Phytochemistry, 1972, Vol. 11, pp. 1819 to 1822. Pergamon Press. Printed in England.

SHORT COMMUNICATION

APORPHINE ALKALOIDS OF *ANNONA SQUAMOSA**

D. S. BHAKUNI, SHOBHA TEWARI and M. M. DHAR

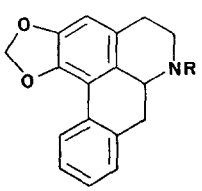
Central Drug Research Institute, P.O. Box 173, Lucknow, India

(*Received* 28 *May* 1971, *in revised form* 20 *October* 1971)

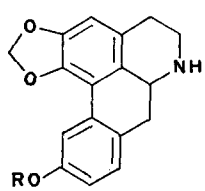
Abstract—The aporphine alkaloids anonaine, roemerine, norcorydine, corydine, norisocorydine, isocorydine and glaucine have been isolated from *Annona squamosa*.

INTRODUCTION

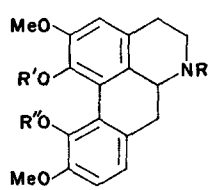
Annona squamosa L. (Annonaceae), a small evergreen tree, occurs wild and is also cultivated throughout India for its fruit. A 50% ethanol extract of the leaves and stem of the plant showed anticancer activity.[1] This finding prompted detailed chemical investigations and has resulted in the isolation of corydine (V), a base reported to have anticancer activity[2] and six other aporphine bases, anonaine (I), roemerine (II), norcorydine (IV), norisocorydine (VI), isocorydine (VII) and glaucine (VIII). Indications have also been obtained for the presence of norlaureline (III), aporphine (IIIa) and the dienone (IX) from NMR and MS of preparative TLC fractions. Anonaine had been isolated earlier from the seeds of this plant.[3]



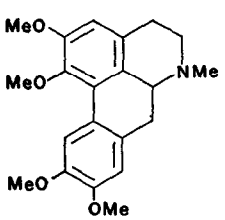
(I) R = H
(II) R = Me



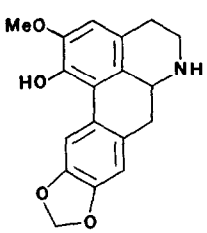
(III) R = Me
(IIIa) R = H



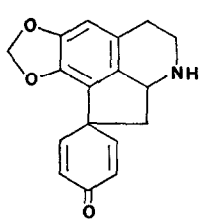
(IV) R = R′ = H ; R″ = Me
(V) R = R″ = Me; R′ = H
(VI) R = R″ = H ; R′ = Me
(VII) R″ = H ; R = R′ = Me



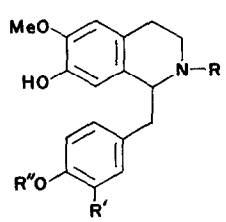
(VIII)



(X)



(IX)



(XI) R = R′ = R″ = H
(XII) R = R″ = Me ; R′ = OH

* Communication No. 1639 from the Central Drug Research Institute.

[1] D. S. BHAKUNI, M. L. DHAR, M. M. DHAR, B. N. DHAWAN and B. N. MEHROTRA, *Indian J. Exptl Biol.* **7**, 250 (1969).

[2] V. PETERS, J. L. HARTWELL, A. J. DALTON, and M. J. SHEAR, *Cancer Res.* **6**, 490 (1946); *Chem. Abs.* **42**, 5540 (1948).

[3] F. R. REYES and A. C. SANTOS, *Philiphine J. Sci.* 409 (1931); *Chem. Abs.* **25**, 2807 (1931).

RESULTS AND DISCUSSION

The bases were separated by chromatography on neutral Al_2O_3 column and preparative TLC. The molecular formulae of the isolated alkaloids were confirmed by their mass spectra. The mass fragmentation pattern (Table 1) of these bases are typical of aporphine alkaloids.[4] The relative intensities of the peaks especially those of M^+ and the base peak (M^+-1) are in conformity with the indicated substitution pattern.

