Journal of Natural Products
Vol. 58, No. 12, pp. 1848-1860, December 1995

MAJAPOLENE A, A CYTOTOXIC PEROXIDE, AND RELATED SESQUITERPENES FROM THE RED ALGA *LAURENCIA MAJUSCULA*

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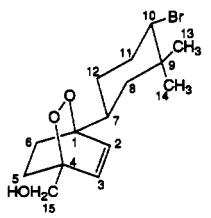
ABSTRACT.—Seven new sesquiterpenes, majapolenes A [**1**] and B [**2**], majapolone [**3**], and majapols A [**4**], B [**5**], C [**6**], and D [**7**], were isolated from a Philippine collection of *Laurencia majuscula*. With the exception of majapolene B [**2**], all compounds were isolated as inseparable diastereomeric mixtures. Structure elucidation was achieved by spectroscopic methods. Majapolene A [**1**], a dioxabicyclo[2.2.2]-alkene, displayed modest activity in the NCI 60-cell line cytotoxicity screen. Majapolene A was also found as a major component of a Philippine collection of *Laurencia caraibica*.

Laurencia is a well-studied genus of the Rhodomelaceae (1,2), although its metabolites have not been extensively investigated for bioactivity. Organic extracts of a sample of *Laurencia majuscula* (Harvey) Lucas, collected off Apo Island, Philippines, displayed modest activity in the NCI 60-cell line human tumor assay (3) with some differential cytotoxicity in certain leukemia, non-small cell lung, and renal cell lines. This observation prompted an investigation of the chemistry of this alga. We report here the isolation and characterization of the major active component, the sesquiterpene peroxide majapolene A [**1**], and six related sesquiterpenoid metabolites [**2–7**]. Each of these compounds, with the exception of **2**, occurred as an inseparable mixture of diastereomers.

Solvent-solvent partitioning of the crude organic extract afforded a CCl_4 fraction in which the activity was concentrated. Sephadex LH-20 gel filtration with hexane-CH_2Cl_2-MeOH (2:5:1) followed by vlc (4,5) on Si gel, gave majapolene B [**2**] in the EtOAc-hexane (1:5) eluent and a mixture of majapolene A [**1**] and **3–7** in the EtOAc-hexane (1:1) eluent. Majapolene A [**1**] was isolated from this mixture by Si gel vlc with EtOAc-$CHCl_3$ (1:9). The remaining mixture [**3–7**] was resolved by Si gel tlc with a variety of solvents (see below). Because majapolene B [**2**] is a single isomer with well defined spectra, it was characterized first, facilitating the subsequent characterization of the diastereomeric mixtures of **1** and **3–7**.

Majapolene B [**2**], $[\alpha]_D$ −14.6°, was isolated in 0.3% yield from the crude extract as a colorless oil that gradually crystallized. Hreims established its molecular formula as $C_{15}H_{21}BrO$, indicating five degrees of unsaturation. Prominent atomic mass unit losses



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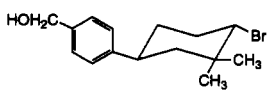

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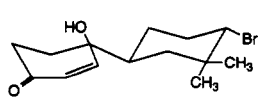

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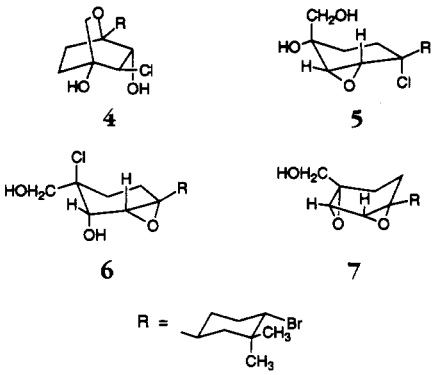


from the molecular ion included 17 (OH), 31 (CH_2OH), and 80 (HBr). Ir spectroscopy confirmed the presence of OH (3355 cm^{-1}) and indicated aromatic character (3012, 1614, 1514, 1470 cm^{-1}). ^1H-, ^{13}C-, and DEPT nmr spectra (Table 1) revealed four different types of aromatic carbons, two of which were substituted (δ 138.81 and 145.28). Doublets at δ 7.28 and 7.17 (J=8 Hz) in the ^1H-nmr spectrum further characterized the aromatic moiety as a para-disubstituted benzene. A methylene carbon at δ 65.11 and a two-proton singlet at δ 4.64 confirmed an isolated -CH_2OH group. A methine carbon at δ 65.72, with its proton at δ 4.03, accommodated the bromine functionality. The remaining seven carbons and thirteen hydrogens were accounted for by two methyls, three methylenes, one methine, and one quaternary carbon.

Both methyl groups appeared as singlets in the ^1H-nmr spectrum at chemical shifts (δ 1.08 and 1.15) that precluded their attachment to the aromatic ring. Therefore, both had to be attached to the lone saturated quaternary center in the molecule, which placed the isolated CH_2OH group on the benzene ring. The aliphatic methine group, with a proton at δ 2.82, was assigned as the second aromatic substituent. This benzylic hydrogen appeared as a triplet of triplets, necessitating the presence of two adjacent

TABLE 1. ^{13}C- (125 MHz) and ^1H- (500 MHz) Nmr Data for Majapolene B [**2**] ($CDCl_3$).

Position	δ_C	δ_H[a] (m, Hz)	^1H-^1H COSY
1	145.28		
2	126.94	7.28 (d, 8.3)	3, 13, 15
3	127.24	7.17 (d, 8.3)	2
4	138.81		
5	127.24	7.17 (d, 8.3)	6
6	126.94	7.28 (d, 8.3)	5, 13, 15
7	38.74	2.82 (tt, 3.5, 3.5, 12.7, 12.7)	8a, 8b, 12a, 12b
8	47.87	1.50 (t, 12.7, 12.7)	7, 8b, 13
		1.83 (dt, 3.0, 3.5, 12.7)	7, 8a, 12b
9	37.07		
10	65.72	4.03 (dd, 4.4, 12.7)	11a, 11b
11	34.49	2.15 (dq, 3.9, 12.7, 12.7, 13.2)	10, 11b, 12a, 12b
		2.24 (dq, 3.9, 3.9, 4.4, 13.2)	10, 11a, 12a, 12b
12	35.44	1.51 (dq, 3.9, 12.7, 12.7, 12.7)	7, 11a, 11b, 12b
		1.85 (d pentets, 3.0, 3.5, 3.9, 3.9, 12.7)	7, 8b, 11a, 11b, 12a
13	31.67	1.15 (s)	2 (6), 8a
14	20.46	1.08 (s)	
15	65.11	4.64 (s)	

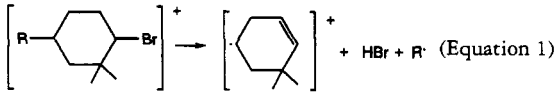
[a]With geminal protons, the smaller δ-value is given the a designation, the larger δ-value is given the b designation.

methylene groups. The ^{1}H-^{1}H COSY nmr spectrum revealed that the protons in one of these methylene groups were coupled to another set of methylene protons (δ 2.15 and 2.24), which, in turn, were coupled to the bromomethine. The bromomethine appeared as a simple doublet of doublets, indicating that the quaternary carbon with the *gem*-dimethyl group was adjacent. With all 15 carbons accounted for, the quaternary center must be bonded to the remaining methylene group flanking the benzylic carbon to give a 1,2,2,4-tetrasubstituted cyclohexyl system.

