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INTERACTION OF BIOGENIC AMINES WITH COMPONENTS OF CIGARETTE SMOKE

FORMATION OF CYANOMETHYLAMINE DERIVATIVES

PETER H. YU,* DAVID A. DURDEN, BRUCE A. DAVIS and ALAN A. BOULTON

Neuropsychiatric Research Unit, Department of Psychiatry, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

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Abstract—A reaction of the biogenic amines 5-hydroxytryptamine, dopamine, histamine, *p*-tyramine, β-phenylethylamine and tryptamine with components of cigarette smoke was observed. The adducts formed from 5-hydroxytryptamine and β-phenylethylamine were purified by chromatographic procedures and identified by high resolution mass spectrometry. The structures of some of these compounds were established as cyanomethylamine derivatives, i.e. $RCH_2CH_2NHCH_2CN$. In the case of 5-hydroxytryptamine, a cyanomethyl-β-1,2,3,4-tetrahydrocarboline product formed via a Pictet–Spengler condensation reaction was isolated. The mass spectra of such adducts and their fragment ions were observed to be identical to those of chemically synthesized cyanomethylamines. Both formaldehyde and cyanide, which are known to be present in cigarette smoke, were involved in the reaction with the primary amines. The reaction was time dependent and was enhanced by an increase in temperature or by incubation under alkaline conditions. Cyanomethyl adduct formation was increased when smoke from cigarettes with higher tar and nicotine content was used. When the amines were incubated with human saliva obtained after cigarette smoking, cyanomethylamine products were readily detected.

A large number of tumorigenic and carcinogenic agents have been identified in cigarette smoke including volatile, non-volatile, and tobacco-specific *N*-nitrosamines [1, 2]. This latter group of substances is formed from amines and nitrogen oxides during smoking [3, 4]. The formation of nitrosamines *in vitro* has been widely investigated [5, 6]. Their formation *in vivo* in cigarette smokers has been considered to be feasible since smoke contains high concentrations of nitric oxides [7, 8]. During a recent study on the effect of cigarette smoke on monoamine oxidase activities, we observed that monoamines such as β-phenylethylamine, *p*-tyramine and 5-hydroxytryptamine could readily interact with some components in the cigarette smoke to produce lipophilic adducts [9]. These amine adducts were not formed via nitrosation with nitric oxides. In this paper we describe the formation and identification of some of these amine derivatives. The possible implications of such new compounds *in vivo* are discussed.

MATERIALS AND METHODS

Preparation of cigarette smoke solution. The cigarette smoke solution was prepared from a brand of filtered cigarettes with a tar content of 16 mg and nicotine, 1.2 mg. Smoking was simulated as one puff/0.5 min with a 2-sec puff duration. The resultant smoke was bubbled through 0.02 M phosphate buffer solution at pH 8.0 (one cigarette/3 ml) as previously described [9].

Formation and detection of amine–smoke adducts. Radio-isotopically labelled amines including 5-[2-^{14}C]hydroxytryptamine, β-[*ethyl*-^{14}C]phenylethylamine, *p*-[1-^{14}C]tyramine, [2-^{14}C]tryptamine and [8-^{3}H(N)]dopamine (New England Nuclear, Boston, MA) were incubated with freshly prepared cigarette smoke solution in a total volume of 200 μl in 0.02 M phosphate buffer (pH 8.0) at 37° for 30 min. The reaction was terminated by adding 250 μl of 2 M citric acid. The formed adducts were extracted into 1 ml of toluene : ethyl acetate (1 : 1, v/v) of which 600 μl was transferred to a counting vial containing 10 ml Omnifluor fluid (New England Nuclear, Boston). The radioactivity was assessed by liquid scintillation spectrometry (Beckman LS 7500, Fullerton, CA).

