.5–156.5 °C. Anal. Calcd for $C_{10}H_{16}N_2Cl_2O_9$: C, 31.68; H, 4.25; N, 7.39. Found: C, 31.78; H, 4.23; N, 7.26.

Standards and Controls. Nicotine *N*-oxide bis(picrate) was dissolved in 75% aqueous ethanol to make a stock solution containing 1.0 mg/mL nicotine *N*-oxide base. The stock solution was diluted with water to prepare aqueous working standards or to spike nonsmokers' urine to concentrations over the range of 50–2000 ng/mL.

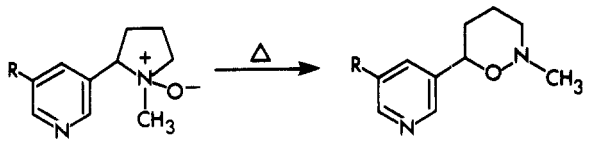
Sample Preparation and Gas Chromatographic Determination. Urine samples were acidified to a pH of 2–3 with HCl and stored frozen until the day of the analysis. Disposable 3-mL silica extraction columns were washed with 2 mL of methanol–57% ammonia (10:1) followed by 2 mL of water and then sucked dry. To 2-mL aliquots of urine were added 100 μL of internal standard (20 μg/mL aqueous 5-methylnicotine *N*-oxide) and 0.2 mL of saturated aqueous sodium acetate. The samples were vortex-mixed and applied to the columns under slight vacuum—enough to produce a flow of about 1 mL/min. By use of a homemade manifold constructed from copper tubing, up to 20 samples can be processed simultaneously. The columns were then washed successively with 2 mL of water and 2 mL of isopropyl alcohol to remove extraneous urinary constituents. Elution of nicotine *N*-oxide and internal standard was accomplished by passing 4 mL of methanol–57% ammonia (10:1) through the columns by gravity. The eluates were evaporated to dryness at about 50 °C with a current of nitrogen in 13 × 100 mm Pyrex culture tubes. Anisole (0.5 mL) was added to each tube and the tubes were capped and then heated at 150–160 °C for 30 min in a heating block. After the tubes were cooled to room temperature, 0.5 mL of 0.5 M sulfuric acid and 2 mL of toluene–isoamyl alcohol (95:5) were added to all tubes, which were then vortex-mixed for 5 min and centrifuged. The aqueous layers were frozen by placing the tubes in a dry ice–acetone bath, and the organic layers were poured off and discarded. Aqueous 3 M sodium hydroxide (0.5 mL) and toluene–isoamyl alcohol (95:5, 0.5 mL) were added, the tubes were vortex-mixed, centrifuged, and placed in a dry ice–acetone bath to freeze the aqueous layers. The organic layers were poured into autosampler vials for GC analysis. For packed column analyses, the autosampler parameters were two prewashes, two pumps, and 3-μL injection volume. At a column oven temperature of 160 °C, the retention times for 2-methyl-6-(3-pyridyl)tetrahydro-1,2-oxazine and the 5-methyl analogue were 1.50 and 2.22 min, respectively (Figure 4). For capillary column analyses (Figure 5), injections (1 μL) were made in the splitless mode, and the oven temperature was programmed from 90 to 275 °C at 25 deg/min after a 0.5-min initial hold. Retention times for the tetrahydro-1,2-oxazines derived from nicotine *N*-oxide and internal standard were 6.09 and 6.74 min, respectively. For both packed and capillary column analyses, the computing integrator was calibrated on a 1000 ng/mL standard using the internal standard method and peak height ratios. Standard curves were linear from 10 to 4000 ng/mL.

