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Note

Thin-layer chromatography of N-nitrosamines

A. A. LESLIE GUNATILAKA

Chemistry Department, University of Sri Lanka, Peradeniya Campus, Peradeniya (Sri Lanka)

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N-Nitrosamines, which are known carcinogens[1], are being used increasingly in synthetic organic chemistry[2–5]. In spite of their importance in these respects, very little attention has been directed to chromatographic studies of this class of compounds, and their thin-layer chromatographic (TLC) behaviour and staining methods for their detection was of interest.

In recent years, the correlation of chemical structure with chromatographic behaviour has received considerable attention. These attempts have so far been restricted to the study of relatively simple compounds and homologous series and have met with varying degrees of success. In 1964, Preussmann and co-workers[6,7] carried out a TLC study on a series of nitrosamines in order to develop suitable spray reagents. Plots of the R_F values for homologous series of nitrosamines *versus* molec-

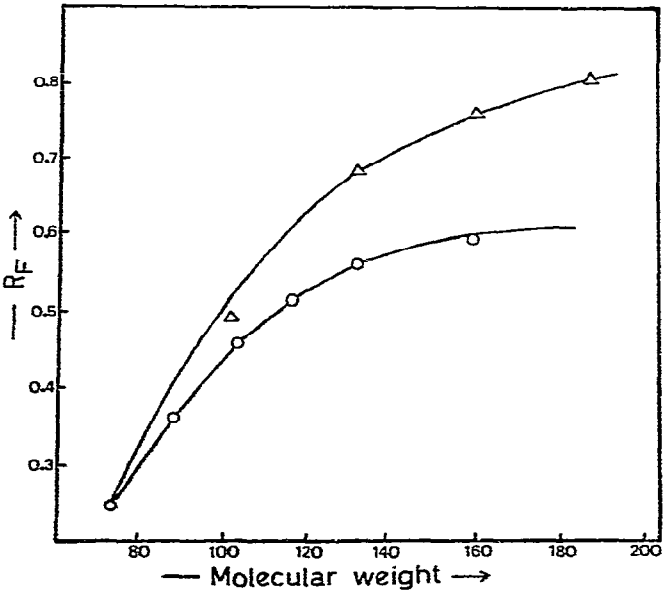


Fig. 1. R_F values of some nitrosamines *versus* molecular weight. ○, Alkylmethyl nitrosamines; △, *sym*-dialkyl nitrosamines. Adsorbent: silica gel G (0.25 mm thick). Developing solvent: *n*-hexane–diethyl ether–dichloromethane (4:3:2).

ular weight were smooth, steadily increasing curves (Fig. 1), and a mathematical description of the effect of side-chains on chromatographic adsorption was attempted. For this purpose, Lerosen and co-workers[8,9] developed an expression similar to that of Martin and Synge[10], the general validity of which in adsorption chromatography was shown by Trueblood and Malmberg[11]. The equation, which takes into consideration the chemical interaction between the adsorbed compound and the adsorbent, is

$$f = (1 - R_F)/R_F = k\,(M_{sc})^{-1}$$

where f is the adsorption affinity, R_F the ratio between the distance moved down the adsorbent column by the adsorbate zone and that by the solvent M_{sc} the molecular weight of the side-chain attached to the functional group and k the sum of the interaction tendencies between the adsorbent and the adsorbed compound.

Fig. 2 shows the approximate linearity of a log–log plot of adsorption affinity $[(1 - R_F)/R_F]$ *versus* the molecular weight of the side-chain (M_{sc}) of alkylmethyl nitrosamines. The results show that the general equation of this straight line is of the type predicted, but it was found that the exponent of M_{sc} is not -1 as assumed in the first approximation. The value of k determined by this type of plot represents the sum of all of the interaction tendencies that cause adsorption and should prove useful in assigning values of donor and acceptor strengths to adsorbents and adsorbed compounds in future work. Alternatively, the constant k can be calculated by inserting the R_F value for *n*-butylmethyl nitrosamine in the above equation. Table I shows the R_F values calculated for other nitrosamines using this k value, and the agreement

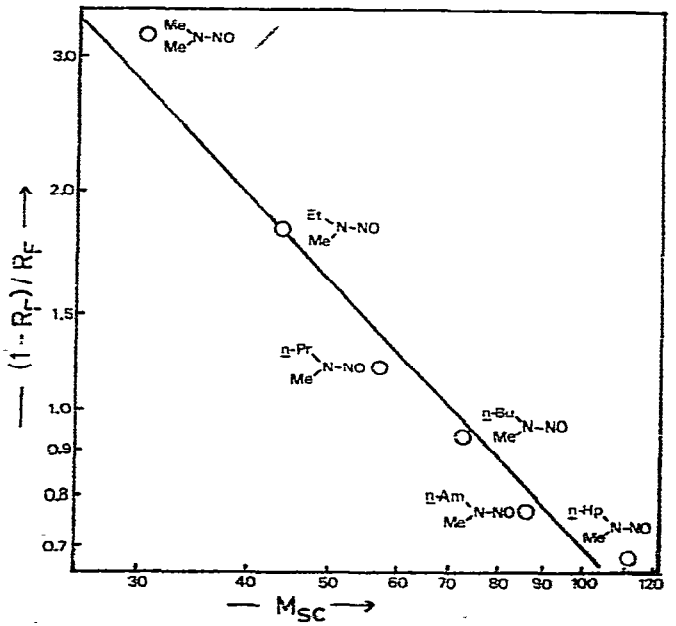
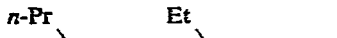
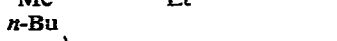
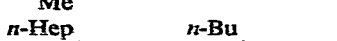
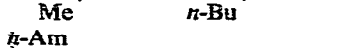


