MELOSMINE AND MELOSMIDINE, 7,7-DIMETHYLTETRADEHYDROAPORPHINE ALKALOIDS FROM *GUATTERIA MELOSMA*

V. Zabel and W. H. Watson

Department of Chemistry, Texas Christian University, Fort Worth, Texas 76129

and

C. H. PHOEBE, JR.,[1] J. E. KNAPP, P. L. SCHIFF, JR., and D. J. SLATKIN*

Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

ABSTRACT.—Fractionation and chromatography of an ethanol extract of the stembark of *Guatteria melosma* Diels (Annonaceae) afforded melosmine (**2**) and melosmidine (**3**), two members of a new class of aporphine alkaloids (7,7-dimethyltetradehydroaporphines). A consideration of the spectral data of melosmine (**2**) and its *O,O*-dimethyl-(**5**), tetrahydro-(**6**), *O,O*-dimethyltetrahydro-(**7**), *N*-methyltetrahydro-(**8**), *N*-methyl-*O,O*-dimethyltetrahydro-(**9**) and *O,O*-diacetyl-(**10**) derivatives suggested the structure to be **2** (2,3-dimethoxy-7,7-dimethyl-7*H*-dibenzo [*de,g*] quinolin-1,9-diol). This was confirmed by x-ray crystallographic analysis. A consideration of the spectral data of melosmidine (**3**) and its *O*-methyl-(**5**), tetrahydro-(**11**), *N*-methyltetrahydro-(**12**) and *O*-acetyl-(**13**) derivatives indicated that melosmidine was **3** (1,2,3-trimethoxy-7,7-dimethyl-7*H*-dibenzo [*de,g*] quinolin-9-ol). *O*-Methylmelosmidine (**5**) is identical to *O,O*-dimethylmelosmine (**5**). In addition, isoboldine (**4**) and two uncharacterized alkaloids, GMA 1 and GMA 2, were isolated.

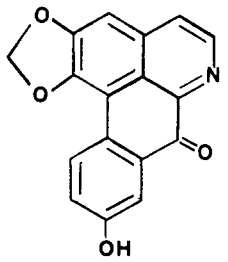
Guatteria melosma, a tree native to South America, is a member of one of the larger genera of the Annonaceae (1). Chemical investigations have been reported on only a few of the 285 species of the genus. To date, oxoaporphine (2–5), alcoholic aporphine (2), noraporphine (6) and bisbenzylisoquinoline (7) alkaloids, as well as propenylbenzenes (8), have been isolated from this genus. In a recent report from our laboratories, oxoanolobine (**1**), a new oxoaporphine alkaloid, has been isolated from the plant that is the subject of this investigation (9).

This paper presents the isolation and characterization of two novel aporphinoid alkaloids (7,7-dimethyltetradehydroaporphines), melosmine (**2**) and melosmidine (**3**). In addition, isoboldine (**4**) and small amounts of two uncharacterized alkaloids, GMA 1 (mp 245°; M^+ *m/e* 307) and GMA 2 (mp 181–2°; M^+ *m/e* 309), were isolated.

An ethanol extract of the stembark of *Guatteria melosma* Diels (Anonaceae) was partitioned between chloroform and aqueous citric acid. The aqueous acid layer was alkalinized with ammonium hydroxide and extracted with chloroform. The chloroform fraction (non-quaternary alkaloid fraction) was concentrated to a residue and chromatographed over silicic acid. Fractions from this column were rechromatographed with the subsequent isolation of six alkaloids.

Melosmine (**2**) was isolated as pale yellow cuboidal crystals (500 mg), mp 104°, M^+ *m/e* 337.1305 for $C_{20}H_{19}NO_4$. It was not optically active and had an uv spectrum unlike simple aporphine or oxoaporphine alkaloids (10,11). The bathochromic shift, upon the addition of base, indicated the phenolic nature of the alkaloid. The ^{1}H-nmr spectrum partially resembled that of a 1,2,3,9-tetrasubstituted oxoaporphine alkaloid. The aromatic AB system at δ8.50 (1H, d, $J=6$ Hz) and 7.71 (1H, d, $J=6$ Hz) was assigned to the C–5 and C–4 protons, respectively. The aromatic AMX system at δ8.91 (1H, d, $J=9$ Hz), 6.89 (1H, dd, $J=9$, 3 Hz) and 7.22 (1H, d, $J=3$ Hz) was assigned to the C–11, C–10 and C–8 protons, respectively. Singlets at δ4.17 (3H) and 4.00 (3H) were assigned to two aromatic methoxy groups, while a remaining singlet [δ1.73 (6H)] indicated the presence of a *gem*-dimethyl group in the molecule.

[1]Present Address: Department of Pharmacognosy and Pharmacology, School of Pharmacy, University of Illinois at the Medical Center, Chicago, Illinois.



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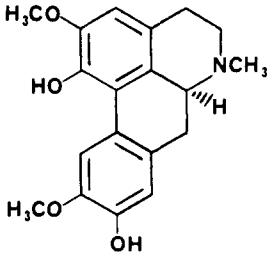

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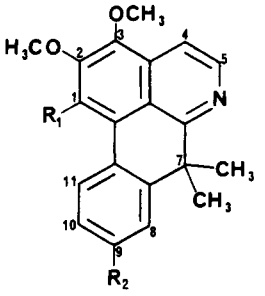
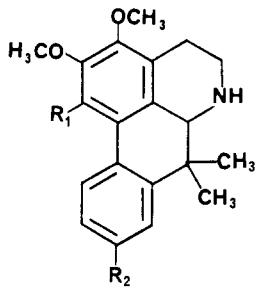
The lack of spectral evidence for the presence of a 7-keto group of an oxoaporphine alkaloid allowed for the assignment of the *gem*-dimethyl group at C–7. Melosmine (**2**) did not contain a carbonyl band (1650 cm^{-1}) in the ir spectrum characteristic of oxoaporphine alkaloids (12), and the mass spectral fragmentation did not show characteristic losses of ring A substituents through conjugative elimination involving the 7-keto group of oxoaporphines (13). All these data suggest that melosmine possessed a 1,2,3,9-tetrasubstituted 7,7-dimethylaporphinoid nucleus.

