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Cytotoxic clerodane diterpene esters from *Laetia corymbulosa*

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Abstract

Three cytotoxic clerodane diterpene esters, corymbulosins A–C, were isolated from an organic extract of the fruit of *Laetia corymbulosa* (Flacourtiaceae) from Peru. The structures were determined by spectroscopic methods as clerodane diterpenes unsaturated at C-3, C-13(16) and C-14. Corymbulosin A was esterified at C–2 with a decadienoate moiety, while corymbulosins B and C were C-2 epimers esterified at C-6 with a decanoate moiety.

Keywords: *Laetia corymbulosa*; Flacourtiaceae; Diterpenoids; Corymbulosin A

1. Introduction

The organic extract of the fruit of *Laetia corymbulosa* Spruce ex Benth. (Flacourtiaceae) was selected for investigation based on modest in vivo activity in the NCI hollow fiber model (Hollingshead et al., 1995). This genus, and several others in the Flacourtiaceae (*Casearia*, *Zuelania*), reportedly contain clerodane or kolovane diterpene esters (Khan et al., 1990; Gibbons et al., 1996), some of which had either cytotoxic (Itokawa et al., 1990), insect antifeedant (Chen and Wiemer, 1991) or LFA-1/ICAM binding inhibitory activities (Hunter et al., 1997).

2. Results and discussion

Partitioning of the crude extract by diol batch elution yielded a cytotoxic CH_2Cl_2 fraction. Cytotoxicity bioassay-guided fractionation of this fraction using gel permeation over Sephadex LH-20, followed by normal phase HPLC on cyano and diol bonded phases, yielded three major metabolites, corymbulosins A, B and C (Scheme 1).

HRFABMS of corymbulosin A (**1**) gave an [M + Cs] adduct ion at 717.2401, corresponding to a molecular formula of $C_{34}H_{48}O_8$. The presence of three carbonyl resonances in the ^{13}C NMR spectrum could be accounted for by two acetates and an unsaturated fatty acid ester, which had to be C_{10} if the core structure was an intact diterpene. The two oxygenated methine carbon signals at δ 96.6 and 99.4 were consistent with a diacetal structure, while two oxymethine signals at δ 72.1 and 74.7 were also present. Two separate diene systems and a single isolated double bond were evident from the NMR spectral data. The first diene system was located in the diterpene side chain based on the occurrence of two pairs of *exo*-methylene protons, with resonances at δ 4.91 and 5.00 and at δ 5.04 and 5.29. A fifth proton at δ 6.45 was coupled to both protons of the latter pair. Data from nOe, COSY and HMBC experiments established the system as a *cis*-diene. The second diene system displayed HMBC correlations to both the carbonyl at δ 167.6 as well as to the resonances of the alkyl chain of the fatty acid, establishing its location at positions 2′–5′ in the fatty acid ester. The single unsaturation was placed at C-3–4 based on HMBC correlations between the proton at δ 5.92 and the acetal carbon at δ 96.6.

A combination of gradient HSQC and HMBC experiments thus permitted the assignment of a clerodane skeleton substituted by a decadienoate fatty acid and two acetates. The oxymethine carbon at δ 74.7 could be assigned to C-6 from HMBC correlations between C-6 and H-19 and H-10. H-19 was likewise correlated to the acetate carbonyl at δ 171.5, while H-18

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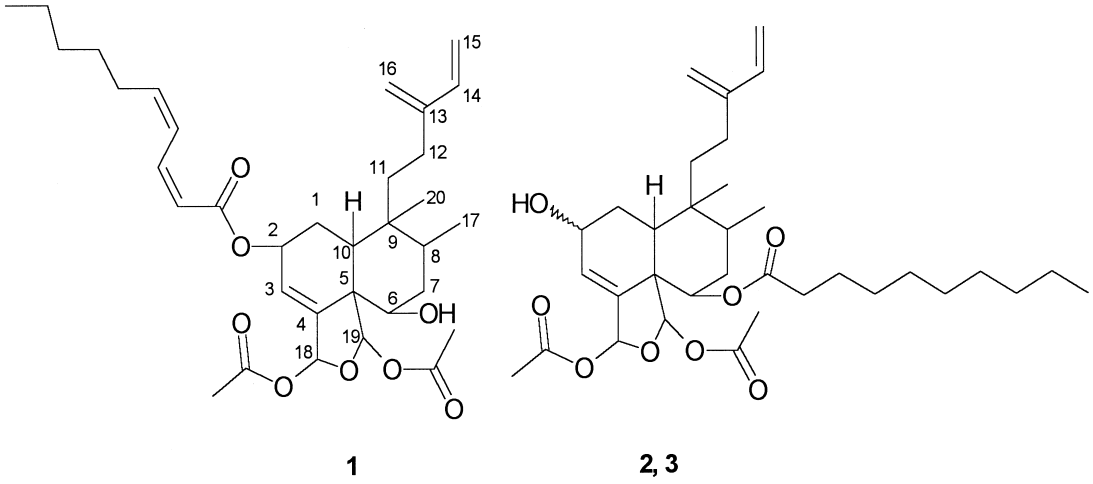

