# SYNTHESIS OF DEUTERIUM-LABELED NITROSOMETHYLETHYLAMINES

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## SUMMARY

Ethyl isocyanate, N-methylacetamide, N-methylacetamide-$d_3$, and N-methylacetamide-$d_6$ were reduced with lithium aluminum deuteride and then nitrosated with sodium nitrite to yield, respectively, nitrosomethyl(-$d_3$)ethylamine, nitrosomethylethyl($\alpha$-$d_2$)amine, nitrosomethylethyl(-$d_5$)amine, and nitrosomethyl(-$d_3$)ethyl($d_5$)amine. The characterization of the compounds by mass spectrometry is described.

Key Words: Deuterium-labeled nitrosomethylethylamines

## INTRODUCTION

Evidence is accumulating that a rate-limiting step in the carcinogenic action of nitrosamines is the removal of a hydrogen atom from an $\alpha$-carbon. Both nitrosodimethylamine-$d_6$ and nitrosomorpholine-3,3,5,5-$d_4$ were significantly less carcinogenic than nitrosodimethylamine (1) and nitrosomorpholine (2). Also 2,5-dimethylnitrosopyrrolidine and 2,6-dimethylnitrosopiperidine, in which access to the $\alpha$-carbon atoms is hindered, are non-carcinogenic (3,4). 2,2,6,6-tetramethylnitrosopiperidine (4), which has no hydrogens on the $\alpha$-carbon atoms is also non-carcinogenic, while the unsubstituted nitrosamines are potent carcinogens. Similarly, nitrosodi-isopropylamine is very weakly carcinogenic compared with nitrosodiethylamine (5).

In the case of unsymmetrical aliphatic nitrosamines, there is some question as to which of the two $\alpha$-carbon atoms participates most in the carcinogenic process, an important factor in elucidating the mechanism of action of this class of carcinogens. To answer this question, nitrosomethylethylamine (1)

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labeled with deuterium in either the methyl (2) or ethyl group (3 or 7), and also in both alkyl groups (8), were prepared so that their relative carcinogenic activity in rats could be established by oral administration at equimolar doses.

## RESULTS AND DISCUSSION

Four nitrosomethylethylamines specifically labeled with deuterium were prepared in high isotopic purity by reduction of ethyl isocyanate or the appropriate N-methylacetamide with lithium aluminum deuteride. Hydrolysis of the product had to be carried out in non-protonated solvent ($P_2O_5$ in $D_2O$) to avoid introduction of unwanted hydrogen. All products were characterized by nmr spectrometry, UV spectrophotometry, and mass spectrometry. The chemical and isotopic purity of the products was deemed satisfactory for the biological testing of their relative carcinogenic effectiveness.

The mass spectra of nitrosomethylethylamine (1), and the four deuterium labeled analogues (2, 3, 7, 8) show patterns which confirm previous conclusions about the fragmentation of the undeuterated compound.

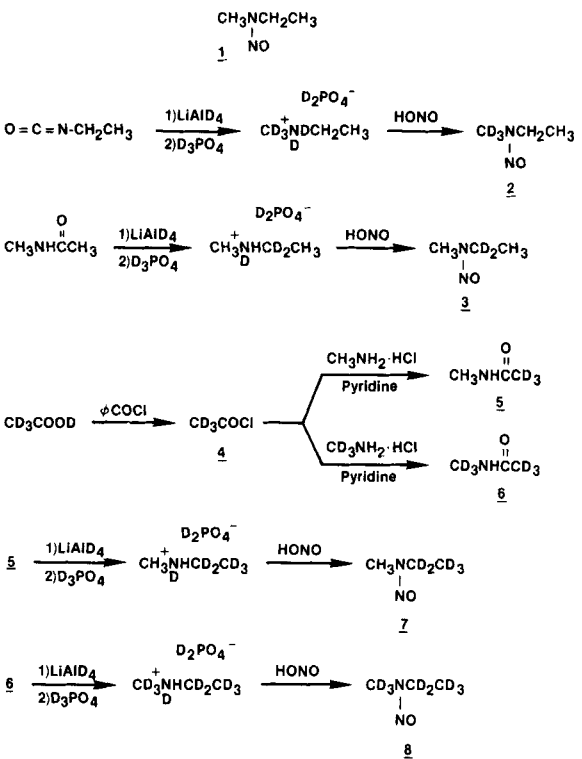
Each of the five compounds shows a substantial molecular ion $(M)^+$ and a $(M-30)^+$ ion, corresponding to loss of NO, which was always 2 to 3% of the molecular ion. All three compounds having an ethyl group with $CH_3$ (1, 2, and 3) show an $(M-15)^+$ ion of relative intensity 7 to 9%, corresponding to loss of that methyl. The two compounds having an ethyl group containing $CD_3$ (7 and 8) show a smaller tendency to lose $CD_3$.

