GAS CHROMATOGRAPHY OF PHENOLIC AND CATECHOLIC AMINES AS THE TRIMETHYLSILYL ETHERS

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Steroids (Vanden Heuvel et al., 1960), alkaloids (Lloyd et al., 1960) and many other compounds of biological interest have been successfully analyzed by gas chromatography. Good methods for the gas chromatographic analysis of physiologically important amines are still lacking. This is largely because these amines are generally polar, nonvolatile and extremely unstable at high temperatures. Fales and Pisano (1962) recently described a method for the separation of many aromatic amines by gas chromatography on an SE-30 column. Their method is good for the analysis of simple aromatic amines and of some phenolic amines but it is not suitable for the analysis of more complicated amines like the catecholamines. This communication describes a method for the gas chromatographic analysis of biologically important amines, such as epinephrine, norepinephrine, dopamine, metanephrine, normetanephrine and 3-methoxytyramine.

After unsuccessful experiments with acetylated derivatives, we found that suitably volatile derivatives of the catecholamines could be prepared by conversion to trimethylsilyl (TMS) ethers. The idea originated from reports of a convenient method for the trimethylsilylation of phenols by Langer et al. (1958), and of sugars by Bentley et al. (1963). The trimethylsilylation may be easily accomplished by treatment of the catecholamine with hexamethyldisilazane (HMDS) in the presence of pyridine as a solvent.

About 10 mg of the amine or its hydrochloride in a 10 ml glass-stoppered test tube were treated with 0.5 ml of pyridine and 1.5 ml of HMDS. The mixture was shaken mechanically for 20 hours at room temperature and then heated in a 95° C water bath for an additional 30 minutes. The small precipitate of ammonium chloride which formed during the reaction did not affect the analysis, and a 1 µl aliquot could be injected directly onto the column without further purification.

A Perkin-Elmer model 800 gas chromatograph equipped with dual columns and a differential flame ionization detector was used. The columns were stainless steel tubes, 6 feet in length, and 1/8 inch internal diameter. The solid support was Chromosorb W, 60-80 mesh, washed first with concentrated hydrochloric acid and then with methanolic potassium hydroxide, and treated with HMDS to reduce adsorptive effects. The solid support was suspended in 6% General Electric SE-30 siloxane polymer dissolved in ether and the excess solvent removed under vacuum at 40° C. The injector temperature was 290° C and the column temperature either 200° C or 220° C. The higher column temperature produced sharper peaks, but the lower temperature gave better separation of the TMS derivatives of different amines. A flow rate of 38 ml/min. of the carrier gas (nitrogen) was used in all cases.

The fact that HMDS reacts quantitatively with phenolic and alcoholic groups to form the respective trimethylsilyl ethers is well established (Langer et al., 1958; Bentley et al., 1963). It was found that the reagent also reacts with amine groups. A 10 mg portion of phenylethylamine was treated with HMDS under the conditions stated earlier, and the reaction mixture was analyzed by gas chromatography on the SE-30 column. The analysis revealed the formation of a new derivative of phenylethylamine, probably the N-trimethylsilyl derivative. The exact nature of the TMS derivative of the different phenolic and catecholic amines is not clear but HMDS probably reacted with all the available reactive centers, namely, the phenolic, alcoholic and amine groups.

All the TMS derivatives tried, chromatographed well with little tailing on this SE-30 column and each produced a single symmetrical peak. A typical chromatogram of a mixture of the TMS derivatives of epinephrine, metanephrine and dopamine is shown in Figure 1, and the relative retention times of the TMS derivatives of the different amines studied are given in Table 1.

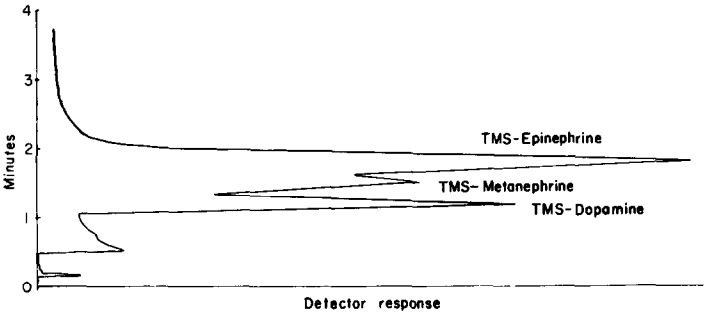

Figure 1. Gas chromatographic separation of trimethylsilyl derivatives of amines. Conditions: Column 6 feet x 1/8 inch I.D. stainless steel tube, 6% SE-30 on 60-80 Chromosorb W; temperature 220° C, nitrogen flow 38 ml/min.

As evident in Table 1, the TMS derivatives of epinephrine and norepinephrine could not be separated one from the other on the SE-30 column nor could the TMS derivatives of metanephrine and normetanephrine. The use of liquid phases (butanediol succinate, Carbowax 20 M, 5% DC-710 oil or 5% Apiezon L on Anakrom ABS) did not improve the separation of these derivatives and sometimes led to the appearance of extensive tails or to the disappearance of peaks. Success in distinguishing epinephrine from norepinephrine and metanephrine from normetanephrine was obtained by paper chromatography in phenol saturated with 1 N hydrochloric acid (Weiss et al., 1963), elution of the separated amines, subsequent conversion to the TMS derivatives and chromatography on an SE-30 column. This procedure allowed analysis for 1 µg amounts of each of the amines studied.

In a typical trial run, a mixture of 1 µg each of epinephrine and norepinephrine was submitted to paper chromatography and the separated

Table 1.

Relative Retention Times of Trimethylsilyl Derivatives of Amines.[a]

Amine	6% SE-30 Column	
	200° C	220° C
Dopamine	1.24	1.2
Epinephrine	2.04	1.84
Norepinephrine	2.04	1.84
DL-Metanephrine	1.66	1.56
DL-Normetanephrine	1.66	1.56
3-Methoxytyramine	1.0 (1.6)[b]	1.0 (1.0)[b]

[a] For experimental conditions see text.

[b] Actual retention time in minutes.

amines eluted with water. The eluants were concentrated to dryness and the residue treated with 1 ml of a 1:1 mixture of pyridine and HMDS under the conditions previously described. Four µl aliquots of each reaction mixture (corresponding to 0.004 µg of the amine) were chromatographed and a peak corresponding to the TMS derivative of epinephrine or norepinephrine could be detected.

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