Since there were four oxygen atoms in the molecule (high-resolution ms) and two methoxy groups noted in the ^{1}H-nmr spectrum, the final two-ring substituents should be phenolic hydroxy groups. To substantiate this fact, melosmine (**2**) was treated with ethereal diazomethane, and the resulting *O,O*-dimethylmelosmine (**5**) was isolated as a yellow oil. The ms of the product (M^+ *m/e* 365) showed the



	R_1	R_2
2	OH	OH
3	OCH_3	OH
5	OCH_3	OCH_3
10	$OCOCH_3$	$OCOCH_3$
13	OCH_3	$OCOCH_3$



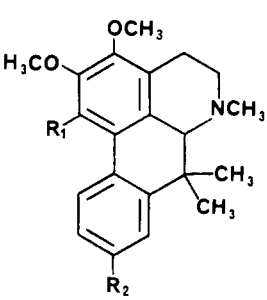
	R_1	R_2
6	OH	OH
7	OCH_3	OCH_3
11	OCH_3	OH

addition of 28 amu, while the ^{1}H-nmr spectrum showed four methoxy singlets (δ4.09, 4.05, 3.92 and 3.89), the aromatic AB and AMX systems, and the singlet for the *gem*-dimethyl group.

To verify the presence of the 7,7-dimethylaporphinoid nucleus, melosmine (**2**) and O,O-dimethylmelosmine (**5**) were reduced with Zn/HCl. Both products [tetrahydromelosmine (**6**) and *O,O*-dimethyltetrahydromelosmine (**7**)] showed the addition of 4 amu in the ms (M^+ *m/e* 341 and 369, respectively) as well as retrograde Diels-Alder rearrangement losses of 29 amu characteristic of noraporphine alkaloids (14). The ^{1}H-nmr spectra of both products indicated the loss of the aromatic AB system present in ring B and the splitting of the six-proton singlet due to the *gem*-dimethyl group into two singlets [δ1.44 (3H) and 0.84 (3H); δ1.45 (3H) and 0.87 (3H), respectively]. The uv spectra of both compounds were similar to those produced by aporphine alkaloids (10).

Both reduction products were *N*-methylated (formaldehyde/sodium borohydride) to yield *N*-methyltetrahydromelosmine (**8**) and *N*-methyl-*O,O*-dimethyltetrahydromelosmine (**9**), respectively. These products showed the addition of 14 amu in the ms (M^+ *m/e* 355 and 383, respectively) as well as Diels-Alder rearrangement losses of 43 amu typical of aporphine alkaloids (14). Their ^{1}H-nmr spectra showed the addition of signals due to the *N*-methyl groups [δ2.53 (3H)] in both compounds. Finally, the uv spectra of these products were similar to those produced by aporphine alkaloids (10).

Thus, melosmine (**2**) should be a 1,2,3,9-tetrasubstituted 7,7-dimethyltetrade-



	R_1	R_2
8	OH	OH
9	OCH_3	OCH_3
12	OCH_3	OH

hydroaporphine with two methoxy and two phenolic hydroxy groups. In order to determine the placement of these substituents, melosmine (**2**) was acetylated (acetic anhydride/pyridine). The product, *O,O*-diacetylmelosmine (**10**), showed a strong absorption in the ir spectrum at 1775 cm^{-1} for the carbonyl of the phenolic acetates. The mass spectrum (M^+ *m/e* 421; with two sequential losses of 42 amu) and ^{1}H-nmr spectrum [δ2.46 (3H,s) and 2.33 (3H,s)] indicated the addition of two acetate groups. A comparison of the ^{1}H-nmr spectra of melosmine (**2**) and its *O,O*-diacetyl derivative (**10**) allowed for the assignment of the methoxy groups at C–2 and C–3 and the hydroxy groups at C–1 and C–9. The C–11 proton was shifted upfield by 0.42 ppm in *O,O*-diacetylmelosmine (**10**) due to the shielding effect of an acetate group at C–1, thus indicating that melosmine (**2**) had a C–1 hydroxy group. This fact was further supported by the upfield shift of the C–2 methoxy from δ4.17 to 4.03 in *O,O*-diacetylmelosmine (**10**). The downfield shift of the protons at C–10 and C–8 (0.25 and 0.23 ppm, respectively) in the acetylated product indicated the presence of an acetoxy group at C–9 in **10** and, therefore, a hydroxy group at C–9 in melosmine (**2**). Thus, melosmine (**2**) is 2,3-dimethoxy-7,7-dimethyl-7*H*-dibenzo[*de,g*] quinolin-1,9-diol.[2] X-ray crystallographic analysis supported this proposal.

Melosmidine (**3**) was isolated as a yellow amorphous solid (mp 170–1°; 150 mg) having no optical activity. Its uv and ir spectra were similar to that of melosmine (**2**). A bathochromic shift in the uv spectrum upon the addition of base indicated that melosmidine (**3**) was probably phenolic. A mass spectrum of this compound showed an M^+ *m/e* 351 which was 14 amu higher than melosmine (**2**) (M^+ *m/e* 337) and 14 amu lower than *O,O*-dimethylmelosmine (**5**) (M^+ *m/e* 365). The ^{1}H-nmr spectrum of melosmidine (**3**) showed the same AB system [δ8.48 (1H, d, $J=5$ Hz) and 7.76 (1H, d, $J=5$ Hz) for C–5 and C–4, respectively], AMX system [δ8.80 (1H, d, $J=9$ Hz), 6.93 (1H, dd, $J=9$, 3 Hz) and 7.25 (1H, d, $J=3$ Hz) for C–11, C–10 and C–8, respectively] and quaternary *gem*-dimethyl group [δ1.72 (6H,s)] as did melosmine (**2**) and *O,O*-dimethylmelosmine (**5**). These data indicated that melosmidine (**3**) was a 1,2,3,9-tetrasubstituted-7,7-dimethyltetradehydroaporphine alkaloid. The only difference between their ^{1}H-nmr spectra was that melosmine (**2**) contained two methoxy signals (δ4.17 and 4.00), melosmidine (**3**) contained three methoxy signals (δ4.09, 4.05 and 3.90) and *O,O*-dimethylmelosmine (**5**) contained four methoxy signals (δ4.09, 4.05, 3.92 and 3.89).

