Pergamon

S0031-9422(96)00181-1

Phytochemistry, Vol. 42, No. 6, pp. 1703–1706, 1996

0031-9422/96 $15.00 + 0.00

A DIOXOAPORPHINE AND OTHER ALKALOIDS OF TWO ANNONACEOUS PLANTS OF SRI LANKA

E. M. KITHSIRI WIJERATNE,* YASUMARU HATANAKA, TOHRU KIKUCHI, YASUHIRO TEZUKA and A. A. LESLIE GUNATILAKA†

Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama 930-01, Japan; †Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0212, U.S.A.

(*Received in revised form* 26 *January* 1996

Key Word Index—*Artabotrys zeylanicus*; *Xylopia championii*; Annonaceae; 4,5-dioxoaporphines; oxoaporphines; 8-methoxyouregidione.

Abstract—A new 4,5-dioxoaporphine alkaloid, 8-methoxyouregidione, along with artabotrine, ouregidione, liriodenine, oxocrebanine, oxobuxifoline, atherospermidine and lanuginosine have been isolated from *Artabotrys zeylanicus*. Investigation of *Xylopia championii* afforded *O*-methylmoschatoline and dicentrinone.

INTRODUCTION

Plants of the Annonaceae are known to elaborate a variety of alkaloids some of which are reported to have interesting pharmacological properties [1]. In Sri Lanka, the family Annonaceae is represented by 46 species distributed in 17 genera [2]; the genera *Artabotrys* and *Xylopia* each contain two and three species, respectively. In the course of our continuing phytochemical studies of the Sri Lankan Annonaceae [3–5], investigation of the alkaloidal constituents of *A. zeylanicus* Hook. F. & Thoms. and *X. championii* Hook. F. & Thoms. were undertaken. In this paper we report the isolation of a new 4,5-dioxoaporphine, 8-methoxyouregidione (**1**), and several known oxoaporphine and 4,5-dioxoaporphine alkaloids from these two plant species.

Previous studies on *A. zeylanicus*, a tall woody climber growing in the moist and adjacent intermediate region of Sri Lanka [6], has resulted in the isolation of two bioactive alkaloids, artabotrine (**2**) and atherospermidine (**7**) [5]. *X. championii* is a slender tree endemic to Sri Lanka [7]. There is no reported phytochemical work on this species.

RESULTS AND DISCUSSION

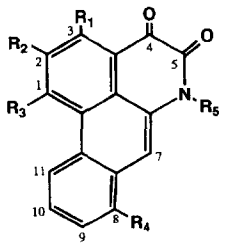
The dried and powdered stem bark of *A. zeylanicus* was successively and exhaustively extracted with hot hexane, chloroform and methanol. TLC investigation indicated the presence of alkaloids only in the chloroform extract, the chromatographic fractionation of which furnished (in order of increasing polarity) artabotrine (**2**) [5], liriodenine (**4**) [8], oxocrebanine (**5**) [9], ouregidione (**3**) [10], oxobuxifoline (**6**) [11], lanuginosine (**8**) [12], atherospermidine (**7**) [5] and a new 4,5-dioxoaporphine alkaloid identified as 8-methoxyouregidione (**1**).

All the above known alkaloids except **3** were identified by comparison of their physical and/or spectral data with those reported in the literature (see Experimental section). Alkaloid **3** was crystallized from methanol as orange needles, mp 262–264°. Its IR spectrum showed the presence of two CO groups (ν_{max} 1660 and 1620 cm^{-1}) in addition to a band due to a NH/OH group at ν_{max} 3000–3500 cm^{-1}. The UV spectrum with λ_{max} at 419, 317.5, 306, 241.5 and 211.5 nm was characteristic of a 4,5-dioxoaporphine [13]. The ^1H NMR spectrum showed a D_2O exchangable singlet at δ 11.45, signals due to three OCH_3 groups at δ 4.21, 4.17 and 4.10, one aromatic singlet at δ 7.79 and four aromatic protons at δ 7.66 (2H, *m*), 7.97 (1H, *m*) and 9.49 (1H, *m*) which were ascribed to the protons of the unsubstituted D ring of the aporphine nucleus [9, 14, 15]. The aromatic proton at δ 7.79 showed an NOE with signals at δ 11.45 and 7.97; the OCH_3 signal at δ 4.21 gave a NOE with the aromatic signal at δ 9.49. Thus, the compound was identified as ouregidione (**3**), which has previously been isolated from *Guatteria ouregou* [10]; however, the reported UV spectral data and the mp (280°) were different.