GC–MS Analysis of Urine Extract Containing Oxazine Derivatives. A smoker's urine specimen containing 1100 ng/mL nicotine *N*-oxide and 1000 ng/mL 5-methylnicotine *N*-oxide was carried through the extraction and thermal rearrangement procedure described above. A 1-μL aliquot was analyzed by GC–MS on a 12 m × 0.21 mm cross-linked methylsilicone column with a capillary direct interface to the mass spectrometer (Hewlett-Packard 5970B). The GC separation was carried out with a temperature program of 70–200 °C at 20 deg/min following a 3-min initial hold. The mass spectral analysis was carried out by using electron impact ionization at 70 eV and scanning from 35 to 210 amu. The mass spectrum of the oxazine derivative of nicotine *N*-oxide, which had a retention time of 8.7 min, displayed major ions at *m/z* 178 (8%, molecular ion), 119 (92%), 118 (68%), and 60 (100%), which is in good agreement with the mass spectral data obtained from the synthetic tetrahydro-1,2-oxazine described above. The oxazine derivative of 5-methylnicotine *N*-oxide, which had a retention time of 9.7 min, had a mass spectrum that was consistent with the proposed structure: *m/z* 192 (7%, molecular ion), 133 (100%), 132 (95%), and 60 (98%).

Determination of Efficiency of Extraction and Thermal Conversion to the Oxazine Derivative. Nonsmokers' urine was spiked with nicotine *N*-oxide to a concentration of 500 ng/mL, extracted, and analyzed as described above. The peak area of the thermal rearrangement product was compared to the area obtained by injecting a solution of synthetic oxazine derivative. For four replicate determinations, the yield ranged from 92 to 97%.

RESULTS AND DISCUSSION

Thermal rearrangement of nicotine *N*-oxide on an analytical scale was first attempted by directly injecting solutions into the gas chromatograph. At injection port temperatures ranging from 150 to 350 °C, we observed roughly equal amounts of reduction back to the nicotine and oxazine formation, as well as the formation of several minor products. This is consistent with a report (*16*) that nicotine *N*-oxide undergoes deoxygenation and dehydration as well as rearrangement to the oxazine when heated in the ion source of a mass spectrometer. When we injected extracts of urine



Nicotine N-Oxide, R = H

Internal Standard, R = CH_3

Figure 2. Thermal rearrangement of nicotine *N*-oxide and internal standard.

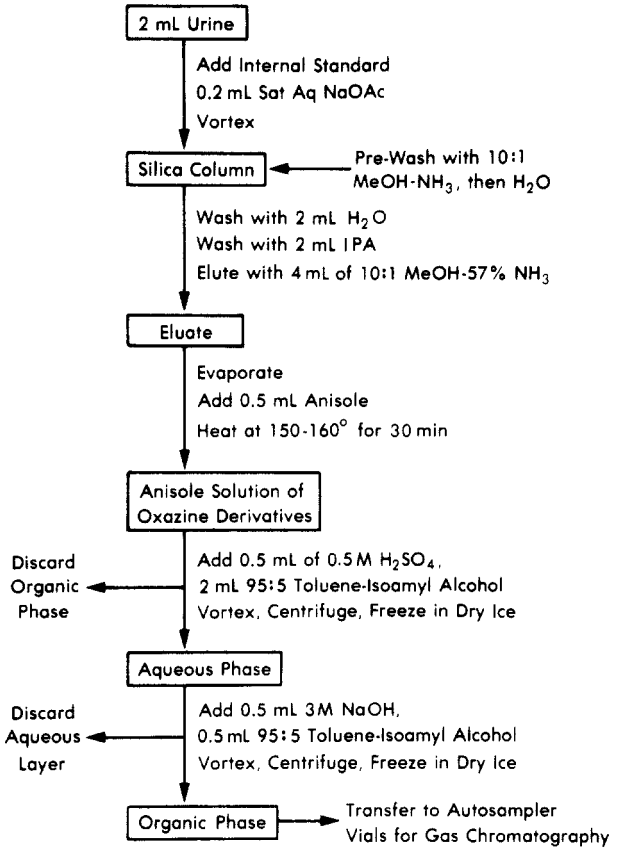


Figure 3. Flow diagram of the extraction procedure.

containing nicotine *N*-oxide, the oxazine was not resolved from other urinary constituents on either packed or capillary columns. Consequently, we turned our attention to methods for effecting the ring-expansion reaction prior to injection into the gas chromatograph in order to include cleanup steps in the extraction procedure.

