# Tumor Inhibitors II: Cytotoxic Alkaloids from *Annona purpurea*

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**Abstract** □ Investigation of the cytotoxic principles of *Annona purpurea* L. resulted in the isolation and characterization of four new alkaloids of the aporphine variety. One of the four is an oxoaporphine which shows borderline activity *in vitro* against the 9-KB tumor test system. The known aporphine isocorydine, oxoaporphine *O*-methylatheroline, and the proaporphines glaziovine and stepharine were also obtained. Glaziovine and *O*-methylatheroline also showed limited tumor inhibition.

**Keyphrases** □ *Annona purpurea*—isolation, characterization of four aporphine alkaloids □ Tumor inhibitors—isolation, characterization of four aporphine alkaloids from *Annona purpurea* □ Aporphine alkaloids, *Annona purpurea*—potential tumor inhibitors

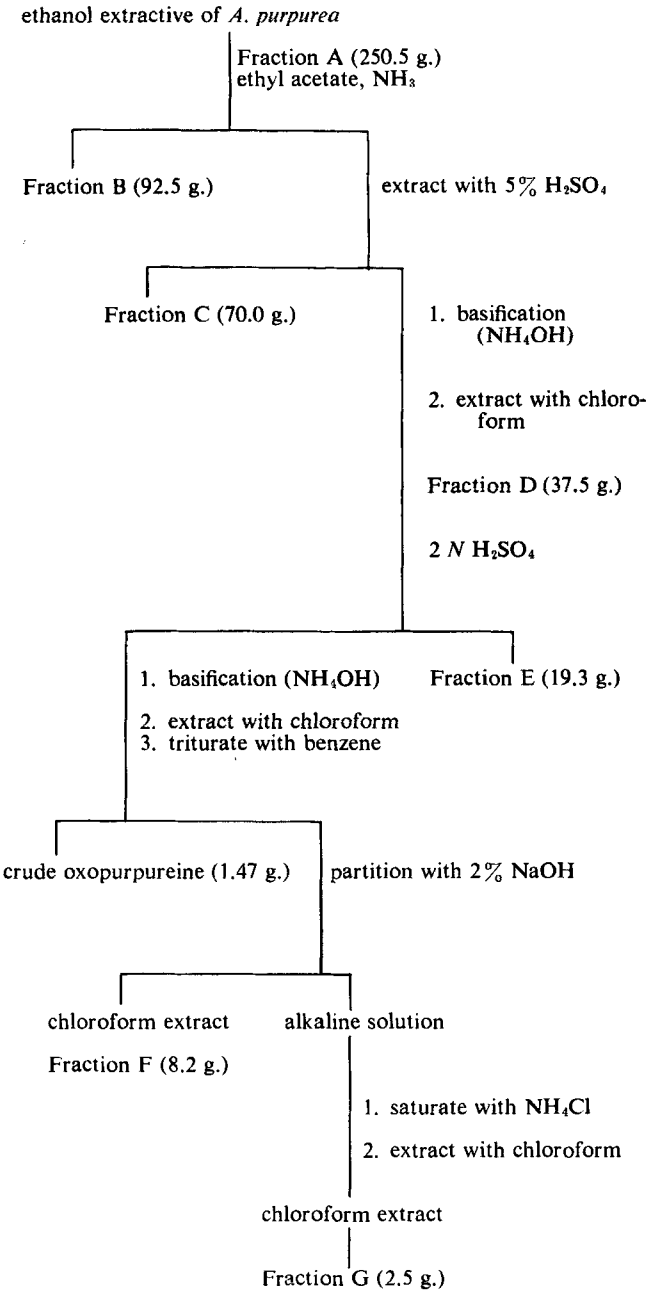
The first paper in this series (1) reported the isolation and identification of liriodenine from wood and stem bark of *Annona glabra* L. The present paper reports the continuation of this search for tumor inhibitors from plant and insect sources. Ethanol extracts of ground stems and leaves of *Annona purpurea* L.[1] have been found to inhibit tumor activity *in vitro* in the test system 9-KB[2]; several cytotoxic alkaloids from these extracts have been isolated and characterized.

## EXPERIMENTAL

The procedure was as follows: 10.9 kg. (24 lb.) of dried plant material was steeped with hexane and extracted with ethanol. The concentrated ethanol extract was then extracted with ammoniacal ethyl acetate. This extract was washed repeatedly with 5% sulfuric acid and then made basic (pH 10) with ammonium hydroxide. It was then extracted with chloroform. The activity proved to be concentrated in the acid-soluble fraction (Scheme I).

