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TWO NEW REARRANGED SESQUITERPENOIDS FROM THE RED ALGA *LAURENCIA OBTUSA*

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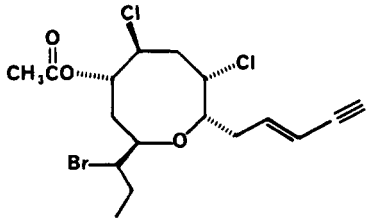
ABSTRACT.—Two new rearranged sesquiterpenoid metabolites, 2,5-epoxybrasil-1(6)-en-9-ol [**3**] and 5,9-dihydroxybrasil-1(6)-en-7-one [**4**], have been isolated along with laurencienyne and brasilenol from the Mediterranean marine alga *Laurencia obtusa*. They possess the unusual skeleton of brasilane. Their structures were determined by detailed spectral analysis. The relative configurations were assigned by NOESY spectroscopy and molecular mechanics calculations.

The red alga *Laurencia obtusa* (Huds.) Lamouroux (Rhodomelaceae) is present in most coastal waters around the world and has received continued attention from several groups of chemists mainly because it is a rather prolific source of metabolites of diverse nature, particularly terpenes and acetogenins (1,2). From material collected along the eastern coast of Sicily one of us had previously isolated a brominated ketal (3), a halogenated diterpenoid (4) and an acetylenic cyclic ether (5) (laurencienyne [**1**]). Two characteristics of this species, observed in a gc-ms study, are the dominance of one major metabolite associated with several minor components which are difficult to separate, and a marked variability of the constituents in the collections from different sites (6).

In this paper we wish to present the isolation and structure elucidation of two novel rearranged sesquiterpenoids present as very minor components and possessing the carbon skeleton of brasilenol [**2**], which was isolated first from *L. obtusa* collected near Athens, Greece, and from the sea hare *Aplysia brasiliana* (7).

Cc and preparative hplc of selected fractions afforded, besides **1** (2.07% extract), the very minor compounds **2**, **3**, and **4** (0.02%, 0.11%, and 0.09% extract, respectively).



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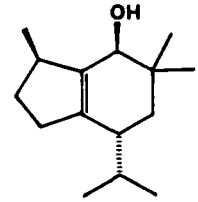

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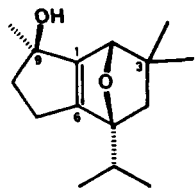

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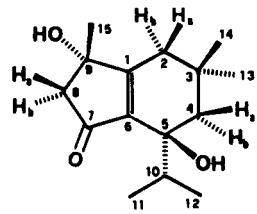

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The molecular formula of $C_{15}H_{24}O_2$ for 2,5-epoxybrasil-1(6)-en-9-ol [**3**] was deduced from its hreims in combination with its ^{1}H- and ^{13}C-nmr spectra. The ir spectrum exhibited significant absorptions (cm^{-1}) for hydroxyl (3440), isolated double bond (1645), and isopropyl (1365 and 1370, same intensity) and indicated the absence of a carbonyl functionality. Only end absorption was noted in the uv spectrum.

The DEPT ^{13}C spectrum (Table 1) displayed two unsaturated quaternary carbons only. Thus, the tricyclic nature of **3** is evident from its four degrees of unsaturation. The rest of the spectrum also showed the presence of three oxygen-bearing carbon atoms [δ 93.5 (s), 83.1 (s), and 80.2 (d)], one more quaternary carbon, and one CH, three CH_2, and five Me groups. Because from the molecular formula there are only two oxygens and the ^{13}C-nmr spectrum shows three carbons which bear an oxygen, there must be one hydroxyl and one ether function. The presence of only one hydroxyl group was confirmed by a differential isotope shift (DIS) experiment (8) (see Experimental) which indicated a shift only for the low field resonance (9,10) at δ 93.5. The ^{1}H spectrum (Table 1) indicated an isopropyl group by the presence of two methyls as doublets (δ 0.99 and 0.98) coupled ($J = 6.9$ Hz) to a methine hydrogen heptet, three methyl singlets (δ 1.52, 1.32, and 0.92), an isolated methylene AB system (δ 1.71 and 1.26, $J = 12.7$ Hz), and an isolated ABCD system due, from the values of the chemical shift and coupling pattern (Table 1), to two vicinal methylene groups. A 2D COSY experiment confirmed the assignments and couplings of all the proton signals.