Heating nicotine *N*-oxide neat or in various solvents at temperatures ranging from 100 to 200 °C resulted in varying amounts of reduction and oxazine formation. Aromatic ethers such as anisole, veratrole, and diphenyl ether gave the best results. With anisole as the solvent, the conversion to oxazine was 90% complete in less than 15 min at 160 °C with negligible reduction and was successful for quantities as little as 20 ng. The identity of the rearrangement product was confirmed by comparison of its retention time to that of material synthesized by the method of Rayburn et al. (*11*) and by its mass spectrum (*15*). In order to correct for losses of nicotine *N*-oxide occurring during extraction, thermal rearrangement and chromatography, we utilized a structural analogue, 5-methylnicotine *N*-oxide (Figure 2) as an internal standard. On thermal treatment, this compound was converted to a derivative which, based on its mass spectrum, is the Meisenheimer rearrangement product 2-methyl-6-(5-methyl-3-pyridyl)tetrahydro-1,2-oxazine.

The procedure developed for analysis of urine samples is shown in Figure 3 and described in detail in the Experimental Section. Following extraction from urine with silica columns and elution with methanolic ammonia, the eluates were evaporated to dryness with a current of nitrogen and heated in anisole at 150–160 °C to effect the ring-expansion reactions. After cooling to room temperature, the samples were cleaned up using standard acid–base partitioning steps. Unlike the *N*-oxides, the tetrahydro-1,2-oxazine derivatives are lipophilic and may be easily extracted from aqueous solutions into organic solvents. The final extracts were transferred to autosampler vials for gas chromatographic analysis. The analyses were carried out using either packed (2% Carbowax 20 M, 2% KOH) or capillary (25-m 5% phenylmethylsilicone fused silica) columns. Chromatograms obtained from extracts of nonsmokers' urines were free of peaks at retention times corresponding to the oxazine derivatives of both nicotine *N*-oxide and internal standard (Figures 4 and 5). We are now carrying out all of our analyses using capillary GC due to the high resolution and stability of modern cross-linked fused silica capillary columns. This requires temperature programming and a longer chromatographic run time but using an automatic sampler over 90 samples can be analyzed during a 24-h period.

The precision of the method is good. By use of packed column analysis, within-run relative standard deviations were between 3 and 5% at concentrations found in smokers' urine (Table I). A good correlation (r = 0.99) was obtained for a set of samples analyzed by both capillary and packed column GC. Twelve representative smokers' urine specimens which had originally been analyzed by packed column GC were reextracted and analyzed by capillary GC 2 years later (Table

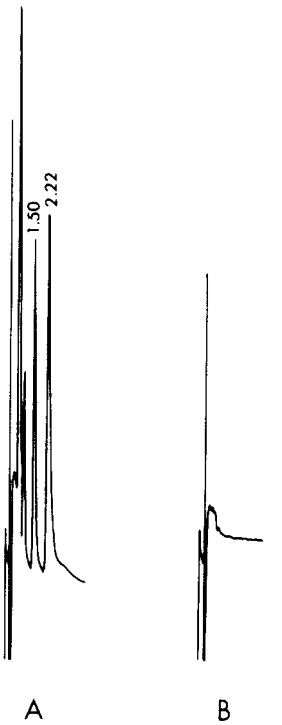


Figure 4. Chromatograms of urine extracts on Carbowax-KOH packed column. Numbers above peaks are retention times in min: tetrahydro-1,2-oxazine derived from nicotine *N*-oxide, 1.50 min; internal standard, 2.22 min. A, smoker's urine containing 540 ng/mL nicotine *N*-oxide. B, nonsmoker's urine.