Fig. 2. Adsorption affinities of some alkylmethyl nitrosamines. General equation: $(1 - R_F)/R_F = k\,(M_{sc})^n$; $n = -1.15$, $k = 45$.

TABLE I

EXPERIMENTAL AND CALCULATED R_F VALUES OF SOME NITROSAMINES

Nitrosamine	*R_F value*	
	*Experimental**	*Calculated***
	0.24	0.30
	0.36	0.39
	0.46; 0.49	0.46
	0.51	—
	0.57; 0.69	0.55
	0.59***	0.59
	0.60; 0.77	0.62
	0.82	0.67

* See ref. 7.

** Calculated using the expression $R_F = M_{sc}/(k + M_{sc})$; k was obtained by inserting the experimental R_F value for *n*-butylmethyl nitrosamine.

*** From the curve in Fig. 1.

between the calculated and experimental R_F values is evident. It further shows that of an isomeric pair, the alkylmethyl nitrosamine is always more strongly bound than the symmetrical counterpart to the adsorbent. In TLC, this property could therefore be used to differentiate between isomeric pairs of nitrosamines.

Two spray reagents currently being used for the detection of nitrosamines are both due to Preussmann and co-workers[6,7] and employ at some stage a photolytic decomposition of the nitrosamine. The staining method described below makes use of the facile acidic cleavage of nitrosamines to their corresponding amines[12], which are detected with iodoplatinate reagent[13,14].

EXPERIMENTAL

Materials

The nitrosamines referred to in Table II were prepared by nitrosation of the corresponding amines with nitrous acid according to the standard procedure[15].

Thin-layer chromatography

Silica gel G (Merck, Darmstadt, G.F.R.) was used for TLC. The developing solvents contained various proportions of *n*-hexane, diethyl ether and dichloromethane (see footnote to Table II).

The spray reagent was similar to that described by Munier[13] except that the potassium iodide solution was prepared with 10% sulphuric acid instead of water. After development, the plates were sprayed with this acidic iodoplatinate reagent, heated at 100° for 1–3 min and then re-sprayed with the same reagent if necessary to increase the sensitivity.

RESULTS AND DISCUSSION

With the above procedure, the nitrosamines appeared as pale grey to bright purple spots (Table II).

During the examination of a mixture of amines and nitrosamines, the chromatograms were first sprayed with iodoplatinate reagent as described by Munier[13],

TABLE II

R_F VALUES OF SOME NITROSAMINES AND THEIR COLOURS WITH ACIDIC IODOPLATINATE SPRAY REAGENT

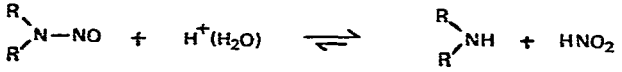
Nitrosamine	*Developing solvent**	R_F**	*Coloration with acidic iodoplatinate*
Dimethyl nitrosamine	A	0.25	pale grey
Ethylmethyl nitrosamine	A	0.40	pale grey
Diethyl nitrosamine	A	0.50	pale grey
Diisopropyl nitrosamine	A	0.65	grey
N-Nitrosopyrrolidine	B	0.40	pale grey
N-Nitrosopiperidine	B	0.60	grey
N-Nitrosomorpholine	B	0.40	grey
Diphenyl nitrosamine	C	0.80	purple
Nitroso-N-methyl-(2-phenethyl)amine	C	0.30	bright purple

* The following *n*-hexane–diethyl ether–dichloromethane systems were used: A, 4:3:2; B, 5:7:10; C, 10:3:2.

** The plates used were impregnated with 0.20–0.25 mm thick silica gel G (Merck).

whereby only the amine spots were stained. Subsequent treatment of the chromatograms with 10% sulphuric acid followed by heat treatment as described above then revealed the nitrosamine spots.

Nitrosamines are known to undergo cleavage in the presence of aqueous mineral acids, resulting in the corresponding amines with the liberation of nitrous acid[12] according to the equation:



Iodoplatinate spray reagent has been employed widely to detect amines on thin-layer chromatograms[13,14].

CONCLUSIONS

The validity of the the Lerosen-type equation for the homologous series of alkylmethyl nitrosamines seems to indicate the general correctness of the assumption that the functional group, in this instance N–NO, is a major factor in adsorption on to silica gel. However, further work is necessary in order to establish the conclusions more definitely.

The staining method described here has proved to be useful in the TLC control of both nitrosation and denitrosation reactions[5]. The high sensitivity shown by the reagent could be applied to the detection of nitrosamine carcinogens in biological samples.

ACKNOWLEDGEMENT

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