An $(M-17)^+$ ion, corresponding to loss of OH, was seen only in the spectra of the three compounds having an ethyl group containing $CH_3$ (1, 2, and 3), suggesting that the hydrogen came from the β-carbon, through formation of a four-membered ring structure, rather than through migration of a hydrogen from an α-carbon. This was confirmed by the relatively large $(M-18)^+$ ion in the spectrum of 8, probably a composite of $(M-OD)^+$ and $(M-CD_3)^+$; a metastable ion corresponding to loss of 18 mass units in both 7 and 8 was analogous with the metastable corresponding to loss of 17 mass units from the molecular ion of the other three compounds. This confirms the loss of hydrogen from the

β-carbon together with the oxygen atom as OH.

There was a substantial $(M-31)^+$ ion from the undeuterated compound, indicating a loss of NOH. All of the nitrosomethylethylamines except 8 showed this ion. It was, however, largest in the spectrum of the undeuterated compound suggesting that the hydrogen could come from either α-carbon. Similarly, the $(M-32)^+$ ion probably arises through loss of $NOH_2$, but the $(M-33)^+$ and $(M-34)^+$ ions cannot yet be explained.

The ratio of the intensities of the molecular ions of each of the deuterated compounds to those of the less-deuterated species present was highest for 3 at 100:3. The ratio was 100:9 for 2, 100:9 for 7 and 100:9 for 8. This degree of purity was considered adequate for the biological studies, since in fewer than 1 molecule in 10 was there a hydrogen instead of deuterium in the labeled position.



## EXPERIMENTAL

### Nitrosomethyl(-$\underline{d}_3$)ethylamine ($\underline{2}$)

A solution of ethyl isocyanate (Aldrich; 3.55g, 0.05 mol) in anhydrous ether (45 ml) was added to lithium aluminum deuteride (Stohler Isotope Chemicals; 4.65g, 0.11 mol) stirred under a nitrogen atmosphere in anhydrous ether (200 ml). The reaction mixture was boiled under reflux for 9 hrs., cooled to room temperature, and hydrolyzed with a phosphoric acid-$\underline{d}_3$ solution. (This solution was prepared by adding $P_2O_5$ (14.2g, 0.1 mol) to $D_2O$ (30g, 1.5 mol) under a nitrogen atmosphere, and then allowing it to stand for 1 hr. before use). When the hydrolysis was complete, the solvent was evaporated and the aqueous layer concentrated *in vacuo* to a volume of approximately 30 ml. Glacial acetic acid (15 ml) and concentrated hydrochloric acid (9 ml) were added with stirring to the cooled sample, followed by a suspension of sodium nitrite (34.5g, 0.5 mol) in water (40 ml). The reaction mixture was stirred overnight, and then diluted with water (100 ml), and saturated with sodium chloride. The mixture was extracted with methylene chloride (150 ml) three times; the organic layers were combined and washed with 5% sodium bicarbonate until the aqueous phase remained basic. The solution in methylene chloride was dried ($MgSO_4$) and then evaporated under a stream of nitrogen to give a yellow oil (2.4g, 52% based on Et-NCO) which showed $\lambda_{max}^{95\%\ EtOH}$ 346 ($\varepsilon$ 84); nmr ($CDCl_3$): 0.96-1.50 ppm (m, 3H), and 3.38-4.36 ppm (m, 2H); mass spectum: (See Table I).

### Nitrosomethylethyl($\alpha$-$\underline{d}_2$)amine ($\underline{3}$)

A solution of N-methylacetamide (5.1g, 0.07 mol) in anhydrous ether (50 ml) was added to a solution of lithium aluminum deuteride (3.7g, 0.09 mol) in anhydrous ether (250 ml) which was stirred under a nitrogen atmosphere. The reaction mixture was boiled under reflux overnight (21 hrs), and then cooled to room temperature, and hydrolyzed with phosphoric acid-$\underline{d}_3$. The concentrated aqueous layer was nitrosated as described for $\underline{2}$ using glacial acetic

acid (21 ml), concentrated hydrochloric acid (12.8 ml), and sodium nitrite (48g, 0.7 mol). The product was a yellow oil (2.9g, 45% based on N-methylacetamide). The UV spectrum showed $\lambda_{max}^{95\% \text{ EtOH}}$ 346 ($\varepsilon$ 67); nmr ($CDCl_3$): 1.10 ppm and 1.38 ppm (anti- and syn- $\beta$-$CH_3$ respectively, in ratio 1:3.76), and 3.04 ppm and 3.73 ppm (syn- and anti- $CH_3$ respectively, in ratio 3.55:1); mass spectrum: (See Table I).

Acetyl Chloride-$d_3$ (4)

Acetyl Chloride-$d_3$ was prepared following the method of Brown (6). Acetic acid-$d_4$ (Merck, Sharpe, and Dohme; 15.7g, 0.25 mol) was added slowly to benzoyl chloride (56g, 0.4 mol). The mixture was heated and acetyl chloride-$d_3$ was distilled through a Vigreaux column. The fraction collected at bp. 40-47° (14.7g, 73%) was a colorless, fuming liquid (lit. bp. 48-51° (6)).

