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ANALYSIS OF NICOTINE AS A TRICHLOROETHYL CARBAMATE BY GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION

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SUMMARY

Nicotine was subjected to reaction at 90° with trichloroethyl chloroformate in the presence of pyridine to form a carbamate in which the pyrrolidine ring was opened. Upon heat treatment, this carbamate partially formed the corresponding olefin. About 10 pg could be detected with an electron-capture detector and 60 pg with an alkali flame-ionization detector.

The extraction was studied with ^{14}C-labelled nicotine. Methylene chloride was suitable for extraction from diluted plasma, whereas toluene containing 5% of heptafluorobutanol was used in a re-extraction step and also as the chloroformate reaction medium.

Due to a nicotine blank the limit for quantitative determinations was 10 ng/ml in plasma (sample volume 1 ml). N-*n*-Propylnornicotine was used as an internal standard. The precision at the 30 ng/ml level was $\pm 8.8\%$ ($n = 7$).

INTRODUCTION

Nicotine is a widespread toxic agent and can be regarded as an environmental pollutant. The analysis of nicotine in biological systems has been performed mainly by gas chromatographic methods[1–7]. Recently, methods based on radioimmunoassay[8,9] and liquid chromatography[10] have appeared. Although nicotine was one of the first alkaloids to be gas chromatographed[11], it has proved difficult to analyse by gas chromatography at low concentrations owing to its adsorptive properties, being a divalent amine. Procedures have been described in which intact nicotine has been chromatographed and detected with a flame-ionization detector[1–3], with an alkali flame-ionization detector in the nitrogen mode[4,5] or with a mass spectrometer[6,7]. The presence of nicotine in the analytical systems as a blank has been observed[1–5]. Even in non-smokers' urine nicotine has been positively demonstrated by atmospheric pressure ionization mass spectrometry without previous gas chromatographic separation[12]. The handling of nicotine after sampling and during gas chromatography

would be facilitated if the adsorptive properties could be reduced in a derivatization step. A previously described procedure used catalytic reduction and pentafluoropropionylation of the piperidine formed. This derivative possessed electron-capture properties but no application to biological samples was reported[13].

The reaction of pentafluorobenzyl and trichloroethyl chloroformates with tertiary amines has opened the possibility of obtaining, in one step, both electron-capture sensitive and non-adsorptive derivatives, the so-called carbamates[14,15]. This paper discusses the conditions for the extraction of nicotine and the preparation and properties of a nicotine carbamate. Analyses in the nanogram range have been made with both electron-capture and alkali flame-ionization detection.

EXPERIMENTAL

Apparatus

Liquid scintillation counting. The measurements on [^{14}C]nicotine were performed in a Nuclear Chicago Mark II liquid scintillation system. The activity was obtained by calculation of the efficiency from the external standard ratio compared with a standard graph. Standard graphs in toluene and water were prepared by measurement of [^{14}C]toluene after addition of 0.1–0.9 ml of methylene chloride to 10 ml of the scintillator liquid.

pH measurements. The measurement of pH was carried out with an Orion Model 701 pH meter.

Gas chromatography. Studies of the conditions in the reaction of trichloroethyl chloroformate with nicotine in the milligrams per millilitre range were performed with a Varian 1400 gas chromatograph equipped with a flame-ionization detector. The glass column (1.5 m × 2 mm I.D.) was filled with 3% OV-17 on Gas-Chrom P (100–120 mesh) and operated at 240°. The injector and detector temperatures were both maintained at 300°. The flow-rate of the carrier gas (nitrogen) was 30 ml/min.

Studies in the nanograms per millilitre range were performed in the same instrument with a ^{63}Ni electron-capture detector working in the d.c. mode. The glass column (2.1 m × 2 mm I.D.) was filled with 3% OV-17 on Gas-Chrom Z (80–100 mesh) (= column B, Table II). The column temperature was 220° and the injector temperature 235°; other conditions were as above. Studies of the derivatives in the alkali flame-ionization detector were made with a Hewlett-Packard 5730 instrument equipped with an 18740A splitless injection system for capillary columns.

Packed columns of various polarity and length were used (see Table II).