The fact that melosmidine (**3**) was tetrasubstituted, showed three methoxy signals in the ^{1}H-nmr spectrum, and showed a bathochromic shift in the uv spectrum upon the addition of base, indicated that the remaining substituent was a phenolic hydroxy group. To confirm this, melosmidine (**3**) was treated with ethereal diazomethane to yield *O*-methylmelosmidine (**5**), which was isolated as a yellow oil. The product showed a M^+ *m/e* 365 (addition of 14 amu) and was identical (uv, ir, ms, ^{1}H-nmr) to *O,O*-dimethylmelosmine (**5**). Thus, melosmidine (**3**) was a monomethyl derivative of melosmine (**2**). Further support of this fact was provided by the reduction and subsequent *N*-methylation of melosmidine (**3**). Reduction with Zn/HCl afforded a tetrahydro-derivative (tetrahydromelosmidine) (**11**), which was then *N*-methylated (formaldehyde/sodium borohydride) to yield *N*-methyltetrahydromelosmidine (**12**). Spectral data obtained on these products (uv, ir, ms, ^{1}H-nmr) were consistent with those obtained with the reduction and subsequent *N*-methylation products of melosmine (**2**) and *O,O*-dimethylmelosmine (**5**). Thus, melosmidine (**3**) is a 1,2,3,9-tetrasubstituted 7,7-dimethyltetradehydroaporphine with three methoxy and one hydroxy group.

In order to determine the placement of the substituents, melosmidine (**3**) was acetylated (acetic anhydride/pyridine). The product, *O*-acetylmelosmidine (**13**), showed an absorption in the ir spectrum at 1765 cm^{-1} for the carbonyl of the

[2]The authors are grateful to Kurt L. Loening, Nomenclature Director, Chemical Abstracts Service, Columbus, Ohio, for providing the correct IUPAC nomenclature.

phenolic acetate. The mass spectrum (M^+ *m/e* 393; with a loss of 42 amu) and ^{1}H-nmr spectrum [δ2.33(3H,s)] indicated the addition of one acetate group. A comparison of the ^{1}H-nmr spectra of melosmidine (**3**) and its *O*-acetyl derivative (**13**) allowed for the assignment of the three methoxy groups at C–1, C–2 and C–3 and the hydroxy group at C–9. The lack of an upfield shift of the proton at C–11 in *O*-acetylmelosmidine (**13**) indicates that C–1 possessed a methoxy rather than an acetoxy group. In addition, since there is no shift of any of the methoxy signals, none of the methoxy groups are ortho to the acetoxy group. This suggested that C–1, C–2 and C–3 had methoxy substituents. Finally, the downfield shift of the protons at C–10 and C–8 (0.19 and 0.16 ppm, respectively) in the acetylated product indicated the presence of an acetoxy group at C–9 in **13** and, therefore, a hydroxy group at C–9 in melosmidine (**3**). Thus, melosmidine (**3**) should be 1,2,3-trimethoxy-7,7-dimethyl-7H-dibenzo[*de,g*]quinolin-9-ol.[2]

EXPERIMENTAL[3]

PLANT MATERIAL.—The plant material used in this investigation consisted of the stembark of *Guatteria melosma* Diels (Anonaceae) and was collected and identified in Peru in July, 1974. A herbarium specimen is deposited at the National Arboretum, U.S.D.A. (Number PR-80718).

EXTRACTION AND FRACTIONATION.—Dried ground stembark (27.2 kg) of *G. melosma* was placed in a stainless steel percolator and extracted exhaustively with ethanol (570 liters) until the extract gave a negative test with Dragendorff's reagent (15). The ethanol was concentrated in a wall evaporator and evaporated to dryness yielding a syrupy residue (1.18 kg). The ethanol extract was dissolved in chloroform (19 liters) and extracted with 2% aqueous citric acid (19 liters x 4). The chloroform was dried over anhydrous sodium sulfate and evaporated to leave a dark brown residue (fraction A–654 g). The combined aqueous acidic layers were basified to pH 9 with concentrated ammonium hydroxide and extracted with chloroform (76 liters x 3). The chloroform extracts were pooled, dried over anhydrous sodium sulfate, and evaporated to leave a brown non-quaternary alkaloid residue (fraction B–37.3 g).

ISOLATION OF ALKALOIDS.—Fraction B was dissolved in chloroform, adsorbed onto silicic acid (85 g) and chromatographed over a silicic acid column (400 g, 5 x 41 cm) prepared from a slurry in hexane-chloroform (1:1). Elution was begun with hexane-chloroform (1:1) and the polarity increased by the addition of chloroform and subsequently methanol (Column A).

Elution with chloroform-hexane (8:2) afforded a fraction (1.72 g) which was dissolved in chloroform and chromatographed over a silica gel column (60 g, 3 x 22 cm) packed with a slurry in chloroform. Elution with chloroform was initiated with 200 fractions (5 ml) being collected. Fractions 169–200 yielded melosmidine (**3**) (150 mg).

Thin-layer chromatographic analysis of the fraction eluted with chloroform (Column A) revealed the presence of two major alkaloidal spots (R_f 0.15 and 0.35; benzene-methanol, 85:15). This fraction (1.18 g) was dissolved in benzene-methanol (85:15) and applied to a preparative high pressure liquid chromatographic silica column which had been equilibrated with the solvent system before the addition of the sample. With a flow rate of 0.2 liters per minute, elution was begun with benzene-methanol (85:15). Fractions were collected according to changes in the refractive index and finally pooled according to thin-layer chromatographic analysis. The first fraction (1,200 ml) yielded, upon crystallization from ethanol, melosmine (**2**) (500 mg); the third fraction (1,800 ml) gave, upon crystallization from methanol, GMA 1 (mp 245°; 40 mg). The second fraction (1,100 ml) contained a mixture of the two alkaloids.

