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Note

Esculentins A & B, two new diterpenes from *Casearia esculenta*

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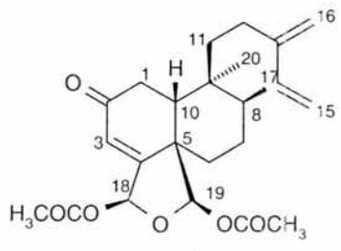
The structures of esculentins A and B, isolated from the twigs of *Casearia esculenta* (*Samydaceae*), have been elucidated. They are found to be 18β,19β-diacetyloxy-18α,19α-epoxy-3,13(16),14-clerodatrien-2-one **1** and 18β,19β-diacetyloxy-18α,19α-epoxy-3,12,14-clerodatrien-2β-isovaleryloxy-6β,7α-diol **2** respectively by the analysis of 1D- and 2D-NMR spectra. Both **1** and **2** show weak cytokine inhibition activity.

In our screening program for biologically active compounds, two new diterpenes esculentin A **1** and esculentin B **2** from the twigs of *Casearia esculenta* (*Samydaceae*) were isolated. The isolation and biological properties were reported earlier[1]. Herein, we report the structure elucidation of **1** and **2** by spectral analysis.



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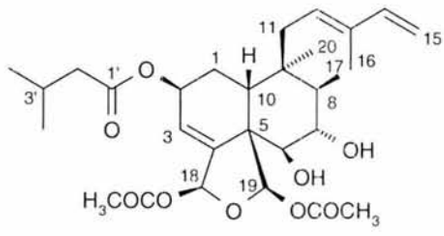

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Results and Discussion

Esculentin A 1. ESI-MS of **1** gave a molecular ion peak at 417 $(M+H)^+$. The IR bands at 1755 (broad) and 1670 cm^{-1} indicated the presence of ester and α,β-unsaturated carbonyl groups respectively in **1**. The ^1H and ^{13}C NMR spectral data of esculentin A **1** are summarized in **Table I**. The ^1H NMR spectrum of **1** showed the presence of four methyl groups, two of which are acetyl groups. The ^{13}C NMR spectrum together with DEPT-135 indicated the presence of seven quaternary carbons [3 × CO, 2×=C, 2×C], six methines [2×=CH, 2×OCHO, 2×CH], seven methylenes [2×=CH_2, 5×CH_2] and four methyls [2×$COCH_3$, 2×CH_3] accounting for 24 carbons. From the foregoing spectral data and elemental analysis, the molecular formula of **1** was determined to be $C_{24}H_{32}O_6$.

The molecular formula of **1** required nine degrees of unsaturation, out of which six were accounted for by three carbonyls and three double bonds, suggesting that esculentin A **1** was a tricyclic compound. Three isolated spin systems *viz.* -CH_2-CH_2-C(=CH_2)-CH=CH_2 (**A**), -CH-CH_2- (**B**) (an ABX system) and -CH_2-CH_2-CH-CH_3 (**C**) were derived from DQF HH COSY spectrum of **1**. The spectral properties were very similar to those reported for 2-oxo-clerodane type of diterpenes[2-5]. The relatively downfield shift of H_2-1 (δ 2.49, 2.56) and the absence of protons at C-2 suggested the presence of an oxo group at C-2. A comparison of the spectral data of **1** with those reported for the related diterpenes[2-5] and the presence of the spin systems **A-C** led to structure **1** for esculentin A.

The relative stereochemistry of the chiral centres in **1** was established by the analysis of 2D NOESY spectrum and coupling constants as well. NOE correlations were observed between H-7, H-11 and H-19, which could be best accommodated by a *cis* A/B ring junction, an αH-19 and an α-equatorial methyl group/β-axial side chain at C-9. This was further supported by the downfield chemical shift of CH_3-20 in the ^{13}C NMR spectrum of **1** (δ_C 25.92)[2], besides an NOE interaction between CH_3-20 and H-1. H-18 showed a weak allylic coupling of about 1 Hz with H-3 requiring an α orientation for H-18. H-10 required an axial configuration as was manifested from the large coupling constant (11.6 Hz) and also

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Table I—^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectral data of esculentin A **1** and esculentin B **2** in $CDCl_3$.