Liquid chromatography. The liquid chromatographic analyses were performed using an LDC Model 711-47 pump and an LDC Model 1205 UV detector at 254 nm. A 150 × 3.2 mm I.D. stainless-steel column packed with LiChrosorb SI 100 (10 μm) was used with *n*-hexane–*n*-butanol (19:1) as the mobile phase. The k' value of the δ-chlorocarbamate of nicotine was 5.5.

Mass spectrometry. The trichloroethyl carbamate formed from nicotine was identified by mass spectral analysis in an LKB 9000 mass spectrometer. The sample was introduced into the mass spectrometer either after a preceding gas chromatographic separation or through the direct inlet with a cool ion source. The ionization energy was 24 eV.

Reagents and chemicals

1-[2'-^{14}C]Nicotine-*d*-bitartrate was purchased from the Radiochemical Centre, Amersham, Great Britain, with a specific activity of 63.3 μCi/mg. It was added in the extraction experiments in a concentration of *ca.* 200 ng/ml, corresponding to 10,000 dpm/ml.

The scintillator liquid for samples in organic solvents was 10 ml of toluene containing 2% of methanol and 0.5% of 2,5-diphenyloxazole as scintillator. For aqueous samples, 10 ml of Instagel® (Packard, Downers Grove, Ill., U.S.A.) was used.

Buffer solutions were carbonate or phosphate buffers with an ionic strength of 1.1.

Heptafluorobutanol was purchased from Fluka (Buchs, Switzerland). Other solvents were of the highest analytical quality and saturated with water before use in the extraction studies.

Methylene chloride of analytical quality was washed with equal volumes of 0.1 *M* orthophosphoric acid, 0.1 *M* sodium hydroxide solution and water and finally distilled. The purified solvent was stored in clean amber-glass bottles which were tightly closed. Toluene of analytical quality was repeatedly shaken with one tenth of its volume of concentrated sulphuric acid until no further colour appeared. Then the solvent was rinsed with 0.1 *M* sodium hydroxide solution and water, followed by distillation. Trichloroethyl chloroformate was used as obtained from EGA Chemie (Rüdesheim bei Heidenheim, G.F.R.). Alcoholic alkali solutions were 0.5 and 1.8 *M* potassium hydroxide in methanol.

Nicotine was purchased from Kebo (Solna, Sweden) and analogues of nicotine were supplied by the Swedish Tobacco Co. (Stockholm, Sweden).

The N-propyl analogue of nicotine, (N-*n*-propylnornicotine) was used as an internal standard. It was synthesized from nornicotine by propionylation and subsequent catalytic reduction. A stock solution at pH 7 containing 160 ng/ml was kept in a refrigerator and used within a month. Nicotine δ-trichloroethylcarbamate, used as a reference compound, was a gift from the Swedish Tobacco Co.

Methods

Determination of partition ratio of nicotine. To 1.0 ml of [^{14}C]nicotine solution in water are added 5.0 ml of buffer (ionic strength 0.2) and 4.0 ml of water. The aqueous phase is equilibrated for 30 min with 10 ml of organic solvent. After centrifugation the organic phase is separated by a capillary siphon. Unlabelled nicotine is added to the receiving flask to prevent adsorption losses. The pH is measured in the aqueous phase and 5.0 ml of each phase are counted in the liquid scintillation counter. The partition ratio, $K_{D(A)}$, is calculated from the ratio of the activity in the organic phase to that in the aqueous phase.

Evaluation of reaction conditions. Trichloroethyl chloroformate (5–50 μl) and in some instances a base are added to nicotine (1 mg/ml) as base in 0.2 ml of solvent. In the solvent 0.4 mg/ml of dotriacontane is present, used as an internal standard. The mixture is heated in a metal block and, after an appropriate time, the mixture is cooled and 0.5 ml of 0.5 *M* alcoholic alkali solution is added. After shaking for 5 min, 0.5 ml of water is added and after shaking for a further 2 min, 1–2 μl of the organic phase is injected into the gas chromatograph equipped with a flame-ionization detector. The peak-height ratio of "formed carbamate" to internal standard is calculated.