The fraction (1.95 g) eluted with 16% methanol-chloroform (Column A) was rechromatographed over a silica gel column packed with a slurry in benzene-methanol (85:15). Elution with the same solvent first afforded GMA 2 (mp 181°, 21 mg) and then isoboldine (**4**) (35 mg).

Finally, upon standing the fraction eluted with 75% methanol-chloroform (Column A) deposited oxoanolobine (**1**) (mp 270–5° dec., 50 mg).

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[3]Melting points were determined on a Thomas-Hoover Uni-melt Capillary Apparatus and are corrected. Optical rotations were measured on a Perkin-Elmer model 241 Polarimeter. Ultraviolet absorption spectra were obtained in methanol on a Perkin-Elmer model 202 Recording Spectrophotometer. Infrared spectra were taken in a potassium bromide pellet or in a chloroform solution on a Perkin-Elmer model 157 Infrared Recording Spectrophotometer. The ^{1}H-nuclear magnetic resonance spectra were taken on a 60MHz Hitachi Perkin-Elmer model R-24 High Resolution Spectrometer with either deuterated chloroform, deuterated methanol or trifluoroacetic acid as solvent with tetramethylsilane as the internal standard and chemical shifts recorded in δ (ppm) units. Low resolution mass spectra were taken on a LKB–900 Mass Spectrometer. High resolution mass spectra were taken on a Varian Mat, model CH5 Mass Spectrometer. High pressure liquid chromatography was done on a Waters-Prep LC/System 500 Liquid Chromatograph with a single Prep PAK-500/Silica cartridge. The adsorbents used for column chromatography were 100 mesh silicic acid (Mallinckrodt) and silica gel D–0 (CAMAG)-acid washed celite (Mallinckrodt) (4:1). Thin layer chromatography was done with 5 x 20 cm or 10 x 20 cm glass plates coated with 0.30 mm of silica gel DF–0 (CAMAG) and activated at 110° for one hour. Alkaloids were visualized by spraying with Dragendorff's reagent (15). All solvents were evaporated under reduced pressure at 40°.

MELOSMINE (**2**).—Melosmine (**2**) was isolated as light yellow cuboidal crystals; mp 104° (EtOH) (500 mg); $[\alpha]^{29}D$ 0° (c 1.0, MeOH); uv λ max (MeOH) 218 nm (sh) (log ε 4.34), 240 (sh) (4.41), 252 (4.44), 309 (3.76), 322 (3.78), 377 (3.96); uv λ max (MeOH+OH^-) 221 nm (log ε 4.41), 241 (sh) (4.41), 278 (4.40), 412 (3.85); uv λ max (MeOH+H^+) 223 nm (log ε 4.48), 240 (sh) (4.47), 279 (4.56), 331 (sh) (3.55), 450 (3.83); ir ν max (KBr) 3450 (br), 3350, 3200 (br), 1610, 1580, 1495, 1450, 1410, 1350, 1300, 1260, 1200, 1180, 1145, 1100, 1030, 975, 945, 822 cm^{-1}; ^1H-nmr ($CDCl_3$) 8.91 (1H, d, J=9 Hz) (C-11), 8.50 (1H, d, J=6 Hz) (C-5), 7.71 (1H, d, J=6 Hz) (C-4), 7.22 (1H, d, J=3 Hz) (C-8), 6.89 (1H, dd, J=9,3 Hz) (C-10), 4.17 (3H, s) (C-2 methoxy), 4.00 (3H, s) (C-3 methoxy), 1.73 (6H, s) (C-7 *gem*-dimethyl); ms *m/e* 338 (8%), 337 (M^+, 37), 323 (22), 322 (100), 307 (5), 306 (4), 292 (4), 290 (4), 289 (17), 264 (8), 261 (5), 208 (4), 161 (5), 153.5 (M^{++}, 6); high resolution mass measurement: found 337.1305, calcd. for $C_{20}H_{19}NO_4$ (337.1314). x-ray: space group C2/c with unit cell dimensions a=18.362 (5), b=13.975 (4), c=17.151 (6) °A, β=113.4 (2)°, V=4040 (2) °A^3 with Z=8. The positioning of the substituents was confirmed by the crystal structure analysis; however, the structure has not been refined sufficiently to completely confirm atom identification.

PREPARATION OF *O,O*-DIMETHYLMELOSMINE (**5**).—Melosmine (**2**) (200 mg) was dissolved in ethanol (10 ml) and treated with ethereal diazomethane[4] for 18 hours. The solution was evaporated to leave a residue, which, upon thin-layer chromatographic analysis (benzene-ethanol, 9:1), showed a single alkaloidal spot (R_f 0.25). The residue was chromatographed on a silica gel column (10 g, 1.5 x 14 cm) packed in benzene for the purpose of removing non-alkaloidal impurities. Elution with benzene and collection of the yellow band afforded *O,O*-dimethylmelosmine (**5**) (180 mg) as a yellow oil; $[\alpha]^{27}D$ 0° (c 1.0, MeOH); uv λ max (MeOH) 212 nm (log ε 4.24), 248 (4.27), 255 (4.23), 313 (sh) (3.73), 326 (3.86), 364 (3.81); uv λ max (MeOH+OH^-) same as MeOH spectrum; uv λ max (MeOH+H^+) 215 nm (log ε 4.23), 240 (4.21), 275 (4.37), 337 (sh) (3.37), 433 (3.72); ir ν max ($CHCl_3$) 2975, 2945, 2845, 1610, 1595, 1573, 1487, 1455, 1382, 1345, 1302, 1150, 1100, 1040, 1012, 970, 917 cm^{-1}; ^1H-nmr ($CDCl_3$) 8.93 (1H, d, J=9 Hz) (C-11), 8.54 (1H, d, J=6 Hz) (C-5), 7.74 (1H, d, J=6 Hz) (C-4), 7.27 (1H, d, J=3 Hz) (C-8), 6.94 (1H, dd, J=9,3 Hz) (C-10), 4.09 (3H, s) (C-2 methoxy), 4.05 (3H, s) (C-3 methoxy), 3.92 (3H, s) (C-9 methoxy), 3.89 (3H, s) (C-1 methoxy), 1.74 (6H, s) (C-7 *gem*-dimethyl); ms *m/e* 366 (10%), 365 (M^-, 46), 351 (26), 350 (100), 337 (4), 336 (9), 335 (5), 321 (6), 320 (24), 306 (5), 292 (6), 249 (10), 221 (4), 182.5 (M^{++}, 6), 175 (11), 167.5 (13), 160 (14).