The absence of olefinic hydrogens, associated with the ^{13}C data, is diagnostic of a tetrasubstituted double bond. At this point, of the 15 carbon atoms, six were accounted for by methyl and isopropyl groups. From the multiplicity of the carbons and the type of the methylene groups, the remaining nine carbons might be accommodated as two fused rings. Furthermore, the observation of five methyl groups excludes the common four-methyl-group composition of most sesquiterpenoids, and a rearranged bicyclic carbon skeleton like that of brasilane can be assumed as a structural base.

The remainder of the ^{1}H spectrum consists of a hydrogen geminal to oxygen (δ 4.28) and an unusual low field (δ 7.75) sharp singlet (D_2O exchangeable) attributable to an intramolecular hydrogen-bonded hydroxyl (11,12) that does not shift upon tenfold dilution. Irradiation of both methyl doublets disclosed all the lines of the ABCD spin system partially overlapped with H-10 heptet. The appearance of this system before and after irradiation is shown in Figure 1, together with the calculated spectrum using the PANIC nmr simulation computer program (see Experimental). The true shifts and the observed coupling constants are given in Table 1. As is readily seen from Figure 1, there is complete agreement between the calculated spectrum and the observed irradiated spectrum.

One-bond HETCOR correlation (Table 1) confirmed the assignment of the protonated carbons in the ^{13}C spectrum of **3**, while HETCOR long-range correlation (Table 1) identified all the quaternary carbons and indicated bond connectivities across them, confirming the carbon skeleton and clarifying the point of attachment of the methyls and isopropyl group to the rings as in structure **3** (apart from the stereochemistry).

Moreover, the hypothetical alternative structure **5** (which shows the same set of variously protonated carbons as well as hydrogen multiplicity) can be ruled out. In fact, the unambiguous assignment of the signal at 144.9 ppm at C-1 was achieved by its long-range cross peaks with deshielded Me-15, with the H-2 singlet, and with H-7. The alternative assignment of this signal at C-6 in structure **3** cannot correlate through four bonds with Me-15. The C-6 signal at δ 146.7 correlates with the A part of the AB spin system of the isolated methylene groups, while this correlation cannot be observed in the case of structure **5**.

The deshielded Me-15 correlates with the methylene protons at δ 2.28 and 1.98,

TABLE 1. ^{1}H- and ^{13}C-nmr Data for Compound **3**.[a]

Position	δ_C	DEPT	δ_H (*J*)	H/C long-range correlation[b]	NOESY[c]
1	144.9	C		H_3-15, H-2, H_a-7	
2	80.2	CH	4.28 s	H_3-13, H_3-14	H_3-13, H_3-14, H_3-15
3	32.4	C		H_3-13, H_3-14, H-2, H_2-4	
4	38.7	CH_2	H_a 1.71 d (12.7) H_b 1.26 d (12.7)	H_3-13, H_3-14, H-2	H_3-14 H_3-14
5	83.1	C		H_3-11, H_3-12, H_2-4	
6	146.7	C		H-2, H_a-4	
7	28.5	CH_2	H_a 2.63 ddd (16.43, 8.84, 4.36) H_b 2.52 ddd (16.43, 8.78, 4.12)		
8	35.8	CH_2	H_a 2.28 ddd (13.73, 8.84, 4.12) H_b 1.98 ddd (13.73, 8.78, 4.36)	H_3-15	
9	93.5	C		H_3-15, H_2-8	
10	31.3	CH	2.07 hept (6.9)	H_3-11, H_3-12, H_b-4	H_3-11, H_3-12
11	18.1	Me	0.99 d (6.9)	H_3-12	
12	16.4	Me	0.98 d (6.9)	H_3-11, H-10	
13	28.3	Me	1.32 s	H_3-14, H_a-4	H-2, H_3-14
14	28.7	Me	0.92 s	H_3-13, H_2-4	H-2, H_3-13, H_2-4
15	21.7	Me	1.52 s	H_2-8	H-2
-OH			7.75 s[d]		

