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Chemical constituents of *Nothapodytes pittosporoides* (Icacinaceae)

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ABSTRACT

A new alkaloid, *O*-acetyl-7-methoxycamptothecin (**1**), was isolated from the roots of *Nothapodytes pittosporoides* (Icacinaceae), together with seventeen known compounds (**2**–**18**). The structures of these compounds were identified by extensive spectroscopic interpretation. Isocoumarins were reported from the investigated genus for the first time. The alkaloids and isocoumarins in *N. pittosporoides* could serve as its chemotaxonomic markers.

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1. Subject and source

Nothapodytes pittosporoides (Oliv.) Sleum (Icacinaceae) is a traditional Chinese herbal medicine mainly distributed in South China (Fang, 1981). Camptothecin and its derivatives (CIDs) have been identified as the characteristic compounds in *Nothapodytes foetida* (Wight) Sleum. The roots of *N. pittosporoides* were collected from Zunyi, Guizhou Province, China. A voucher specimen (Cai20110805) has been preserved in the Yada Pharmaceutical Co., Ltd., Chengdu, Sichuan Province, China.

2. Previous work

Camptothecin, 9-methoxycamptothecin, 10-hydroxycamptothecin, 9-methoxymappicine-20-*O*-*β*-D-glucopyranoside, mappicine-20-*O*-*β*-D-glucopyranoside, (3*S*)-pumiloside, (−)-(3*S*)-1, 2, 3, 4-tetrahydro-*β*-carboline-3-carboxylic acid, *β*-sitosterol, *β*-daucosterol, 7-oxo-sitosterol, *β*-sitosteryl-3-*O*-*β*-D-glucopyranoside-2′-*O*-palmitate, 6*β*-dihydroxydaucosterol, lupeol, 3-acetoxy-12-oleanen-28-ol have been isolated from *N. pittosporoides* (Bai and Song, 2014).

3. Present study

The dry roots of *N. pittosporoides* (10 kg) were powdered and extracted twice with 1% HCl aqueous solution at room temperature. The percolate was concentrated to a syrup (240 g), which was divided into two fractions (I, II) by silica gel

Abbreviation: CIDs, camptothecin and its derivatives.

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column chromatography (CC) with gradient mixtures of $CHCl_3$—MeOH. A total of 18 compounds were isolated from these fractions by repeated silica gel, ODS-A and Sephadex LH-20 CC, as well as preparative HPLC. Among them, (*R*)-mellein (**13**, 9.1 mg) (Hirschmann et al., 2005), (*R*)-6-methoxymellein (**14**, 2 mg) (Govindachari et al., 1971), (*R*)-7-hydroxymellein (**15**, 12.1 mg) (Oliveira et al., 2011), 8-hydroxy-6,7-dimethoxy-3-methyl isocoumarin (**16**, 4 mg) (Boonlarppradab et al., 2011), stigmast-4-en-6β-ol-3-one (**17**, 21 mg) (Shao et al., 2013), and (+)-medioresinol (**18**, 50 mg) (Yu et al., 2012), were isolated from fraction I. A new alkaloid, *O*-acetyl-7-methoxy-camptothecin (**1**, 8 mg), together with eleven known compounds, 10-hydroxy-9-methoxycamptothecin (**2**, 79 mg) (Aimi et al., 1992), 7-methoxycamptothecin (**3**, 10 mg) (Arbain et al., 1993), 10-hydroxycamptothecin (**4**, 17 mg) (Lin and Cordell 1990), 9-methoxycamptothecin (**5**, 8g) (Pirillo et al., 1995), camptothecin (**6**, 376 mg) (Ezell and Smith 1991), *O*-acetylcamptothecin (**7**, 8 mg) (Wu et al., 1995), 5-hydroxycamptothecin (**8a**/**8b** racemic mixture, 45 mg) (Wu et al., 2008), 5-hydroxy-9-methoxycamptothecin (**9a**/**9b** racemic mixture, 8 mg) (Wu et al., 2008), mappicine (**10**, 93 mg) (Govindachari et al., 1974), 9-methoxymappicine (**11**, 60 mg) (Das and Madhusudan, 1999), and dihydrocamptothecin (**12**, 38 mg) (Govindachari et al., 1974), were yielded from fraction II.

