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SEVERIBUXINE, A NEW QUINOLIN-2,4-DIONE AND OTHER CONSTITUENTS FROM *SEVERINIA BUXIFOLIA*

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Key Word Index—*Severinia buxifolia*; Rutaceae; root bark; cytotoxicity; severibuxine.

Abstract—A new quinolin-2,4-dione alkaloid, severibuxine, together with 23 known compounds were isolated from the root bark of *Severinia buxifolia*. The structure of these compounds were determined by spectral and chemical methods. Most of them showed cytotoxic activity against P-388. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Severinia buxifolia (*Atalantia buxifolia*) is a Chinese folk medicine which is said to be useful for the treatment of chronic rheumatism, paralysis, snakebite and malaria [1]. Essential oils, coumarins, acridone alkaloids, tricyclene type sesquiterpenoids and tetranorterpenoids had been reported from the leaves, fruits and root of this plant [2–10]. As a result of our continuing search for novel bioactive natural products, screening work for cytotoxicity was carried out. The chloroform extract of the root bark of *S. buxifolia* was found to show cytotoxicity. Further bioassay-directed fractionation led to the isolation and characterization of a new quinolone alkaloid, severibuxine (**1**), and 23 known compounds. This paper describes the isolation, structural elucidation and the cytotoxic activity of these compounds.

RESULTS AND DISCUSSION

Severibuxine (**1**) was assigned the molecular formula $C_{29}H_{39}NO_3$ (elemental analysis). Its UV spectrum was characteristic of the 2,4-quinolindione system [11]. The presence of a phenolic hydroxyl group and an amide group in the molecule was inferred by the IR bands and two signals with D_2O exchangeable in ^1H NMR spectrum, together with a positive $FeCl_3$ reaction. The aromatic region of the ^1H NMR spectrum contained an ABX type pattern attributable to H-5, H-7 and H-8. In the aliphatic region, two sets of geranyl groups were observed and this was confirmed by mass fragments at m/z 380 $[M\text{-}C_5H_9]^+$, 312 $[M\text{-}C_{10}H_{17}]^+$ and 244 $[M\text{-}C_{10}H_{17}\text{-}C_5H_9+1]^+$.

To confirm the location of the hydroxyl group, **1** was acetylated with pyridine and acetic anhydride to give **1a**. In the ^1H NMR spectrum of **1a**, the signals of H-5 and H-7 were shifted to δ 7.70 and 7.22, respectively. This result suggested the location of the hydroxyl group at C-6. This was supported by NOESY (Fig. 1) and HMBC experiments (Fig. 2). On the basis of the above results, the structure of severibuxine could be represented by **1**.

The known compounds, severinolide (**2**) [10], cycloseverinolide (**3**) [10], atalantin (**4**) [10], dehydroatalantin (**5**) [10], cycloepiatalantin (**6**) [10], atalantolide (**7**) [10], severifoline (**8**) [6], *N*-methyl severifoline (**9**) [6], 5-hydroxy-*N*-methylseverifoline (**10**) [6], atalaphylline (**11**) [6], *N*-methylatalaphylline (**12**) [6], atalaphyllinine (**13**) [6], α-santalen-11-one (**14**) [7], dihydro-α-santalen-12-one (**15**) [7], 12, 13-epoxy-α-santalene (**16**) [7], α-photosantalol (**17**) [7], $\Delta^{13,14}$ iso-α-santalol (**18**) [7], α-santalene (**19**) [7], (*E*)-5-(2,3-dimethyl-3-nortricyclyl)pent-3-en-2-one (**20**) [7], umbelliferone (**21**) [12], auraptene (**22**) [13], geranyl scopoletin (**23**) [14] and asparagine (**24**) [15] were also isolated and characterized by comparison of their spectroscopic data (UV, IR, NMR and mass spectrometry) with literature values.

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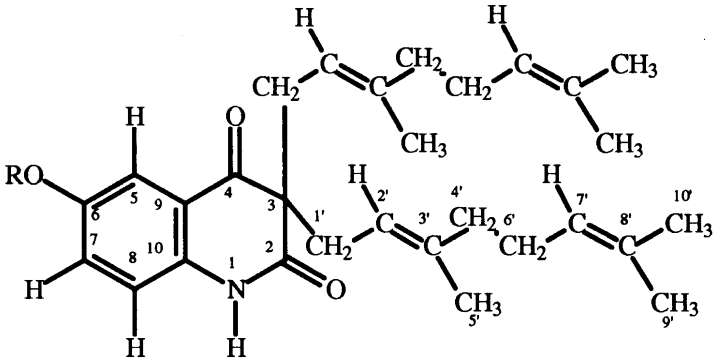

1: R=H
1a: R=Ac

The isolated compounds were assayed for their cytotoxic activity. The results are summarized in Table 1. Most of them showed strong cytotoxic activity against P-388. Severifoline (**8**) and atalaphyllinine (**13**) also revealed significant cytotoxic activity against Hep. G2, 2, 15 at 0.1 and 3.78 μg/ml, respectively. Dehydroatalantin (**5**) displayed cytotoxic activity against Hep. G2 at 1.39 μg/ml.