Purification of the amine–smoke adducts. Cigarette smoke solution (60 ml) was prepared as described above from twenty cigarettes. We observed that the active components in the cigarette smoke which interacts with amines cannot be extracted by toluene : ethyl acetate, whereas the cigarette smoke–amine adducts can be extracted by the same solvent. To reduce the complexity for structural determination, we prepurified the cigarette smoke solution by solvent extraction, i.e. shaking vigorously three times with an equal volume of toluene : ethyl acetate. The pH value of the extracted aqueous solution (25 ml) was then adjusted to 8.0 with Na_2CO_3, and β-phenylethylamine or 5-hydroxyphenylethylamine (10 mg of each) was added. The mixture was then incubated at 60° for 60 min, and then 10 ml of 2 N citric acid was added. The amine adducts were then extracted twice with toluene : ethyl acetate (1 : 1, v/v). The organic extracts were then pooled, concentrated under vacuum, and finally

* Author to whom correspondence should be addressed.

dried under a stream of nitrogen. The amine adducts were further purified by preparative thin-layer chromatography. The β-phenylethylamine adduct was separated on a reverse phase plate (Whatman KC-18) in the solvent system acetonitrile:ethanol:H_2O (25:35:40, by vol.). The 5-hydroxytryptamine adducts were separated on a silica gel plate (Merck Keiselgel 60) in the solvent system ethyl acetate:toluene (3:1, v/v). The parts of the plates corresponding to radioactively labelled authentic amine-adducts run in parallel were delineated, removed, and eluted with acetonitrile. These isolated compounds were then analyzed by mass spectrometry. In the control experiments, 25 ml of cigarette smoke solution in the absence of amines was prepared and analyzed as described above.

Mass spectrometery. Mass spectra were obtained using a VG 70-70F double focussing mass spectrometer equipped with an HP 5700 gas chromatograph containing a J and W 60 m × 0.32 mm i.d. DB1 bonded phase capillary column (helium flow, 32 cm/sec), a J and W 15 × 50 DB1 megabore capillary column (helium flow, 5 ml/min), and a direct insertion probe. Electron impact low and high resolution spectra were recorded at 800 and 3000 resolution respectively. The phenylethylamine adduct was admitted via the capillary column (GC program 100° 4 min, 6 degrees/min to 290°, retention time 19 min, 54 sec) and the *t*-butyl-dimethylsilyl (TBDS) derivative of the 5-HT adduct via the megabore column (conditions, R_f). The 5-hydroxytryptamine adduct and the 1-dimethylaminonaphthalene-5-sulfonyl derivative of the phenylethylamine adduct were admitted using the direct probe. Low resolution ammonia chemical ionization spectra of the phenylethylamine complex were also measured.

Chemical synthesis of N-cyanomethyl derivatives of β-phenylethylamine and 5-hydroxytryptamine. To confirm the structure of the amine–cigarette smoke adducts, the *N*-cyanomethylamine derivatives were chemically synthesized according to Winstead *et al.* [10]. To a solution of 37% formaldehyde (37.5 nmole) and sodium bisulfite (37.5 mmol) in 2.5 ml water was added phenylethylamine (free base, 25 mmol), followed by sodium cyanide (1 equivalent) in 2.5 ml water. After stirring for 1 hr at room temperature, the product was extracted with ether, the extract was dried over anhydrous sodium sulfate, and the solvent was removed. Distillation of the product (117–119°/30 mm) gave a viscous, colorless liquid, the mass spectrum of which showed major ions at *m/e* 160 (M^+), 132, 105, 91, 69 (CH_2NHCH_2CN) and 42. This is consistent with *N*-cyanomethylphenylethylamine. No β-phenylethylamine was present.

When 5-hydroxtryptamine creatinine sulfate salt was treated as described above with formaldehyde and cyanide, three products were isolated. These were separated by HPLC and identified by mass spectrometery as 6-hydroxy-1,2,3,4-tetrahydrocarboline ($M^+ = 188$), 6-hydroxy-2-cyanomethyl-1,2,3,4-tetrahydrocarboline ($M^+ = 227$) and *N*-cyanomethyl-5-hydroxytryptamine ($M^+ = 215$). In aqueous solution, the major product was 6-hydroxytetrahydrocarboline which precipitated before addition of cyanide. The approximate ratio of the three above-mentioned products was 2:1:1 (based on the relative intensities of the molecular ions). Pure 6-hydroxy-2-cyanomethyltetrahydrocarboline was prepared by treating a methanol–water (2:1) solution of 6-hydroxytetrahydrocarboline hydrochloride with equimolar amounts of formaldehyde and cyanide. The product precipitates on dilution with water.