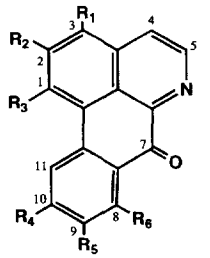
The new alkaloid, $C_{20}H_{17}O_6N$ (HREI mass spectrum), was crystallized from methanol as an orange powder, mp 276–277°. Its IR spectrum indicated the presence of OH/NH (3000–3500 cm^{-1}) and two CO (1680 and 1650 cm^{-1}) functionalities. The presence of UV maxima at 449, 388, 325, 311, 256, 240 and

*Author to whom correspondence should be addressed. Permanent address: Division of Natural Products Chemistry, Medical Research Institute, P.O. Box 527, Colombo 08, Sri Lanka.

210 nm suggested a 4,5-dioxoaporphine structure [13]. The ^{1}H NMR spectrum showed a D_2O exchangeable 1H broad singlet at δ 8.91, four singlets of 3H each at δ 4.18, 4.15, 4.08 and 4.07, and one aromatic singlet at δ 7.99. The remaining signals were found in the aromatic region [δ 9.09 (1H, *d*, J = 8.85 Hz), 7.58 (1H, *dd*, J = 8.85, 7.94 Hz) and 7.07 (1H, *d*, J = 7.94 Hz)], which showed an ABX pattern. An ABX system can be accommodated either in ring A or D of the 4,5-dioxoaporphine nucleus, which leaves ring A completely unsubstituted and ring D fully substituted or ring A fully substituted and ring D monosubstituted with the substituents at C-8 or C-11. Out of the four OCH_3 groups in **1** two were located at biogenetically favourable C-1 and C-2 of the ring A of the 4,5-dioxoaporphine nucleus, which suggested the possible presence of the former substitution pattern. The NH at δ 8.91 showed a NOE with the aromatic singlet at δ 7.99 and the latter was therefore assigned to H-7. Based on its chemical shift the aromatic doublet at δ 9.09 was assigned to H-11 and this showed a NOE with the OCH_3 signal at δ 4.18, allowing the assignment of this to C-1. The remaining doublet of the ABX system at δ 7.07 showed a NOE with the OCH_3 signal at δ 4.07. Thus, this OCH_3 should be located at C-8. This confirmed the presence of only one OCH_3 group in ring D. On the basis of the above data, the structure of this new alkaloid was 8-methoxyouregidione (**1**).



	R_1	R_2	R_3	R_4	R_5
1	OMe	OMe	OMe	OMe	H
2	H	O–CH_2–O		H	OMe
3	OMe	OMe	OMe	H	H



	R_1	R_2	R_3	R_4	R_5	R_6
4	H	O–CH_2–O		H	H	H
5	H	O–CH_2–O		H	OMe	OMe
6	OMe	O–CH_2–O		H	OMe	H
7	OMe	O–CH_2–O		H	H	H
8	H	O–CH_2–O		H	OMe	H
9	OMe	OMe	OMe	H	H	H
10	H	O–CH_2–O		OMe	OMe	H

Chromatographic separation of the alkaloidal fraction derived from the chloroform extract of *X. championii* yielded *O*-methylmoschatoline (**9**) [16] and dicentrinone (**10**) [17], the structures of which were established by comparison of their UV and ^{1}H NMR data with the literature data. The structure of **10** was further supported by NOE experiments which showed NOEs between the OCH_3 group at δ 3.99 with the aromatic singlet at δ 8.64 (H-11) and the OCH_3 group at δ 4.08 with the aromatic singlet at δ 7.97 (H-8).