EXPERIMENTAL

Mps: uncorr, ^{1}H NMR (100, 200, 400 MHz): $CDCl_3$ (except where noted), with TMS as an int. standard; MS: were direct inlet; UV: MeOH; IR: $CHCl_3$ soln.

Plant material

Severinia buxifolia (Pior.) Tenore was collected from Tainan, Taiwan and identified by Professor Kuoh. A voucher specimen (NCKU-WU-810405) is deposited in the Herbarium of National Cheng Kung University, Tainan.

Extraction and separation

The procedure of extraction and separation was reported in a previous paper [6]. The *n*-hexane elute fraction was directly chromatographed on silica gel and eluted with *n*-hexane and *i*-Pr_2O to give **14** (0.5 g), **15** (0.2 g), **16** (0.1 g), **17** (6.3 g), **18** (0.3 g), **19** (0.1 g) and **20** (1.2 g). The benzene elute fraction was also rechromatographed on silica gel and eluted with *n*-hexane-benzene (1:1), benzene, then benzene-Me_2CO (1:1) to obtain **1** (100 mg), **4** (0.7 g), **5** (80 mg), **8** (50 mg), **9** (50 mg), **10** (2.1 g), **11** (40 mg), **12** (40 mg), **22** (1.2 g), **23** (0.4 g) and steroids (3.2 g), successively. Fraction 3 was treated in a similar method as fr. 1 to afford **2** (5.1 g), **3** (0.6 g), **6** (0.9 g), **7** (1.2 g), **13** (2.4 g) and **21** (0.3 g), respectively. The H_2O layer was filtered to obtain **24** (5.6 g).

Severibuxine (**1**)

Yellowish needles (*n*-hexane), mp 113–115°. Anal. Calcd. for $C_{29}H_{39}NO_3$: found: C, 77.24; H, 9.03; N, 3.09%, required: C, 77.46; H, 9.03; N, 3.12%. UV λmax nm: 209, 242, 265, 382; IR νmax cm^{-1}: 3560, 3380, 1680, 1640, 1480; EIMS *m/z*: 449[M]$^+$, 380,

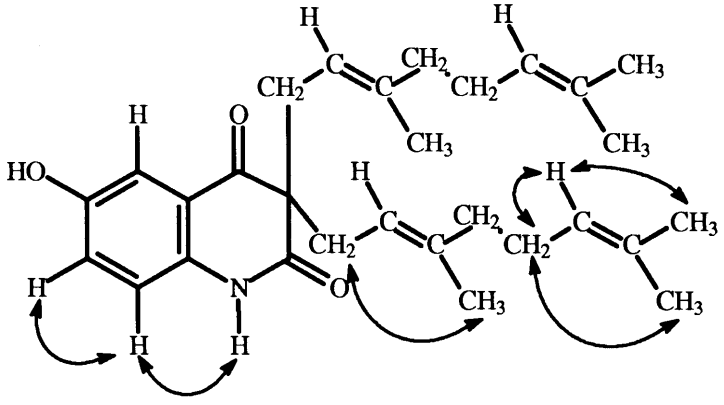

Fig. 1. NOE correlations of severibuxine (**1**).

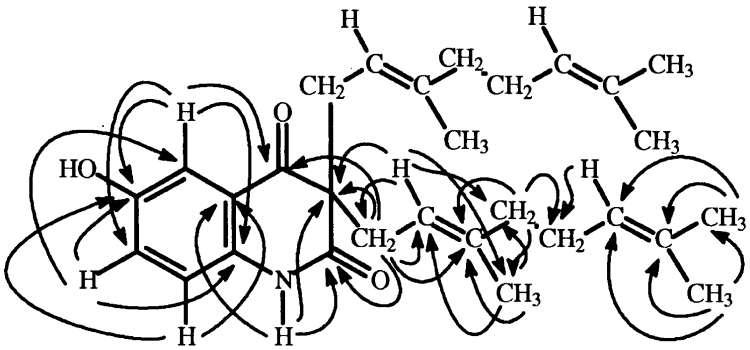

Fig. 2. $^{2}J,^{3}J$-correlations (HMBC) of severibuxine (**1**).

