2,4-dimethoxy-5-ethoxybenzoic acid by direct comparison (mp, mmp, co-TLC and co-IR) with a synthetic sample.

REFERENCES

1. Chibber, S. S. and Sharma, R. P. (1979) *Phytochemistry* (in press).
2. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
3. Ollis, W. D., Rhodes, C. A. and Sutherland, I. O. (1967) *Tetrahedron* **23**, 4741.
4. Campbell, R. V. M., Harper, S. H. and Kemp, A. D. (1969) *J. Chem. Soc. C* 1787.
5. Gopinath, K. W., Kidwai, A. R. and Prakash, L. (1961) *Tetrahedron* **16**, 201.
6. Rajagopalan, S., Seshadri, T. R. and Varadarajan, S. (1949) *Proc. Indian Acad. Sci. Sect. A* **30** 265.

Phytochemistry, 1979. Vol. 18, pp. 1584–1586. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0901-1584 $02.00/0

ALKALOIDS FROM LEAVES OF *ANNONA SQUAMOSA*

P. K. Bhaumik*, B. Mukherjee†, J. P. Juneau‡, N. S. Bhacca‡ and R. Mukherjee§

* Department of Chemistry, Krishnagar College, West Bengal, India; † Department of Pharmacology, B. C. Roy Post-Graduate Institute of Basic Medical Sciences, Calcutta-700020, India; ‡ Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, U.S.A. and § Faculdade de Farmacia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

(*Revised received* 26 *January* 1979)

Key Word Index—*Annona squamosa*; Annonaceae; alkaloids; (−)-xylopine; (+)-*O*-methylarmepavine; lanuginosine.

INTRODUCTION

Annona squamosa L. has been in use in folk medicine [1] for quite some time and an EtOH extract of the leaves and stems is reported to have anti-cancer activity [2]. Isolation of a number of alkaloids [3, 4], terpene derivatives [5] and a novel diazepine, squamolone [6] from this plant has been reported. However, detailed chemical and pharmacological investigations on the leaves are still awaited. Recent pharmacological screening on the total bases from the leaves revealed a strong acetylcholine-like activity[‖] which prompted us to undertake a complete chemical investigation of the crude extract. This resulted in the isolation of friedelin and the alkaloids (−)-xylopine, (+)-*O*-methylarmepavine and lanuginosine for the first time from this source.

‖ Preliminary pharmacological investigations have been carried out in the Department of Pharmacology, B. C. Roy Post-Graduate Institute of Basic Medical Sciences, Calcutta 700020, India.

RESULTS AND DISCUSSION

The compounds were isolated by solvent extraction, chromatography over Brockmann alumina and subsequent purification of different fractions.

The first alkaloid **1**, $C_{18}H_{17}NO_3$ (M^+ 295), mp 123°, showed UV absorption and MS fragmentation pattern typical of aporphine alkaloids [7]. The ^1H NMR spectrum, with stepwise irradiation of selective absorptions and the use of nuclear Overhauser effect (NOE) (Table 1) was in agreement with structure **2**, providing additional means for assigning the substituents. Direct comparison with an authentic sample revealed its identity with (−)-xylopine [8, 9].

The polar liquid alkaloid **2**, $C_{20}H_{25}NO_3$ (M^+ 327),

Table 1. ^1H NMR spectral analysis of (−)-xylopine

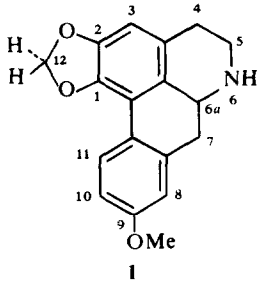
Chemical shift δ	Appearance of signal and other observations	Assignment and other conclusions	
6.83 (A)	An ABX pattern (J_{AB} = 2.5 Hz (*meta*), J_{AX} =	A → H-10	(line broadening in X was ob-
6.83 (B)	8.5 Hz (*ortho*), J_{BX} = 0 Hz (*para*)), A and B adja-	B → H-8	served because of overlap of A
7.88 (X)	cent to OMe inferred because of their high field positions. Low field position of H-11 is typical of the aporphines [10].	X → H-11	and B resonances)
3.78 (M_3)	Three-proton singlet, when M_3 irradiated, A and B exhibit 15–20% NOE enhancements.	M_3 → OMe	(so OMe is at C-9)
6.54 (E)	One proton singlet in aromatic region, exhibits (~25%) NOE enhancements when benzylic methylene resonance region is irradiated.	E → H-3	
5.92 (M)	A two-proton four-line pattern (J_{MN} = 1.5 Hz)	M → H-12	
6.05 (N)	representing methylenedioxy group protons [10].	N → H-12	
3.70 (T)	A wide (18 Hz) one proton pattern exhibiting splittings of 12 and 6 Hz.	T → H-6_a	(the proton has very likely an axial orientation)

showed UV absorption indicative of benzylisoquinoline alkaloids. The molecular formula was confirmed by chemical ionization MS, as the M^+ peak at 327 was negligible compared to the base peak at *m/e* 206 (fragment ion 4), which is not uncommon in this series of alkaloids [11]. The oily alkaloid was identified as (+)-*O*-methylarmepavine [12].