The relative stereochemistry of **2** was easily deduced from the coupling constants of the bromomethines and benzylic methines. The bromomethine (dd) displayed *J* values of 4.4 and 12.7 Hz, establishing its axial orientation and placing the bromine equatorially. The benzylic methine (tt) showed two axial-equatorial couplings (J=3.5 Hz) and two axial-axial couplings (J=12.7 Hz); thus, it too was axial. The phenyl substituent, then, was equatorial and trans to the bromine. Of interest were the COSY-detected long-range couplings of H-2/H-6 with the H-15 and the equatorial H-13 methyl protons. These were co-planar extended *W*-couplings over five and seven bonds, respectively.

Majapolene A [**1**], $[\alpha]_D$ −20.0°, was isolated as a crystalline solid in 1.7% yield from the crude extract. Hreims established a molecular formula of $C_{15}H_{23}BrO_3$ for **1**, implying four degrees of unsaturation. The base peak in the mass spectrum was derived from the loss of 33 atomic mass units, which corresponded to loss of OOH, as indicated by an accurate mass measurement using a thermal desorption probe. This facile loss of a hydroperoxy group is characteristic of dioxabicyclo-[2.2.2]alkenes and dioxabicyclo[n.2.2]alkanes in general (6). Additional atomic mass unit losses of 17, 18, 31, and 80 supported the presence of OH, CH_2OH, and Br moieties. A prominent fragment also occurred at *m/z* 109 ($C_8H_{13}^+$). Ir spectroscopy confirmed the presence of alcohol (3282, 1055 cm^{-1}) and alkene (3056, 1629 cm^{-1}) groups. In the ^{1}H-nmr spectrum, a pair of mutually coupled vinyl hydrogens (δ 6.5 and 6.6) with a J value of 8.8 Hz indicated that the alkene was cis-disubstituted. The ^{13}C-nmr spectrum revealed that majapolene A [**1**] was an equimolar mixture of diastereomers, as all but three of the fifteen carbon resonances were doubled, and these three single peaks were of sufficient intensity to accommodate two carbons each. Although the presence of isomeric mixtures complicated the spectral interpretation, especially with overlapping proton resonances, it was still possible to make definitive assignments in most cases. The tables for such mixtures list values for both isomers where sufficient resolution made individual assignments possible. In the discussion which follows, each diastereomeric mixture is treated as a single compound.

The DEPT spectrum of majapolene A [**1**] identified the multiplicities of each carbon and the HMQC spectrum permitted the assignment of the carbons to their attached protons, confirming the earlier structural assignments derived from ms and ir data. These data, together with the ^{1}H- and ^{1}H-^{1}H COSY nmr spectra, defined an isolated -CH_2CH_2- group and the 4-bromo-3,3-dimethylcyclohexyl moiety previously characterized in majapolene B [**2**]. The prominent ion at *m/z* 109 in the ms can be attributed to the loss of HBr and cleavage of the molecule at the C-1/C-7 bond (Equation 1):



(Equation 1)

The assembly of the various partial structures into the final whole was made possible by HMBC analysis. Especially helpful were the long-range correlations exhibited by the

quaternary peroxy carbons. Both of these carbons (δ 79.3 and 77.9) showed correlations to both olefinic protons (δ 6.6 and 6.5) and to the downfield protons (δ 2.01 and 2.02) of both carbons of the isolated -CH_2CH_2-group. Thus, the peroxy carbons must serve as links between the vinyl carbons and the isolated -CH_2CH_2- group. In addition, the peroxy carbon at δ 77.9 (C-4) displayed coupling to the methylene hydrogens (δ 3.72 and 3.77) of the primary alcohol group (C-15), making this group a substituent on that peroxy carbon. In support of this assignment, these same methylene hydrogens showed coupling to one vinyl carbon (δ 132, C-3) and to one of the carbons (δ 24, C-5) of the isolated -CH_2CH_2- group. The last remaining C-C bond assignment must then be between the peroxy carbon at δ 79 (C-1) and the 4-bromo-3,3-dimethylcyclohexyl moiety. Correlations between the cyclohexyl carbon at δ 37 (C-7) and the olefinic proton at δ 6.5 (H-2), as well as the deshielded methylene hydrogen at δ 2.02 ($H\text{-}6_{syn}$), confirmed this final skeletal link. Additional confirmatory long-range C-H correlations for structure **1** are found in Table 2.

Repeated Si gel tlc with EtOAc-hexane (1:3), followed by MeOH-$CHCl_3$ (1:4), separated majapolone [**3**] and majapol A [**4**] from **5–7**. Majapolone [**3**], isolated in 0.02% yield from the crude extract, analyzed for $C_{14}H_{21}BrO_2$ by hrfabms, implying four degrees of unsaturation. An alcohol was suggested by atomic mass unit losses of 17 and 18, and the base peak at *m/z* 109 indicated the presence of the bromodimethylcyclohexyl group. The ir spectrum of majapolone [**3**] displayed OH and conjugated carbonyl absorptions. A λ max of 216 nm (ε 8600) in the uv spectrum suggested an unsubstituted cyclohexenone chromophore. ^{13}C-, DEPT, HMQC, and ^1H-nmr spectra added further support, with mutually coupled vinyl hydrogens at δ 6.7 and 5.9, vinyl carbons at δ 152 and 129, and a carbonyl carbon at δ 198. The alcohol group was defined as tertiary by the DEPT spectrum. An isolated -CH_2CH_2- group and a 4-bromo-3,3-dimethylcyclohexyl moiety were firmly established by the ^1H-^1H COSY, ^{13}C-, and ^1H-nmr data.

In the HMBC spectrum of **3**, the carbonyl carbon (C-4) showed correlations to the protons on both carbons of the isolated -CH_2CH_2- group as well as to the vinyl hydrogen

TABLE 2. ^{13}C- (125 MHz) and ^1H- (500 MHz) Nmr Data for Majapolene A [**1**] ($CDCl_3$).

