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Chemical Constituents of *Cocculus carolinus* D.C. (Menispermaceae)

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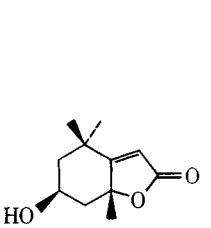
Abstract □ A phytochemical investigation of an ethanolic extract of the stems and leaves of *Cocculus carolinus* D.C. (Menispermaceae) resulted in the isolation and characterization of six compounds: the cyclitols, (+)-quercitol and (−)-viburnitol; the lactone, loliolide; and the alkaloids, sinoacutine, magnoflorine, and palmatine. In each case, the identity of the constituent was confirmed by spectral and mixed melting-point comparison with authentic samples.

Keyphrases □ *Cocculus carolinus* D.C. (Menispermaceae)—isolation and identification of chemical constituents □ (+)-Quercitol—isolated and identified from *Cocculus carolinus* D.C. □ (−)-Viburnitol—isolated and identified from *Cocculus carolinus* D.C. □ Loliolide—isolated and identified from *Cocculus carolinus* D.C. □ Sinoacutine—isolated and identified from *Cocculus carolinus* D.C. □ Magnoflorine—isolated and identified from *Cocculus carolinus* D.C. □ Palmatine—isolated and identified from *Cocculus carolinus* D.C.

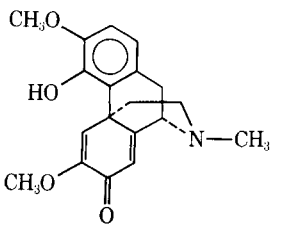
Cocculus carolinus D.C., a species native to the southeastern United States, belongs to a family (Menispermaceae) from which over 99 alkaloid constituents have been isolated (1). In a preliminary phytochemical study, Wall *et al.* (2) indicated the presence of alkaloids in the leaves of this species. Thus, a systematic phytochemical investigation was initiated on this species.

An ethanol extract of the stems and leaves was fractionated into nonquaternary alkaloids (phenolic and nonphenolic), quaternary alkaloids, and acid–neutral fractions. On concentration of the ethanolic extract, a crystalline mixture deposited. Column chromatography of this material on cellulose resulted in the isolation and identification of the cyclitols, (+)-quercitol and (−)-viburnitol. (+)-Quercitol has been previously isolated from several other members of the Menispermaceae: *C. trilobus* D.C. and *C. laurifolius* D.C. (3), *Legnephora moorei* Miers (4), *Cissampelos pareira* L. (5), and *Tiliacora racemosa* Colebr. (6). (−)-Viburnitol has been isolated from several plant families, including two members of the Menispermaceae: *Stephania hernandifolia* Walp. (7) and *Menispermum canadense* L. (8).

Chromatography of the nonquaternary nonphenolic alkaloid fraction resulted in the isolation and characterization of the nonalkaloid, loliolide (I). Loliolide was



I



II

first isolated in 1964 by Hodges and Porte (9) from an ether extract of *Lolium perenne* L. (Graminae). In that same year, Wada and Satoh (10) obtained this same compound from the leaves of *Digitalis purpurea* L. (Scrophulariaceae). In 1969, Pailer and Haschke-Hofmeister (11) isolated loliolide from *Plantago major* L. (Plantaginaceae). This is the first reported occurrence of this compound in the Menispermaceae.

Chromatography of the nonquaternary phenolic alkaloid fraction resulted in the isolation and identification of sinoacutine (II). This alkaloid was first isolated from the Chinese drug "Ching-feng-teng," *Sinomenium acutum* Rehd *et* Wils. (Menispermaceae) (12). Sinoacutine has since been isolated from three other species: *Cassytha pubescens* R.Br. (Lauraceae) (13), *Croton flavens* L. (Euphorbiaceae) (14), and *Corydalis pallida* var. *tenuis* (Fumariaceae) (15). This is the first reported occurrence of this alkaloid in the genus *Cocculus*.

Ion-exchange and adsorption chromatography of the quaternary alkaloid fraction resulted in the isolation and identification of magnoflorine and palmatine.

