

## Chemical Components from the Light Petroleum Soluble Fraction of *Uvaria cordata* (Dunal) Alston

Kamaliah Mahmood, Hapipah Mohd Ali,  
Rohana Yusof, A. Hamid Hadi and Mary Pais<sup>1</sup>

Department of Chemistry  
Universiti Malaya  
59100 Kuala Lumpur, Malaysia

<sup>1</sup>Center National De La Recherche Scientifique  
91198, Gif-sur-Yvette, France

Received 19 February 1993

### ABSTRAK

Pemisahan dengan menggunakan kaedah kromatografi ke atas ekstrak petroleum ringan kulit batang pokok *Uvaria cordata* (Dunal) Alston menghasilkan triterpenoid, glutinol dan taraxerol. Disamping itu terbitan sikloheksena, pipoksida berserta klorohidrinnya dan juga sedikit benzil benzoat dihasilkan.

### ABSTRACT

Chromatographic separation of the light petroleum extract from the stem bark of *Uvaria cordata* (Dunal) Alston led to the isolation of the triterpenoids glutinol and taraxerol in addition to the cyclohexene derivatives, pipoxide and its chlorohydrin. A small amount of benzyl benzoate was also isolated.

**Keywords:** Annonaceae, *Uvaria cordata*, triterpenoids, cyclohexene, derivatives

### INTRODUCTION

Plants of the genus *Uvaria*, a member of the Annonaceae, have provided a fascinating array of secondary metabolites, several of which have shown interesting antibacterial, antifungal, antimalarial and antitumour activity (Leboeuf *et al.* 1982; Waterman and Muhammad 1984; Nkunya *et al.* 1987). As part of an ongoing programme to elucidate the chemical components of the Annonaceae, *U. cordata* (Dunal) Alston was investigated since no previous work has been reported on this species. This paper reports the isolation of triterpenoids and of cyclohexene derivatives from *U. cordata*.

## MATERIALS AND METHODS

### General

*Uvaria cordata*, a large climber, was collected at the Lengor Forest Reserve, Mersing, Johore in September 1988. A voucher specimen (KL 3705) was deposited at the Herbarium of the Chemistry Department, University of Malaya.

Melting points were determined with an electrothermal melting point apparatus and are uncorrected. UV spectra were obtained on a Varian Superscan 3 UV-vis spectrometer and IR spectra were measured on a Beckman Acculab IR spectrometer.  $^1\text{H}$  NMR spectra were recorded at 400 MHz (unless otherwise stated) on a Bruker spectrometer with TMS as the internal standard. EIMS were obtained on a VG Micromass 70 instrument. Chromatography was carried out using Merck kieselgel 60 (230 - 400) for flash chromatography and Merck kieselgel 60 F<sub>254</sub> for preparative tlc.

### Isolation of Components

Dried and milled stem bark (940 g) was soxhlet-extracted exhaustively with light petroleum (b.p. 60-80°C). The concentrated crude extract (4.3 g) was fractionated by silica gel chromatography using a mixture of petroleum ether (60-80°C) and diethyl ether (9:1). The fractions collected were grouped into a series of fractions after monitoring with tlc. Each series was treated separately to isolate and purify the compounds by column chromatography and prep. tlc.

Glutinol (1) : m.p. 206-208°C [(lit. Matsunaga *et al.* 1988) m.p. 208-210°C]; IR  $\nu$  cm<sup>-1</sup> : 3430, 1040;  $^1\text{H}$  NMR :  $\delta$  0.83, 0.96, 1.01, 1.05, 1.10, 1.14, 1.17 (each 3H, s, 8Me), 3.37 (1H, s), 5.63 (1H, m); EIMS m/z (rel. int.) : 426 [M<sup>+</sup>] (10), 408 [M - H<sub>2</sub>O]<sup>+</sup> (5), 274 [fragment a] (100), 259 [a - Me] (80), 245 (15), 205 (30).

