Table I—Formation of 2-Chlorothioxanthen-9-one (II) from Chlorprothixene (I) under Various Conditions

Exposure to Light	Solvent	Temperature	Days	Amount of II Formed[a], %
Darkness	2 *M* HCl	80°	9	ND
	2 *M* KOH	80°	9	ND
	1 *M* NaOH	22°	18	ND
	5 *M* NaOH	22°	18	0.2
	Gastric fluid, simulated[b]	80°	1	ND
	Gastric fluid I (pH 2.4)[c]	37°	31	0.6
	Gastric fluid II (pH 1.5)[c]	37°	38	1.5
	Gastric fluid III (pH 6.5)[c]	37°	31	0.6
	Chloroform–isopropanol (95:5 v/v)	4°	32	ND
	Chloroform–isopropanol (95:5 v/v)	4°	580	0.5
	Chloroform–isopropanol (95:5 v/v)	22°	32	ND
	Chloroform–isopropanol (95:5 v/v)	22°	580	3.5
Lamp light	Methanol	22°	34	ND[d]
	Chloroform–isopropanol (95:5 v/v)	22°	34	ND[d]
UV light (254 nm) + lamp light	Methanol	22°	4 + 16	32[d]
	Chloroform–isopropanol (95:5 v/v)	22°	4 + 16	31[d]

[a] The formation of II was measured by GLC; see *Experimental*. ND = not detectable (<0.2%). [b] Prepared according to the USP method (5). [c] Obtained by gastric aspiration in three healthy adults. [d] Considerable quantities (5–40%) of degradation products of I other than II.

Exact mass measurements in a high-resolution mass spectrometer showed that the empirical formula of the ion at *m/z* 246 was $C_{13}H_7ClOS$ (theoretical mass, 245.9906; observed mass, 245.9906). Similarly, the ions at *m/z* 218 and 183 were shown to be $C_{12}H_7ClS$ (theoretical mass, 217.9957; observed mass, 217.9959) and $C_{12}H_7S$ (theoretical mass, 183.0268; observed mass, 183.0264), respectively.

From these data, the unknown compound was identified tentatively as 2-chlorothioxanthen-9-one (II). This compound was synthesized by two methods. The synthetic compounds cochromatographed with the unknown in GLC, and their mass spectra were identical. Thus, the identity of the unknown compound was established as II.

Table I shows the formation of II from chlorprothixene (I) under various storage conditions. Only negligible amounts were formed when the drug was stored in the dark or in lamp light. However, when I was treated with UV light for 4 days followed by storage in lamp light for 16 days, ~30% of I was converted to II. Moreover, considerable quantities of unidentified degradation products of I were formed when I was exposed to lamp light and UV light. These findings are in accordance with unpublished data (6). Therefore, samples from cases of acute drug poisoning should be stored in the dark.

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GLC Analysis of Phenylalkyl Primary Amines Using Nitrogen Detector

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Abstract □ A comprehensive method for the analysis of several phenylalkyl primary amines of biological interest was developed. The amines were derivatized with perfluoro acid anhydrides or carbon disulfide in ethyl acetate, and the respective acyl derivatives or isothiocyanate derivatives were analyzed by GLC using nitrogen-specific detection. The described procedure was used to measure the amphetamine concentrations in rat serum, brain, and liver after intraperitoneal injection (5 mg/kg).

Keyphrases □ Phenylalkyl primary amines—GLC analysis using nitrogen detection, rat serum, brain, and liver □ Amphetamines—phenylalkyl primary amines, GLC analysis using nitrogen detection, rat serum, brain, and liver □ GLC—nitrogen detection, analysis, phenylalkyl primary amines in rat serum, brain, and liver

The use of nitrogen–phosphorus detection in GLC has many applications in biomedicine for the trace analyses of nitrogen-containing compounds. The nitrogen–phosphorus detector is generally 10–100 times more sensitive than the flame-ionization detector and has very little response to organic compounds that do not contain phosphorus or nitrogen atoms. Thus, therapeutic levels of many drugs containing a secondary or tertiary nitrogen, *e.g.*, anticonvulsants (1, 2), analgesics (3), tricyclic antidepressants (4–6), antipsychotics (7, 8), and drugs of abuse (9–11), can be measured. However, this approach has not yet been exploited for the analysis of primary amines. The use of GLC with nitrogen detection was suggested for the identification of amphetamine in forensic toxicology (9).