Potential anticancer activity exhibited by oxoaporphines [5, 18] and 4,5-dioxoaporphines [5] in our previous studies prompted us to subject **1**, **3**, **5** and **10** to our mechanism-based screen utilizing genetically engineered mutants of the yeast *Saccharomyces cerevisiae* [19, 20]. Compound **10** was found to be inactive even at a dose of 100 μg ml^{-1} whereas other alkaloids tested showed preferential toxicity towards DNA repair-deficient strains, RAD 52Y and RS 321, compared with the wild-type DNA repair-proficient strain, RAD$^+$, suggesting their potential as anticancer agents [18–21]. The bioactivity data of these and several other oxo- and 4,5-dioxo-aporphines will be published elsewhere.

EXPERIMENTAL

General. Mps (uncorr.) were determined on a Yanaco micro melting point apparatus. UV spectra were recorded in EtOH with a Shimadzu UV 160 spectrometer and IR spectra with a Shimadzu IR 408 spectrometer. The ^{1}H NMR spectra were recorded on a JEOL JNM-6X 400 FT NMR spectrometer with TMS as int. standard. EIMS and HREIMS were recorded on JEOL JMS D-300 spectrometer. Unless otherwise stated chromatographic sepns were carried out on silica gel, and TLC and prep. TLC on silica gel G. TLC spots due to alkaloids were detected under UV (254 and 365 nm) and spraying the plates with Dragendorff's reagent.

Plant material. Stem bark of *A. zeylanicus* was collected at Madugoda in the Central Province of Sri Lanka, and identified by Mr D.A.S. Wijesundera of the Peradeniya Royal Botanic Garden, Sri Lanka. The stem bark of *X. championii* was collected by Mr E.M.H.G.S. Ekanayake (Institute of Fundamental Studies, Kandy, Sri Lanka) at Ratnapura in the Sabaragamuwa Province of Sri Lanka. The voucher specimens of both plants have been deposited at the Medical Research Institute of Sri Lanka.

Extraction and fractionation. Dried and powdered stem bark (4.5 kg) of *A. zeylanicus* was successively and exhaustively extracted with hot hexane, $CHCl_3$ and MeOH. Evapn of solvents *in vacuo* afforded hexane (22 g), $CHCl_3$ (50 g), and MeOH (65 g) extracts. A portion (40 g) of the $CHCl_3$ extract was subjected to CC on silica gel (70–23 mesh, 800 g) made up in CH_2Cl_2 and eluted with CH_2Cl_2 followed by CH_2Cl_2 containing increasing amounts of MeOH. Three major alkaloid containing frs were collected and labelled as A (eluted with 2.5% MeOH in CH_2Cl_2), B (10% MeOH in CH_2Cl_2—early frs) and C (10% MeOH in CH_2Cl_2—

late fractions). Fr. A on further purification by MPLC on silica gel (G-60, 10 g) and elution with 0.5% MeOH in $CHCl_3$ yielded **2** as an orange–yellow solid (220 mg). Fr. B on further purification by MPLC over silica gel (3% MeOH in CH_2Cl_2), followed by 3 successive prep. TLC sepns (EtOAc–MeOH, 4:1; $CHCl_3$–MeOH, 19:1; CH_2Cl_2–*iso*-PrOH, 19:1) afforded **4** (6.1 mg). The late frs of the above MPLC eluted with 3% MeOH in CH_2Cl_2 was subjected to further purification by prep. TLC ($CHCl_3$–MeOH, 19:1) yielding **5** (20.0 mg).

Fr. C was further purified by MPLC (1–4% MeOH in CH_2Cl_2). The frs eluted with 2% MeOH in CH_2Cl_2 and 3% MeOH in CH_2Cl_2 were found to contain alkaloids and were designated C_1 and C_2, respectively. Fr. C_1 was subjected to prep. TLC (CH_2Cl_2–*iso*-PrOH, 19:1; triple elution) and 4 bands were collected. The band with R_f 0.31 (6.1 mg) on further purification by prep. TLC ($CHCl_3$–MeOH, 19:1) gave **3** (5.2 mg). The TLC band with R_f 0.66 (12.8 mg) was sepd by prep. TLC (alumina, Merck, 0.25 mm) (CH_2Cl_2–*iso*-PrOH, 19:1) yielding a further quantity of **3** (8.2 mg) and crude **6** (1.4 mg). The latter was passed through a short column of silica gel. Elution with 4% MeOH in $CHCl_3$ afforded pure **6** (1.2 mg). Fr. C_2 (60 mg) was sepd into 5 bands by prep. TLC (CH_2Cl_2–*iso*-PrOH, 19:1; triple elution). The band with R_f 0.51 (16.3 mg) on prep. TLC ($CHCl_3$–MeOH, 19:1) gave a further quantity of **4** (2.1 mg) and **7** (5.2 mg). The next polar band with R_f 0.42 (10.1 mg) was further sepd by prep. TLC ($CHCl_3$–MeOH, 19:1; triple elution) affording the new oxoaporphine alkaloid, **1** (0.8 mg) in addition to more **4** (4.6 mg) and **7** (2.3 mg). The most polar band (R_f 0.36; 12.8 mg) when purified by prep. TLC (CH_2Cl_2–Et_2O–MeOH, 19:19:2; triple elution) gave **8** (2.3 mg).

