Four New Clerodane Diterpenes from the Leaves of *Casearia guianensis* Which Inhibit the Interaction of Leukocyte Function Antigen 1 with Intercellular Adhesion Molecule 1

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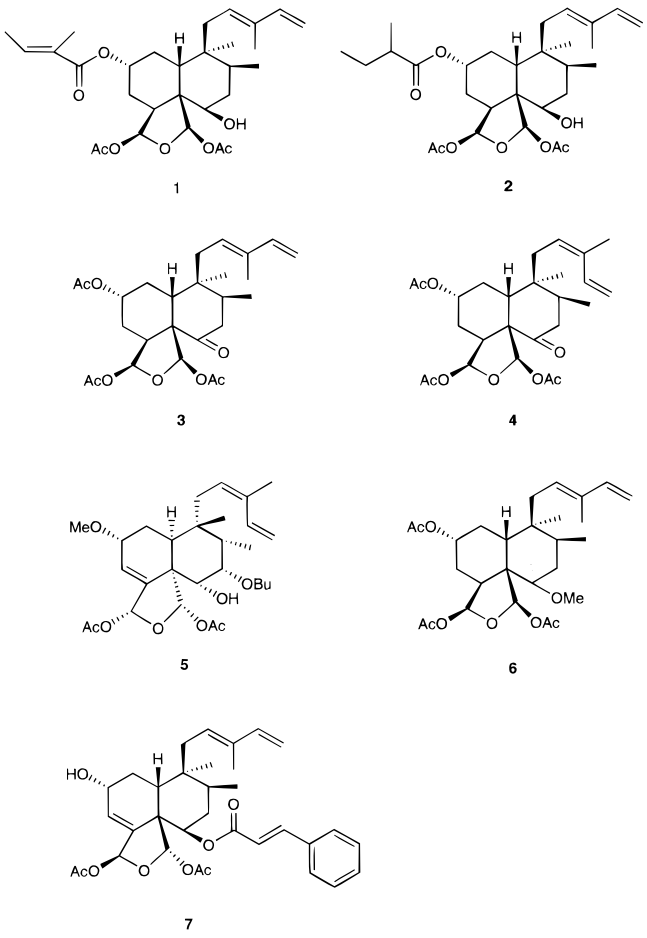
Four new clerodane diterpenes, casearinols A and B (**1** and **2**) and casearinones A and B (**3** and **4**), were isolated from the leaves of *Casearia guianensis*. These immunomodulatory compounds have been structurally elucidated primarily on the basis of 2D NMR analysis and spectral data comparison with known compounds. These compounds inhibited the binding of T-cell leukocyte function antigen 1 to intercellular adhesion molecule 1.

Activation of T-cells upon exposure to antigen requires the interaction of one or more stimulatory ligand/receptor pairs. T-cell leukocyte function antigen 1 (LFA-1) interaction with intercellular adhesion molecule 1 (ICAM-1) present on the surface of antigen presenting cells mediates adhesion and results in T-cell activation and proliferation.[1] Selective inhibition of LFA-1 binding to ICAM is a rational approach to finding immunosuppressive therapeutics for the treatment of transplant rejection.[2] A series of plant extracts was screened as part of an effort to identify small molecule antagonists of the LFA-1/ICAM interaction. Four new 18,19-diacetoxyclerodane 18,19-oxide acetals (**1**−**4**) were isolated from an extract of *Casearia guianensis* J. R. Johnston (Flacourtiaceae) that inhibited LFA-1 binding to ICAM-1. Comparison was made to other clerodane acetals, previously isolated from *C. sylvestris*,[3,4] *Zuelania guidonia*,[5,6] *C. corymbosa*,[7,8] *C. tremula*,[9] and *Laetia procera*.[10] In most of these cases, however, ring A contained a Δ^3 double bond, whereas the clerodane diterpenes reported herein (**1**−**4**) are all saturated in ring A. In this communication, we report the isolation and structure elucidation of these new clerodane diterpenes.



