# Precursors of *N*-Nitrosodimethylamine in Malted Barley. 1. Determination of Hordenine and Gramine

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*N*-Nitrosodimethylamine (NDMA) in beer has been traced to direct-fired malt. This study separated and quantified the malt alkaloid *N*-methyltyramine as well as the suspected NDMA precursors, hordenine and gramine. However, laboratory nitrosation of malt samples resulted in hordenine accounting for only minor amounts of NDMA and gramine, almost none. Thus, a large portion of NDMA in malt must be due to one or more other precursors. Studies indicate that dimethylamine may be a likely candidate.

## INTRODUCTION

Over a decade ago, investigators around the world reported *N*-nitrosodimethylamine (NDMA) in beer, generally in the range 1–5 μg/kg. Mangino et al. (1981) summarized their findings. The source of NDMA was malt dried by the direct-fire kilning process (Spiegelhalder et al., 1980; Hardwick et al., 1981). Oxides of nitrogen formed from combustion become incorporated into the drying air where they reacted with amines in the malt. O'Brien et al. (1980) conclusively demonstrated that NDMA formed during the kilning (drying) step of malt manufacturing (Figure 1). In response to this finding, many in the malting industry significantly reduced NDMA formation in their processes by converting to indirect-fire kilns and/or by introducing sulfur dioxide during kilning (O'Brien et al., 1980).

Following changes in the malt drying process, Scanlan et al. (1990), in a survey of approximately 200 U.S. and Canadian beers, reported that average NDMA levels had dropped to 0.074 μg/kg, about 2–3% of the amounts previously found. Many other investigators have reported similar findings for beer in other countries (Frommberger, 1989; Gavinelli et al., 1988; Kubacki et al., 1989; Mavelle et al., 1991; Österdahl, 1988; Tricker and Preussmann, 1991).

Over the past decade, researchers have speculated on the identity of the amine precursor(s) in malt for NDMA (Mangino et al., 1981; Wainright, 1986a,b). Two candidates were hordenine and gramine, tertiary amine alkaloids biosynthesized from tyrosine (Lette and Marion, 1953; Mann et al., 1963; Steinhart et al., 1964) and tryptophan (Bowden and Marion, 1951; Spenser, 1968), respectively, during the germination step of the malt manufacturing process. Mangino and Scanlan (1982, 1985) demonstrated NDMA formation from both hordenine and gramine.

The purpose of this study was to determine the levels of hordenine and gramine in malt, as well as those of their immediate precursors *N*-methyltyramine and *N*-methyl-3-(aminomethyl)indole formed during the malting process (see Figure 2). Furthermore, our aim was to determine the relative roles of hordenine and gramine as precursors to NDMA during kilning.

## EXPERIMENTAL PROCEDURES

**Chemicals.** Commercial sources of chemicals are given in Table I. Abbreviations used throughout this paper are also given in Table I. *N*-Methyltyramine and *N*-methyl-3-(aminomethyl)-indole were synthesized according to the procedure of Mangino (1983). Solutions of standard amines were prepared in MeOH.

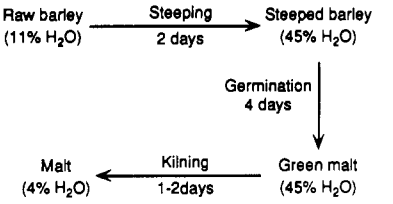


**Figure 1.** Unit operations in a typical malting process.

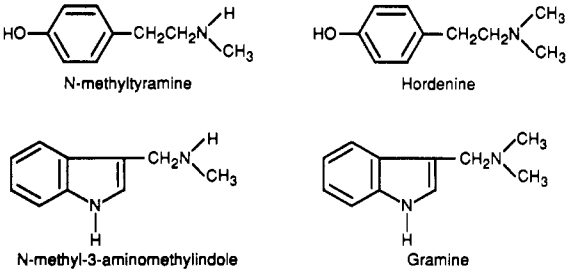


**Figure 2.** Malt alkaloids.

**Barley and Malt Samples.** Commercial samples of a number of varieties of raw barley, green malt, and kilned malt were obtained from Great Western Malting Co., Vancouver, WA. Figure 1 illustrates a typical commercial malting process. For more detail about the malting process, see Briggs et al. (1981).