Dried and powdered stem bark of *X. championii* (1.0 kg) was successively and exhaustively extracted with hexane, $CHCl_3$ and MeOH. Removal of solvents under red. pres. yielded hexane (30 g), $CHCl_3$ (8 g) and MeOH (23 g) extracts. A portion (7 g) of the $CHCl_3$ extract was subjected to usual acid–base extraction. The resulting alkaloidal fr. (0.3 g) was fractionated by MPLC over silica gel (10 g) using 1% MeOH in $CHCl_3$ as the mobile phase. The early frs were combined and purified by prep. TLC ($CHCl_3$–MeOH, 19:1) giving **9** (8.0 mg). The combined middle frs. when subjected to further purification by prep. TLC ($CHCl_3$–MeOH, 9:1) afforded **10** (12.0 mg).

8-*Methoxyorugidione* (**1**). Orange solid, mp 276–277°; IR ν_{max}^{KBr} cm^{-1}: 3500–3000, 1680, 1650, 1580, 1410, 1270; UV λ_{max}^{EtOH} nm: 210, 240, 256, 311, 325, 388, 449; ^1H NMR: δ 4.07 (3H, *s*, OMe), 4.08 (3H, *s*, OMe), 4.15 (3H, *s*, OMe), 4.18 (3H, *s*, OMe), 7.07 (1H, *d*, J = 7.94 Hz, H-9), 7.58 (1H, *dd*, J = 8.85, 7.94 Hz, H-10), 7.99 (1H, *s*, H-7), 8.91 (1H, *br s*, D_2O exchangeable, NH), 9.09 (1H, *d*, J = 8.85 Hz, H-11); HREIMS: *m/z* (rel. int.) $[M]^+$ 367.10503 (33) [$C_{20}H_{17}O_6N$ requires 367.10558], 352 (9) $[M-Me]^+$, 335 (100) $[M-Me-OH]^+$, 324 (16), 305 (10).

Artabotrine (**2**). Orange–yellow rods, mp 287–289°; identified by direct comparison (co-TLC, mmp, ^1H NMR) with an authentic sample [5].

Ouregidione (**3**). Orange needles, mp 262–264°; IR ν_{max}^{KBr} cm^{-1}: 3500–3000, 1660, 1620, 1550, 1500, 1450, 1380, 1260, 1200; UV λ_{max}^{EtOH} nm: 211.5, 241.5, 306, 317.5, 419; ^1H NMR: δ 4.10 (3H, *s*, OMe), 4.17 (3H, *s*, OMe), 4.21 (3H, *s*, OMe), 7.66 (2H, *m*, H-9, H-10), 7.79 (1H, *s*, H-7), 7.97 (1H, *m*, H-8), 9.49 (1H, *m*, H-11), 11.45 (1H, *s*, D_2O exchangeable, NH); EIMS: *m/z* 337 $[M]^+$, 322 $[M-Me]^+$, 308 $[M-OMe]^+$, 294.

Liriodenine (**4**). Yellow powder, mp 279–281°; UV λ_{max}^{EtOH} nm: 219 sh, 247, 270, 306, 410; ^1H NMR: δ 6.36 (2H, *s*, OCH_2O), 7.16 (1H, *s*, H-3), 7.57 (1H, *dt*, J = 7.93, 1.22 Hz, H-9), 7.72 (1H, *dt*, J = 7.94, 1.22 Hz, H-10), 7.74 (1H, *d*, J = 5.19 Hz, H-4), 8.57 (1H, *dd*, J = 7.93, 1.22 Hz, H-8), 8.60 (1H, *d*, J = 7.94 Hz, H-11), 8.87 (1H, *d*, J = 5.19 Hz, H-5).

