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THE ALKALOIDS OF AMERICAN LOTUS, *NELUMBO LUTEA*[1,2]

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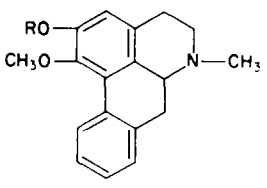
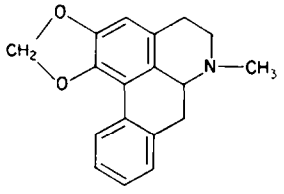
Abstract—The leaves and stems of *Nelumbo lutea* (Willd.) Pers. from Wisconsin yielded the alkaloids nuciferine, (±)-armepavine, and two apparently new alkaloids to which we have assigned structures V ((−)N-norarmepavine) and XII ((−)-N-nornuciferine).

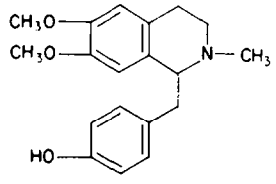
RECENT reports have described the isolation from Asiatic lotus, *Nelumbo nucifera* Gaertn. (Nymphaeaceae), of the alkaloids nuciferine (I),[3,4,6] roemerine (II),[4,6] nor-nuciferine (III) [4-6] and (±)-armepavine (IV).[7]



I R = CH_3
III R = H



II



IV

The alkaloids nuciferine, (±)-armepavine, and two apparently new alkaloids to which we have assigned structures V ((−)-N-norarmepavine) and XII ((−)-N-nornuciferine) for the reasons detailed below, have been isolated from American lotus, *Nelumbo lutea* (Willd.) Pers. (Nymphaeaceae).

Coarsely ground *N. lutea*[8] was extracted with methanol and the methanolic extract was treated to separate non-phenolic from phenolic bases. The non-phenolic base fraction yielded nuciferine (0·0466%) and (−)-N-nornuciferine (0·0134%). The phenolic base fraction yielded (−)-N-norarmepavine (0·0554%) and (±)-armepavine (0·0046%).

[1] The investigation which forms the subject of this paper was first outlined in part in a preliminary communication: *J. Pharm. Sci.* **51**, 599 (1962). This work was supported by research grants (H-2952 and CY-4500) from the National Institutes of Health.

[2] The work was presented at the Second I.U.P.A.C. Symposium, *Chemistry of Natural Products* Prague, August (1962).

[3] H. R. Arthur and H. T. Cheung, *J. Chem. Soc.* 2306 (1959).

[4] M. Tomita, Y. Watanabe, M. Tomita and H. Furukawa, *J. Pharm. Soc., Japan* **81**, 469 (1961).

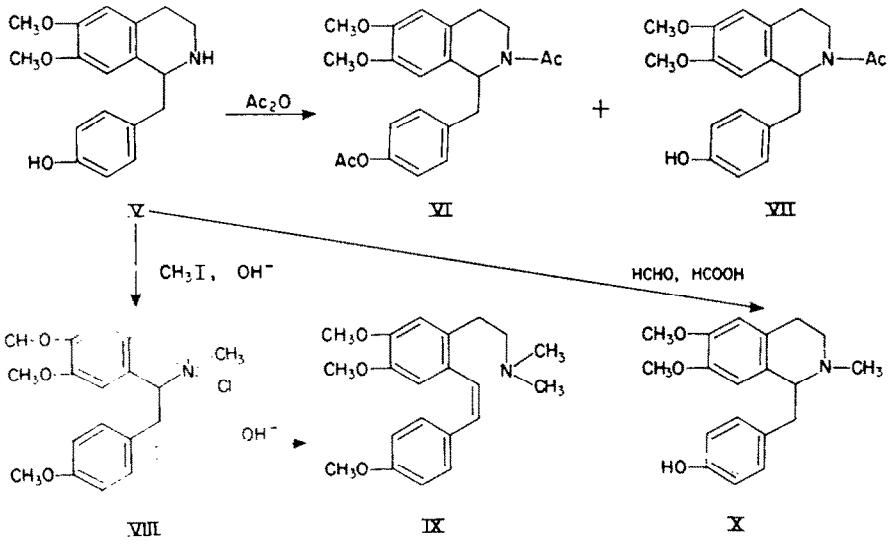
[5] M. Tomita, Y. Watanabe and H. Furukawa, *J. Pharm. Soc., Japan* **81**, 942 (1961).

