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GAS CHROMATOGRAPHIC SEPARATION OF 4'-NITROAZOBENZENE-4-CARBOXAMIDES OF PRIMARY AND SECONDARY AMINES

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SUMMARY

Primary and secondary amines can be directly separated as 4'-nitroazobenzene-4-carboxamides by gas–liquid chromatography. Using silicone grease as a liquid phase and a constant temperature of 270°, the separation was performed with derivatives of primary and secondary aliphatic, alicyclic, and aromatic amines up to C_{12} and several tobacco alkaloids. A preparation technique is described whereby pure compounds are obtained from complex mixtures ready for mass spectrometric identification. A short review is given with respect to the examination of tobacco and tobacco smoke.

Recent publications have indicated the use of direct gas chromatographic separation techniques for crystallizable derivatives of carbonyl compounds including oximes[1,2] and 2,4-dinitrophenylhydrazones[3–5]. The use of 4'-nitroazobenzene-4-carboxamides (NABSA) for the separation and identification of amines by thin-layer chromatography has been reported earlier[6].

In 1955, the basic reagent 4'-nitroazobenzene-4-carboxylic acid chloride was prepared by HECKER[7], for the first time, and later on used as a reagent for hydroxyl compounds by BUTENANDT *et al.* during investigations on "bombicol"[8,9].

The reaction equations for hydroxyl compounds and primary and secondary amines are shown in Fig. 1.

Derivatives obtained by this method crystallize well, and are strongly coloured substances with exact melting points.

Direct gas chromatography of derivatives proved to be very helpful in the identification of both amines and hydroxyl compounds. Concerning the gas chromatography of hydroxyl derivatives a detailed report will be published, in which retention times of about 100 compounds including alcohols and phenols will be given*.

* This report has been published in the meantime (see ref. 10).

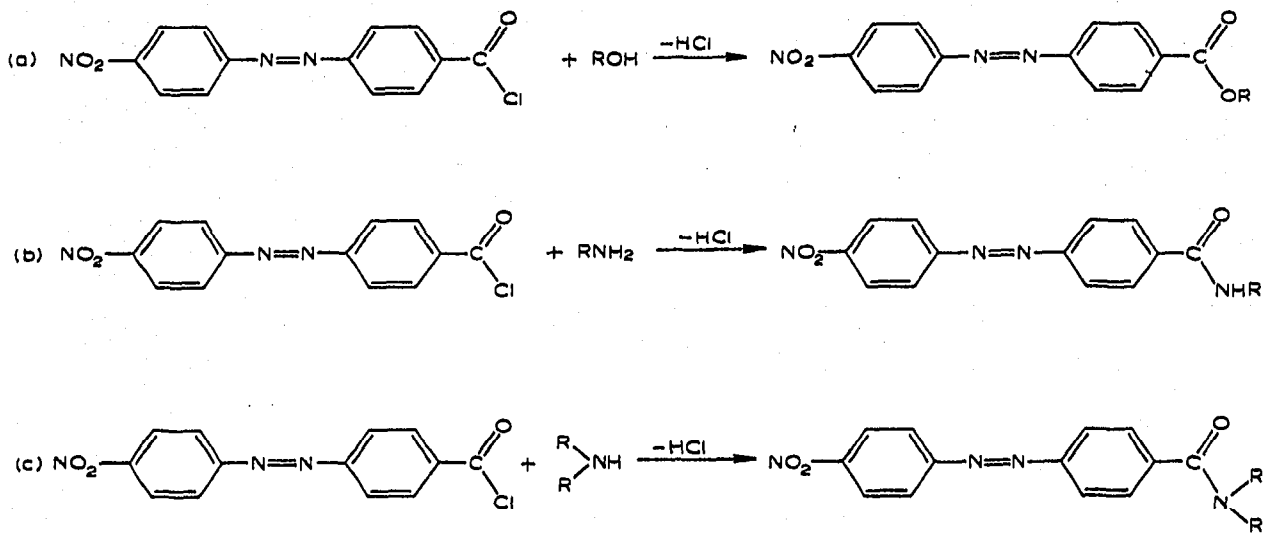

Fig. 1. The reaction equations for: (a) hydroxyl compounds; (b) primary amines; (c) secondary amines.

