SYNTHESIS OF CARBON-14 AND DEUTERIUM LABELED N-NITROSO-2(3',7'-DIMETHYL-2',6'-OCTADIENYL)AMINOETHANOLS

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SUMMARY

Methods of preparation of carbon-14 and deuterium labeled N-nitroso-2(3',7'-dimethyl-2',6'-octadienyl)aminoethanols are described. The primary synthetic method involved alkylation of ethanolamine or ethylglycine with suitable chlorides and subsequent mild nitrosation. Isomeric ^{14}C-nitrosamines were also prepared by selective α-cleavage of the di-substituted ethanolamine with nitrous acid.

Key words: Cis- and trans-N-nitroso-2(3',7'-dimethyl-2',6'-octadienyl)aminoethanols, Carbon-14, Deuterium, Geraniol-1,1-$^2H_2$, and Geranyl chloride-1,1-$^2H_2$.

INTRODUCTION

The increasing concern with the occurrence of nitrosamines in the environment and the potential hazard they pose for humans have prompted us to investigate the possibility of their formation from certain nitrogenous fishery chemicals. 2-Bis (3',7'-dimethyl-2',6'-octadienyl)aminoethanol (GD-174), one of the known anesthetic drugs structurally related to terpenes, is at present an experimental fish toxicant. It has been shown at the National Fishery Research Laboratory to possess selectivity toward carp and is therefore a candidate control agent for this species. The commercial production of this compound (as a mixture of cis and trans isomers) often results in the formation of small amounts of secondary amine by-products. It is known that, in addition to secondary amines, tertiary amines and quaternary ammonium salts (1, 2, 3) can also serve as precursors of nitrosamines in the presence of suitable nitrosating agents. We have in fact detected mutagenic cis- and trans-N-nitroso-2(3',7'-dimethyl-2',6'-octadienyl)amino-

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0362-4803/81/081215-11$01.10

Received July 14, 1980
Revised August 15, 1980

ethanols in samples of commercial formulations of GD-174 containing a mixture of the secondary and tertiary aminoethanols or containing only the pure tertiary amine, following the nitrosation of each sample at ambient temperature (4, 5). In connection with our studies on the determination of trace amounts of these nitrosamines and their metabolism in model animals, we needed both radioactive and stable isotope labeled nitroso-compounds. With the latter compounds in hand, our goal was to attain the required precision in the analytical methodology and to gain further insight into the metabolic fate of the hitherto unknown unsaturated nitrosamines. This paper presents details of the synthesis of the labeled compounds required in these studies.

RESULTS AND DISCUSSION

Two basically different methods were employed for the synthesis of respective trans-N-nitroso-2(3',7'-dimethyl-2',6'-octadienyl)aminoethanol-1,2-$^{14}C_2$, 2a, and cis- and trans-N-nitroso-2(3',7'-dimethyl-2',6'-octadienyl)aminoethanols-1,2-$^{14}C_2$, 2b. In method A (Scheme 1), the approach was to utilize the commercially available, uniformly radiolabeled ethanolamine. Reaction of this radioactive starting material with equivalent amounts of both trans-3,7-dimethyl-2,6-octadienyl chloride (geranyl chloride) and triethylamine -- an efficient hydrogen chloride acceptor -- afforded geranylethanolamine-1,2-$^{14}C_2$, 1, in moderate yield. There was also obtained, as the sole side product, the di-substituted ethanolamine 3a whose yields could be minimized to about 10% by careful control of reaction variables. The concomitant formation of this compound during the alkylation did not pose problems, because it could easily be separated from reaction products by thin layer chromatography and reused in reactions under method B. The mono-substituted ethanolamine 1 was isolated and found to be free of other ethanolamines by thin layer chromatography. It was then treated with sodium nitrite in aqueous acetic acid to afford the isomerically pure trans-nitrosamine 2a. After purification on a silica gel plate, aliquot samples were injected simultaneously into two independent gas chromatographic columns packed individually with Carbowax 20M and a methyl silicon stationary phase. Under normal preparative conditions, the compound obtained in this manner was of sufficiently high purity as evidenced by

