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URSANE TRITERPENOIDS FROM LEAVES OF *MELALEUCA LEUCADENDRON*

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Key Word Index—*Melaleuca leucadendron*; Myrtaceae; leaves; ursane; triterpenoids; 3*β*-*cis*-*p*-coumaroyloxy-2*α*-hydroxyursa-12,20(30)-dien-28-oic acid, *cis*- and *trans*- 3*β*-caffeoyloxy-2*α*-hydroxyurs-12-en-28-oic acids.

Abstract—Three novel triterpenoids esters were isolated from the leaves of *Melaleuca leucadendron* in addition to the five known triterpenes ursolaldehyde, ursolic acid, 2*α*-hydroxyursolic acid, 3*β*-*cis*-*p*-coumaroyloxy-2*α*-hydroxyurs-12-en-28-oic acid and 3*β*-*trans*-*p*-coumaroyloxy-2*α*-hydroxyurs-12-en-28-oic acid. The structures of the new compounds were determined by spectral methods to be 3*β*-*cis*-*p*-coumaroyloxy-2*α*-hydroxyursa-12,20(30)-dien-28-oic acid, *cis*- and *trans*- 3*β*-caffeoyloxy-2*α*-hydroxyurs-12-en-28-oic acids.

INTRODUCTION

In a previous paper we have reported the isolation of aromadendane and lupane derivatives from the leaves of *Melaleuca leucadendron* L. [1]. In a continuation of the chemical studies on the same plant, the triterpenoids in the leaves have been investigated. Three novel ursane derivatives (**6**–**8**), along with five known triterpenoids (**1**–**5**), were identified by comparison of their spectral data with those in the literature. The present paper deals with the structural investigations of the three novel triterpenoid cinnamates.

RESULTS AND DISCUSSION

The ethyl acetate fraction of the acetone extract of the leaves of *M. leucadendron* was subjected to repeated chromatography (CC and HPLC) which led to the isolation of three novel pentacyclic triterpenoids (**6**–**8**), in addition to five known triterpenoids which were identified by comparison of their spectroscopic data with published values for the compounds themselves or their derivatives, as ursolaldehyde (**1**), ursolic acid (**2**), 2*α*-hydroxyursolic acid (**3**), 3*β*-*cis*-*p*-coumaroyloxy-2*α*-hydroxyurs-12-en-28-oic acid (**4**), 3*β*-*trans*-*p*-coumaroyloxy-2*α*-hydroxyurs-12-en-28-oic acid (**5**), respectively (Table 1).

The triterpenoid coumarate (**6**) was obtained as a mixture with the triterpenoid acids (**7**, **8**). This mixture was first methylated and then subjected to a high-performance liquid chromatography to yield **6a**, **7a** and **8a**.

Compound **6** was obtained as a dimethyl derivative **6a**. The accurate mass spectrum demonstrated the elemental composition $C_{41}H_{56}O_6$ for compound **6a** and showed significant fragments at *m*/*z* 260 and 201. The IR spectrum revealed the presence of a hydroxy group (3475 cm^{-1}). The ^1H NMR spectrum of **6a** showed five tertiary methyl signals, a secondary methyl, a *cis*-*p*-coumaroyloxy groups [*δ* 7.69 and 6.85 (AA′BB′ system), 6.89 and 5.89 (AB system, $J = 12.7$ Hz)] and two oxygenated methine protons [*δ* 3.81 (*m*), 4.55 (*d*, $J = 10$ Hz)]. The last signals were assigned to H-2, H-3, from the COSY-90 spectrum and were consistent with a *trans*-diequatorial (2*α*, 3*β*) configuration of two oxygen functions. The coumaroyl moiety was located at C-3 from the HMBC spectrum which showed that H-3 (*d*, *δ* 4.55, $J = 10.1$ Hz) was correlated to C-23 (*δ* 28.6), C-24 (*δ* 17.7) and C-1′ (*δ* 167.7). The ^{13}C NMR signals corresponding to rings B, C, D, E were similar to those of methyl 2*α*,3*β*-dihydroxyursa-12,20(30)-dien-28-oate [2], while signals at *δ* 152.8 (*s*) and 105.1 (*t*) showed the presence of an exomethylene group. ^1H NMR data of **6a** showed that a doublet methyl signal has been replaced by an exomethylene (*δ* 4.67 and 4.62, each *br s*) signal. Since the doublet signal of H-18 was observed at *δ* 2.34, a C-29 methyl group must be present in **6a**.