EXPERIMENTAL

Apparatus

For chromatography, 1-m and 2-m stainless steel tubes (4-mm I.D.) packed with 2.5 % w/w silicone grease (E. Merck AG., Darmstadt, Germany) on 60 to 80 mesh Chromosorb G, acid washed and DMCS treated, were used in fractometers, types F6 and F7 (Perkin Elmer Bodenseewerk, Überlingen, Germany). The fractometers were equipped with flame-ionization detectors and a 2.5-mV recorder (Siemens-Kompensograph L 288 × 288) with a paper feed of 0.5 cm/min.

Conditions

The operations were carried out under the following general conditions:
Constant column temperature: 270°
Injection temperature: 300°
Carrier gas: helium
Flow rate: 30 ml/min, without splitting after the column.

Procedure

The 4'-nitroazobenzene-4-carboxamides of the amines were prepared as described earlier[6] and showed the melting points listed there. Because of their slight solubility the samples were dissolved in dichloromethane and 1 to 5 μg in about 5 μl of the solvent were injected into the apparatus. When a new column is used for the first time or, for example, the injection is interrupted overnight, the column must be pre-loaded with amides in order to get any results. After a number of injections of an amide solution these difficulties were overcome.

Another problem is the small recovery of the injected amides, certainly dependent on the above-mentioned effect; 95 % of the sample seem to decompose while passing through the first part of the column. If a glass column is used a grey zone of 3 or 4 cm length can be seen after one or two injections. Nevertheless, the columns are normally useful for about 4–6 weeks.

A slight peak distortion caused by some bleeding of silicone grease, due to the high temperature used, and deposition of silicone dioxide on the FID electrode, was overcome by using a relatively high hydrogen flow rate to the FID (about 30 ml/min).

Preparation technique

In spite of the great loss of derivatives after injection mentioned above, a preparation technique to obtain traces of pure compounds for mass spectrometric measurements was developed.

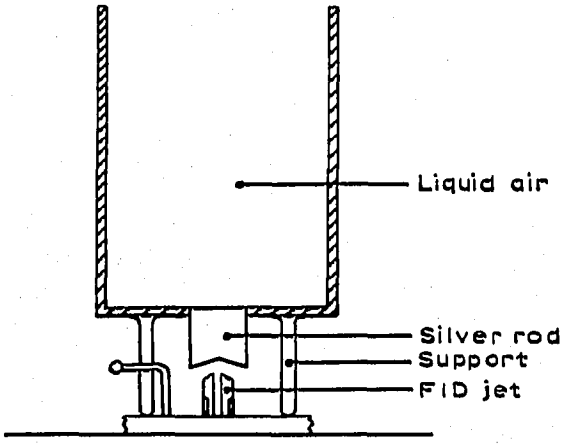

Fig. 2. Plan of the arrangement for obtaining traces of pure compounds.

Fig. 2 gives a key plan of this arrangement. After extinguishing the FID flame the opposite electrode was turned away from the FID jet in the manner shown in Fig. 2; the compounds can then be condensed into the recess of a liquid air cooled silver rod, according to their predetermined retention times.

The condensed derivatives can be recovered by means of fine glass wool moistened with acetone and directly inserted into the sample glass of the mass spectrometer. The smallest sample size injected which gives a distinct mass spectrum is about 5 μg.

RESULTS AND DISCUSSION

Fig. 3 shows the gas chromatographic separation of the NABSA of primary amines. The separation of the *n*- and *iso*-forms is only possible up to C_4, while *n*- and iso-pentylamine cannot be separated.

Fig. 4 gives a corresponding fractogram of the symmetric secondary amines. These secondary amines indicate the increasing influence of the larger molecules with respect to the separation of the *n*- and *iso*-forms.

