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CYTOTOXIC COMPONENTS OF *DIOSPYROS MORRISIANA**

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Key Word Index—*Diospyros morrisiana*; Ebenaceae; isodiospyrin; β-amyrin; olean-12-en-3-one; β-amyrin acetate; X-ray crystal structure

Abstract—Two cytotoxic compounds, isodiospyrin and β-amyrin, in addition to an inactive triterpene, olean-12-en-3-one, have been isolated from *Diospyros morrisiana*. The cytotoxic activity of isodiospyrin, which has ED_{50} values of 4.9 and 0.59 μg/ml, respectively, against HCT-8 colon tumour and P-388 lymphocytic leukemia, was demonstrated for the first time. Results of single-crystal X-ray analyses of β-amyrin acetate and olean-12-en-3-one are also reported.

INTRODUCTION

Diospyros morrisiana Hance (Ebenaceae), known as 'Shan Hung Shih' in the herbal medicine of Taiwan, has been claimed to possess antibiotic activity [1]. Previous investigations of the roots of this plant led to the isolation of isodiospyrin [2, 3] and betulinic acid [3], as well as some natural naphthoquinone pigments [4]. In the course of our continuing search for novel potent plant antitumour agents, the hexane extract of the stem parts of this plant was found to show significant cytotoxicity against *in vitro* tissue culture cells of human KB and A-549 lung carcinoma, HCT-8 colon tumour and murine P-388 and L-1210 lymphocytic leukemia. Bioassay-directed fractionation of the foregoing extract led to the isolation and characterization of two cytotoxic components, isodiospyrin (**1**) and β-amyrin (**2**), as well as an inactive triterpene, olean-12-en-3-one (**3**). The binaphthoquinone, isodiospyrin (**1**), with ED_{50} values of 4.9 and 0.59 μg/ml, was shown for the first time to be cytotoxic in the HCT-8 and P-388 screens. That β-amyrin (**2**) was also moderately cytotoxic was indicated by its ED_{50} values of 3.5 and 4.6 μg/ml in KB and A-549 screens. X-Ray crystal structure analysis established the constitution of olean-12-en-3-one (**3**) while a corresponding study of β-amyrin acetate (**4**) confirmed the identity of β-amyrin (**2**). Views of the solid-state conformations of **3** and **4** are presented in Figs 1 and 2, respectively.

EXPERIMENTAL

Mps: uncorr. Optical rotations were taken in $CHCl_3$ sol. ^1H NMR spectra were run in $CDCl_3$ at 400 MHz with chemical shifts recorded in ppm, TMS as int. standard.

Plant material. The plant *D. morrisiana* used in this investigation was collected from Mt. Yang.-Ming, Taipei, Taiwan, in Feb. 1987. A voucher specimen is kept in the School of Agriculture, Chinese Culture University, Taipei, Taiwan, R.O.C.

Extraction and isolation. The dried stems of *D. morrisiana* (3 kg) were extracted with MeOH. Removal of solvent gave a syrup (125 g) which was then dissolved in MeOH–H_2O (3:1) and extracted exhaustively with hexane. The hexane extract (18 g) was chromatographed on silica gel (800 g) and eluted successively with $CHCl_3$ and then with $CHCl_3$–Me_2CO mixtures containing increasing amounts of Me_2CO to yield fractions A (3.6 g), B (2.4 g), C (1.4 g), and D (68 mg).

*Part 106 in the series 'Antitumor Agent' For part 105, see Y. H. Kuo, C. H. Chen, L. M. Yang Kuo, M. L. King, T. S. Wu, S. T. Lu, I. S. Chen, D. R. McPhail, A. T. McPhail and K. H. Lee (1989) *Heterocycles* (submitted).