Magnoflorine has been found in several members of the Menispermaceae, including *Sinomenium acutum* Rehd *et* Wils. (16) and two members of the genus *Cocculus: C. laurifolius* D.C. (17) and *C. trilobus* D.C. (18). Palmatine was first isolated from columba root, *Jatrorrhiza palmata* Miers (Menispermaceae). It has since been found in other members of this family, including *Stephania glabra* Miers (19) and *C. leabae* D.C. (20).

EXPERIMENTAL[1]

Plant Material—Stems and leaves of *C. carolinus* D.C. (Menispermaceae) were used in this study[2].

Extraction—Air-dried ground stems and leaves of *C. carolinus* D.C. (9.25 kg.) were percolated at room temperature with a total of 203 l. of ethanol. The extract was evaporated, *in vacuo*, at 40° to leave a green-black syrup (1.25 kg.). During the concentration, a white crystalline material precipitated (53.4 g.). TLC on precoated cellulose plates[3], using a solvent system of acetone–water (4:1) and visualizing with potassium permanganate spray reagent (21), indicated the presence of two components, R_f 0.28 and 0.17

Isolation of Cyclitols—Chromatography of this crystalline mixture (4 g.) on a column of cellulose powder[4] (75 g.) [prepared according to Angyal *et al.* (22)] gave a fraction that eluted with acetone–water (4:1)[5] which yielded (+)-quercitol (181 mg.). Recrystallization of this cyclitol from 80% ethanol gave colorless crystals (62 mg.), m.p. 232–234° [lit. (23) m.p. 233–234°]; $[\alpha]_D^{25}$ +19.9° (c 0.73, water) [lit. (23) $[\alpha]_D^{15}$ +24.37° (c 8.0, water)].

Anal.—Calc. for $C_6H_{12}O_5$: C, 43.90; H, 7.37. Found: C, 43.72; H, 7.57.

An IR spectrum showed characteristic signals at ν_{max}^{KBr} 3310 and 3240 cm.$^{-1}$ (broad O—H stretch), 2910 and 2940 cm.$^{-1}$ (C—H stretch), 1420 cm.$^{-1}$ (C—H bend), and 1050 and 1075 cm.$^{-1}$ (C—O stretch). A low resolution mass spectrum did not exhibit the expected molecular ion of *m/e* 164 but showed significant peaks at *m/e* 146(1), $M^+ - H_2O$; 128(7), $M^+ - 2H_2O$; 110(5), $M^+ - 3H_2O$; and 73 (100), $C_3H_5O_2^+$. There was no depression of melting point when mixed with authentic (+)-quercitol[6], and IR spectra were superimposable.

Further elution with acetone–water (4:1) gave a fraction (515 mg.) which, on recrystallization from ethanol, yielded white needles of (−)-viburnitol (380 mg.), m.p. 179–180° [lit. (24) m.p. 181.5°]; $[\alpha]_D^{26}$ −55.4° (c 0.46, water) [lit. (24) $[\alpha]_D^{20}$ −50.0° (c 4.0, water)].

Anal.—Calc. for $C_6H_{12}O_5$: C, 43.90; H, 7.37. Found: C, 43.71; H, 7.57.

An IR spectrum showed signals at ν_{max}^{KBr} 3300 cm.$^{-1}$ (broad O—H stretch), 2940 cm.$^{-1}$ (C—H stretch), and 1040 and 1025 cm.$^{-1}$ (C—O stretch). A low resolution mass spectrum did not contain a molecular ion. Peaks due to major fragmentations were seen at *m/e* 146(1), $M^+ - H_2O$; 128(17), $M^+ - 2H_2O$; 110(7), $M^+ - 3H_2O$; and 73(100), $C_3H_5O_2^+$. The IR spectrum of this compound and authentic (−)-viburnitol[6] were superimposable, and there was no depression of melting point upon mixture of the two.

Fractionation (Scheme I)—The dried ethanolic extract (1.25 kg.) was stirred with 1% hydrochloric acid (2.5 l.). The residue was then dissolved in ether, dried, and evaporated to yield a residue of acidic and neutral substances (551 g., Fraction A). The aqueous acidic solution was basified to pH 8 with concentrated ammonium hydroxide solution and extracted with ether (9 × 3.0 l.). The ether solution was dried over sodium sulfate and evaporated to give a dark-red nonquaternary alkaloid fraction (25.0 g., Fraction B).