Procedure for the determination of nicotine in plasma. A plasma sample ($\leq$ 1 ml) is mixed with 1.0 ml of internal standard solution (N-*n*-propylnornicotine), then 1.0 ml of 3 *M* sodium hydroxide solution and water are added to give a final volume of 5 ml. This aqueous solution is shaken for 30 min with 5 ml of methylene chloride. After centrifugation, the organic layer is transferred into another tube with 1.0 ml of 0.1 *M* orthophosphoric acid and shaken for 10 min. The organic phase is discarded and, after alkalinization, the amines are extracted for 10 min with 0.25 ml of toluene containing 5% of heptafluorobutanol. The organic phase is transferred into another tube fitted with an air condenser. Trichloroethyl chloroformate (25 μl) and pyridine (10 μl) are added and the mixture is heated for 45 min in a metal block at 90°. The reaction mixture is shaken with 0.5 ml of 0.5 *M* alcoholic alkali solution for 5 min and, after addition of 0.5 ml of water and shaking, the aqueous phase is discarded. Then 1.8 *M* alcoholic alkali solution (0.5 ml) is added and, after vigorous shaking for 15 sec and addition of 0.5 ml of water, 1–5 μl of the organic phase are injected into the gas chromatograph equipped with an electron-capture detector. The same procedure was used with alkali flame-ionization detection.

A standard curve is prepared by addition of known amounts of nicotine to blank porcine plasma and treatment according to the procedure above.

RESULTS AND DISCUSSION

Extraction of nicotine

Partition into different organic solvents. The extraction of [^{14}C]nicotine from aqueous solutions, buffered to pH 10, was studied with radiometric technique. The partition ratio, $K_{D(A)}$, and the percentage extraction into different organic solvents using equal phase volumes are given in Table I. A quantitative extraction [$K_{D(A)} > 100$, corresponding to >99% in the organic phase] could not be achieved with any of the solvents. The highest degree of extraction was obtained with methylene chloride (*ca.* 97% extraction). The radiometric results gave a $-\log K_{D(A)}K'_{HA}$ value of 6.5, which is in accordance with the results of Borg *et al.*[18].

Partition into toluene containing an alcohol. The addition of a solvent with proton-donating properties to toluene increased the extraction of nicotine, probably owing to adduct formation. *n*-Butanol increased the degree of extraction from 80 to 90%. With 5% of heptafluorobutanol, being a stronger proton donor, the extraction increased to 98% (Table IB).

Extraction of nicotine from plasma. Methylene chloride extracted 97 $\pm$ 0.7% (n = 10) of nicotine from plasma diluted 1:5 using equal volumes of aqueous phase and methylene chloride. Extraction with toluene under the same conditions gave 85% extraction. Toluene containing 5% of heptafluorobutanol extracted 94 $\pm$ 1% (n = 8) of nicotine from an alkaline plasma. It was necessary to dilute the plasma sample about 4-fold, otherwise the extraction yield of nicotine decreased. In the method methylene chloride was used in the initial extraction owing to its higher degree of extraction in this step compared with toluene.

Formation and properties of the trichloroethyl carbamate of nicotine

Two chloroformates, *viz.*, trichloroethyl and pentafluorobenzyl chloroformate, have been used to prepare electrophore derivatives (carbamates) from tertiary

TABLE I

PARTITION OF NICOTINE

[A] = Total concentration of nicotine in the actual phase; aq = aqueous phase; org = organic phase. $K_{D(A)}$ = [A]org/[A]aq = partition ratio. pK_{HA} for nicotine = 8.0 (ref. 16). Concentration of [^{14}C]nicotine = $1.5 \cdot 10^{-5}$ *M*.

A	*Solvent*	$K_{D(A)}$ *at pH 10*	*% in the organic phase**	
	Ethyl acetate	6.7	87	
	Methyl isobutyl ketone	5.5	85	
	Diethyl ether	3.7	79	
	Methylene chloride	35	97	
	Toluene	5.0	80	
	n-Hexane	1.8	64	
B	*Solvent*	$-\log K_{D(A)}K'_{HA}$**	*% in the organic phase at pH >9**	*<1% in the organic phase* at pH*
	Methylene chloride	6.50	97	4.5
	Toluene	7.4	80	5.4
	Toluene + 5% *n*-butanol	7.0	90	5.0
	Toluene + 5% heptafluorobutanol	6.35	98	4.3
	Toluene + 10% heptafluorobutanol	6.0	>99	4.0

* Equal phase volumes.