[a] ^{1}H and ^{13}C-nmr spectra were recorded in $CDCl_3$ at 205.13 and 62.9 MHz, respectively; TMS as internal standard; coupling constants are given in Hz.
[b] Long-range correlations were obtained with polarization transfer optimized for $J = 7.5$ and 5.0 Hz.
[c] Recorded in degassed $CDCl_3$ solution.
[d] H-bonded with the ethereal oxygen (see text).

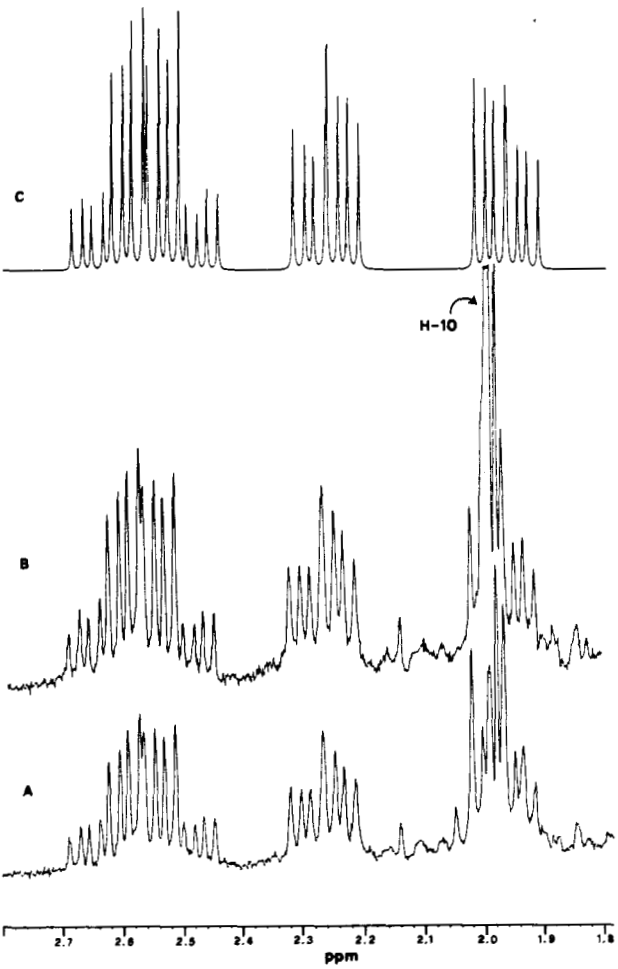
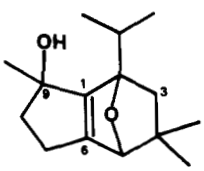


FIGURE 1. Observed ABCD spin system of **3** in ^{1}H-nmr spectrum (A); after irradiation on both methyl doublets at δ 0.985 (B); simulated with PANIC-85 program (C).

thereby establishing the assignment of H-8. The presence of two geminal methyls is evident from the connectivity between H_3-13 and Me-14 and vice versa. The correlation of the oxygenated C-5 at δ 83.1 with Me-11 and Me-12 located the isopropyl group, ruling out the C-2 position. Interpretation of the other ^{1}H/^{13}C long-range correlated data also confirmed the assignment of the secondary ^{13}C signals at δ 38.7, 28.5, and 35.8.