[6] M. Tomita, Y. Watanabe and H. Furukawa, *J. Pharm. Soc., Japan* **81**, 1202 (1961).

[7] M. Tomita, Y. Watanabe and H. Furukawa, *J. Pharm. Soc., Japan* **81**, 1644 (1961).

[8] Air-dried leaves and stems, collected in Wisconsin during the summers of 1959, 1960 and 1961. We thank Professor H. H. Iltis of the University of Wisconsin for confirming the identity of the plant. A voucher specimen is deposited in the University of Wisconsin Herbarium.

The major phenolic alkaloid (V, m.p. 152–153°, $[\alpha]_D^{25}$ −23° (*c*, 1·33, chf.), $[\alpha]_{546}^{26}$ −40° (*c*, 1·03, chf.), λ_{max}^{EtOH} 228 mμ (ε 15,400), 282 mμ (ε 5,000), 287 mμ (ε 4,000)) was shown to have the formula $C_{16}H_{15}ON(OCH_3)_2$ by determination of equivalent weight and analysis of the alkaloid and several of its salts. Acetylation of V with acetic anhydride–pyridine, followed by chromatography on neutral alumina, gave an amorphous O,N-diacetate (VI) (IR bands at 5·70, 6·18 μ) and a crystalline N-acetate (VII), m.p. 237–238°, IR band at 6·20 μ but none in the 5·6–6·0 μ region, soluble in dilute sodium hydroxide. The foregoing facts established that V possesses a phenolic hydroxyl group and an acylable amino group.



Exhaustive methylation of V gave the 1-(4′-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline methiodide (VIII), m.p. 127–129°.[9] Treatment with alkali yielded the methine IX, m.p. 86–87°, characterized by direct comparison with an authentic sample.[10] The latter experiments established that V is a 4′,6,7-trisubstituted benzylisoquinoline alkaloid in which two of the substituents are methoxyl and one hydroxyl. The NMR spectrum fully supported the postulated structure, showing (in $CDCl_3$) six aromatic hydrogens (τ = 3·0–3·4), two labile hydrogens (τ = 4·8, representing the result of exchange among two labile hydrogens and a small amount of water present in the solution), one tertiary hydrogen (quadruplet centered at τ = 5·85), six methoxyl hydrogens (doublet at τ = 6·18) and six ring methylene hydrogens (broad doublet centering at τ = 7·05).[11]

Methylation of V with formalin and formic acid yielded (+)-armepavine (X), m.p. 139–140°, $[\alpha]_D^{25}$ +91° (*c*, 1·19, chf.). The IR spectrum of the methylation product in chloroform was superimposable upon that of a sample of (−)-armepavine, m.p.

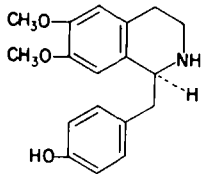
[9] R. Konovalova, S. Yunusov and A. Orekhov, *Ber. Dtsch. Chem. Ges.* **68**, 2158, 2277 (1935); L. Marion, L. Lemay and V. Portelance, *J. Org. Chem.* **15**, 216 (1950); H. King, *J. Chem. Soc.* 737, (1940); M. Tomita, E. Fujita and F. Murai, *J. Pharm. Soc., Japan* **71**, 226 (1951).

[10] E. Fujita and T. Tomimatsu, *J. Pharm. Soc., Japan* **79**, 1260 (1959).

[11] The NMR spectrum was run by Varian Associates on a solution in deuterated chloroform with tetramethylsilane added as an internal reference on an HR-60 spectrometer at 60 mc/sec.

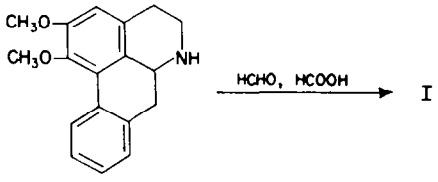
140–141°, prepared by sodium–liquid ammonia reduction of cycleanine.[12] Mixture of equal quantities of (+)- and (−)-armepavine and crystallization from acetone yielded (±)-armepavine, m.p. 166–167°. The melting point was undepressed by admixture of the sample isolated from *N. lutea*, and the infrared spectra of the respective samples in chloroform were identical. Hence, the name (−)-N-norarmepavine is proposed for the new phenolic secondary base (V). A total synthesis of (±)-1-(4′-hydroxybenzyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline has been reported.[13]

Diazomethane methylation of (+)-armepavine (derived from (−)-N-norarmepavine) yielded a non-phenolicbase, m.p. 62–63°, $[\alpha]_D^{28}$ +79° (*c*, 0·84, chf.) which was identified as (+)-1-(4′-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline by direct comparison with an authentic sample.[9] The latter compound has recently been shown to possess the (−)-configuration at C-1.[14] Consequently, the absolute configuration of (−)-(−)-N-norarmepavine may be represented as in XI.