The basic aqueous layer was acidified to pH 3 with concentrated hydrochloric acid. The quarternary alkaloids were precipitated with a saturated solution of ammonium reineckate and filtered. The crude reineckate was suspended in water (600 ml.) and acidified to pH 5 with concentrated hydrochloric acid. Anion-exchange resin[7] (75 g.) was added and the mixture was stirred overnight. The resulting suspension was filtered and the filtrate was evaporated to leave a brown residue of quaternary alkaloidal chlorides (7.2 g., Fraction C).

The nonquaternary alkaloid fraction (Fraction B) was fractionated into phenolic and nonphenolic constituents (Scheme II). Fraction B (25.0 g.) was dissolved in ether (500 ml.) and shaken with 5% sodium hydroxide solution (4 × 300 ml.). The ether layer was washed with water (900 ml.), dried, and evaporated to yield a brown nonquaternary nonphenolic alkaloid fraction (1.2 g., Fraction B-1). The aqueous sodium hydroxide solution (1.2 l.) was acidified to pH 5 with concentrated hydrochloric acid and then rebasified with concentrated ammonium hydroxide solution to pH 8. The resulting solution was extracted with ether (3 × 1.2 l.), dried, and evaporated to give a red nonquaternary phenolic alkaloid fraction (14.3 g., Fraction B-2).

Isolation of Loliolide—Fraction B-1 was chromatographed on silicic acid[8] (100 mesh)–diatomaceous earth[9] (4:1) (150 g.). Elution with benzene–chloroform (1:4) and recrystallization from petroleum ether (30–60°)–chloroform yielded white needles of loliolide (12 mg.), m.p. 148–149° [lit. (25) m.p. 149–151°]; UV λ_{max}^{EtOH} 218 nm. (log ε 4.2) [lit. (25) UV λ_{max}^{EtOH} 214 nm. (log ε 4.2)]. The IR spectrum showed major absorptions at ν_{max}^{KBr} 3440 cm.$^{-1}$ (O—H stretch), 2980, 2950, 2925, and 2885 cm.$^{-1}$ (C—H stretch), 1730 and 1720 cm.$^{-1}$ (C=O stretch), 1620 cm.$^{-1}$ (C=C stretch), and 1273 and 1100 cm.$^{-1}$ (C—O stretch). The molecular formula, determined by high resolution mass spectrometry, was found to be $C_{11}H_{16}O_3$, with a molecular ion of 196.1092 (calc. 196.1099). The fragmentations were in agreement with those described by Pailer and Haschke-Hofmeister (11). There was no depression of melting point when mixed with authentic loliolide[10], and IR spectra were superimposable.

Isolation of Sinoacutine—Column chromatography of Fraction B-2 on silicic acid–diatomaceous earth (4:1) (175 g.) yielded a fraction that eluted with 1% methanol–chloroform, which was rechromatographed on a column of neutral alumina[11], grade V (620 g.). Elution with benzene and recrystallization from acetone yielded colorless prisms of sinoacutine (135 mg.), m.p. 199–200° dec. (lit. (14) m.p. 197–199°]; $[\alpha]_D^{28}$ −115.8° (c 1.0, ethanol) [lit. (14) $[\alpha]_D^{23}$ −115° (c 1.03, ethanol)]; UV λ_{max}^{EtOH}; 214 (log ε 4.4), 245 (log ε 4.3), and 280 nm. (log ε 3.8) [lit. (13) UV λ_{max}^{EtOH}: 240 (log ε 4.3) and 277 nm. (log ε 3.8)]. The IR spectrum showed major absorptions at ν_{max}^{KBr} 3450 cm.$^{-1}$ (broad O—H stretch), 1675 cm.$^{-1}$ (conjugated C=O stretch), and 1647 and 1618 cm.$^{-1}$ (conjugated C=C stretch). The NMR spectrum showed the presence of two methoxy groups (3.92 and 3.80 δ), one *N*-methyl group (2.50 δ), two olefinic protons (C-5, 7.63 δ; C-8, 6.37 δ), and a two-proton aromatic *AB* system (6.69 and 6.81 δ, J = 9.0 Hz.). The mass spectrum of the isolated sinoacutine gave significant peaks at *m/e* 327(100), M^+; 312(32), $M^+ - CH_3$; 299(24), $M^+ - CO$; and 284(44), $M^+ - CO - CH_3$; it was in agreement with that of salutaridine, its enantiomer, reported by Wheeler *et al.* (26). The IR spectra of this compound and of authentic sinoacutine[12] were superimposable, and there was no depression of melting point upon their mixture.