N-Methylacetamide-$d_3$ (5)

Methylamine hydrochloride (13.5g, 0.2 mol) was added to a solution of pyridine (32g, 0.4 mol) in dry benzene (150 ml). The mixture was stirred for 1.5 hrs., then acetyl chloride-$d_3$ (4, 13g, 0.16 mol) was added dropwise with stirring and cooling. After 4 hrs., the reaction mixture was allowed to warm to room temperature and the solvent was removed by rotary evaporation (40°, 50-150 mm) leaving a thick paste, which was exhaustively extracted with ether (Soxhlet). Evaporation of the solvent gave a crude oil from which N-methylacetamide-$d_3$ was distilled as a colorless oil (bp. 103-112°/25mm) which solidified on cooling. The IR spectrum was consistent with that of a deuterium-containing N-methylacetamide.

Nitrosomethylethyl(-$d_5$)amine (7)

A solution of 5 (6.1g, 0.08 mol) in anhydrous ether (50 ml) was added to a solution of lithium aluminum deuteride (5g, 0.12 mol) in anhydrous ether (275 ml) which was stirred under a nitrogen atmosphere. The reaction was boiled under reflux for 20 hrs., hydrolyzed with phosphoric acid-$d_3$ and

nitrosated as described for 3. The product was a yellow oil (3.3g, 46% based on 5) with $\lambda_{max}^{95\% \text{ EtOH}}$ 346 (ε 84); mass spectrum: (See Table I).

N-Methylacetamide-$d_6$ (6)

Methylamine-$d_3$ hydrochloride (Stohler Isotope Chemicals, 7.75g, 0.11 mol) was added to a solution of pyridine (20g, 0.25 mol) in dry benzene (125 ml). The mixture was stirred for 1.5 hrs, then acetyl chloride-$d_3$ (4, 10g, 0.12 mol) was added dropwise with stirring and cooling. The reaction mixture was worked-up and the product isolated as described for 5. The crude oil was distilled to give N-methylacetamide-$d_6$ a colorless oil (bp. 100-112°/22 mm) which solidified on cooling. The IR spectrum was that expected for this N-methylacetamide.

Nitrosomethyl(-$d_3$)ethyl(-$d_5$)amine (8)

A solution of 6 (5.5g, 0.07 mol) was reduced and nitrosated as described above to give a yellow oil (3.5g, 52% based on 6) which showed $\lambda_{max}^{95\% \text{ EtOH}}$ 346 (ε 69); mass spectrum: (See Table I).

TABLE I. MASS SPECTRA OF N-NITROSOMETHYLETHYLAMINES

| | m/e (Relative Intensity) | | | |
|---|---|---|---|---|
| *$CH_3CH_2\underset{\underset{NO}{|}}{N}CH_3$(1) | $CH_3CH_2\underset{\underset{NO}{|}}{N}CD_3$(2) | $CH_3CD_2\underset{\underset{NO}{|}}{N}CH_3$(3) | $CD_3CD_2\underset{\underset{NO}{|}}{N}CH_3$(7) | $CD_3CD_2\underset{\underset{NO}{|}}{N}CD_3$(8) |
| 88 (100) | 91 (100) | 90 (100) | 93 (100) | 96 (99) |
| 73 (8) | 90 (4) | 89 (2) | 92 (6) | 95 (8) |
| 71 (9) | 89 (2) | 88 (1) | 91 (1) | 94 (1) |
| 57 (14) | 88 (3) | 75 (9) | 90 (1) | 78 (12) |
| 56 (20) | 76 (7) | 73 (5) | 88 (1) | 64 (7) |
| 44 (5) | 74 (8) | 72 (4) | 75 (1) | 63 (16) |
| 43 (8) | 60 (10) | 69 (5) | 62 (6) | 62 (13) |
| 42 (97) | 59 (16) | 59 (8) | 61 (7) | 48 (69) |
| 41 (6) | 46 (64) | 58 (10) | 46 (11) | 46 (100) |
| | 45 (78) | 46 (54) | 45 (62) | 45 (22) |
| | 44 (15) | 45 (79) | 44 (29) | 43 (24) |
| | 43 (21) | 44 (30) | 43 (60) | 34 (76) |
| | 42 (19) | 43 (79) | | |

*Synthesized by same method as (2), but using lithium aluminum hydride.

All nmr spectra were obtained with a Varian XL-100 with Nicolet TT-100 FT System. Mass spectra were obtained at 70 ev. on a single-focusing mass spectrometer (30.5-cm. radius, 90° magnetic sector) constructed in the Analytical Chemistry Division of the Oak Ridge National Laboratory.

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REFERENCES

1. Keefer L., Lijinsky W., and Garcia H., -J. Natl. Cancer Inst. 51:299, (1973).
2. Lijinsky W., Taylor H.W., and Keefer L. - J. Natl. Cancer Inst., 57: 1311, (1976).
3. Lijinsky W., and Taylor H.W. - Cancer Res., 36:1988, (1976).
4. Lijinsky W., and Taylor H.W. - Int. J. Cancer, 16:318, (1975).
5. Druckrey H., Preussmann R., Ivanokovic S., and Schmähl D. - Z. Krebsforsch. 69:103 (1967).
6. Brown H.C. - J. Am. Chem. Soc., 60:1325, (1938).