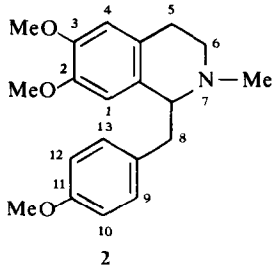
The third alkaloid, $C_{18}H_{11}NO_4$ (M^+ 305), mp 315–317° (decomp), was crystallized from $CHCl_3$ as bright orange needles. It exhibited a green fluorescence in $CHCl_3$ solution and formed a deep red colour in mineral acids. Its UV spectrum indicated characteristics of oxoaporphine alkaloids [9] and IR indicated the presence of a methylenedioxy group and a highly conjugated carbonyl function. The alkaloid was identified as lanuginosine 3 by direct comparison with an authentic sample [13].

The compounds obtained from *Annona squamosa* are of biogenetic interest. Isolation of michelabine, anonaine and roemerine, along with the corresponding oxoaporphine, liriodenine [3, 4] and xylopine with the corresponding oxoaporphine, lanuginosine from *Annona squamosa* lends support to the contention that oxoaporphines are formed in the plants from the corresponding aporphines [14].

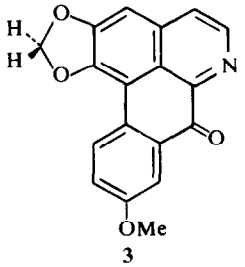
The occurrence of the same aporphine and oxoaporphine alkaloids, viz. anonaine, xylopine, liriodenine and lanuginosine in *Xylopia brasiliensis* [9] and *Annona squamosa*, suggests chemotaxonomic relationship between these two species.



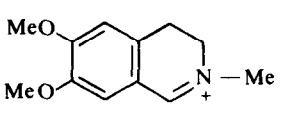
1



2



3



4

EXPERIMENTAL

Mps are uncorr. IR, UV, 100 MHz ^{1}H NMR spectra were recorded in KBr, EtOH, and $CDCl_3$ and DMSO-d_6 with TMS as internal standard, respectively. Si gel G plates were used for TLC with $CHCl_3$–MeOH–C_6H_6 (3:1:1) as developer. Non-aq. solvents were routinely dried over Na_2SO_4 before use. High resolution MS were recorded at 70 eV using a direct inlet system.

Isolation. Air-dried finely ground leaves (2.5 kg), collected from Rupdaha, Nadia District, West Bengal, in winter, were defatted with petrol (bp 60–80°) for 18 hr in a Soxhlet and then percolated with 95% EtOH containing 5% HOAc acid for a prolonged period. The solvent was removed under red. pres. and the resulting dark viscous residue was extracted with 3% HOAc (5 × 150 ml). The acidic extract was defatted with petrol (40–60°, 5 × 250 ml) and then extracted with C_6H_6 (5 × 250 ml) and $CHCl_3$ (5 × 250 ml) in succession. The C_6H_6 and $CHCl_3$ extracts were each made alkaline with dil NH_4OH, washed with H_2O, dried and the solvents removed to yield a gummy residue. On addition of Me_2CO to the gum obtained from the $CHCl_3$ extract, a solid separated (A) which was filtered and the filtrate, on removal of solvent, again gave an oily mass. This and the residue obtained from the C_6H_6 extract showed an identical alkaloid composition on TLC and were combined and the mixture (B, 2.1 g) chromatographed over Brockmann Al_2O_3 (125 g). The column was successively eluted with petrol (60–80°), petrol–C_6H_6 (7:3), petrol–C_6H_6 (1:1), C_6H_6, C_6H_6–$CHCl_3$ (1:1) and $CHCl_3$.