Scheme 1. Structures of corymbulosins A–C.

was correlated to the other acetate carbonyl at δ 171.9. The lack of an HMBC correlation between either oxymethine signal and the fatty acid carbonyl (δ 167.6 ppm) was problematic, however the substantial downfield shift for H-2 compared to H-6 (δ 5.56 vs. 3.97) pointed to C-2 as the site of esterification.

Corymbulosin B (**2**) had a formula of $C_{34}H_{52}O_8$ by HRFABMS ([M+Cs] 721.2745), indicating that it lacked two of the unsaturations of **1**. NMR spectral analysis indicated that the fatty acid ester substituent in **2** was a saturated caproic acid. The esterification site was determined by an HMBC correlation from H-6 (δ

Table 1
^{1}H NMR spectral data for corymbulosins A (d_4-MeOH, 500 MHz, J in brackets), B and C ($CDCl_3$)

	1	2	3
1a	1.78 *m*	1.97 *m*	1.67 *m*
1b	2.22 *m*		2.19 *m*
2	5.56 *brt* (7.5)	4.38 *t* (4.7)	4.42 *t* (8.1)
3	5.92 *brs*	5.95 *d* (3.4)	5.91 *s*
6	3.97 *dd* (4.2, 11.8)	4.93 *dd* (5.5, 10.7)	5.13 *d* (5.1)
7a	1.67 *m*	1.64 *m*	1.67 *m*
7b	1.73 *m*		
8	1.89 *m*	1.85 *m*	1.91 *m*
10	2.39 *dd* (13.9, 2.8)	2.30 *m*	2.30 *m*
11a	1.27 *m*	1.48 *m*	1.17 *m*
11b	1.50 *m*		1.45 *m*
12	2.13 *m*	2.06 *t* (7.8)	2.04 *m*
14	6.45 *dd* (11.0, 17.5)	6.38 *dd* (10.8,17.6)	6.39 *dd* (10.8, 17.5)
15a	5.04 *d* (10.8)	4.98 *d* (10.8)	4.98 *d* (17.5)
15b	5.29 *d* (17.5)	5.16 *d* (17.6)	5.19 *d* (10.8)
16a	4.91 *s*	4.89 *s*	4.89 *s*
16b	5.00 *s*	5.00 *s*	5.01 *s*
17	0.94 3H *d* (6.8)	0.87 3H *d* (6.8)	0.88 3H *d* (6.9)
18	6.62 *s*	6.45 *t* (1.5)	6.40 *t* (1.3)
19	6.39 *s*	6.49 *s*	6.43 *s*
20	0.98 3H *s*	0.94 3H *s*	0.94 3H *s*
2′	5.69 *d* (11.8)	1.58 *m*	1.57 *m*
3′	7.07 *dt* (11.6, 1.1)	2.25 *m*	2.27 *m*
4′	7.27 *t* (12.0)	1.20–1.26 *br*	1.20–1.26 *br*
5′	5.96 *d* (8.4)	1.20–1.26 *br*	1.20–1.26 *br*
6′	2.30 *dq* (1.3, 7.8)	1.20–1.26 *br*	1.20–1.26 *br*
7′	1.44 *m*	1.20–1.26 *br*	1.20–1.26 *br*
8′	1.33 *m*	1.19 m	1.20 *m*
9′	1.33 *m*	1.22 m	1.20 *m*
10′	0.91 *t*	0.83 3H *t*	0.83 3H *t* (6.9)
18-OAc Me	2.05 3H *s*	2.01 3H *s*	2.01 3H *s*
19-OAc Me	1.86 3H *s*	1.85 3H *s*	1.85 3H *s*

4.93) to the ester carbonyl at δ 173.2, and by the location of the H-6 proton signal downfield of the H-2 signal (δ 4.38).

Corymbulosin C (**3**) was identical in molecular formula to **2**, however its optical rotation was quite different (-51°) compared to **2** ($+0.7^\circ$). The ^{13}C NMR spectrum of **3** primarily differed from **2** in the shifts assigned to C-2, C-3, and C-10, consistent with **3** being the C-2 epimer of **2**.

