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UNCARINIC ACIDS: PHOSPHOLIPASE Cγ1 INHIBITORS FROM HOOKS OF *UNCARIA RHYNCHOPHYLLA*

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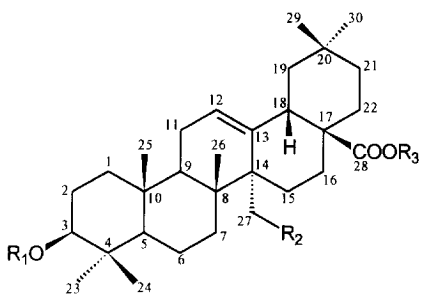
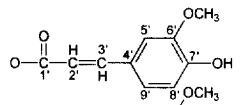
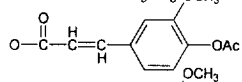
Abstract: Bioactivity-guided fractionation of the $CHCl_3$ extract from hooks of *Uncaria rhynchophylla* led to the isolation of two triterpene esters, namely uncarinic acids A (**1**) and B (**2**). Their structures were established by spectroscopic and chemical methods. These compounds inhibited phospholipase Cγ1 with IC_{50} values of 35.66 and 44.55 μM, respectively.

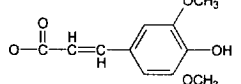
Phosphatidylinositol-specific phospholipase C (PI-PLC) is the key enzyme involved in the signal transduction of growth factors, neurotransmitters and hormones. The activation of this enzyme causes the hydrolysis of phosphatidylinositol 4,5-biphosphate, which generates inositol 1,4,5-triphosphate and diacylglycerol. These two second messengers induce the increase of intracellular free Ca^{2+} concentration and the activation of protein kinase C, which lead to a series of events that culminate in the DNA synthesis and cell proliferation. It has been reported that PI-PLC activity was increased in a number of human cancer cells, suggesting that PI-PLC, especially γ isoforms, would be a good target for the development of anticancer agents[1].

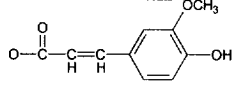
In the previous paper[2], we have isolated the PI-PLCγ1 inhibitors with cytotoxic activity against several human cancer cells from the sarcotestas of *Ginkgo biloba*. Our continued interest to find the natural product inhibitors of PLCγ1 has led to the isolation of two new triterpene esters, uncarinic acid A (**1**) and its isomer, uncarinic acid B (**2**) from the hooks of *Uncaria rhynchophylla* (Miquel) Jackson (Rubiaceae). The bioactivity-guided isolation, structure elucidation, PI-PLCγ1 inhibitory activity and cytotoxic activity of the compounds are described.



1 R_1 = H R_2 =  R_3 = H

1a R_1 = Ac R_2 =  R_3 = H

1b R_1 = H R_2 =  R_3 = CH_3

2 R_1 = H R_2 =  R_3 = H

3 R_1 = H R_2 = OH R_3 = H

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PII: S0960-894X(99)00211-5

Isolation

The methanolic extract (800 g) from hooks (20 kg) of *U. rhynchophylla* was partitioned between H_2O and $CHCl_3$. $CHCl_3$ layer was evaporated *in vacuo* to give the $CHCl_3$ extract (600 g), exhibited PI-PLCγ1 inhibitory activity. Bioactivity-guided fractionation of the $CHCl_3$ extract by silica gel column chromatography led to the isolation of crude mixture of triterpene esters **1** and **2**, which was further purified by sequential column chromatography over Sephadex LH-20 and RP-HPLC to afford **1** (52.3 mg)[3] and **2** (63.2 mg)[3] as white amorphous powders.

Structure Elucidation

Uncarinic acid A (1): The molecular formula of **1**, $C_{40}H_{56}O_7$, was determined by FABHRMS (*m/z* 671.3956 $[M+Na]^+$). The IR spectrum of **1** exhibited characteristic bands at 3423 (OH), 1694 (C=O), 1630 (olefinic C=C), 1595 (aromatic C=C) and 970 (*trans* CH=CH) cm^{-1}. Compound **1** yielded the acetate (**1a**) and the methyl ester (**1b**) on reaction with Ac_2O/pyridine and CH_2N_2, respectively. The ^{1}H-NMR spectra of **1a** and **1b** displayed signals of δ 2.25 and 1.98, and a methoxycarbonyl at δ 3.50, respectively, indicating the presence of two hydroxyl groups, and one free carboxylic acid moiety in **1**.