RESULTS

Reaction of biogenic amines with components in the cigarette smoke solution. As can be seen in Fig. 1, A and B, when ^{14}C-labelled amines such as *p*-tyramine, β-phenylethylamine, dopamine, tryptamine and serotonin were incubated with different concentrations of cigarette smoke solution, labelled adducts were formed in direct proportion to the amount of smoke components, and these could be separated from the parent amines by extraction with the organic solvent mixture toluene:ethyl acetate (1:1, v/v). Histamine also reacted with the cigarette smoke solution to form adducts in a similar manner (data not shown). We also observed that, when an aqueous extract of cigarette tobacco was incubated with the labelled amines, adducts were not formed (results not shown).

The formation of the adducts was dependent on pH. For example, the linkage of 5-hydroxytryptamine and β-phenylethylamine with the cigarette smoke components occurred favourably in an alkaline condition; below pH 4.0 the adducts were not formed (Fig. 2A). Their formation was also temperature dependent with more adducts being formed at higher temperatures (Fig. 2B).

The reactions of the amines with cigarette smoke from nine different brands of filtered cigarettes were

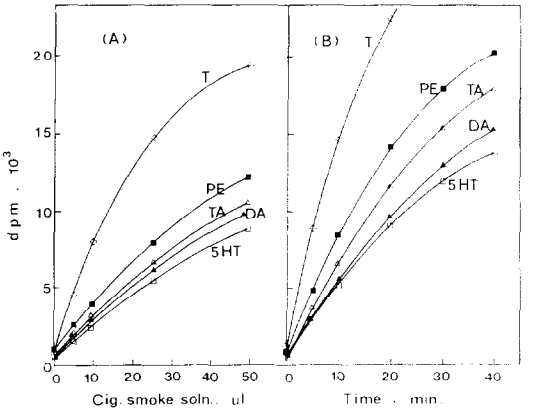


Fig. 1. Reactions of biogenic amines with components of cigarette smoke. (A) Radioactively labelled amines (0.1 nmol, 0.1 μCi) [*p*-tyramine (TA), 5-hydroxytryptamine (5-HT), tryptamine (T), dopamine (DA) and β-phenylethylamine (PE)] were incubated with increasing amounts of cigarette smoke solution at pH 8.0, 37° for 20 min, and the labelled adducts formed were isolated by extraction with toluene:ethyl acetate (1:1, v/v) and counted in a liquid scintillation counter. (B) Time course of the reaction. The same amines were incubated with the cigarette smoke solution (50 μl), and the reactions were terminated after different time intervals.

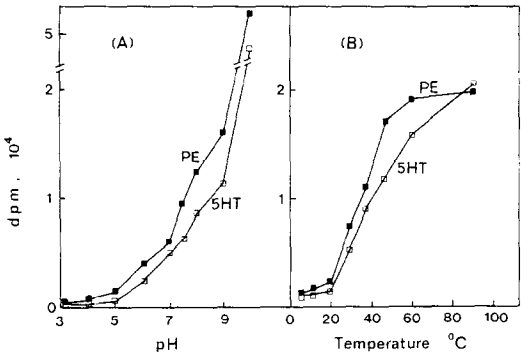


Fig. 2. Effects of pH and temperature on the formation of amine adducts. (A) 5-Hydroxytryptamine (5-HT) and β-phenylethylamine (PE) were incubated with the cigarette smoke solution in buffers at different pH values [borate buffer (pH 9–10), phosphate buffer (pH 6–8) and sodium citrate buffer (pH 3–5)] at 37° for 20 min. (B) Labelled 5-HT and PE were incubated in phosphate buffer, pH 8.0, at different temperatures for 20 min, the reactions were terminated by addition of citric acid, and the labelled products were extracted immediately with toluene:ethyl acetate (1:1, v/v).

compared. As listed in Table 1, the smoke from all of these brands interacted with 5-hydroxytryptamine and β-phenylethylamine. Apparently more amine adducts were formed as the tar and nicotine content increased.

Formation of cyanomethylamine derivatives from amines and cigarette smoke constituents. Several toxic substances, known to be major constituents of cigarette smoke [2], as listed in Table 2, were incubated with ^{14}C-labelled β-phenylethylamine and 5-hydroxytryptamine. Any formed lipophilic-labelled adducts were extracted with the toluene:ethyl acetate solvent, and their radioactivity was assessed. As can be seen from Table 2, the highest recovery of radioactivity was found when the amines were incubated with a mixture of formaldehyde and potassium cyanide. Very little radioactivity was extracted when any of the other substances listed in Table 2 were used except for acetaldehyde and cyanide.