For certain experiments, 300-g lots of barley were steeped, germinated, and indirect-fire-dried in a Seeger electric malt kiln. The pilot-kilning procedure approximated the commercial kilning process, except that since electric heat was used in the pilot kiln, the samples were not exposed to nitrosative conditions, i.e., oxides of nitrogen.

Freeze dehydration of pilot-germinated samples of green malt was accomplished in a Hull pilot plant scale unit that maintained 18 °C throughout the drying operation.

Malt byproducts, consisting mainly of rootlets but also some chaff and acrospires (shoot tips), were manually removed from pilot-kilned and freeze-dried samples on a precision sieve (slotted, 0.09 × 9.75) to produce clean malt.

**Extraction and Purification.** The alkaloids were recovered from samples of barley, clean malt, and malt byproducts by the process described in Table II.

**Separation and Quantification.** The high-performance liquid chromatograph (HPLC) used for the separation of the isolated amines was a Spectra-Physics Model 8000 with a 10-μL injection loop. For *N*-methyltyramine, hordenine, and *N*-methyl-3-(aminomethyl)indole, a 250 × 4.6 mm i.d., 10-μm μBondapak-Phenyl column monitored with a fixed-wavelength SP 8200 detector at 280 nm was used. The mobile phase consisted of, solvent A, 0.05 M sodium monobasic phosphate adjusted to pH 3.00 with concentrated $H_3PO_4$ and, solvent B, MeOH; the flow

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**Table I. Commercial Sources of Chemicals**

glass-distilled chemicals from Burdick and Jackson Laboratories, Inc.
- chloroform ($CHCl_3$)
- dichloromethane (DCM)
- ethyl acetate (EtOAc)
- methanol (MeOH)
- petroleum ether (pet. ether)

Sigma Chemical Co.
- dansyl chloride
- fluorescamine
- gramine
- hordenine hemisulfate
- iodoplatinate
- tyramine

Aldrich Chemical Co.
- dimethylamine hydrochloride (DMA)

J. T. Baker Chemical Co.
- 28% ammonium hydroxide ($NH_4OH$)
- ammonium sulfamate
- anhydrous sodium sulfate ($Na_2SO_4$)
- diethyl ether ($Et_2O$)
- glacial acetic acid (AcOH)
- hydrochloric acid (HCl)
- phosphoric acid ($H_3PO_4$)
- sodium hydroxide (NaOH)
- sodium nitrite (food grade)
- sodium phosphate (monohydrate and monobasic)

**Table II. Extraction Steps and Procedures**

| step | process | equipment and method |
|---|---|---|
| 1. grinding | grind malt | Osterizer blender |
| | 100-g samples | 1-L Erlenmeyer flasks |
| 2. ether extraction | defat | 400 mL of pet. ether, for 3 h, magnetic stirrer |
| | filter | Whatman No. 1 paper in Büchner funnel |
| | wash residue | 3 × 130 mL of pet. ether and air-dry |
| | extract and purge | 400 mL of 5% $NH_4OH$ in MeOH, with $N_2$, 24 h, room temp |
| | filter | Whatman No. 1 paper in Büchner funnel |
| | wash | 3 × 130 mL, 5% $NH_4OH$ in MeOH |
| 3. acidic extraction | combine filtrates and evaporate to remove MeOH | rotary evaporator at 40–50 °C |
| | residue extractions | (1) 25 mL of 1 N HCl |
| | | (2, 3) 25 mL of DCM and 1 N HCl mix 1:1 |
| | | (4) 25 mL of 1 N HCl |
| | combine extracts and shake gently to form two layers | 250-mL separatory funnel |
| | discard | DCM phase |
| | extract aqueous phase | 3 × 40 mL of DCM |
| | | 5 × 40 mL of $CHCl_3$ |
| | discard | DCM and $CHCl_3$ extracts |
| 4. basic extraction | raise pH to 9.0 and saturate | 250-mL beaker, concentrated $NH_4OH$; NaCl |
| | extract | 5 × 40 mL of EtOAc |
| | raise pH to 10.15 and saturate | 250-mL beaker, concentrated $NH_4OH$; NaCl |
| | extract | 5 × 40 mL of EtOAc |
| | combine and dry extracts and filter through | anh $Na_2SO_4$ |
| | evaporate and dry | rotary evaporator at 40 °C, stream of $N_2$ gas |
| 5. final purification | dissolve | 1–2 mL of MeOH |
| | activate alumina | 115 °C overnight and cool to room temp |
| | load slurry into column | $CHCl_3$ slurry containing 15 g of activated alumina |
| | rinse column with 50 mL of $CHCl_3$ | |
| | apply MeOH containing alkaloids to column | |
| | elute from $Al_2O_3$ column | 200 mL of MeOH |
| | evaporate to 2 mL | rotary evaporator |
| | reduce to 1 mL | $N_2$ stream |
| | filter | 0.45-μm disposable Acrodisc filter |