Chromatography of Quaternary Alkaloids—Fraction C was chromatographed on neutral alumina[11], grade V (60 g.), packed in chloroform.

Isolation of Palmatine—Elution with 2% methanol–chloroform afforded a fraction (90 mg.) which, following rechromatography on

[1] Melting points were determined on a Thomas-Hoover Uni-melt melting-point apparatus and are corrected. IR spectra were run in KBr using a Perkin-Elmer 257. Optical rotations were determined on a Perkin-Elmer 141 polarimeter. UV spectra were run on a Perkin-Elmer 202. NMR spectra were obtained in deuterated chloroform on a Jeol C-60HL, with tetramethylsilane as the internal standard. Low resolution mass spectra were taken by the Graduate School of Public Health, University of Pittsburgh, on a LKB-9000 spectrometer. High resolution spectra were obtained on an E. I. duPont de Nemours 21-492 spectrometer at the School of Pharmacy, University of Mississippi. Microanalyses were carried out by Midwest Microlab, Ltd., Indianapolis, Ind.

[2] Collected in Starkville, Miss., by Dr. Coy Box, and in Oxford, Miss., during the summer of 1970. Voucher specimens are deposited in the Herbarium of the School of Pharmacy, University of Mississippi.

[3] E. Merck, 0.10 mm.

[4] Whatman standard grade.

[5] Instrumentation Specialties Co., Inc., Fraction Collector, 20-ml. fractions.

[6] Dr. S. J. Angyal, School of Chemistry, University of New South Wales, Kensington, Australia.

[7] IRA-400(C1), Mallinckrodt.

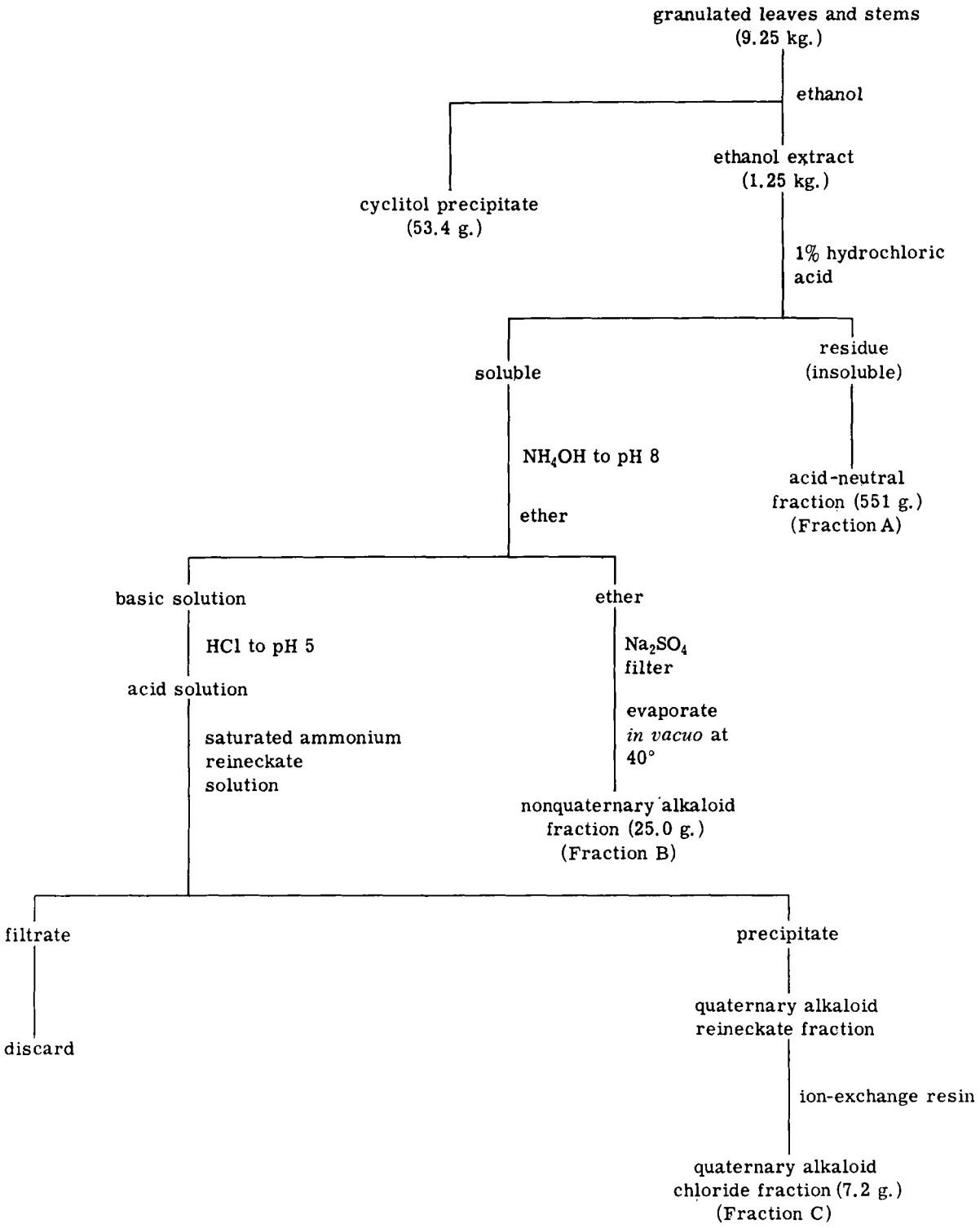
[8] Mallinckrodt.

