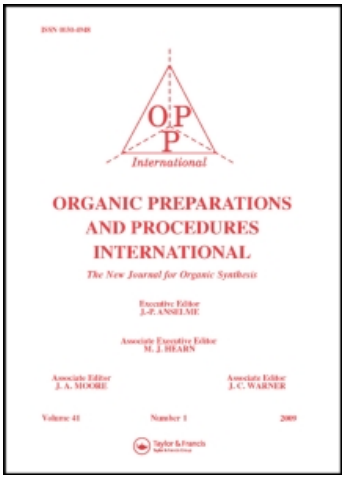

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SYNTHESES OF CARCINOGENIC β-KETONITROSAMINES FOUND IN MOLDY MILLET, WHEAT FLOUR AND CORNBREAD†

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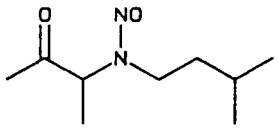
It has been reported that Linxian, a county in northwest Henan province, People's Republic of China has the world's highest recorded incidence of esophageal cancer.[1] It has also been found that the food contains high levels of N-nitrosodimethylamine, N-nitrosodiethylamine and N-nitrosopyrrolidine as well as elevated amounts of secondary amine precursors together with nitrite.[2] In addition to these components, two



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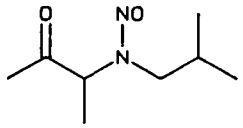

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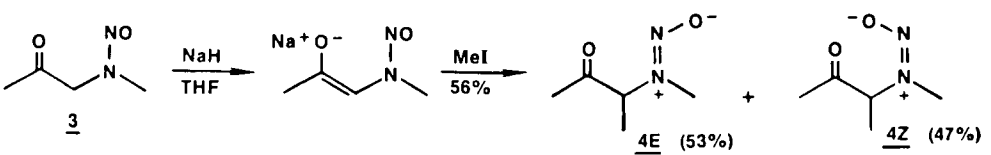
nitrosamines of unusual structure bearing a β-keto group (acetonyl) have been isolated from moldy foodstuffs of Linxian. N-(1-methylacetonyl)-N-(3-methylbutyl)nitrosamine (NMAMBA, 1) has been found in moldy cornbread, millet and wheat flour,[3] while N-(1-methylacetonyl)-N-(2-methylpropyl)-nitrosamine (NMAMPA, 2) was isolated only from moldy millet and wheat flour.[4] It was suggested by Ji et al.[4] that Fusarium moniliforme, a species of fungus which commonly contaminates foods in that area, plays a key role in the formation of these unusual nitrosamines. The biosyntheses of these compounds can result from the occurrence of isoamylamine or of isobutylamine and 3-hydroxy-2-butanone, a constituent of corn flavor. Condensation of the above materials, followed by enzymatic reduction

to the alkanolamine, oxidation and finally nitrosation would then produce NMAMBA (1) and NMAMPA (2) respectively. Singer et al.[5] have demonstrated that oxidative nitrosation in dilute sulfuric acid, leads directly to small, but significant amounts of the β-ketonitrosamine from the alkanolamine. Compound 1 induced forestomach carcinomas as well as liver tumors in mice and rats upon gastric intubation.[6] Though not yet tested in animals, the analogous compound 2 is expected to produce a similar spectrum of tumors. Jiang et al.[7] reported a two-step synthesis of 1 and Ji et al.[4] prepared 2 by a similar method. Very little is known about the chemistry and metabolism of these compounds and synthetic methods permitting the easy introduction of carbon-14 were needed. We now report the syntheses of 1 and 2 by a method based on the alkylation of doubly activated β-ketonitrosamines.