The amine adducts formed after reaction with the cigarette smoke and extracted in the toluene:ethyl acetate solvent mixture were concentrated under a stream of N_2 at 45° and separated by thin-layer chromatography. In the case of the β-phenylethylamine adduct, as can be seen in Fig. 3A, a single zone at an R_f value of 0.67 appeared to contain all the radioactivity. When labelled β-phenylethylamine was reacted with 0.25% formaldehyde and 0.25% sodium cyanide, or when unlabelled β-phenylethylamine was incubated with [^{14}C]sodium cyanide and formaldehyde, the adducts formed were chromatographically identical with the β-phenylethylamine adduct obtained after reaction with the cigarette smoke.

When the smoke adducts formed from 5-hydroxytryptamine and the products of the 5-HT–formaldehyde–KCN reaction were chromatographed (see Fig. 3B), a major zone at the R_f value of 0.53 was revealed along with at least two minor zones at R_f values of 0 and 0.75 respectively.

Determination of the chemical structures of the amine–cigarette smoke adducts by mass spectrometry. Figure 4a shows the mass spectrum of the amine adducts formed from reaction of the smoke extract with an equimolar mixture of phenylethylamine and 2,2-$^2H_2$-phenylethylamine (PE and PE-d_2 respectively), and Fig. 4b shows the spectrum of the protio compound alone. The ion pairs of masses 160 and 162, 130 and 132, and 91 and 93 contained the benzyl fragments which are from PE and PE-d_2, whereas ions of m/z 69 and 42 contained only the new part of the molecule. High resolution mass analysis gave the following exact mass measurements and elemental compositions, m/z 69.0466 ($C_3H_5N_2$, 69.0452), m/z 91.0548 (C_7H_7, 91.0458), m/z 93.0666 ($C_7H_5{}^2H_2$, 93.0673), m/z 160.0987 ($C_{10}H_{12}N_2$, 160.1000), and m/z 162.1111 ($C_{10}H_{10}{}^2H_2N_2$,

Table 1. Formation of lipophilic adducts of β-phenylethylamine and 5-hydroxytryptamine with cigarette smoke obtained from different commercially available brands of cigarettes

Cigarette brand	Tar content* (mg)	Nicotine content (mg)	Adduct formation† (dpm × 10^{-3})	
			5-Hydroxytryptamine	β-Phenylethylamine
1	16	1.2	48.3 ± 4.1	39.0 ± 4.4
2	15	1.2	47.7 ± 4.1	32.0 ± 1.5
3	13	1.0	43.0 ± 1.0	32.0 ± 0.3
4	13	1.0	36.7 ± 1.3	25.7 ± 0.9
5	10	0.8	34.7 ± 0.9	22.7 ± 1.3
6	9	0.8	31.0 ± 1.0	19.7 ± 0.9
7	8	0.8	23.3 ± 1.4	12.3 ± 0.3
8‡	8	0.8	33.7 ± 2.4	22.7 ± 2.3
9	4	0.4	26.3 ± 3.2	16.7 ± 3.3

* Tar and nicotine contents were indicated on the packs of cigarettes.

† ^{14}C-Labelled β-phenylethylamine or 5-hydroxytryptamine (1×10^{-5} M, 0.1 μCi) dissolved in 100 μl of 0.1 M phosphate buffer (pH 8.0) was incubated at 60° for 30 min with 100 μl of cigarette smoke solution obtained from different brands of cigarettes. At the end of the incubation period, 1 ml of a toluene: ethyl acetate (1:1, v/v) mixture was added to each tube and, after vigorous shaking followed by centrifugation, 600 μl of the organic phase was removed for scintillation counting. Each value is the mean ± SE of three experiments.

‡ A menthol cigarette.