(−)-*Xylopine* 1. The solid A obtained from $CHCl_3$ extraction was soluble in hot H_2O. The aq. soln, on basification with NH_4OH, extraction with $CHCl_3$ and work-up in the usual way, gave (−)-xylopine (110 mg), mp 123°; $[\alpha]_D$ −21.5° (MeOH); λ_{max} nm: 280 (log ε 4.32); MS (*m/e*) M^+ (295), M^+ −1 (294), M^{2+} (147.5), M^+ −15 (280), M^+ −29 (266) and M^+ −30 (265). It was identified as (−)-xylopine by direct comparison with an authentic sample by TLC, mp, UV, IR, ^{1}H NMR and CD.

(+)-O-*Methylarmepavine* 2. The petrol–C_6H_6 (7:3) eluate of B, after chromatography over neutral Al_2O_3, yielded (+)-*O*-methylarmepavine (200 mg) as an oil; $[\alpha]_D$ +92.5° (MeOH); λ_{max} nm: 280 (log ε 3.78); ^{1}H NMR: singlets at δ 3.85, 3.78 and 3.57 ppm (3 × Ar-OMe), 2.52 (N-CH_3), 7 and 6.8 (4 × Ar-H in an A_2B_2 pattern at C-9, 10, 12 and 13) and singlets at 6.56 (C-4H) and 6.02 (C-1H, unusual high field position indicating that this proton lies below the plane of the benzene ring). It was identified as (+)-*O*-methylarmepavine by comparing UV, IR and ^{1}H NMR data and by direct GLC comparison with an authentic sample.

Lanuginosine 3. The $CHCl_3$ eluate of B furnished lanuginosine (30 mg), mp 315–317° (dec); λ_{max} nm: 246, 273 and 315 (log ε 4.52, 4.42 and 3.88); ν_{max}^{KBr} cm^{-1}: 1665 (conjugated C=O), 1495, 1420, 1360, 1262, 1135, 1045, 965 and 945 (CH_2<O—, —O>). Its identity with lanuginosine was established by direct comparison of TLC, IR, UV, ^{1}H NMR and MS with an authentic sample.

Acknowledgements—The authors wish to express their sincere thanks to Drs. B. Das, France (MS), Mrs. B. Talapatra, Calcutta (lanuginosine), P. Sengupta, Kalyani (friedelin), J. Schmutz, Switzerland ((−)-xylopine), G. J. Kapadia, U.S.A. (GLC comparison with (+)-*O*-methylarmepavine) and C. Casagrande, Italy (xylopine HCl and ^{1}H NMR curve of lanuginosine). The Calcutta group wishes to thank Dr. S. Sikdar, Head of the Department of Pharmacology, for his interest and active cooperation, and the U.G.C., New Delhi, for financial assistance. R. M. thanks CNPq, Brazil, for a research grant.

REFERENCES

1. Watt, G. (1972) *A Dictionary of the Economic Products of India*, Vol. 1, p. 260. Periodical Experts, New Delhi.
2. Bhakuni, D. S., Dhar, M. L., Dhar, M. M., Dhawan, B. N. and Mehrotra, B. N. (1969) *Indian J. Exp. Biol.* 7, 250.
3. Yang, T. H. and Chi-Ming, C. (1970) *J. Chin. Chem. Soc. (Taipei)* **17**, 243.
4. Bhakuni, D. S., Tewari, S. and Dhar, M. M. (1972) *Phytochemistry* **11**, 1819.
5. Bohlmann, F. and Rao, N. (1973) *Chem. Ber.* **106**, 84.
6. Yang, T. H. and Chi-Ming, C. (1972) *J. Chin. Chem. Soc. (Taipei)* **19**, 149.
7. Ohashi, M., Wilson, J. M., Budzikiewicz, H., Shamma, M., Slusarchyk, W. A. and Djerassi, C. (1970) *J. Am. Chem. Soc.* **85**, 2807.
8. Schmutz, J. (1959) *Helv. Chim. Acta* **42**, 335.
9. Casagrande, C. and Merotti, C. (1970) *Farmaco Ed. Sci.* **25**, 799.
10. Bhacca, N. S., Johnson, L. F. and Shoolery, J. N. (1962) *NMR Spectra Catalog*, Spectra 333 and 342. Varian Associates, Palo Alto, California.
11. Shamma, M. (1972) *The Isoquinoline Alkaloids, Chemistry and Pharmacology*, p. 80. Academic Press, New York.
12. Kapadia, G. J., Saha, J. N. and Highet, J. R. (1964) *J. Pharm. Sci.* **53**, 1140.
13. Talapatra, S. K., Patra, A. and Talapatra, B. (1975) *Tetrahedron* **31**, 1105.
14. Shamma, M. (1972) *The Isoquinoline Alkaloids, Chemistry and Pharmacology*, p. 246. Academic Press, New York.