Position	δ_C[a]	δ_H[a,b] (m, Hz)	^1H-^1H COSY	HMBC (^1H Correlation)
1	79.32, 79.34			2, 3, 5b, 6b
2	133.28, 133.58	6.50, 6.51 (d, 8.8)	3	3, 6b
3	132.88, 132.94	6.62, 6.63 (d, 8.8)	2	5, 15a, 15b
4	77.91			2, 3, 5b, 6b, 15a, 15b
5	24.38, 24.46	1.36 (dt, 3.0, 12.2, 12.7)	5b, 6a, 6b	6a, 6b, 15b
		2.01 (m)	5a, 6a	
6	25.03, 25.26	1.48, 1.49 (dt, 3.0, 12.2, 12.7)	5a, 5b, 6b	5a, 5b
		2.02 (m)	5a, 6a	
7	37.03, 37.20	1.90 (m)	8a, 8b, 12a, 12b	2, 6b, 8a, 8b, 12a, 12b
8	40.30	1.19, 1.24 (m)	7, 8b, 14	12a, 12b
		1.72, 1.78 (m)	7, 8a	
9	36.44, 36.53			10, 11a, 14
10	65.16, 65.19	3.90, 3.91 (dd, 4.4, 12.7)	11a, 11b	8b, 11a, 12b, 14
11	33.67, 33.71	2.02 (m)	10, 11b, 12a, 12b	10, 12a
		2.19 (m)	10, 11a, 12a, 12b	
12	28.26, 28.30	1.19, 1.24 (m)	7, 11a, 11b, 12b	
		1.73, 1.80 (m)	7, 11a, 11b, 12a	
13	31.62, 31.68	1.04, 1.05 (s)		8a, 10
14	20.20, 20.29	1.02, 1.03 (s)	8a	8a, 10
15	64.40	3.72 (d, 12.7)	15b	5b
		3.77 (d, 12.7)	15a	

[a]Values are listed for both isomers, if resolved.

[b]With geminal protons, the smaller δ-value is given the a designation, the larger δ-value is given the b designation.

at δ 6.7 (H-2). The tertiary alcohol carbon (δ 71, C-1) likewise correlated with the protons on both carbons of the isolated ethylene group and to the vinyl hydrogen at δ 5.95 (H-3). Furthermore, the cyclohexyl carbon at δ 41 (C-7) displayed a correlation with one of the hydrogens on the isolated $-CH_2CH_2-$ group (H-6a). These data can only be accommodated by structure **3**. The cyclohexenone moiety was assigned as equatorial on C-7 of the dimethylcyclohexyl ring, and trans to the equatorial C-10 bromine.

Majapol A [**4**], isolated in 0.04% yield from the crude extract, displayed OH absorptions (3416, 1049 cm^{-1}) in the ir spectrum and analyzed for $C_{15}H_{24}BrClO_3$ by hrfabms, specifying three degrees of unsaturation. Prominent $(M-H_2O)^+$ and $(M-HCl)^+$ peaks were evident in the mass spectrum. The strong *m/z* 109 fragment again suggested that the 4-bromo-3,3-dimethylcyclohexyl group was present. This was confirmed by ^1H-, ^{13}C-, HMQC, and ^1H-^1H COSY nmr spectra. An isolated $-CH_2CH_2-$ group was also established. In addition to the bromomethine of the 3,3-dimethylcyclohexyl moiety, majapol A contained five other deshielded carbons: two quaternary, two methine, and one methylene. Two of the deshielded carbons carried OH groups, as determined by D_2O exchange in the ^1H-nmr spectrum. The remaining three deshielded carbons were accounted for by the chlorine substituent and an ether linkage.

The HMBC spectrum was used to construct the skeletal framework of **4**. Both deshielded quaternary carbons (δ 74, C-1 and δ 68, C-4) were coupled to the protons of both carbons of the isolated ethylene group; thus, this latter group forms a bridge between the two deshielded quaternary carbons. The proton attached to the δ 78 carbon (C-2) correlated with the δ 23 carbon (C-6) of the isolated ethylene group and with the quaternary C-1 (δ 74). Hence, the δ 78 methine must be connected to C-1. The proton attached to the δ 72 carbon (C-3) correlated with the δ 29 carbon (C-5) of the isolated $-CH_2CH_2-$ group and with the quaternary C-4 (δ 68). Therefore, this δ 72 methine was attached to C-4. As the ^1H-^1H COSY nmr spectrum showed a strong cross-peak between the δ 78 methine proton (H-2) and the δ 72 methine proton (H-3), they had to be on adjacent carbons. This was confirmed by HMBC correlations between the δ 4.12 proton (H-2) and the δ 72 carbon (C-3) and between the δ 3.96 proton (H-3) and the δ 78 carbon (C-2). The protons (δ 3.4 and 3.7) of the deshielded methylene group at δ 66 (C-15) showed long-range coupling to both quaternary carbons (δ 74, C-1 and δ 68, C-4), to the methine carbon at δ 72 (C-3) and to the δ 29 carbon (C-5) of the isolated $-CH_2CH_2-$ group. These data could only be accommodated by linking the δ 66 methylene (C-15) to the δ 68 quaternary carbon (C-4) and creating an ether bridge between the δ 66 methylene (C-15) and the δ 74 quaternary carbon (C-1). The 4-bromo-3,3-dimethylcyclohexyl group was also placed on the δ 74 carbon (C-1) as depicted in **4**.

The placement of the OH and Cl substituents was achieved by nmr studies in DMSO-d_6. In this solvent, the ^1H-nmr spectrum of majapol A [**4**] displayed an OH doublet at δ 5.7 (J=5.8 Hz) and an OH singlet at δ 5.1. The latter OH must be tertiary and was assigned to C-4. The placement of the secondary OH at C-2 was made possible by HMQC and HMBC spectra in DMSO-d_6, which also confirmed the tertiary OH location. Thus, in this solvent, the tertiary OH showed correlations to C-3, C-4, and C-5, while the secondary OH showed correlations to C-1, C-2, and C-3. The secondary OH must be bonded at C-2, which left the chlorine to occupy the C-3 position.