rate was 1.8 mL/min at 35 °C. All solvents were freshly prepared before use, and all mobile-phase solutions were filtered through 0.45-μm Millipore filters. The elution program was a 0–30% linear gradient with solvent B for 12 min, holding with 30% solvent B for an additional 12 min.

Because gramine coeluted with an unknown malt compound on the μBondapak-Phenyl column, gramine was separated on a 250 × 4.6 mm i.d., 10-μm Spherisorb 10-ODS column, monitored with a variable-wavelength Model SP 8440 UV–vis detector at 280 nm. The mobile phase consisted of, solvent A, an ion-pairing solution, 0.1 M trichloroacetic acid adjusted to pH 3.00 with 6 N NaOH and, solvent B, MeOH; the flow rate was 1.8 mL/min at 35 °C. The elution program was a 20–45% linear gradient with solvent B for 12 min, holding with 45% solvent B for an additional 12 min.

**Confirmation of Identity.** The three alkaloids detected were *N*-methyltyramine, hordenine, and gramine; no *N*-methyl-3-(aminomethyl)indole was detected. Thin-layer chromatography (TLC), ultraviolet spectroscopy (UV), mass spectrometry (MS), and retention times on HPLC were used for confirmation of identity of the three alkaloids detected. Extracts from a variety of barley, Wintermalt, were used for these experiments.

*TLC.* Twenty microliters of malt extract (Table II) was spotted on a precoated TLC plate (silica gel 60 F-254 from EM Laboratories, Inc.). Standard amines in the following amounts were also spotted and cochromatographed: 91 μg of *N*-methyltyramine, 100 μg of hordenine, 45 μg of gramine, and 45 μg of tyramine. The mobile phase was $Et_2O$/MeOH/28% $NH_4OH$ (17:2:1 v/v/v), freshly prepared for each run. The developed plate was air-dried and then sprayed, first with fluorescamine, second with dansyl chloride, and finally with iodoplatinate. After each spray application, the plate was examined under UV light, except for the last one in which the spots could be seen with visible light. The $R_f$ values and the color developed for each spot were compared with those of standard amines.

*UV.* Fractions corresponding to the peaks for the three alkaloids identified were collected from the HPLC column. Employing a 50-μL sample loop, the μBondapak-Phenyl column was used for *N*-methyltyramine and hordenine. For gramine, the Spherisorb 10-ODS column was used. Each collected fraction was analyzed by UV, and the spectrum was compared to the spectra of the standard amines.

*MS.* For MS analysis, fractions corresponding to the peaks for the alkaloids were collected from HPLC columns, as described for UV analysis. Low-resolution MS analyses were performed on a Finnigan 1015C quadrupole spectrometer, interfaced to a Riber 400 data system, including a Digital Equipment Corp. PDP 8/E minicomputer, a Diablo 31 disk system, and a Tektronix 4010-1 display terminal.

**Nitrosamine Determination.** NDMA was determined in malt samples using the procedure described by Havery et al. (1984). Essentially, malt is ground, mixed with water and Celite, and loaded into a glass column. NDMA is eluted with DCM which is subsequently concentrated to 1 mL. Separation and

Table III. Ranges of Amines Added to Four Malt Samples, Mean Recoveries, and Detection Limits in Malt

| | fortification range, μg/g | mean recovery, % | detection limit, μg/g |
|---|---|---|---|
| *N*-methyltyramine | 4.9–51.7 | 77.4 | 0.2 |
| hordenine | 5.2–52.8 | 92.0 | 0.2 |
| *N*-methyl-3-(aminomethyl)indole | 1.2–12.0 | 74.0 | 0.1 |
| gramine | 1.2–9.1 | 80.5 | 0.1 |

quantification is by gas chromatography-thermal energy analyzer (GC-TEA). The GC column was a 10 ft × 1/8 in. i.d. stainless steel column packed with 20% Carbowax 20M plus 2% NaOH coated on 100/120-mesh Chromosorb W-AW as the support. The column was operated at 170 °C, the injection port was 170 °C, and the helium flow rate was 30 mL/min.