Fig. 5 shows the fractogram of a mixture of some asymmetric secondary amines.

Fig. 6 shows the slope of the retention times of primary and secondary *n*-alkylamines plotted against the number of C-atoms. It is evident that symmetric secondary amines have much lower retention times than derivatives of primary amines containing the same number of C-atoms. The decreasing effect of methyl and ethyl groups in asymmetric secondary amines is not as strong as that produced by

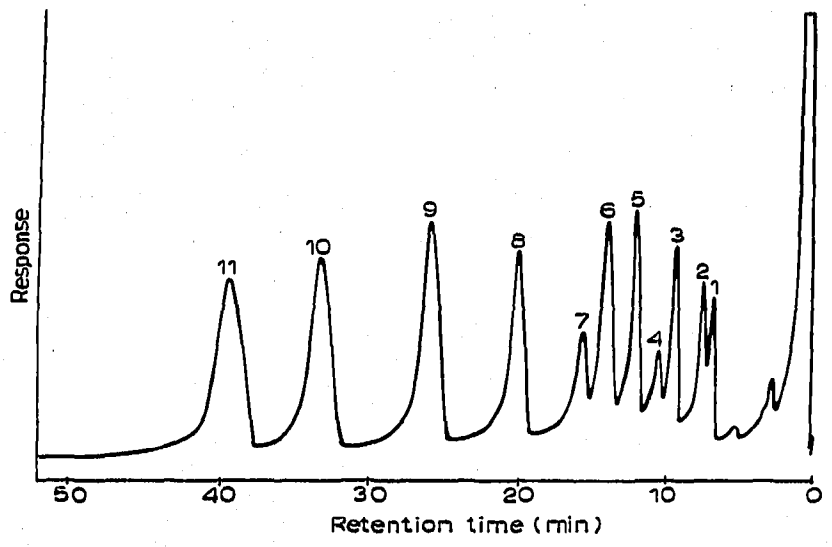

Fig. 3. Gas chromatographic separation of the NABSA of primary amines, using general conditions. 1 = Methyl + ethylamine; 2 = isopropylamine; 3 = *n*-propylamine; 4 = isobutylamine; 5 = *n*-butylamine; 6 = *n*- + isopentylamine; 7 = isopentenylamine; 8 = *n*-hexylamine; 9 = *n*-heptylamine; 10 = *n*-octylamine; 11 = *β*-phenethylamine.

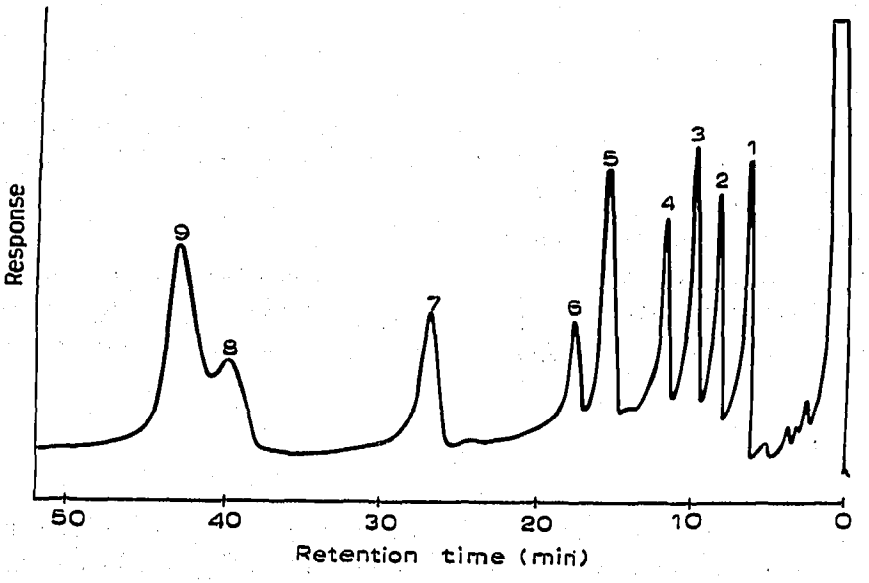

Fig. 4. Gas chromatographic separation of the NABSA of symmetric secondary amines, using general conditions. 1 = Dimethylamine; 2 = diethylamine; 3 = diisopropylamine; 4 = dipropylamine; 5 = dibutylamine; 6 = diisopentylamine; 7 = dipentylamine; 8 = dicyclopentylamine; 9 = dihexylamine.