The ^{1}H- and ^{13}C-NMR spectral data suggested that **1** was a triterpene ester possessing three functionalities, namely, a carboxylic acid unit, a secondary hydroxyl group, and a *trans*-ferulic acid moiety esterified at a hydroxymethyl substituent. It was further supported by the appearance of prominent peaks at *m/z* 194.0585 ($C_{10}H_{10}O_4$, M^+-454) for ferulic acid moiety, and at *m/z* 454.3441 ($C_{30}H_{46}O_3$, M^+-194) for triterpenoid part in the EI mass spectrum. In addition, the presence of significant peaks at *m/z* 201 (246-CO_2H) and 189 (207-H_2O), resulting from the sequential cleavage of the *retro*-Diels-Alder fragments at *m/z* 246 and 207, suggested that **1** was a Δ^{12}-unsaturated triterpene ester with a hydroxyl group in ring A or B, and a free carboxylic acid unit in ring D or E[4,5].

The ^{1}H-NMR spectrum of **1** showed characteristic signals for the *trans*-ferulic substituent, e.g. 1,2,4-trisubstitued aromatic [δ 6.89 (d, J = 8.1 Hz), 6.98 (d, J = 1.8 Hz) and 7.02 (dd, J = 8.1, 1.8 Hz)], *trans*-oriented vinylic [δ 6.18 and 7.50 (each d, J = 15.9 Hz)], and aromatic methoxy [δ 3.88 (s)] protons. Comparison of the six tertiary methyl signals in the ^{1}H-NMR spectrum of **1**, as well as its ^{13}C-NMR data with published values, allowed the triterpenoid carbon skeleton of Δ^{12}-oleanene[6]. Basic hydrolysis of **1** afforded the known compounds, 3β,27-dihydroxyolean-12-en-28-oic acid (**3**) and *trans*-ferulic acid, and identity of these compounds were established by comparison of their physical and spectral data with the literature values[7,8].

In the ^{13}C-NMR spectrum of **1**, the downfield shift of C-27 signal and the upfield shift of C-14 signal were observed when compared with those of **3** (Table 1). Furthermore, the HMBC spectrum of **1** showed distinct correlation through two and three bonds from δ 4.14 and 4.29 (H_2-27) to δ 39.88 (C-8), 137.20 (C-13), 45.20 (C-14) and 23.40 (C-15). These findings indicated that the ferulic acid moiety in **1** is linked to the C-27 hydroxyl group of **3**. From all the above data, the structure of **1** was elucidated as 3β-hydroxy-27-*E*-feruloyloxyolean-12-en-28-oic acid.

Table 1. NMR Chemical Shifts of the Triterpenoids **1-3**[a, b].

Position	**1** (δ C)	**1** (δ H)	**2** (δ C)	**2** (δ H)	**3** (δ C)
1	38.84		38.12		37.96
2	26.72		26.69		27.04
3	78.50	3.15 dd (8.0, 7.5)	78.47	3.15 dd (8.1, 7.4)	78.72
4	38.64		38.50		38.67
5	55.12		54.98		54.73
6	18.24		18.14		18.15
7	33.10		32.80		32.38
8	39.88		39.75		39.64
9	48.77		48.45		48.29
10	37.13		37.00		37.04
11	23.90		23.71		24.11
12	126.90	5.57 t (3.5)	126.97	5.51 t (3.3)	129.56
13	137.20		137.25		137.70
14	45.20		44.99		47.47
15	23.40		23.34		24.46
16	22.82		22.68		22.37
17	46.12		46.00		46.06
18	41.00	2.86 dd (13.8, 3.9)	40.70	2.81 dd (14.0, 3.3)	40.31
19	44.80	0.72 m	44.81	0.70 m	44.87
		1.05 dd (13.8, 2.5)		1.01 dd (14.0, 2.5)	
20	30.57		30.50		30.75
21	33.67		33.58		33.40
22	32.43		32.37		32.23
23	27.91	0.93 s	27.81	0.86 s	27.97
24	15.56	0.72 s	15.44	0.67 s	15.74
25	15.51	0.88 s	15.37	0.77 s	15.46
26	17.92	0.74 s	17.96	0.68 s	18.52
27	65.91	4.14 d (12.6)	65.67	4.14 d (12.7)	62.99
		4.29 d (12.6)		4.25 d (12.7)	
28	181.23		181.30		183.25
29	32.91	0.83 s	32.89	0.74 s	33.00
30	23.53	0.88 s	23.47	0.80 s	23.80
1'	166.58		167.39		
2'	116.58	6.18 d (15.9)	115.34	5.69 d (13.0)	
3'	143.35	7.50 d (15.9)	144.96	6.73 d (13.0)	
4'	125.47		122.99		
5'	113.05	6.98 d (1.8)	109.76	7.79 d (1.9)	
6'	146.20		144.96		
7'	147.17		147.31		
8'	114.13	6.89 d (8.1)	115.73	6.83 d (8.2)	
9'	126.90	7.02 dd (8.1, 1.8)	126.60	7.10 dd (8.2, 1.9)	
$-OCH_3$	55.90	3.88 s	55.88	3.88 s	