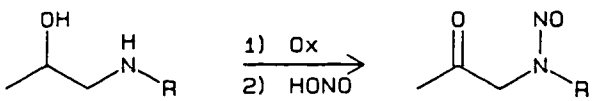
Since the α-methylene group of this type of compound is activated both by the nitroso and the carbonyl group, enolization occurs readily and exclusively towards the nitrogen atom.[8] N-(Acetonyl)-N-methylnitrosamine 3 is a representative compound in this series. Anion formation is carried out at 25° with sodium hydride in THF and trapped with methyl iodide to give N-(1-methylacetonyl)-N-methylnitrosamine 4 in 56% yield. This compound exists as a mixture of 4E:4Z rotational isomers in 1.13:1 ratio, the separation of which was accomplished on HPLC (Eq. 1).[9]



(1)

The technique developed to produce the enolate anion of 3 was applied to the synthesis of the two compounds found in the Linxian staples. Reductive alkylation of 1-amino-2-propanol with isovaleraldehyde and sodium borohydride gave 1-(isoamylamino)-2-propanol 5 in 81% yield.10 Oxidation

of 5 with chromium trioxide-sulfuric acid, followed by *in situ* nitrosation gave a 65% yield of N-(acetonyl)-N-isoamylnitrosamine 6. The enolate anion of 6 was formed in THF at about 0° with sodium hydride. The anion was trapped with methyl iodide to give a 74% yield of N-(1-methylacetonyl)-N-(3-methylbutyl)nitrosamine 1. Proton magnetic resonance analysis in $CDCl_3$ indicated that 1 exists as a mixture of two rotational isomers 1Z (65%) and 1E (35%). The structural congener N-(1-methylacetonyl)-N-(2-methylpropyl)nitrosamine 2 was prepared by a similar procedure. Initially, 1-(isobutylamino)-2-propanol 7 was oxidized and nitrosated in 66% yield to the corresponding nitrosoketone 8. Enolate formation with sodium hydride in tetrahydrofuran followed by alkylation with methyl iodide gave 2 in 86% yield. Analysis of the product by NMR indicated a 2:1 ratio of 2Z:2E rotational isomers.



5 (R=isoamyl) 6 (R=isoamyl)

7 (R=isobutyl) 8 (R=isobutyl)

EXPERIMENTAL SECTION

Proton and carbon NMR spectra were recorded using a Nicolet NT-300 spectrometer or a Varian XL-200. Low and high resolution mass spectral measurements were carried out on a VG-Micromass ZAB-2F spectrometer equipped with a VG data system, Model 2035, or a VG-Micromass Model 7070 spectrometer. Elemental analyses were done at Galbraith Laboratories, Inc., Knoxville, TN.

N-(1-Methylacetonyl)-N-methylnitrosamine (4).- To a slurry of 0.746 g (0.031 mol) of sodium hydride in 10 ml of tetrahydrofuran cooled to 0° under nitrogen, was added a 0.5 M solution of 3.28 g (0.028 mol) of N-(acetonyl)-N-methylnitrosamine 3[11] in tetrahydrofuran. The ice-bath was removed and the mixture stirred at 25° under nitrogen for 1 hr. The reaction mixture was cooled once again to 0° and 6.71 g (0.047 mol) of

methyl iodide were added. The mixture allowed to warm up to room temperature and stirred for 30 min. Water (0.5 ml) was added and the solvent removed on a rotary evaporator. The residue was extracted with methylene chloride, filtered through a layer of magnesium sulfate and the solvent removed on a rotary evaporator. Purification of the crude oil was carried out on dry-packed silica gel and eluted with 5:1 methylene chloride:ethyl acetate to give 2.1 g (56%) of 4, bp. 72° at 15 mm Hg; IR (film): 2990, 2935, 1720, 1435, 1340, 1072, 1040 cm^{-1}; ^1H NMR ($CDCl_3$): E isomer (53%): δ 1.61 (d, 3H), 2.22 (s, 3H), 2.99 (s, 3H), 5.4 (q, 1H); Z isomer (47%): δ 1.38 (d, 3H), 2.11 (s, 3H), 3.79 (s, 3H), 5.10 (q, 1H); ^{13}C NMR ($CDCl_3$): E isomer: 14.07 ppm, 26.75, 29.63, 66.80, 204.32; Z isomer: 11.69 ppm, 26.64, 36.42, 58.24, 202.21; MS, m/z (relative intensity): 130 (M^+, 3), 129 (30), 116 (28), 115 (11), 112 (5), 101 (7), 100 (39), 98 (84), 86 (21), 85 (8), 84 (20), 82 (7), 74 (28), 70 (16), 67 (27), 57 (22), 55 (21), 43 (100); Exact mass (M^+): 130.0767; calcd for $C_5H_{10}N_2O_2$ (M^+): 130.0378.