the symmetric substitution resulting in a medium position of their slopes not given in Fig. 6.

Table I lists the retention times of all the derivatives tested on the 2-m and 1-m columns under the conditions mentioned above. The retention time of the reagent 4′-nitroazobenzene-4-carboxylic acid chloride is 3 min, thus excluding any interference with the derivatives.

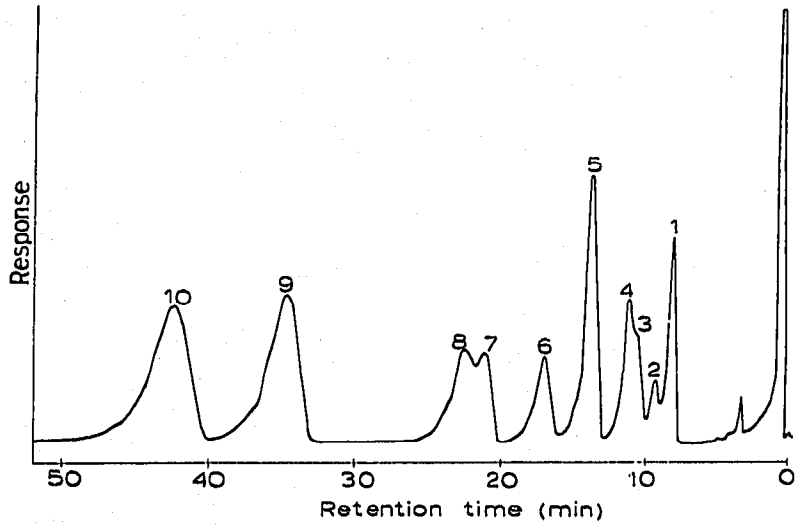

Fig. 5. Gas chromatographic separation of the NABSA of asymmetric secondary amines, using general conditions. 1 = Dimethylamine; 2 = N-methyl-ethylamine; 3 = N-methyl-isopropylamine; 4 = N-methyl-propylamine; 5 = N-methyl-butylamine; 6 = N-methyl-pentylamine; 7 = N-methylaniline; 8 = N-ethylaniline; 9 = N-methyl-benzylamine; 10 = N-methyl-N-β-phenethylamine.

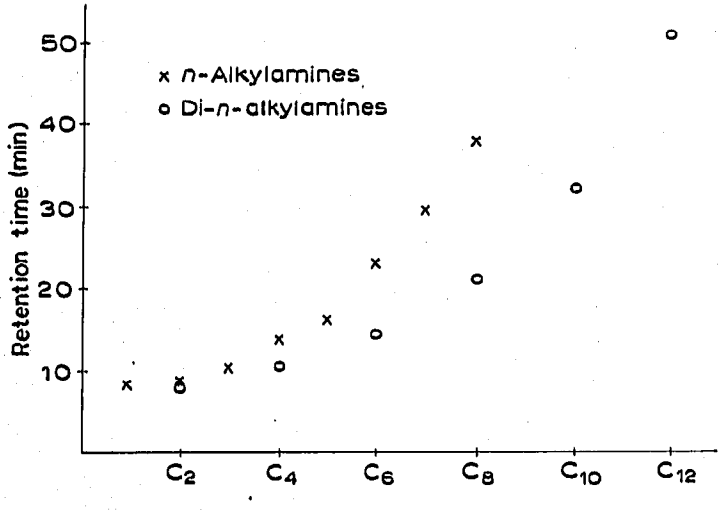

Fig. 6. Retention times on a 2-m column plotted against the number of C-atoms (conditions given in Experimental section).