In this report, a comprehensive method for the analysis of several phenylalkyl primary amines is described. The amines were derivatized with acid anhydrides or carbon disulfide, and the *N*-acyl and isothiocyanate derivatives were analyzed by GLC with nitrogen detection.

EXPERIMENTAL

Materials—All of the phenylalkyl primary amines were obtained

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commercially[1]. Glass-distilled[2] methanol, ethyl acetate, and hexane were used. Trifluoroacetic anhydride, pentafluoropropionic anhydride, and heptafluorobutyric anhydride were used as received[3]. Carbon disulfide and acetic anhydride were analytical grade.

The gas chromatograph[4] was equipped with a dual nitrogen–phosphorus detector. The instrument was set according to the instruction manual provided by the manufacturer, except that the offset current applied to the alkali metal bead was adjusted to give a recorder deflection of 20% of the full scale at a range of 1 and an attenuation of 32. The voltage input can be increased further to improve sensitivity to a point when the detector response to baseline noise ratio is optimum.

The 0.9-m coiled glass (2-mm i.d.) column was packed with 3% OV-17 on 80–100-mesh WHP Supelcoport. The gas flow rates were as follows: hydrogen, 3 ml/min; air, 50 ml/min; and helium (carrier gas), 30 ml/min. Oven temperature of 130° isothermal and injection port and detector temperatures of 200 and 300°, respectively, were used when trifluoroacetic anhydride was the derivatizing reagent.

Procedures—The stock solutions of standard reference compounds were made in methanol to give 1-mg (as free base) concentrations. The salts were extracted from 1 ml of 1 *N* NaOH solutions with 0.5 ml of hexane or ethyl acetate. Primary amines without derivatization eluted as broad peaks because of the polar amino group. Several derivatizing reagents were tried to find optimum conditions. Amphetamine, β-phenethylamine, and tyramine were used as prototype amines for determining these conditions. These primary amines, when underivatized, eluted immediately as broad peaks from 3% OV-17 at 130°.

Derivatization with Carbon Disulfide—Isothiocyanate derivatives were prepared according to a previously published method (12). Extracted solutions of amphetamine, phenethylamine, and tyramine were transferred to a reaction vial, and 0.5 ml of carbon disulfide was added. The mixture was shaken for 15 min, dried under nitrogen, redissolved in ethyl acetate, and injected into the chromatograph. Under these conditions, the derivatized amphetamine and phenethylamine eluted as symmetrical peaks with retention times of ~4.6 and 4.8 min, respectively, relative to the injection time.

Derivatization with Acid Anhydrides—To the amine solutions in the reaction vial was added 0.5 ml of the acid anhydride, and the mixture was shaken and left at room temperature for 1 hr. Then, the vials were either dried under nitrogen and redissolved in hexane for chromatographic analysis or washed with water and ammonium hydroxide as follows. For this purpose, the reaction was carried out in glass centrifuge tubes. After the reaction, 1 ml of distilled water was added carefully with constant mixing, the tubes were shaken for ~30 sec, and the water was discarded. Then 1 ml of 2 *N* NH_4OH was added, the tubes were shaken for 5 min, and the hexane layer was separated and injected into the chromatograph.

Amphetamine Levels in Biological Tissues—The applicability of the method to biological fluids was determined by measuring amphetamine levels in rat serum, brain, and liver after amphetamine administration. The rats were injected intraperitoneally with amphetamine sulfate (5 mg/kg) and sacrificed 30 min later. Blood was collected, and the serum was separated by centrifugation. The brain and liver were removed and homogenized in 0.4 *N* HCl (1:10 w/v). The homogenate was centrifuged, and the amphetamine concentration was measured in the acidic layer after extraction.