** For definition and calculation, see ref. 17.

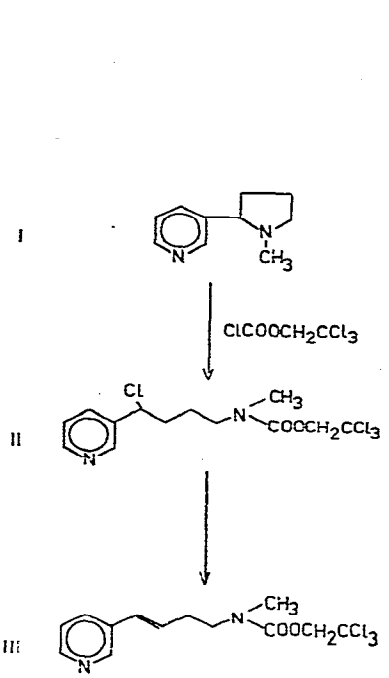
amines[15,19]. Although the carbamates were comparable in detectability in the electron-capture detector, trichloroethyl chloroformate was preferred as it gave rise to fewer disturbing components[15].

Identity of derivatives. The reaction of nicotine with several types of chloroformates has been discussed by Hootelé and Lenders[20]. The reaction with trichloroethyl chloroformate would accordingly result in the products given in Fig. 1. The opening of the pyrrolidine ring upon reaction with chloroformates is well established after recent studies on some cyclic tertiary amines[21]. For other cyclic tertiary amines dealkylation seems to be favoured[15].

The δ-chlorocarbamate (II) formed upon ring opening contains a chlorine atom in the side-chain. This compound, on excessive heat treatment, will form the corresponding olefin (III). Attempts to confirm structure II by mass spectral analysis with or without previous gas chromatographic separation always resulted in a spectrum corresponding to III owing to the loss of hydrogen chloride in the heated ion source. Use of the direct inlet system and a cool ion source demonstrated that II was the main component in the reaction of nicotine with trichloroethyl chloroformate. When the purified reference compound II was studied by liquid chromatography, only one component could be demonstrated.

Opening of the pyridine ring in nicotine was proposed by Hootelé and Lenders[20]. As the total yield of the two carbamates was about 80%, this might indicate that in addition to the carbamates some other degradation products might appear. Reaction of the pyridine ring in compounds such as brompheniramine with pentafluorobenzyl chloroformate has recently been reported[22].

The present experience of the reaction of tertiary methylamines with chloro-



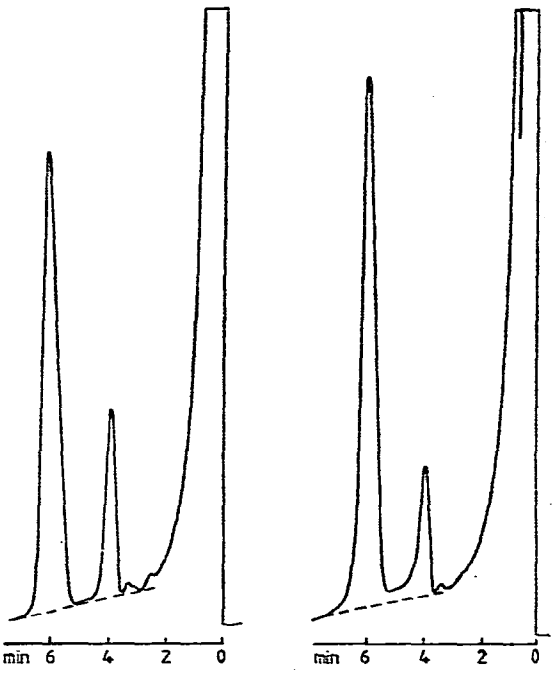


Fig. 1. Reaction scheme of nicotine with trichloroethyl chloroformate. I = Nicotine; II = δ-chlorocarbamate of nicotine; III = olefinic carbamate of nicotine.

Fig. 2. Gas chromatogram of the carbamates of nicotine. Right: first injection in the day. Left: twelfth injection. Column A (Table II), injector temperature 217°, flame-ionization detector at 325°.

formates is that dealkylation cannot be generally predicted as slight structural changes can lead to other products[15,19]. A practical demonstration of a useful side reaction with methyl chloroformate has been given for the tertiary amine N,N-dimethyl-dibenzo(*b*,*f*)thiepin-10-methylamine, where a chloromethylene derivative was obtained[23].