The crude alkaloids were triturated with benzene, and the triturate was separated into base-soluble alkaloids (Fraction E) and base-insoluble alkaloids (Fraction F). Each group was then further fractionated with McIlvaine buffer solutions. Finally, each was subjected to column chromatography on various grades of neutral alumina until pure compounds, judged by TLC, were obtained.

An orange solid was obtained from the benzene-insoluble fraction, which was resolved by further column chromatography on Activity I alumina with benzene and chloroform into an orange compound and a small amount of a yellow compound. The yellow compound proved to be identical with *O*-methylatheroline (I*a*). The sample melted at 220–226°[3]; reported melting points are 225–227° (2), 227–229° (3), and 235–236° (4). The IR spectrum[4] was identical to the published spectrum for I*a* (4), and the UV (Table I) and NMR spectral data were, likewise, in accord with published data (3–5).




| Fraction | $ED_{50}$, mcg./ml. |
|---|---|
| A | 100 |
| B | >100 |
| C | >100 |
| D | 20.0 |
| E | 21.0 |
| F | 25.0 |
| G | 8.2 |

*Scheme I—Fractionation of cytotoxic principles of* A. purpurea *and cytotoxicity of the fractions*

[1] Collected in Puerto Rico in 1969.

[2] Cytotoxicity was assayed, under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, U. S. Public Health Service, against Eagle's KB strain of cells derived from human carcinosarcoma of the nasopharynx [H. Eagle and G. E. Foley, *Amer. J. Med.*, **21**, 739(1956); *Cancer Res.*, **18**, 1017(1958)].

[3] Melting points are uncorrected and were obtained on a Fisher-Johns melting-point apparatus. Mention of a proprietary item in this paper does not constitute an endorsement of this product by the U. S. Department of Agriculture.

[4] IR spectra were obtained with Perkin-Elmer 137 and 521 spectrophotometers.

Table I—UV Spectral Data[a]

| Compound | $\lambda_{max.}$, nm. | Log ε | Reference |
|---|---|---|---|
| Ia: *O*-Methylatheroline | 246, 277, 363 | 4.59, 4.58, 4.16 | —[b] |
| | 242, 272, 355 | 4.52, 4.53, 3.99 | 3 |
| Ia: $H^+$ | 260, 284, 385 | 4.65, 4.63, 4.20 | —[b] |
| Ib: Oxopurpureine | 251, 282, 354, 392, 456 | 4.37, 4.54, 3.86, 3.94, 3.78 | —[b] |
| II: Cassamedine | 252, 281, 324, 364, 460 | 4.47, 4.53, 4.12, 3.97, 3.76 | 6 |
| Ib: $H^+$ | 260, 287, 425 | 4.40, 4.49, 3.86 | —[b] |
| II: $H^+$ | 272, 286, 408, 534 | 4.49, 4.50, 4.10, 3.40 | 6 |
| IIIa: Glaziovine | 286, 315sh | 3.59, 2.93 | —[b] |
| | 288 | 3.57 | 9 |
| IIIb: Stepharine | 278 | 3.78 | —[b] |
| | 284 | 3.48 | 14 |
| IVa: Norpurpureine | 272sh, 280, 300, 311sh | 4.13, 4.20, 4.13, 4.07 | —[b] |
| IVb: Purpureine | 273sh, 282, 303, 312sh | 4.26, 4.36, 4.33, 4.29 | —[b] |
| V: Isocorydine | 267, 271sh, 305 | 4.03, 4.02, 3.69 | —[b] |
| | 266, 302 | 4.26, 3.82 | —[7] |
| VI: *O*-Demethylpurpureine | 272sh, 283, 302, 312sh | 4.11, 4.25, 4.21, 4.15 | —[b] |
| VIIa: *O*-Methylcassyfiline | 283, 302, 312 | 4.31, 4.30, 4.27 | 6 |
| VIIb: Ocoteine | 283, 302 | 4.24, 4.25 | 14 |

[a] Spectra were determined in ethanol solvent with a Cary 14 recording spectrophotometer. [b] This work.

After recrystallization from toluene, the orange compound was analyzed for $C_{21}H_{19}NO_6 \cdot {}^1/_2\, C_7H_8$, m.p. 198–202° dec. The absence of NH and alkyl $CH_2$ stretching absorptions in its IR spectrum, the presence of a strong 1640 $cm.^{-1}$ band, and the formation of a cherry-red solution in dilute acid suggested an oxoaporphine nucleus. The NMR spectrum (Table II) indicated five methoxyl groups; the UV spectra, both in neutral and in acidic solutions, showed some similarity to those of cassamedine (II) (6). From the NMR spectrum, positions C-4 and C-5 were clearly unsubstituted. The remaining pair of aryl protons was unsplit and could, therefore, be present *para* to each other at positions C-8 and C-11 as in Structure I*b*. Further evidence for this structure is presented later.

