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THIN-LAYER CHROMATOGRAPHIC SEPARATION OF THE Z AND E ROTATIONAL ISOMERS OF α-N-NITROSO-N-ALKYLAMINO ACIDS

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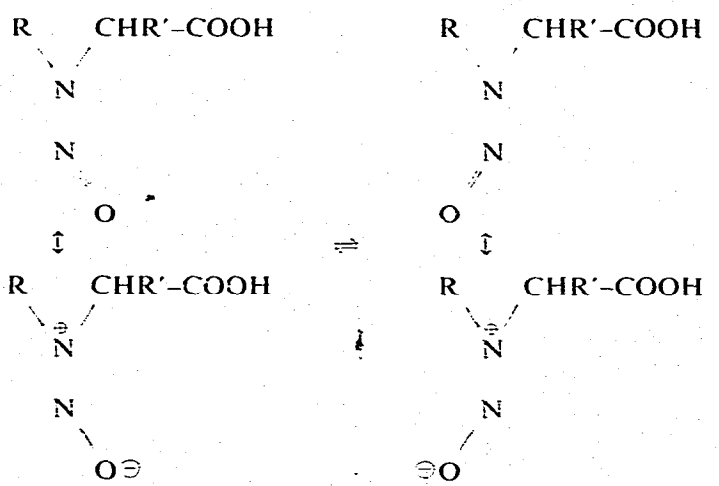
SUMMARY

Thin-layer chromatography at 0–2 C affords a simple and rapid method for the detection and separation of the Z and E isomers of N-nitroso-N-alkylamino acids. The procedure enables conformational purity of the crystals to be determined and conformational Z⇌E interconversions to be studied.

INTRODUCTION

The partial double bond character of the N–N linkage in N-nitroso-N-alkylamino acids leads to the formation of the Z and E isomers*, which are in dynamic equilibrium[1–4]:



Interconverting rotational isomers can be separated if their mean lifetime is of the order of at least several hours. This means that the free enthalpy of activation ΔG^*, which must be supplied in order to convert the Z isomer into the E isomer or *vice-versa*, is greater than 20–23 kcal/mole (refs. 5 and 6). Several instances of success-

* The Z and E system for specifying double bond isomers unambiguously is employed[6].

ful separations of the Z and E isomers of amides, thioamides and N-nitrosoamines have appeared in the literature[2,4,7–16].

Chromatographic separations of the Z and E isomers of N-nitroso-N-alkylamino acids have been studied on thin layers[4]. It was found that thin-layer chromatography (TLC) offers not only a rapid and simple method for the detection and separation of the isomers, but also a method for the determination of their conformational purity and for the study of conformational interconversions.

EXPERIMENTAL

Thin-layer chromatographic plates

The TLC plates consisted of a 0.25-mm layer of silica gel G (Merck, Darmstadt, G.F.R.) coated on glass supports (20 × 10 cm).

Developing solvents

The following solvent systems were used: S1, *n*-butanol–25% ammonia–water (75:6:75, upper layer); S2, *n*-butanol–25% ammonia (6:1); S3, light petroleum (b.p. 45–58°)–ethyl acetate (5:2); S4, light petroleum (b.p. 45–58°)–ethyl acetate (3:2); S5, light petroleum (b.p. 45–58°)–ethyl acetate (1:1). Solvent systems S3–S5 were used for the separation of isomers of esters of N-nitroso-N-alkylamino acids.

Detection reagents

A 0.1% solution of bromocresol green in methanol was used for spraying chromatograms of N-nitroso-N-alkylamino acids. An iodine tank was used for N-nitrosoamino acids and their esters.

Temperature

In order to obtain compact and well defined spots, it is advisable to run chromatograms at 0–2 °C by placing the tank in a refrigerator. Variations in temperature during the run cause deterioration of the chromatogram. The higher the development, the more diffuse are the spots and the more tailing occurs.

Procedures

Solid recrystallized N-nitroso-N-alkylamino acids were dissolved in cold (0 °C) water, chloroform or acetone and applied with a micropipette 3 cm from the lower edge of the TLC plate. For checking the conformational purity of the crystals, the materials were applied to TLC plates immediately after dissolution. Each solution of a sample was then stored at room temperature for equilibration of the isomers and aliquots, withdrawn at appropriate intervals, and applied to the TLC plate for further development.

Solid recrystallized *p*-nitrobenzyl esters of N-nitrosoamino acids were dissolved in cold (0 °C) ethyl acetate, and immediately after dissolution were applied to the TLC plate. Each solution was then stored at room temperature for equilibration of the isomers and aliquots were withdrawn at appropriate intervals for further development.

RESULTS AND DISCUSSION

The results of the chromatographic separation of Z and E isomers are presented in Tables I and II.

R_F values for the Z and E isomers of eleven N-nitroso-N-alkylamino acids are shown in Table I and for four *p*-nitrobenzyl esters in Table II. All separation experiments were performed with solid starting materials, which were recrystallized before development and which had previously been studied by us at least by nuclear magnetic resonance (NMR) spectroscopy[2–4]. Hence the results presented for the separation of Z and E isomers by TLC can be discussed jointly with the NMR spectroscopic evidence.