TABLE 1. MS OF ALKALOIDS FROM *Annona squamosa*

	Alkaloids	M^+	M^{2+}	M^+-1	M^+-15	M^+-17	M^+-29	M^+-31	M^+-43	M^+-58	M^+-74
(I)	$C_{17}H_{15}NO_2$	265	132·5	264	250	—	236	235(M^+-30)	—	—	—
(II)	$C_{18}H_{17}NO_2$	279	139·5	278	264	—	—	249(M^+-30)	—	—	—
(III)	$C_{18}H_{17}NO_3$	295	—	294	280	—	266	265(M^+-30)	—	—	—
(IIIa)	$C_{17}H_{15}NO_3$	281	—	280	266	264	252	251(M^+-30)	—	—	—
(IV)	$C_{19}H_{21}NO_4$	327	163·5	326	312	310	298	296	—	—	253
(V)	$C_{20}H_{23}NO_4$	341	170·5	340	326	324	—	310	298	383	267
(VI)	$C_{19}H_{21}NO_4$	327	163·5	326	312	310	298	296	—	—	253
(VII)	$C_{20}H_{23}NO_4$	341	170·5	340	326	324	—	310	298	283	267
(VIII)	$C_{21}H_{25}NO_4$	355	177·5	354	340	338	—	324	312	297	281
(IX)	$C_{17}H_{15}NO_3$	281	—	280	—	—	252	—	—	—	—
(X)	$C_{18}H_{19}NO_4$	311	155·5	310	296	294	282	280	—	253	237

The UV spectra of I and II have λ_{max} at 231–235, 270–272 and 310–315 of III; at 273–276 and 304–316 of IV to VII at 220, 268–270 and 302–308 of VIII at 218, 280–282 and 302 nm. These maxima are in agreement for those aporphines[5,6] lacking substitution in ring D; aporphines substituted at position 10; aporphines substituted at position 10 and 11; and aporphines substituted at positions 9 and 10 respectively.

TABLE 2. CHEMICAL SHIFTS (τ) OF THE PROTONS OF *Annona squamosa* BASES

Alkaloid	N-6	C_1	C_2	C_3	C_8	C_9	C_{10}	C_{11}
(I)	—	3·82 (d)	3·97 (d)	3·31	2·67	2·67	2·67	2·30 (m)
(II)	7·30	3·96 (d)	4·00 (d)	3·32	2·64	2·64	2·64	2·18 (m)
(IV)	—	—	6·00	3·32	3·00	2·78	6·02	6·21
(V)	7·41	—	6·05	3·19	3·05	3·05	6·05	6·24
(VI)	—	6·45	6·08	3·27	2·97	3·00	6·18	—
(VII)	7·42	6·39	6·18	3·32	3·30	3·42	6·16	—
(VIII)	7·32	6·30	6·02	3·29	3·12	6·04	6·06	1·79

d—doublet; m—multiplet.

The NMR spectra (Table 2) of these compounds were very informative. A low field proton signal, characteristic of a deshielded proton at position 11 of the aporphine system,[7] was present at τ 1·79 in the spectrum of base VIII. A low field one proton multiplet at τ 2·18–2·30 and a characteristic AB quartet of a methylenedioxy group[7] located at position

[4] A. H. JACKSON and J. A. MARTIN, *J. Chem. Soc.* **C**, 2181 (1966); M. OHASHI, J. M. WILSON, H. BUDZIKIEWICZ, M. SHAMMA, W. A. SLUSARCHYK and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 2807 (1963).

[5] M. SHAMMA, *Experientia* **16**, 484 (1960).

[6] A. W. SANGSTER and K. L. STUART, *Chem. Rev.* **65**, 69 (1965).

[7] W. H. BAARSCHERS, R. R. ARNDT, K. PACHLER, J. A. WEISBACH and B. DOUGLAS, *J. Chem. Soc.* 4778 (1964); I. R. C. BICK, J. H. MASON, N. SHEPPARD and M. J. VERNENGO, *J. Chem. Soc.* 1896 (1961); S. GOODWIN, J. N. SHOOLERY and L. F. JOHNSON, *Proc. Chem. Soc.* 306 (1958).

1, 2 was discernible at τ 3·92–4·00, $\Delta\lambda$ 8·5 Hz; $J = 2$ Hz in the NMR spectra of bases I and II. A three proton signal for a shielded methoxyl group at position 1, appeared at τ 6·30–6·45 in the spectra of VI, VII and VIII. A comparatively less shielded methoxyl group present at position 11 gave rise to a signal at τ 6·21–6·24 in the spectra of IV and V. The signals for methoxyl groups present at positions 2, 9 and 10 appeared with the usual chemical shift at τ 6·00–6·10.

