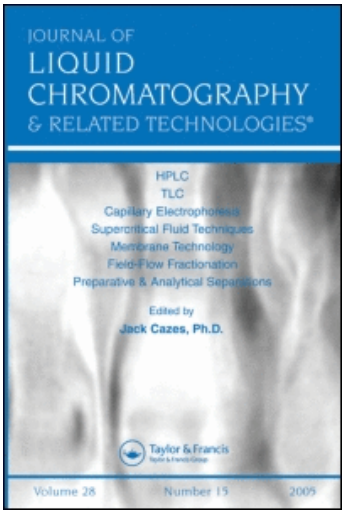

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Sandra S. Singer[a]; George M. Singer[a]
[a] Carcinogenesis Program NCI Frederick Cancer Research Center,

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ISOLATION OF GRAM QUANTITIES OF CONFIGURATIONAL ISOMERS OF CYCLIC NITROSAMINES BY PREPARATIVE LIQUID CHROMATOGRAPHY

Sandra S. Singer and George M. Singer
Carcinogenesis Program
NCI Frederick Cancer Research Center

ABSTRACT

Substituted cyclic nitrosamines, e.g., 2,6-dimethyl-N-nitrosomorpholine and 3,5-dimethyl-N-nitrosopiperidine are separable into their component configurational isomers by preparative hplc. Gram quantities of the individual isomers sufficient for animal testing were obtained. The identification of contaminants in commercial 2,6-dimethylmorpholine of purported 99% is discussed.

INTRODUCTION

The N-nitroso derivatives of many cyclic amines are carcinogenic (1). Aliphatic heterocycles with more than one methyl group, e.g. 3,5-dimethylpiperidine and 2,6-dimethylmorpholine are mixtures of configurational isomers (cis- and trans- methyl groups). When the nitroso derivatives of these compounds are prepared, the product is a mixture of configurational and conformational (E-Z) isomers. While the separation of nitrosamines into component conformational isomers can be achieved for special cases such as nitrosamino acids (2,3), it has not generally been possible to separate and maintain the nitroso E-Z conformers in

the pure state due to the relatively low barrier to rotation about the N-NO bond. The separation of configurational isomers, on the other hand, should be possible, and, under favorable conditions, gram quantities (sufficient for animal testing) might be obtained.

MATERIAL AND METHODS

Apparatus

A Waters Associates Liquid Chromatograph equipped with a Model 440 Absorbance Detector, two Model 6000A pumps, and Model 660 solvent programmer and a U6K injector (Waters Assoc., Milford Mass.) was used for analytical hplc.

A Waters Associates PrepLC/500 (Waters Assoc., Milford Mass.) equipped with two silica columns was used for preparative work.

Hplc Procedure

Analysis of nitrosamines was carried out on a Waters μPorasil 3.9 mm x 25 cm column using either 30% $CHCl_3$/hexane at 1.5 ml/min or 20% ethyl acetate/hexane at 1.5 ml/min.

Glc Procedure

Gas chromatographic analysis of the amines was carried out on a 10 ft x 2 mm 10% carbowax 20M + 2% KOH on Chromosorb W, 75°, 60 ml/min He. The instrument used was a Tracor MT-220.

Chemicals

Solvents for analytical hplc were Burdick and Jackson (Muskegon, MI) "Distilled in Glass". They were filtered through 0.5 μ filters before use, but not otherwise purified. Solvents for preparative lc were Eastman Reagent Grade and were used as received.

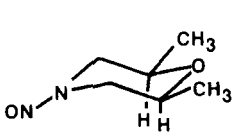
2,6-Dimethyl-N-nitrosomorpholine was prepared as described elsewhere (4).

3,5-Dimethyl-N-nitrosopiperidine

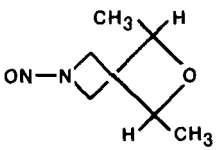
3,5-Lutidine (Aldrich, 95%) was distilled and the fraction with bp 173-174° was collected. The purified 3,5-lutidine (10g) was dissolved in 50% acetic acid (100 ml) and hydrogenated over PtO_2 (0.2 g) at 50 lbs pressure. When hydrogen uptake was completed, the reaction mixture was filtered, diluted with water 75 ml), acidified (12 N HCl), and cooled in an ice bath during the addition of a solution of $NaNO_2$ (22g, ca. 4 eq.) in H_2O (75 ml). The reaction mixture was stirred for 90 min, basified with KOH, and extracted with CH_2Cl_2 (3 x 200 ml). The CH_2Cl_2 extract was washed with 1N HCl (2 x 150 ml) and brine (1 x 200 ml), dried ($MgSO_4$) and evaporated in vacuo to a yellow oil (11 g, 87%) whose infrared spectrum was identical with an authentic sample of 8.