Anal. Calcd for $C_5H_{10}N_2O_2$: C 46.14%, H 7.74%, N 21.52%

Found: C 46.44%, H 7.70%, N 21.37%

N-(Acetonyl)-N-isoamylnitrosamine (6).- A solution of 6.5 g (0.0435 mol) of 1-(isoamylamino)-2-propanol 5[10] in 65 ml of water was cooled to 0°C, and 12 ml of conc. sulfuric acid was added. To the resulting solution was added 7 g (0.07 mol) of chromium trioxide in 20 ml of water, and stirred at 25°C for 12 hrs. The reaction mixture was cooled to 0°C, 75 ml of methylene chloride were added followed by the dropwise addition of 6.9 g (0.1 mol) of sodium nitrite in 20 ml of water and the two-phase mixture stirred at 25° for 3 hrs. The organic layer was separated, and the aqueous layer extracted three times with methylene chloride. The combined organic solutions were dried over anhydrous potassium carbonate, filtered through a layer of magnesium sulfate and evaporated in vacuo. The crude

product was purified on silica-gel column chromatography using 8:2:1 methylene chloride:acetone:ethyl acetate as the eluent to give 4.7 g (61%) of 6, bp. 80° at 0.3 mm Hg, lit.[12] bp. 80° at 0.3 mm Hg; IR (film): 2950, 2865, 1730, 1450, 1350, 1178, 1100, 1035 cm^{-1}; NMR ($CDCl_3$): Z isomer (73%): δ 0.92 (d, 6H), 1.55 (m, 1H), 1.63 (m, 2H), 2.16 (s, 3H), 4.21 (s, 2H), 4.228 (m, 2H); E isomer (27%): δ 0.92 (d, 6H), 1.32 (m, 2H), 1.55 (m, 1H), 2.22 (s, 3H), 3.60 (m, 2H), 4.96 (s, 2H); MS, m/z, (relative intensity): 172.12 (M^+, 2), 129 (30), 124 (5), 120 (5), 116 (28), 115 (11), 101 (7), 100 (40), 98 (84), 86 (21), 85 (8), 84 (20), 82 (7), 71 (28), 70 (16), 67 (27), 57 (22), 55 (21), 43 (100). Exact mass (M^+): 172.1188; calcd for $C_8H_{16}N_2O_2$ (M^+): 172.1211.

N-(1-Methylacetonyl)-N-(3-methylbutyl)nitrosamine, NMAMBA (1).- To a slurry of 150 mg (6.25 mmol) of sodium hydride in 12 ml of anhydrous tetrahydrofuran at 0°C was added, dropwise, a 1 M solution of 788 mg (4.5 mmol) of N-(acetonyl)-N-isoamylnitrosamine 6 in tetrahydrofuran and stirred at room temperature for 30 min. Methyl iodide (1.3 g, 9.15 mmol) was added rapidly and the stirring continued at room temperature under nitrogen for an additional 30 min. The reaction was quenched with saturated ammonium chloride solution (ca. 5 ml) and the organic solvent removed on a rotary evaporator. The remaining mixture was extracted with methylene chloride, dried over sodium sulfate and filtered through a pad of magnesium sulfate. Evaporation of the solvent gave 834 mg of crude product, 1, which was purified by silica-gel column chromatography using 8:2:1 methylene chloride:ethyl acetate:hexane as the eluant to give 630 mg (74%) of N-(1-methylacetonyl)-N-(3-methylbutyl)nitrosamine, NMAMBA, 1. Mass and C-13 NMR spectral properties of this compound are identical to authentic sample prepared by Singer et al.[5] The proton magnetic resonance spectrum is reported here for the first time: NMR ($CDCl_3$): E isomer (35%): δ 0.913 (d, 6H), 1.59 (m, 1H), 1.635 (d, 3H), 1.73 (m, 2H), 2.206

(s, 3H), 3.601 (m, 2H), 5.14 (q, 1H); Z isomer (65%): δ 1.005 (d, 6H) 1.367 (d, 3H), 1.59 (m, 1H), 1.73 (m, 2H), 2.206 (s, 3H), 4.184 (m, 2H), 4.456 (q, 1H).