Based on the NMR spectroscopic evidence, the TLC results for the separation of the isomers are grouped into five categories (A, B, C, D and F) for discussion.

TABLE I

R_F VALUES OF Z AND E ISOMERS OF N-NITROSO-N-ALKYLAMINO ACIDS ON SILICA GEL G PLATES

Solvent systems: S1 = *n*-butanol–25% ammonia–water (75:6:75); S2 = *n*-butanol–25% ammonia (6:1). Temperature, 0–2 C.

Compound	*NMR category*	*S1*		*S2*	
		Z	*E*	*Z*	*E*
N-nitrososarcosine	A	–	–	0.19	0.24
N-nitroso-N-ethylglycine	A	–	–	0.19	0.24
N-nitroso-N-propylglycine	A	0.21	0.31	–	–
N-nitroso-N-isopropylglycine	C	0.20	–	–	–
N-nitroso-N-methyl-DL-alanine	B	0.20	0.25	–	–
N-nitroso-N-ethyl-DL-alanine	A	0.22	0.32	–	–
N-nitroso-N-propyl-DL-alanine	A	0.24	0.32	–	–
N-nitroso-N-isopropyl-DL-alanine	C	0.21	–	–	–
N-nitroso-N-methyl-DL-phenylalanine	D	–	–	0.29	0.35
N-nitroso-N-methyl-L-valine	D	–	–	0.25	0.31
N-nitroso-L-proline	F	–	–	0.16	0.20

TABLE II

R_F VALUES OF Z AND E ISOMERS OF *p*-NITROBENZYL ESTERS OF N-NITROSO-N-ALKYLAMINO ACIDS ON SILICA GEL G PLATES

Solvent systems: S3 = light petroleum–ethyl acetate (5:2); S4 = light petroleum–ethyl acetate (3:2); S5 = light petroleum–ethyl acetate (1:1). Temperature, 0–2 C.

p-Nitrobenzyl ester	*NMR category*	*S3*		*S4*		*S5*	
		Z	*E*	*Z*	*E*	*Z*	*E*
N-nitrososarcosine	B	0.11	0.16	0.24	0.35	–	–
N-nitroso-N-ethylglycine	F	0.21	0.29	–	–	–	–
N-nitroso-N-methyl-DL-alanine	F	0.18	0.25	–	–	–	–
N-nitroso-L-proline	F	–	–	–	–	0.36	0.42

Some of the N-nitroso-N-alkylamino acids studied show a tendency to assume the Z or E conformation in the crystalline form. On dissolution of the original isomer present in the crystal, partial conversion into the other isomer gradually takes place until equilibrium is established[1–4].

According to the NMR spectroscopic evidence, compounds in category A in Tables I and II crystallized in the Z conformation. Chromatograms run on a freshly prepared solution of a sample revealed only a single spot with a lower R_F value. On standing at room temperature, all of these solutions showed the gradual appearance of an upper spot of the E isomer produced during the approach to equilibrium. The ratio of the areas and of the intensities of the colours of the upper and lower spots gradually increased until equilibrium was established.

Compounds in category B crystallized in the E conformation. On dissolution, the Z isomer was gradually produced from the original E isomer until equilibrium was established. Chromatograms run on freshly prepared solutions of these B compounds revealed the presence of only the upper spot. On standing at room temperature, these solutions showed the gradual appearance of a second spot with a lower R_F value. The ratio of the areas and of the intensities of the colours of the lower and upper spots gradually increased until equilibrium was established.

Comparative studies revealed that TLC at 0–2 °C offers a more sensitive method of checking the conformational parity of isomers than does NMR spectroscopy: both for compounds that crystallize in the Z conformation (category A) and those that crystallize in the E conformation (category B), a small extent of contamination by the other isomer could be detected more easily by TLC than by NMR spectroscopy.

N-Nitroso-N-isopropylglycine and N-nitroso-N-isopropyl-DL-alanine (category C) are conformationally pure in the crystal. NMR spectroscopic studies revealed that after dissolution of the original isomer present in the crystal, this isomer was not converted into another isomer. Chemical shifts in the resonances indicated that the Z conformation was present. Both category C compounds gave only one spot on thin-layer chromatograms, and no traces of a second spot were visible even after leaving an aqueous solution of the sample at room temperature for 7 days or heating this solution for 2 h at 90 °C. R_F values for the "lower" spots are in agreement with the NMR evidence that these compounds exist only as the Z isomers.