To 1 ml of the serum and the brain and liver acid extracts was added 500 ng of benzylamine as the internal standard. The pH was adjusted to 10 by the addition of 0.1 ml of 5 *N* NaOH, and the mixture was extracted with 1 ml of ethyl acetate. Trifluoroacetic anhydride (0.5 ml) was added directly to this extract, and the mixture was left at room temperature for 1 hr. Then it was dried under a nitrogen stream, redissolved in 1 ml of hexane, and injected into the chromatograph.

RESULTS AND DISCUSSION

Without derivatization, the phenylalkyl primary amines were eluted from the 3% OV-17 column at low temperatures as broad tailing peaks. However, after derivatization, the peaks were symmetrical. Figure 1 shows a representative chromatogram of six amines when analyzed after derivatization with trifluoroacetic anhydride. Since the GLC separation of free amines generally results in peak tailing due to spurious adsorption onto the column, several approaches were used for derivatization of the

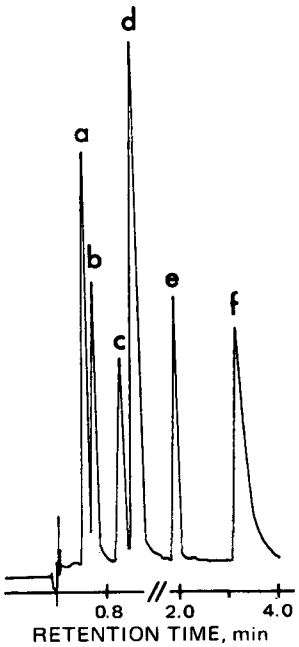
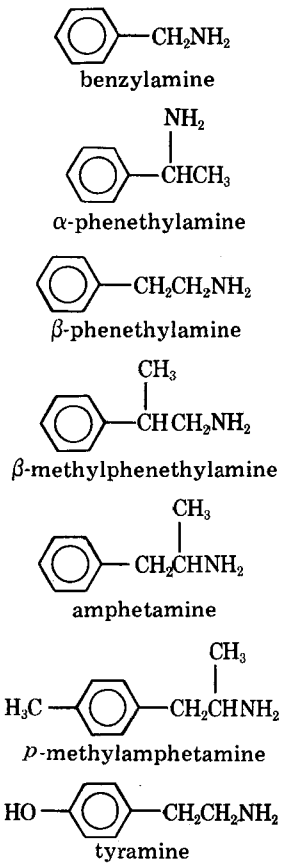


Figure 1—*Representative chromatogram showing the separation of six phenylalkyl primary amines as their trifluoroacyl derivatives. Key: a, α-phenethylamine; b, benzylamine; c, amphetamine; d, β-phenethylamine; e, p-methylamphetamine; and f, tyramine.*

amino groups (13, 14). The isothiocyanate derivatives of phenethylamine and amphetamine resulted in symmetrical peaks, whereas that of tyramine was not eluted. This result was probably due to the presence of the underivatized hydroxyl group in tyramine because Narasimhachari and Vouros (12) were able to analyze the isothiocyanate derivative of tyramine after silylation.

Acyl derivatives of amines, because of their ease of preparation, have been used successfully for GLC analysis. In this study, the trifluoroacetyl and heptafluorobutyryl derivatives of phenylalkyl primary amines were evaluated for GLC analysis with nitrogen detection. The best results were obtained when these amines were derivatized with trifluoroacetic anhydride. All of the primary amines, including tyramine, eluted as sym-



[1] Sigma Chemicals, St. Louis, MO 63178.
[2] Burdick & Jackson Laboratories, Muskegon, MI 49442.
[3] Regis Chemical Co., Morton Grove, IL 60053.
[4] Model 5730-A, Hewlett-Packard, Avondale, PA 19311.

