Alkaloids of *Nelumbo lutea* (Willd.) pers. (Nymphaeaceae)

STEVEN G. ZELENSKI

Abstract □ A phytochemical investigation of an alcoholic extract of the petioles of *Nelumbo lutea* resulted in the identification of the alkaloids *N*-methylasimilobine, anonaine, and roemerine. The alkaloids nuciferine, armepavine, *N*-nornuciferine, and *N*-norarmepavine, previously reported in the whole plant, were also identified.

Keyphrases □ *Nelumbo lutea*—alcoholic extract of petioles, various alkaloids identified □ Alkaloids, various—identified in alcoholic extract of petioles of *Nelumbo lutea*

Nelumbo lutea (Willd.) pers. (Nymphaeaceae), or American lotus, is widely distributed in the United States and can be found from Louisiana north to Wisconsin. In previous phytochemical investigations, the alkaloids nuciferine, armepavine, *N*-nornuciferine, and *N*-norarmepavine were isolated from leaves and stems of this species (1).

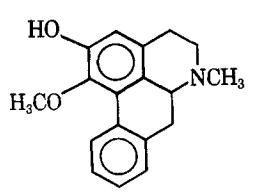
A 2-propanol extract of the petioles was fractionated to yield an alkaloid fraction. Chromatography of this fraction was followed by combined GLC–mass spectrometry of the alkaloids. Analysis of the spectra and comparison with standard materials resulted in the identification of the benzylisoquinoline alkaloids *N*-methylasimilobine (I), anonaine (II), and roemerine (III).

The previously reported alkaloids (nuciferine, armepavine, *N*-nornuciferine, and *N*-norarmepavine) also were identified in this extract. Since only trace amounts of each alkaloid were isolated by semipreparative GLC (2) for melting-point determinations or derivatization, no information is available on their stereochemistry. All of these alkaloids previously were isolated from the Asiatic lotus, *N. nucifera* Gaertn. (Nymphaeaceae) (3). The alkaloid nuciferine has been shown to possess an inhibitory action toward acetylcholine in rat Renshaw cells (4).

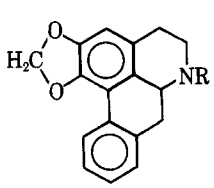
EXPERIMENTAL

Plant Material—The petioles of *N. lutea* were collected in July 1975[1].

Extraction—Fresh plant material of *N. lutea* (18 kg) was homogenized in a large blender at 5° with 10 liters of 2-propanol. The extract was filtered, and the marc was allowed to macerate with another 20 liters of 2-propanol. The combined filtrates were evaporated *in vacuo* at 37° to leave a dark-green syrupy extract (1.2 kg).



I



II: R = H
III: R = CH_3

Fractionation—This extract was acidified with 2 liters of 10% acetic acid and extracted with 400 ml each of ether, ethyl acetate, and chloroform. The acidic aqueous layer was made basic with 28% NH_4OH and extracted with 2 × 500 ml of chloroform. The chloroform residue was evaporated *in vacuo* to yield a tan crystalline solid (225 mg). This material was further purified by silica gel[2] column chromatography (140 g, 2 × 65 cm) and elution with increasing concentrations of methanol in chloroform to which 1% pyridine had been added.

Procedures—*Methylation of* N-*Nornuciferine and* N-*Norarmepavine*—Isolated *N*-nornuciferine and *N*-norarmepavine were methylated with formaldehyde–formic acid (5) to yield nuciferine and armepavine, respectively.

Combined GLC–Mass Spectrometry—The fractions from silica gel chromatography were injected directly into a 2-m × 2-mm i.d. glass column containing 1.5% OV-17–1.95% QF-1 on Varaport 30. The column temperature was programmed from 160 to 260° at 2°/min. The injection temperature was 230°. The carrier gas was helium at 28 ml/min. The effluent from the column entered the mass spectrometer through a glass jet separator maintained at 220°, and the ion source temperature was maintained at 220°. The ionizing voltage was 70 ev, and the accelerating voltage was 12,600 to 900 ev. Spectra were recorded every 10 sec on a low-resolution mass spectrometer interfaced with a data reduction system[3].

Identification of I—The GLC retention time was 800 sec (standard 800 sec). The mass spectrum showed ions at M^+ *m/e* 281 (87%), 280 (100), 266 (77), 250 (48), 178 (57), 165 (40), and 152 (25) [standard M^+ *m/e* 281 (85%), 280 (100), 266 (70), 250 (52), 178 (57), 165 (37), and 152 (25)]. The melting point was 194–195° [lit. (6) mp 195–196°].