Position	Esculentin A		Esculentin B	
	δ_C [a]	δ_H [b]	δ_C [a]	δ_H [b]
1	36.16 (t)	2.56 (m	27.57 (t)	1.95 (m)
		2.49 (dd, 14.6, 4.8 Hz)		
2	199.86 (s)	-	67.01 (d)	5.46 (bd, 2.4 Hz)
3	124.62 (d)	6.07 (bs)	122.71 (d)	6.06 (d, 2.4 Hz)
4	145.78 (s)	-	145.30 (s)	-
5	51.16 (s)	-	53.51 (s)	-
6	30.64 (t)	1.86 (m)	77.39 (d)	3.55 (d, 10.9 Hz)
		1.70 (m)		
7	28.38 (t)	1.65 (m)	73.94 (d)	3.57 (t, 10.9 Hz)
		1.57 (m)		
8	37.41 (t)	1.76 (m)	37.11 (d)	1.65 (m)
9	38.47 (s)	-	39.68 (s)	-
10	39.84 (d)	2.64 (dd, 11.6, 4.8 Hz)	43.13 (d)	2.37 (m)
11	27.76 (t)	1.52 (m)	32.32 (t)	2.41 (m)
		1.25 (m)		1.62 (m)
12	24.39 (t)	2.07 (m)	129.69 (d)	5.37 (d, 6.1 Hz)
13	145.78 (s)	-	136.48 (s)	-
14	140.90 (d)	6.43 (dd, 17, 10.9 Hz)	141.95 (d)	6.28 (dd, 17.1, 10.9 Hz)
15	113.68 (t)	5.21 (d, 17 Hz)	111.90 (t)	5.11 (d, 17.1 Hz)
		5.03 (d, 10.9 Hz)		4.96 (d, 10.9 Hz)
16	116.06 (t)	5.04 (bs)	12.72 (q)	1.67 (s)
		4.92 (bs)		
17	16.33 (q)	0.93 (d, 7.3 Hz)	11.80 (q)	1.06 (d, 7.3 Hz)
18	94.42 (d)	6.83 (d, 1 Hz)	96.50 (d)	6.74 (s)
19	100.19 (d)	6.39 (s)	98.36 (d)	6.50 (s)
20	25.92 (q)	0.94 (s)	26.17 (q)	0.86 (s)
1'			173.31 (s)	-
2'			44.42 (t)	2.28 (d, 7.3 Hz)
3'			26.90 (d)	2.15 (m)
4'			23.18 (q)	1.02 (d, 7.3 Hz)
5'			23.12 (q)	1.03 (d, 7.3 Hz)
Ac-CO	170.38 (s)	-	171.23 (s)	-
	166.23 (s)	-	170.13 (s)	-
Ac-CH_3	22.05 (q)	1.91 (s)	22.37 (q)	1.93 (s)
	21.74 (q)	2.08 (s)	21.96 (q)	2.09 (s)
6-OH				2.82 (bs)
7-OH				2.76 (bs)

[a] The carbon multiplicities were determined by the analysis of DEPT-135 spectrum, the protonated carbons by the analysis of CH COSY experiment and the quaternary carbons by comparison with the related 2-oxo-clerodanes[3-6].
[b] The protons were assigned by the analysis of phase sensitive double quantum filtered HH COSY experiment.

NOE correlation between H-10 and H-1. CH_3-17 was assigned an equatorial configuration based on the observation of NOE interactions between CH_3-17 and H-11 and rather a weak correlation between H-1 and H-8. Thus, the structure of esculentin A (**1**) was eastiblished as 18β,19β-diacetyloxy-18α,19α-epoxy-3,13(16),14-clerodatrien-2-one.

Esculentin B 2. ESI-MS spectrum of **2** showed a molecular ion peak at 535 $(M+H)^+$. The IR spectrum of **2** showed bands at 3420, 1770 and 1750 cm^{-1} indicating the presence of hydroxyls and ester carbonyls respectively. The ^{1}H NMR spectral data (**Table I**) of **2** showed the presence of seven methyls including two acetyl groups and D_2O exchangeable

protons. The analysis of ^{13}C NMR and DEPT-135 spectra of **2** (**Table I**) indicated the presence of seven quaternary carbons [3×CO, 2×=C, 2×C], eleven methines [3×=CH, 2×OCHO, 3×OCH, 3×CH], four methylenes [1×=CH_2, 3×CH_2] and seven methyls [2×$COCH_3$, 1×=CCH_3, 4×CH_3] accounting for 29 carbons. The foregoing data in combination with elemental analysis gave a molecular formula of $C_{29}H_{42}O_9$ for **2**.