Table 2. Formation of lipophilic adducts of β-phenylethylamine and 5-hydroxytryptamine with some volatile substances known to be present [2] in cigarette smoke

	5-Hydroxytryptamine (dpm)	β-Phenylethylamine (dpm)
H_2O	392	315
Formaldehyde (0.25%)	325	515
Formaldehyde (0.25%) + KCN (0.25%)	10,151	4,553
Acetaldehyde (0.25%)	310	343
Acetaldehyde (0.25%) + KCN (0.25%)	1,226	3,815
KCN (0.25%)	341	407
H_2O_2 (0.025%)	391	451
$NaNO_2$ (0.25%)	609	268
Acetonitrile (0.25%)	326	429
Ammonia hydroxide (0.25%)	417	279

^{14}C-Labelled β-phenylethylamine or 5-hydroxtryptamine (1×10^{14} M, 0.1 μCi) dissolved in 0.02 M phosphate buffer (pH 8.0) (50 μl) at 37° for 30 min was incubated with the substances listed in the above table. At the end of the incubation period, a toluene:acetate (1:1, v/v) mixture (1 ml) was added to each tube and, after vigorous shaking followed by centrifugation, an aliquot (600 μl) of the organic phase was removed for scintillation counting. The values listed are the means of duplicate experiments.

162.1126). Thus, the molecular ion of the protio compound appears to be *m/z* 160, $C_{10}H_{12}N_2$ which fragments into two ions, *m/z* 91 (C_7H_7) and *m/z* 69 ($C_3H_5N_2$) through beta cleavage of the alkyl chain. Ammonia chemical ionization gave a somewhat similar spectrum to Fig. 4a with ions at *m/z* 161 and 163 for the protio and deutero compounds respectively.

In addition, the spectrum of the dansyl derivative of the adduct (results not shown) from the smoke extract that reacted with PE and PE-d_2 contained highest mass ions of *m/z* 393.1450 ($C_{22}H_{23}N_3SO_2$) and 395.1597 ($C_{22}H_{21}{}^2H_2N_3SO_2$) respectively.

Figure 5a illustrates the spectrum produced by reaction of a cigarette smoke solution with a 1:1

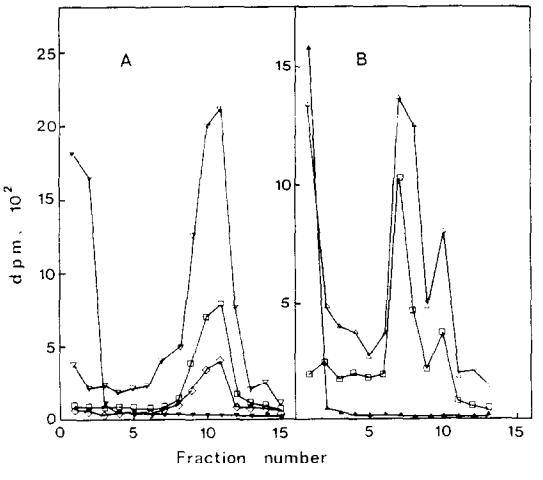


Fig. 3. Thin-layer chromatographic separation of amine–cigarette smoke solution adducts. (A) ^{14}C-Labelled phenylethylamine (▼), and adducts prepared after reactions of [^{14}C]phenylethylamine with cigarette smoke (▽) or in 0.25% sodium cyanide and 0.25% formaldehyde (□) as well as unlabelled phenylethylamine incubated with 0.1 μCi of ^{14}C-labelled sodium cyanide (0.25%) and unlabelled 0.25% formaldehyde (◇), were separated on a KC-18 reverse phase thin-layer plate and were developed in a solvent system of acetonitrile:ethanol:water (25:35:40, by vol.). (B) ^{14}C-Labelled 5-hydroxytryptamine (▲) and adducts prepared after reactions of [^{14}C]5-hydroxytryptamine with cigarette smoke (△) or in 0.25% sodium cyanide and 0.25% formaldehyde (□) were separated on a silica gel plate in a solvent system of toluene:ethyl acetate (3:1, v/v).