RESULTS AND DISCUSSION

A wide variety of cyclic nitrosamines was examined by nmr and hplc. While the nmr spectra indicated the presence of more than one conformational isomer in most instances, and the presence of configurational isomers as well in some cases, only a few of these compounds have thus far been found to be amenable to hplc separation into the component configurational isomers. Of principal interest was the potent carcinogen 2,6-dimethyl-N-nitrosomorpholine (4). The nitrosamine was made from amine of purported 99% purity obtained from two commercial sources. Sample A was analyzed by glc and had three components in the ratio 66:1:33. The other sample (B) also had three components, but in the ratio 80:1:19. The major peak corresponds to the cis isomer, and the other large peak corresponds to the trans-isomer. The identity of the minor peak will be discussed (vide infra).



1 (cis-)



2 (trans-)

When nitrosamines were prepared from these amines and analyzed by hplc, sample A showed four peaks in the ratio 60:4:34.5:.5 (Figure 1), while sample B showed four peaks in the ratio 70:9.5:20:0.5. The major peak was shown by nmr to be cis-2,6-dimethyl-N-nitrosomorpholine (*1*). The second largest peak was shown to be trans-2,6-dimethyl-N-nitrosomorpholine (*2*), which the nmr spectrum clearly showed to be in the twist-boat form (5). The third peak (*3*) that was present as 4% of the mixture in sample A and 9.5% in sample B is apparently responsible for the discrepancy between the glc and hplc results. The parent amine for this compound

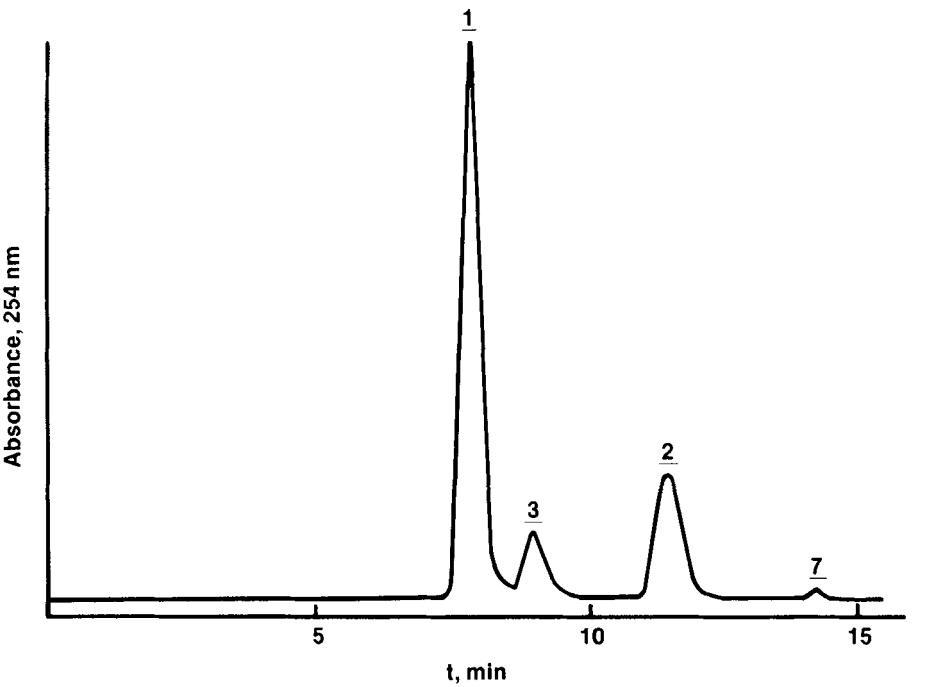
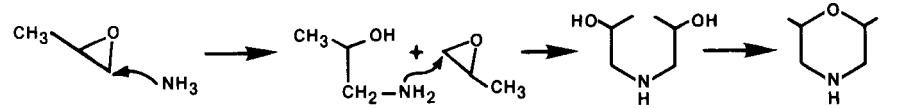


Figure 1.

