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ANNOQUINONE-A, AN ANTIMICROBIAL AND CYTOTOXIC PRINCIPLE FROM *ANNONA MONTANA*

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Key Word Index—*Annona montana*; Annonaceae; phenanthrene-1,4-quinone; annoquinone-A; anthraquinone; antibacterial activity; cytotoxicity.

Abstract—A new naturally occurring phenanthrene-1,4-quinone, annoquinone-A, along with parietin (physcion) and β-sitostenone were isolated from the stem bark of *Annona montana*. The structure of annoquinone-A was elucidated by spectral methods and synthesis. Annoquinone-A demonstrated potent antimicrobial activity against *Bacillus subtilis* and *Micrococcus luteus* as well as cytotoxicity in the KB (ED_{50} = 0.16 μg/ml) tissue culture assay. β-Sitostenone also showed significant cytotoxicity.

INTRODUCTION

Annona montana Macf. is a small evergreen tree which is widely distributed from the West Indies to southern Brazil and is cultured for its fruit in Taiwan [1]. Yang *et al.* [2], Wu *et al.* [3], and Cavé *et al.* [4, 5] have reported the isolation of alkaloids from the leaves, stem and root bark of this plant. We report here on the isolation, structural elucidation, and the antimicrobial and cytotoxic activities of a new phenanthrene-1,4-quinone, annoquinone-A (**1**), as well as the known compounds, parietin (physcion, **3**) and β-sitostenone (**5**) from the stem bark of *A. montana* collected in Taiwan.

RESULTS AND DISCUSSION

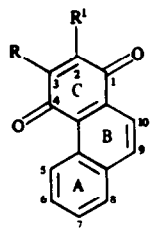
The mass spectrum of annoquinone (**1**) showed a molecular ion peak at *m/z* 238. The presence of a phenanthrene-1,4-quinone nucleus was suggested by UV absorption maxima at 228, 279, 283, 317 and 370 nm [6, 7], IR bands at 1670 and 1635 cm^{-1} and two carbonyl carbon signals at δ182.4 and 185.3 in the ^{13}C NMR spectrum. The ^1H NMR spectrum of **1** contained AB type proton signals at δ8.17 and 7.89 (J = 10 Hz) which were attributed to the *ortho*-located protons on a tetra-substituted aromatic ring. The lower field signal was assigned to H-10 as it was deshielded by the carbonyl moiety. A double doublet centred at δ9.40 was characteristic of the C-5 proton in phenanthrenes [8]. The presence of signals at δ7.50–7.74 (2H, *m*) and 7.90 (1H, *dd*, J = 2 and 8 Hz) were assigned to H-6, 7 and 8, respectively, indicating that ring-A was unsubstituted. A sharp three-proton singlet (δ3.92) was assigned to a methoxy group while a singlet (δ6.11) was assigned to a lone olefinic proton (H-2 or H-3). The above data were in excellent accord with the structure of **1** or **2** for annoquinone-A.

Final proof for the structure of annoquinone-A was provided by the demonstration that it could be synthesized by condensation of styrene with methoxy-*p*-benzoquinone in a sealed tube at 100°.

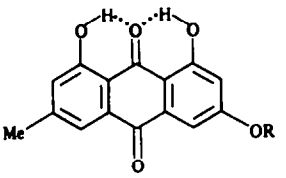
This is the first report of the occurrence of **1** in a natural source despite the fact that it was synthesized by Kakisawa in 1971 [9].

The known compounds parietin (**3**) [10, 11] and β-sitostenone (**5**) [12, 13] were isolated and identified by IR, ^1H NMR and mixed melting point determination with authentic samples. This is the first report of the isolation of an anthraquinone (parietin) from Annonaceae.

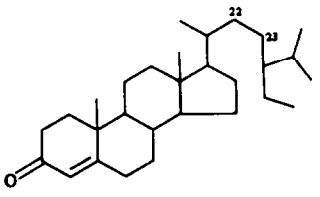
The compounds isolated in this study were tested for antimicrobial [14] (Table 1) and cytotoxic activities.