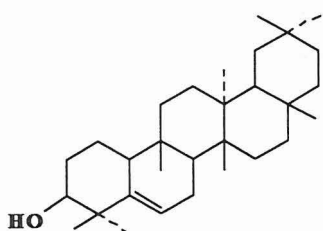
Taraxerol (2) : m.p. 283-285°C [(lit. Ogihara *et al.* 1987) m.p. 283-285°C]; IR  $\nu$  cm<sup>-1</sup> 3440, 3080, 1650, 880;  $^1\text{H}$  NMR ;  $\delta$  0.80, 0.92, 0.95 (each 6H, s), 0.98, 1.11 (each 3H, s), 3.15 (1H, m), 5.53 (1H, dd, J = 4, 7 Hz); EIMS m/z (rel int.) : 426 [M<sup>+</sup>] (58), 411 [M - Me]<sup>+</sup> (30), 393 (5), 302 (60), 287 (54), 204 (100).

Pipoxide (3) : m.p. 154-155°C [(lit. Singh *et al.* 1970) m.p. 152-154°C]; UV  $\lambda_{\text{max}}$  nm (log  $\epsilon$ ): 228 (4.45), 274 (3.30), 280 (3.15); IR  $\nu$  cm<sup>-1</sup>: 3440, 1722, 1680, 1601, 1280, 1060, 895;  $^1\text{H}$  NMR:  $\delta$  3.10 (1H, d, J = 6 Hz, OH), 3.56 (1H, dd, J = 3.4, 1.8 Hz, H-6), 4.30 (1H, dd, J = 8, 6 Hz, H-2), 4.48, 5.1 (2H, AB system, J = 12 Hz, CH<sub>2</sub>-O-Ar), 5.65 (1H, J = 8, 2, 2.5 Hz, 5.86 (1H, dt, J = 10, 2, 1.8 Hz, H-4), 6.06 (1H, dt, J = 10, 3.8, 2.5 Hz, H-5), 7.3 - 8.1 (10H, m, aromatic H); EIMS m/z (rel. int.): 366 [M<sup>+</sup>] (5), 335 (25),

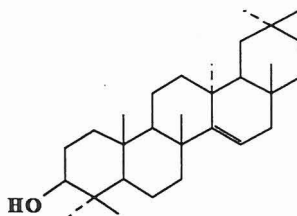
244 (90), 122 (100), 105 (98), 77 (80).

Pipoxide chlorohydrin (4) : m.p. 199-201°C [(lit. Singh *et al.* 1970) m.p. 201-203°C]; UV  $\lambda_{\max}$  nm (log  $\epsilon$ ) : 230 (4.40), 274 (3.25), 281 (3.18); IR  $\nu$   $\text{cm}^{-1}$ : 3540, 1688, 1600, 1580;  $^1\text{H NMR}$ :  $\delta$  4.18 (1H, dd,  $J = 8, 6$  Hz, H-2), 4.53, 4.63 (2H, AB system,  $J = 12$  Hz,  $\text{CH}_2 - \text{O} - \text{Ar}$ ), 4.83 (1H, d,  $J = 5, 4$ , H-6), 5.73 (1H, t,  $J = 6$  Hz, H-4), 5.81 (1H, dd,  $J = 11, 9$ , H-3), 6.05 (1H, ddd,  $J = 10, 3, 2.8$  Hz, H-5), 7.5 - 8.2 (10H, m, aromatic H); EIMS  $m/z$  (rel. int.): 403  $[\text{M} + 1]^+$  (10), 163 (50), 122 (85), 105 (80), 77 (90).

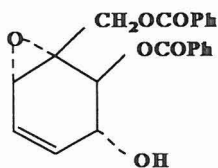
Benzyl benzoate (5) : viscous oil ; IR  $\nu$   $\text{cm}^{-1}$  : 1705, 1600, 1581, 1445, 751 - 733, 709 - 694;  $^1\text{H NMR}$  (90 MHz):  $\delta$  5.29 (2H, s,  $\text{Ar-CH}_2\text{-OR}$ ), 7.30 - 7.38 (7H, m, aromatic H), 8.01 - 8.09 (2H, m, aromatic H). A direct comparison of the oil with an authentic sample (Aldrich) of benzyl benzoate showed the two samples to be the same.