Separation of 2,6-dimethyl-N-nitrosomorpholine into 4 components on hplc. *1* = cis isomer; *2* = trans- isomer; *3* and *7* are positional isomers present as contaminants. (Waters μPorasil, 30% $CHCl_3/C_6H_{14}$, 2 ml/min)

apparently co-chromatographs with the cis-dimethylmorpholine on glc, but the corresponding nitrosamine (*3*) is separated out on hplc. It is difficult to obtain *3* free of *1*, but quantities sufficient for spectral analysis were obtained. The infrared spectrum of the compound was very similar to that of *1* and *3*. The nmr spectrum was consistent with that of a dimethyl-N-nitrosomorpholine, in particular the 2,5-derivative.



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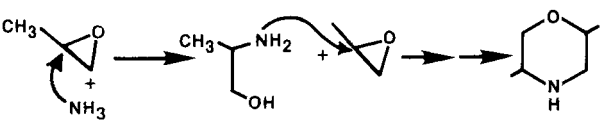
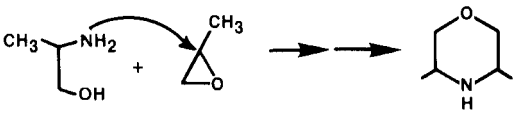




6 7

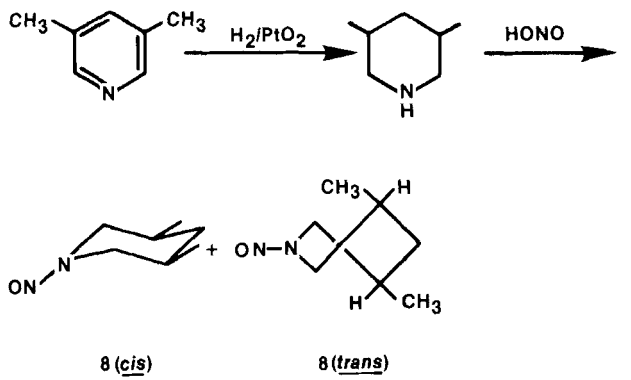
The parent amine for this compound could arise readily in the industrial synthesis of 2,6-dimethylmorpholine. The industrial synthesis of 2,6-dimethylmorpholine employs the reaction of propylene oxide with ammonia. The major pathway for ring opening, attack at C-2, gives 2-hydroxypropylamine (*5*). A second similar condensation gives the desired product, 2,6-dimethylmorpholine (eq *1*). Ring opening at C-1 can also occur as shown in eq. *2* to give 1-hydroxy-2-propylamine (*6*) which then can condense C-2 of propylene oxide to give the 2,5-dimethylmorpholine (*3*) or, *6* can attack at C-1 another propylene oxide molecule to give 3,5-dimethylmorpholine (*7*). The latter may be the smallest peak in the glc and HPLC traces, but this compound has not been isolated or characterized.

We first separated large quantities of *1* and *2* by open column chromatography on silica gel with hexane-ethyl acetate as eluent. This was successful but very time-consuming. The separation on a Waters PrepLC/500 using two silica cartridges with 70% chloroform-hexane as eluent (250 ml/min) proceeded rapidly. A typical trace is shown in Figure 2 (top).

While the resolution indicated on the trace obtained from the refractive index detector was not very impressive, subsequent analysis of the collected fractions by analytical hplc showed that some separation and enrichment had been achieved. The analysis of each fraction is indicated in Figure 2. Recovery of material was greater than 95%. Changing the solvent mixture to 20% ethyl acetate-hexane gave a marked improvement in peak shape and resolution (see Figure 2, bottom). The fractions rich in *2* were rechromatographed to obtain pure *2*. In order to obtain several grams of pure *2*, the *2*-rich fractions must be chromatographed 2-4 more times, each time shaving off as much pure *1* and *2* as possible. The recycle mode of the PrepLC/500 can be used to accomplish this once all of *7* is removed.