**Nitrosation of Malt Spiked with Hordenine, Gramine, and DMA.** The purpose of this experiment was to determine the percentage yield of NDMA from hordenine, gramine, and DMA in malt under controlled laboratory nitrosation conditions. Samples from a clean, freeze-dried Morex malt were separately spiked with equimolar amounts of standard amines (30 μg of hordenine, 31.5 μg of gramine, and 14.8 μg of DMA per g of malt) that were allowed to be absorbed by the ground malt. Unspiked malt served as a control. Samples were nitrosated in quadruplicate.

For the controlled nitrosation reaction, 1 g of ground malt spiked with the amine was placed in a 250-mL beaker, and 50 mL of 15% AcOH was added. (The AcOH was prepared by adjusting 15% AcOH to pH 3.2 with 6 N NaOH.) Ten milliliters of a 0.5 g/mL solution of $NaNO_2$ was added, and the mixture was placed under a fume hood at room temperature. After reacting for 18 h, each mixture was acidified with concentrated $H_2SO_4$ to pH 1.5, and 12 mL of a 0.8 g/mL ammonium sulfamate solution was added to stop the nitrosation reaction. Each mixture was filtered through glass wool into a 250-mL separatory funnel, and the residue and container were rinsed with 3 × 15 mL of deionized water. Each filtrate along with its combined rinsings was extracted with 3 × 30 mL of DCM. The combined DCM extracts for each reaction were extracted with 50 mL of 2 N NaOH, and the DCM fraction was dried by passage through anhydrous $Na_2SO_4$ and placed in a Kuderna-Danish apparatus connected to a condenser. The DCM extract of each reaction was concentrated to 1 mL and analyzed by GC-TEA for NDMA. The percentage yield of NDMA from the known amounts of hordenine, gramine, and DMA was calculated after the amount of NDMA in the control was subtracted.

**Determination of the Relative Roles of Hordenine and Gramine in NDMA Production.** Five varieties of kilned, clean malt were nitrosated under controlled laboratory conditions and extracted as described above. After extraction, NDMA levels were determined by GC-TEA. Using the NDMA values, the amounts of hordenine and gramine in each sample, and the yield of NDMA from the alkaloids, the relative contribution of the two alkaloids to the formation of NDMA was estimated.

## RESULTS

**Separation and Quantification.** HPLC was used to separate and quantify *N*-methyltyramine, hordenine, *N*-methyl-3-(aminomethyl)indole, and gramine. A linear response ($r > 0.99$) over the range of interest was established for each alkaloid. Quantification was accomplished by comparing peak areas of malt alkaloids with those of standard alkaloids injected immediately before and after each sample injection.

**Extraction and Purification.** *N*-Methyltyramine, hordenine, *N*-methyl-3-(aminomethyl)indole, and gramine were efficiently extracted from malt using the procedure described in Table II. Alkaloids at four levels were added to freeze-dried Morex malt over the ranges given in Table III. Mean recoveries, corrected for any indigenous amounts the malt contained, are also shown in Table III, along with the alkaloid detection limits. The tertiary amines, hordenine and gramine, had greater recoveries than the secondary amines, suggesting that more of the latter two was lost in the extraction and purification process.

Table IV. Amounts of Alkaloids Extracted from Five Samples of the Same Malt[a]

| sample | *N*-methyltyramine, μg/g (dry wt) | hordenine, μg/g (dry wt) |
|---|---|---|
| 1 | 29.4 | 13.9 |
| 2 | 27.4 | 12.2 |
| 3 | 28.5 | 12.8 |
| 4 | 29.5 | 13.1 |
| 5 | 29.1 | 13.7 |
| mean | 28.8 | 13.1 |
| SD | 0.77 | 0.62 |

[a] Freeze-dried 81-Morex malt from Idaho.