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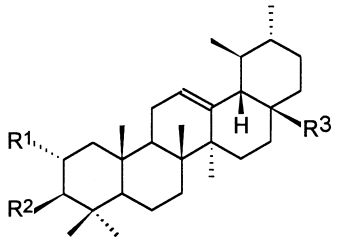
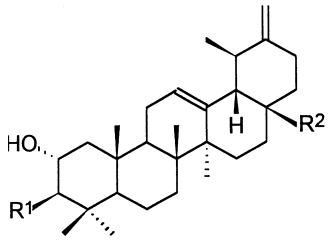


	R1	R2	R3
1	H	OH	CHO
2	H	OH	COOH
3	OH	OH	COOH
4	OH	*cis-p*-coumaroyloxy	COOH
5	OH	*trans-p*-coumaroyloxy	COOH
7	OH	*cis*-caffeoyloxy	COOH
7a	OH	di-*O*-methyl-*cis*-caffeoyloxy	COOMe
8	OH	*trans*-caffeoyloxy	COOH
8a	OH	di-*O*-methyl-*trans*-caffeoyloxy	COOMe



	R1	R2
6	cis-*p*-coumaroyloxy	COOH
6a	cis-*p*-*O*-methyl-coumaroyloxy	COOMe

Table 1. ^{1}H and ^{13}C NMR data of compound **6a** and ^{13}C NMR data for **7a**, **8a** (300 and 75 MHz, $CDCl_3$)

	6a δ (mult.)*		**7a** δ (mult.)*	**8a** δ (mult.)*		**6a** δ (mult.)*		**7a** δ (mult.)*	**8a** δ (mult.)*
No.	^{13}C	^{1}H	^{13}C	^{13}C	No.	^{13}C	^{1}H	^{13}C	^{13}C
1	47.9 *t*	1.06 *t* (12.5 Hz) 2.09 *dd* (12.5, 4.5 Hz)	47.8 *t*	47.9 *t*	22	38.7 *t*	1.61 *m* 1.79 *m*	36.6 *t*	36.4 *t*
2	67.6 *d*	3.81 *m*	67.5 *d*	67.3 *d*	23	28.6 *q*	0.88 *s*	28.6 *q*	28.5 *q*
3	84.8 *d*	4.55 *d* (10.1 Hz)	84.7 *d*	84.7 *d*	24	17.7 *q*	0.81 *s*	17.6 *q*	17.7 *q*
4	39.4 *s*		39.5 *s*	39.4 *s*	25	16.2 *q*	0.98 *s*	17.0 *q*	16.7 *q*
5	55.2 *d*	0.92 *m*	55.1 *d*	55.0 *d*	26	16.9 *q*	0.72 *s*	16.9 *q*	16.9 *q*
6	18.3 *t*	1.38 *m* 1.53 *m*	18.3 *t*	18.2 *t*	27	23.5 *q*	1.12 *s*	23.5 *q*	23.5 *q*
7	32.7 *t*	1.34 *m* 1.50 *m*	32.8 *t*	32.6 *t*	28	177.3 *s*		178.0 *s*	177.8 *s*
8	39.5 *s*		39.4 *s*	39.4 *s*	29	16.6 *q*	0.99 *d* (6.3 Hz)	16.6 *q*	16.5 *q*
9	47.5 *d*	1.59 *m*	47.5 *d*	47.3 *d*	30	105.1 106.0 *t*	4.62 *br s* 4.67 *br s*	21.1 *q*	21.0 *q*
10	38.1 *s*		38.1 *s*	38.0 *s*	1′	167.6 *s*		167.5 *s*	168.1 *s*
11	23.4 *t*	1.94 *dd* (8.8, 3.5 Hz)	23.3 *t*	23.2 *t*	2′	117.0 *d*	5.89 *d* (12.7 Hz)	117.0 *d*	115.5 *d*
12	125.7 *d*	5.26 *t* (3.4 Hz)	125.2 *d*	125.1 *d*	3′	144.2 *d*	6.89 *d* (12.7 Hz)	144.1 *d*	145.0 *d*
13	137.9 *s*		138.2 *s*	138.1 *s*	1″	127.4 *s*		127.6 *s*	127.2 *s*
14	42.1 *s*		42.0 *s*	41.9 *s*	2″	132.2 *d*	7.69 *d* (8.7 Hz)	113.2 *d*	109.4 *d*
15	27.9 *t*	1.10 *m* 1.78 *m*	27.9 *t*	27.8 *t*	3″	113.4 *d*	6.85 *d* (8.7 Hz)	148.2 *s*	149.0 *s*
16	24.3 *t*	1.76 *m* 2.17 *m*	24.1 *t*	24.0 *t*	4″	160.9 *s*		150.1 *s*	151.0 *s*
17	48.2 *t*		48.0 *s*	47.6 *s*	5″	113.4 *d*	6.85 *d* (8.7 Hz)	110.2 *d*	110.8 *d*
18	54.8 *d*	2.34 *br s*	52.8 *d*	52.6 *d*	6″	132.2 *d*	7.69 *d* (8.7 Hz)	124.8 *d*	122.6 *d*
19	37.3 *d*	2.32 *m*	39.0 *d*	38.9 *d*	OMe	51.6 *s*	3.58 *s*	51.4 *s*	51.3 *s*
20	152.8 *s*		38.8 *d*	38.7 *d*	OMe	55.3 *s*	3.80 *s*	55.8 *s*	55.7 *s*
21	32.2 *t*	2.20 *m* 2.27 *m*	30.6 *t*	30.5 *t*	OMe			55.8 *s*	55.8 *s*