Results and Discussion

Leaves of *C. guianensis* were lyophilized and extracted with ethanol and were detannified by passage over DEAE-cellulose. The tannin-purified extract was chromatographed by reversed-phase HPLC, and fractions were tested for inhibition of LFA-1 binding to ICAM-1. Three regions of activity were identified. From fractions corresponding to one of these regions, **1** was isolated. Fractions corresponding to the other two regions were further purified on silica gel HPLC, from which **2**−**4** were isolated.

Casearinol A (**1**) was obtained as a colorless oil, which was shown to have molecular formula of $C_{29}H_{42}O_8$ by HRMS, suggesting nine degrees of unsaturation. Infrared analysis of **1** showed absorptions at 1752 and 1729 cm^{-1}, indicating the presence of ester functions. The structure was further elucidated by examination of the ^1H, ^{13}C, COSY, NOESY, HMQC, and HMBC NMR spectra (Table 1). The ^1H NMR spectrum indicated the presence of seven methyl groups, of which one was attached to a saturated carbon (δ 0.82), one was attached to a methine carbon (δ 0.89, J = 6.4 Hz), three were attached to unsaturated carbons (δ 1.63, 1.78, and 1.81), and two were part of acetate functions (δ 1.93 and 2.02). The carbonyls of the acetate groups were observed in the ^{13}C NMR spectrum (Table 1) at δ 169.5 and 169.8, together with another ester carbonyl at δ 167.9. The presence of a terminal unsaturated methylene was suggested by broad doublets at δ 4.93 and 5.08 in the ^1H NMR spectrum. This terminal methylene, along with COSY, HMQC, and HMBC correlations

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Table 1. NMR Data for Casearinol A (**1**) (500 MHz, $CDCl_3$)

position	δ ^{13}C	δ ^{1}H (mult, J = Hz)	COSY	HMBC (H to C)	NOESY
1	27.5	α, 1.29 (ddd, 14.0, 7.3, 14.3)	1*β*, 2, 10	2, 5, 6, 10	1*β*, 6
		β, 2.26 (ddd, 14.0, 9.3, 2.3)	1α, 2, 10	2, 5	1α, 2, 10, 20
2	70.0	5.31 (m)	1α, 1*β*, 3*β*	4, 1′	1*β*, 3*β*, 10
3	26.6	α, 1.65 (m)	3*β*, 4	2, 4, 5	3*β*, 4
		β, 2.09 (ddd, 5.7, 15.2, 14.6)	2, 3α, 4	2, 4, 5	2, 3α
4	43.8	2.69 (ddd, 4.0, 14.6, 7.2)	3α, 3*β*, 18	6	3α, 6, 18
5	54.6				
6	74.9	3.79 (dd, 3.7, 11.9)	7α, 7*β*	4, 5, 19	1α, 4, 7α
7	37.1	α, 1.72 (m)	7*β*	6, 8	6, 7*β*, 8
		β, 1.55 (m, 11.9)	7α, 8	4, 8	7α, 19
8	37.2	1.68 (m, 6.4)	7*β*, 17	6, 10	7α, 17
9	37.7				
10	37.8	2.10 (dd, 14.3, 2.3)	1α, 1*β*	1, 4, 6, 8, 9, 11	1*β*, 2, 12
11	30.4	a, 1.64 (m)	11b, 12	10, 12, 13	7*β*, 11b, 19
		b, 2.17 (dd, 16.6, 8.0)	11a, 12	10, 12, 13	11a, 17
12	129.0	5.34 (m)	11a, 11b	14	10, 11a, 14, 20
13	136.0				
14	141.3	6.31 (dd, 10.7, 17.2)	15a, 15b	13, 12	12, 15a
15	112.2	a, 4.93 (d, 10.7)	14	13	14, 15b
		b, 5.08 (d, 17.2)	14	13, 14	15a, 16
16	12.4	1.63 (s)		12, 13, 14	15b
17	15.6	0.89 (d, 6.4)	8	7, 9	8, 11b, 20 (w)
18	98.9	6.50 (d, 7.2)	4	4, 5, 19, CO	4
19	97.5	6.34 (s)		4, 5, 6,18, CO	7*β*, 11a
20	25.8	0.82 (s)		8, 9, 10, 11	1*β*, 12, 17 (w)
1′	167.9				
2′	128.9				
3′	137.7	6.82 (q, 6.91)	4′, 5′	1′, 4′, 5′	4′
4′	14.5	1.78 (bd, 6.91)	3′	2′, 3′	3′, 5′
5′	12.4	1.81 (s)		1′, 2′, 3′	4′
aCO	169.8				
aCH_3	21.2	2.02 (s)		aCO (Ac)	
bCO	169.5				
bCH_3	21.7	1.93 (s)		bCO (Ac)	