PREPARATION OF TETRAHYDROMELOSMINE (**6**).—A solution of melosmine (**2**) (50 mg) in acetic acid-water (2:1, 2 ml) was treated with zinc powder (3 g) and concentrated hydrochloric acid (6 ml) (16). The reaction mixture was heated with stirring at 100° for 1.5 hours. The acidic solution was then basified to pH 8 with concentrated ammonium hydroxide solution and extracted with chloroform (30 ml x 4). Crystallization of the residue from methanol afforded tetrahydromelosmine (**6**) as pale yellow crystals (40 mg); mp 223°; $[\alpha]^{29}D$ 0° (c 1.0, $CHCl_3$); uv λ max (MeOH) 218 nm (log ε 3.90), 235 (sh) (3.69), 272 (sh) (3.71), 282 (3.75), 300 (sh) (3.59), 312 (sh) (3.51); uv λ max (MeOH+OH^-) 218 nm (log ε 3.90), 235 (sh) (3.69), 272 (sh) (3.67), 283 (3.73), 301 (3.65), 312 (3.60); uv λ max (MeOH+H^+) 218 nm (log ε 3.90), 235 (sh) (3.69), 272 (sh), (3.71), 283 (3.75), 302 (sh) (3.58), 316 (3.57); ir ν max (KBr) 3500–3300 (br), 2945, 2840, 1610, 1595, 1465, 1420, 1380, 1365, 1342, 1295, 1245, 1200, 1164, 1137, 1090, 1075, 1030, 1005, 925, 865, 825 cm^{-1}; ^1H-nmr ($CDCl_3$-CD_3OD, 1:1) 8.23 (1H, d, J=9 Hz) (C-11), 6.92 (1H, d, J=3 Hz) (C-8), 6.75 (1H, dd, J=9,3 Hz) (C-10), 3.96 (3H, s) (C-2 methoxy), 3.87 (3H, s) (C-3 methoxy), 1.44 (3H, s) (C-7 methyl), 0.84 (3H, s) (C-7 methyl); ms *m/e* 341 (M^+, 100%), 340 (82), 326 (24), 325 (32), 324 (64), 322 (8), 312 (11), 311 (13), 310 (30), 309 (6), 308 (8), 298 (30), 297 (17), 280 (6), 294 (7), 170.5 (M^{++}, 4).

PREPARATION OF *O,O*-DIMETHYLTETRAHYDROMELOSMINE (**7**).—*O,O*-Dimethylmelosmine (100 mg), dissolved in glacial acetic acid-water (2:1, 2 ml), was heated at 100° with stirring for 1.5 hours with zinc powder (3 g) and concentrated hydrochloric acid (6 ml) (16). The solution was basified with concentrated ammonium hydroxide to pH 8 and extracted with chloroform (20 ml x 4). The chloroform extracts were pooled, dried over anhydrous sodium sulfate, filtered, and evaporated to yield *O,O*-dimethyltetrahydromelosmine (**7**) (90 mg) as a light yellow oil; $[\alpha]^{29}D$ 0° (c 1.0, $CHCl_3$); uv λ max (MeOH) 222 nm (log ε 4.34), 236 (sh) (4.16), 274 (sh) (4.20), 286 (4.28), 301 (sh) (4.13); uv λ max (MeOH+OH^-) same as MeOH spectrum; uv λ max (MeOH+H^+) 224 nm (log ε 4.41), 288 (4.29), 303 (sh) (4.13); ir ν max ($CHCl_3$) 2945, 2845, 1610, 1588, 1465, 1418, 1378, 1364, 1340, 1300, 1090, 1075, 1050, 1030, 1002, 970, 945, 905 cm^{-1}; ^1H-nmr ($CDCl_3$) 8.25 (1H, d, J=9 Hz) (C-11), 7.00 (1H, d, J=3 Hz) (C-8), 6.83 (1H, d, J=9,3 Hz) (C-10), 3.98 (3H, s) (C-2 methoxy), 3.92 (3H, s) (C-3 methoxy), 3.86 (3H, s) (C-9 methoxy), 3.71 (3H, s) (C-1 methoxy), 1.45 (3H, s) (C-7 methyl), 0.87 (3H, s) (C-7 methyl); ms *m/e* 370 (19%), 369 (M^+, 79) 368 (58), 367 (8), 355 (5), 354 (17), 353 (15), 352 (11), 340 (9), 339 (27), 338 (100), 326 (13), 325 (12), 324 (6), 323 (8), 322 (7), 309 (4), 308 (9), 280 (4), 222 (4), 184.5 (M^{++}, 2).

PREPARATION OF *N*-METHYLTETRAHYDROMELOSMINE (**8**).—Formalin (37%, 0.5 ml) was added dropwise with stirring to a solution of tetrahydromelosmine (**6**) (30 mg) in methanol (5 ml). After one hour of stirring, sodium borohydride (100 mg) was added slowly to the solution, and stirring was continued for four hours. After evaporation, the resulting residue was diluted with water (20 ml) and acidified with dilute hydrochloric acid to pH 3. It was then basified with ammonium hydroxide to pH 9, and extracted with chloroform (30 ml x 3). The combined chloroform extracts were dried over anhydrous sodium sulfate and evaporated to a residue. The residue was placed on a silica gel column (4 g, 1 x 9.5 cm) and eluted with chloroform-methanol (10:1). Thirty (1 ml) fractions were collected. Fractions 12–18 were combined

[4]Diazald-Aldrich Chemical Co.