The relative stereochemistry at C-9 was straightforwardly deduced from a NOESY spectrum (Table 1), which showed intense interactions among H-2 (δ 4.28, s), Me-15 (δ 1.52, s), and both Me-13 and Me-14 (δ 1.32 and 0.92, s) suggesting that the former



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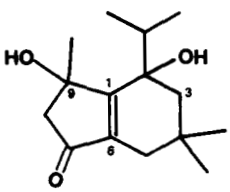

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TABLE 2. ^{1}H- and ^{13}C-nmr Data for Compound **4**.[a]

Position	δ_C	DEPT	δ_H (*J*)	H/C long-range correlation[b]	NOESY[c]
1	175.0	C		H_2-2, H_2-8, H_3-15	
2	36.5	CH_2	H_a 2.16 dd (17.8, 1.5) H_b 2.06 d (17.8)	H_3-14, H_3-13	H_3-15 H_3-13, H_3-14
3	30.5	C		H_2-2, H_2-4, H_3-13, H_3-14	
4	40.7	CH_2	H_a 1.44 dd (14.5, 1.5) H_b 1.46 d (14.5)	H_3-14	
5	73.4	C		H_3-11, H_3-12, H-10	
6	139.3	C		H_2-2, H_2-4	
7	204.5	C		H_2-8	
8	52.6	CH_2	H_a 2.60 d (17.8) H_b 2.66 d (17.8)	H_3-15	H_3-15
9	75.3	C		H_3-15, H_2-8	
10	36.3	CH	2.38 hept (7.0)	H_3-11, H_3-12	H_3-11, H_3-12
11	15.0	Me	0.97 d (7.0)	H_3-12	H_3-12, H-10
12	18.2	Me	0.67 d (7.0)	H_3-11	H_3-11, H-10
13	25.9	Me	0.95 s	H_3-14	H_3-14, H_b-2
14	32.8	Me	1.13 s	H_3-13	H_3-13, H_3-15, H_b-2
15	25.8	Me	1.48 s	H_a-8	H_3-14, H_a-2, H_a-8
-OH			2.35 s		

[a] ^{1}H and ^{13}C-nmr spectra were recorded in $CDCl_3$ at 205.13 and 62.9 MHz, respectively; TMS as internal standard; coupling constants are given in Hz.
[b] Long-range correlations were obtained with polarization transfer optimized for $J = 7.5$ and 5.0 Hz.
[c] Recorded in degassed $CDCl_3$ solution.

is cis to the Me-15. Because of their close steric proximity, similar interaction was observed among Me-14 (δ 0.92, s) and both H-4 (δ 1.71 and 1.26, AB system). Thus the relative configuration of **3** can be defined as 2*S**, 5*S**, and 9*R**.

The second new metabolite isolated from *L. obtusa*, 5,9-dihydroxybrasil-1(6)-en-7-one [**4**], displayed a very weak molecular ion in eims. However a clear $[M + H]^+$ was observed in fabms. Hreims established the elemental composition $C_{15}H_{22}O_2$ for the $[M - H_2O]^+$ fragment. Its uv spectrum showed an α,β,γ-alkyl-substituted cyclopentenone [λ max 236 (observed), 239 nm (calcd)] (13), while the ir spectrum showed absorption bands (cm^{-1}) characteristic of hydroxyl (3380), an α,β-unsaturated ketone (1690 and 1640), and an isopropyl group (1362 and 1368, same intensity).

Comparison of the ^{13}C spectrum of **3** and **4** (Tables 1 and 2, respectively) showed significant differences in chemical shifts, indicating the presence of different substituents. The DEPT ^{13}C spectrum (Table 2) displayed one carbonyl group and two unsaturated quaternary carbons. Thus, from this information and from the four degrees of unsaturation, the compound is bicyclic.