for the methylene group at δ 1.96 and 2.54, alkene protons at δ 5.30 and 6.61, and a singlet methyl group at δ 1.63 (Table 1) represents a six-carbon side chain and rationalized the observed UV absorption of a conjugated diene (λ max 224 nm, ϵ 13 800). The quaternary carbon at C-9 showed HMBC correlations to the C-20 methyl singlet at δ 0.82 and the C-17 methyl doublet at δ 0.89 and became the logical point of attachment for the diene side chain. An ester side chain was implied at C-2 by the chemical shift of the H-2 methine (δ 5.32) and the HMBC correlation to the C-1′ carbonyl at δ 167.9. The side chain is unsaturated with one olefinic proton (δ 6.82) and two methyl groups, one of which was coupled to the δ 6.82 proton and the other appeared as a broad singlet, which was HMBC-correlated to the ester carbonyl at δ 167.9 (Table 1). These data fit the tiglate structure as depicted in **1**. Comparison of the remaining core structure was made with known casearins,[3,4] e.g., **5**; corymbotins,[8] e.g., **6**; and zuelanins,[5−7] e.g., **7**. From the obtained spectral information in this investigation and these comparisons, structure **1** was proposed. The diacetal ring was confirmed in the HMBC experiment by cross-correlations between the H-18 acetal proton with C-19, and the H-19 acetal proton with C-18, as well as the HMBC correlations with each acetal proton and their corresponding acetate carbonyl (Table 1). The major structural difference between **1** and most of those reported earlier is that ring A is saturated. This difference was substantiated by COSY correlations observed between H-18 and H-4 and between H-4 and a methylene assigned as H-3α and H-3*β*. H-3*β* was further coupled to the tiglate ester H-2 methine at δ 5.31. This spin system continued from H-2 to both H-1 methylene protons (δ 1.29, 2.26) and terminated at the bridgehead

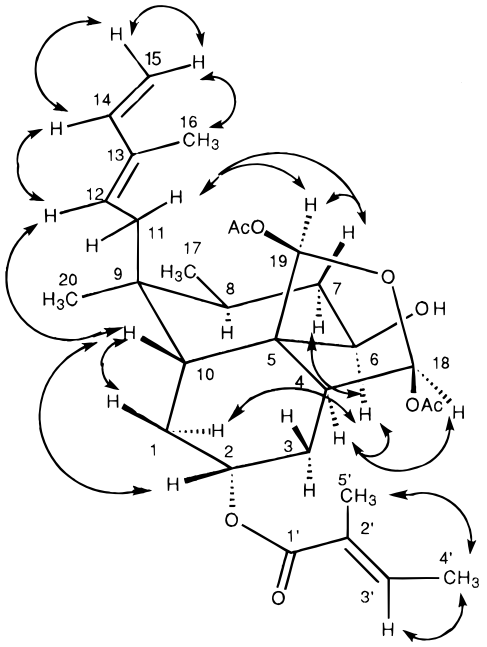

Figure 1. Selected NOESY correlations for **1**.

H-10 proton (δ 2.10). The A/B bridgehead position of this proton was confirmed by its HMBC correlations to C-1, C-4, C-6, C-8, C-9, and C-11. The remaining COSY spin system contained a hydroxyl-bearing H-6 methine at δ 3.79 coupled to the H-7 methylene at δ 1.72 and 1.55 which further coupled to the H-8 methine at δ 1.68 and terminated with the C-17 methyl doublet at δ 0.89.