The stereochemistry of **4** could be deduced from a combination of data. That the bromine at C-10 was equatorial was established by J values of 4.3 and 12.7 Hz for H-10. The bulky substituent at C-7 was presumed to reside in the preferred equatorial orientation, which rendered it trans to the bromine. The trans-relationship of H-2 and H-3 was established by their small coupling constant—both appeared as broad singlets in the ^1H-nmr spectrum. cis-Protons in a [2.2.2]bicyclooctyl system would be expected

to exhibit *J* values of 8–10 Hz (7). In the ^{1}H-^{1}H COSY nmr spectrum, H-2 displayed a cross-peak with H-6_{syn} as expected if H-2 were also *syn* and, therefore, in a co-planar *W*-relationship with H-6_{syn}. No such cross-peak was seen between the H-3 and H-5 proton, which placed H-3 *anti* to the oxide bridge. In addition, the chemical shift difference between C-5 and C-6 supported a γ-gauche shielding effect of OH-2 on C-6. The chlorine atom at C-3 is not properly oriented to exert such a shielding effect on C-5 (8,9).

The assignment of the individual methylene protons of the oxabicyclooctyl system followed from the ^{1}H-^{1}H COSY nmr data. Long-range *W*-couplings were displayed between H-2 and H-6_{syn} (δ 1.92), between H-3 and the δ 3.4 hydrogen at C-15, and between H-5_{anti} (δ 1.76) and the δ 3.7 hydrogen at C-15. These assignments were confirmed by the HMBC spectrum, which showed stronger cross-peaks for $^3J_{C\text{-}H}$ when the H and C involved were trans, as was the case for C-2 and H-6_{syn}, C-3 and H-5_{syn}, C-5, and the δ 3.7 hydrogen at C-15, and C-15 and H-3.

Majapols A–D [**5**–**7**] were separated by successive Si gel tlc with EtOAc-$CHCl_3$ (1:3), EtOAc-hexane (1:3), and MeOH-$CHCl_3$ (1:30). Majapol B [**5**] was obtained in 0.3% yield from the crude extract. A molecular formula of $C_{15}H_{24}BrClO_2$ (three degrees of unsaturation) for **5** was established by hrfabms. Both ms $(M-H_2O)^+$ and ir (3443, 1066 cm^{-1}) analyses identified an alcohol functionality. The base peak at *m/z* 109 in the ms indicated a bromodimethylcyclohexyl system, and this was confirmed by the ^{1}H, ^{13}C, DEPT, HMQC, and ^{1}H-^{1}H COSY nmr spectra. An isolated -CH_2CH_2- group was also established. A D_2O-exchange ^{1}H-nmr experiment revealed that majapol B [**5**] contained two OH groups, which were identified as primary and tertiary when the solvent was changed to DMSO-d_6 (δ 4.63, t and δ 4.75, s). The third oxygen was ascribed to an epoxide ring in consideration of two methine carbons at δ 54 and 61 in the ^{13}C-nmr spectrum and the absence of a third exchangeable proton. In addition to the quaternary carbon-bearing the OH group, a second deshielded quaternary carbon was present. This carbon must bear the chlorine substituent, since all other deshielded methines and methylene carbons were occupied by bromine and/or oxygen functionalities.

Construction of the carbon skeleton was achieved by analysis of HMBC spectra. In addition to the 4-bromo-3,3-dimethylcyclohexyl and epoxide rings, a third ring was necessary to satisfy the molecular formula, inasmuch as there was no unsaturation in the molecule. That the isolated ethylene group bridged the two deshielded quaternary carbons was seen from the long-range correlation between each quaternary carbon and the protons of both carbons of the -CH_2CH_2- group. The quaternary carbon at δ 73 (C-4) also correlated with both carbons of the -CH_2CH_2- group. The quaternary carbon at δ 73 (C-4) also correlated with both epoxide protons and the protons of the primary alcohol group. Thus, both the epoxy group and the -CH_2OH group had to be attached to this quaternary carbon (C-4). The quaternary carbon at δ 64 (C-1) displayed coupling to one of the epoxy protons (δ 4.35, H-3). Placement of a bond between this carbon (C-1) and the remaining open epoxy carbon (δ 61, C-2) accommodated both this coupling and ring closure. The 4-bromo-3,3-dimethylcyclohexyl ring also had to be attached to the δ 64 quaternary carbon (C-1), because this was the only site remaining for a non-heteroatom. Location of the OH and Cl substituents at C-4 and C-1, respectively, was achieved by HMQC and HMBC spectra in DMSO-d_6. In this solvent, the tertiary OH (s, δ 4.75) showed long-range correlations with C-3, C-4, C-5, and C-15, firmly establishing its attachment at C-4 (δ 73), and, by default, placing the chlorine atom group at C-1 and δ 64.

With respect to the relative stereochemistry of **5**, the bromine at C-10 was equatorial, as H-10 displayed *J* values of 4.0 and 12.7 Hz. The large C-7 substituent was

also assigned to an equatorial position because of thermodynamic preference. NOe studies in DMSO-d_6 (Figure 1) defined the configuration at C-1 relative to C-4. Irradiation of the δ 3.27 methylene proton of the -CH_2OH group (H-15a) resulted in an enhancement of the signal for the axial proton at C-6 (δ 1.85) and vice versa. This necessitated the axial placement of the -CH_2OH group on C-4. Based on the premise that the larger substituent at C-1 appropriates the equatorial position, the -CH_2OH group would be cis-related to that C-1 substituent. The equatorial nature of the bromodimethylcyclohexyl substituent at C-1 was supported by the observation of a weak nOe enhancement of H-7 upon irradiation of the axial hydrogen at C-6. No correlations were observed that would permit definitive assignment of the configuration of the epoxide group. Although drawn and proposed as α in **5**, its relative stereochemistry remains undetermined. Additional nOe correlations are given in Figure 1.

Majapol C [**6**] was isolated in 0.6% yield from the crude extract. Hrfabms established its molecular formula as $C_{15}H_{24}BrClO_3$ (three degrees of unsaturation). The ms fragmentation pattern was very similar to that of **5**, implying alcohol and bromodimethylcyclohexyl groups. The ir spectrum confirmed alcohol functionality (3425, 1124, 1060 cm^{-1}). The ^1H-^1H COSY spectrum revealed an isolated -CH_2CH_2- group. Secondary (δ 4.99, d) and primary (δ 4.83, t) OH groups were identified in nmr spectra run in DMSO-d_6. The third oxygen as assigned to a trisubstituted epoxide ring with its methine carbon appearing at δ 57 and its quaternary carbon at δ 66 in the ^{13}C- and HMQC nmr spectra. The C-10 bromomethine was at its usual methine position of δ 65; therefore, the one remaining deshielded (and quaternary) carbon at δ 73 was bonded to the chlorine atom.