One other cyclic nitrosamine that we have found to be amenable to chromatographic separation into component configurational isomers is 3,5-dimethyl-N-nitrosopiperidine (*8*). This compound is prepared by the reduction of 3,5-lutidine (which has been distilled to remove other isomeric lutidines that may be present) and sub-

Eq 4



8 (*cis*) 8 (*trans*)

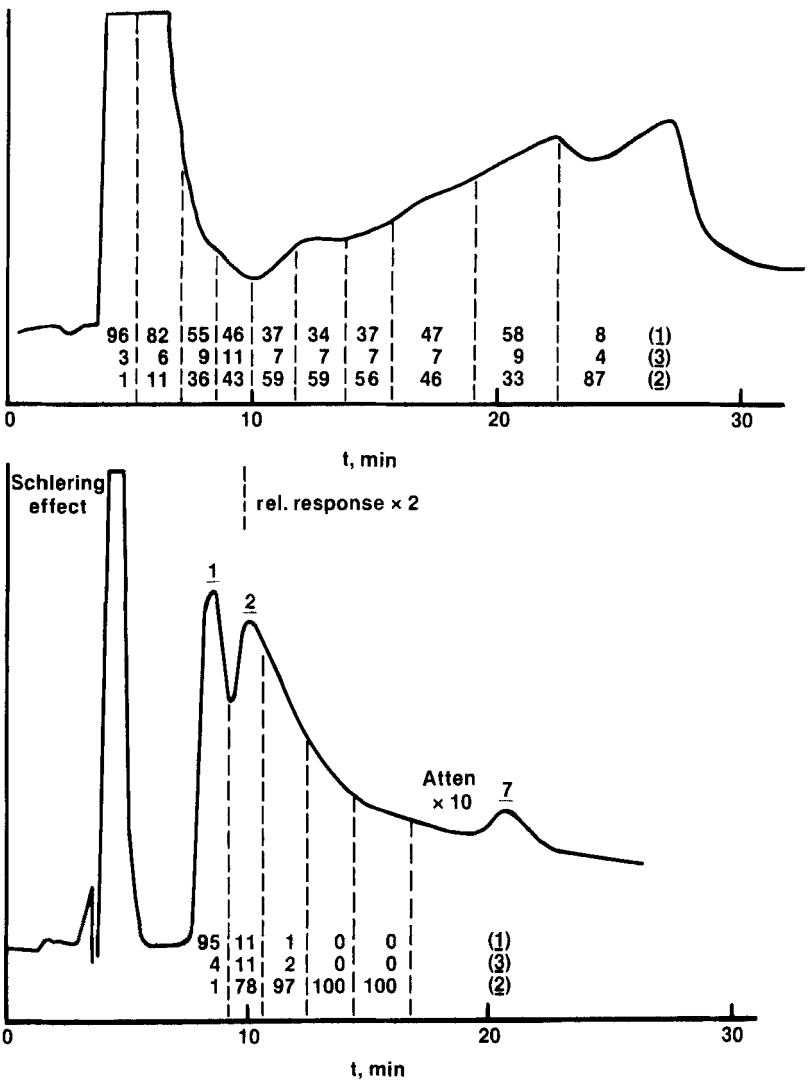


Figure 2.

PrepLC500 separation of configurational isomers of 2,6-dimethyl-N-nitrosomorpholine and removal of contaminants. TOP: 70% $CHCl_3/C_6H_{14}$, 250 ml/min - 18 g of nitrosamine mixture. BOTTOM: 20% CH_3CO_2Et/C_6H_{14}, 250 ml/min, reinjection of fraction rich in isomer 2 (trans). Dotted lines indicate where fractions were taken. Relative percentages of the isomers in each fraction are shown. Detector: refractive index, relative response x5.

sequent nitrosation of the resulting piperidine. The product (*8*) is about 80% cis and 20% trans, although this ratio fluctuates slightly with any given reduction.

The two component mixture of *8* presents a difficult separation problem. Excellent analytical separation is achieved on a μPorasil column using 10% ethyl acetate-hexane, 1.5 ml/min as eluent (see Figure 3), but on the PrepLC 500, *8*-cis tails badly and is difficult to separate completely from *8*-trans. In a typical run of a 9 ml injection in 15% ethyl acetate-hexane at 250 ml/min, all of the compound eluted in 16 minutes. The first fraction (250 ml) contained 4.5 g pure *8*-cis. Later fractions (500 ml) were recombined and reinjected with 10% ethyl acetate-hexane as eluent. The first fraction (8 min retention time, 250 ml) contained 1 g of pure *8*-cis. All fractions rich in *8*-trans were recombined and rechroma-