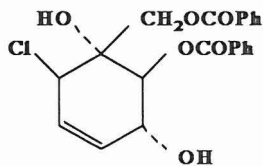
(1)



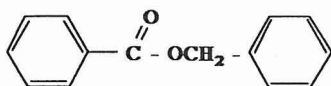
(2)



(3)



(4)



(5)

## RESULTS AND DISCUSSION

The light petroleum extract of the stem bark of *U. cordata* was subjected to chromatography to afford the pentacyclic triterpenoids, glutinol (1) and taraxerol (2) in addition to the cyclohexene derivatives, pipoxide (3) and pipoxide chlorohydrin (4). A small amount of the ester benzyl benzoate (5) was also isolated.

The pentacyclic triterpenoid (1) crystallized as colourless needles from MeOH-CHCl<sub>3</sub>, m.p. 206-208°C. Its IR spectrum showed absorption peaks at 3430 and 1040 cm<sup>-1</sup>. The proton <sup>1</sup>H NMR spectrum of (3) displayed signals due to eight methyl groups (δ 0.85-1.17), a hydroxyl group (δ 3.37) and a trisubstituted double bond (δ 5.63). In its mass spectrum, a molecular ion at 426 was exhibited, thus giving a possible molecular formula of C<sub>30</sub>H<sub>50</sub>O. Two intense peaks arising from cleavage of the B-ring at m/z 274 (fragment a) and 259 (a - Me) characteristic of a triterpene-5-ene skeleton (Budzikiewicz *et al.* 1965) were also observed. The spectral data suggested that the triterpenoid was glutinol and this was verified by comparison with literature values (Hui *et al.* 1975; Gaid *et al.* 1976, Matsunaga *et al.* 1988).

Compound (2) was obtained from CHCl<sub>3</sub>-MeOH, m.p. 283-285°C. The IR spectrum showed peaks at 3440, 3080, 1650 and 880 cm<sup>-1</sup>. The <sup>1</sup>H NMR spectrum of (2) exhibited signals due to eight methyl groups at δ 0.80 - 1.11, a hydroxyl group at δ 3.15 and a well-defined double doublet due to an olefinic proton at δ 5.53. The molecular formula of C<sub>30</sub>H<sub>50</sub>O was suggested by the molecular ion at m/z 426. A fragment peak at m/z 411 was consistent with the loss of [M<sup>+</sup> - Me]. Two intense peaks which appeared at m/z 302 and m/z 287 indicated that the compound was a taraxer-14-ene derivative (Djerassi *et al.* 1962). This observation suggested that compound (2) was taraxerol. The identification of (2) as taraxerol was confirmed by comparison of the reported spectral data with the compound (Ogihara *et al.* 1987).

Compound (3) was isolated from benzene as colourless plates, m.p. 154 - 155°C. Its UV spectrum λ<sub>max</sub> 228, 274 and 280 nm indicated that it possessed a CO group attached to a benzenoid system (Singh *et al.* 1970). Its IR spectrum displayed peaks at 3440, 1722, 1680, 1601, 1280, 1260, 1060 and 895 cm<sup>-1</sup>. The molecular ion at m/z 366 suggested a molecular formula of C<sub>21</sub>H<sub>18</sub>O<sub>6</sub>. Fragments ion consistent with losses of (M<sup>+</sup> - CH<sub>2</sub>OH) and (M<sup>+</sup> - C<sub>6</sub>H<sub>5</sub>COOH) appeared at m/z 335 and m/z 244 respectively. The other important peaks appeared at m/z 122 (C<sub>6</sub>H<sub>5</sub>COOH), m/z 105 (C<sub>6</sub>H<sub>5</sub>CO) and m/z 77 (C<sub>6</sub>H<sub>5</sub>). The <sup>1</sup>H NMR spectral parameters of (3) are identical to those of the cyclohexene derivative, pipoxide, which was reported in the literature (Joshi *et al.* 1979; Nkunya *et al.* 1987). Comparison of these data with the reported values suggested compound (3) to be pipoxide.