Moreover, two quaternary carbons bear oxygen (δ 73.4 and 75.3), and a DIS experiment (see Experimental) showed a significant shift in these resonances. This information assured the presence of two hydroxy groups in compound **4**. The ^{13}C spectrum also showed the presence of five methyl groups, suggesting again the presence of a nonisoprenoid sesquiterpene skeleton, as in brasilenol and compound **3**. Of these methyls, two belong to an isopropyl group, appearing as doublets in the ^1H spectrum (δ 0.97 and 0.67, $J = 7.0$ Hz) and correlating in 2D COSY spectrum to a methine hydrogen (δ 2.38, $J = 7.0$ Hz). The rest of the ^{13}C spectrum also displayed three CH_2 groups and one more quaternary carbon. The ^1H-nmr spectrum also indicated a downfield methyl geminal to a hydroxylated carbon (δ 1.48, s), two methyl singlets (δ 1.13 and 0.95), and three isolated methylene AB groups. One of the last appeared strongly deshielded in both ^1H and ^{13}C spectra and would be vicinal to the carbonyl group.

The two-dimensional spectra (one-bond and long-range $^1H/^{13}C$ correlations, Table 2) confirmed the assignment of all the carbons and established bond connections across them. In particular correlation between C-13 and H_3-14 and between C-14 and H_3-13 established the presence of a *gem*-dimethyl group. Moreover, connectivity of the carbonyl with the hydrogens of the downfield methylene was observed. This methylene, in turn, showed a three-bond connectivity to the deshielded Me-15; thus, the isopropyl group has to be located in the six-membered ring and not in the 9-position. The ^{13}C resonance at δ 175.0 is correlated with the Me-15 signal, and it can be unambiguously assigned to C-1 instead of to C-6, which lacks this three-bond connectivity. It is reported that in an enone system the carbon α to the carbonyl exhibits a higher field resonance with respect to the carbonyl, on the β carbon (14). At this point, however, construction of the structure **6** is still possible. Because the ^{13}C signal at δ 139.3 was assigned to C-6 vicinal to the carbonyl the proposed structure **4** is consistent with the three-bond connectivity of this resonance with H_2-2 and H_2-4, while in the alternative structure **6**, C-6 carbon cannot be correlated with both methylene groups of the cyclohexene ring. In this fashion, structure **6** is ruled out. The location of the isopropyl group at C-5 rather than at C-3 is confirmed by the correlation of C-5 with Me-11, Me-12, and H-10.

The stereochemistry of compound **4** was difficult to assign by the NOESY spectrum in $CDCl_3$ (Table 2); this experiment, in fact, did not provide a clear correlation between the H_a-4 and methyl(s) attached at C-3 because it was almost impossible to distinguish clearly the H_a-4 signal at δ 1.44 from the very close H_b-4 signal (δ 1.46) in the 2D cross peak map.

To better understand the NOESY correlations and to get some insight into the preferred conformation of the cyclohexene ring, a computational analysis was carried out

using the MacroModel program (MM2 force field, see Experimental) (15,16). A stereoscopic view of this conformation is shown in Figure 2, which reports a diaxial trans configuration between the two hydroxyls at C-9 and C-5. A similar preferred conformation possessing the pseudo-equatorial isopropyl group was found for the diastereomer pos-

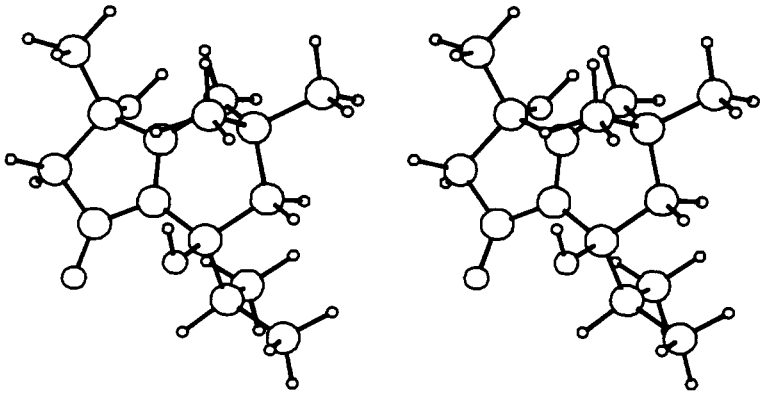

FIGURE 2. Stereostructure plot of lowest-energy conformation calculated using MacroModel program (MM2 force field) for trans diastereomer of compound **4**.