One can distinguish the derivatives of primary and secondary amines by treatment of the mixture with nitrous gases, after which the derivatives of the primary amines disappear whilst those of the secondary amines still persist.

A mixture of two primary and two secondary amines is shown in the fractogram of Fig. 7; the same mixture is shown in Fig. 8 after treatment with nitrous gases for about 40 min.

Finally, the separation of basic constituents of tobacco and tobacco smoke will be briefly reviewed. Tobacco[11] and tobacco smoke[12] were examined for volatile

TABLE I

RETENTION TIMES OF THE NABSA OF VARIOUS AMINES

2-m column

Parent amine	*Retention time (min)*
Methylamine	8.0
Dimethylamine	8.0
Ethylamine	8.2
N-Methyl-ethylamine	8.6
Diethylamine	10.4
n-Propylamine	10.4
N-Methyl-propylamine	12.8
N-Ethyl-propylamine	12.6
Dipropylamine	14.4
Isopropylamine	8.4
N-Methyl-isopropylamine	11.0
N-Ethyl-isopropylamine	12.0
Diisopropylamine	12.2
n-Butylamine	13.8
N-Methyl-butylamine	14.2
N-Ethyl-butylamine	15.2
Dibutylamine	21.2
sec.-Butylamine	11.2
N-Methyl-*sec.*-butylamine	12.6
N-Ethyl-*sec.*-butylamine	13.8
Di-*sec.*-butylamine	18.4
Isobutylamine	13.0
N-Methyl-isobutylamine	12.6
N-Ethyl-isobutylamine	13.6
Diisobutylamine	17.4
tert.-Butylamine	9.0
N-Methyl-*tert.*-butylamine	11.2
N-Ethyl-*tert.*-butylamine	12.6
n-Pentylamine	16.8
N-Ethyl-pentylamine	17.6
Dipentylamine	32.2
α-Ethylpropyl-amine	14.8
2-Methyl-butylamine	16.2
1,2-Dimethyl-propylamine	13.4
Isopentylamine	16.2
N-Ethyl-isopentylamine	16.4
Diisopentylamine	25.8
n-Hexylamine	23.4
Dihexylamine	51.6
n-Heptylamine	29.6
n-Octylamine	38.0
Allylamine	10.8
N-Ethyl-allylamine	11.6
Diallylamine	14.0
2-Butenylamine	14.6
3-Methyl-2-butenylamine	18.8
Diisopentenylamine	17.8
2-Methoxy-ethylamine	14.0
2-Amino-ethanethiol	9.8
Cyclopropylamine	13.4
Cyclopropane-methylamine	16.4
Cyclopentylamine	21.6

(*continued on p. 211*)

TABLE I (*continued*)

2-m column

Parent amine	*Retention time (min)*
Cyclohexylamine	27.4
3-Cyclohexen-1-yl-amine	28.1
Dicyclopentylamine	46.6
N-Methylaniline	20.8
N-Ethylaniline	22.6
N-Butylaniline	30.8
Diphenylamine	64.0
o-Toluidine	21.2
m-Toluidine	45.0
p-Toluidine	47.4
Benzylamine	39.0
Pyrrolidine	18.2
Piperidine	20.0
2-Methylpyrrolidine	17.4
2-Pipecoline	22.0
3-Pipecoline	20.6
4-Pipecoline	22.6
2-Aminopyridine	28.5
3-Methylamino-pyridine	25.4
2-(Methylaminomethyl)-pyridine	33.4
4-(Methylaminomethyl)-pyridine	41.2
Morpholine (Tetrahydro-2H-1,4-oxazine)	17.8