The relative stereochemistry of **1** was assigned on the basis of coupling constants (Table 1) and NOESY data (Figure 1). The coupling of H-1α to H-10 was 14.3 Hz,

Table 2. NMR Data for Casearinol B (**2**) (500 MHz, $CDCl_3$)

position	δ ^{13}C	δ ^{1}H (mult, J = Hz)	COSY	HMBC (H to C)	NOESY
1	26.9	α, 1.25 (ddd, 14.0, 7.3, 14.5)	1*β*, 2, 10	2, 5, 10, 11	1*β*, 6
		β, 2.22 (ddd, 14.0, 9.2, 2.4)	1α, 2, 10	2, 3, 5, 10	1α, 2, 10, 20
2	69.5	5.26 (m)	1α, 1*β*, 3*β*	4, 1′	1*β*, 3*β*, 10
3	26.1	α, 1.57 (dd, 14.7, 3.7)	3*β*, 4	2, 4, 5	3*β*, 4
		β, 2.06 (ddd, 5.9, 14.7, 14.5)	2, 3α, 4	2, 4, 5	2, 3α
4	43.6	2.66 (ddd, 3.7, 14.5, 7.2)	3α, 3*β*, 18	6, 5 (w)	3α, 6, 18
5	54.5				
6	74.8	3.77 (dd, 3.8, 12.1)	7α, 7*β*	4, 5, 19	1α, 4, 7α
7	36.8	α, 1.72 (ddd, 3.8, 12.8, 3.1)	6, 7*β*, 8	4, 6, 9	6, 7*β*, 8
		β, 1.56 (m)	6, 7α	8	7α, 19
8	36.9	1.69 (m)	7*β*, 17	6, 10	7α, 17
9	37.3				
10	37.7	2.07 (dd, 14.5, 2.4)	1α, 1*β*	4, 6, 8, 9, 19	1*β*, 2, 12
11	30.0	a, 1.62 (m)	11b, 12	12, 13	7*β*, 11b, 19
		b, 2.17 (dd, 17.4, 7.8)	11a, 12	10, 12, 13	11a, 17
12	129.0	5.33 (dd, 3.3, 7.8)	11a, 11b	14	10, 11a, 14
13	136.2				
14	141.4	6.30 (dd, 10.7, 17.3)	15a, 15b	13, 12	12, 15a
15	111.1	a, 4.92 (d, 10.7)	14	13	14, 15b
		b, 5.08 (d, 17.3)	14	13, 14	15a, 16
16	12.4	1.63 (s)		12, 13, 14	15b
17	15.6	0.90 (d, 5.6)	8	7	8, 11b, 20 (w)
18	98.9	6.47 (d, 7.2)	4	4, 5, 19, aCO	4
19	97.6	6.34 (s)		4, 5, 6, 18, bCO	7*β*, 11a
20	25.7	0.81 (s)		9, 11	1*β*, 12, 17 (w)
1′	176.5				
2′	41.2	2.31 (qt, 7.0, 6.9)	5′	1′, 3′, 5′	3′a, 3′b, 5′
3′	26.5	a1.43 (m)	3′b, 4′	1′, 2′, 4′, 5′	2′, 3′b, 4′
		b1.63 (m)	3′a, 4′	1′, 2′, 4′, 5′	2′, 3′a
4′	11.8	0.89 (t, 7.5)	3′	2′, 3′	3′a, 5′
5′	16.4	1.11 (d, 6.9)	2′	1′, 2′, 3′	2′, 4′
aCO	169.6				
aCH_3	21.1	2.01 (s)		aCO (Ac)	
bCO	169.3				
bCH_3	21.5	1.93 (s)		bCO (Ac)	