Table I. Precision (%) of the Method for Spiked Urine Samples

	100 ng/mL	500 ng/mL	1000 ng/mL
within-run rel std dev (n = 6)	3.5	3.7	4.3
between-run rel std dev (n = 3)	9.0	1.0	5.6

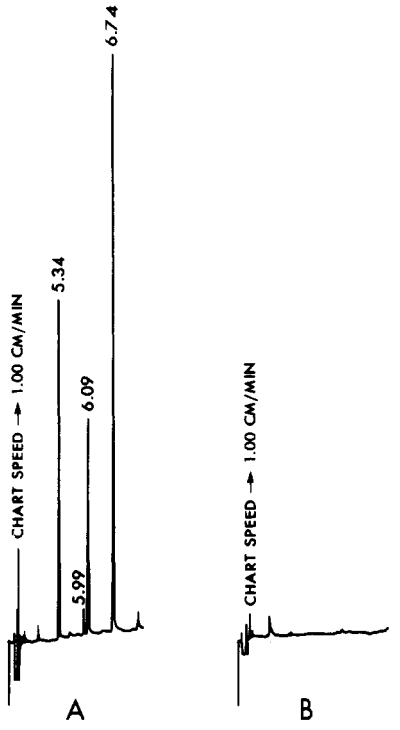


Figure 5. Chromatograms of urine extracts on 25 m × 0.31 mm 5% phenylmethylsilicone capillary column. Numbers above peaks are retention times in min: tetrahydro-1,2-oxazine derived from nicotine *N*-oxide, 6.09 min; internal standard, 6.74 min. A, smoker's urine containing 244 ng/mL nicotine *N*-oxide. B, nonsmoker's urine.

Table II. Concentration of Nicotine *N*-Oxide in Urine of 12 Smokers Determined on Packed and Capillary Columns[a]

	nicotine *N*-oxide concn,[a] ng/mL	
sample	packed column[b]	capillary column[c]
HB	410	445
MJ	1090	1260
LD	1040	1190
SS	909	1090
SK	242	236
JD	282	395
DM	363	300
ND	113	98
AL	86	81
JM	304	364
EB	370	335
SC	990	963
mean	525	562

[a] Correlation coefficient $r = 0.99$. [b] Extraction and GC determination carried out in January 1984, using a 2-m Carbowax-KOH column. [c] Extraction and GC determination carried out in March 1986, using a 25 m × 0.132 mm 5% phenylmethylsilicone column.

II). Values obtained by capillary GC averaged 7% higher, but the difference was not statistically significant by paired *t* test.

This method has been used in our laboratory for the analysis of approximately 250 urine specimens obtained from smokers. Urinary excretion of nicotine *N*-oxide in 26 smokers averaged 0.56 mg per 24 hr, which is similar to published data (*2*, *17*, *18*). Nicotine and cotinine excretion were also determined (*19*) in these subjects and was found to be 2- to 3-fold greater than nicotine *N*-oxide (Table III).

The previously reported gas chromatographic methods (*2*, *20*) for determining nicotine *N*-oxide in smoker's urine require reduction of the *N*-oxide to nicotine. In these procedures nicotine, present in much higher concentrations than the

Table III. Urinary Excretion[a] of Nicotine and Metabolites by 26 Smokers

	mean	std dev	range
nicotine concn, ng/mL	2230	1800	159–6540
nicotine excretion, mg/24 h	1.12	0.88	0.076–3.18
cotinine concn, ng/mL	2800	1600	438–6170
cotinine excretion, mg/24 h	1.39	0.66	0.174–2.78
nicotine *N*-oxide concn, ng/mL	1060	665	102–3120
nicotine *N*-oxide excretion, mg/24	0.56	0.37	0.041–1.59

[a] Urine was collected in 24-h blocks on a research ward.

N-oxide in most biological samples, must be removed first by several extractions. Our method, which utilizes the Meisenheimer rearrangement of nicotine *N*-oxide to the tetrahydro-1,2-oxazine derivative, does not require prior removal of nicotine. We have not yet explored the generality of the method, but it appears that analogous procedures would be applicable for the determination of other tertiary amine *N*-oxides.

ACKNOWLEDGMENT

The authors are grateful to Kaye Welch, Beverly Busa, and Guy Flores for their assistance in this research and to Lisa Peterson for providing some of the mass spectral data.