to yield *N*-methyltetrahydromelosmine (**8**) as a yellow oil (18 mg); $[\alpha]^{29}D$ 0° (c 0.8, MeOH); uv λ max (MeOH) 220 nm (log ε 4.43), 270 (sh) (4.13), 286 (4.19), 301 (sh) (4.15), 311 (sh) (4.06); uv λ max (MeOH+OH^-) 220 nm (log ε 4.43), 288 (sh) (4.17), 302 (4.17), 312 (sh) (4.15); uv λ max (MeOH+H^+) 220 nm (log ε 4.43), 275 (sh) (4.22), 282 (4.23), 304 (4.11), 315 (4.11); ir ν max (KBr) 3500–3400 (br), 2945, 2860, 1610, 1595, 1464, 1420, 1374, 1333, 1295, 1200, 1160, 1150, 1122, 1090, 1070, 1005, 923, 895, 758 cm^{-1}; ^1H-nmr ($CDCl_3$) 8.26 (1H, d, J=9 Hz) (C-11), 6.92–6.62 (2H, m) (C-8, 10), 3.96 (3H, s) (C-2 methoxy), 3.87 (3H, s) (C-3 methoxy), 3.15 (1H, s), 3.06–2.83 (3H, m), 2.67 (1H, s), 2.53 (3H, s) (N-methyl), 1.54 (3H, s) (C-7 methyl), 0.87 (3H, s) (C-7 methyl); ms *m/e* 357 (3%), 356 (17), 355 (M^+, 68), 354 (16), 340 (17), 339 (23), 338 (100), 337 (7), 336 (9), 326 (9), 325 (7), 324 (24), 323 (6), 322 (8), 313 (10), 312 (43), 309 (5), 308 (8), 298 (4), 297 (11), 296 (6), 294 (7), 281 (4).

PREPARATION OF *N*-METHYL-*O*,*O*-DIMETHYLTETRAHYDROMELOSMINE (**9**).—*O*,*O*-Dimethyltetrahydromelosmine (**7**) (50 mg) was treated with formalin (37%, 0.5 ml) and sodium borohydride (100 mg) as previously described. The resultant residue was chromatographed on a silica gel column (4 g, 1.3 x 10 cm) packed in chloroform. Twenty fractions (2 ml) were collected, and fractions 4–9 were combined to give *N*-methyl-*O*,*O*-dimethyltetrahydromelosmine (**9**) (30 mg) as a light yellow oil; $[\alpha]^{30}D$ 0° (c 0.9, MeOH); uv λ max (MeOH) 220 nm (log ε 4.46), 273 (sh) (4.15), 288 (4.29), 303 (sh) (4.16); uv λ max (MeOH+OH^-) same as MeOH spectrum; uv λ max (MeOH+H^+) 221 nm (log ε 4.46), 235 (sh) (4.23), 288 (4.38), 303 (4.21); ir ν max ($CHCl_3$) 2945, 1608, 1585, 1460, 1415, 1370, 1348, 1330, 1300, 1095, 1070, 1025, 1000 cm^1; ^1H-nmr ($CDCl_3$) 8.32 (1H, d, J=9 Hz) (C-11), 7.01 (1H, d, J=3 Hz) (C-8), 6.82 (1H, dd, J=9,3 Hz) (C-10), 4.00 (3H, s) (C-2 methoxy), 3.93 (3H, s) (C-3 methoxy), 3.87 (3H, s) (C-9 methoxy), 3.71 (3H, s) (C-1 methoxy), 3.10 (1H, s), 2.8–3.0 (3H, s), 2.68 (1H, s), 2.53 (3H, s) (N-methyl), 1.56 (3H, s) (C-7 methyl), 0.88 (3H, s) (C-7 methyl); ms *m/e* 384 (6%), 383 (M^+, 26), 382 (5), 368 (4), 354 (4), 353 (26), 352 (100), 340 (9), 337 (3), 336 (5), 322 (6).

PREPARATION OF *O*,*O*-DIACETYLMELOSMINE (**10**).—Acetic anhydride (1 ml) was added to a solution of melosmine (**2**) (70 mg) in pyridine (1 ml) and set aside at room temperature for 24 hours. The solution was then chilled, and cold distilled water (5 ml) was added. The aqueous solution was then basified with concentrated ammonium hydroxide to pH 9 and extracted with chloroform (5 ml x 3). The chloroform extracts were combined, dried over anhydrous sodium sulfate, and evaporated to an oily residue. The residue was placed on a silica gel column (10 g, 1.5 x 14 cm) and eluted with chloroform-methanol (99:1). One-hundred fractions (1 ml) were collected. Fractions 11–20 yielded *O*, *O*-diacetylmelosmine (**10**) as a yellow oil (32 mg); $[\alpha]^{25}D$ 0° (c 1.4, $CHCl_3$); uv λ max (MeOH) 207 nm (sh) (log ε 4.17), 239 (4.33), 251 (sh) (4.29), 323 (3.93), 354 (3.91); uv λ max (MeOH+OH^-) 212 nm (log ε 4.31), 238, (4.38), 257 (sh) (4.10), 280 (sh) (3.91), 325 (sh) (3.77), 355 (3.82), 378 (3.80); uv λ max (MeOH+H^+) 214 nm (log ε 4.27), 238 (4.19), 272 (4.43), 332 (sh) (3.38), 405 (3.69); ir ν max (KBr) 2970, 2940, 2860, 1775, 1610, 1595, 1560, 1490, 1450, 1392, 1372, 1345, 1305, 1200, 1090, 1030, 1012, 980, 955, 875, 840, 755 cm^{-1}; ^1H-nmr ($CDCl_3$) 8.56 (1H, d, J=6 Hz) (C-5), 8.49 (1H, d, J=9 Hz) (C-11), 7.77 (1H, d, J=6 Hz) (C-4), 7.45 (1H, d, J=2 Hz) (C-8), 7.14 (1H, dd, J=9, 2 Hz) (C-10), 4.03 (6H, s) (C-2,3 methoxy), 2.46 (3H, s) (C-1 acetoxy), 2.33 (3H, s) (C-9 acetoxy), 1.75 (6H, s) (C-7 *gem*-dimethyl); ms *m/e* 421 (M^+, 32%), 379 (45), 364 (100), 354 (4), 337 (11), 336 (10), 322 (38), 307 (6), 306 (11), 290 (5), 289 (11), 278 (6), 264 (6), 261 (5), 235 (5), 208 (4), 207 (4), 149 (5), 87 (6), 85 (36), 83 (53), 57 (10), 55 (9).