Table V. Alkaloids in Clean Malt from 10 Barley Varieties

| | *N*-methyltyramine, μg/g (dry wt) | hordenine, μg/g (dry wt) | gramine, μg/g (dry wt) |
|---|---|---|---|
| pilot malting process | | | |
| Wintermalt | 28.3 | 26.0 | 10.0 |
| Shabet | 18.5 | 29.7 | nd[a] |
| Manker | 29.1 | 14.2 | nd |
| Klages | 25.3 | 29.9 | nd |
| Karla | 29.9 | 8.9 | nd |
| Piroline | 22.4 | 26.2 | nd |
| Glenn | 40.4 | 23.2 | nd |
| commercial malting process | | | |
| Advance, lot A | 26.1 | 27.0 | nd |
| Advance, lot B | 28.5 | 31.0 | nd |
| Larker, lot A | 33.8 | 30.8 | nd |
| Larker, lot B | 36.6 | 37.6 | nd |
| Morex, lot A | 40.2 | 37.6 | nd |
| Morex, lot B | 48.5 | 42.4 | nd |

[a] Not detected.

**Confirmation of Identities.** The identities of *N*-methyltyramine, hordenine, and gramine extracted from malt were confirmed by comparing with standard compounds their respective (1) retention times on the HPLC, (2) UV spectra, (3) $R_f$ values on TLC, and (4) mass spectra. Recall that *N*-methyl-3-(aminomethyl)indole, the precursor to gramine, was not detected in any of the malt samples analyzed.

The mass spectrum of each alkaloid and that of its corresponding standard showed excellent agreement. Each of the three alkaloid mass spectra was also compared with standard spectra published in collections of MS data (*1988 Registry of Mass Spectral Data*, 1988; NIST, EPA, MSDC, 1990). The data agreed well with the spectra in the registry, but the NIST library was of very limited use due to its poor spectral quality index for the three alkaloids. (The quality index for the NIST standards ranged from 346/1000 for *N*-methyltyramine to 694/1000 for gramine, with hordenine in between at 515/1000.)

**Alkaloids in Barley and Malt.** To verify the reproducibility of the extraction procedure, five samples of the same Morex malt were extracted for *N*-methyltyramine and hordenine (Table IV). Results indicate that the extraction procedure has a relatively low variance. The same methods were used to quantify the alkaloids in barleys and malts.

Clean malts from one sample of each of 10 varieties of barleys were analyzed for their alkaloid content (Table V). The first seven malt samples were prepared in our laboratory, while the last six were drawn from different lots of a commercially malted product. Gramine was detected only in Wintermalt. *N*-Methyltyramine ranged from 18.5 μg/g in Shabet to 48.5 μg/g in Morex, lot B; hordenine ranged from 8.9 μg/g in Karla to 42.4 μg/g in Morex, lot B.

To isolate the differential effects of malt variety from those of location of production and other differences in

**Table VI. Alkaloid Levels in Raw Barley, Clean Malt, and Malt Byproducts in Five Barley Varieties Grown and Malted under Nearly Identical Conditions**

| | *N*-methyltyramine, μg/g (dry wt) | hordenine, μg/g (dry wt) | gramine, μg/g (dry wt) |
|---|---|---|---|
| Karla | | | |
| raw barley | 0.7 | 0.7 | nd[a] |
| clean malt | 25.6 (9.8%)[b] | 25.0 (8.4%) | nd |
| malt byproducts | 2798.4 (90.2%) | 3245.3 (91.6%) | nd |
| total[c] | 241.6 | 275.9 | nd |
| Morex | | | |
| raw barley | 11.4 | nd | nd |
| clean malt | 32.9 (13.4%) | 38.8 (7.9%) | nd |
| malt byproducts | 2903.4 (86.6%) | 4447.2 (92.1%) | nd |
| total[c] | 222.0 | 443.5 | nd |
| Klages | | | |
| raw barley | 0.3 | nd | nd |
| clean malt | 15.8 (9.4%) | 38.0 (7.7%) | nd |
| malt byproducts | 1717.4 (90.6%) | 5180.1 (92.3%) | nd |
| total[c] | 154.1 | 456.1 | nd |
| Piroline | | | |
| raw barley | 0.4 | nd | nd |
| clean malt | 15.9 (9.3%) | 28.7 (7.2%) | nd |
| malt byproducts | 1967.0 (90.7%) | 4794.2 (92.8%) | nd |
| total[c] | 155.9 | 371.4 | nd |
| Steptoe | | | |
| raw barley | 0.7 | nd | nd |
| clean malt | 13.0 (13.0%) | 19.4 (9.0%) | 4.9 |
| malt byproducts | 1683.7 (87.0%) | 3760.5 (91.0%) | 13.1 |
| total[c] | 95.4 | 203.8 | 5.3 |