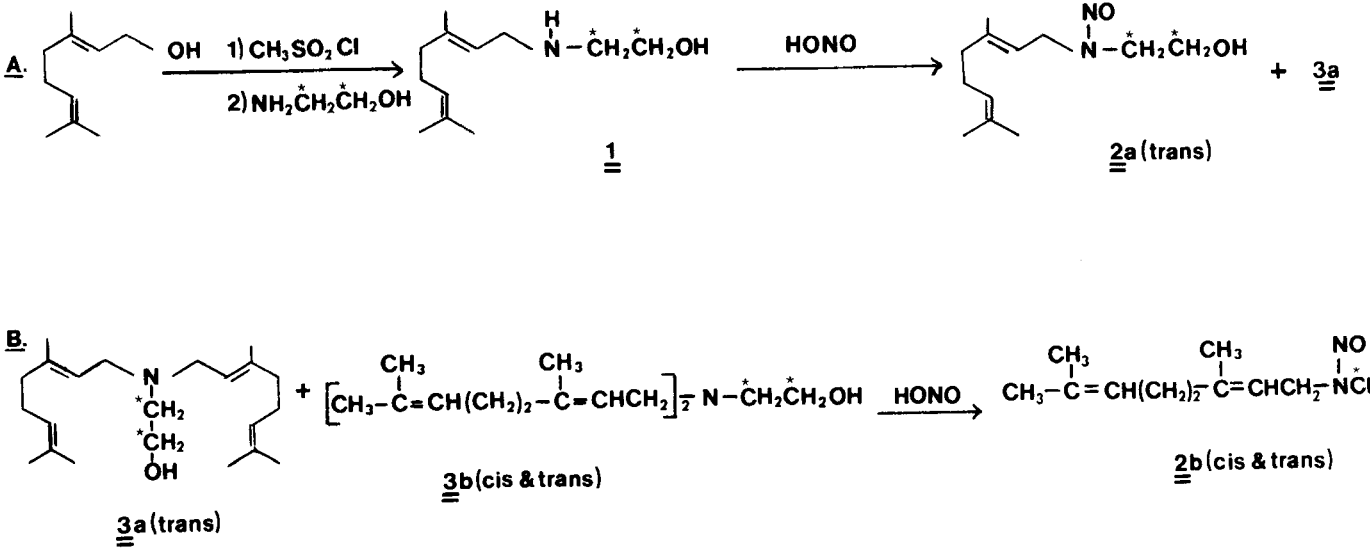
the appearance of a single peak on each of the chromatograms. The structural identity of the compound was established by gas chromatography-mass spectrometry and co-chromatography with an authentic sample. The use of a pure radioactive geometrical isomer is of particular importance in studying the stereospecific metabolic pathways by which each isomer proceeds to interact with biological substrates.

In method B, compound 3a and the cis and trans mixture 3b (which was made available through custom synthesis) were combined and nitrosated directly under optimized conditions to facilitate selective α-cleavage at the C_1'-N bond of the terpene group to form a mixture of ^{14}C-labeled nitrosamine 2b. Yields ranged from satisfactory to excellent. Attempts to purify the crude product on a small silica gel column were unsuccessful. However, an elaborate technique using a consecutive dry column and thin layer chromatographic system allowed effective separation of the desired product from other by-products. The overall recovery was quantitative. The radiochemical purity of the final isolated material exceeded 98%. Comparison of the mass spectra of 2a and 2b revealed identical spectral characteristics. Gas chromatography of the latter compound showed, beside a peak superimposable with that of 2a, an additional peak ascribable to the cis-component which was found to co-chromatograph perfectly with a sample of an authentic cis-compound.