Table 1. Cytotoxic activities of compounds from the root bark of *S. buxifolia*

Compounds	Cell line (IC_{50} μg/ml)			
	Hep. G2	Hep. G2, 2, 15	KB	P388
Severibuxine (**1**)	22.80	6.40	3.90	0.10
Severinolide (**2**)	14.97	17.48	8.49	0.07
Cycloseverinolide (**3**)	16.01	27.36	3.94	1.13
Atalantin (**4**)	19.40	39.92	3.21	1.13
Dehydroatalantin (**5**)	1.39	5.67	3.25	1.17
Cycloepiatalantin (**6**)	64.78	38.76	8.55	0.10
Atalantolide (**7**)	51.78	15.25	3.49	1.00
Severifoline (**8**)	68.18	0.10	4.35	1.03
5-Hydroxy-*N*-methylseverifoline (**10**)	5.73	7.18	3.29	0.08
Atalaphylline (**11**)	10.11	6.94	1.15	0.99
N-methylatalaphylline (**12**)	23.78	25.74	0.79	0.15
Atalaphyllinine (**13**)	7.11	3.78	5.88	0.07
Umbelliferone (**21**)	19.76	8.84	1.73	1.07
Auraptene (**22**)	7.92	5.80	4.07	0.71
Geranylscopoletin (**23**)	22.10	7.90	4.31	0.08

312, 244, 190, 69, 41; ^{1}H NMR δ: 8.97 (1H, *br.s*, NH), 7.40 (1H, *d*, J = 2.4 Hz, H-5), 7.07 (1H, *dd*, J = 8.4, 2.4 Hz, H-7), 6.82 (1H, *d*, J = 8.4 Hz, H-8), 6.32 (1H, *br.s*, OH), 4.91 (2H, *t*, J = 7.6 Hz, H-2′, 2″), 4.88 (2H, *m*, H-7′, 7″), 2.78 (2H, *dd*, J = 13.2, 7.6 Hz, H-1′, 1″), 2.72 (2H, *dd*, J = 13.2, 7.6 Hz, H-1′, 1″), 1.77 (8H, *br.s*, H-4′, 4″, 6′, 6″), 1.57 (6H, *s*, H-5′, 5″), 1.52 (6H, *s*, H-10′, 10″), 1.45 (6H, *s*, H-9′, 9″); ^{13}C NMR δ: 198.4 (C-4), 174.2 (C-2), 152.1 (C-6), 139.5 (C-3′, 3″), 134.8 (C-10), 131.3 (C-8′, 8″), 124.2 (C-7), 123.9 (C-7′, 7″), 120.6 (C-9), 117.5 (C-2′, 2″), 117.3 (C-8), 111.7 (C-5), 61.5 (C-3), 39.7 (C-4′, 4″), 37.7 (C-1′, 1″), 26.5 (C-6′, 6″), 25.6 (C-10′, 10″), 17.6 (C-9′, 9″), 16.2 (C-5′, 5″).

Acetylation of severibuxine (**1**)

Severibuxine (**1**, 10 mg) was treated with Ac_2O (1 ml) and pyridine (1 ml) and the mixture allowed to stand overnight. The soln was evapd to dryness *in vacuo* and the residue was recrystallized from *n*-hexane to give pale yellowish needles of **1a** (9 mg). Pale yellowish needles (*n*-hexane), mp 49–51°. UV λmax nm: 235(sh), 238, 259, 349; IR νmax cm^{-1}: 1745, 1683, 1647, 1484, 1362, 1180; EIMS *m*/*z*: 491$[M]^{+}$, 448, 422, 354, 312, 298, 286, 244, 232, 228, 202, 190, 69, 41; ^{1}H NMR δ: 10.0 (1H, *br.s*, NH), 7.70 (1H, *d*, J = 2.8 Hz, H-5), 7.22 (1H, *dd*, J = 8.4, 2.8 Hz, H-7), 6.95 (1H, *d*, J = 8.4 Hz, H-8), 4.90 (4H, *m*, H-2′, 2″, 6′, 6″), 2.75 (4H, *m*, H-1′, 1″), 2.35 (3H, *s*, $OCOCH_3$), 1.79 (8H, *s*, H-4′, 4″, 5′, 5″), 1.80 (6H, *s*, $2XCH_3$), 1.60 (6H, *s*, $2XCH_3$), 1.44 (6H, *s*, $2XCH_3$).

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