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Note

Gas chromatographic determination of the lower aliphatic primary amines as their Schiff bases

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The identification and quantitative determination of the lower aliphatic and related amines (*e.g.* in food, tobacco and tobacco smoke, drugs, natural products, and biological systems) is a commonly encountered problem in organic analysis. The gas chromatographic (GC) determination of these amines at low concentrations is limited by adsorption and decomposition in the column, ghosting phenomena, badly tailed elution peaks, and the low sensitivity of the compounds in the gas chromatographic detector.

A common method of overcoming these limitations is to mask the polar group by derivative formation or to convert it into a derivative that has a selective sensitivity increase in the GC detector, *e.g.*, an electron capture detector (ECD). Several derivatives, such as trimethylsilyl ethers[1], Schiff bases[2–6], *p*-tosyl amides[7], pentafluorobenzoyl amides[8–10], 2,4-dinitrophenyl[11], isothiocyanates[12,13], carbamates[14] and trifluoroacetates[15–17] have been reported for this purpose.

In GC involving Schiff base formation reactions, VandenHeuvel *et al.*[2] have chromatographed biologically important amines, such as *n*-dodecyl amine and cyclododecyl amine, as their Schiff bases, the derivatives being subsequently prepared by condensation with ketones. Uno *et al.*[5] have also reported the application of the GC technique to the determination of non-volatile aromatic amines through the use of Schiff bases formation reactions and the GC determination of the activation energy of Schiff base formation reactions[6]. However, the qualitative analysis of complex mixtures of amines by this technique is difficult.

Moffat and Horning[3,4] have used pentafluorobenzaldehyde as a derivatizing agent for GC–ECD of picogram quantities of several primary amines.

In this study, in order to attain the selective and sensitive GC analysis of the lower aliphatic primary amines, the amines were converted into corresponding Schiff bases by reaction with benzaldehyde, and the Schiff bases produced were detected directly by GC. The reaction is illustrated in the following scheme

$$C_6H_5CHO + RNH_2 \rightarrow C_6H_5CH{=}NR + H_2O$$

where R = CH_3, C_2H_5, *n*-C_3H_7, *iso*-C_3H_7, $CH_2{=}CHCH_2$, *n*-C_4H_9, *iso*-C_4H_9, *sec.*-C_4H_9, *tert.*-C_4H_9, *n*-C_5H_{11}, *iso*-C_5H_{11}, *n*-C_6H_{13}, *n*-C_7H_{15}. This method has resulted in

harper peaks, and a quantitative precision of higher selectivity and sensitivity than the GC analysis with free amines.

EXPERIMENTAL

Preparation of Schiff base

The procedure for the preparation of all the Schiff bases listed in Table I was as follows. The amines (0.1–0.3 mole) and benzaldehyde (0.1–0.3 mole) were mixed directly, whence the reaction mixture was bubbled with nitrogen at room temperature for 0.5 h and then distilled.

The purities of the derivatives were checked by GC; all were found to be about 99% by area percent computation.

Gas chromatography

The gas chromatograph was a Shimadzu GC5AP$_5$F equipped with a flame ionization detector. The GC column consisted of a 3 m × 3 mm I.D. glass column packed with 5% SE-30 on Shimalite W, 60–80 mesh, acid washed and silanized. The chromatographic conditions for the flame ionization detector were: carrier gas (nitrogen) flow-rate, 45 ml/min; column temperature, 100°; air and hydrogen flow-rate, 1.0 l/min and 50 ml/min, respectively; injection and detector temperature, 150°.

Determination of the response factors and the relative retention times of the Schiff bases

In the determination of the response factors of the Schiff bases listed in Table I, the sample solution was prepared by dissolving the Schiff base (1×10^{-4} mole) and ethyl benzene (1×10^{-4} mole), as internal standard, in 5 ml of ethanol.

The peak area of each Schiff base and internal standard was determined by a digital integrator, and the response factor (F_i) was calculated from the following equation[18,19]:

$$F_i = A_s/W_s \times W_i/A_i$$

where A_s and W_s are the peak area (log) and weight of the internal standard, respectively, and A_i and W_i are the area (log) and weight of the Schiff bases, respectively.

The relative retention times of the Schiff bases listed in Table I were calculated using ethylbenzene as internal standard.

RESULTS AND DISCUSSION

The formation reactions of six representative Schiff bases proceeded readily and exothermically at room temperature with the minor possibility of side reactions. as shown in Table I, the response factors of these Schiff bases were uniform with a high reproducibility over a series of several homologues. A good uniformity of response for the Schiff bases is considered to be associated with their stability in the GC system.