whereas the coupling of H-1*β* to H-10 was 2.3 Hz. This required both H-1α and H-10 to be axial. The moderately large coupling constants between H-2 and H-1α and H-1*β* (7.3 and 9.3 Hz) implied that H-2 was pseudoaxial and the C-2 ester function was pseudoequatorial. The axial orientation of H-2 was confirmed by the strong NOE between H-2 and the equatorial proton assigned as H-1*β*, and the lack of an NOE between it and the axial proton assigned as H-1α. Also consistent with the axial orientation of H-2 was a weak NOE observed with the axial proton at C-10. The coupling constants between H-4 and H-3*β* (14.6 Hz) and between H-4 and H-3α (4.0 Hz) implied that both H-4 and H-3*β* were axial. This was confirmed by the strong NOE between H-4 and H-3α. The coupling constant between H-6 and H-7*β* was 11.9 Hz, implying that both protons were axial, and placed the C-6 hydroxyl group in an equatorial position. Homonuclear decoupling of the methyl doublet (C-17) revealed a large coupling constant for H-8 with H-7*β* (10.3 Hz) and hence required H-8 to be axial and the C-17 methyl group to be equatorial. Strong NOE correlations between H-19 and H-7*β*, between H-1α and H-6, between H-2 and H-10, and between H-4 and H-6 were consistent with a *cis* A/B ring juncture with ring A in a boat conformation and ring B in a chair conformation. The strong NOE correlations between H-10 and H-12 and between H-11a and H-19 demonstrated that the unsaturated side chain was axial and the C-20 methyl group was equatorial. The acetal proton H-18 was assigned as α based on the strong NOE to H-4. The Δ^{12} double bond was assigned an *E* configuration on the basis of the NOE observed between H-12 and H-14. The methyl groups (C-4′ and C-5′) of the tiglate ester side chain were assigned as *cis* on the basis of the strong NOE between them and the lack of an NOE between H-3′ and the C-5′ methyl. The lack of an NOE between the diequatorial methyl groups at C-17 and C-20 initially caused concern. However when an optimal mixing time of 264 ms was used, a small NOE was observed. Thus these observations lead to the assignment of the relative stereochemistry proposed for **1**.

Casearinol B (**2**) was isolated as a colorless oil and determined to have a molecular formula of $C_{29}H_{44}O_8$ by HRMS, indicating eight degrees of unsaturation. Spectral data for **2** were almost identical to **1** except for saturation in the C-2 tiglate side chain (Table 2). The ^{13}C NMR signal of the tiglate carbonyl ester was observed at lower field (δ 176.5), and was HMBC-correlated to the H-2 acetal methine at δ 5.26, the H-2′ methine at δ 2.31, the H-3′ methylene at δ 1.43 and 1.63, and the H-5′ methyl at δ 1.11. The terminal H-4′ methyl triplet (δ 0.89, J = 7.5 Hz) was COSY-correlated with the H-3′ methylene. The relative stereochemistry of **2** was the same as **1** based on comparative coupling constants. This was confirmed by the NOESY experiment (Table 2), which showed the same NOE correlations as for **1** (Table 1), except for the C-2 side chain, where data were consistent with it being saturated (Table 2).

Casearinone A (**3**) was isolated as a colorless oil and yielded a formula of $C_{26}H_{36}O_8$ by HRMS, indicating nine degrees of unsaturation. The presence of a ketone function was supported by the strong IR absorption at 1695 cm^{-1} and the ^{13}C NMR signal at δ 207.7 (Table 3). Comparison of the NMR spectral data with those of **1** indicated the presence of a third acetate replacing the tiglate moiety. The placement of the acetate at C-2

Table 3. NMR Data for Casearinone A (**3**) (500 MHz, $CDCl_3$)