Investigation of the base-insoluble Fraction F provided two new aporphine alkaloids (IV*a* and IV*b*) and the known proaporphine, stepharine (III*b*). Stepharine was identified by direct comparison with authentic (+)-stepharine (IR and mixed melting point). Compound IV*a*, m.p. 115–117°, was identified as a pentamethoxynoraporphine from its NMR spectrum; it also gave an *N*-methyl methiodide, m.p. 227–229°, identical with the methiodide of the pentamethoxyaporphine (IV*b*). Oxidation of Compound IV*b* with chromium trioxide pyridine provided a small sample (~2 mg.) of the corresponding oxoaporphine which was identical to Compound I*b* (TLC and IR).

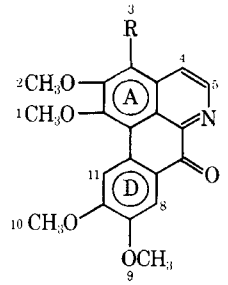
The base-soluble Fraction G provided the phenolic aporphine, isocorydine (V), m.p, 183–185°: [lit (7) m.p. 186°]. The NMR, UV, and IR data (7) were identical with those obtained for the alkaloid isolated from *A. purpurea*. The methiodide showed m.p. 231–232° (darkening at 224°) [lit. (8) m.p. 231–232° when heated rapidly]. This alkaloid was finally identified with a known sample of (+)-isocorydine (IR and mixed melting point). A phenolic proaporphine, m.p. 227–231° dec., was also obtained; its NMR and UV spectra compared well with those of glaziovine (III*a*), m.p. 235–237° dec. (9). The picrate of Compound III*a* had m.p. 196.5–200° [lit. (9) m.p. 199–203°]. This alkaloid was also identified with an authentic sample of (+)-glaziovine (IR).

A phenolic tetramethoxyaporphine (VI) was also isolated; complete methylation generated the same methiodide described for Compounds IV*a* and IV*b*. The pattern of oxygenation must, therefore, be the same for the phenol, Compounds IV*a* and IV*b*, and for the oxoaporphine, Compound I*b*. The phenol (VI) did not incorporate deuterium when treated under conditions that normally cause protons *ortho* and *para* to phenolic hydroxyl groups to be exchanged

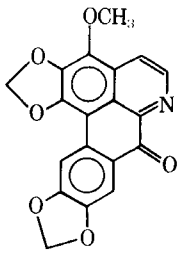
Table II—NMR Data[a]

| Compound | Solvent | Aryl Protons C4 | C5 | C8 | C9 | C11 | Other Protons | Reference |
|---|---|---|---|---|---|---|---|---|
| Ib: Oxopurpureine | $CDCl_3$ | 8.13d(5.4) | 8.91d | 7.94s | — | 8.65s | $OCH_3$: 4.03, 4.06, 4.07, 4.10, 4.15 | —[b] |
| | TFA | 8.87d(6.3) | 9.01d | 8.08s | — | 8.98s | $OCH_3$: 4.18, 4.26, 4.34, 4.38, 4.43 | —[b] |
| II: Cassamedine | TFA | 7.83d | 8.85d | 8.19s | — | 8.85s | $OCH_3$: 4.48; $OCH_2O$: 6.23, 6.62 | 6 |
| IVa: Norpurpureine | $CDCl_3$ | — | — | 6.74s | — | 7.98s | $OCH_3$: 3.72, 3.90(3), 3.94 | —[b] |
| IVb: Purpureine | $CDCl_3$ | — | — | 6.77s | — | 7.95s | $NCH_3$: 2.54; $OCH_3$: 3.71, 3.88, 3.91(2), 3.94 | —[b] |
| IVb: Methiodide | Dimethyl sulfoxide | — | — | 7.02s | — | 7.79s | $NCH_3$: 3.36, 3.40; $OCH_3$: 3.73, 3.78, 3.82, 3.89(2) | —[b] |
| VI: *O*-Demethylpurpureine | $CDCl_3$ | — | — | 6.76s | — | 7.89s | $NCH_3$: 2.54; $OCH_3$: 3.70, 3.90(2), 3.96 | —[b] |
| | Dimethyl sulfoxide | — | — | 6.90s | — | 7.76s | $NCH_3$: 2.44; $OCH_3$: 3.66, 3.77(3) | —[b] |
| | Dimethyl sulfoxide–NaOMe | — | — | 6.75 | — | 7.70 | | —[b] |
| V: Isocorydine | Dimethyl sulfoxide | — | — | 6.84,6.87[c] | 6.84,6.87[c] | — | C3H: 6.83; $NCH_3$: 2.49; $OCH_3$: 3.58, 3.76, 3.82 | —[b] |
| | Dimethyl sulfoxide–NaOMe | — | — | 6.44[d] | 5.89[d] | | C3H: 6.53 | —[b] |