Compound **1** was obtained as a yellow amorphous powder. The molecular formula $C_{23}H_{20}N_2O_6$ was determined by a quasi-molecular ion peak at *m*/*z* 421.1395 $[M+H]^+$ in the positive HRESIMS. The degree of unsaturation was 15 on the basis of this formula. The IR absorption bands at 3440, 1667 and 1749 cm^{-1} showed the existence of amide and lactone group, respectively. The ^{13}C NMR spectrum showed 23 carbon signals, which were classified by the chemical shifts and HSQC spectrum as two methyl carbons (δ_C 7.6, 20.6), one methoxy carbon (δ_C 58.6), three methylene carbons (δ_C 30.2, 50.4, 66.3), five methane carbons (δ_C 94.5, 121.2, 127.0, 128.6, 130.8), and twelve quaternary (including three carbonyl) carbons (δ_C 75.9, 111.1, 118.6, 119.9, 145.7, 145.9, 149.1, 154.1, 156.4, 157.7, 167.4, 169.7). The ^1H NMR spectrum showed signals of three triplets, five singlets, two doublets and a multiplet at δ_H 0.91 (3H, t, $J=7.2$ Hz), 7.65 (1H, t, $J=7.8$ Hz), 7.82 (1H, t, $J=7.8$ Hz), 2.22 (3H, s), 4.44 (3H, s), 5.49 (2H, s), 5.72 (2H, s), 7.03 (1H, s), 8.07 (1H, d, $J=7.8$ Hz), 8.24 (1H, d, $J=7.8$ Hz), 2.14 (2H, m), respectively. The ^1H NMR, ^{13}C NMR and DEPT spectra of **1** indicated a camptothecin skeleton (Lin and Cordell, 1990; Ezell and Smith, 1991). Comparative analysis of the ^{13}C NMR, ^1H NMR and 2D NMR data of **1** with the literature data showed that **1** was similar to **3** (Fig. A.1) (Arbain et al., 1993). The key distinction was the surplus NMR signals of an acetyl group [δ_C 169.7 (s), δ_C 20.6 (q), δ_H 2.22 (3H, s)], and the absence signal of δ_H 6.59 (1H, s, 20-O**H** in **3**). The downfield chemical shift of the ethyl group proton signals at δ_H 0.87 (CH_3-18 of **3**, t) and 1.86 (H-19 of **3**, m), to δ_H 0.91 (CH_3-18, t) and 2.14 (H_2-19, m), as well as carbon signal of δ_C 72.4 (C-20 of **3**) to δ_C 75.9 (C-20), suggested an acetoxy group at C-20 (Wu et al., 1995). A careful analysis on the remaining ^1H and ^{13}C NMR spectral data indicated the same E ring structure of **1** as that of **7** (Fig. A.1). On the basis of the above analysis, compound **1** was identified as *O*-acetyl-7-methoxycamptothecin.

By combined analysis of NMR and HRESIMS data, as well as by comparing the ^1H NMR data with those in the literature (Aimi et al., 1992), compound **2** was determined as 10-hydroxy-9-methoxycamptothecin. In this work, its ^{13}C NMR, optical rotation, IR and mass spectral data were reported for the first time.

O-Acetyl-7-methoxycamptothecin (**1**), $C_{23}H_{20}N_2O_6$, yellow amorphous powder; positive HRESIMS *m*/*z* 421.1395 (calcd. for $C_{23}H_{21}N_2O_6$ $[M+H]^+$, 421.1400); IR (KBr) cm^{-1}: 3440, 2927, 1749, 1667, 1620, 1571, 1505, 1454, 1400, 1385, 1338, 1236, 1162, 1119, 1110, 1091, 1053, 1026, 568; ^1H and ^{13}C NMR data (DMSO-d_6) see Table A.1.