MELOSMIDINE (**3**).—Melosmidine (**3**) was isolated as a yellow amorphous solid (150 mg); mp 170–71°; $[\alpha]^{29}D$ 0° (c 1.0, MeOH); uv λ max (MeOH) 219 nm (sh) (log ε 3.90), 242 (4.03), 328 (3.30), 365 (3.32); uv λ max (MeOH+OH^-) 224 nm (sh) (log ε 3.99), 240 (4.05), 282 (sh) (3.58), 394 (3.32); uv λ max (MeOH+H^+) 229 nm (sh) (log ε 3.98), 239 (4.05), 277 (3.84), 438 (3.15); ir ν max (KBr) 3500–3300 (br), 2920, 2845, 1608, 1595, 1570, 1490, 1475, 1450, 1385, 1305, 1245, 1200, 1140, 1085, 1038, 1008, 970, 950, 820, 805 cm^{-1}; ^1H-nmr ($CDCl_3$) 8.80 (1H, d, J=9 Hz) (C-11), 8.48 (1H, d, J=5 Hz) (C-5), 7.76 (1H, d, J=5 Hz) (C-4), 7.25 (1H, d, J=3 Hz) (C-8), 6.93 (1H, dd, J=9,3 Hz) (C-10), 4.09 (3H, s) (C-2 methoxy), 4.05 (3H, s) (C-3 methoxy), 3.90 (3H, s) (C-1 methoxy), 1.72 (6H, s) (C-7 *gem*-dimethyl); ms *m/e* 352 (12%), 351 (M^+, 44), 337 (24), 336 (100), 320 (5), 307 (8), 306 (3), 292 (6), 278 (7), 235 (18), 207 (10), 175.5 (M^{++}, 1), 153 (5), 151 (9).

PREPARATION OF *O*-METHYLMELOSMIDINE (**5**).—Treatment of melosmidine (**3**) (30 mg) with excess ethereal diazomethane[4] for 24 hours followed by evaporation yielded a residue which was chromatographed on a silica gel column (15 g, 1.5 x 20 cm) packed in chloroform-methanol (99:1). Elution with chloroform-methanol (99:1) and collection of the yellow band afforded *O*-methylmelosmidine (**5**) as a yellow oil (10 mg); $[\alpha]^{29}D$ 0° (c 1.0, MeOH); ir ν max ($CHCl_3$) 2975, 2945, 2845, 1610, 1595, 1573, 1487, 1455, 1382, 1345, 1302, 1150, 1100, 1040, 1012, 970, 917 cm^{-1}; ms *m/e* 366 (11%), 365 (M^+, 44), 351 (23), 350 (100), 337 (1), 336 (2), 335 (4), 321 (6), 320 (24), 306 (4), 292 (5), 249 (12), 221 (3), 182.5 (M^{++}, 9), 175 (14), 167 (16), 160 (18). The product was identical (uv, ir, ms, ^1H-nmr) with *O*,*O*-dimethylmelosmine (**5**).

PREPARATION OF TETRAHYDROMELOSMIDINE (**11**).—Melosmidine (**3**) (50 mg) in acetic acid-water (2:1, 2 ml) was treated with zinc powder (3 g) and concentrated hydrochloric acid (6 ml) as previously described (16). The resulting residue was crystallized from methanol to yield tetrahydromelosmidine (**11**) (46 mg) as pale yellow needles; mp 236°; $[\alpha]^{30}D$ 0° (c 0.5, MeOH); uv λ max (MeOH) 218 nm (log ε 4.52), 237 (sh) (4.24), 273 (sh) (4.31), 284 (4.39), 300 (sh) (4.25); uv λ max (MeOH+OH^-) 218 nm (log ε 4.52), 237 (sh) (4.24), 291 (4.33), 320 (4.03); uv λ max (MeOH+H^+) 218 nm (log ε 4.52), 237 (sh) (4.24), 275 (sh) (4.29), 287 (4.39), 302 (sh) (4.23); ir ν max (KBr) 3315, 3260, 2970, 2940, 2870, 2830, 1608, 1588, 1460, 1418, 1405, 1375, 1342, 1295, 1240, 1220, 1205, 1165, 1145, 1120, 1095, 1075, 1062, 1030, 1008, 970, 950, 920, 860, 820, 792 cm^{-1};

^{1}H-nmr ($CDCl_3$) 8.14 (1H, d, J=9 Hz) (C-11), 6.94 (1H, d, J=2 Hz) (C-8), 6.73 (1H, dd, J=9, 2 Hz) (C-10), 3.96 (3H, s) (C-2 methoxy), 3.91 (3H, s) (C-3 methoxy), 3.70 (3H, s) (C-1 methoxy), 1.44 (3H, s) (C-7 methyl), 0.84 (3H, s) (C-7 methyl); ms *m/e* 356 (16%) 355 (M^+, 70), 354 (58), 353 (6), 341 (7), 340 (17), 339 (14), 338 (7), 326 (10), 325 (26), 324 (100), 312 (12), 311 (12), 310 (9), 309 (8), 308 (7), 296 (5), 295 (5), 294 (9), 281 (4), 280 (5), 266 (4), 222 (4), 177.5 (M^{++}, 2), 177 (5).