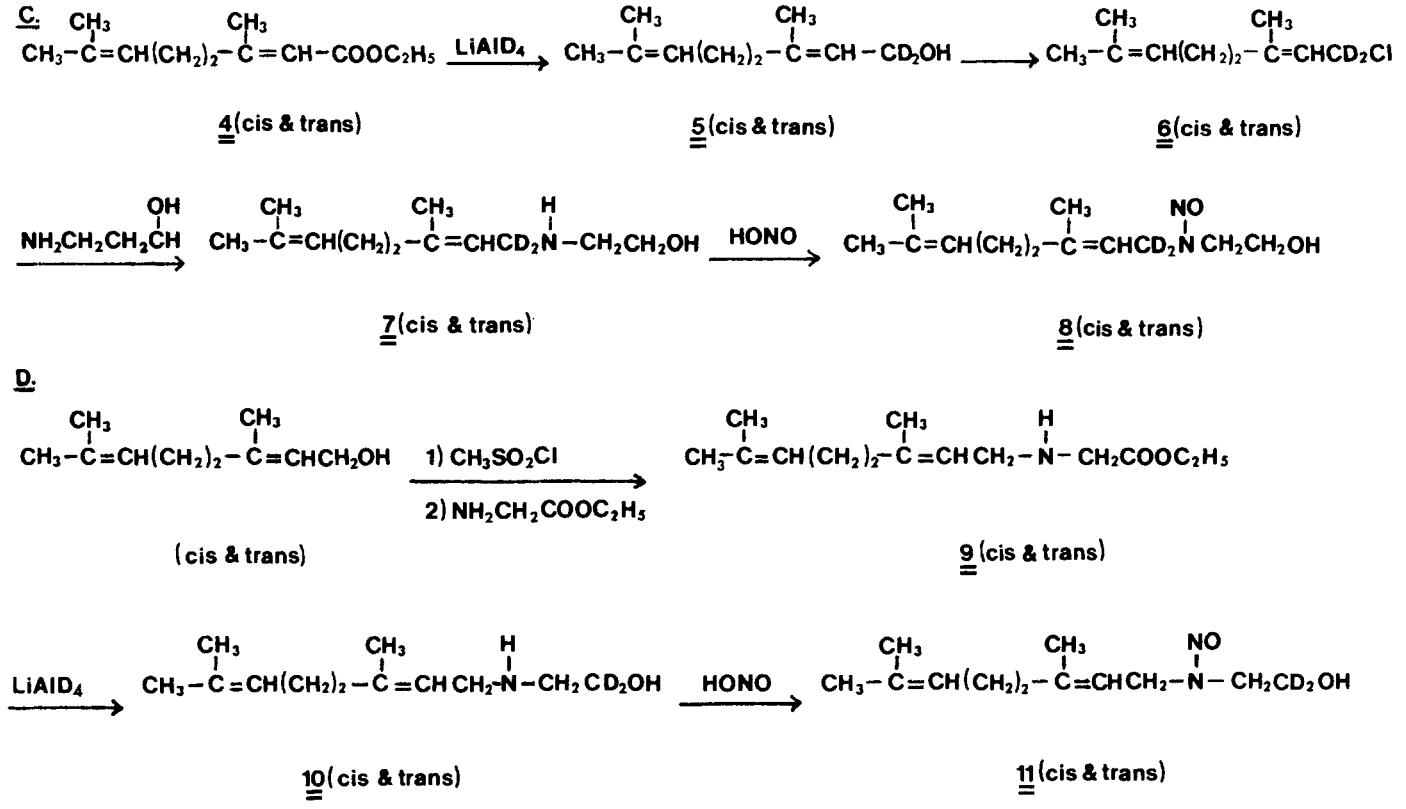
The synthesis of deuterium labeled nitrosamines 8 and 11 involved rather simple and straightforward reactions as illustrated in Scheme 2. In both preparations, lithium aluminum deuteride was used as the deuterium source. The deuteriochloride 6, generated by the reduction of the acid 4 with lithium aluminum deuteride followed by mild chlorination of the resulting deuterio-alcohol 5, was coupled to ethanolamine consuming one equivalent of the excess amine for the removal of evolving hydrogen chloride and afforded 7. Subsequent treatment of 7 with nitrite at room temperature led to a product which was identified as 8. It was necessary to use column chromatography for the isolation of pure 8. Sequential elution of the crude mixture with suitable solvents through a silica gel column afforded fractions that were monitored gas chromatographically on two parallel columns. The compound acquired by this procedure had the required purity. The extent of deuterium incorporation in 8 was greater than 96% based on its spectral