[a] Not detected. [b] % of total as defined in footnote *c*. [c] Total gives the amount in the clean malt plus its byproducts, e.g., total hordenine in Karla is (25.0 × 0.9221) + (3245.3 × 0.0779) = 275.9, where 0.9221 and 0.0779 are the proportionate weights of clean malt and malt byproducts in 1 g of unclean sample. The respective weights of clean malt and its byproducts differ for each variety, but the method of calculating "total" was the same.

growing conditions (e.g., temperature, moisture, fertilization), five varieties of barley grown under nearly identical conditions were selected. Raw barley from each variety was analyzed for alkaloid content. Samples of these five barleys were also steeped, germinated, and pilot-kilned by identical processes and analyzed for the alkaloids. Results are reported in Table VI.

Very small amounts of *N*-methyltyramine were found in all five varieties of raw barley; hordenine was only detected in raw Karla, at very low levels. Despite literature reports to the contrary (Lette and Marion, 1953; Mann et al., 1963; Hosoi et al., 1970), finding these alkaloids in raw barley suggests some enzymatic activity, perhaps during storage (from L-tyrosine to tyramine to *N*-methyltyramine and, in the case of Karla, to hordenine).

No gramine was detected except for small amounts in Steptoe malt, an animal feed. Of the total amount of *N*-methyltyramine or hordenine in clean malt plus its malt byproducts, most was found in the byproducts (mainly rootlets), ranging from 86.6% of the total for *N*-methyltyramine in Morex to 92.8% of the total for hordenine in Piroline. The results confirmed the reported finding that alkaloids are mainly biosynthesized during the germination step of malt manufacturing.

Another experiment was carried out to determine if, and by how much, kilning affected the levels of alkaloids in malt. The alkaloid levels in malts from five barley varieties processed by freeze-drying (moisture removed without heat) were compared to those of malts processed in a pilot electric kiln. The assumption was that any change in the alkaloid content of pilot electric kilned malt compared to that of freeze-dried malt would be due to heat during the early stages of kilning. Results are presented in Table VII.

**Table VII. Alkaloid Levels in Five Varieties of Raw Barley, Freeze-Dried Clean Malt and Malt Byproducts, and Kilned Clean Malt and Malt Byproducts**

| | *N*-methyltyramine, μg/g (dry wt) | hordenine, μg/g (dry wt) | gramine, μg/g (dry wt) |
|---|---|---|---|
| Wintermalt | | | |
| raw barley | 13.4 | 0.5 | nd[a] |
| freeze-dried | | | |
| clean malt | 24.7 | 18.2 | 7.3 |
| malt byproducts | 962.3 | 1956.2 | 19.5 |
| total[b] | 74.3 | 120.7 | 7.9 |
| kilned | | | |
| clean malt | 17.4 | 13.9 | 7.9 |
| malt byproducts | 1819.7 | 3954.0 | 19.0 |
| total[b] | 102.5 | 199.9 | 8.4 |
| Morex | | | |
| raw barley | 17.9 | 0.7 | nd |
| freeze-dried | | | |
| clean malt | 37.5 | 30.2 | nd |
| malt byproducts | 2091.1 | 3671.9 | nd |
| total[b] | 182.8 | 288.1 | nd |
| kilned | | | |
| clean malt | 34.4 | 31.5 | nd |
| malt byproducts | 2337.6 | 3972.4 | nd |
| total[b] | 210.6 | 333.0 | nd |
| Klages | | | |
| raw barley | 0.4 | 1.0 | nd |
| freeze-dried | | | |
| clean malt | 21.0 | 35.5 | nd |
| malt byproducts | 2039.4 | 4744.3 | nd |
| total[b] | 135.0 | 301.6 | nd |
| kilned | | | |
| clean malt | 23.0 | 41.6 | nd |
| malt byproducts | 2060.9 | 4664.7 | nd |
| total[b] | 146.5 | 321.8 | nd |
| Piroline | | | |
| raw barley | 0.4 | nd | nd |
| freeze-dried | | | |
| clean malt | 12.0 | 9.6 | nd |
| malt byproducts | 1761.7 | 3696.9 | nd |
| total[b] | 64.8 | 120.9 | nd |
| kilned | | | |
| clean malt | 15.8 | 21.7 | nd |
| malt byproducts | 1491.5 | 3904.0 | nd |
| total[b] | 183.7 | 463.5 | nd |
| Steptoe | | | |
| raw barley | 1.8 | 0.5 | nd |
| freeze-dried | | | |
| clean malt | 11.6 | 12.5 | 4.7 |
| malt byproducts | 1078.1 | 2747.1 | 85.7 |
| total[b] | 58.3 | 132.3 | 8.2 |
| kilned | | | |
| clean malt | 15.7 | 22.3 | 6.7 |
| malt byproducts | 1358.4 | 2741.2 | 120.2 |
| total[b] | 75.0 | 142.2 | 11.7 |