position	δ ^{13}C	δ ^{1}H (mult, J = Hz)	COSY	HMBC (H to C)	NOESY
1	27.6	α, 0.92 (ddd, 13.4, 8.5, 13.7)	1*β*, 2, 10	2, 5, 9, 10	1*β*
		β, 2.16 (ddd, 13.4, 8.5, 1.3)	1α, 2, 10	2, 3, 5	1α, 2, 10
2	69.0	5.15 (ddd, 8.5, 8.5, 6.3)	1α, 1*β*, 3*β*	4, aCO (Ac)	1*β*, 3*β*, 10 (w)
3	25.9	α, 1.59 (dd, 15.2, 4.1)	3*β*, 4	1, 2, 4, 5	3*β*, 4
		β, 2.04 (ddd, 6.3, 15.2, 14.9)	2, 3α, 4	2, 4	2, 3α
4	37.5	3.27 (ddd, 4.1, 14.9, 7.2)	3α, 3*β*, 18	2, 3, 5, 6, 19	3α, 18
5	65.5				
6	207.7				
7	43.2	α, 2.31 (dd, 14.4, 3.4)	7*β*, 8	6, 8, 9, 17	7*β*
		β, 2.62 (dd, 14.4, 14.4)	7α, 8	6, 9	7α, 11a, 17, 19
8	38.2	1.94 (m)	7*β*, 17	6	17, 20
9	37.6				
10	40.3	2.24 (dd, 13.7, 1.3)	1α, 1*β*	1, 2, 4, 5, 6, 8	1*β*, 2, 11a, 12
11	30.3	a, 2.01 (m)	11b, 12		11b, 19
		b, 2.40 (dd, 17.2, 7.5)	11a, 12	9, 10, 12, 13	11a, 17
12	127.3	5.41 (dd, 4.8, 7.5)	11a, 11b	14, 16	10, 14, 20
13	136.7				
14	140.9	6.32 (dd, 10.7, 17.4)	15a, 15b	12, 13, 16	15a
15	111.6	a, 4.96 (d, 10.7)	14	13	14, 15b
		b, 5.12 (d, 17.4)	14	13, 14	15a, 16
16	12.1	1.68 (s)		12, 13, 14	15b
17	15.7	0.96 (d, 6.8)	8	7, 9	8, 7*β*, 11b
18	98.6	6.48 (d, 7.2)	4	4, 5, 19, CO	4
19	96.7	6.28 (s)		4, 5, 6,18, CO	7*β*, 11a
20	25.1	0.91 (s)		9, 10, 11	8, 12
aCO	170.8				
aCH_3	21.0	2.01		aCO (Ac)	
bCO	169.4				
bCH_3	21.0	2.00 (s)		bCO (Ac)	
cCO	168.9				
cCH_3	21.0	1.93 (s)		cCO (Ac)	

was confirmed by the HMBC correlations of both the H-2 methine and the acetate methyl at δ 2.01 to the acetate carbonyl at δ 170.8. The spin system from H-7α, H-7*β*, H-8, and Me-17 could be observed from the ^{1}H NMR and the COSY spectra (Table 3). The downfield chemical shift of H-7α and H-7*β* (δ 2.31 and 2.62 respectively), compared to **1**, implied the keto group to be at C-6. This was confirmed by the HMBC experiment (Table 3), which showed correlations between C-6 and H-4, H-7α, H-7*β*, H-8, and H-10. The relative stereochemistry of **3** was found to be identical with **1** based on evaluation of coupling constants and NOE data (Table 3).

Casearinone B (**4**) was isolated as a colorless oil, which was determined to have the same molecular formula, $C_{26}H_{36}O_8$, as **3**. Comparison of the NMR data of **4** with **3** indicated the isomeric difference was in the diene side chain. The ^{1}H NMR signals from the vinylic H-14 proton and the two terminal methylene protons, H-15a and H-15b, were shifted downfield, as was the signal from the H-16 methyl group. Furthermore, the NOE between H-12 and H-14, observed in **3**, was not present; instead there was a strong NOE between H-12 and the H-16 methyl group, and a medium NOE between the proton assigned as H-11a and H-14. Therefore the Δ^{12} double bond was assigned a *Z* configuration as was determined in the casearins.[3,4]

The relative stereochemistries of **1**−**4** were internally consistent, based on analysis of coupling constants and NOE data, and are also in agreement with the corymbotins[7] (e.g., **6**). As a class, however, the 18,19-diacetoxyclerodane 18,19-oxide acetals have shown considerable variations in their configurations (e.g., **5**, **7**).[3−8]

Compounds **1**−**4** inhibited the binding of LFA-1 to ICAM-1. Quantitative data were obtained for casearinol A (**1**), which inhibited the binding of LFA-1 to ICAM-1 in a dose-responsive manner, yielding an IC_{50} of 50 μM. This is the first report of immunomodulatory activity for this class of diterpenes.