XI

The minor non-phenolic alkaloid (XII, m.p. 128–129°, $[\alpha]_D^{28}$ −145° (*c*, 0·98, EtOH); λ_{max} 230 mμ (ε 20,500), 272 mμ (ε 16,800), 310 mμ (ε 2,200) was shown by analysis to have the formula $C_{18}H_{19}O_2N$. The UV and IR spectral characteristics of the compound resembled those of nuciferine; these facts and the apparent (IR) secondary amine-nature of the alkaloid led to the hypothesis that the compound was (−)-N-nornuciferine (XII). The hypothesis was confirmed by N-methylation of XII with formalin and formic acid, whereupon nuciferine was obtained.[15]



XII

(−)-N-norarmepavine was tested for biological activity in three pharmacological

[12] E. Fujita and F. Murai, *J. Pharm. Soc., Japan* **71**, 1043 (1951).

[13] H. Yamaguchi and K. Nakano, *J. Pharm. Soc., Japan* **79**, 1106 (1959).

[14] M. Tomita and J. Kunitomo, *J. Pharm. Soc., Japan* **82**, 734 (1962). We thank Professor Tomita cordially for informing us of these results prior to publication.

[15] After completion of our work, a total synthesis of (±)-N-nornuciferine has been reported; J. A. Weisbach and B. Douglas, *J. Org. Chem.* **27**, 3738 (1962). The IR spectrum of a sample of the synthetic (±)-N-nornuciferine in chloroform was found to be identical with that of our (−)-N-nornuciferine. We thank Drs. Weisbach and Douglas cordially for a comparison sample of (±)-N-nornuciferine and for informing us of their results prior to publication. We subscribe to the latter authors' suggestion that, in the future, the prefix *nor* be used only to indicate a des-N-methyl-relationship.

screening procedures.[16] In the mouse, this alkaloid produced mydriasis, bradypnea and a slight decrease in spontaneous motor activity after oral doses of 200 mg/Kg. The dose of 100 mg/Kg caused only slight mydriasis in one out of two animals. Intraperitoneally, doses of 50 mg/Kg caused writhing in the animal, however, this effect is probably related to the low pH necessary to solubilize the compound. (−)-N-norarmepavine in doses of 50 mg/Kg orally exerted a very weak analgetic action in rats but failed to produce anti-pyresis or diminish edema in the Randall and Selitto anti-inflammatory test. In the ether-chloralose anesthetized cat (−)-N-norarmepavine produced transient depressor effects after doses as high as 10 mg/Kg intravenously but failed to alter the responses to standard agents affecting the autonomic nervous system.

EXPERIMENTAL[17]

Extraction of alkaloids from Nelumbo lutea

Coarsely ground *N. lutea* (air-dried leaves and stems, 3 kg) was extracted with methanol by percolation at room temp. The plant material was covered with fresh charges of solvent 10 times over a period of 7 days. Evaporation of the whole extract under red. press. left a semisolid residue. The residue was triturated with 2% sulfuric acid with mechanical stirring. The acid solution was filtered and the insoluble solid was again extracted with 2% sulfuric acid as above. The combined acid extract (ca. 7 l.) was washed with ether (2 300 ml portions) to remove non-basic material. The acid solution was then made alkaline with ammonium hydroxide, and the precipitated base was extracted with ether. Evaporation of the ether left an oily residue, which was dissolved in chloroform (180 ml) and extracted with 2% sodium hydroxide solution (3 300 ml portions). The alkaline solution was acidified with acetic acid, made alkaline with ammonium hydroxide, and extracted with ether. The ethereal extract was washed with water and dried (Na_2SO_4). Evaporation of the ether left the total phenolic alkaloid fraction as a brown powder (7·9 g).