N-Isobutyl-N-2-hydroxypropylamine (7).- A solution of 18.8 g (0.26 mol) of isobutylamine dissolved in 200 ml of water was cooled to 0°C. To the solution was added 8.9 ml (0.13 mol) of propylene oxide and the mixture was stirred at 25°C overnight. Excess isobutylamine was removed on a rotary evaporator and the product extracted with dichloromethane. The organic layer was dried over sodium sulfate, filtered through a pad of magnesium sulfate, and the solvent removed in vacuo. The crude product was vacuum distilled to give 10.5 g (63%) of N-isobutyl-N-2-hydroxypropylamine 7: b.p. 46-7°C at 10 mm Hg; IR (film): 3440, 3180, 2980, 2886, 1456, 1370, 1112, 928 cm^{-1}; NMR ($CDCl_3$): δ 0.9 (d, 6H), 1.14 (d, 3H), 1.73 (m, 1H), 2.40 (m, 2H), 2.67 (m, 2H), 3.79 (m, 1H); MS, m/z, (relative intensity): 131 (M^+, 5), 116 (5), 100 (3), 98 (8), 88 (62), 86 (100), 70 (49), 58 (6), 57 (34), 56 (10), 42 (23), 41 (33); Exact mass (M^+): 131.1304; calcd for $C_7H_{17}NO$ (M^+): 131.1310.

Anal. calcd for $C_7H_{17}NO$: C 64.08%, H 13.06%, N 10.67%

Found: C 64.31%, H 13.36%, N 10.01%

N-(Acetonyl)-N-isobutylnitrosamine (8).- Oxidation and in situ nitrosation of 13.14 g (0.1 mol) of N-isobutyl-N-2-hydroxypropylamine 7 was carried out as described above to give, after purification, 11.8 g (74%) of N-(acetonyl)-N-isobutylnitrosamine 8: IR (film): 2962, 2939, 1730, 1450, 1430, 1346, 1310, 1217, 1171, 1096, 1035, 710 cm^{-1}; NMR ($CDCL_3$): Z isomer (87%): δ 0.999 (s, 6H), 2.038 (m, 1H), 2.198 (s, 3H), 4.013 (d, 2H), 4.229 (s, 1H); E isomer (13%): δ 0.842 (d, 6H), 2.004 (m, 1H), 2.26 (s, 3H), 3.423 (d, 2H), 4.977 (s, 2H); MS, m/z, (relative intensity): 159 ($M^+ + 1$, 100), 144 (2), 143 (4), 141 (2), 135 (4), 130 (8.33); Exact mass (MH+): 159.1133; calcd for $C_7H_{15}N_2O_2$ (MH+): 159.1125.12

N-(1-Methylacetonyl)-N-(2-methylpropyl)nitrosamine, NMAMPA (2).- Enolate formation and alkylation of 1.466 g (9.28 mmol) of N-nitrosoisobutyl-N-2-hydroxypropylamine 8 was carried out as described above for the preparation of congeners 1. N-(1-Methylacetonyl)-N-(2-methylpropyl)nitrosamine 2 was obtained in 86% yield: IR (film): 2966, 1721, 1465, 1425, 1385, 1356, 1305, 1101, 948 cm^{-1}; NMR ($CDCl_3$): E isomer (33%): δ 0.856 (d, 3H), 0.866 (d, 3H), 1.694 (d, 3H), 1.970 (m, 1H), 2.197 (s, 3H), 3.35 (m, 2H), 4.887 (q, 1H); Z isomer (67%): δ 1.034 (d, 3H), 1.053 (d, 3H), 1.366 (d, 3H), 2.090 (s, 3H), 2.132 (m, 1H), 3.954 (m, 2H), 4.342 (q, 1H); MS, m/z, (relative intensity): 173 (M^+, 100), 144 (16), 142 (11), 100 (53), 95 (9), 82 (10), 81 (10), 72 (6), 71 (5), 69 (4), 56 (11), 55 (7), 42 (10); Exact mass (MH^+): 173.1290; required for $C_8H_{17}N_2O_2$ (MH^+): 173.1278. This compound was identical to the one prepared by Ji et al.[4]

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