Gas chromatographic properties. A gas chromatogram of a reaction mixture showed one major peak corresponding to II and one minor peak (*ca.* 20%) with a shorter retention time corresponding to III. Very often an on-column conversion of II to III with a typical decomposition band in between was observed. This was more pronounced in the low concentration range. If the column had not been in use for some hours, the first chromatograms (microgram amounts) showed a considerable decomposition band (see Fig. 2). After a few injections this phenomenon almost disappeared. The influence of the column packing on the elution pattern was demonstrated in a few experiments with a capillary column with SE-30 as stationary phase. In this instance the decomposition band between compounds II and III was almost negligible (see Table II for retention data).

The conversion of II into III in the injector was considerable at temperatures above 250° (see Fig. 3). With an injector temperature of 220° ten injections of the same sample gave 21 ± 1.1% of the olefinic compound III (flame-ionization detection). The same behaviour was observed for the internal standard, N-*n*-propylnornicotine.

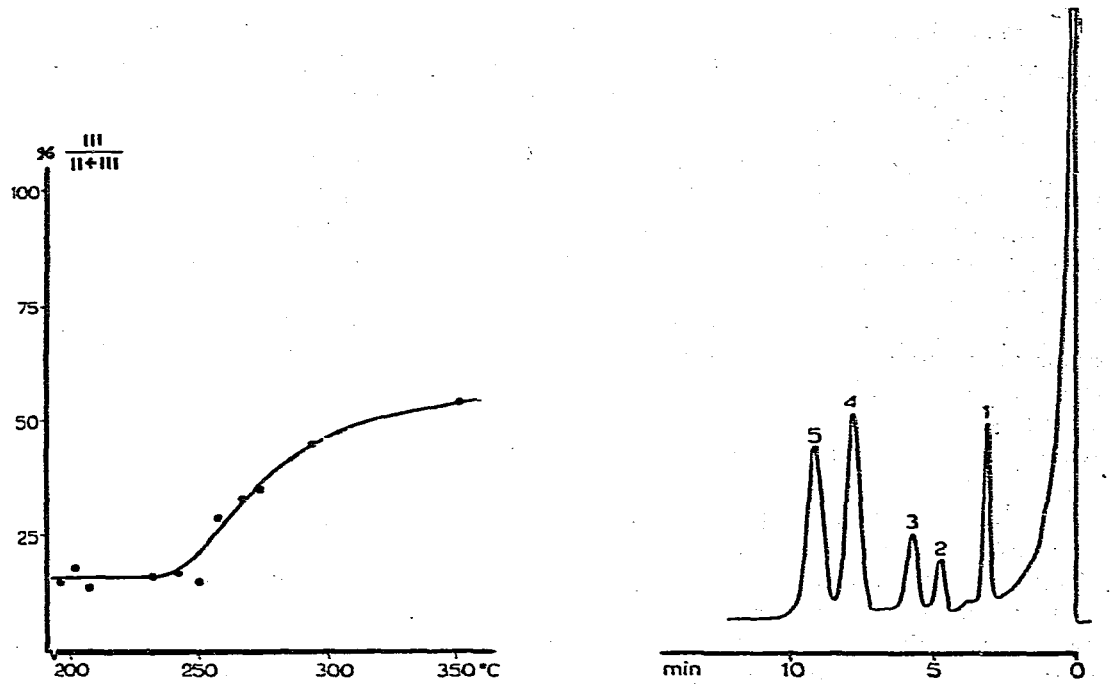


Fig. 3. Effect of injection temperature on the formation of the olefinic nicotine carbamate (III) from the δ-chlorocarbamate.

Fig. 4. Gas chromatogram of the carbamates on OV-225. Column C (Table II) with alkali flame-ionization detection. 1 = Trichlorethylcarbamate of nornicotine; 2 = olefin carbamate of nicotine; 3 = olefin carbamate of internal standard; 4 = δ-chlorocarbamate of nicotine; 5 = δ-chlorocarbamate of internal standard.