Identification of II—The GLC retention time was 1040 sec (standard 1033 sec). The mass spectrum showed ions at M^+ *m/e* 265 (15%), 264 (82), 176 (9), 151 (49), 102 (100), and 88 (60) [standard M^+ *m/e* 265 (10%), 264 (84), 176 (11), 151 (51), 102 (100), and 88 (57)]. The melting point was 120–121° [lit. (3) mp 118–121°].

Identification of III—The GLC retention time was 900 sec (standard 903 sec). The mass spectrum showed ions at M^+ *m/e* 279 (24%), 278 (100), 262 (13), 236 (9), 204 (5), 178 (67), and 165 (24) [standard M^+ *m/e* 279 (20%), 278 (100), 262 (11), 236 (11), 204 (5), 178 (69), and 165 (25)]. The melting point was 100–101° [lit. (6) mp 100–101°].

Identification of Nuciferine—The GLC retention time was 1430 sec (standard 1410 sec). The mass spectrum showed ions at M^+ *m/e* 295 (100%), 294 (56), 280 (38), 265 (38), 252 (23), and 139 (11) [standard M^+ *m/e* 295 (100%), 294 (55), 280 (35), 265 (35), 252 (23), and 139 (10)]. The melting point was 164–165° [lit. (6) mp 162–163°].

Identification of Armepavine—The GLC retention time was 1028 sec (standard 1030 sec). The mass spectrum showed ions at M^+ *m/e* 313 (0.09%), 206 (49), 190 (10), 177 (6), 132 (50), 118 (18), 107 (77), and 91 (43) [standard M^+ *m/e* 313 (0.08%), 206 (48), 190 (11), 177 (6), 132 (52), 118 (18), 107 (75), and 91 (45)]. The melting point was 167–168° [lit. (1) mp 166–167°].

Identification of *N*-Nornuciferine—The GLC retention time was 930 sec. The mass spectrum showed ions at M^+ *m/e* 281 (49%), 280 (78), 266 (20), 264 (60), 251 (17), 221 (23), 207 (27), 194 (52), and 165 (100). Methylation of this compound yielded a compound whose mass spectrum was identical with nuciferine.

Identification of *N*-Norarmepavine—The GLC retention time was 1150 sec. The mass spectrum showed ions at M^+ *m/e* 299 (0.15%), 192 (93), 176 (100), 159 (22), 148 (68), 146 (21), and 131 (35). Methylation of this compound yielded a compound whose mass spectrum was identical with armepavine.

[1] The plant material was identified as *N. lutea* (Willd.) pers. (Nymphaeaceae) by Dr. Dale Thomas, Department of Botany, Northeast Louisiana University. A voucher specimen (SZ-03:069) is available for inspection at the Herbarium of the Department of Pharmacy, Northeast Louisiana University.

[2] Hi-Flosil, 60–200 mesh, Applied Science Laboratories.

[3] Du Pont 321 Dimespec and 320 data system.

REFERENCES

(1) S. M. Kupchan, B. Dasgupta, E. Jujita, and M. L. King, *Tetrahedron*, **19**, 227 (1963).

(2) S. G. Zelenski, Ph.D. thesis, University of Rhode Island, Kingston, R.I., 1973.

(3) J. Kunitomo, Y. Nagai, Y. Okamoto, and H. Furukawa, *J. Pharm. Soc. Jpn.*, **90** (9), 165 (1970).

(4) A. W. Duggan, D. Lodge, T. J. Bixcoe, and P. M. Headley, *Arch. Int. Pharmacodyn. Ther.*, **204** (1), 147 (1973).

(5) R. N. Icke and B. B. Wisegarver, "Organic Synthesis," coll. vol. III, Wiley, New York, N.Y., 1955, p. 723.

(6) M. Tomita, Y. Watanabe, M. Tomita, and H. Furukawa, *J. Pharm. Soc. Jpn.*, **81**, 469 (1961).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 25, 1976, from the *School of Pharmacy, Northeast Louisiana University, Monroe, LA 71203.*

Accepted for publication January 31, 1977.

The author thanks Dr. Jun-ichi Kunitomo for samples of roemerine, *O*-nornuciferine, anonaine, nuciferine, and armepavine.