data. The shift by two mass numbers from mass fragments m/e 137 (M_H-89) to m/e 139 (M_D-89) as displayed in the respective mass spectra of nondeuterated and deuterated compounds is indicative of the complete deuteration of the labeled material. The fact that the mass fragment at m/e 138 was absent precludes the possibility of partial deuteration or isotope exchange throughout the sequence of reactions. We anticipated the logical pathway to the preparation of 11 to start with ethanolamine-1,1-$^2H_2$ followed by the same scheme of conversions leading to 8. We encountered difficulty in preparing ethanolamines-1,1-$^2H_2$ in sufficient quantity by reducing ethyl glycine with lithium aluminum deuteride or lithium triethylborodeuteride. Apparently the problem lies in the comparatively low partition coefficient of ethanolamine in an organic solvent in relation to water. Continuous liquid-liquid extraction of the heterogeneous hydride reaction mixture with ether improved the yields to some extent but was inconvenient and tedious. We, therefore, diverted our attention to a more expedient route to compound 11 as outlined in method D. Initially, ethyl glycine was reacted with the isomeric C_{10}-terpene chlorides to give 9. Reduction of this terpene substituted ethyl glycine with lithium aluminum deuteride afforded the intermediate 10. The usual nitrosation of the latter proceeded smoothly to give cis- and trans-N-nitroso-2(3',7'-dimethyl-2',6'-octadienyl)aminoethanols-1,1-$^2H_2$, 11. In general, the yields of the nitrosamines of interest were observed to decrease substantially in cases where a large excess of nitrite was employed and the nitrosation reaction was continued for a prolonged period of time. A considerable amount of intractable polymeric matter was consequently produced. While the mechanism of polymerization is unclear, the heavy dependence of nitrosamine formation on the nitrite concentration would suggest the likely involvement of the double bonds of the terpene moiety in the reaction with the excess nitrosating agent. The results of NMR analysis illustrate the exceptional facility with which the structures of isomers can be differentiated by ^{13}C NMR (6). In contrast, the ^1H NMR spectra of the cis and trans isomers of the nitrosamine under study were relatively uninformative, although they served to verify successfully the labeling of deuteriocompounds.

SCHEME 1


A.
OH
1) CH3SO2Cl
2) NH2*CH2*CH2OH
H
N–*CH2*CH2OH
1
HONO
NO
N–*CH2*CH2OH
2a (trans)
+ 3a
B.
N
*CH2
*CH2
OH
3a (trans)
+
CH3
CH3
CH3–C=CH(CH2)2–C=CHCH2
2
N–*CH2*CH2OH
3b (cis & trans)
HONO
CH3
CH3
NO
CH3–C=CH(CH2)2–C=CHCH2–N*CH2*CH2OH
2b (cis & trans)

SCHEME 2


C.
LiAlD4
4(cis & trans)
5(cis & trans)
6(cis & trans)
NH2CH2CH2OH
HONO
7(cis & trans)
8(cis & trans)
D.
1) CH3SO2Cl
2) NH2CH2COOC2H5
(cis & trans)
9(cis & trans)
LiAlD4
HONO
10(cis & trans)
11(cis & trans)

In summary, the use of these new labeled compounds including labeled intermediates prepared by the convenient methods presented here greatly facilitates trace analyses and metabolism experiments on the title nitrosamine -- a potent environmental mutagen -- and certain anesthetic drugs.