Neither the relative nor the absolute stereochemistry of the corymbulosins could be definitively assigned, despite several experimental efforts with that goal. Gradient difference 1-D NOESY studies of **1** gave ambiguous results as to relative stereochemistry (Table 3).

An H-3 coupling of 3.4 Hz for **2**, contrasting with the lack of an observable coupling constant for H-3 in **3**, suggested that H-2 was equatorial in **2** and axial in **3**. A transaxial NOE was detected between H-10 and H-2 for **3**, but not **2**, supporting the identification of **2** and **3** as C-2 epimers. No NOE was detected between H-10 and H-6 for either compound. The optical rotations of the corymbulosins A and C were negative, in contrast with the large positive rotations measured for the casearborins and casearins (Itokawa et al., 1990; Beutler et al., 2000). Preparation of a crystal for X-ray studies was precluded by the tendency of the corymbulosins to precipitate from solution as oils, in contrast to the casearborins (Beutler et al., 2000), which readily crystallized.

The corymbulosins share common structural features with previously reported diterpenes from the Flacourtiaceae. Corymbulosin A (**1**) is isomeric with pitumbin (Guittet et al., 1988), a diterpene isolated from *Casearia pitumba* which was reported to be a C-6 decadienoate ester. A comparison of NMR spectra data for **1** in d_6-acetone with the literature showed that the compounds are not identical. In particular, the C-2 ^{13}C NMR signal occurs at δ 64 in pitumbin, whereas for **1** it is at δ 71, both measured in the same solvent.

Corymbulosin A (**1**) was the most cytotoxic of these three compounds, with IC_{50} values ranging from 0.6 μM in SF539 human CNS tumor cells to 8 μM in the LOX melanoma cell line in 2-day cytotoxicity tests. Compounds **2** and **3** were approximately 10-fold less potent. The mechanism of cytotoxicity of the clerodane acetal

Table 2
^{13}C NMR spectral data for corymbulosins A (d_4-MeOH, 125 MHz), B and C ($CDCL_3$)

	1	2	3
1	27.6 *t*	29.4 *t*	30.4 *t*
2	72.1 *d*	63.7 *d*	68.3 *d*
3	124.9 *d*	126.4 *d*	129.3 *d*
4	146.1 *s*	142.3 *s*	141.4 *s*
5	55.1 *s*	52.1 *s*	52.3 *s*
6	74.7 *d*	73.7 *d*	74.9 *d*
7	38.1 *t*	33.0 *t*	33.3 *t*
8	38.5 *d*	36.6 *d*	36.8 *d*
9	39.3 *s*	37.3 *s*	37.9 *s*
10	42.6 *d*	36.1 *d*	42.1 *d*
11	29.0 *t*	27.9 *t*	27.4 *t*
12	25.1 *t*	23.7 *t*	23.7 *t*
13	147.1 *s*	145.1 *s*	145.0 *s*
14	141.5 *d*	140.2 *d*	140.2 *d*
15	113.2 *t*	112.5 *t*	112.4 *t*
16	115.5 *t*	115.3 *t*	115.3 *t*
17	16. *q*	15.5 *q*	15.5 *q*
18	96.6 *d*	95.2 *d*	94.6 *d*
19	99.4 *d*	98.2 *d*	97.7 *d*
20	25.8 *q*	25.4 *q*	25.5 *q*
1′	167.1 *s*	173.2 *s*	173.4 *s*
2′	117.8 *d*	24.7 *t*	24.7 *t*
3′	140.9 *d*	34.6 *t*	34.7 *t*
4′	125.6 *d*	29.2 *t*[a]	29.2 *t*[a]
5′	143.1 *d*	29.2 *t*[a]	29.2 *t*[a]
6′	28.3 *t*	29.2 *t*[a]	29.2 *t*[a]
7′	30.2 *t*	29.0 *t*	29.2 *t*[a]
8′	32.5 *t*	31.7 *t*	31.8 *t*
9′	23.5 *t*	22.6 *t*	22.6 *t*
10′	14.4 *q*	14.0 *q*	14.0 *q*
18-OAc Me	21.1 *q*	21.2 *q*	21.1 *q*
18-OAc C=O	171.9 *s*	170.0 *s*	169.8 *s*
19-OAc Me	21.9 *q*	21.6 *q*	21.6 *q*
19-OAc C=O	171.5 *s*	169.9 *s*	169.8 *s*

[a] Resonances in vertical columns may be interchanged.