The analysis of DQF HH COSY spectrum of **2** gave four isolated spin systems *viz.* =CH-CH(OCO)-CH_2-CH- (**D**), -CH(OH)-CH(OH)-CH-CH_3 (**E**), -CH_2-CH-$(CH_3)_2$ (**F**) and -CH_2-CH=C(CH_3)-CH=CH_2 (**G**). As in the case of **1**, the spectral properties of **2** showed strong similarities to those reported for 2-oxo-clerodane type of diterpenes[2-5]. A comparison of the spectral data of **2** with those reported for the related diterpenes[2-5] and the presence of the spin systems **D-G** led to structure **2** for esculentin B.

The relative stereochemistry of the chiral centres was established by the analysis of 2D NOESY spectrum of **1** and also coupling constants. NOE correlations, observed between H-7, H-11 and H-19 and between CH_3-20 and H-1 and the downfield chemical shift of CH_3-20 in the ^{13}C NMR spectrum of **2** (δ_C 26.17)[2] suggested a *cis* A/B ring junction, αH-19 and an α-equatorial methyl group/β-axial side chain at C-9. H-18 showed a weak allylic coupling (1 Hz) with H-3 indicating an α orientation for H-18. A small coupling constant of 2.4 Hz for H-2 with H-1 suggested an equatorial orientation. A large coupling constant (J = 10.9 Hz) between H-6 and H-7 is due to diaxial coupling attributing equatorial configurations for the two hydroxyls at C-6 and C-7. The Δ^{12}-double bond was found to have *E*-configuration by the NOE correlation between H-12 and H-14 and was further substantiated by the upfield shift of CH_3-16 in the ^{13}C NMR spectrum (δ_C 12.17)[2]. NOE interactions observed for H-8 with H-1 and C-7 hydroxyl group required an axial configuration for H-8. Thus, the structure of esculentin B **2** was established as 18β,19β-diacetyloxy-18α,19α-epoxy-3,12,14-clerodatrien-2β-isovaleryloxy-6β,7α-diol.

Esculentin A **1** and Esculentin B **2** showed very weak cytokine inhibition activity at a concentration of 5000 ppm against LPS-stimulated release of IL-1 alpha and TNF-alpha in human mononuclear cells in Perper's model[6] of adjuvant arthritis.

Experimental Section

General. Melting points are uncorrected. UV spectra were recorded on a Jasco V 550 and IR spectra on a Perkin-Elmer 782 spectrophotometer. Optical rotations were measured on a Rudolph AP III 589 polarimeter. NMR spectra were recorded on a Bruker ACP 300 spectrometer using concentrations of 5 mg/ml and 10 mg/ml in $CDCl_3$ (chemical shifts in δ, ppm) and mass spectra on a VG BIO-Q spectrometer.

Plant material

The twigs of *Casearia esculenta* were collected around Mumbai, India. The identification of the plant species was as per the morphological details given in literature[7]. A voucher specimen of the species is preserved at the Herbarium of the Research Centre, Hoechst Marion Roussel Limited, Mulund (W), Mumbai 400 080, India.

Esculentin A 1: White crystalline powder; mp 176-78°C; $[\alpha]_D$-137.67° (*c* 0.32, $CHCl_3$); UV (MeOH): 224 nm; IR (KBr): 1755, 1670, 1380, 1225, 1070, 1030, 950 and 900 cm^{-1}; ESI-MS: *m/z* 417 $(M+H)^+$; Anal. Found: C 69.96, H 7.61. Calcd. for $C_{24}H_{32}O_6$: C 69.23, H 7.69.

Esculentin B 2: White crystalline powder; mp 145-47°C; $[\alpha]_D$ + 56.15° (*c* 0.32, $CHCl_3$); UV (MeOH): 232 nm; IR (KBr): 3420, 1770, 1750, 1390, 1250, 1090, 1045, 1015, 940 and 910 cm^{-1} ; ESI-MS: *m/z* 534 $(M+H)^+$; Anal. Found: C 65.71, H 7.79. Calcd. for $C_{29}H_{42}O_9$: C 65.16, H 7.86.

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