EXPERIMENTAL

Perkin-Elmer 337 and Beckman DB-GT instruments were used to obtain IR and UV spectra, respectively. FT ^{1}H (^{13}C) NMR spectra were determined on JEOL FX-90 (22.5) spectrometer. Chemical shifts are given as δ values with reference to tetramethylsilane in deuterated chloroform. Analytical gas chromatography was carried out using Varian 3700 and 2100 gas chromatographs. Mass spectral measurements were made on a Varian MAT 112 magnetic-sector, double focusing mass spectrometer equipped with a dual electron impact/chemical ionization source. The mass spectrometer was coupled to a Varian 1440 gas chromatograph through a Varian MAT dual capillary-glass jet interface unit. All compounds reported here were homogeneous by thin layer chromatography analysis [Analtech Uniplate (0.025 X 5 X 20 cm) silica gel GHLF]. Beckman LS-8000 liquid scintillation counter was used for measuring radioactivity. 2-Bis (3',7'-dimethyl-2',6'-octadienyl)aminoethanol-1,2-$^{14}C_2$ was purchased from New England Nuclear (Boston, Massachusetts).

3,7-Dimethyl-2,6-octadienyl chlorides -- The pure trans compound (geranyl chloride) and the mixture of cis and trans isomers were prepared from the appropriate alcohols and methanesulfonyl chloride as described by Bunton and co-workers (7). Geranyl chloride: 75% yield, b.p. 59-60°C (0.03 mm Hg) [lit. (7), b.p. 64-65°C (0.04 mm Hg)]. Cis and trans mixture: 79% yield, b.p. 60-70°C (0.1 mm Hg). Gas chromatography showed two peaks in a ratio of 3:1. The major peak corresponded to the trans isomer.

Trans-2(3',7'-dimethyl-2',6'-octadienyl)aminoethanol-1,2-$^{14}C_2$, (1) -- In a 5 ml Reacti-vial fitted with a screw cap Teflon faced Mininert valve were placed ethanolamine-1,2-$^{14}C_2$ hydrochloride (2.7 mCi, 0.01 mmol) and triethylamine (16.2 mg, 0.16 mmol). The vial was heated to 50°C in a metal heat block. The warm solution was stirred while a solution of freshly distilled geranyl chloride (13.8 mg, 0.08 mmol) in 1 ml tetrahydrofuran was added dropwise by means of a

syringe. The Mininert valve was closed immediately after the complete addition of the chloride. The reaction mixture was stirred continuously for 12 hours at 65°C. The mixture was allowed to cool to room temperature and then evaporated under reduced pressure to leave a residue. This material was purified by preparative thin layer chromatography (1.0 mm thickness precoated silica gel plates developed with hexane–ether–methanol–methylene chloride, 2:2:1:2). There was obtained 12 mg (2.2 mCi, 80% yield) of 1. R_f = 0.34 (benzene–ether–methanol–methylene chloride, 2:2:1:2), radiochemical purity, 99.5% by TLC; GLC, 6 min (5% Carbowax 20 M, 150°C).

A small amount of a less polar minor product was also isolated and characterized to be 3a.