Compound (4) was obtained as colourless plates from EtOAc - benzene, m.p. 199 - 201°C. Its UV spectrum exhibited absorption bands at  $\lambda_{\max}$  230, 274 and 281 nm. The IR spectrum of (4) displayed peaks at 3540, 1688, 1600 and 1580  $\text{cm}^{-1}$ . A visible molecular ion could not be obtained in its mass spectrum, but the (M + 1) peak at 403 and other peaks at m/z 280 ( $M^+ - 122$ ), m/z 163, m/z 122, m/z 105 and m/z 77 were significant. On the basis of the above data (4) was identified as pipoxide chlorohydrin. The structure of (4) was further confirmed by comparison of its  $^1\text{H}$  NMR spectrum with that reported in literature (Singh *et al.* 1970; Joshi *et al.* 1979).

Compound (5) was obtained as a viscous oil. Its IR spectrum revealed peaks at 1705, 1600, 1581, 1445, 751 - 733 and 709 - 694  $\text{cm}^{-1}$ . Its  $^1\text{H}$  NMR spectrum displayed signals at  $\delta$  5.34 which are attributable to the methylene protons and in the region of  $\delta$  7.20-7.55 and 8.07 - 8.25, indicative of the aromatic protons. Compound (5) was identified as benzyl benzoate and its identity was confirmed by direct comparison with an authentic sample.

#### ACKNOWLEDGEMENTS

Support from the University of Malaya and IRPA grant No. 3-07-04-13 is gratefully acknowledged. Thanks are also due to the CNRS, France for the NMR spectral data.

#### REFERENCES

- BUDZIKIEWICZ, H., J.I. BRAUMAM and C. DJERASSI. 1965. Massenspektrometrie und Anwendung auf Strukturelle und Stereochemische Probleme-LXVII. *Tetrahedron* **21**: 1855-1879.
- DJERASSI, C., H. BUDZIKIEWICZ and J.M. WILSON. 1962. Mass spectrometry in structural and stereochemical problems: unsaturated pentacyclic triterpenoids. *Tetrahedron Letters* 263-270.
- GAIND, K.N., A.K. SINGLA, R.B. BOAR and D.B. COPSEY. 1976. Triterpenoids and sterols of *Kalanchoe spathulata*. *Phytochem.* **15**: 1999-2000.
- HUI, W.H., D.S. KO, Y-C. LEE, L-M. LI and H.R. ARTHUR. 1975. Triterpenoids from ten *Lithocarpus* species of Hong Kong. *Phytochem.* **14**: 1063-1066.
- JOSHI, B.S., D.H. GAWAD and H. FUHRER. 1979. Revised structures of pipoxide and pipoxide chlorohydrin. *Tetrahedron Letters* 2427-2430.
- LEBOEUF, M., A. CAVE, P.K. BHAUMIK, B. MUKHERJEE and R. MUKHERJEE. 1989. The phytochemistry of the Annonaceae. *Phytochemistry* **21**: 2783-2813.
- MATSUNAGA, S., R. TANAKA and M. AKAGI. 1988. Triterpenoids from *Euphorbia maculata*. *Phytochem.* **27**: 535-537.

- NKUNYA, M.H.H., H WEENAN, N.J. KOYI, L. THIJIS and B. ZWANENBURG 1987. Cyclohexene epoxides, (+)-pandoxide, (+)-beta-senepoxide and (-)-pipoxide from *Uvaria pandensis*. *Phytochem.* **26**: 2563-2565.
- OGIHARA, K., M. HIGA and T. SUGA. 1987. Triterpenes from the leaves of *Parsonia laevigata*. *Phytochem.* **26**: 783-785.
- SINGH, J., K.L. DHAR and C.K. ATAL. 1970. Studies on the genus *Piper*-X. Structure of pipoxide. A new cyclohexene epoxide from *P. hookeri* Linn. *Tetrahedron* **26**: 4403-4406.
- WATERMAN, P.G. and I. MUHAMMAD. 1984. Chemistry of Annonaceae. Structures of uvarindoles A-D, four new benzylated indole alkaloids from *Uvaria angloensis*. *J. Chem. Soc. Chem. Comm.* 1280-1281.