10-Hydroxy-9-methoxycamptothecin (**2**), $C_{21}H_{18}N_2O_6$, yellow amorphous powder; $[\alpha]_D^{20}$ −25.8977 (*c* 0.0013, DMSO); positive HRESIMS *m*/*z* 395.1236 (calcd. for $C_{21}H_{19}N_2O_6$ $[M+H]^+$, 395.1243); IR (KBr) cm^{-1}: 3442, 1738, 1657, 1634, 1580, 1563, 1503, 1468, 1394, 1376, 1324, 1253, 1235, 1157, 1138, 1113, 1502, 1010, 836; ^1H and ^{13}C NMR data (DMSO-d_6) see Table A.1.

The IR spectra were obtained by a Tensor 27 spectrophotometer using KBr pellets. Optical rotation was measured on a P-1020 Polarimeter (JASCO, Tokyo, Japan). The 1D and 2D spectra were recorded on a Bruker AV-600 spectrometer with TMS as internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. The HRESIMS data were recorded on an Agilent G6230 TOF MS.

4. Chemotaxonomic significance

Camptothecin, a quinoline pentacyclic alkaloid, was first reported from *Camptotheca acuminate* Decne. (Nyssaceae) (Wall et al., 1966). This compound exhibited anticancer activity by inhibiting DNA topoisomerase I (Hsiang et al., 1985). Subsequently, CIDs were isolated from various kinds of plants mainly including *Ervatamia heyneana* (Wall.) T. Cooke (Apocynaceae) (Gunasekera et al., 1979), *Merrilliodendron megacarpum* (Hemsl.) Sleumer (Icacinaceae) (Arisawa et al., 1981), *Mostuea brunonis* Didr. (Gelsemiaceae) (Dai et al., 1999), *Ophiorrhiza filistipula* Bl. (Arbain et al., 1993), *Ophiorrhiza liukiuensis* Hayata (Kitajima et al., 2005), *Ophiorrhiza mungos* Linn. (Tafur et al., 1976), *Ophiorrhiza pumila* Champ. ex Benth. (Aimi et al., 1990), *Ophiorrhiza trichocarpon* Blume (Klausmeyer et al., 2007) (Rubiaceae), and *Pyrenacantha klaineana* Pierre ex Exell & Mendonça (Zhou et al., 2000) (Icacinaceae).

CIDs were also reported from *N. foetida* (Govindachari and Viswanathan, 1972) and *N. pittosporoides* (Lv et al., 2010; Zeng et al., 2013; Bai and Song, 2014). Interestingly, CIDs have never been reported from any other species of *Nothapodytes*. It is worth noting that CIDs with 5-hydroxy group have only been reported from *N. foetida* (Lorence and Nessler, 2004; Wu et al., 2008; Ramawat and Merillon, 2013) and *N. pittosporoides*. Moreover, both of the species contain a special E-ring fission CIDs, mappicine and its derivatives, which are rarely distributed in plants. To date, only 19-hydroxymappicine has been reported from *C. acuminate* (Lin and Cordell, 1989). The presence of CIDs provides a close chemotaxonomic relationship between *N. foetida* and *N. pittosporoides*. However, the present study shows that *N. pittosporoides* contains four isocoumarins (**13**–**16**), whereas *N. foetida* contains coumarins, but not isocoumarins (Wu et al., 1995). Therefore, isocoumarins could serve as the

chemical marker to distinguish *N. pittosporoides* from *N. foetida*. Further research is needed to identify other compounds that differentiate these species from other species of *Nothapodytes*.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2015.06.039.

Appendix

Table A.1
^{1}H NMR (600 MHz) and ^{13}C NMR (125 MHz) spectral data of 1 and 2 (in DMSO-d_6, δ in ppm, J in Hz).