N-Nitroso-2(3',7'-dimethyl-2',6'-octadienyl)aminoethanol-1,2-$^{14}C_2$, (2a, 2b) -- Method A (2a): Geranylethanolamine, 1, (1.8 mCi, 10 mg, 0.05 mmol) in 5 ml glacial acetic acid was treated with sodium nitrite (10 mg, 0.15 mmol) in 0.5 ml water. The solution was stirred overnight at room temperature. Water–ether (1:1, 10 ml) was then added. After shaking the mixture thoroughly, the layers were separated. The aqueous phase was extracted with two additional 5 ml portions of ether. The combined ether extracts were washed successively with 5 ml water, 10 ml 10% potassium carbonate solution, 5 ml sodium chloride solution, dried over anhydrous sodium sulfate and finally evaporated. The residue after preparative thin layer chromatography (silica gel, 0.5 mm, benzene–acetone, 1:1) yielded 8.6 mg (1.4 mCi, 75%) of trans-N-nitroso-2(3',7'-dimethyl-2',6'-octadienyl)aminoethanol-1,2-$^{14}C_2$, 2a. R_f = 0.73 (methylene chloride–methanol, 2:1). GLC, 21 min (5% Carbowax 20 M, 220°C). GC-CI-MS m/e (I%): 227 (M^+ + 1, 52), 196 (25), and 137 (100).

Method B (2b): A mixture of cis- and trans-2-bis(3',7'-dimethyl-2',6'-octadienyl) aminoethanols, 3b, (2 mCi, 72 mg, 0.21 mmol) and the trans isomer 3a (0.5 mCi, 4.6 mg, 0.014 mmol) were placed in a 50 ml round bottom flask attached to a reflux condenser. To this a solution of 10 ml 80% acetic acid in water (buffered at pH 4.5 with sodium acetate) was added followed by 1 ml solution of sodium nitrite (145 mg, 2.1 mmol) in water. The mixture was stirred magnetically,

heated to 90°C and maintained at this temperature for 2 hours. The reaction was then allowed to continue for an additional 2 hours at room temperature. After workup according to the preceding procedure, the mixture gave 46 mg of the crude product. This material was chromatographed on a silica gel dry column (8) (ICN Pharmaceutical Inc., Cleveland, Ohio) using benzene–ethyl acetate–methanol (4:1:0.5) as developing solvent. The radioactive fractions were collected, combined, and further purified by thin layer chromatography (silica gel, 1 mm, benzene–chloroform–methanol–acetic acid, 8:5:1:1) to give 39 mg (1.7 mCi, 68%) of 2b. R_f = 0.43 and 0.46 (hexane–ether–methanol–methylene chloride) or R_f = 0.72 and 0.76 (methylene chloride–methanol, 2:1), radiochemical purity, 99.7% by TLC; GLC, 19 and 21 min (5% Carbowax 20M, 220°C). GC-CI-MS m/e (1% cis, trans) 227 (M^+ + 1, 28, 55) 196 (14, 25), and 137 (100, 100).

N-Nitroso-2(3',7'-dimethyl-2',6'-octadienyl)aminoethanol-1',1'-$^2H_2$, (8) -- A solution of 3,7-dimethyl-2,6-octadienoic acid ethyl ester (a gift from Givaudan, Clifton, New Jersey; 25 g, 0.13 mol) in 100 ml anhydrous ether was added slowly through a dropping funnel to a stirred suspension of lithium aluminum deuteride (2.5 g, 0.11 mol) in 100 ml anhydrous ether. After stirring at room temperature overnight, the mixture was worked up in the usual way and distilled to give 19 g (92%) of colorless oil which was identified as 5 [b.p. 115-118°C (12 mm Hg), IR, 3400 cm^{-1}(OH)]. This compound was used to prepare the nitrosamine 8 via the deuterated chloride 6 [b.p. 65-72°C (0.2 mm Hg)] and the substituted ethanolamine 7 [b.p. 105-120°C (0.1 mm Hg), IR, 3400 cm^{-1}(OH), 3200 cm^{-1}(NH)] following the methods as described earlier under the preparation of 2a. Thus reaction of 7 (16 g, 0.081 mol) with sodium nitrite (13.8 g, 0.2 mol) in 500 ml 90% aqueous acetic acid yielded crude material of 8 (16.6 g, 91%) which was passed onto a silica gel column and eluted in sequence with 200 ml each of hexane, hexane–ether (4:1, 2:1, 1:1, 1:2, 1:4), ether, ether–methylene chloride (4:1, 2:1, 1:1, 1:2, 1:4) and methylene chloride. Fifteen ml fractions were collected and each fraction was analyzed for the compound 8 by gas chromatography on 5% Carbowax 20 M and 3% OV-17 columns. The desired nitrosamine was normally found in the hexane–ether eluates (13 g, 73%). Attempts to distill 8 under high vacuum resulted in