The chloroform solution after extraction of the phenolic alkaloids was washed with water and dried (Na_2So_4). Evaporation of the chloroform left the amorphous total non-phenolic alkaloid fraction (4·5 g). Upon paper chromatography,[18] the phenolic alkaloid fraction was shown to consist of one major alkaloid and a few minor alkaloids. The non-phenolic fraction was shown to consist of two major components and a few minor alkaloids.

Isolation of (−)-*nuciferine* (I) *and* (−)-N-*nornuciferine* (XII)

A solution of the non-phenolic alkaloids (4·5 g) in benzene was chromatographed on neutral alumina (44 g, Woelm). The first benzene eluate (170 ml) left a crystalline residue upon evaporation. Recrystallization from aqueous methanol yielded prisms (1·398 g), m.p. 166–168°; $[\alpha]_D^{24}$ −149° (*c*, 1·06, EtOH); λ_{max} 230 mμ (ε 18,300), 272 mμ (ε 15,400) 310 mμ (ε 2,100). The m.p. was not depressed by admixture of a sample of (−)-nuciferine (m.p. 164–167°) isolated from *Nelumbo nucifera*, and the IR spectra of the respective samples in chloroform solution were identical.

The next eluates (benzene, 220 ml; benzene–acetone, 49:1, 110 ml; 48:2, 210 ml; 45:5, 550 ml; 25:25, 440 ml) were combined on the basis of their paper chromatographic patterns. Evaporation to dryness left a crystalline residue (0·404 g), m.p. 120–127°. Two recrystallizations from ether afforded

[16] We thank Mr. Edward Macko of the Smith, Kline and French Laboratories, Philadelphia, Pa., for the pharmacological results reported herein.

[17] M.p.s are corrected. Values of $[\alpha]_D$ have been approximated to the nearest degree. UV absorption spectra were determined in 95% ethanol on a Cary recording spectrophotometer (Model 11 MS). IR spectra were determined on solutions in chloroform on a Beckmann Model IR5 spectrophotometer with NaCl prism and plates, using 0·1 NaCl cells. Microanalyses were carried out by Dr. S. M. Nagy and his associates at the Massachusetts Institute of Technology.

[18] Paper chromatography was conducted on Whatman filter paper (No. 4) which was treated with phosphate–citric acid buffer (pH 3·5), using as eluant the organic phase of a mixture of n-butanol: n-butyl acetate: pyridine: water (30:15:10:50 by volume). After drying, a chloroform solution of Bromphenol Blue was sprayed to detect the alkaloids.

colorless small needles, m.p. 128–129°, $[\alpha]_D^{28}$ −145° (*c*, 0·98 EtOH); λ_{max} 230 mμ (ε 20,500), 272 mμ (ε 16,800), 310 mμ (ε 2,200). (Found: C, 76·67; H, 6·64; N, 4·90. Calc. for $C_{18}H_{19}O_2N$: C, 76·84; H, 6·81; N, 4·98%).

N-Methylation of (−)-N-*noruciferine* (XII)

A solution of XII (74 mg, m.p. 123–124°) in 40% formalin (1 ml) and 88% formic acid (1 ml) was heated under reflux on a steam bath for 5 hr. The reaction mixture was evaporated to dryness under red. press. To remove all traces of formaldehyde, the residue was repeatedly dissolved in dil. hydrochloric acid and evaporated to dryness. The residue was dissolved in 2% sulfuric acid and the solution was made alkaline with ammonium hydroxide and extracted with ether. The ether extract was washed with water, dried (Na_2SO_4) and evaporated to dryness. Recrystallization of the residue from ether gave prisms (56 mg), m.p. 167–168°. Admixture with an authentic sample of (−)-nuciferine of m.p. 164–167° gave a mixture of m.p. 164–167°. The IR spectra of the respective samples in chloroform solution were identical.

Isolation of (−)-N-*norarmepavine* (V)