1 R = OMe, R^1 = H
2 R = H, R^1 = OMe



3 R = Me
4 R = H



5
6 Δ^{22}

Table 1. Antibacterial activities of compounds 1, 4 and 6

Organism	Annoquinone-A (1) 100*	50	10	1	4 100	6 100
Gram positive bacteria						
Staphylococcus aureus ATCC 6538 P	+	+	–	–	–	–
Staphylococcus epidermidis ATCC 12228	–	–	–	–	–	–
Bacillus subtilis ATCC 6633	+	+	+	–	–	–
Streptococcus faecium ATCC 10541	–	–	–	–	–	–
Gram negative bacteria						
Micrococcus luteus ATCC 9341	+	+	+	–	–	–
Escherichia coli ATCC 10536	–	–	–	–	–	–
Klebsiella pneumoniae ATCC 10031	–	–	–	–	–	–
Pseudomonas aeruginosa ATCC 25619	–	–	–	–	–	–
Bordetella bronchiseptica ATCC 4617	–	–	–	–	–	–
Salmonella typhi ATCC 6539	–	–	–	–	–	–

+ Complete growth inhibition, measured after 48 hr.
– Ineffective, normal growth occurred after 24 hr.
*μg/ml.

Annoquinone-A (1) brought about the complete inhibition of growth of *Bacillus subtilis* ATCC 6633 and *Micrococcus luteus* ATCC 9341 at $\leqslant 10\ \mu g/ml$ and *Staphylococcus aureus* ATCC 6538-p at $\leqslant 50\ \mu g/ml$, and also exhibited potent cytotoxicity ($ED_{50} = 0.16\ \mu g/ml$) in the KB tissue culture assay [15]. Compound 4 showed significant cytotoxicity ($ED_{50} = 4.0\ \mu g/ml$) in the KB system.

EXPERIMENTAL

Mps are uncorr; ^1H NMR (100 MHz, 400 MHz) and ^{13}C NMR (25.0 MHz): $CDCl_3$, except where noted, TMS as int. standard; MS: direct inlet.

Plant material. The stem bark of *A. montana* was collected in Tainan Hsien, Taiwan and identified by Prof. C.-S. Kuoh. The voucher specimen is deposited in the Herbarum of Chia-Nan Junior College of Pharmacy, Tainan, Taiwan.

Extraction and separation. The powdered stem bark (1 kg) was extracted successively with n-C_6H_{12}, $CHCl_3$ and MeOH in a Soxhlet extractor. The n-C_6H_{12}-extract, after removal of the solvent by evapn, was subjected to CC on silica gel eluted with n-C_6H_{12} to afford β-sitostenone (155 mg). The $CHCl_3$ extract on CC on silica gel eluted with n-C_6H_{12}–EtOAc (5:1) gave 1 (4 mg) and 3 (1 mg), respectively.

Annoquinone-A (1). Orange microneedles from Me_2CO, mp 170–172°. $C_{15}H_{10}O_3$; UV λ_{max}^{MeOH} nm: 228, 279, 283, 317, 370; IR ν_{max}^{KBr} cm^{-1} 1670, 1635, 1620, 1610, 1560; MS *m/z* (rel. int.) 238 $[M]^+$ (53), 223 (20), 210 (8), 209 (8), 208 (8), 181 (13), 167 (15), 152 (30), 139 (100), 126 (31); ^1H NMR ($CDCl_3 + (CD_3)_2CO$): δ3.92 (3H, *s*, OMe), 6.11 (1H, *s*, H-2), 7.50–7.74 (2H, *m*, H-6, 7), 7.90 (1H, *dd*, J = 2, 8 Hz, H-8), 7.98 (1H, *d*, J = 10 Hz, H-9), 8.17 (1H, *d*, J = 10 Hz, H-10), 9.40 (1H, *dd*, J = 2, 8 Hz, H-5); $CDCl_3$: δ3.98 (3H, *s*, OMe), 6.19 (1H, *s*, H-2), 7.66–8.00 (3H, *m*, H-6, 7, 8), 8.24 (2H, *s*, H-9, 10), 9.62 (1H, *dd*, J = 2, 8 Hz, H-5); ^{13}C NMR: δ56.6 (*q*), 107.2 (*d*), 121.8 (*d*), 126.0 (*s*), 127.6 (*d*), 128.4 (*d*), 128.8 (*d*), 130.0 (*s*), 130.3 (*d*), 132.6 (*s*), 135.6 (*d*), 136.3 (*s*), 160.8 (*s*), 182.4 (*s*), 185.3 (*s*).