sessing a diaxial cis configuration between the two hydroxyls; it is shown in Figure 3. In both diastereomers, the half-chair conformation of the cyclohexene ring was calculated to have a much lower energy with respect to the boat conformation where the isopropyl group assumes a pseudo-axial position. The NOESY correlations in Tables 2 and 3 were thus examined considering only the half-chair (not the boat) conformation for the cyclohexene ring and made it possible to distinguish between the configurational alternatives in Figures 2 and 3. In the 2D spectrum in $CDCl_3$, an nOe correlation was observed between the signals due to the Me-15 and Me-14 (δ 1.13, see Table 2). This enhancement is possible only in the structure **4** and Figure 2 where the Me-15 shows a cis interaction at 2.9 Å with Me-14, ruling out the alternative diastereomer shown in Figure 3 where Me-15 is without the normal distance of nOe correlation with the methyls attached at C-3. To assign unequivocally the diastereotopic methyls at C-3 and the hy-

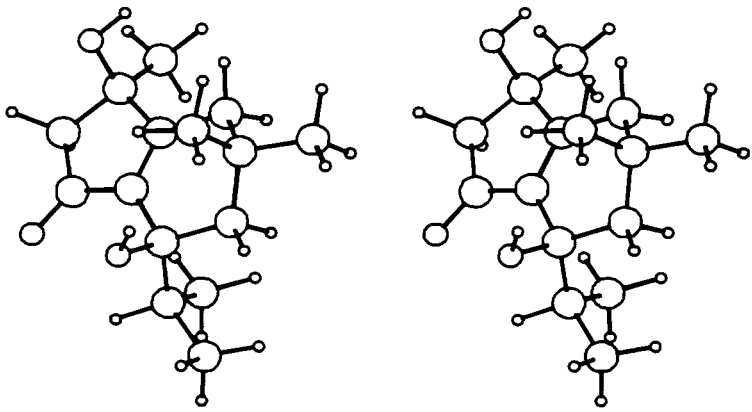

FIGURE 3. Stereostructure plot of lowest-energy conformation calculated by MM2 for cis diastereomer of compound **4**.

TABLE 3. ^{1}H-nmr and NOESY data for Compound **4** in C_5D_5N.[a]

Position	δ_H (J)	NOESY
2	H_a 2.42 dd (18.0, 1.7)	H_3-13, H_3-14, H_3-15
	H_b 2.35 d (18.0)	H_3-13
4	H_a 1.68 dd (14.5, 1.7)	
	H_b 1.61 d (14.5)	H_3-12, H_3-13
8	H_a 2.77 d (17.8)	H_3-15
	H_b 3.03 d (17.8)	
10	3.06 hept (7.0)	H_3-11, H_3-12
11	1.13 d (7.0)	H_3-12, H-10
12	0.68 d (7.0)	H_3-11, H_b-4, H-10
13	1.12 s	H_3-14, H_2-2, H_b-4
14	1.24 s	H_3-13, H_a-2
15	1.55 s	H_3-14, H_a-2, H_a-8

[a] ^{1}H-nmr spectrum was recorded in C_5D_5N at 205.13 MHz; TMS as internal standard; coupling constants are given in Hz.