The HMQC and HMBC spectra established the skeletal framework of **6**. The epoxide quaternary carbon (δ 66, C-1) and the chlorine-bearing quaternary carbon (δ 73, C-4) both displayed long-range correlations to protons on both carbons of the isolated -CH_2CH_2- group, indicating that each quaternary carbon was bonded to one of these methylene carbons. One of the methylene protons of the -CH_2OH group (H-15b) displayed coupling to the δ 22 carbon (C-5) of the isolated -CH_2CH_2- group, while the other (H-15a) correlated with the chlorine-bearing carbon (δ 73, C-4). Therefore, the -CH_2OH group must be attached to the chlorine-bearing carbon. The methylene protons of the primary alcohol were further coupled to the methine carbinol, which, in turn, was coupled to the epoxy proton at δ 3.2 (H-2), thus completing a cyclohexyl ring through the quaternary epoxy carbon at δ 66 (C-1). The 4-bromo-3,3-dimethylcyclohexyl group was assigned to C-1, the last remaining open site.

The relative stereochemistry of the groups at C-7 and C-10 was established as trans-diequatorial as described for **5**. The relative stereochemistry of the groups on the epoxycyclohexyl system was established as follows. Long-range ^1H-^1H coupling of H-

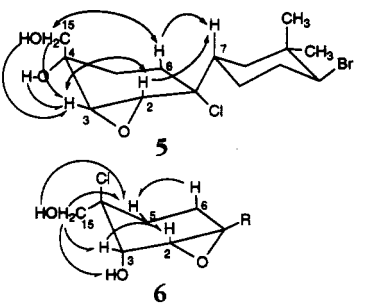


FIGURE 1. Selected observed nOe effects in **5** and **6** (DMSO-d_6).

3 with H-5a was observed in the COSY nmr spectrum, suggesting that these two protons are equatorial (co-planar *W*geometry) and thereby making the OH-3 group axial. A coupling constant of 5.4 Hz between H-2 and H-3 defined these protons as cis; a near 90° dihedral angle and ca. 0 Hz coupling between these protons would be observed if they were trans (8,10). Thus, the secondary OH and the epoxide were cis-related, and the large C-1 substituent was trans to the secondary OH. The relative stereochemistry of the $-CH_2OH$ group at C-4 was assigned on the basis of nOe studies in DMSO-d_6. Irradiation of either H-15a or H-15b produced an enhancement of the signals for the C-3 OH proton. Irradiation of H-15b also resulted in a weak enhancement of the axial proton (δ 1.74) on C-5. No effect was observed on the axial hydrogen at C-6. This placed the $-CH_2OH$ group equatorial at C-4, cis to OH-3 and the epoxide oxygen as depicted in **6** (see Figure 1 for additional nOe correlations).

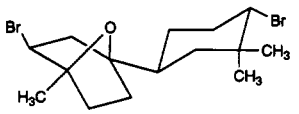
Majapol D [7] was isolated in 0.05% yield from the crude extract. Hreims established its molecular formula as $C_{15}H_{23}BrO_3$, indicating four degrees of unsaturation. Prominent atomic mass unit losses included 17 (OH), 18 (H_2O), and 80 (HBr), with $C_8H_{13}^+$ (*m/z* 109) again appearing as the base peak. The ir spectrum confirmed the presence of an OH group (3441, 1057 cm^{-1}); the ^1H-, ^{13}C-, HMQC, HMBC, and ^1H-^1H COSY nmr spectra verified the presence of a 4-bromo-3,3-dimethylcyclohexyl moiety and an isolated $-CH_2CH_2-$ group. Two trisubstituted epoxide functionalities were identified by the ^{13}C- and HMQC nmr spectra, which displayed quaternary carbons at δ 57 and 60 and methine carbons at δ 51 and 52. The other deshielded carbons were accounted for by a bromomethine (δ 65) and a primary alcohol group (δ 62).

Construction of the carbon skeleton followed from the HMBC spectrum. The bridging of the quaternary epoxide carbons (δ 57, C-4 and δ 60, C-1) by the isolated $-CH_2CH_2-$ group was demonstrated by correlations of each quaternary epoxy carbon with the protons of both carbons of the $-CH_2CH_2-$ group. The $-CH_2OH$ carbon (δ 62, C-15) showed correlations with the protons on the δ 22 carbon (C-5) of the isolated ethylene group. In addition, correlation between the methylene protons of the $-CH_2OH$ group (H-15a and/or H-15b) with the quaternary (δ 57, C-4) and methine (δ 51, C-3) carbons of one epoxy group, placed the $-CH_2OH$ group on that quaternary epoxy carbon. Further correlation of the methine epoxy carbon at δ 51 (C-3) with the epoxy methine hydrogen at δ 3.1 (C-2), together with correlations of the quaternary epoxy carbon at δ 57 (C-4) with both epoxy methine hydrogens, linked the two epoxides through their methine carbons. This placed the 4-bromo-3,3-dimethylcyclohexyl group on the δ 60 quaternary epoxy carbon (C-1) as depicted in **7**.

The stereochemistry of the C-7 and C-10 substituents on the dimethylcyclohexyl ring was determined to be trans-diequatorial as discussed for the other numbers of the series. The relative stereochemistry of the two epoxy groups was established as cis by the *J* value (2.7 Hz) of their adjacent methine hydrogens (8,10), as well as from the probable genesis of **7** from **1** as discussed below.

Compounds **1–7** may be viewed as cyclized bisabolene derivatives. Only one other report of a *Laurencia*-derived compound of this skeletal type, the dibromoether **8**, has appeared in the literature (11). The fact that all compounds isolated, except the aromatic representative **2**, occur as diastereomeric mixtures suggests that the source of isomerism is in the oxygenated ring. It is likely that this ring, and its enantiomer, are attached to a 4-bromo-3,3-dimethylcyclohexane that is of consistent stereochemistry. In support of this conjecture, the carbons and protons at positions 8 and 12 exhibit greater chemical shift variability between the individual diastereomers than any other atoms in the molecule.

The occurrence of isomeric mixtures in this alga raises the question of whether these



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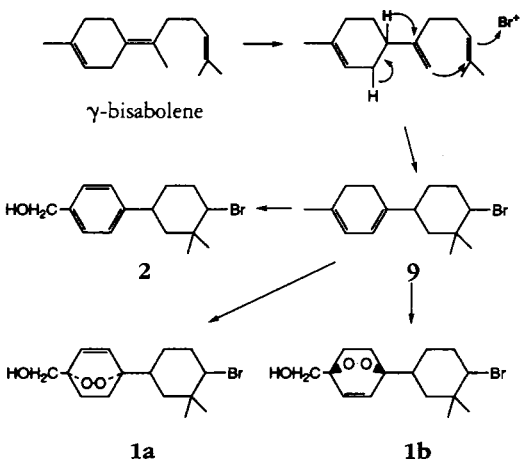
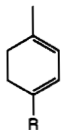
compounds are produced entirely by enzymatic processes. A reasonable immediate precursor to **1** and **2** is the 1,3-cyclohexadiene **9**, presumably derived from bromonium-induced cyclization of a bisabolene system as shown in Scheme 1. Oxidation of the allylic methyl group and 1,4-oxygen addition to both faces of the diene system would give rise to **1a** and **1b** in roughly equivalent amounts. There is no such opportunity for isomerism with **2**.