* Signals without multiplicity assigned were picked up from HMQC.

This suggested that **6a** had a 20(30)-ene structure. Thus, the structure of **6a** was determined to be methyl 3*β*-*cis*-*p*-*O*-methylcoumaroyloxy-2*α*-hydroxyursa-12,20(30)-dien-28-oate.

Compound **7a** was obtained as a trimethyl derivative (**7**). The EI mass spectral data of **7a** indicated a molecular ion peak at *m*/*z* 676. The 42 carbon signals observed in the ^{13}C NMR spectrum were characterized by a DEPT experiment, which indicated that **7a** has seven methyls, eight methylenes, thirteen methines and eleven quaternary carbons. Furthermore, the chemical shifts of two of the methine carbon signals (δ 67.5 and 84.7) suggested the presence of two carbons bearing oxygen in addition to signals at δ 51.4 and 55.8 ($\times 2$) which showed the presence of three methoxyl groups. From these data, the empirical formula, $C_{42}H_{60}O_7$, was deduced. In the ^{1}H NMR spectrum, the appearance of five signals δ 5.89 (*d*, $J = 12.8$ Hz), 6.80 (*d*, $J = 8.4$ Hz), 6.83 (*d*, $J = 12.8$ Hz), 7.17 (*dd*, $J = 8.2$, 1.8 Hz), 7.66 (*d*, $J = 1.8$ Hz) suggested a *cis*-caffeoyl group. The carbon signals of the rest of the molecule were similar to those of compound **3a** (methylation of **3**). Thus compound **7a** was proposed to be methyl 3*β*-*cis*-di-*O*-methylcaffeoyloxy-2*α*-hydroxyurs-12-en-28-oate.

The general spectral features of compound **8a**, $C_{42}H_{60}O_7$, mp 136–138°, $[\alpha]_D^{25}$ +5.6 ($c = 0.35$, MeOH), molecular ion peak at *m*/*z* 676, closely resembled those of **7a** expect for the signals of the caffeoyl group in the ^{1}H NMR and ^{13}C NMR spectra (Table 1). In the ^{1}H NMR spectrum of **8a**, *trans*-conjugated olefinic protons (δ 6.29 and 7.58, each *d*, $J = 15.9$ Hz) were apparent in contrast to the analogous *cis*-conjugated signals (δ 5.89 and 6.83, each *d*, $J = 12.8$ Hz) observed in the ^{1}H NMR spectrum of **7a**. Therefore, compound **8a** was assigned the structure methyl 3*β*-*trans*-di-*O*-methylcaffeoyloxy-2*α*-hydroxyurs-12-en-28-oate.

Since related compounds from *Pyracantha crenulata* were found to possess potent anti-inflammatory activity [5], the biological activities of these three compounds are of interest and will be further investigated.

EXPERIMENTAL

Plant material

The leaves (10 kg) of *M. leucadendron* were collected at the campus of the National Taiwan University in July 1994 and was identified by Shing-Fan Huang, a technician of the Department of Botany of the National Taiwan University. Voucher specimens are deposited at the Department of Chemistry, National Taiwan University (C.C.M.T., accession #8307).

Extraction and isolation

The fresh leaves were extracted exhaustively with cold Me_2CO (70 l $\times$ 3). The extract was concd *in vacuo* and the filtrate was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble portion was concd and chromatographed on a silica gel column by elution with increasing polar mixture of EtOAc–hexane. The appropriate frs were combined and further separated or purified by HPLC to give **1** (4 mg), **2** (26 mg), **3** (15 mg), **4** (21 mg), **5** (7 mg), **6** (3 mg), **7** (12 mg) and **8** (10 mg). The known compounds **2** and **3** were identified by direct comparison with authentic samples. The other known compounds **1** [3], **4** [4] and **5** [4] were identified by comparison with published spectral data.