drogens at C-4, and to confirm the pseudo-equatorial conformation of the isopropyl group, a NOESY experiment in pyridine-d_5 was performed. In this solvent the H_2-4 resonances were clearly separated (Table 3) due to a different solute-solvent interaction (17). The Me-15 is nOe correlated with the pseudo-equatorial H_a-2 (δ 2.42) which is, in turn, correlated to the Me-14. It was diagnostic of a 4-bond W coupling (1.7 Hz) between H_a-2 (δ 2.42) and H_a-4 (δ 1.68), which confirmed the equatorial coplanar position of these protons (18). The observation of an nOe correlation of the pseudo-axial H_b-4 with one of the *gem*-dimethyl groups at C-10 distinguished a pseudo-equatorial from a pseudo-axial isopropyl group. Only the former would be expected to show this observed correlation. The computational analysis in Figure 2 showed, in addition, a restricted rotation of the isopropyl group leading to a steric proximity of only Me-12 with H_b-4. A straightforward correlation was also observed in the NOESY spectrum between the H_b-4 (δ 1.61) and the Me-13 (δ 1.12), confirming again their close cis (ax-eq) steric proximity.

Thus, based on the convergent computational analysis and NOESY findings, the relative configuration of **4** can be defined as 5*S**, 9*S**.

The finding that the structures of **3** and **4** bear the methyl and isopropyl groups in the same position as the brasilane skeleton, differing only for the oxygenated groups, corroborates the hypothesis (1) that brasilanes are rearranged products generated from a 1,3 shift in a poitane and stimulates the search for further brasilane representatives.

EXPERIMENTAL

GENERAL METHODS.—Mp's were taken on a Kofler block and are uncorrected. Eims (18 eV), low and high resolution, were obtained on a Kratos MS 50 mass spectrometer. Fab spectra in positive mode were obtained using an ION-TECH gun in a matrix of 2,4-dinitrobenzylalcohol. Ir and uv spectra were obtained on a Perkin-Elmer model 684 and a Perkin-Elmer model 330 spectrophotometer, respectively. ^{1}H-nmr and ^{13}C-nmr spectra were measured on a Bruker AC-250 instrument operating at 250.13 MHz and 62.9 MHz, respectively, using $CDCl_3$ as solvent. Chemical shifts are quoted in ppm (δ) relative to TMS. DEPT, COSY, HETCOR, and NOESY experiments were performed using standard Bruker microprograms. Long-Range HETCOR was performed using a modified version of the Freeman-Morris pulse sequence (19) with the introduction of one-step low-pass J filter, to suppress one-bond correlations, and a BIRD pulse to eliminate one-bond modulations of long-range cross-peak intensities, as described by Zektzer *et al.* (20) and Salazar *et al.* (21); $\Delta_1 = 100$, $\Delta_2 = 50$, $\tau = 3.3$ ms for $J = 5$ Hz and $\Delta_1 = 66.6$, $\Delta_2 = 33.3$, $\tau = 3.3$ ms for $J = 7.5$ Hz were used. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Preparative liquid chromatography (plc) was carried out on a Jobin-Yvon LC Miniprep instrument, using Kieselgel 60 (Merck) as stationary phase. Molecular Modeling and Force Field calculations (MM2) were performed on a Digital Vax-Station 2000 computer using MacroModel Version 2.5 pro-

gram. Simulation and iteration of nmr spectra were performed with a PANIC 85 Bruker program running on a Bruker Aspect 3000 computer.

PLANT MATERIAL.—*L. obtusa* was collected at Castelluccio, near Augusta, eastern Sicily, in October 1988, in littoral zones. A voucher specimen was deposited in the Herbarium of the Institute of Botany, Catania, Italy.