It is possible that **3** and **7** are directly derived from **1**. Nitz and co-workers (12) have reported the conversion of **10** to endoperoxide **11** and *bis*-epoxide **12** during isolation of **10** from parsley leaves. The *bis*-epoxide [**12**] was derived from endoperoxide **11** by heat, irradiation, or polar phases. It is well known that ascaridole [**13**] and related endoperoxides stereospecifically rearrange to the corresponding *bis*-epoxides [**12**] when treated with metal ions (13–15). In addition, fragmentation of the R group can occur with formation of the hydroxyenone **14** analogous to **3** (13,16). In the present case, very small quantities of **3** and **7** were found, which may lend support to their being derived from **1** during isolation. On the other hand, **1** is a major component and is clearly present in the organic extracts as demonstrated by their ^{1}H-nmr spectra. However, this does not exclude the possibility that **1** was formed during storage or extraction of the alga prior to solvent partitioning, although both alga and extract were stored at −20°.

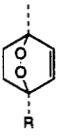
Compound **1** represents the major and only active component of the *L. majuscula* extract. It displayed modest mean response parameter values for all NCI 60-cell lines of 0.4 μM for GI_{50} (50% net growth inhibition, relative to controls), 0.9 μM for TGI (net total growth inhibition) and 2.8 μM for LC_{50} (50% net cell death). According to COMPARE analyses (17,18), the NCI in vitro screening profile of **1** showed considerable similarity to the screening profiles obtained with several other *Laurencia* extracts. In particular, a *L. caraibica* P.C. Silva (Philippines) extract gave correlation coefficients of 0.70 (GI_{50}), 0.79 (TGI), and 0.76 (LC_{50}), prompting an investigation of this alga. Solvent



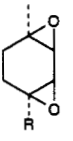
SCHEME 1



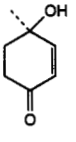
10 R= -C(CH$_3$)=CH$_2$



11 R= -C(CH$_3$)=CH$_2$
13 R= -CH(CH$_3$)$_2$



12



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partitioning of the extract afforded CCl_4 and $CHCl_3$ fractions whose tlc data and ^{1}H-nmr spectra suggested the presence of **1** as a major component. Prep. tlc of the $CHCl_3$ extract afforded **1**, again as an inseparable mixture of diastereomers. It is interesting to note that the differential cytotoxicity profile of **1** is markedly different from the patterns (19) produced by halogenated chamigrenes from Australian *L. majuscula*.

EXPERIMENTAL

PLANT MATERIAL.—*Laurencia majuscula* was collected by scuba at 50–70-foot depths off Apo Island, near the southern tip of Negros Island, Central Visayas, Philippines, in June 1988, and kept frozen until processing. *Laurencia caraibica* was collected in the same locality in June 1986. The algae were identified by Dr. Ernani Menez and voucher specimens were deposited at the Smithsonian Institution.

EXTRACTION AND ISOLATION.—The frozen algal mass of *L. majuscula* was ground with dry ice, extracted with H_2O at 3° for 4 h, filtered, and freeze-dried. The dried marc was extracted with MeOH-CH_2Cl_2 (1:1), then MeOH, at 25° for 16 h; the filtered extracts were then combined and concentrated *in vacuo* to give 6.57 g of extract (8.0% yield from the marc). *L. majuscula* crude extract (6.5 g) was partitioned between 90% aqueous MeOH and hexane. After adjusting the MeOH layer to 80% MeOH, it was extracted with CCl_4 to yield 679 mg of CCl_4-soluble material. Gel permeation of the CCl_4 extract on Sephadex LH-20 in hexane-CH_2Cl_2-MeOH (2:5:1) gave a cytotoxic fraction (295 mg). Vlc on Si gel yielded 20 mg of **2** in the EtOAc-hexane (1:4) eluent and 154 mg of a mixture of **1** and **3–7** in the EtOAc-hexane (1:1) eluent.

A second Si gel vlc with EtOAc-$CHCl_3$ (1:9) separated **1** (108 mg) from **3–7**. Prep. Si gel tlc of the latter mixture with EtOAc-hexane (1:3) resolved **3**, **4** from **5–7**. Compounds **3**, 1.5 mg, and **4**, 2.4 mg, were separated by prep. Si gel tlc with MeOH-$CHCl_3$ (1:40). Prep. Si gel tlc with EtOAc-$CHCl_3$ (1:3), followed by EtOAc-hexane (1:3), afforded 14.3 mg of **5**, 4.2 mg of **6**, and , after Si gel tlc purification with MeOH-$CHCl_3$ (1:30), 1.8 mg of **7**.

L. caraibica crude extract (250 mg) was partitioned as described above for *L. majuscula* to yield 23.5 mg of CCl_4-soluble material. The aqueous MeOH phase was adjusted to 60% MeOH and was extracted with $CHCl_3$ to yield 13.7 mg of extract. Both extracts contained **1** as judged by tlc with EtOAc-$CHCl_3$ (1:9). The $CHCl_3$ extract was subjected to Si gel prep. tlc with EtOAc-$CHCl_3$ (1:9) to yield 1 mg majapolene A [**1**], identified by tlc, ^{1}H-nmr data, and 60-cell differential response pattern comparisons with **1** from *L. majuscula*.

Majapolene A [**1**].—Crystalline solid; $[\alpha]_D$ −20.0° (c=0.2, $CHCl_3$); ir ν max (film) 3282, 3056, 2944, 2866, 1629, 1453, 1388, 1319, 1267, 1212, 1111, 1055, 967, 934, 878, 735 cm^{-1}; ^{1}H- and ^{13}C-nmr data ($CDCl_3$), see Table 2; fabms (noba) *m/z* 333/331 $[MH]^+$ (14), 315 (12), 314 (10), 313 (10), 301 (200), 300 (98), 299 (41), 298 (100) 297 (28), 286 (47), 284 (50), 281 (20), 124 (19), 121 (19), 120 (26), 115 (19), 109 (76), 107 (54), 93 (28), 91 (50), 90 (30), 89 (46), 81 (19), 79 (33), 77 (53), 67 (27), 41 (24); hreims *m/z* 330.0823 (calcd for $C_{15}H_{23}{}^{79}BrO_3$, 330.0825).