[9] Celite, Johns-Manville Co.

[10] Reference sample provided by Dr. Takayuki Wada, Shionogi and Co., Ltd., Osaka, Japan.

[11] Woelm.

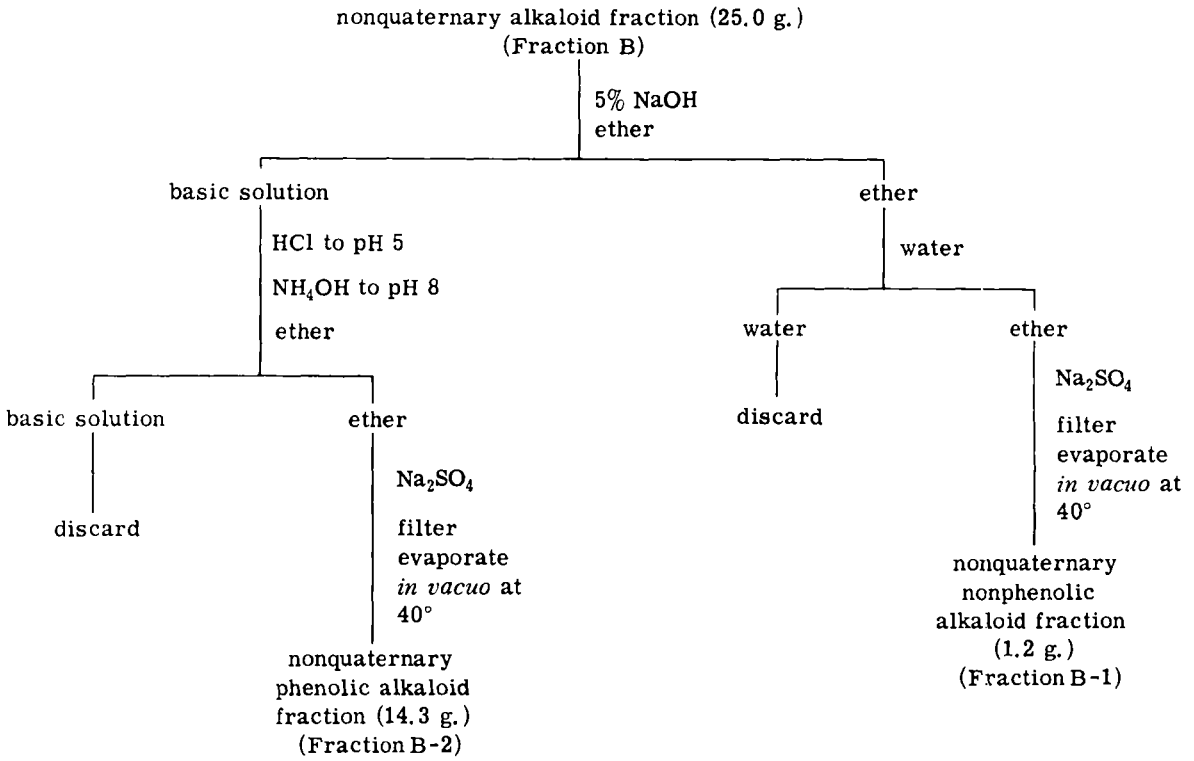
[12] The reference sample was provided by Dr. K. L. Stuart, Chemistry Department, University of the West Indies, Kingston, Jamaica.