[a] JEOL LA 300 or Bruker AMX 500 spectrometer, $CDCl_3$-CD_3OD 9:1.
[b] Coupling constants are in parentheses.

Uncarinic acid B (2): The spectral data of **2** were very similar to those of **1** (Table 1). However, the ^{1}H-NMR spectrum of **2** exhibited the *cis*-conjugated olefinic proton signals at δ 5.69 and 6.73 (each d, J = 13.0 Hz), indicating the presence of *cis*-ferulic acid moiety in **2**. Through the same assignment as that of **1**, the structure of **2** was established as 3β-hydroxy-27-*Z*-feruloyloxyolean-12-en-28-oic acid. Dissolved in MeOH, **1** and **2** equilibrate with a ratio of 65% (*trans*) to 35% (*cis*).

Biological Activity

The PI-PLCγ1 and cytotoxicity assays were performed by the methods of Rhee *et al*[9] and NCI[10], respectively. Uncarinic acids A (**1**) and B (**2**) inhibited phospholipase Cγ1 with IC_{50} values of 35.66 and 44.55 μM, respectively. Moreover, these compounds inhibited growth of several human cancer cells with IC_{50} values of 0.73~3.53 μg/mL (Table 2).

Table 2. Growth Inhibitory Effects of Uncarinic acids A (**1**) and B (**2**) on Human Cancer Cell Lines.

Compound	IC_{50} (μg/mL)			
	A-549[a]	HCT-15[b]	MCF-7[c]	HT-1197[d]
1	0.73	1.41	2.03	3.53
2	1.79	1.44	2.59	2.34

[a] human lung adenocarcinoma, [b] human colon adenocarcinoma, [c] human breast adenocarcinoma, [d] human bladder adenocarcinoma.

Acknowledgments

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3. Physical data (**1**): white amorphous powder, mp 263-266 °C (dec); UV: λ_{max} (isopropyl alcohol) nm (log ε): 203 (4.21), 236 (3.95), 327 (4.20); IR: ν_{max} (KBr): 3423, 1694, 1630, 1595, 1159, 1032, 970, 845 cm^{-1}; FABHRMS (positive): *m/z* 671.3956 (calcd for $C_{40}H_{56}O_7Na$: 671.3924); EIMS: *m/z* (rel.int.): 454.3441 (M^+-$C_{10}H_{10}O_4$, 15), 421 (5), 375 (3), 300 (5), 299 (6), 285 (10), 255 (8), 246 (15), 239 (15), 207 (8), 201 (13), 194.0585 (M^+-$C_{30}H_{46}O_3$, 100), 189 (5), 179 (25), 133 (32), 100 (32), 77 (32), 69 (42), 55 (50). (**2**): white amorphous powder, mp 163-165 °C (dec); IR: ν_{max} (KBr): 3422, 1699, 1629, 1595, 1277, 1163 cm^{-1}; FABHRMS (positive): *m/z* 671.3923 (calcd for $C_{40}H_{56}O_7Na$: 671.3924).
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