TABLE II

RELATIVE RETENTION OF THE TRICHLOROETHYL CARBAMATES FROM NICOTINE AND N-*n*-PROPYLNORNICOTINE

Columns: A: 0.9 m, 3% OV-1 + 3% OV-17, Chromosorb W (80–100 mesh), 210°. B: 2.1 m, 3% OV-17, Gas-Chrom Z (80–100 mesh), 225°. C: 1.2 m, 3% OV-225, Gas-Chrom Q (100–120 mesh), 220°. D: 1.2 m, 3% OV-17 + 0.6% Carbowax 20M terephthalic acid, Gas-Chrom Q (80–100 mesh), 200°. E: 10 m × 0.77 mm I.D. SE-30 wide-bore glass capillary column, 200°, flow-rate of carrier gas (helium), 4.6 ml/min.

Carbamate		*Column*				
		A	*B*	*C*	*D*	*E*
Nicotine	δ-Chloro (II)	1.00	1.00	1.00	1.00	1.00
	Olefinic (III)	0.64	0.71	0.62	0.63	0.73
N-*n*-propylnornicotine	δ-Chloro (II)	1.27	1.25	1.16	—	—
	Olefinic (III)	0.88	0.87	0.74	—	—
Absolute retention time of δ-chlorocarbamate of nicotine (min)		6.3	7.9	8.1	49.5	14

The retention data for the derivatives II and III on stationary phases of various polarity are shown in Table II. A polar phase such as OV-225 gave excellent peak performance, although the column temperature had to be higher than for non-polar stationary phases (Fig. 4).

Adsorption of nicotine and its carbamate. The adsorption losses of nicotine, even in the micrograms per millilitre range, were considerable on the glass walls in the reaction tubes, in the injection syringe and in the gas chromatographic system. Adsorption losses were not observed with the carbamate of nicotine (II) when dissolved in toluene and injected into the gas chromatograph.

Reaction conditions. Toluene was used as a reaction medium. The presence of heptafluorobutanol (5%) did not influence the reaction rate or the yield of the derivatives. Earlier studies of the reaction of trichloroethyl chloroformate with tertiary amines showed that fairly high temperatures were required[15]. In this study, 10% of reagent at 90° was found to be suitable. The addition of sodium carbonate to the reaction mixture did not influence the reaction rate[24].

Initially, the attempts to form a carbamate from nicotine resulted in low and very varying yields. Under the same reaction conditions N-methylpyrrolidine required 70 min for a single and stable derivative to be obtained. As this compound resembles the aliphatic moiety of nicotine, it was then suspected that the problems with nicotine were due to the pyridine part of the molecule. The carbamates of the secondary amines, nornicotine and anabasine, were prepared and no decrease in yield of the carbamates with time upon treatment with trichloroethyl chloroformate was observed. This indicated that in the carbamate of nornicotine with the pyrrolidine ring intact pyridinolysis[20] was unimportant.

However, as the carbamate of one nicotine analogue (compound 3, Table III) with a 4-chlorophenyl group instead of pyridine seemed to be formed in reproducible yields, this might indicate that it is the pyridine moiety of the nicotine carbamate which is sensitive to further reaction.

The addition of pyridine or some pyridine derivatives to the reaction mixture increased the yield of the carbamate from nicotine. Pyridine was studied at concentrations of 1, 5 and 10% (Fig. 5). Not only were the yields improved but also the precision (from a relative standard deviation of 20 to 4%). With 5% of pyridine the yield was constant from 30 to 360 min. The same positive effect was observed with the addition of triethylamine. For nicotine a reaction time of 30 min was sufficient

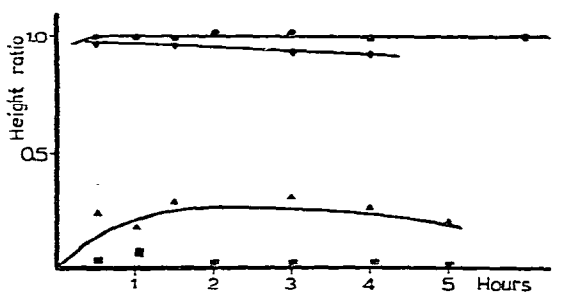


Fig. 5. Time course of the formation of II from nicotine. Conditions: nicotine (1 mg/ml) in 0.2 ml of toluene treated with 20 µl of trichloroethyl chloroformate at 90°. Amount of pyridine added: ♦, 10%; ●, 5%; ▲, 1%; ■, none.

whereas the internal standard required 45 min. The reason for this favourable effect of the bases it not clear.