[a] Not detected. [b] Total—see footnote *c* in Table VI.

**Table VIII. Percentage Increase in Total Alkaloid Content during Kilning[a]**

| variety | *N*-methyltyramine | hordenine | gramine |
|---|---|---|---|
| Wintermalt | 38.0 | 65.6 | 6.3 |
| Morex | 15.2 | 15.6 | –[b] |
| Klages | 8.5 | 6.7 | – |
| Piroline | 183.5 | 283.4 | – |
| Steptoe | 28.6 | 7.5 | 42.7 |

[a] Computed from totals in Table VII: [(total kilned – total green)/total green] × 100. [b] Not detected before or after kilning.

Table VII confirms some of the results in Table VI. Raw barley samples contained very small amounts of *N*-methyltyramine and hordenine, gramine was detected only in small amounts in the Wintermalt and Steptoe varieties, and malt byproducts contained many times more of the alkaloids than did the clean malts. However, the main finding of this experiment was the significant increase in the total alkaloid content of clean malt and its

Table IX. NDMA[a] Obtained from Nitrosating Kilned Clean Malts Containing Known Amounts of Hordenine and Gramine Compared with Expected NDMA Yields

| variety | amounts, μg/g (dry wt), in malt samples: hordenine | amounts, μg/g (dry wt), in malt samples: gramine | NDMA obtained, μg/kg (dry wt) | expected NDMA,[b] μg/kg (dry wt), from: hordenine | expected NDMA,[b] μg/kg (dry wt), from: gramine | % NDMA accounted for by known quantities of the two alkaloids[c] |
|---|---|---|---|---|---|---|
| Wintermalt | 13.9 | 7.9 | 10012 | 169 | 2953 | 31.2 |
| Morex | 31.5 | nd[d] | 4484 | 382 | | 8.5 |
| Klages | 41.6 | nd | 4496 | 504 | | 11.2 |
| Piroline | 21.7 | nd | 7878 | 263 | | 3.3 |
| Steptoe | 22.3 | 6.7 | 3599 | 270 | 2497 | 76.9 |

[a] *N*-Nitrosodimethylamine. [b] Expected values of NDMA are calculated by multiplying the amount of alkaloid times the yield of NDMA and adjusting for the respective molecular weights of NDMA and the alkaloid, e.g., for hordenine in Wintermalt: [(13930 × 74)/165] × 0.027 = 169, where 74 and 165 are the respective molecular weights of NDMA and hordenine, and 2.7% is the average NDMA net yield from hordenine under the nitrosation conditions used in this study. [c] Expected NDMA from hordenine and gramine divided by the NDMA obtained, e.g., for the two alkaloids in Wintermalt: [(169 + 2953)/10012] × 100 = 31.2%. [d] Not detected.

byproducts in kiln-dried malt when compared to that of freeze-dried malt. Table VIII summarizes these results. The increase in alkaloid levels following kilning ranged from a 6.3% increase of gramine in Wintermalt to a 283% increase of hordenine in Piroline. It is likely that biosynthesis of alkaloids occurs early in the kilning step before the biosynthetic enzymes are denaturated by the high temperatures reached toward the end of the kilning process (O'Brien et al., 1980).