Experimental Section

General Experimental Procedures. Analytical HPLC was performed on a Thermo Separation Products P4000 pump, AS3000 injector, and Spectra Focus forward optics UV scanning detector, using a Rainin Microsorb 3 μm C_{18} 4.6 × 150 mm column. Preparative HPLC used the same equipment as analytical HPLC except that the pumps were fitted with preparative heads, and columns were interchanged with a Rainin Microsorb C_{18} 8 μm 21.4 × 250 mm column or a Rainin Microsorb silica 8 μm 21.4 × 250 mm column. ^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were collected on an Varian VXR-500 spectrometer. The samples were dissolved in $CDCl_3$ or DMSO-d_6 100 atom % (MSD Isotopes, St. Louis, MO) at a concentration of 0.1−0.7 wt % and placed in 5 mm NMR tubes (Wilmad Glass, Buena, NJ). Mass spectrometry was performed using a SCIEX (Thornhill, Ontario, Canada) API-III mass spectrometer utilizing an electrospray interface. High-resolution mass spectra were obtained by electron impact on a MAT 90 instrument using electrocalibration with PEG and samples dissolved in H_2O/MeOH (50:50), with 0.01% LiCl to produce MLi^+ ions for analysis. UV absorption spectra were taken on a Hewlett-Packard 8451A diode array spectrophotometer. Optical rotation was obtained on a Perkin-Elmer 241 polarimeter using a 1.0 mL microcell. FTIR spectra were recorded on a Nicolet 800 FTIR coupled to a Spectra-Tech IR microscope. Gamma scintillation counting was obtained on a LKB Clinigamma 1272 (Wallac Oy, Turku, Finland).

Plant Material. Leaves of *C. guianensis* were collected from Puerta Rico in the spring of 1992 by the Missouri Botanical Garden. A voucher specimen has been deposited at the Missouri Botanical Garden, St.

Table 4. NMR Data for Casearinone B (**4**) (500 MHz, $CDCl_3$)

position	δ ^{13}C	δ ^{1}H (mult, J = Hz)	COSY	HMBC (H to C)	NOESY
1	27.6	α, 0.92 (ddd, 13.6, 8.5, 13.6)	1β, 2, 10	2, 5, 9, 10	1β
		β, 2.16 (ddd, 13.4, 8.5, 1.3)	1α, 2, 10	2, 3, 5	1α, 2, 10
2	69.0	5.16 (ddd, 8.5, 8.6, 6.1)	1α, 1β, 3β	4, aCO (Ac)	1β, 3β, 10 (w)
3	25.7	α, 1.60 (dd, 15.2, 4.1)	3β, 4	1, 2, 5	3β, 4
		β, 2.05 (ddd, 6.1, 15.2, 15.0)	2, 3α, 4	2, 4	2, 3α
4	37.6	3.28 (ddd, 4.1, 15.0, 7.2)	3α, 3β, 18	2, 6	3α, 18
5	65.0				
6	208.6				
7	43.3	α, 2.29 (dd, 14.6, 3.4)	7β, 8	6, 8, 9, 17	7β
		β, 2.61 (dd, 14.6, 14.6)	7α, 8	6, 9	7α, 11a, 17, 19
8	38.1	1.93 (m)	7β, 17		17, 20
9	37.9				
10	40.3	2.28 (dd, 13.6, 1.3)	1α, 1β	1, 2, 4, 5, 6, 9, 19	1β, 2, 11a, 12
11	29.3	a, 1.96 (m)	11b, 12	13 (w)	11b, 19
		b, 2.54 (dd, 16.0, 9.0)	11a, 12	9, 10, 12, 13	11a, 14, 17
12	124.7	5.30 (broad d, 9.0)	11a, 11b	14	10, 16, 20
13	134.8				
14	133.4	6.61 (dd, 10.8, 17.1)	15a, 15b	13, 16	11b, 15a
15	114.8	a, 5.14 (d, 10.8)	14	13	14, 15b
		b, 5.23 (d, 17.1)	14	13	15a, 16
16	20.3	1.84 (s)		12, 14	12, 15b
17	15.7	0.96 (d, 6.8)	8	7, 9	7β, 8, 11b
18	98.5	6.48 (d, 7.2)	4	3, 5, 19, CO	4
19	97.2	6.29 (s)		4, 5, 6,18, CO	7β, 11a
20	25.0	0.87 (s)		9, 10, 11	8, 12
aCO	171.0				
aCH_3	21.0	2.00		aCO (Ac)	
bCO	169.1				
bCH_3	21.0	2.00 (s)		bCO (Ac)	
cCO	168.6				
cCH_3	21.0	2.02 (s)		cCO (Ac)	

Louis, MO. The leaf material was frozen in liquid nitrogen and stored at −30 °C until freeze-dried.