The relative positions of the hydroxyl and methoxyl groups in VII was established by base catalysed deuterium exchange experiments.[8] The proton at position 8 was almost completely replaced by deuterium. The fact that I, IV and VI are norbases was confirmed by converting them into the corresponding *N*-methyl derivatives by treatment with formaldehyde followed by $NaBH_4$.[9]

It is of biogenetic interest that *Annona squamosa* produces aporphine such as anonaine (I) which lack substitution in ring D as well as aporphines substituted at positions 1, 2, 10 (norlaureline); at positions 1, 2, 9, 10 (glaucine) and at positions 1, 2, 10 and 11 (corydine and isocorydine).

EXPERIMENTAL

IR, UV and 60 Mcs NMR spectra were recorded in KBr, EtOH and $CDCl_3$ with TMS as internal standard respectively and $[\alpha]_D$ in EtOH. Silica gel-G plates were used for TLC, with $CHCl_3$–MeOH (19:1) and (9:1) and C_6H_6–Et_3NH–EtOAC (7:1:2). Non-aqueous solutions were dried over anhydrous Na_2SO_4.

Isolation of bases. Air dried leaves and tender stems (16 kg) of the plant, collected in Madhya Pradesh in December, were extracted with 95% EtOH (6 × 30 l.) and solvent removed below 60°. The resulting dark green viscous mass was extracted with 5% HCl (5 × 150 ml), the acidic solution defatted with pet-ether (4 × 250 ml), basified with 5% NaOH and extracted with Et_2O (5 × 200 ml). The Et_2O extract was washed with water, dried and evaporated to give alkaloidal mixture-A (800 mg). The remaining alkaline solution was acidified with dil. HCl and then basified with $NaHCO_3$. The liberated bases were extracted with $CHCl_3$ (5 × 200 ml), washed with water, dried and the solvent removed to give alkaloidal mixture-B (1·8 g).

Alkaloidal mixture-A. (800 mg) was chromatographed on neutral Al_2O_3 (50 g). The column was successively eluated with C_6H_6 and C_6H_6–$CHCl_3$ (9:1), (4:1) and (1:1) and $CHCl_3$. Elution was followed by TLC.

Glaucine (VIII). Contents of the C_6H_6–$CHCl_3$ (9:1) eluate were subjected to preparative TLC to yield glaucine (40 mg) m.p. 118°; (Lit.[10] 120°). λ_{max} 218, 281, and 303 nm (log ϵ 4·58, 4·18 and 4·16). The identity of the base with glaucine was established by comparison (TLC, m.p., m.m.p., IR, UV, NMR and MS) with a sample prepared from boldine by treatment with CH_2N_2.

Roemerine (II). The C_6H_6–$CHCl_3$ (4:1) eluate when further purified by preparative TLC afforded roemerine (20 mg), m.p. 96–98°; $[\alpha]_D$ −65° (Lit.[11] m.p. 100–101°; $[\alpha]_D$ −72·5°). λ_{max} 234, 272 and 312 nm (log ϵ 4·17, 4·21 and 3·52). Base HI m.p. 240°. The base was found identical (TLC, m.p., UV, IR, NMR and MS) with a sample of roemerine prepared from anonaine.

Anonaine (I). The $CHCl_3$–C_6H_6 (4:1) eluate gave anonaine (60 mg), m.p. 120–122°; $[\alpha]_D$ −50° (Lit.[12] m.p. 122–123°; $[\alpha]_D$ −52°). Base HCl m.p. 270°; λ_{max} 233, 273 and 314 nm (log ϵ 4·12, 4·20 and 3·53). The base was found identical (TLC, m.p., UV, IR, NMR and MS) when compared with an authentic sample of anonaine. A mixture of the base (30 mg), HCHO (1 ml) and HCOOH (1 ml) was heated at 100° for 30 min. The resulting mixture yielded roemerine m.p. 98–99°.

The alkaloidal mixture-B. 1·6 g was chromatographed on neutral Al_2O_3 (100 g). The column was eluted successfully with C_6H_6, C_6H_6–$CHCl_3$ (9:1), (4:1), (7:3), (1:1), (1:2), $CHCl_3$ and $CHCl_3$–MeOH (99:1), (49:1), (19:1) and (9:1). Elution being followed by TLC.