Table 3
Gradient 1-D NOESY difference data for **1** (d_4-MeOH)

Proton	NOE to protons
H-1a	None
H-1b	None
H-2	H-1b, H-10, H-5′
H-3	H-2, H10, H-18
H-6	H-1a, H-7a, H-8
H-7a	None
H-7b	None
H-8	None
H-10	H-2, H-12, H-19, H-20, 19-OAc
H-11a	None
H-11b	None
H-12	None
H-14	H-15a, 19-OAc
H-15a	H-14, H-15b, H-16a, 19-OAc
H-15b	H-12, H-15a
H-16a	H-11b, H-14(w), H-16b, 19-OAc
H-16b	H-14, H-15b, H-16a, 19-OAc
H-17	H-1b, H-8, H-11b, H-12
H-18	H-3
H-19	H-7b, H-15a, H-18
H-20	H-1b, H-8, H-10, H-12
H-2′	H-3′
H-3′	H-2′, H-6′
H-4′	H-5′
H-5′	H-10, H-4′, H-7′, H-8′/9′
H-6′	H-3′, H-4′, H-7′, H-8′/9′
H-7′	None
H-8′/9′	None
H-10′	None
18-OAc	None
19-OAc	H-6, H-12, H-15b, H-19

diterpenes remains unknown. The masked dialdehyde may generate a reactive intermediate capable of damaging cellular constituents, however the observed structure–cytotoxicity variations among corymbulosins, casearborins and casearins suggest that other mechanisms may also be involved.

3. Experimental

Fruits of *Laetia corymbulosa* Spruce ex Benth. were collected from the municipality of Sapue, Department of Loreto, Province of Requena, Peru by Douglas C. Daly on 16 February 1988 and identified by the collector. A voucher specimen is on deposit in the Botany Department, Museum of Natural History, Smithsonian Institution, voucher Daly 5658 (NCI accession no. Q65T0388). A sample (127 g) of dried, ground fruit was percolated with CH_2Cl_2–MeOH (1:1 v/v) overnight at room temperature, drained, and rinsed with MeOH. The combined solvent extracts were evaporated to yield a crude extract (20 g). This extract (5 g) was coated on flash grade diol media and eluted successively with hexane, CH_2Cl_2, and MeOH (1 L). The CH_2Cl_2 eluate (2.2 g) was evaporated and permeated on Sephadex LH-20 in CH_2Cl_2–MeOH (1:1, v/v) to yield three fractions, the second of which was evaporated to yield an oily solid (1.3 g). HPLC on a cyano bonded phase column using a gradient of *iso*-PrOH in hexane (10–50% *iso*-PrOH) yielded pure **1** (180 mg), as well as a mixture of **2** and **3**. This mixture was resolved by HPLC on a diol column using a gradient of *iso*-PrOH in hexane (5–20% *iso*-PrOH) to give **2** (100 mg) and **3** (73 mg) in pure form.

Corymbulosin A (**1**), NSC#705695, oily solid, $[\alpha]_D$ -111° ($c=1.0$, $CHCl_3$); UV $\lambda_{max}^{CH_3CN}$ 265 nm (log ϵ 4.26); IR ν_{max} cm^{-1}: 3522, 2929, 1757, 1712, 1630, 1595, 1442, 1372, 1227, 1168; HRFABMS m/z 717.2401 (M + Cs) obs, calcd for $C_{34}H_{48}O_8Cs$, 717.2404. ^1H NMR, see Table 1. ^{13}C NMR, see Table 2.

Corymbulosin B (**2**), NSC#705696, $[\alpha]_D$ $+0.7^\circ$ ($c=1.0$, $CHCl_3$); UV $\lambda_{max}^{iso\text{-}PrOH}$ 222 nm (log ϵ 4.11); IR ν_{max} cm^{-1}: 3487, 2929, 1745, 1601, 1455, 1372, 1225, 1105; HRFABMS m/z 721.2745 (M + Cs) obs, calcd for $C_{34}H_{52}O_8Cs$ 721.2717. ^1H NMR, see Table 1. ^{13}C NMR, see Table 2.

Corymbulosin C (**3**) NSC#705697, $[\alpha]_D$ -51° ($c=1.0$, $CHCl_3$); UV $\lambda_{max}^{iso\text{-}PrOH}$ 222 nm (log ϵ 4.13); IR ν_{max} cm^{-1}:3466, 2929, 2354, 1744, 1456, 1373, 1223, 1107; HRFABMS m/z 721.2736 obs, calcd for $C_{34}H_{52}O_8Cs$ 721.2717. For ^1H NMR and ^{13}C NMR spectral analysis, see Tables 1 and 2, respectively.

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