Synthesis of annoquinone-A (1). A mixture of styrene (300 mg) and methoxybenzoquinone (1.3 g) in a sealed tube was heated at 100° for 15 hr. The resulting dark red oil was chromatographed on silica gel CC and eluted with C_6H_6 to afford 2 (5 mg), 1 (198 mg) and an unknown compound (8 mg). Compound 1 was identified by comparison (^1H NMR, IR and mmp) with annoquinone-A. Compound 2: orange needles from Me_2CO, mp 169–170°. IR ν_{max}^{KBr} cm^{-1}: 1675, 1640, 1620, 1585, MS *m/z*: 238 $[M]^+$ (100%), 223, 210, 195, 182, 181, 180, 167, 152, 139, 126; ^1H NMR ($CDCl_3 + (CD_3)_2CO$): δ3.92 (3H, *s*, OMe), 6.14 (1H, *s*, H-3), 7.50–7.90 (3H, *m*, H-6, 7, 8), 8.03 (1H, *d*, J = 10 Hz, H-9), 8.13 (1H, *d*, J = 10 Hz, H-10), 9.52 (1H, *m*, H-5). The identity of 2 with 2-methoxyphenanthrene-1,4-quinone [9] was established by identical IR spectra with authentic sample.

Parietin (*physcion*, 3). Orange needles from Me_2CO, mp 206–208°. UV λ_{max}^{MeOH} nm: 225, 254, 268, 286, 436, 453 (sh); $\lambda_{max}^{AlCl_3}$ nm 225, 255, 268, 304, 482, 510; IR ν_{max}^{KBr} cm^{-1}: 1675, 1630, 1610, 1565; MS *m/z* 284 $[M]^+$ (100%), 255, 241, 227, 226, 213, 198, 185; ^1H NMR (400 MHz): δ2.46 (3H, *s*, 7-Me), 3.94 (3H, *s*, OMe), 6.69 (1H, *d*, J = 2.4 Hz, H-2), 7.09 (1H, *d*, J = 1.4 Hz, H-7), 7.38 (1H, *d*, J = 2.4 Hz, H-4), 7.64 (1H, *d*, J = 1.4 Hz, H-5), 12.13 (1H, *s*, OH) and 12.32 (1H, *s*, OH).

Methylation of emodin (4). A mixture of emodin (4, 50 mg) in Et_2O (50 ml) was treated with excess CH_2N_2. The mixture was allowed to stand overnight, after which time the solvent was evapd to leave an orange crystal. Recrystallization from Me_2CO furnished orange needles (47 mg), mp 207–209°, identified as parietin (3) by comparison (^1H NMR, IR and mmp) with an authentic sample.

β-Sitostenone (*stigmast-4-en-3-one*) (5) *and stigmasta-4,22-dien-3-one* (6). Obtained as needles from MeOH, mp 85–89° UV λ_{max}^{MeOH} nm 242; IR ν_{max}^{KBr} cm^{-1}: 1675, 1620, 1460, 1385 and 1375; MS *m/z*: 412 ($[M]^+$ of 5) and 410 ($[M]^+$ of 6); ^1H NMR: δ0.72, 0.79, 0.86, 0.88, 1.18 (6 × Me) and 5.72 (*s*(*br*), H-4). The major component in the mixture was characterized as β-sitosterone (5) by comparison (IR, UV, TLC and mmp) with an authentic sample. The ^1H NMR (δ5.04–5.14, *m*) and MS $[M]^+$ (410) spectra indicated that the needles contaminated stigmasta-4,22-dien-3-one (6) as a minor component (5:6, 91:9 by integration).

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