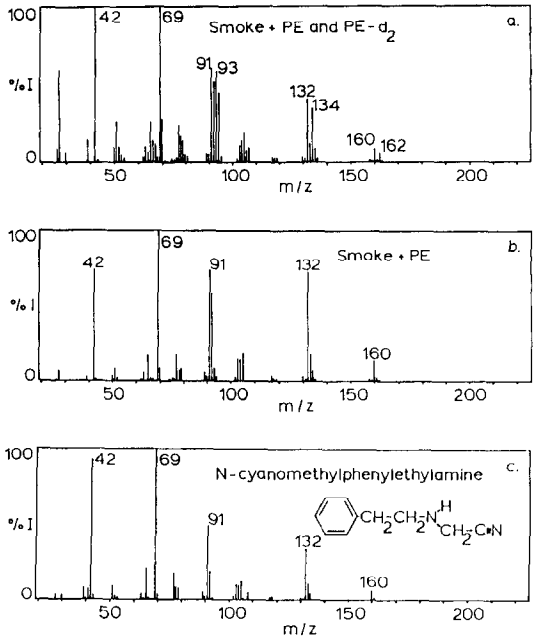


Fig. 4. Mass spectra of: (a) cigarette smoke adduct and an equimolar mixture of PE and PE-β,β-d_2; (b) cigarette smoke adduct and PE; and (c) synthetic cyanomethyl-β-phenylethylamine. Mass spectra were obtained using a VG 70-70F double focussing mass spectrometer equipped with an HP 5700 gas chromatograph containing a J and W 60 m × 0.32 mm i.d. DB1 bonded phase capillary column (helium flow, 32 cm/sec), a J and W 15 m × 50 DB1 megabore capillary column (helium flow, 5 ml/min) and a direct insertion probe. Electron impact low and high resolution spectra were recorded at 800 and 3000 resolution respectively. The phenylethylamine adduct was admitted via the capillary column (GC program 100° 4 min, 6 degrees/min to 290°, retention time 19 min, 54 sec).

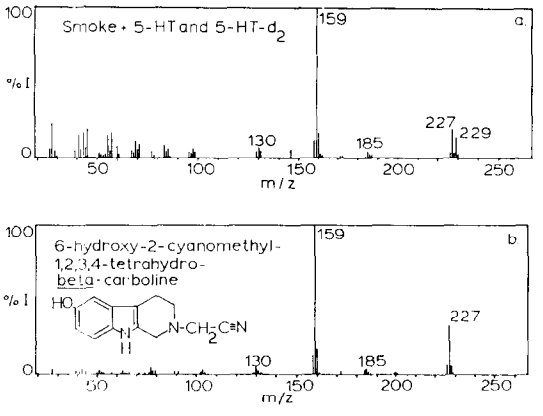


Fig. 5. Mass spectra of: (a) cigarette smoke adduct formed from an equimolar mixture of 5-hydroxytryptamine and 1,1-dideuterated 5-hydroxytryptamine; and (b) synthetic 6-hydroxy-2-cyanomethyl-1,2,3,4-tetrahydro-β-carboline. Samples were admitted via the direct insertion probe.

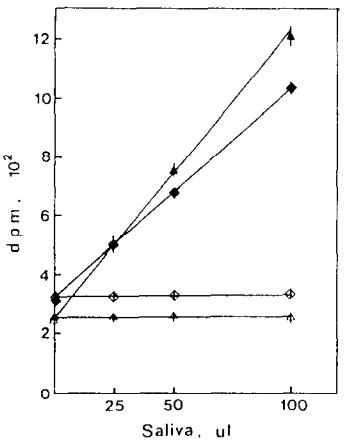


Fig. 6. Formation of cyanomethylamine adducts by incubating labelled β-phenylethylamine (△) and *p*-tyramine (◇) with saliva obtained before (open) and after (closed) smoking a single cigarette. The saliva was centrifuged, and different volumes of the supernatant fraction were incubated with labelled β-phenylethylamine and *p*-tyramine at 37°.

mixture of 5-HT and 1,1-$^2H_2$-5-hydroxytryptamine (5-HT-d_2). Only the highest mass ions m/z 227 and 229 contained the deuterium label. The ions were mass measured as 227.1060 ($C_{13}H_{13}N_3O$) and 229.1179 ($C_{13}H_{11}N_3O^2H_2$). The base peak m/z 159.0706 ($C_{10}H_9NO$) lost the deuterium atoms since they were originally in the alpha position of the alkyl chain. The spectrum of the TBDS derivative contained high mass ions m/z 341 and 343 with precise mass values and elemental compositions which agree with the above measurements: 341.1946 ($C_{19}H_{27}N_3O$ Si) and 343.2000 ($C_{19}H_{25}{}^2H_2N_3O$ Si) as well as the corresponding M-57 ions.