EXTRACTION AND PURIFICATION.—The fresh alga (approximately 15 kg wet wt) was immediately soaked in iPrOH and left steeping for 3 months. The material was then filtered, and the algal residue was repeatedly homogenized with iPrOH in a Waring blender and filtered. The exhausted dried powder residue weighed 720 g. The iPrOH/H_2O solution was concentrated under vacuum and partitioned with Et_2O and $NaNO_3$-saturated solution. The Et_2O layer was dried with Na_2SO_4 and evaporated to give 30 g of dark green oil. The extract was applied to an open column (3 × 100 cm) of Si gel and eluted with increasing concentrations of Et_2O in petroleum ether. Fractions of 250 ml were collected, and those exhibiting similar tlc profiles were combined. Fraction 7 was subjected to plc [eluent systems, in order, Et_2O-C_6H_{14} (3:47) and CH_2Cl_2-C_6H_{14} (1:3)] to yield brasilenol (7 mg, 0.02% extract), identified by comparison of its physical and spectral data, including $[\alpha]^{25}D$ 38.2° (c = 0.6, $CHCl_3$) (7). Fraction 12 gave, without other purification, laurencienyne (620 mg, 2.07% extract) identified by comparison of its physical and spectral data, including $[\alpha]^{25}D$ 48.2° (c = 1.5, $CHCl_3$) (5). Fraction 13 was subjected to plc (gradient of CH_2Cl_2 in C_6H_{14} from 1:1 to 3:2) to yield 2,5-epoxybrasil-1(6)-en-9-ol [**3**] (32 mg, 0.11% extract). Fractions 26–30 were subjected to plc [Et_2O-CH_2Cl_2 (1:3)] to yield 5,9-dihydroxybrasil-1(6)-en-7-one [**4**] (26 mg, 0.09% extract).

2,5-Epoxybrasil-1(6)-en-9-ol [**3**] was obtained as colorless oil which was crystallized from cyclohexane-Et_2O (1:1): mp 121–123°; $[\alpha]^{25}$ (λ) +5.0° (589), +4.5° (578), +5.2° (546), +7.0° (436) (c = 2.5, EtOH); ir ν max (liquid film, before crystallization) cm^{-1} 3440, 1645, 1370, 1365, 1080; hrms $[M]^+$ 236.1782 (calcd for $C_{15}H_{24}O_2$, 236.1776); ms *m/z* (%) 236 (5), 218 (24), 190 (9), 167 (34), 163 (77), 149 (30), 145 (39), 121 (39), 107 (29), 95 (28), 71 (40), 55 (27), 43 (100); ^1H and ^{13}C nmr see Table 1.

5,9-Dihydroxybrasil-1(6)-en-7-one [**4**] was obtained as pale yellow oil and was crystallized from cyclohexane-Et_2O (1:1): mp 126–128°; $[\alpha]^{25}$ (λ) +7.1° (589), +3.3° (578), +1.6° (546) (c = 3.0, EtOH); ir ν max (liquid film, before crystallization) cm^{-1} 3470, 3385, 1690, 1640, 1368, 1362, 1260, 1180, 1140; uv λ max (EtOH) 236 nm (ε = 7160); hrms $[M-H_2O]^+$ 234.1626 (calcd for $C_{15}H_{22}O_2$, 234.1620); ms *m/z* (%) 234 (33), 219 (18), 209 (100), 191 (24), 167 (13), 153 (11), 139 (7), 121 (5), 111 (8), 83 (6), 42 (8); ^1H and ^{13}C nmr see Table 2.

DIFFERENTIAL ISOTOPE SHIFT (DIS) OF **3**.—Differential spectra were obtained with a 5 mm o.d. coaxial dual cell. The inner and outer tube contained the same amount of a solution obtained by dissolving 20 mg of **3** in 0.3 ml of $CDCl_3$. H_2O (50 μl) was added to the inner tube, while 50 μl of D_2O were added to the outer. Chemical shifts were quoted relative to the central signal of $CDCl_3$ (77.0 ppm). The measured DIS value ($\delta^{13}C_{CDCl_3/H_2O}$-$\delta^{13}C_{CDCl_3/D_2O}$) was 0.08 ppm for C-9. No shifts were observed for all the remaining carbons.

DIFFERENTIAL ISOTOPE SHIFT (DIS) OF **4**.—Differential spectra of **4** were obtained as above using a solution of 18 mg in 0.3 ml of $CDCl_3$. The observed DIS values were 0.12 ppm for C-9 and 0.09 ppm for C-5. Negligible or no shifts were observed for the remaining carbons.

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