Registry No. Nicotine *N*-oxide, 2820-55-5; 2-methyl-6-(3-pyridyl)tetrahydro-1,2-oxazine, 15769-88-7; ethyl 5-methylnicotinate, 20826-02-2; *N*-vinylpyrrolidin-2-one, 88-12-0; 5-methylmyosmine, 102780-52-9; 5-methylnicotine, 77629-31-3; 5-methylnicotine *N*-oxide bis(picrate), 102780-54-1; nicotine *N*-oxide bis(picrate), 102780-55-2.

LITERATURE CITED

(1) Rose, J.; Castagnoli, N., Jr. *Med. Res. Rev.* **1983**, *3*, 73–88.
(2) Beckett, A. H.; Gorrod, J. W.; Jenner, P. *J. Pharm. Pharmacol.* **1971**, *23*, 55S–61S.
(3) Benowitz, N. L.; Kuyt, F.; Jacob, P., III; Jones, R. T.; Osman, A.-L. *Clin. Pharmacol. Ther. (St. Louis)* **1983**, *34*, 604–611.
(4) Booth, J.; Boyland, E. *Biochem. Pharmacol.* **1970**, *19*, 733–742.
(5) Kyerematen, G. A.; Damiano, M. D.; Dvorchek. B. H.; Vesell, E. S. *Clin. Pharmacol. Ther. (St. Louis)* **1982**, *32*, 769–780.
(6) Thompson, J. A.; Norris, K. J.; Petersen, D. R. *J. Chromatogr.* **1985**, *341*, 349–359.
(7) Castagnoli, N., Jr.; Craig, J. C.; Melikian, A. P.; Ray, S. K. *Tetrahedron* **1970**, *26*, 4319–4327.
(8) Cowan, D. A. In *Biological Oxidation of Nitrogen*; Gorrod, J. W., Ed.; Elsevier/North Holland Biomedical Press: Amsterdam, 1978; pp 263–268.
(9) Hallstrom, G.; Nguyen, C. H.; Cho, A. K. *Acta Pharm. Suec.* **1983**, *20*, 181–192.
(10) Chan, K.; Kendall, M. J.; Mitchard, M. *J. Chromatogr.* **1974**, *89*, 169–176.
(11) Rayburn, C. H.; Harlan, W. R.; Hanmer, H. R. *J. Am. Chem. Soc.* **1950**, *72*, 1721–1723.
(12) Deady, L. W.; Shanks, R. A.; Campbell, A. D.; Chooi, S. Y. *Aust. J. Chem.* **1971**, *24*, 385.
(13) Jacob, P., III *J. Org. Chem.* **1982**, *47*, 4165–4167.
(14) Taylor, E. C.; Boyer, N. E. *J. Org. Chem.* **1959**, *24*, 275–277.
(15) Phillipson, J. D.; Handa, S. S. *Phytochemistry* **1975**, *14*, 2683–2690.
(16) Shuller, D.; Harker, H.-P. *Org. Mass Spectrom.* **1973**, *7*, 839–843.
(17) Gorrod, J. W.; Jenner, P.; Keyrell, G. R.; Mikhael, B. R. *J. Natl. Cancer Inst. (U.S.)* **1974**, *52*, 1421–1424.
(18) Klein, A. E.; Gorrod, J. W. *Eur. J. Drug Metab. Pharmacokinet.* **1978**, 87–93.
(19) Jacob, P., III; Wilson, M.; Benowitz, N. L. *J. Chromatogr.* **1981**, *222*, 61–70.
(20) Stehlik, G.; Kainzbauer, J.; Tausch, H.; Richter, O. *J. Chromatogr.* **1982**, *232*, 295–303.

RECEIVED for review June 10, 1985. Resubmitted April 21, 1986. Accepted April 21, 1986. This research was supported in part by Public Health Service Grants DA02277, DA01696, CA32389, and HL29476. The clinical studies were carried out at the San Francisco General Hospital Clinical Research Center (RR-00083) with support from the Division of Research Resources, National Institutes of Health.