Scheme I—Flowsheet for Fractionation of C. carolinus

polyamide[11] (70 g.) and elution with concentrated ammonium hydroxide–water–ethanol (1:1:9), afforded, on evaporation, a fraction (10 mg.) to which was added a saturated solution of methanolic potassium iodide (5 drops). This yielded yellow needles of palmatine iodide (1 mg.), m.p. 220–221° dec.; UV λ_{max}^{EtOH}: 239 (log ϵ 4.8), 272 (log ϵ 4.3), 350 (log ϵ 4.4), and 430 nm. (log ϵ 4.0) [lit. (27) λ_{max}^{EtOH}: 265 (log ϵ 4.4), 355 (log ϵ 4.5), and 425 nm. (log ϵ 4.0)].

Preparation of Tetrahydropalmatine—The residue left on evaporation of the mother liquor from the crystallization of palmatine iodide (8 mg.) was dissolved in methanol (3 ml.), and sodium borohydride was added slowly until the solution was colorless (3 mg.). The reaction mixture was evaporated, the residue was dissolved in ether (5 ml.), and the solution was extracted with 1% hydrochloric acid (3 × 10 ml.). The aqueous acid solution was basified to pH 8 with concentrated ammonium hydroxide solution and extracted with chloroform (3 × 30 ml.). The chloroform solution was dried and evaporated to leave a residue of tetrahydropalmatine (3 mg.); λ_{max}^{EtOH}: 230 (log ϵ 4.2) and 283 nm. (log ϵ 3.8) [lit. (27) λ_{max}^{EtOH}: 230 sh (log ϵ 4.3) and 281 nm. (log ϵ 3.8)]. The mass spectrum showed a molecular ion at *m/e* 355(70) and other characteristic fragments at



Scheme II—Fractionation of the Nonquaternary Alkaloid Fraction

m/e 340(20), 192(15), 190(35), 164(100), and 149(60), which are in complete agreement with those reported for tetrahydropalmatine (28).

Isolation of Magnoflorine- Elution with 4% methanol-chloroform afforded a fraction (1.1 g.). Repeated chromatography of this fraction on polyamide[11], using an eluting solvent of concentrated ammonium hydroxide–water (1:10), gave a fraction (105 mg.) which, after treatment with saturated methanolic potassium iodide solution (1 ml.), yielded crystals of magnoflorine iodide. Recrystallization from methanol yielded white needles (65 mg.), m.p. 249–251° dec. [lit. (29) m.p. 248–249° dec.], $[\alpha]_D^{27}$ +182.9° (c 0.146, methanol) [lit. (30) $[\alpha]_D$ +200° (methanol)]; UV λ_{max}^{EtOH}: 232 (log ϵ 4.4), 272 (log ϵ 3.9), and 312 nm. (log ϵ 3.8) [lit. (31) UV λ_{max}^{EtOH}: 227 (log ϵ 4.7), 271 (log ϵ 3.9), and 310 nm. (log ϵ 3.8)]. The IR spectra of this compound and of authentic magnoflorine were superimposable, and there was no depression of melting point upon their mixture.