Corydine (V). The residue from the C_6H_6 eluates was rechromatographed on SiO_2 to give corydine

[8] L. J. HAYNES, K. L. STUART, D. H. R. BARTON and G. W. KIRBY, *J. Chem. Soc.* C, 1676 (1966).
[9] M. TOMITA and M. KOZUKA, *J. Pharm. Soc.* Japan **85**, 77 (1965).
[10] R. H. F. MANSKE, in *The Alkaloids* (edited by MANSKE and HOLMES), Vol. IV, p. 120, Academic Press, New York (1954).
[11] J. SLAVIK, *Coll. Czech. Chem. Commun.* **28**, 1738 (1963); M. SHAMMA and W. A. SLUSARCHYK, *Chem. Rev.* **64**, 62 (1964).
[12] K. W. GOPINATH, T. R. GOPINATH, T. R. GOVINDACHARI, B. R. PAI and N. VISWANATHAN, *Chem. Ber.* **92**, 776 (1959).

(45 mg), m.p. 148°; $[\alpha]_D$ +198° (Lit.[13] m.p. 149°; $[\alpha]_D$ + 205°), λ_{max} 270, and 310 nm (log ϵ 4·12 and 3·8). Base HCl m.p. 254–255°. The free base was found identical with corydine (TLC, UV, NMR and MS).

Isocorydine (VII). The C_6H_6–$CHCl_3$ (3:1) eluate gave isocorydine (70 mg) m.p. 184°; $[\alpha]_D$ + 170° (Lit.[14] m.p. 186°; $[\alpha]_D$ +185°). λ_{max} 220, 266 and 305 nm (log ϵ 4·70, 4·13 and 3·81). Base, HI m.p. 223°. The free base was identical with isocorydine (UV, NMR and MS).

Deuteration of isocorydine. Isocorydine (50 mg), K-*t*-butoxide (110 mg) and D_2O (0·5 ml) were heated in a sealed tube at 100° in N_2 for 100 hr. The resulting solution was cooled, NH_4Cl added and the liberated base extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried and solvent removed. The deuterated compound was identical with isocorydine (TLC). The NMR spectrum of this compound was identical with that of isocorydine except that the signal for an aromatic proton at position 8 had almost disappeared.

Norisocorydine (VI). The $CHCl_3$–MeOH (1:1) eluate gave norisocorydine (45 mg), homogenous on TLC but could not be crystallized. λ_{max} 223, 267 and 308 nm (log ϵ 4·73, 4·14 and 3·79). Base HBr m.p. 202–204° (Lit.[15] 203–205°). A mixture of the base (30 mg) HCHO (1 ml), HCOOH (1 ml) when heated at 100° for 0·5 hr afforded isocorydine m.p. 183°.

Norcorydine (IV). The $CHCl_3$–MeOH (49:1) eluate after preparative TLC yielded norcorydine (43 mg). This product was homogenous on TLC but could not be crystallized. λ_{max} 269 and 309 nm (log ϵ 4·12 and 3·78).

A mixture of the base (35 mg) HCHO (1 ml), and HCOOH (1 ml) was heated at 100° for 0·5 hr. The *N*-methyl derivative thus obtained was identical with corydine (TLC and MS).

The $CHCl_3$–MeOH (49:1) eluate was a mixture of at least 3 compounds (TLC). This mixture was resolved by preparative TLC. The MS and NMR spectral analysis of the fractions revealed the presence of aporphine (III), (IIIa) and dienone (IX).

[13] J. SLAVIK, *Coll. Czech. Chem. Commun.* **24**, 3999 (1959).
[14] O. E. EDWARDS and K. L. HANDA, *Can. J. Chem.* **39**, 1801 (1961); R. R. ARNDT and W. H. BAARSCHERS, *J. Chem. Soc.* 2244 (1964).
[15] A. RUEGGER, *Helv. Chim. Acta.* **42**, 754 (1959).

Acknowledgement—Financial aid from the National Institute of Health. U.S.A. (Grant No. PL-480-134304) is gratefully acknowledged.

Key Word Index—Annona squamosa; Annonaceae; aporphine alkaloids.