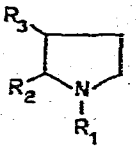
By comparison with a synthesized and purified reference compound the yield of the carbamates was estimated to be of the order of 80%. Of the amount injected into the gas chromatograph, the δ-chlorocarbamate (II) typically comprised 80%, giving a yield of about 65% of II. On gas chromatography the reference compound gave the same ratio of II:III as in the reaction mixture.

Choice of internal standard. The compounds listed in Table III were tested as suitable internal standards in the determination of nicotine. Most of the compounds gave products that were eluted too close to the carbamate of nicotine. They also reacted much faster than nicotine, which is a disadvantage if the internal standard should indicate reaction failures[25]. With 6-methylnicotine (compound 4) no derivative was found.

TABLE III

ANALOGUES OF NICOTINE REACTED WITH TRICHLOROETHYL CHLOROFORMATE AND THE RELATIVE RETENTIONS OF THE δ-CHLOROCARBAMATE DERIVATIVES

Structure of compound before the reaction:



Retention time of trichloroethyl δ-chlorocarbamate of nicotine (II) on column A: 7 min.

No.	R_1	R_2	R_3	*Relative retention*
1	CH_3	3-Pyridyl	H	1.00
2	C_2H_5	3-Pyridyl	H	0.99
3	C_3H_7	3-Pyridyl	H	1.25
4	CH_3	3-(6-Methyl)pyridyl	H	—*
5	CH_3	4-Methylphenyl	H	0.89
6	CH_3	4-Chlorophenyl	H	1.26
7	CH_3	H	4-Chlorophenyl	0.96

* No derivative formed.

Compound 6 was used as an internal standard in the early part of this study. The yields of carbamate from this compound were always consistent even if the nicotine values were irreproducible.

The final choice of internal standard was N-*n*-propylnornicotine (compound 3), which gave a derivative with a suitable retention relative to the δ-chlorocarbamate of nicotine (II) and with about the same formation time.

Analysis of nicotine in the nanograms per millilitre range

Minimum detectable concentration. The high detectability reported earlier for this type of derivative in the electron-capture detector[15] was also found for the δ-chlorocarbamate of nicotine. The purified reference compound gave a minimum detectable concentration (MDC) of $9 \cdot 10^{-16}$ moles/sec on column B. This corresponds to about 10 pg of nicotine δ-chlorocarbamate injected. In this experiment the δ-

chlorocarbamate peak comprised 60% of the total area for the nicotine derivatives (δ-chloro- and olefin carbamate including the area between the peaks).

The MDC value for the δ-chlorocarbamate of nicotine was also evaluated in the alkali flame-ionization detector. With column D an MDC of $6 \cdot 10^{-15}$ moles/sec was obtained.

With thermionic detection a more volatile nicotine carbamate than that with trichloroethyl can be used. The retention data of some nicotine carbamates are given in Table IV. It was not possible to utilize the excellent performance on the OV-225 column with the highest sensitivity as the background signal became waveformed, probably owing to phase bleeding and temperature variations in the thermionic detector.

TABLE IV

RELATIVE RETENTIONS OF SOME CARBAMATES OF NICOTINE

Retention time of δ-chlorotrichloroethyl carbamate on column D: 49.5 min.

Alkyl group in the carbamate moiety	*δ-Chloro*	*Olefin*
CH_3	0.19	0.13
C_2H_5	0.22	0.15
iso-C_4H_9	0.34	0.24
Cl_3CCH_2	1.00	0.63

Purification of reagents. It has been observed that nicotine can be transferred via room air to non-smokers[12]. This means that trapping of nicotine in solvents and other reagents is possible. The organic solvents used in this study had to be washed with both acid and alkali before distillation[5].

Purification of the reaction mixture. The excess of the reagent, trichloroethyl chloroformate, and by-products formed in the reaction were previously removed with an alcoholic alkali treatment[15]. With nicotine it was necessary to use a stronger alcoholic alkali solution in order to remove completely early eluting components from the reaction mixture. The carbamate of nicotine was not sensitive to this treatment. The derivatives were too lipophilic to permit purification by re-extraction into acid.