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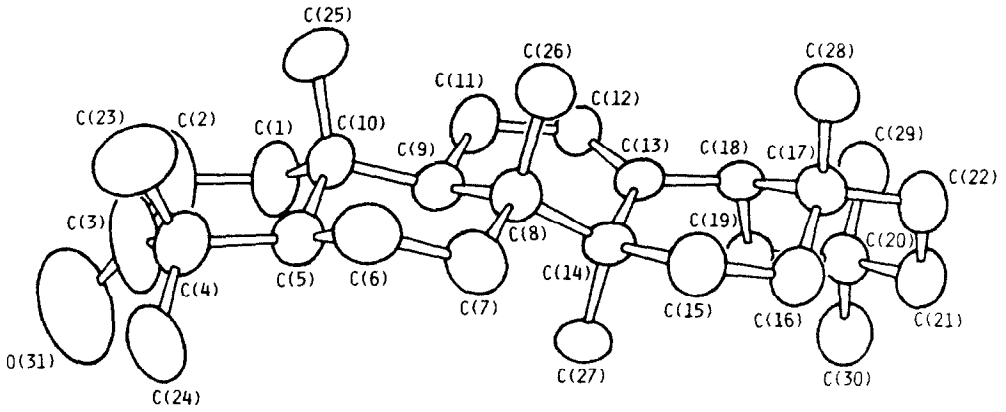


Fig. 1. Structure and solid-state conformation of olean-12-en-3-one (**3**); hydrogen atoms have been omitted for clarity.

Isodiospyrin (**1**). Repeated recrystallization of fraction D from Me_2CO afforded compound **1** as red needles: 12 mg; mp 229–230°; $[\alpha]_D^{25} - 138°$ ($CHCl_3$; *c* 0.25). This compound was identified by direct comparison (mmp, ^1H NMR, UV, and IR) with an authentic sample in our laboratory.

β-Amyrin (**2**). Compound **2** {mp 174–176°; $[\alpha]_D^{25} + 57.2°$ ($CHCl_3$; *c* 0.5)} was obtained from fractions A and B.

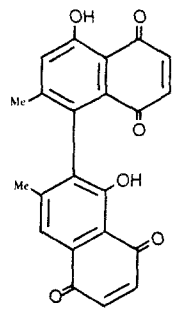
Olean-12-en-3-one (**3**). Compound **3** was obtained from fraction C. Recrystallization from Et_2O and Me_2CO gave 26 mg of a microcrystalline powder: mp 175–177°; $[\alpha]_D^{25} + 124.4°$ ($CHCl_3$; *c* 0.5); EIMS *m/z* 424.3705 (10), $[M]^+$, calcd. for $C_{30}H_{48}O$: 424.3715, and 218 (10); ^1H NMR ($CDCl_3$): δ 0.8–1.3 (*s*, 8 × Me), 2.37 (*ddd*, *J* = 15.9, 6.8 and 3.4 Hz, H-2), and 2.55 (*ddd*, *J* = 15.9, 7.3 and 11.0 Hz, H-2). The constitution of **3** was defined unequivocally by X-ray analysis (*vide infra*) of a crystal grown from CH_2Cl_2–heptane.

β-Amyrin acetate (*olean-12-en-3β-yl acetate*) (**4**). Compound **4** (mp 236–238°) was prepared by acetylation of **2** with Ac_2O in pyridine, and its structure was confirmed by single-crystal X-ray analysis (*vide infra*).

X-Ray crystal structure analyses of compounds **3** *and* **4** *at* 27°.* Crystal data: olean-12-en-3-one (**3**), $C_{30}H_{48}O$, $M_r = 424.72$, monoclinic, $a = 15.481$ (5) Å, $b = 11.700$ (3) Å, $c = 7.319$ (4) Å, $\beta = 103.13$ (3)° (from 25 orientation reflections, $38° < \theta < 41°$), $V = 1291.0$ Å^3, $Z = 2$, $D_{calcd} = 1.092$ g/cm^3, μ(Cu-Kα radiation, $\lambda = 1.5418$ Å) = 4.4 cm^{-1}. Space group $P2_1$ (C_2^2) from the systematically absent reflections, $0k0$ when $k \neq 2n$, and **3** is chiral. Sample dimensions: 0.03 × 0.18 × 0.50 mm.

β-Amyrin acetate (**4**), $C_{32}H_{52}O_2$, $M_r = 468.77$, monoclinic, $a = 11.517$ (3) Å, $b = 16.471$ (8) Å, $c = 7.507$ (1) Å, $\beta = 92.64$ (2)° (from 25 orientation reflections, $23° < \theta < 34°$), $V = 1422.5$ Å^3, $Z = 2$, $D_{calcd.} = 1.094$ g/cm^3, μ(Cu-Kα radiation) = 4.7 cm^{-1}. Space group $p2_1$ (C_2^2) as for **3**. Sample dimensions: 0.06 × 0.06 × 0.85 mm.

