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CONSTITUENTS AND CYTOTOXIC PRINCIPLES OF *NOTHAPODYTES FOETIDA*

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Key Word Index—*Nothapodytes foetida*; Icacinaceae; camptothecins; steroids; cytotoxicity.

Abstract—A new naturally occurring alkaloid, acetylcamptothecin, together with 17 known compounds, (+)-1-hydroxypinoresinol, ω-hydroxypropioguaiacone, *p*-hydroxybenzaldehyde, scopoletin, uracil, thymine, sitosterol, sitosteryl-β-D-glucoside, 3β-hydroxy-stigmast-5-en-7-one, stigmast-5-en-3β,7α-diol, 6β-hydroxystigmast-4-en-3-one, sitost-4-en-3-one, linoleic acid, trigonelline, camptothecin, 9-*O*-methoxycamptothecin and pumiloside were isolated and characterized from the stem of *Nothapodytes foetida*. Among them, scopoletin, camptothecin, 9-*O*-methoxycamptothecin and *O*-acetylcamptothecin showed significant cytotoxic activity.

INTRODUCTION

Nothapodytes foetida (Wight) Sleumer (*Mappia ovata* Miers; *M. ovata var. insularis* Matsum; *Stemonurus foetidus* Wight) is a tree which is widely distributed in South India, Ceylon, Cambodia and the Ryukyu islands. This plant is the only species of *Nothapodytes* native to Orchid island and cultured in Taiton Hsien, Taiwan [1, 2]. It is apparently unused in traditional medicine in Taiwan. Govindachari *et al.* [3, 4] have reported the isolation of alkaloids and triterpenoid from the bark, stem, root and leaf of this plant. As a result of our continuing search for novel bioactive natural products, the ethanol extract of the stem of *N. foetida* was found to show significant cytotoxicity in the human KB tissue culture assay. Bioassay-directed fractionation of the plant extract led to the isolation and characterization of *O*-acetylcamptothecin (**1**), camptothecin (**2**), 9-methoxycamptothecin (**3**) and scopoletin (**7**) as the cytotoxic principles from the chloroform soluble fraction. We now describe the structural elucidation of a new naturally occurring alkaloid, *O*-acetylcamptothecin (**1**) together with 17 known compounds which were isolated from the stem of *N. foetida* and their cytotoxic activity.

RESULTS AND DISCUSSION

O-Acetylcamptothecin (**1**) was isolated as yellowish needles, mp 272–275° and its high resolution mass spectrum indicated a molecular formula $C_{22}H_{18}N_2O_5$ $[M]^+$ at *m/z* 390.1217. Its UV spectrum with maxima absorption at 218.6, 248.6 (sh), 253.4, 288.4, 337.2 (sh), 358.2 and 368.6 nm was very close to that of camptothecin (**2**) [3], which was also isolated in this study, and thus indicated the presence of a camptothecin skeleton for **1**. The ^1H NMR spectrum of **1** differed from that of **2** only by the presence of a signal at δ2.22 (3H, *s*) for acetyl group instead of an alcoholic hydroxyl group at δ6.50 in **2**. This fact was further supported by the IR band at 1745 cm^{-1} for an ester carbonyl group and mass spectral fragment ions at *m/z* 390 $[M]^+$ and 330 $[M - MeCOOH]^+$. In addition, the downfield shift of the ethyl group at δ0.88 (3H, *t*, $J = 7.4$ Hz) and 1.88 (2H, *m*) in **2** to δ0.98 (3H, *t*, $J = 7.6$ Hz), 2.15 (1H, *dd*, $J = 13.4, 7.6$ Hz) and 2.29 (1H, *dd*, $J = 13.4, 7.6$ Hz) in **1**, suggested the acetyl group to be located at C-20. To confirm this proposition, acetylation of **2** with pyridine and acetic anhydride afforded yellowish needles of a product which was identical with **1** by comparison of the spectral data and mixed mp. On the basis of the above results, the structure of *O*-acetylcamptothecin can be represented by **1**. This is the first report of the occurrence of **1** in a natural source although it was prepared by Govindachari [3]. Compound **1** exhibited potent cytotoxicity (Table 1) in the KB, P-388, A-549, HT-29 and HL-60 tissue culture assays [5].

In this study, we found that *N. foetida* represents the most convenient source of camptothecin (**2**) and 9-*O*-methoxycamptothecin (**3**). The known compounds, (+)-1-hydroxypinoresinol (**4**) [6], ω-hydroxypropioguaiacone (**5**) [7], *p*-hydroxybenzaldehyde (**6**), scopoletin (**7**) [8], uracil (**8**), thymine (**9**), sitosterol (**10**), sitosteryl-β-D-glucoside (**11**), 3β-hydroxy-stigmast-5-en-7-one (**12**)

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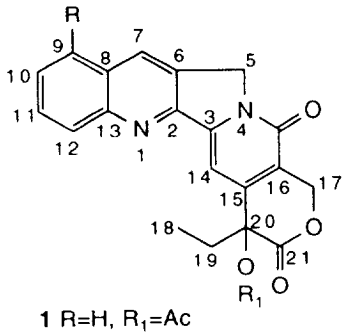

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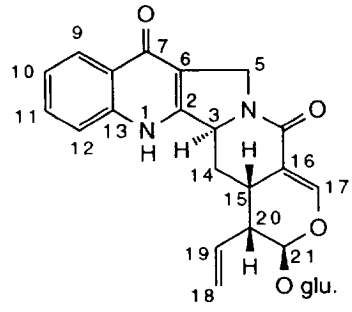