GLC Microanalyses of Phenacetin and Acetaminophen Plasma Levels

M. A. EVANS and R. D. HARBISON ˣ

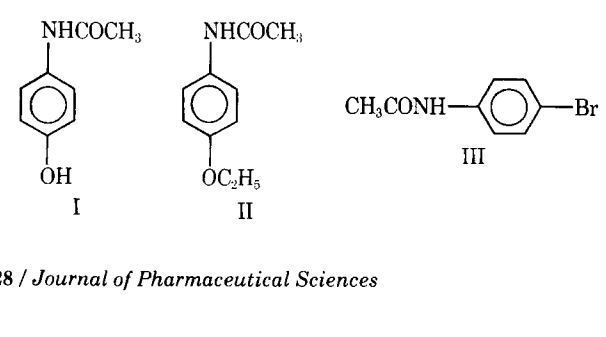
Abstract □ A GLC method utilizing a flame-ionization detector is described for the simultaneous analysis of acetaminophen and phenacetin in plasma. *p*-Bromoacetanilide is used as an internal standard. The drugs are extracted with ether from plasma diluted with 1 *M* phosphate buffer (pH 7.4). The ether extract is evaporated to dryness under nitrogen, and the residue is dissolved in 300 μl of ethyl acetate. The ethyl acetate is transferred to a microcentrifuge tube (0.4 ml), and the sample is evaporated in a vacuum centrifuge. Then the residue is redissolved in 0.2 *M* trimethylanilinium hydroxide in methanol for GLC analysis. Extraction efficiency of added phenacetin and acetaminophen in plasma at concentrations of 1–10 μg/ml was complete, and the limit of detection in plasma was less than 0.1 μg.

Keyphrases □ Phenacetin—GLC analysis in presence of acetaminophen, plasma □ Acetaminophen—GLC analysis in presence of phenacetin, plasma □ GLC—simultaneous analyses, phenacetin and acetaminophen in plasma □ Analgesics—phenacetin and acetaminophen, simultaneous GLC analyses in plasma

Acetaminophen (I) and phenacetin (II) are commonly used analgesics available without prescription. Acute overdose with either compound can produce a dose-dependent, potentially fatal hepatic necrosis (1). Renal tubular necrosis and hypoglycemic coma also can occur (2). Chronic overdosage of I or II has been reported to produce blood dyscrasia including methemoglobinemia, hemolytic anemia, and thrombocytopenia (3). Thus, toxicity coupled with widespread use necessitates specific and rapid detection of the compounds in biological specimens.

A sensitive and specific method for the estimations of I and II in plasma and urine by a GLC technique using trimethylsilyl derivatives was reported recently (4). However, it has a significant time requisite and absolute dryness is required to achieve effective silylating reactions. Furthermore, the continued use of silylating agents leads to rapid detector contamination. A GLC method for I based on sequential alkylation, followed by on-column derivatization with trimethylanilinium hydroxide, also was demonstrated (5). This method is time consuming and specific only for I (5).

Other reported methods for the estimation of I or II in biological specimens are nonspecific, lack sensitivity, or require a large sample volume (6–9). Therefore, a GLC method was developed for the rapid analysis of small amounts of I and II in plasma using an internal standard for quantitation. The sensitivity of this method was greater than 0.1 μg/ml of plasma for both compounds, and no interference was observed with peak identification.



I II III

EXPERIMENTAL

Apparatus—A gas chromatograph[1] equipped with a flame-ionization detector was maintained with gas flows of 5, 30, and 150 ml/min for nitrogen, hydrogen, and air, respectively. A column oven temperature of 165° was used; the injection port and detector were maintained at 300°.

Column—A glass column, 2 m × 2 mm, was packed with 3% SP 2250 on 80–100-mesh Chromosorb W, AW/DMCS[2]. Before packing, the column was rinsed with methanol and acetone, dried, and conditioned 6 hr with a 10% solution of dimethyldichlorosilane[3] in toluene to silylate reactive sites. Following silylation, the column was again rinsed with acetone and dried.

Analytical Procedures—Plasma, 0.1–0.5 ml, was transferred to a 13-ml glass-stoppered centrifuge tube, mixed with the internal standard *p*-bromoacetanilide[4] (III) (10 μg/ml), and diluted with an equal volume of 1 *M* phosphate buffer (pH 7.4). The plasma was extracted with reagent grade ether (7 ml) by stirring on a vortex mixer (30 sec). The ether was transferred by disposable pipet to a dry, clean, 12-ml centrifuge tube and evaporated to dryness in a water bath (45°) using a nitrogen flow. Then the residue was dissolved in 300 μl of spectrophotometric grade ethyl acetate[5] and transferred to a microcentrifuge tube[6] (0.4 ml).

The sample was taken to dryness under vacuum in a rotary vacuum centrifuge[7] and redissolved in 4–8 μl of trimethylanilinium hydroxide (0.2 *M* in methanol)[8]. Samples of 1–3 μl were injected into the gas chro-

[1] Varian model 2100.
[2] Supelco, Bellefonte, Pa.
[3] Applied Science Laboratories, State College, Pa.
[4] Aldrich Chemicals, Atlanta, Ga.
[5] Mallinckrodt Chemicals, St. Louis, Mo.
[6] Brinkmann Instruments, Westbury, N.Y.
[7] Speed Vac concentrator, Savant Instruments, Hicksville, N.Y.
[8] Methulate, Pierce Chemicals, Rockford, Ill.