1-m column

Parent amine	*Retention time (min)*
Dihexylamine	13.6
N,N-Dimethyl-1,3-propanediamine	64.0
2-Ethoxy-ethylamine	4.8
Dicyclohexylamine	21.0
Dicyclopentylamine	13.4
N-Phenyl-benzylamine	22.6
N,N-Dibenzylamine	36.6
3,4-Xylidine	17.6
2-Naphthylamine	43.6
3-Hydroxypiperidine	10.2
2-Amino-3-picoline	∞
3-(4-Methylamino-butyl)-pyridine	29.6
2-(Methylaminomethyl)-pyridine	10.6
3-(Methylaminomethyl)-pyridine	13.0
4-(Methylaminomethyl)-pyridine	13.0
6-Methyl-2-(methylaminomethyl)-pyridine	11.2
3-(4-Methylamino-1-butenyl)-pyridine	31.6
Nornicotine	23.0
Anabasine	30.4
3-(2-Pyrrolin-2-yl)-pyridine (Myosmine)	20.0

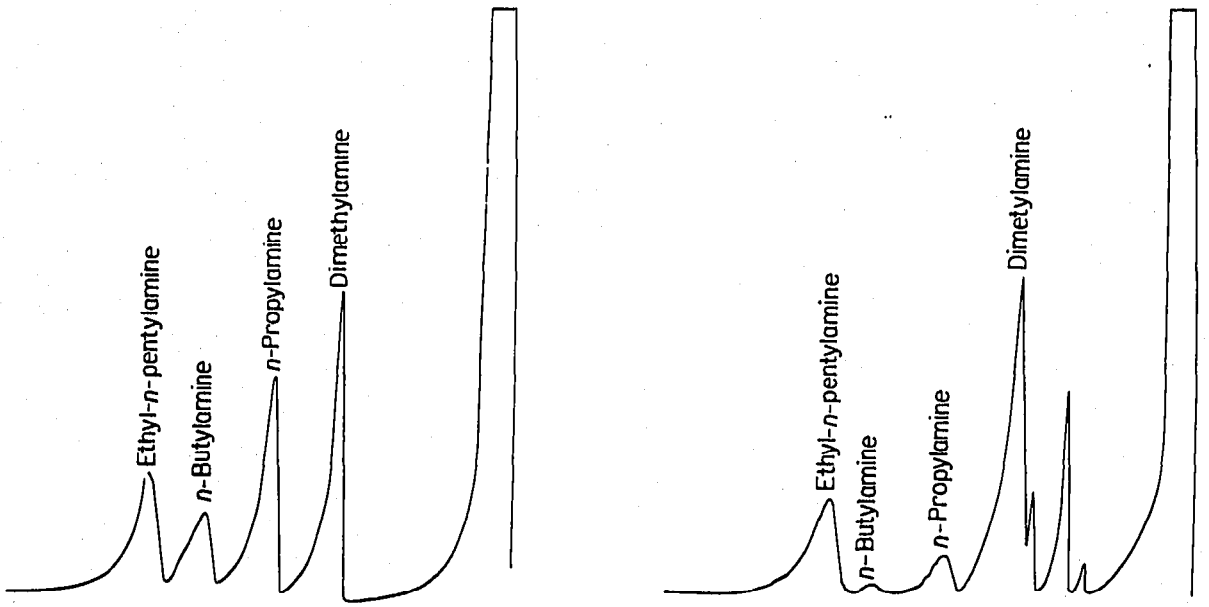

Fig. 7. Mixture of two primary and two secondary amines.

Fig. 8. The same mixture after treatment with nitrous gases for about 40 min.

basic compounds under general analytical conditions. The reaction of the resulting hydrochlorides with 4'-nitroazobenzene-4-carboxylic acid chloride was performed by heating with pyridine. After preseparation on a silica gel column the fractions were separated by thin-layer chromatography. Traces of additional bases in single spots could often be detected by running a gas fractogram thus aiding purification procedures.

Together with other characteristics of the derivatives, *e.g.* melting points, R_F values, elemental analyses, I.R.-spectra, and mass spectra, the retention times are very important for the identification of amines.

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