The relative retention times of six Schiff bases under the analytical conditions listed in Table I indicated that, with the use of a 3 m × 3 mm I.D. glass column

TABLE I

RESPONSE FACTORS F_t, STANDARD DEVIATIONS AND RELATIVE RETENTION TIMES R_{tR} OF SCHIFF BASES

GC conditions: 5% SE-30 on Shimalite W (AW, DMCS), 60–80 mesh; 3 m × 3 mm I.D. glass column; N_2 flow-rate, 45 ml/min; H_2 flow-rate, 50 ml/min; air flow-rate 1.0 l/min; column temperature 100°; injection and detector temperature 150°; FID.

Amine of Schiff base	*B.p. (°C)*		*F_t values and standard deviations*	*R_{tR}***
	*Obsd.**	*Ref. 20*		
Methyl amine	182	180	1.16 ± 0.01	2.32
Ethyl amine	195	195	1.29 ± 0.01	3.37
n-Propyl amine	213	208–210	1.40 ± 0.02	5.70
iso-Propyl amine	197	—	1.40 ± 0.04	3.99
n-Butyl amine	227	—	1.52 ± 0.01	10.25
iso-Butyl amine	218	217–218	1.52 ± 0.01	7.74

* Atomospheric pressure.
** Retention times relative to that of ethyl benzene (2.04 min).

packed with SE-30, the direct GC analysis of the amines offers the best potential for clear separation of the individual amines commonly encountered in organic analysis.

Preparation of standard curves

The detector response produces a straight-line relationship in the range 0.2–32 μg of each Schiff base. The minimum detectable quantities of the Schiff bases were

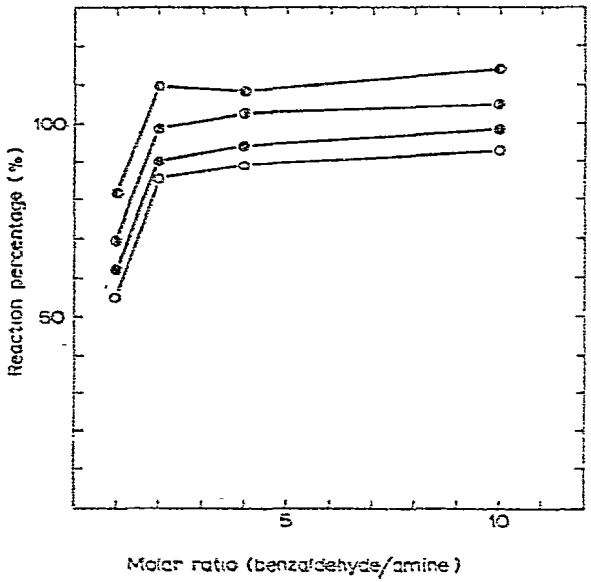

Fig. 1. Effect of molar ratio of benzaldehyde to amine (0.1 mmole in 5 ml ethanol, respectively) upon reaction conditions: 25°, 1 h; ●, methyl amine; ◐, ethyl amine; ◑, *n*-propyl amine, *iso*-propyl amine and *iso*-butyl amine; ○, *n*-butyl amine.

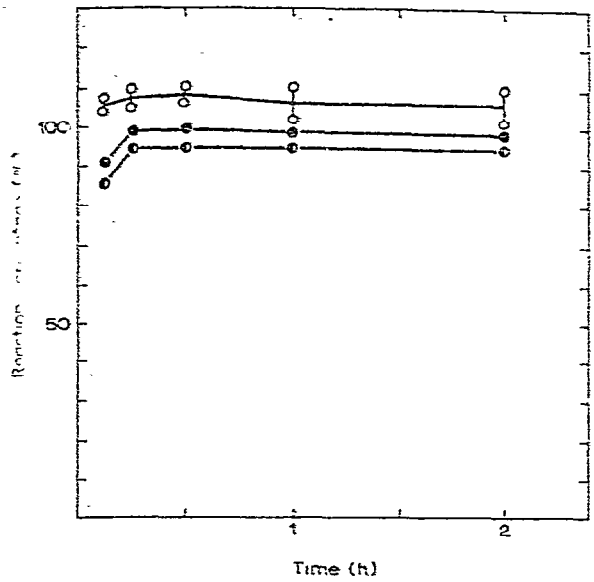

Fig. 2. Effect of reaction time; amine, 0.1 mmole in 5 ml ethanol; benzaldehyde, 0.4 mmoles; reaction temperature, 25°; ⊛, methyl amine; ○, ethyl amine, *n*-propyl amine, *iso*-propyl amine and *iso*-butyl amine; ◐, *n*-butyl amine.