The phenolic alkaloid fraction (7·9 g) was extracted with ether in a Soxhlet extractor for ca. 20 hr. The filtered yellow ethereal extract was evaporated to yield a yellow solid residue (7·3 g). A solution of the yellow solid in acetone was chromatographed on neutral alumina (60 g, Woelm). Acetone (5 120 ml portions) and methanol (5 120 ml portions) eluates were combined on the basis of their paper chromatographic patterns. The residue obtained upon evaporation was dissolved in ethanol and treated with an ethanolic solution of oxalic acid. Concentration of the solution yielded a crystalline oxalate (2·8 g), m.p. 233–240°. Treatment of the crystalline oxalate with dil alkali afforded a free base which was crystallized from acetone to yield colorless small needles (0·931 g), m.p. 150–153°. The acetone mother liquor alkaloids upon chromatography on neutral alumina afforded an additional crop of needles (0·733 g), m.p. 152–154°. Recrystallization from acetone gave an analytical sample, m.p. 152–153°; $[\alpha]_D^{25}$ −23° (*c*, 1·33, chf.), $[\alpha]_{546}^{26}$ −40° (*c*, 1·03, chf.); λ_{max} 228 mμ (ε 15,400), 282 mμ (ε 5,000), 287 mμ (ε 4,900). (Found: C, 72·06, 71·70, 71·68; H, 7·02, 6·71, 6·73; N, 4·89, 4·77; OMe, 20·15, 19·45; M.W., 322 (Rast); Eq. wt., 304 ($HClO_4$ titration). Calc. for $C_{18}H_{21}O_3N$: C, 72·21; H, 7·07; N, 4·68; 2 OMe, 20·7%; M.W. 299). The material gave a negative Gibbs[19] test. Recrystallization of the aforementioned oxalate from ethanol gave needles, m.p. 237–238°. (Found: C, 61·19; H, 6·04; N, 3·73. Calc. for $C_{20}H_{23}O_7N$: C, 61·69; H, 5·95; N, 3·60%). The perchlorate was crystallized from ethanol as plates, m.p. 212–215°. (Found: C, 54·29; H, 5·72; N, 3·68. Calc. for $C_{18}H_{21}O_3N \cdot HClO_4$: C, 54·06; H, 5·25; N, 3·50%). The hydriodide was obtained from ethanol rods, m.p. 242–245°. (Found: C, 50·53; H, 5·10; N, 3·19. Calc. for $C_{18}H_{21}O_3N \cdot HI$: C, 50·58; H, 4·91; N, 3·27%). The hydrochloride, crystallized from methanol, showed m.p. 159–162°.

Acetylation of (−)-N-*norarmepavine* (V)

A solution of the phenolic base (0·330 g) in acetic anhydride (2 ml) and pyridine (2 drops) was heated under reflux for 4 hr. The reaction mixture was diluted with water, made alkaline with ammonium hydroxide, and extracted with chloroform. The chloroform extract was washed with water, dried (Na_2SO_4) and evaporated to dryness. A solution of the residue in benzene was chromatographed on neutral alumina (10 g, Woelm). The benzene–chloroform (4:1) eluate yielded a homogeneous but non-crystallizable residue (0·165 g, λ_{max} 5·70 μ, 6·18 μ). Chloroform–methanol (9:1) yielded a residue (0·12 g) which was crystallized from methanol to yield plates (37 mg), m.p. 237–238°, λ_{max} 6·20 μ. (Found: C, 69·95; H, 6·82; N, 3·92. Calc. for $C_{20}H_{23}O_4N$: C, 70·36; H, 6·79; N, 4·10%).

Hofmann degradation of O,N-*dimethyl*-(−)-N-*norarmepavine methiodide* (VIII)

A solution of (−)-N-norarmepavine (0·502 g), in 0·5 N methanolic potassium hydroxide (6·3 ml) and methyl iodide (3·8 ml) was heated under reflux for 3 hr. Evaporation of the methanol and excess methyl iodide left a yellowish-brown resinous residue which was crystallized from 20% aqueous methanol to yield yellowish-brown rods. Recrystallization from methanol yielded yellowish rods (0·658 g), m.p. 127–129°. The product was added to a solution of sodium hydroxide (2·6 g) in methanol (33 ml) and the mixture was heated on a steam bath for 5 hr. The reaction mixture was evaporated to dryness, water (60 ml) was added, and the suspension was extracted with ether. The ether extract was

[19] H. D. Gibbs, *J. Biol. Chem.* **72**, 649 (1927).

dried (KOH pellets) and evaporated. The colorless oily residue (0·228 g) was crystallized from pet. ether as fine colorless needles (0·136 g), m.p. 86–87°. The m.p. was not depressed by admixture with an authentic sample of O,O,N-trimethylcoclaurine methyl methine,[10] and the IR spectra of the respective samples in chloroform were identical.