Formation of cyanomethylamine derivatives from reaction of amines with saliva collected after cigarette smoking. Saliva before and after smoking a single cigarette from a non-smoker was collected and incubated with ^{14}C-labelled β-phenylethylamine and *p*-tyramine. A small but significant amount of labelled cyanomethyl derivatives of β-phenylethylamine and *p*-tyramine was found as shown (Fig. 6). The labelled products were chromatographically identical to cyanomethyl-*p*-tyramine and cyanomethylphenylethylamine respectively.

DISCUSSION

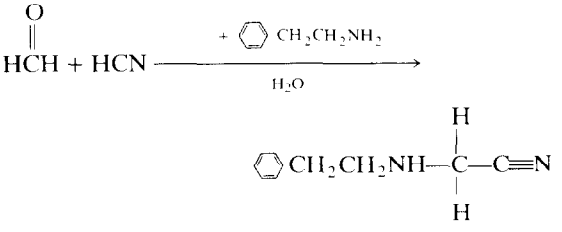
This present study has demonstrated that some components of cigarette smoke can react directly with biogenic amines in aqueous solution under physiological conditions. It proved possible to isolate, purify and identify some of these amine–smoke complexes. Since the active components of a cigarette smoke solution were not extracted by a toluene:ethyl acetate solvent mixture, the complexity of the smoke solution could be reduced considerably by three successive extractions with this solvent mixture. When labelled β-phenylethylamine or 5-hydroxytryptamine was added to, and incubated with, this "washed" cigarette smoke solution, labelled amine-adducts were quickly and easily formed, and they could then be simply extracted with the same organic solvent mixture. After preparative thin-layer chromatography in one dimension, the compounds became reasonably pure and suitable for mass spectrometric analysis.

The identification of these amine derivatives was based on a strategy of reducing the problem to its simplest form. From the chromatographic and extraction data it appeared that the reaction involved the primary amine group from which we would expect a secondary or tertiary amine compound to be formed. One of the simplest aromatic biogenic amines is phenylethylamine since it possesses no other functional groups that could cause secondary reactions or affect the chromatographic properties of the formed adduct. We concentrated first, therefore, on identifying the phenylethylamine–smoke adduct and then we applied the information gained to the analyses of the other amine–smoke complexes. The phenylethylamine–smoke adduct would be expected to retain its phenylethyl chain which in the mass spectrometer would give rise to an intense ion at m/z 91 due to alpha–beta cleavage of the alkyl side chain. Furthermore, by using a mixture of phenylethylamine and its isotopomer labelled with the stable isotope deuterium, in the beta position (i.e. β,β-$^2H_2$-phenylethylamine), we would expect to observe two ions, m/z 91 and 93, of approximately equal intensity (see Fig. 4a) (i.e. the "twin ion" technique of Knapp *et al.* [11]. Using this strategy, it was possible to select from the many mass spectra of the total GC analysis only those compounds originating from phenylethylamine and PE-d_2 (i.e. all fragments containing two ions of approximately equal intensity and differing by 2 milli mass units). Mass measurement of m/z 91 and 93 (Fig. 4a) confirmed their identities as $C_7H_7^+$ and $C_7H_5{}^2H_2^+$ as expected. It was established that m/z 160 (and 162) was the molecular ion by the use of chemical ionization and by preparation of the dansyl derivative (spectra not shown); its elemental composition was measured as $C_{10}H_{12}N_2$ and confirmed by the elemental composition of 162 ($C_{10}H_{10}{}^2H_2N_2$) arising from the deuterated isotopomer and m/z 69 ($C_3H_5N_2$).

This structure was consistent with formation of the ion m/z 69 and of an ion m/z 132 ($M-H_2CN$). In addition, the compound chemically synthesized from an aqueous mixture of β-phenylethylamine, formaldehyde and KCN gave an identical spectrum (see Fig. 4c). The data are consistent with the structure *N*(cyanomethyl)-phenylethylamine, similar to structures reported previously by Winstead *et al.* [10] following reaction of an amine with formaldehyde and cyanide.