Preliminary unit-cell parameters and space group information were derived from oscillation and Weissenberg photographs. Intensity data ($+h$, $+k$, $\pm l$, $\theta_{max} = 67°$; 2427 and 2633 nonequivalent reflections for **3** and **4**, respectively) were recoredd on an Enraf-Nonius CAD-4 diffractometer (Cu.Kα radiation, incident-beam graphite monochromator; ω–2θ scans). The usual



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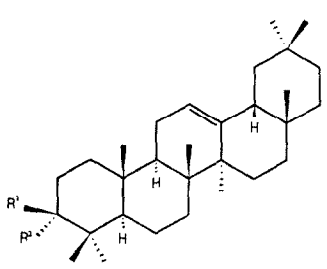

2 R¹ = OH, R² = H
3 R¹, R² = O
4 R¹ = OAc, R² = H

*The results of a low-temperature study (134 K) of **4** have been recently reported in lit. [6]. Corresponding crystal data follow: $a = 11.394(3)$ Å, $b = 16.466(3)$ Å, $c = 7.388(7)$ Å, $\beta = 92.83(4)°$, $V = 1375.1$ Å^3, $Z = 2$, $D_{calcd} = 1.13$ g/cm^3. Bond lengths and angles derived from the present, ambient temperature study are generally in good agreement with the values obtained at 134 K.

†Crystallographic calculations were performed on PDP11/44 and MicroVAX II computers by use of the Enraf–Nonius Structure Determination Package incorporating the direct methods program MULTAN11/82.

‡$R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$.

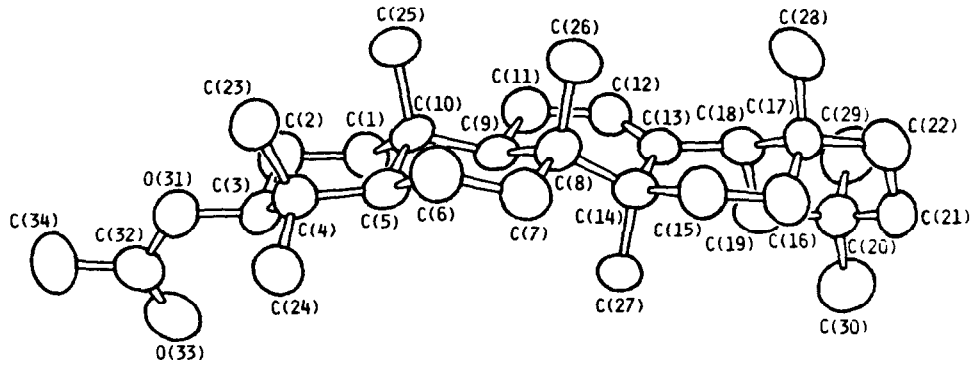

Fig. 2. Structure and solid-state conformation of β-amyrin acetate (**4**); hydrogen atoms have been omitted for clarity.

Lorentz and polarization corrections were applied to these data, and those 1373 and 1233 reflections with $I > 3.0\,(I)$ for **3** and **4**, respectively, were retained for the analyses.

Both crystal structures were solved by direct methods.† Approximate non-hydrogen atoms positions were obtained from *E*-maps. Hydrogen atoms were located in difference Fourier syntheses evaluated following several rounds of full-matrix least-squares refinement of non-hydrogen atom positional and anisotropic temperature factor parameters, and they were included at their calculated positions in the later iterations which converged (max. shift:esd < 0.03) at $R = 0.059$ ($R_w = 0.078$)‡ for **3** and $R = 0.056$ ($R_w = 0.070$) for **4**. Non-hydrogen atom positional and anisotropic temp. factor parameters, hydrogen atom parameters, bond lengths, bond angles, torsion angles, and lists of observed and calculated structure amplitudes for **3** and **4** have been deposited with the Cambridge Crystallographic Data Centre. Neutral atom scattering factors used in all structure-factor calculations were taken from lit. [6]. In the least-squares iterations, $\Sigma w\Delta^2[w = 1/\sigma^2(|F_o|), \Delta = (|F_o| - |F_c|)]$ was minimized.

Biological evaluation. Cytotoxicity assays were carried out according to a procedure described in the lit. [7].

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