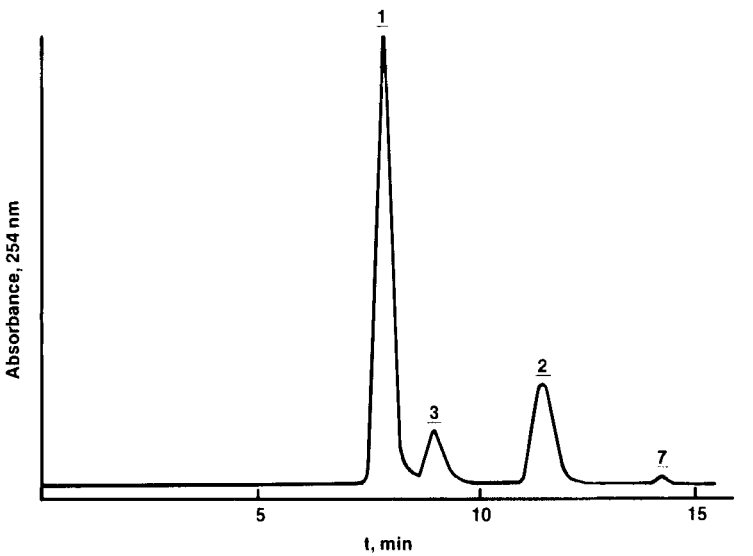


Figure 3.

Separation of 3,5-dimethyl-N-nitrosopiperidine into configurational isomers by analytical hplc (10% $CH_3COOC_2H_5/C_6H_{14}$, 2 ml/min, Waters μPorasil).

tographed using the recycle mode. On the first recycle a 0.4 g fraction that was 98% *δ*-trans was obtained. Lowering the percentage of ethyl acetate in the eluent to 2.5% or even 1% resulted in much longer retention times without any improvement in the separation, since the peaks became broader and *δ*-cis tailed badly into the *δ*-trans peak, making the isolation of *δ*-trans of greater than 90% purity impossible.

The use of the PrepLC/500 has enabled us to obtain quantities of configurational isomers of two nitrosamines sufficient for animal testing with relative ease, compared with open column chromatography. While the PrepLC/500 was operated at 250 ml/min the solvent consumption was economical compared with open column chromatography. In the latter case, the maximum load (of the morpholine derivative) was 8.8 g of mixture on 250 g of silica gel. Eight liters of solvent were necessary to complete elution, and the process required 2-3 working days. One run on the PrepLC/500 with a 15 g load takes 20 minutes of solvent consumption time and therefore uses 5-6 liters of solvent. For the piperidine derivative, the maximum load possible on an open column was 2 g per 250 g of silica gel, and the separation was very poor. Analysis and evaporation of the fractions collected from the PrepLC/500 is time consuming, but the same procedure is necessary for open column chromatography.

Gingell, Nagel, and Kupper (6) have reported on the differential metabolism of the isomers of 2,6-dimethyl-N-nitrosomorpholine. They were unable to separate the nitrosamine isomers on open column chromatography, but chose instead to separate the amines by fractional distillation with subsequent chromatographic clean-up of nitrosamine obtained from the trans isomer. They also observed a very minor impurity with glc retention time intermediate between the cis and trans isomers which had the same molecular ion and mass fragmentation pattern as the major components. We believe this corresponds to 7, the 3,5-dimethyl-N-nitrosomorpholine.

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REFERENCES

1. Druckrey, H., Preussmann, R., Ivankovic, S., and Schmähl, D. Organotrope carcinogene Wirkungen bei 65 verschiedenen N-nitrosoverbindungen an BD-Ratten, Z. Krebsforsch., 69, 103, 1967.

2. Iwaoka, W.T., Hansen, T., Hsieh, S.-T., and Archer, M.C., Chromatographic separation of conformers of substituted asymmetric nitrosamines, J. Chromatog., 103, 349, 1975.

3. Vöter, H., and Helmchen, G., Enantiomers as a result of restricted rotation about partial double bonds: resolution of N-nitroso-, (N-formyl)-, and N-thioformyl-4-piperidine carboxylic acids, Tetrahedron Lett., 1251, 1978.

4. Lijinsky, W., and Taylor, H.W., Increased carcinogenicity of 2,6-dimethylnitrosomorpholine compared with nitrosomorpholine in rats, Cancer Res., 35, 2123, 1975.

5. Singer, G.M. and Singer, S.S. In preparation.

6. Gingell, R., Nagel, D., and Kupper, R., Differential metabolism of geometrical isomers of N-nitroso-2,6-dimethylmorpholine in the hamster, Xenobiotica, 8, 439, 1978.