Table I—Retention Times and Detector Response for Trifluoroacetyl and Isothiocyanate Derivatives of Phenylalkyl Primary Amines

Amine	Nanomoles Injected	Retention Time, min		Detector Response	
		Trifluoroacetyl Derivative	Isothiocyanate Derivative	Trifluoroacetyl Derivative	Isothiocyanate Derivative
α-Phenethylamine	0.165	0.80	2.56	96	34
Benzylamine	0.187	0.96	2.56	32	17
Amphetamine	0.148	1.44	4.64	212	31
β-Phenethylamine	0.165	1.60	4.80	88	60
Tyramine	0.146	4.16	No peak	40	—

metrical peaks under the described conditions. Unlike the isothiocyanate derivative, a second reaction was not needed to derivatize the phenolic hydroxyl group in tyramine. The stereoisomers of trifluoroacetyl derivatives of primary amines did not separate under the described conditions since *d*- and *l*-amphetamines eluted with the same retention times, as did the *d*- and *l*-α-phenethylamines.

The nitrogen detector response to the trifluoroacetyl derivatives was generally better than that to the corresponding isothiocyanate derivatives. Table I gives the comparison of trifluoroacetyl and isothiocyanate derivatives of some primary amines and also shows that the nitrogen detector response varied with different compounds and was not related to the amount of nitrogen present in the compound. For example, on a mole per mole basis, amphetamine has less nitrogen than benzylamine, but amphetamine as the trifluoroacetyl derivative had a 6.5 times better response than did benzylamine. This differential response probably depends on the formation of cyanide radicals after pyrolysis. Although the exact mechanism of the nitrogen detector response is not known, it was suggested (15) that the organic molecules containing nitrogen are pyrolyzed to form cyano radicals, which then remove an electron from the atomized alkali metal bead. These cyanide ions then can combine at the collector to form hydrogen cyanide, thus generating an output signal. Hence, the differential response of nitrogen-containing compounds may be due to their characteristics for producing cyanide radicals after pyrolysis.

The linearity of the nitrogen detector response was determined by derivatizing various concentrations of amphetamine (0.5–2.0 μg/ml), phenethylamine (0.5–2.0 μg/ml), and tyramine (1–10 μg/ml) in duplicate and injecting into the chromatograph. There was a linear response with all three primary amines. The regression equations by the least-squares method were: amphetamine, $y = 16.8x + 0$, $r = 0.990$; phenethylamine, $y = 27.8x - 3.25$, $r = 0.997$; and tyramine, $y = 5.76x + 1.5$, $r = 0.988$.

The excess derivatizing reagent was removed either by drying under nitrogen or by washing as described. When washing was used, the average coefficient of variation for amphetamine analysis of duplicate samples over the 0.5–2.0-μg/ml range was 4.1%, compared to an average coefficient of variation of 14.1% when the reacted samples were dried under nitrogen. However, the hydroxylated phenylalkyl primary amine (tyramine) peak disappeared if the reaction mixture was washed to remove excess trifluoroacetic anhydride. This finding suggests that *N*-acyl derivatives are more stable than *O*-acyl derivatives. This fact could be used to improve the chromatogram and reduce the analysis time by reducing the number of peaks if only a quantitation of nonhydroxylated primary amines in biological fluids is needed. The variability in the analysis of duplicate samples due to the loss during drying could be minimized by using the internal standard method.

Table II—Amphetamine Concentrations in Different Rat Tissues 30 min after Intraperitoneal Injection (5 mg/kg) [a]

Tissue [b]	Amphetamine, ng/ml or g	*SEM*
Serum	512	±81
Brain	4484	±885
Liver	5267	±299

[a] For details, see the procedures. [b] Three rats.

The amphetamine concentrations in rat serum, brain, and liver were determined (Table II). Thus, the method can be used to determine the phenylalkyl primary amines in biological fluids. However, the extraction procedure, specificity, and sensitivity of each compound must be established experimentally for each tissue.

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