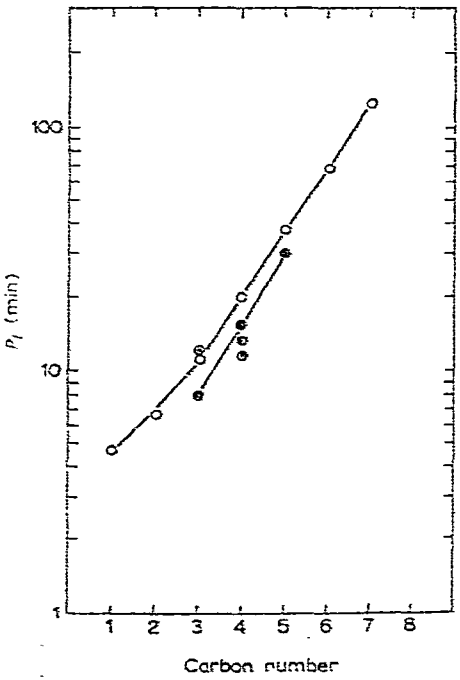

Fig. 3. Plots of retention time of Schiff base *versus* carbon number of alkyl group in Schiff base: ○, *n*-alkyl type; ⊛, *iso*-alkyl type; ⊙, allyl amine; ◐, *sec.*-butyl amine; ◑, *tert.*-butyl amine.

of the order of about 60 ng. The sensitivity to Schiff base as compared with free amin was about 10 times higher.

Evaluation of reaction conditions

The reaction percentages for the formation of the Schiff bases are plotte against the mole ratio of benzaldehyde to the amines and the reaction time.

As shown in Figs. 1 and 2, a completely quantitative reaction took place unde a reaction condition of benzaldehyde/amine mole ratio 2:1 and a reaction time of 1 min at room temperature.

Effects of ammonia and other amines

In this study, the effect of ammonia and other amines on the preparation o the Schiff base and the subsequent GC analysis was examined for six primary amines. In the presence of 100 molar equivalents of ammonia and 20 molar equivalents of dimethyl amine, diethyl amine, trimethyl amine and triethyl amine, no evidence was found for the interference of these compounds in the formation of Schiff bases.

Application to other primary aliphatic amines

For the purpose of exactly identifying the Schiff bases, the retention times of the homologous series of Schiff bases were plotted against the carbon numbers. As shown in Fig. 3, the plots produce a straight-line relationship for the *n*- and *iso*-type of aliphatic primary amine, and a typical gas chromatogram of the 13 Schiff bases is shown Fig. 4.

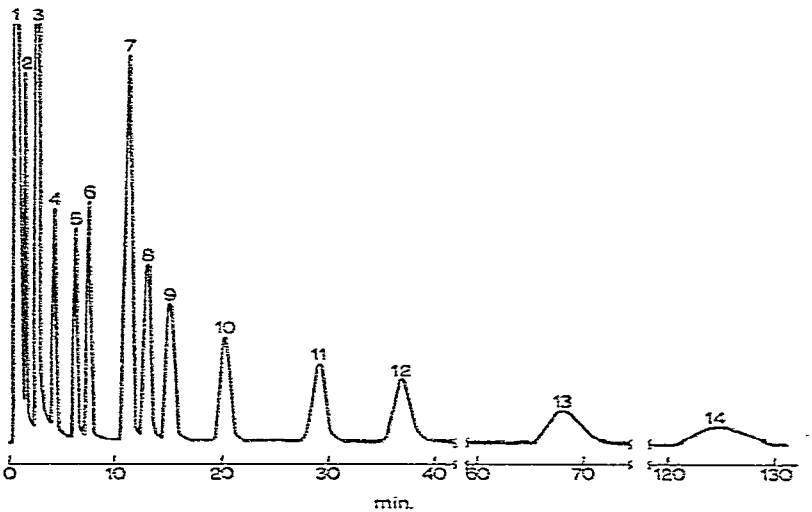

Fig. 4. A typical gas chromatogram for 13 Schiff bases of lower aliphatic primary amines. 1 = Eth nol; 2 = ethylbenzene (internal standard): 3 = excess C_6H_5CHO; 4 = CH_3NH_2; 5 = $C_2H_5NH_2$; 6 = *iso*-$C_3H_7NH_2$; 7 = *n*-$C_3H_7NH_2$; CH_2=$CHCH_2NH_2$, and *tert*.-$C_4H_9NH_2$; 8 = *sec*.-$C_4H_9NH_2$; 9 = *iso*-$C_4H_9NH_2$; 10 = *n*-$C_4H_9NH_2$; 11 = *iso*-$C_5H_{11}NH_2$; 12 = *n*-$C_5H_{11}NH_2$; 13 = *n*-$C_6H_{13}NH_2$; 14 = *n*-$C_7H_{15}NH_2$. The sample contains a mixture of benzaldehyde (3 mmoles) and amines (0.1 mmol in 5 ml ethanol; reaction temperature, 25°; reaction time, 15 min; sample volume, 1 μl; FID sens tivity, 10^5 ($\times$ 10^6 Ω); range 64 ($\times$ 0.01 V). Other GC conditions are the same as in Table I.

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