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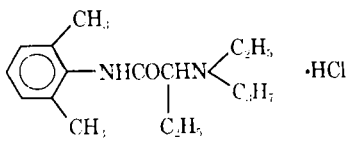
Local Anesthetic Activity and Acute Toxicity of (±)-2-(*N*-Ethylpropylamino)-2′,6′-butyroxylidide, a New Long-Acting Agent

H. JACK ADAMS▲, GEORGE H. KRONBERG, and BERTIL H. TAKMAN

Abstract □ This paper is concerned with the local anesthetic activity and acute toxicity of (±)-2-(*N*-ethylpropylamino)-2′,6′-butyroxylidide (I). Testing in rat sciatic nerve blocks and guinea pig intradermal wheals showed that the compound has rapid onset, excellent frequency, and long durations of block. These observations were confirmed in studies of peridural anesthesia in the cat in which the durations of block were comparable to those of the long-acting agent bupivacaine. Although the compound is more irritating and more toxic than lidocaine, it is not more so than bupivacaine and tetracaine. These studies indicate that the overall pharmacological and toxicological profile of Compound I more closely resembles those of bupivacaine and tetracaine than that of lidocaine.

Keyphrases □ (±)-2-(*N*-Ethylpropylamino)-2′,6′-butyroxylidide—local anesthetic activity and acute toxicity □ Anesthetic activity, local—(±)-2-(*N*-ethylpropylamino)-2′,6′-butyroxylidide □ Toxicity, acute—(±)-2-(*N*-ethylpropylamino)-2′,6′-butyroxylidide

A series of α-aminobutyroxylidide derivatives was synthesized and tested for local anesthetic activity. This paper describes the local anesthetic activity and acute toxicity of one of the most interesting compounds in this series. It is chemically designated as (±)-2-(*N*-ethylpropylamino)-2′,6′-butyroxylidide[1] (I) and has the structural formula shown here.



I

In the studies reported here, Compound I was compared with lidocaine, an agent of intermediate duration, and two long-acting agents, bupivacaine[2] and tetracaine.

[1] W19053.
[2] Marcaine.

METHODS

Rat Sciatic Nerve Blocks—Conduction block in a peripheral nerve trunk was studied in the female albino rat. The method was described in detail by Camougis and Takman (1). Precisely 0.2 ml. of drug solution or vehicle was injected into the midthigh region of the animal, so that it was deposited around the sciatic nerve trunk. After the injections, the animals were examined at frequent intervals for onset, depth, and duration of motor block. Frequencies of complete and of partial blocks were recorded, and overt systemic effects were noted. Mean durations and standard deviations were calculated from the durations of the complete blocks only. Groups of five rats were used at each concentration tested, and injections were made into both hind limbs.

Guinea Pig Intradermal Wheals—To evaluate infiltration anesthesia, the local anesthetic activity of graded concentrations of the agents was studied in the guinea pig intradermal wheal (1). Each wheal was made by injecting 0.1 ml. of drug solution or vehicle intradermally on the shaved backs of guinea pigs, and 12 wheals were made for each concentration. The presence or absence of anesthesia was determined by means of the response to pinpricks.

Peridural Anesthesia in the Cat—Surgical procedures and the evaluation of peridural anesthesia in cats were reported by Duce *et al.* (2). In brief, the procedure requires surgical implantation of a plastic catheter into one of the lumbar vertebrae so that local anesthetic solutions can be introduced into the peridural space. After administration of the local anesthetic solution, animals were examined at frequent intervals for onset of block. The principal endpoints recorded were: block and recovery of the animal's ability to support itself on its hind limbs, block and recovery of the flexor reflex (withdrawal of the limb when pressure is applied to the paw), and loss and complete recovery of normal motor function. Animals were also observed for effects that may result from the spread of the local anesthetic solution and from absorption into the blood.

A total of seven animals received 2% lidocaine and 0.5 and 1.0% Compound I; five animals received 0.5% bupivacaine.

Irritation Studies—The irritation liabilities of Compound I, lidocaine, bupivacaine, and tetracaine were evaluated by means of intradermal wheals in rabbits (1). The animals' backs were shaved and a series of wheals was made by injecting 0.1 ml. of solution or vehicle intradermally at each site. Twenty-four hours later, each wheal was examined and graded for the degree of redness, degree of edema, and presence or absence of a central zone of discolor (indicative of necrosis). Grading was done on an arbitrary scale from 0 to 12, and a mean score was obtained for all wheals made at