Oxocrebanine (**5**). Orange needles, mp 256–258°; UV λ_{max}^{EtOH} nm: 211.5, 248, 274, 438; ^1H NMR: δ 3.94 (3H, *s*, 9-OMe), 4.01 (3H, *s*, 8-OMe), 6.30 (2H, *s*, OCH_2O), 7.00 (1H, *s*, H-3), 7.13 (1H, *d*, J = 8.54 Hz, H-10), 7.63 (1H, *d*, J = 5.19 Hz, H-4), 8.25 (1H, *d*, J = 8.54 Hz, H-11), 8.78 (1H, *d*, J = 5.19 Hz, H-5); EIMS: *m/z* 335 $[M]^+$, 320 $[M-Me]^+$, 306.

Oxobuxifoline (**6**). Dark brown needles, mp 265–267°; UV λ_{max}^{EtOH} nm: 213, 247, 271 sh, 280, 332; ^1H NMR: δ 3.98 (3H, *s*, OMe), 4.28 (3H, *s*, OMe), 6.31 (2H, *s*, OCH_2O), 7.29 (1H, *dd*, J = 8.85, 3.05 Hz, H-10), 8.01 (1H, *d*, J = 3.05 Hz, H-8), 8.18 (1H, *d*, J = 5.19 Hz, H-4), 8.52 (1H, *d*, J = 8.85 Hz, H-11), 8.92 (1H, *d*, J = 5.19 Hz, H-5); EIMS: *m/z* 335 $[M]^+$ 305 $[M-CH_2O]^+$, 275.

Atherospermidine (**7**). Dark orange solid, mp 284–285°; Identified by direct comparison (co-TLC, UV, ^1H NMR) with an authentic sample [5].

Lanuginosine (**8**). Dark brown solid, mp >300°; UV λ_{max}^{EtOH} nm: 248, 271, 317; ^1H NMR: δ 3.99 (3H, *s*, OMe), 6.33 (2H, *s*, OCH_2O), 7.08 (1H, *s*, H-3), 7.24 (1H, *dd*, J = 8.85, 2.24 Hz, H-10), 7.73 (1H, *d*, J = 5.18 Hz, H-4), 7.98 (1H, *d*, J = 2.44 Hz, H-8), 8.48 (1H, *d*, J = 8.85 Hz, H-11), 8.86 (1H, *d*, J = 5.18 Hz, H-5); EIMS: *m/z* 305 $[M^+]$, 275 $[M-CH_2O]^+$.

O-*Methylmoschatoline* (**9**). Orange amorphous solid; UV λ_{max}^{EtOH} nm: 211, 226, 271, 313, 435; ^1H NMR: δ 4.08 (3H, *s*, OMe), 4.11 (3H, *s*, OMe), 4.19 (3H, *s*, OMe), 7.54 (1H, *t*, J = 7.94 Hz, H-9), 7.75 (1H, *dt*, J = 7.93, 1.22 Hz, H-10), 8.21 (1H, *d*, J = 5.49 Hz, H-4), 8.58 (1H, *dd*, J = 7.93, 1.22 Hz, H-8), 8.97 (1H, *d*, J = 5.49 Hz, H-5), 9.10 (1H, *d*, J = 7.93 Hz, H-11).

Dicentrinone (**10**). Dark brown solid, mp >300°; UV λ_{max}^{EtOH} nm 213, 250, 272, 310, 352, 386, 433; ^1H NMR: δ 3.99 (3H, *s*, OMe), 4.08 (3H, *s*, OMe), 6.13 (2H, *s*, OCH_2O), 7.16 (1H, *s*, H-3), 7.74 (1H, *d*, J = 5.49 Hz, H-4), 7.97 (1H, *s*, H-8), 8.64 (1H, *s*, H-11), 8.86 (1H, *d*, J = 5.49 Hz, H-5).