Majapolene B [**2**].—Crystalline solid; $[\alpha]_D$ −14.6° (c=3.8, $CHCl_3$); ir ν max (film) 3355, 3012, 2923, 1614, 1514, 1470, 1454, 1422, 1388, 1368, 1218, 1176, 1133, 1016, 965, 820, 750, 683 cm^{-1}; ^{1}H- and ^{13}C-nmr data ($CDCl_3$), see Table 1; eims *m/z* 298/296 (M^+, 100), 267 (6), 265 (7), 216 (7), 199 (13), 187 (35), 185 (61), 173 (89), 145 (26), 143 (26), 134 (32), 121 (47), 117 (54), 109 (26), 107 (47), 105 (66), 91 (54), 69 (28), 55 (19); hreims *m/z* 296.0778 (calcd for $C_{15}H_{21}{}^{79}BrO$, 296.0771).

Majapolone [**3**].—Oil; uv λ max (MeOH) 216 nm (ε 8600); ir ν max (film) 3418, 2952, 1671, 1455, 1388, 1368, 1222, 1169, 1056, 963, 942, 840, 742 cm^{-1}; ^{1}H- and ^{13}C-nmr data ($CDCl_3$), see Tables 3 and 4; fabms (glycerol) *m/z* 303/301 $[MH]^+$ (70), 286 (42), 285 (43), 284 (44), 283 (35), 205 (22), 204 (20), 203 (42), 130 (22), 121 (29), 112 (56), 111 (48), 110 (32), 109 (100), 107 (49), 105 (26); hrfabms *m/z* $[MH]^+$ 301.0807 (calcd for $C_{14}H_{22}{}^{79}BrO_2$, 301.0797).

Majapol A [**4**].—Oil; ir ν max (film) 3416, 2952, 2870, 1454, 1367, 1139, 1049, 964, 879, 798, 750

TABLE 3. ^{13}C- (125 MHz, $CDCl_3$) Nmr Data for Majapolone [**3**] and Majapols A [**4**], B [**5**], C [**6**], and D [**7**].[a]

Carbon	Compound				
	3	**4**	**5**	**6**	**7**
1	71.25	74.83, 74.91	64.36, 64.54	66.37, 66.57	60.04, 60.21
2	152.32, 152.63	78.04, 78.23	61.64, 61.66	57.92, 57.95	52.56, 52.71
3	129.39, 129.42	72.59	54.23	68.55, 68.67	51.11, 51.13
4	198.72, 198.80	68.39	73.59	73.68	57.87, 57.90
5	33.77	29.77	23.05, 23.07	22.54, 22.57	22.34
6	31.46, 31.49	23.34, 23.36	20.48, 20.61	20.96, 21.01	22.02, 22.21
7	41.33, 41.44	35.92, 36.13	39.59, 39.63	39.34, 39.35	38.33, 38.50
8	39.90, 40.72	39.90, 40.08	40.50, 41.43	41.36, 41.62	41.19, 41.56
9	36.58, 36.66	36.13, 36.57	36.27, 36.36	36.44, 36.49	36.38, 36.42
10	65.00, 65.07	65.90, 65.92	64.69, 64.74	65.02, 65.07	65.07, 65.15
11	33.74, 33.82	34.05, 34.08	33.28, 33.34	33.62, 33.64	33.50, 33.55
12	27.76, 28.47	27.72, 27.94	28.43, 29.35	29.38, 29.65	29.13, 29.40
13	31.79	31.87	31.62, 31.63	31.69	31.68
14	20.44, 20.55	20.36, 20.39	20.27, 20.34	20.30, 20.35	20.34
15		66.83	67.49	68.67	62.97

[a]Values are listed for both isomers, if resolved.

cm^{-1}; ^1H- and ^{13}C-nmr data ($CDCl_3$), see Tables 3 and 4; ^1H nmr (DMSO-d_6) δ 0.93 (3H, s), 0.97, 0.98 (3H, s), 1.16–1.29 (2H, dq), 1.56–1.66 (4H, m), 1.67–1.78 (3H, m), 1.85, 1.90 (1H, dq), 2.04 (1H, dq), 3.3 (1H, obscured by solvent, H_2O), 3.61, 3.62 (1H, d), 3.82 (2H, m), 4.05 (1H, dd), 5.13 (1H, s), 5.74, 5.78 (1H, d); HMQC nmr (DMSO-d_6) ^{13}C δ 20.6 (C-14), 23.6 (C-6), 27.9 (C-12), 31.4, 31.5 (C-5), 31.9 (C-13), 34.3, 34.4 (C-11), 35.8, 36.0 (C-7), 39.7 (C-8), 66.4 (C-15), 68.2 (C-10), 73.2 (C-3), 77.7 (C-2); HMBC nmr (DMSO-d_6) ^{13}C δ (quaternary carbons) 36.6 (C-9), 67.8 (C-4), 75.0, 75.6 (C-1); fabms (glycerol) *m/z* 369/367 $[MH]^+$ (4), 351 (2), 349 (3), 333 (13), 331 (13), 315 (13), 313 (14), 297 (77), 257 (36), 242 (63), 241 (94), 227 (49), 223 (5), 221 (24), 187 (68), 165 (48), 150 (86), 149 (100), 135 (80), 131 (87), 129 (78), 119 (97), 117 (84), 109 (86), 107 (47), 103 (83); hrfabms *m/z* $[MH]^+$ 367.0681 (calcd for $C_{15}H_{25}{}^{79}Br^{35}ClO_3$, 367.0670).

Majapol B [**5**].—Oil; ir ν max (film) 3443, 2950, 1454, 1390, 1368, 1219, 1066, 965, 905, 865, 811, 758, 679, 688 cm^{-1}; ^1H- and ^{13}C-nmr data ($CDCl_3$), see Tables 3 and 4; ^1H nmr (DMSO-d_6) δ 0.94, 0.95 (3H, s), 0.99 (3H, s), 1.13–1.26 (3H, m), 1.58–1.64 (1H, m), 1.67–1.98 (4H, m), 2.04 (1H, m), 3.05, 3.09 (1H, s), 3.25, 3.27 (1H, dd), 3.46 (1H, dd), 4.05 (1H, s), 4.12 (1H, dd), 4.62, 4.63 (1H, t), 4.74, 4.75 (1H, s); HMQC nmr (DMSO-d_6) ^{13}C nmr δ 19.9 (C-14), 21.4 (C-6), 26.3 (C-5), 28.5, 28.8 (C-12), 31.0 (C-13), 33.1 (C-11), 37.6 (C-7), 40.3, 40.4 (C-8), 61.1, 61.3 (C-2), 62.6 (C-15), 64.0 (C-3), 66.5 (C-10); HMBC nmr (DMSO-d_6) ^{13}C δ (quaternary carbons) 36.1 (C-9), 64.2 (C-1), 72.2 (C-4); fabms (glycerol) *m/z* 369/367 [MH] (32), 351 (23), 349 (22), 333 (14), 331 (13), 315 (14), 313 (13), 303 (5), 301 (5), 297 (15), 295 (8), 287 (16), 269 (17), 251 (20), 233 (13), 215 (6), 143 (20), 135 (20), 120 (32), 119 (32), 109 (100), 107 (55); hrfabms *m/z* $[MH]^+$ 367.0684 (calcd for $C_{15}H_{25}{}^{79}Br^{35}ClO_3$, 367.0670).