No.	1			2		
	δ_C	δ_H	HMBC ($^{1}H-^{13}C$)	δ_C	δ_H	HMBC ($^{1}H-^{13}C$)
2	154.1s			149.8s		
3	145.9s			145.9s		
5	50.4t	5.72 (1H, s)	C-2, 6	50.5t	5.25 (1H, s)	C-2, 3, 6, 8
6	111.1s			129.8s		
7	157.7s			124.5d	8.65 (1H, s)	C-2, 5, 9, 13
8	119.9s			124.2s		
9	121.2d	8.24 (1H, d, 7.8)	C-7, 11, 13	139.2s		
10	127.0d	7.65 (1H, t, 7.8)	C-8, 12	147.5s		
11	130.8d	7.82 (1H, t, 7.8)	C-9, 13	123.6d	7.53 (1H, d, 9.0)	C-8, 9, 13
12	128.6d	8.07 (1H, d, 7.8)	C-8, 10	125.6d	7.83 (1H, d, 9.0)	C-8, 9, 10
13	149.1s			143.3s		
14	94.5d	7.03 (1H, s)	C-2, 3, 16, 20	96.2d	7.26 (1H, s)	C-2, 3,16, 20
15	145.7s			150.1s		
16	118.6s			118.4s		
17	66.3t	5.49 (2H, s)	C-15, 16, 22	65.3t	5.41 (2H, s)	C-3, 14, 15, 16, 19, 20, 21, 22
18	7.6q	0.91 (3H, t, 7.2)	C-19, 20	7.9q	0.87 (3H, t, 7.2)	C-19, 20
19	30.2t	2.14 (2H, m)	C-15, 18, 20, 22	30.3t	1.85 (2H, m)	C-15, 18, 20, 22
20	75.9s			72.5s		
21	156.4s			157.0s		
22	167.4s			172.7s		
OCH_3	58.6q	4.44 (3H, s)	C-7	60.7q	3.93 (3H, s)	C-9
CH_3COO	20.6q	2.22 (3H, s)				
	169.7s					
O**H**					6.53 (1H, s)	C-15, 19, 20, 22

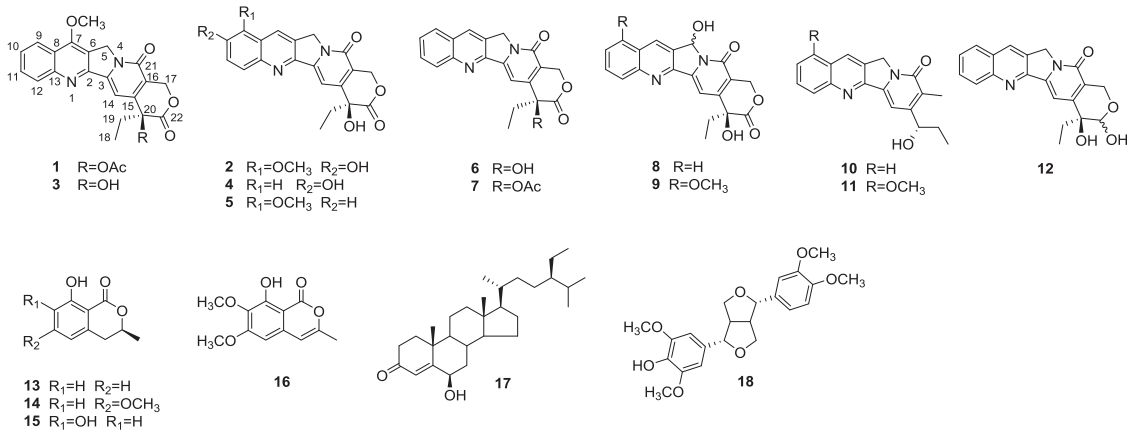

Fig. A.1. Structures of **1**–**18**.

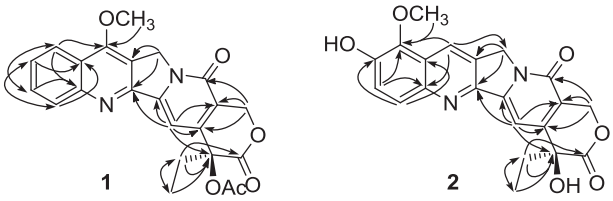

Fig. A.2. HMBC ($^{1}H \rightarrow {}^{13}C$) correlations of **1** and **2**.

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