Acknowledgements—We thank Japan International Cooperation Agency (JICA) for the award of a training

fellowship to E.M.K.W., Mr D.A.S. Wijesundera (Peradeniya Royal Botanic Gardens, Sri Lanka) for authentication of *A. zeylanicus*, and Mr E.M.H.G.S. Ekanayake (Institute of Fundamental Studies, Kandy, Sri Lanka) for authentication and collection of *X. championii*.

REFERENCES

1. Buckingham, J. (1994) *Dictionary of Natural Products*. Chapman & Hall, London.
2. Abeywickrema, B. A. (1959) *Ceylon J. Sci. (Biol. Sci.)* **2**, 119.
3. Wijeratne, E. M. K., De Silva, L. B., Kikuchi, T., Tezuka, Y., Gunatilaka, A. A. L. and Kingston, D. G. I. (1995) *J. Nat. Prod.* **58**, 459.
4. Wijeratne, E. M. K., De Silva, L. B., Tezuka, Y. and Kikuchi, T. (1995) *Phytochemistry* **39**, 443.
5. Wijeratne, E. M. K., Gunatilaka, A. A. L. Kingston, D. G. I., Haltiwanger, R. C. and Eggleston, D. S. (1995) *Tetrahedron* **51**, 7877.
6. Huber, H. (1985) in *A Revised Handbook to the Flora of Ceylon* (Dassanayake, M. D. and Fosberg, F. R., eds), Vol. V, p. 52. Amerind Publishing, New Delhi.
7. Huber, H. (1985) in *A Revised Handbook to the Flora of Ceylon* (Dassanayake, M. D. and Fosberg, F. R., eds), Vol. V, p. 58. Amerind Publishing, New Delhi.
8. Guinaudeau, H., Lebouf, M. and Cave, A. (1975) *Lloydia* **38**, 275.
9. Kunimoto, J., Munakami, Y., Oshikata, M., Shingu, T., Lu, S. T., Chen, I. S. and Asaku, M. (1981) *Yakugaku Zasshi* **101**, 431.
10. Cortes, D., Hocquemiller, R., Leboeuf, M., Cave, A. and Moretti, C. (1986) *J. Nat. Prod.* **49**, 878.
11. Roblot, F., Hocquemiller, R., Cave, A. and Moretti, C. (1983) *J. Nat. Prod.* **46**, 862.
12. Talapatra, S. K., Patra, A. and Talapatra, B. (1975) *Tetrahedron* **31**, 1105.
13. Achenbach, H., Frey, D. and Waibel, R. (1991) *J. Nat. Prod.* **54**, 1331.
14. Guinaudeau, H., Lebouf, M. and Cave, A. (1979) *J. Nat. Prod.* **42**, 325.
15. Guinaudeau, H., Lebouf, M. and Cave, A. (1983) *J. Nat. Prod.* **46**, 761.
16. Gottlieb, O. R., Magalhaes, A. F., Magalhaes, E. G., Masa, J. G. S. and Marsaioli, A. J. (1978) *Phytochemistry* **17**, 837.
17. Cava, M. P. and Venkateswarlu, A. (1971) *Tetrahedron* **27**, 2637.
18. Harrigan, G. G., Gunatilaka, A. A. L., Kingston, D. G. I., Chan, G. and Johnson, R. K. (1994) *J. Nat. Prod.* **57**, 68.
19. Johnson, R. K., Bartus, H. F., Hofmann, G. A., Bartus, J. O., Mong, S.-M., Faucette, L. F., McCabe, F. L., Chan, J. A. and Mirabelli, C. K. (1986) in *In Vitro and In Vivo Models for Detection of New Antitumor Drugs* (Hanka, L. J., Kondo, T. and White, R. J., eds), pp. 15–26. Organizing Committee of the 14th International Congress of Chemotherapy, Kyoto.
20. Gunatilaka, A. A. L., Samaranayake, G., Kingston, D. G. I., Hofmann, G. and Johnson, R. K. (1992) *J. Nat. Prod.* **55**, 1648.
21. Gunatilaka, A. A. L., Kingston, D. G. I. and Johnson, R. K. (1994) *Pure Appl. Chem.* **66**, 2219.