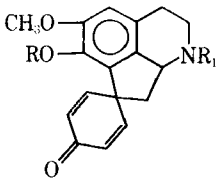
[a] NMR spectra were recorded with a Varian HA-100 spectrometer. Data are given in parts per million measured from tetramethylsilane as an internal standard. Standard abbreviations used are s = singlet, d = doublet. [b] This work. [c] Coupling constant $J_{AB}$ 8 Hz. [d] $J_{AX}$ 8 Hz.



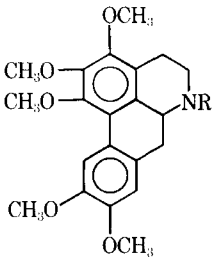
Ia: R = H
Ib: R = $OCH_3$



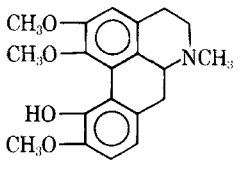
II



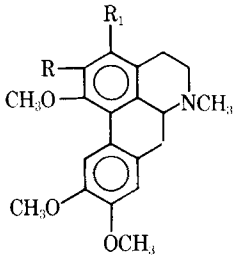
IIIa: R = H, $R_1$ = $CH_3$
IIIb: R = $CH_3$, $R_1$ = H



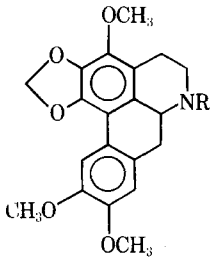
IVa: R = H
IVb: R = $CH_3$



V



VIa: R = OH, $R_1$ = $OCH_3$
VIb: R = $OCH_3$, $R_1$ = OH



VIIa: R = H
VIIb: R = $CH_3$

(10). A comparison of the NMR spectra of the phenol in dimethyl sulfoxide, with and without added base, showed no large upfield shift of either aryl proton in the basified solution, although a shift of 0.4–0.6 p.p.m. can be expected for protons *meta* to a phenolic hydroxyl (11). An example of such a shift is provided in Table II (isocorydine). The observations, therefore, indicated that the phenolic hydroxyl was situated on a fully substituted ring so the two aryl protons must be on the same ring. Since they are not visibly coupled, they must be *para* to one another and can only be assigned to positions C-8 and C-11 in ring D. Then, since the oxygenation pattern of the four new compounds is the same, the structures of the nonphenolic alkaloids, Compounds I*b*, IV*a*, and IV*b*, were determined. The hydroxyl group of the new phenol must be present in ring A. Position C-1 can be eliminated since no downfield shift of the proton at C-11 occurred in the NMR spectrum of the phenol anion (11). Structure VI*a* or VI*b*, in which the hydroxyl group is either on C-2 or C-3, can be assigned to this phenol. Insufficient material prevented a degradative study to distinguish the two alternatives. The UV spectra of *O*-methylcassifiline (VII*a*) and ocoteine (VII*b*) were included in Table I for comparison with IV*a* and IV*b*.

**Table III**—Inhibition Test Data

| Compound | Amount Isolated, mg. | $ED_{50}$, mcg./ml. |
|---|---|---|
| I*a*: *O*-Methylatheroline | 21 | 5.1 |
| I*b*: Oxopurpureine | 300 | 5.8 |
| III*a*: Glaziovine | 159 | 2.6 |
| III*b*: Stepharine | 107 | 17 |
| IV*a*: Norpurpureine | 33 | 22 |
| IV*b*: Purpureine | 293 | >100 |
| V: Isocorydine | 163 | 87 |
| VI: *O*-Demethylpurpureine | 104 | 22 |

The results of the cytotoxicity tests for the isolated alkaloids are summarized in Table III. The oxoaporphines, Compounds I*a* and I*b*, and the proaporphine, glaziovine, showed borderline inhibitory activity. Corydine hydrochloride, an aporphine, was reported to be active against Sarcoma 37 when intramuscularly implanted in hybrid mice (12); liriodenine, an oxoaporphine, is also cytotoxic (1). The proaporphines, which have the susceptibility to nucleophilic attack inherent in the cyclohexadienone structure, are therefore good candidates for tumor screening (13) and should be further investigated. The following names are suggested for the new alkaloids: I*b*, oxopurpureine; IV*a*, norpurpureine; IV*b*, purpureine; and VI, *O*-demethylpurpureine.

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