Bioanalytical applications. In earlier applications of carbamates in the determination of tertiary amines in biological fluids, it was necessary to remove the corresponding secondary amine as this metabolic product yields the same carbamate[26]. N-Methylpyrrolidine compounds, like nicotine, however, undergo ring opening in the chloroformate reaction whereas with nornicotine the ring remains intact. This means that nornicotine does not have to be removed before the chloroformate reaction. The carbamate from nornicotine is eluted with a retention of 0.48 relative to the carbamate of nicotine (II) on OV-17 and 0.42 on OV-225 (see Fig. 4). Interference from other nicotine metabolites is not likely[27].

The relative standard deviation of the method was 8.8% ($n = 7$) when samples spiked with 29 ng of nicotine per millilitre of pig plasma were analysed. A chromatogram is shown in Fig. 6.

The nicotine blank. Several studies have been published in which the presence of nicotine in the blanks has been demonstrated. Falkman *et al.*[1] observed a blank

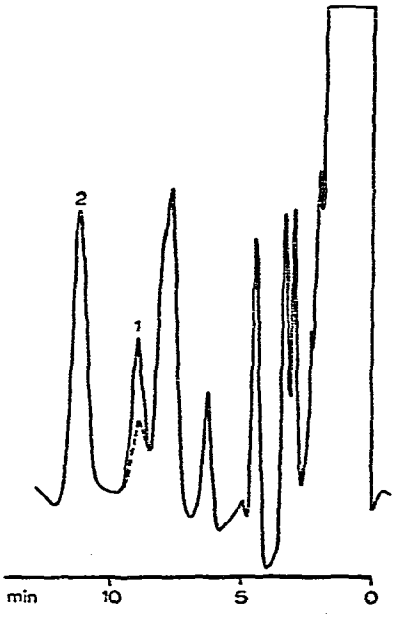


Fig. 6. Gas chromatogram of derivatized extract from plasma. 29 ng of nicotine added to 1 ml of plasma and analysed according to the procedure. Column B and electron-capture detection. 1 = Carbamate II of nicotine; 2 = internal standard. The broken line indicates the nicotine blank.

value corresponding to about 3 ng/ml of nicotine. By mass spectrometry the blank was verified to be nicotine. Isaac and Rand[3] found a nicotine blank of about 3 ng/ml in their investigation of non-smokers' plasma. Feyerabend *et al.*[2] assayed nicotine in areas where smoking was forbidden and the blank value was found to be 0.5 ng/ml. Their observation that the same samples gave a blank value of 4 ng/ml when analysed in areas where smoking was only partially restricted is interesting. They suggested that blank plasma samples may absorb nicotine from the air.

This route of contamination is supported by the work of Horning *et al.*[12], who found that the most probable route of transfer of nicotine was the air. They demonstrated the presence of nicotine in non-smokers' urine. When the same simple extraction technique was applied to laboratory water no nicotine was found. Blank values have also been reported in radioimmunological methods. Haines *et al.*[8] found about 4 ng/ml of nicotine in control plasma.

From the above-mentioned reports, it is obvious that nicotine is present in non-smokers' urine and even in plasma. There is also reason to believe that with handling of extracts in the analytical procedure a nicotine blank will appear.

In this study the blank problem has been an obstacle to low-level analyses. Although the carbamate of nicotine has a high detectability in the electron-capture detector, in practice it has not been possible to work quantitatively below about 10 ng/ml as the blank value has been around 5 ng/ml.

A series of experiments were performed in a room in which no one had smoked for at least 3 years. There it was possible to construct standard graphs that did pass through the origin, indicating a very low background. However, even here from time to time there were higher blank values.

CONCLUSIONS

The δ-trichloroethylcarbamate of nicotine has excellent sensitivity in the electron-capture detector, making picogram amounts detectable. The δ-chlorocarbamate was about six times less sensitive in the thermionic detector. The adsorptive properties of the derivative are lower than that of nicotine. This is the major advantage of the method, combined with the fact that once the derivative has been formed, the influence from environmental nicotine is unimportant. The formation of the olefinic carbamate due to the partial thermal dehydrohalogenation in the injector and on-column is a disadvantage of the procedure.

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