Methyl 3*β*-*cis*-*p*-O-*methylcoumaroyloxy*-2*α*-*hydroxyursa*-12,20(30)-*dien*-28-*oate* (**6a**). Amorphous powder, mp 119–121° (MeOH). $[\alpha]_D^{25}$ +59.2 (MeOH, *c* 0.03). IR ν_{max}^{neat} cm^{-1}: 3475, 2924, 2851, 1713, 1652, 1512, 1255, 1163, 1022, 753. UV λ_{max}^{MeOH} (log ε): 305 (4.04). MS *m*/*z* (rel. int.): 644 [M]$^+$ (3), 626 (0.6), 585 (1), 483 (2), 448 (3), 407 (3), 260 (11), 201 (47), 161 (100). ^{1}H and ^{13}C NMR (δ $CDCl_3$): Table 1.

Methyl 3*β*-*cis*-*di*-O-*methylcoumaroyloxy*-2*α*-*hydroxyurs*-12-*en*-28-*oate* (**7a**). Amorphous powder, mp 115.5–117.5° (MeOH). $[\alpha]_D^{25}$ +13.0 (MeOH, *c* 0.62). IR ν_{max}^{neat} cm^{-1}: 3511, 2948, 2872, 1720, 1515, 1455, 1261, 1174, 1143, 1027, 912, 732. UV λ_{max}^{MeOH} (log ε): 233 (3.66), 288 (3.63), 317 (3.68). MS *m*/*z* (rel. int.): 676 [M]$^+$ (4), 648 (1), 450 (1), 414 (17), 262 (16), 208 (30), 203 (42), 191 (100). ^{1}H NMR (500 Hz, $CDCl_3$): δ 0.71 (3H, *s*), 0.82 (3H, *s*), 0.83 (3H, *d*, 6.5 Hz), 0.88 (3H, *s*), 0.90 (3H, *d*, 5.0 Hz), 0.97 (3H, *s*), 1.05 (3H, *s*), 2.21 (1H, *d*, 11.2 Hz, H-18), 3.57 (3H, *s*, OMe), 3.88 (6H, *s*, OMe $\times$ 2), 4.55 (1H, *d*, 10.0 Hz, H-3), 5.22 (1H, *t*, 3.5 Hz, H-12), 5.89 (1H, *d*, 12.8 Hz, H-2′), 6.80 (1H, *d*, 8.4 Hz, H-5″), 6.83 (1H, *d*, 12.8 Hz, H-3′), 7.17 (1H, *dd*, 8.2, 1.8 Hz, H-6″), 7.66 (1H, *d*, 1.8 Hz, H-2″). ^{13}C NMR: Table 1.

Methyl 3*β*-*trans*-*di*-O-*methylcaffeoyloxy*-2*α*-*hydroxyurs*-12-*en*-28-*oate* (**8a**). Amorphous powder, IR ν_{max}^{neat} cm^{-1}: 3503, 2947, 2872, 1720, 1599, 1513, 1261, 1159, 1140, 1024, 756. UV λ_{max}^{MeOH} (log ε): 234 (3.55), 294 (3.62), 320 (3.69). MS *m*/*z* (rel. int.): 676 [M]$^+$ (4), 616 (1), 468 (1), 414 (35), 262 (24), 208 (42), 203 (34), 191 (100). ^{1}H NMR (300 Hz, $CDCl_3$): δ 0.69 (3H, *s*), 0.82 (3H, *s*), 0.83 (3H, *d*, 6.5 Hz), 0.88 (3H, *s*), 0.90 (3H, *d*, 5.0 Hz), 0.97 (3H, *s*), 1.05 (3H, *s*), 2.17 (1H, *d*, 11.1 Hz, H-18), 3.54 (3H, *s*, OMe), 3.70 (1H, *m*, H-2), 3.83 (6H, *s*, OMe $\times$ 2), 4.59 (1H, *d*, 9.9 Hz, H-3), 5.19 (1H, *br s*, H-12), 6.29 (1H, *d*, 15.9 Hz, H-2′), 6.78 (1H, *d*, 8.2 Hz, H-5″), 6.98 (1H, *br s*, H-3″), 7.01 (1H, *br d*, 8.2 Hz, H-6″), 7.58 (1H, *d*, 15.9 Hz, H-3′). ^{13}C NMR: Table 1.

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