The first NMR spectrum after dissolution of a sample of N-nitroso-N-methyl-DL-phenylalanine in perdeuterated acetone revealed the presence of a major proportion of the Z isomer and a minor proportion of the E isomer[3]. The proportions of the isomers in solution did not change even after prolonged (7 days) storage at room temperature. The same results were obtained for N-nitroso-N-methyl-L-valine: the proportions of the isomers did not change on storing the solution but according to the NMR spectroscopic evidence a major proportion exists in the form of E isomer and a minor proportion as the Z isomer[3]. Both of these compounds, which are in category D in Table I, gave two spots in each chromatogram and the ratio of their areas and of the intensities of the colours did not change even on prolonged storage of the solution at room temperature. In agreement with the NMR spectroscopic evidence, the main spot for N-nitroso-N-methyl-DL-phenylalanine was that with the lower R_F value, while for N-nitroso-N-methyl-L-valine it was that with the higher R_F value.

The compounds in category F showed a preference for crystallization in the Z conformation. Contrary to the category A compounds these compounds were not usually conformationally pure in the crystal, and immediately after dissolution of a sample of a solid material only a solution with the equilibrium displaced towards the Z isomer resulted. The approach to equilibrium, involving gradual interconversion of the Z into the E isomer, could be studied by repetitive scanning of the NMR spectra[2–4]. Thin-layer chromatograms run for freshly prepared solutions of these category F compounds revealed two spots. These solutions, when stored at room temperature, showed a gradual increase in the ratio of the areas and of the intensities of the colours of the upper and lower spots until equilibrium was established.

It is evident that the Z (*syn*) isomers always gave spots with lower R_F values, and this applied both to N-nitroso-N-alkylamino acids and their *p*-nitrobenzyl esters.

Some oily N-nitroso-N-alkylamino acids and some oily alkyl esters of N-nitrosoamino acids were studied by TLC, but the results of their separation into two spots are not presented here as such oily compounds were usually mixtures of both Z and E isomers and their isomerism has not been studied in detail by other techniques.

The most suitable solvents for the separation of the Z and E isomers of N-nitroso-N-alkylamino acids contained ammonia. The presence of ammonia in the separating solvent systems is of the utmost importance as it was found that interconversions of the Z and E isomers are substantially slower for salts[4]. Thus, in the solvent systems S1 and S2, which gave the best separation results, the Z and E isomers of N-nitroso-N-alkylamino acids are separated mainly in the form of carboxylate anions, and the mean lifetimes of the Z and E anions are substantially longer than those of the corresponding unionized species.

In conclusion, it can be stated that TLC at low temperatures is capable not only of separating the Z and E isomers of N-nitroso-N-alkylamino acids and their esters, but also, because of the high separation efficiency, it enables the conformational purity to be checked, very small amounts of "conformational" contamination of the Z isomer by the E isomer or *vice-versa* to be detected, and the dynamic aspect of the conformational isomerism of this class of compounds to be studied.

Cautionary note

While N-nitroso-N-alkylamino acids are easy to prepare and to handle, it should be remembered that many N-nitrosamines are potent carcinogens. Proper precautions against contact with the skin should therefore always be taken.

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REFERENCES

1 W. Lijinsky, L. Keefer and J. Loo, *Tetrahedron*, 26 (1970) 5137.
2 B. Liberek, J. Ciarkowski, K. Steporowska, K. Stachowiak and E. Jereczek, *Rocz. Chem.*, 46 (1972) 1157.
3 B. Liberek, J. Ciarkowski, K. Steporowska, K. Stachowiak and E. Jereczek, *Rocz. Chem.*, 46 (1972) 1457; 47 (1973) 291 and 2221.

4 B. Liberek, J. Ciarkowski, K. Steporowska, K. Stachowiak and E. Jereczek, *Rocz. Chem.*, 46 (1972) 1895.
5 H. Kessler, *Angew. Chem., Int. Ed. Engl.*, 9 (1970) 219.
6 H.-O. Kalinowski and H. Kessler, in N. L. Allinger and E. L. Eliel (Editors), *Topics in Stereochemistry*, Wiley, New York, 1972, Vol. 7, p. 295.
7 W. E. Stewart and T. H. Siddall, III, *Chem. Rev.*, 70 (1970) 517.
8 H. Kessler and A. Rieker, *Justus Liebigs Ann. Chem.*, 708 (1967) 57.
9 H. A. Staab and D. Lauer, *Tetrahedron Lett.*, (1966) 4593.
10 W. Walter and E. Schaumann, *Chem. Ber.*, 104 (1971) 336.
11 W. Walter and G. Maerten, *Justus Liebigs Ann. Chem.*, 715 (1968) 35.
12 W. Walter and F. Becker, *Justus Liebigs Ann. Chem.*, 753 (1971) 187.
13 A. Mannschreck, A. Mattheus and G. Rissmann, *J. Mol. Spectrosc.*, 23 (1967) 15.
14 A. Mannschreck, *Angew. Chem., Int. Ed. Engl.*, 4 (1965) 985.
15 A. Mannschreck, H. Muensch and A. Mattheus, *Angew. Chem., Int. Ed. Engl.*, 5 (1966) 728.
16 A. Mannschreck and H. Muensch, *Angew. Chem., Int. Ed. Engl.*, 6 (1967) 984.