The spectrum of the 5-HT–smoke adduct (see Fig. 5a) was consistent with the authentic 6-hydroxy-2-cyanomethyl-1.2.3.4-tetrahydro-β-carboline (Fig. 5b). The TBDS derivative (of the phenolic group) gave a spectrum with an elemental composition which confirms the identity.

The formation of cyanomethylamine derivatives is conceivable since both formaldehyde and cyanide are present in significant amounts in cigarette smoke [12].The mechanism of formation of cyanomethylamine derivatives in the presence of sodium bisulfite has been proposed previously [10]. In the present experiments we observed that the reactions can readily proceed in the absence of sodium bisulfite in aqueous solution (Table 1) as indicated in the scheme:



It does not rule out that other components in the cigarette smoke may be involved in the intermediate step for the synthesis of cyanomethylamine compounds. In the case of the reaction of 5-HT with formaldehyde and cyanide, 6-hydroxy-β-carbolines can easily be formed by a Pictet–Spengler condensation reaction, which is well known [13].

It is not yet known whether such a chemical reaction as described above actually occurs *in vivo*. The fact that saliva after the smoking of a single cigarette did trap components which facilitated the formation of cyanomethylamine derivatives (Fig. 6), however, suggests that the occurrence of such reactions is quite possible, at least locally (i.e. in the lung). In the case of human smoking, the exposure of the lung tissues to the smoke could be quite extensive. The total surface areas of the respiratory tract and pulmonary alveoli are quite large. Cigarette smoke components may easily diffuse into these tissues and into the bloodstream. It is known that the concentrations of various primary amines (5-hydroxytryptamine and histamine, for example) are very high in the lung [14]. The lungs, in contrast to other organs, receive the total venous return and have been proposed to play a role in regulating the concentration of amines in venous blood before they reach the arterial circulation where amines exhibit profound effects [15, 16]. The interaction of lung amines and perhaps circulating amines with cigarette smoke may be implicated in many pathophysiological conditions.

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REFERENCES

1. D. Hoffmann and J. D. Adams, *Cancer Res.* **41**, 4305 (1981).
2. United States Department of Health and Human Services, *USPHS Publication No. 82-50179*, p. 322. USPHS, Washington, DC (1982).
3. J. P. Adams, S. J. Lee, N. Vinehkoski, A. Castonguay and D. Hoffmann, *Cancer Lett.* **17**, 339 (1983).
4. D. Hoffmann, J. D. Adam, K. D. Brunnemann and S. S. Hecht, in *American Chemical Society Symposium Series* (Eds. R. A. Scanlan and S. R. Tannenbaum), Vol. 174, p. 247. American Chemical Society, Washington, DC (1981).
5. S. S. Mirvish, *Banbury Rep.* **12**, 227 (1982).
6. H. Ohshima and H. Bartsch, *Cancer Res.* **41**, 3658 (1981).
7. T. B. Williams, *Betr. Tabakforsch.* **10**, 91 (1980).
8. D. Hoffmann and K. D. Brunnemann, *Cancer Res.* **43**, 5570 (1983).
9. P. H. Yu and A. A. Boulton, *Life Sci.* **41**, 675 (1987).
10. M. B. Winstead, C. A. Ciccarelli and H. S. Winchell, *J. Pharmac. exp. Ther.* **205**, 751 (1978).
11. D. R. Knapp, N. H. Holcombe, A. Kruegers and P. J. Privitera, *Drug Metab. Dispos.* **4**, 164 (1976).
12. D. Hoffmann and E. L. Wynder, in *Zentrbl. Bakt. ParasitKde Abt. I*, Orig. B **166**, 113 (1978).
13. W. M. Whaley and T. R. Govindachari, in *Organic Reactions* (Eds. R. Adams, H. Adkins, A. M. Blatt and A. M. Cope), Vol. VI, p. 151. John Wiley, New York (1951).
14. C. Sadavongvivad, *Br. J. Pharmac.* **38**, 353 (1970).
15. V. A. Albaster, in *Metabolic Functions of the Lung* (Eds. Y. S. Bakkle and J. R. Vane), p. 3. Marcel Dekker, New York (1977).
16. A. F. Juno, in *Handbook of Physiology, Section 3, The Respiratory System* (Eds. A. P. Fishman, A. B. Fisher and S. R. Geiger), Vol. 1, p. 337. Waverly Press, Baltimore, MD (1985).