its decomposition. However, the O-methyl derivative was distillable, b.p. 125-126°C (0.6 mm Hg); [unlabeled O-methyl compound, b.p. 124-125°C (0.6 mm Hg). Anal. Calcd. for $C_{13}H_{24}N_2O_2$: C, 65.00; H, 10.00; N, 11.67. Found: C, 65.11; H, 10.18; N, 11.50]. UV, 232 nm (ε1.0 X 10^4). IR, 3400 cm^{-1}(OH), 1474, 1439, 1285, and 1037 cm^{-1}(N-N=O) (9), and 1675 cm^{-1}(C=C). ^1H NMR δ 1.68 [s, 9H, =C(CH_3)], 2.03-2.12 [m, 4H, =C(CH_2)], 3.68 (s, 1H, OH), 3.94 (t, 2H, -CH_2OH), 4.12-4.32 [m, 2H, N(NO)-CH_2-], and 4.81 [d, 2H, N(NO)-CH_2C=], absent in the deuterio-compound 8. ^{13}C NMR, superimposable with that of 11. GC-CI-MS m/e (1%, cis, trans), 229 (M^+ + 1, 35, 60), 198 (20, 35), and 139 (100, 100).

N-Nitroso-2(3',7'-dimethyl-2',6'-octadienyl)aminoethanol-1,1-$^2H_2$, (11) -- 2(3',7'-dimethyl-2',6'-octadienyl)aminoacetic acid ethyl ester, 9, [(23.9 g, 0.1 mol), b.p. 150-160°C (0.05 mm Hg). IR, 3200 cm^{-1}(NH), 1765 cm^{-1}(C=O)] prepared by similar alkylation of ethylglycine with the corresponding cis and trans chlorides, was dissolved in 100 ml anhydrous ether and reacted at room temperature with lithium aluminum deuteride (4.2 g, 0.1 mol) in 100 ml anhydrous ether to yield 18.9 g (95%) of 10. Without further purification, 10 (15 g, 0.075 mol) was nitrosated as usual with sodium nitrite (13 g, 0.18 mol) in 500 ml 90% aqueous acetic acid to yield 15.1 g (88%) of the crude nitrosamine. After purification as before by column chromatography, the pure deuterio-nitrosamine 11 was obtained as a light yellow liquid (12 g, 69%) [its O-methyl derivative: b.p. 123-124°C (0.5 mm Hg)]. A sample of this material showed identical chromatographic characteristics as the unlabeled materials. ^1H NMR was strikingly similar to that of 8 except that the resonance around δ 3.94 was not present. UV, 233 nm (ε1.1 X 10^4). IR, 3400 cm^{-1}(OH) 1475, 1440, 1283, and 1035 cm^{-1}(N-N=O), and 1674 cm^{-1}(C=C). ^{13}C NMR δ 16.47(3'-CH_3, trans), 22.86 (3'-CH_3, cis), 51.67 (1'-CH_2-), 46.25 (2-CH_2-), 58.88 (1-CH_2-), 39.69 (4'-CH_2-), 117.2 (2'-C=), 123.4 (6'-C=), 131.5 (3'-C=), 141.2 (7'-C=), 26.51 (5'-CH_2-), and 50.87 (2'-CH_2-). GC-CI-MS m/e (1%, cis, trans), 229 (M^+ + 1, 30, 55), 198 (17, 30), and 137 (100, 100).

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