**Nitrosation of Malt Spiked with Hordenine, Gramine, and DMA.** Nitrosation of these same five varieties of kilned, clean malt, containing known concentrations of hordenine, gramine, and DMA, was conducted to determine the relative contribution of these alkaloids to the production of NDMA in malt. The yields of NDMA obtained from these samples were compared with expected yields calculated from standard hordenine, gramine, and DMA nitrosated under the same reaction conditions.

To obtain expected values of NDMA, samples of freeze-dried, clean Morex malt were spiked with standard hordenine, gramine, or DMA and nitrosated. The four-trial average NDMA obtained from the malts spiked with hordenine was 6028 μg/kg, the average NDMA obtained from the control sample (unspiked) was 5670 μg/kg, and the theoretical amount of NDMA from 100% conversion of spiked amine was 13 444 μg/kg. Thus, the net yield of NDMA from spiked hordenine was 2.7%: [(6028 − 5670)/13444] × 100. Similarly, the net yield of NDMA from freeze-dried malt spiked with gramine was 87.9%: [(17440 − 5670)/13384] × 100. The net yield of NDMA from freeze-dried malt spiked with DMA was 92.3%: [(18064 − 5670)/13430] × 100.

**Relative Roles of Hordenine and Gramine in Producing NDMA.** Results of the nitrosation of five varieties of kilned clean malts are reported in Table IX. The percentages of NDMA obtained from nitrosation *not* accounted for by the known alkaloids ranged from 23.1% in Steptoe malt to 96.7% in Piroline malt. The data clearly indicate that, besides hordenine and/or gramine, there are one or more unknown NDMA precursors in these malt samples.

## DISCUSSION

Mangino and Scanlan (1985) determined that the tertiary amine, gramine, rapidly forms NDMA upon nitrosation. In the present study, gramine was only detected in Steptoe malt, a feed barley, and in Wintermalt, a barley variety no longer used for malt production. Surprisingly, none of the more common barley varieties used for commercial malt production contained detectable amounts of gramine.

Schneider and Wightman (1974) and Hanson et al. (1981) established that gramine biosynthesis takes place in the acrospire of a germinating barley seed. Acrospires were included in the malt byproducts we analyzed. Our results suggest that acrospire development during commercial malt manufacturing does not progress sufficiently to allow detectable amounts of gramine to be formed. Only extremely small amounts of gramine were found in the byproducts of Steptoe and Wintermalt. Again, byproducts of more common malting varieties did not contain gramine at detectable levels.

The levels of NDMA obtained from the nitrosation of malt were found to be far greater than quantities of NDMA expected for the levels of hordenine and gramine they were known to contain. This suggests that the malt samples must contain one or more NDMA precursors besides hordenine and gramine.

Mangino and Scanlan (1982) reported NDMA yields from nitrosation under laboratory conditions for dimethylamine (DMA), trimethylamine (TMA), hordenine, gramine, *N*-methyltyramine, sarcosine, and choline and concluded that the first four amines were potential precursors of NDMA. The present study suggests that there must be other precursors in malt besides hordenine and gramine.

DMA had been reported in malt by Slaughter and Uvgard (1971), Drews et al. (1957), and French et al. (1982). French et al. extracted and quantified DMA from five malted barleys, including Klages. When they converted the 2.9 μg/g of acid-extractable DMA in Klages to NDMA, their yield was 3035 μg/kg. This is very close to the 3992 μg/kg NDMA in Klages from some other source in this study (see Table IX, where NDMA obtained was 4496 μg/kg and NDMA expected from hordenine is 504 μg/kg). Thus, DMA appears to be a candidate precursor of NDMA in malt.

These results lead directly to the next paper in this series of two (Yoo et al., 1992).

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**Registry No.** NDMA, 62-75-9; DMA, 124-40-3; hordenine, 539-15-1; gramine, 87-52-5; *N*-methyltyramine, 370-98-9; *N*-methyl-3-(aminomethyl)indole, 19293-60-8.