Majapol C [**6**].—Oil; ir ν max (film) 3425, 2928, 1453, 1389, 1368, 1242, 1124, 1060, 965, 858, 823, 754 cm^{-1}; ^1H- and ^{13}C-nmr data ($CDCl_3$), see Tables 3 and 4; ^1H nmr (DMSO-d_6) δ 0.96 (3H, s), 0.99 (3H, s), 1.18–1.32 (2H, m), 1.43–1.54 (2H, m), 1.55–1.78 (4H, m), 1.88–1.99 (2H, m), 2.02–2.10 (1H, m), 3.06, 3.09 (1H, d), 3.48 (1H, dd), 3.61 (1H, dd), 3.95 (1H, dd), 4.13 (1H, dd), 4.83 (1H, t), 4.98, 4.99 (1H, d); fabms (glycerol) *m/z* 369/367 $[MH]^+$ (5), 351 (7), 349 (7), 333 (3), 331 (3), 316 (6), 315 (4), 314 (6), 313 (4), 297 (6), 287 (7), 269 (6), 251 (5), 233 (10), 167 (59), 147 (89), 145 (43), 121 (20), 109 (100), 107 (30); hrfabms *m/z* [MH] 367.0674 (calcd for $C_{15}H_{25}{}^{79}Br^{35}ClO_3$, 367.0670).

Majapol D [**7**].—Oil; ir ν max (film) 3441, 2943, 1454, 1389, 1368, 1220, 1057, 965, 904, 867, 758, 677 cm^{-1}; ^1H- and ^{13}C-nmr data ($CDCl_3$), see Tables 3 and 4; eims *m/z* 315/313 $[M^+-OH]$ (5), 314 (4), 312 (4), 301 (6), 299 (6), 273 (2), 271 (2), 233 (3), 219 (5), 215 (5), 175 (7), 141 (9), 135 (11), 133 (10), 123 (19), 111 (11), 110 (11), 109 (100), 107 (19); hreims *m/z* $[M]^+$ 330.0831 (calcd for $C_{15}H_{23}{}^{79}BrO_3$, 330.0824).

BIOLOGICAL TESTING.—Two cell lines from the NCI melanoma screening panel (KM-12 and SK-MEL-5) were chosen for a two-day cytotoxicity assay (20) to monitor fractionation. The 60-cell in vitro human tumor screening panel tests have been described previously (3). Differential selectivity pattern comparisons were performed employing the COMPARE algorithm (17,18).

TABLE 4. ^{1}H- (500 MHz, $CDCl_3$) Nmr Data for Majapolone [**3**] and Majapols A [**4**], B [**5**], C [**6**], and D [**7**].[a,b]

Proton	Compound				
	3	**4**	**5**	**6**	**7**
2	6.71, 6.73 d, 10.3	4.12 br s	3.32, 3.34 d, 2.4	3.22, 3.25 d, 5.4	3.13, 3.15 d, 2.9
3	5.95 d, 10.3	3.96 br s	4.35 br s	4.14 dd 5.4, 1.5	3.40, 3.41 d, 2.4
5a	2.38, 2.42 ddd, 17.1, 6.8, 4.9	1.76 m	1.39, 1.43 m	1.56 m	1.57 m
5b	2.62, 2.63 ddd, 17.1, 9.8, 4.9	1.95 m	1.66 m	1.74 m	1.78 m
6a	1.94 m	1.86 m	1.98 m	1.93 m	1.72 m
6b	2.15 ddd, 13.2, 9.3, 4.4	1.92, 1.94 m	2.02 m	2.08, 2.11 m	1.72 m
7	1.84 m	1.67, 1.69 m	1.53 tt	1.56 m	1.58 m
8a	1.24, 1.26 m	1.27, 1.31 m	1.17, 1.20 m	1.21, 1.24 m	1.16, 1.20 m
8b	1.65, 1.79 dt, 12.6, 3.4, 2.9	1.58, 1.66 m	1.65, 1.72 m	1.69, 1.74 m	1.66 m
10	3.89 dd, 3.9, 12.7	3.88 dd 4.3, 12.7	3.86 dd, 4.0, 12.7	3.87 dd 3.9, 12.7	3.86 dd 4.0, 12.7
11a	1.99, 2.01 dq 13.2, 13.2, 13.2, 4.4	1.95 m	1.96 m	1.97 dq 3.9, 12.5, 12.5, 12.7	1.96 dq 13.2, 13.2, 13.2, 4.0
11b	2.22 m	2.15 m	2.17 dq 3.9, 3.9, 3.9, 13.2	2.18 m	2.17 m
12a	1.24, 1.26 m	1.27, 1.31 m	1.18, 1.21 m	1.20, 1.23 m	1.16, 1.20 m
12b	1.72, 1.83 d quintets, 13.2, 3.4, 3.4, 3.4, 2.9	1.64, 1.66 m	1.70, 1.76 m	1.74, 1.79 m	1.70 m
13	1.07, 1.08 s	1.04 s	1.07 s	1.06, 1.07 s	1.05 s
14	1.03, 1.05 s	0.99 s	1.01, 1.02 s	1.02 s	1.00 s
15a		3.47, 3.49 dd 8.3, 1.5	3.44 d, 11.7	3.74 d, 12.2	3.59, 3.61 d, 12.7
15b		3.77, 3.79 dd 8.3, 3.5	3.64 d, 11.7	3.83 d, 12.2	3.75, 3.76 d, 12.7

[a]Values are listed for both isomers, if resolved.
[b]Multiplicity and *J* values (Hz) are given.

ACKNOWLEDGMENTS

We thank E. Menez for collections, T. McCloud for extractions, T. Prather for the 2-cell line screening assays, and A. Monks and D. Scudiero for the 60-cell screening assays.

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Received 16 May 1995