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Table 1. Cytotoxic activities of camptothecin derivatives and scopoletin

Compounds	Cell line ED_{50}($\mu g\,ml^{-1}$) KB	P-388	A-549	HT-69	HL-60
O-Acetylcamptothecin (**1**)	3.5×10^{-1}	3.5×10^{-1}	1.5×10^{-2}	8.0×10^{-3}	1.3×10^{-2}
Camptothecin (**2**)	2.7×10^{-4}	6.9×10^{-5}	9.8×10^{-6}	3.9×10^{-6}	5.4×10^{-6}
9-Methoxy-camptothecin (**3**)	1.8×10^{-4}	9.9×10^{-5}	3.6×10^{-5}	3.0×10^{-5}	1.5×10^{-5}
Pumiloside (**18**)	> 40	> 40	> 40	> 40	> 40
Scopoletin (**7**)	4.0	—	—	—	—

[9], stigmast-5-en-3β,7α-diol (**13**) [10], 6β-hydroxy-stigmast-4-en-3-one (**14**) [11], sitost-4-en-3-one (**15**) [12], linoleic acid (**16**), trigonelline (**17**) [13], camptothecin (**2**) [3], 9-*O*-methoxycamptothecin (**3**) [3] and pumiloside (**18**) [14] were isolated and characterized by comparison of their spectroscopic data and/or mmp with authentic samples.

Cytotoxic activities of camptothecin derivatives and scopoletin against human epidermoid cascinoma KB cells, P-388, A-549, HT-29 and HL-60 *in vitro* were examined and are shown in Table 1.

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR (200 and 400 MHz) were recorded in $CDCl_3$ except where noted. Chemical shift values are shown in ppm (δ) with TMS as int. standard. MS were recorded using a direct inlet system. UV were determined in MeOH and IR recorded in KBr disc.

Plant material. Nothapodytes foetida was collected from Taiton Hsien, Taiwan in July 1989 and verified by Prof. C. S. Kuoh. A voucher specimen is deposited in the Herbarium of Cheng Kung University, Tainan, Taiwan, R.O.C.

Extraction and separation. Air-dried stem of *N. foetida* (5 kg) was chipped and extracted with EtOH at room temp. After filtration and evapn of solvent to afford a brown syrup, the EtOH extract was partitioned between $CHCl_3$ and H_2O, then *n*-BuOH, successively. The $CHCl_3$ layer was dried over Na_2SO_4 and then concd under red. pres. to leave a brown syrup which was directly chromatographed on silica gel and eluted with a gradient of $CHCl_3$ and Me_2CO to give 13 fractions. Fr. 5 was rechromatographed on a silica gel column using C_6H_6–Me_2CO (9:1) as eluant to obtain **10** (1.2 g), **6** (4 mg), **12** (3 mg), unknown **a** (2 mg), **1** (1.2 mg), **15** (5 mg), **14** (7 mg), **b** (1 mg), and **13** (2 mg), successively. Fr. 9 was repeatedly chromatographed on silica gel column and eluted with $CHCl_3$–MeOH (9:1) to give **7** (5 mg), **16** (6 mg), **c** (1 mg), **d** (1.5 mg), **f** (1.2 mg), **g** (2 mg), **5** (3 mg), **4** (2 mg), **h** (1 mg), **3** (8.3 g), **2** (12.4 g), i (3 mg) and **11** (42 mg), successively. Fr. 13 was treated in the same way as fr. 9 to afford **18** (0.2 g). The BuOH layer was sepd by Sephadex LH-20 CC eluting with H_2O to give 7 fractions. Fr. 6 was rechromatographed on a Sephadex LH-20 CC using H_2O as eluant to obtain **8** (6 mg), and **17** (3 mg), respectively. Fr. 7 was treated in the same way as fr. 6 to afford **j** (1 mg) and **9** (3 mg), respectively.

O-Acetylcamptothecin (**1**). Yellowish needles, mp 272–275°. Found: $[M]^+$ at *m/z* 390.1217; $C_{22}H_{18}N_2O_5$, requires, 390.1216. UV λ_{max} nm: 218.6, 248.6 (sh), 253.4, 288.4, 337.2 (sh), 358.2, 368.6. IR ν_{max} cm^{-1}: 1745, 1665, 1620. EIMS *m/z* (rel. int.): 390 $[M]^+$ (41), 330 (91), 315 (40), 302 (100), 287 (46), 275 (15). ^{1}H NMR: δ8.40 (1H, *s*, H-7), 8.23 (1H, *dd*, *J* = 8.0, 1.2 Hz, H-9), 7.94 (1H, *dd*, *J* = 8.0, 1.2 Hz, H-12), 7.84 (1H, *td*, *J* = 8.0, 1.2 Hz, H-11), 7.67 (1H, *td*, *J* = 8.0, 1.2 Hz, H-10), 7.23 (1H, *s*, H-14), 5.68, 5.41 (each 1H, *d*, *J* = 17.2 Hz, H-17), 5.39 (2H, *s*,

H-5), 2.29, 2.15 (each 1H, *q*, $J = 7.6$ Hz, H-19), 2.22 (3H, *s*, OAc), 0.98(3H, *t*, $J = 7.6$ Hz, H-18).

Acetylation of camptothecin (**2**). Camptothecin (**2**, 0.2 g) was treated with Ac_2O (5 ml) and pyridine (5 ml) and the mixt. allowed to stand overnight. The soln was evapd to dryness *in vacuo* and the residue was recrystallized from Me_2CO to give pale yellowish needles, mp 271–273°, which was identified with **1** by comparison of their spectral data and mmp.

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