PREPARATION OF *N*-METHYLTETRAHYDROMELOSMIDINE (**12**).—Tetrahydromelosmidine (**11**) (40 mg) was treated with formalin (37%, 0.5 ml) and sodium borohydride (100 mg) as previously described. The resulting residue (33 mg) was chromatographed on a silica gel column (4 g, 1 x 9.5 cm) packed in chloroform-methanol (10:1). Thirty (1 ml) fractions were collected and fractions 10–15 were combined to give *N*-methyltetrahydromelosmidine (**12**) (29 mg) as a pale yellow oil; $[\alpha]^{23}D$ 0° (c 1.4, $CHCl_3$): uv λ max (MeOH) 220 nm (log ε 4.26), 270 (sh) (3.02), 288 (4.11), 301 (sh) (4.02); uv λ max (MeOH+OH^-) 220 nm (log ε 4.26), 300 (4.10), 321 (sh) (3.85); uv λ max (MeOH+H^+) 223 nm (log ε 4.26), 231 (sh) (4.11), 290 (4.19), 303 (sh) (4.03); ir ν max (KBr) 3400 (br), 2960, 2935, 2860, 1610, 1585, 1460, 1415, 1365, 1345, 1330, 1295, 1205, 1125, 1090, 1070, 1022, 998, 940, 920, 755 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 8.24 (1H, d, J=9 Hz) (C-11), 6.93 (1H, d, J=2 Hz) (C-8), 6.74 (1H, dd, J=9, 2 Hz) (C-10), 3.99 (3H, s) (C-2 methoxy), 3.92 (3H, s) (C-3 methoxy), 3.68 (3H, s) (C-1 methoxy), 3.10 (1H, s), 3.0–2.75 (3H, m), 2.67 (1H, s), 2.53 (3H, s) (N-methyl), 1.52 (3H, s) (C-7 methyl), 0.87 (3H, s) (C-7 methyl); ms *m/e* 370 (8%), 369 (M^+, 31), 368 (6), 354 (4), 340 (7), 339 (26), 338 (100), 326 (9), 322 (5), 311 (4), 308 (6), 295 (2).

PREPARATION OF *O*-ACETYLMELOSMIDINE (**13**).—Melosmidine (**3**) (30 mg) in pyridine (1 ml) was treated with acetic anhydride (1 ml) as previously described. The residue was chromatographed on a silica gel column (4 g, 1 x 9.5 cm) packed in chloroform-methanol (10:1). Twenty fractions (1 ml) were collected and fractions 4–7 were combined to give *O*-acetylmelosmidine (**13**) (15 mg) as a pale yellow oil; $[\alpha]^{29}D$ 0° (c 1.0, $CHCl_3$); uv λ max (MeOH) 210 nm (sh) (log ε 4.20), 241 (4.39), 255 (sh) (4.33), 308 (sh) (3.90), 322 (4.02), 345 (sh) (3.93), 358 (3.96); uv λ max (MeOH+OH^-) 211 nm (sh) (log ε 4.31), 237 (4.40), 258 (sh) (4.24), 329 (3.87), 366 (3.92); uv λ max (MeOH+H^+) 227 nm (log ε 4.30), 237 (4.21), 274 (4.46), 324 (sh) (3.57), 409 (3.82); ir ν max (KBr) 2965, 2940, 2855, 1765, 1608, 1590, 1488, 1455, 1388, 1345, 1300, 1288, 1210 (br), 1100, 1042, 1014, 970, 960, 938, 910, 835 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 8.95 (1H, d, J=9 Hz) (C-11), 8.53 (1H, d, J=6 Hz) (C-5), 7.73 (1H, d, J=6 Hz) (C-4), 7.41 (1H, d, J=2 Hz) (C-8), 7.12 (1H, dd, J=9, 2 Hz) (C-10), 4.09 (3H, s) (C-2 methoxy), 4.08 (3H, s) (C-3 methoxy), 3.94 (3H, s) (C-1 methoxy), 2.33 (3H, s) (C-9 acetoxy), 1.72 (6H, s) (C-7 *gem*-dimethyl); ms *m/e* 393 (M^+, 50%), 378 (100), 351 (8), 350 (10), 348 (5), 336 (41), 321 (6), 320 (80), 307 (13), 306 (25), 292 (11), 279 (4), 278 (11), 277 (8), 262 (5), 249 (7), 235 (13), 207 (6), 206 (6), 178 (6), 43 (44).

ISOBOLDINE (**4**).—Isoboldine was isolated as colorless cuboidal crystals (35 mg); mp 125–6°; $[\alpha]^{28}D$+58° (c 1.0, EtOH). The substance was identical (mp, mmp, ir, ^{1}H-nmr, ms) with an authentic sample available in our laboratory.

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LITERATURE CITED

1. "Gray Herbarium Index", Vol. 5, G. K. Hall and Co., Boston, 1968, pp. 180–88.
2. W. H. Harris and T. A. Geisman, *J. Org. Chem.*, **30**, 432 (1965).
3. M. Hasegawa, M. Soyo and A. Lira, *Acta Cient. Venez.*, **23**, 165 (1972).
4. J. W. Skiles and M. P. Cava, *J. Org. Chem.*, **44**, 409 (1979).
5. C. C. Hsu, R. H. Dobberstein, G. A. Cordell and N. Farnsworth, *Lloydia*, **40**, 505 (1977).
6. *Ibid.*, p. 152.
7. G. Corrado, G. B. Marini-Bettalo and D. Vecchi, *Gazz. Chim. Ital.*, **105**, 1207 (1975).
8. R. G. Enqiquez, M. A. Chavez and F. Jauregui, *Phytochemistry*, **19**, 2024 (1980).
9. C. H. Phoebe, Jr., P. L. Schiff, Jr., J. E. Knapp and D. J. Slatkin, *Heterocycles*, **14**, 1977 (1980).
10. M. Shamma, "The Isoquinoline Alkaloids", Academic Press, New York, 1972, pp. 221–2.
11. *Ibid.*, pp. 251–5.
12. H. Guinaudeau, M. Leboeuf and A. Cave, *Lloydia*, **38**, 275 (1975).
13. M. Shamma and R. L. Castenson, "The Alkaloids", Vol. 14, R. H. F. Manske, Ed., Academic Press, New York, 1973, pp. 257–61.
14. H. Budzikiewicz, C. Djerassi and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Vol. 1, Holden-Day, San Francisco, 1964, pp. 178–9.
15. R. Munier and M. Maceboeuf, *Bull. Soc. Chim. Biol.*, **33**, 846 (1951).
16. S. M. Kupchan, M. I. Suffness and E. M. Gordon, *J. Org. Chem.*, **35**, 1683 (1970).