N-Methylation of (−)-N-*norarmepavine* (V)

A solution of the base (0·178 g, m.p. 151–153°) in 88% formic acid (2 ml) and 40% formalin (2 ml) was heated under reflux for 3 hr. The reaction mixture was diluted with water, made alkaline (pH 9) with sodium carbonate, and extracted with chloroform. The chloroform extract was washed once with water, dried (Na_2SO_4) and evaporated to dryness under red. press. The residue was dissolved in acetone and filtered through neutral alumina (3 g, Woelm). The oily residue obtained after evaporation of acetone was dissolved in dilute hydrochloric acid. The acid solution was washed with ether, basified with sodium carbonate and extracted with ether. The ethereal extract was dried (K_2Co_3) and evaporated to dryness to yield a residue which was crystallized from acetone–petroleum ether to yield rods (74 mg), m.p. 138–141°. Recrystallization from the same solvent yielded colorless rods, m.p. 139–140°, $[\alpha]_D^{25}$ +91° (*c*, 1·19, chf.). The IR spectrum in chloroform was superimposable upon that of a sample of (−)-armepavine, m.p. 140–141°, which was prepared by sodium-liquid ammonia cleavage of cycleanine.[12]

(±)-*Armepavine*

(a) *From* Nelumbo lutea. Coarsely ground *N. lutea* (3 kg) was extracted as above, and the phenolic fraction was processed as above to remove (−)-N-norarmepavine. The residual phenolic alkaloids (1·770 g) were fractionated by preparative paper chromatography on 16 sheets of Whatman No. 4 filter paper (22 × 9″) with the same solvents used in the analytical work.[18] After spraying with Bromphenol Blue, the main alkaloid band was cut from the sheets and extracted with methanol–chloroform (1:1) in a Soxhlet extractor. The solvent was evaporated to yield a residue which was dissolved in 2% hydrochloric acid. The acid solution was washed with ether, made alkaline with ammonium hydroxide and extracted with chloroform. The chloroform extract was washed and dried as usual and evaporated to leave a pink powdery residue (0·857 g). A solution of the residue in acetone was chromatographed on neutral alumina (20 g, Woelm). The first acetone eluate (100 ml) yielded a solid residue (0·279 g) which was crystallized from acetone as plates (0·139 g). Recrystallization from acetone gave hexagonal plates (0·104 g), m.p. 162–163°, $[\alpha]_D^{25}$ ±0° (*c*, 1·19, chf.). The mixed m.p. with a sample of (±)-armepavine (prepared as below by mixture of the antipodes) of m.p. 166–167° was found to be 165–166°. The IR spectra of the respective samples were identical.

The second acetone (100 ml) eluate and the following methanol (100 ml) eluate were combined and evaporated. The residue was crystallized from acetone to yield needles (0·175 g). Recrystallization from acetone gave (−)-N-norarmepavine in the form of colorless needles (88 mg.), m.p. 153–155°.

(b) *From* (+)- *and* (−)-*armepavine*. A mixture of (+)-armepavine (5·3 m.g. m.p. 139–140°) and (−)-armepavine[12] (5·3 mg., m.p. 140–141°) was crystallized from acetone to yield (±)-armepavine as colorless plates, m.p. 166–167°.

Methylation of (+)-*armepavine with diazomethane*

A solution of (+)-armepavine (0·116 g), obtained by N-methylation of L-N-norarmepavine (V), in methanol (10 ml) was added to an ethereal solution of diazomethane prepared from nitrosomethylurea (6 g). After 2 days at room temp. acetic acid was added to destroy excess diazomethane and the solution was evaporated to dryness. The residue was dissolved in 5% hydrochloric acid. The acid solution was made alkaline with sodium hydroxide and the suspension was extracted with ether. The ether solution was dried (Na_2SO_4) and evaporated to leave a crude non-phenolic basic oil (76 mg). A solution of the oil in benzene was chromatographed on neutral alumina (0·780 g, Woelm). The column yielded to benzene a yellowish oil (56 mg), which was crystallized from benzene–petroleum ether to yield colorless needles (21 mg), m.p. 62–63°, $[\alpha]_D^{28}$ +79° (*c*, 0·84 chf.). The subsequent fractions eluted from the column yielded additional crystalline product (30 mg). The product was characterized as (+)-1-(4′-methoxybenzyl)-6,7-dimethoxy-2-methyl 1,2,3,4-tetrahydroisoquinoline by mixed m.p. and IR spectral comparison with an authentic sample m.p. 61–62°, $[\alpha]_D^{28}$ +82° (*c*, 1·00, chf.).[10]