Extraction and Isolation. A 10 g pulverized freeze-dried leaf sample was extracted with 100 mL of 95% EtOH. The extract was filtered and diluted with water (200 mL) and passed through DEAE-cellulose (20 g) (Sigma) to remove tannin and polyphenolic compounds. The column was washed with water−ethanol (2:1, 400 mL) and evaporated to dryness. The recovered oil (1.7 g) was purified by reversed-phase semiprep HPLC with a mobile phase gradient of 50−60% CH_3CN in H_2O with 0.05% TFA over 2 h. Fractions were tested for their ability to inhibit the binding of LFA-1 to ICAM-1 immobilized on plastic. Three zones with inhibitory activity were collected separately at 49.0−52.0, 103.0−107.0, and 112.0−115.5 min and taken to dryness. From fractions 103.0−107.0 min, **1** (5.6 mg) was isolated. Fractions 49.0−52.0 and 112.0−115.5 min were further purified on a Rainin Microsorb 10 × 250 mm semiprep silica column using isocratic mobile phase 99.3% hexane−0.7% IPA, yielding **2** (1.4 mg) from purified fractions 112.0−115.5 min, and **3** (1.8 mg) and **4** (0.4 mg) from purified fractions 49.0−52.0 min.

LFA-/ICAM-1 Binding Assay. The LFA-1 binding assay was performed as previously described[11] with modifications as described below. Wells of Removeawell strips (Dynatech Immulon 4, Chantilly, VA) were coated with the full length ectodomain of recombinant sICAM-1[12] at a concentration of 20 μg/mL TS buffer (150 mM NaCl, 25 mM Tris, 5 mM $MgCl_2$) overnight at 4 °C. Wells were washed with TS buffer and blocked with TS buffer containing 1% BSA. The [^{125}I]LFA-1 was diluted to 100 000 cpm/50 μL with TS buffer containing 1% BSA and 50 μL/well was added to the plates containing various amounts of plant extract or column fractions previously diluted in TS buffer. The plates were incubated overnight at 4 °C and then washed three times with TS buffer to remove the unbound [^{125}I]LFA-1. The wells were then counted on a gamma scintillation counter.

Casearinol A (1): colorless oil; $[\alpha]^{25}_D$ +9.06° (*c* 0.0034 g/mL, $CHCl_3$); UV (MeOH) λ_{max} 224 nm (ϵ 13 800); FTIR ν_{max} 3449, 3348, 1752, 1729, 1650, 1446, 1141, 1003−1100, 737 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HR FABMS *m*/*z* [M + Li] 525.3022 ($C_{29}H_{42}O_8Li$ requires 525.3040).

Casearinol B (2): colorless oil; UV (MeOH) λ_{max} 226 nm (ϵ 11 900); FTIR ν_{max} 3432, 3351, 1752, 1725, 1640, 1469, 1151, 1003, 788 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 2; HR FABMS *m*/*z* [M + Li] 527.369 ($C_{29}H_{44}O_8Li$ requires 527.3196).

Casearinone A (3): colorless oil; UV (MeOH) λ_{max} 232 nm (ϵ 15 750); FTIR ν_{max} 3441, 3348, 1754, 1695, 1622, 1468, 1236, 1156, 790 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 3; HR FABMS *m*/*z* [M + Li] 483.2568 ($C_{26}H_{36}O_8Li$ requires 483.2569).

Casearinone B (4): colorless oil; UV (MeOH) λ_{max} 234 nm (ϵ 15 750); FTIR ν_{max} 3442, 3345, 1750, 1692, 1623, 1465, 1237, 1156, 1015, 788 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 4; HR FABMS *m*/*z* [M + Li] 483.2554 ($C_{26}H_{36}O_8Li$ requires 483.2569).

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