# Sesquiterpenes from the Red Alga *Laurencia tristicha*

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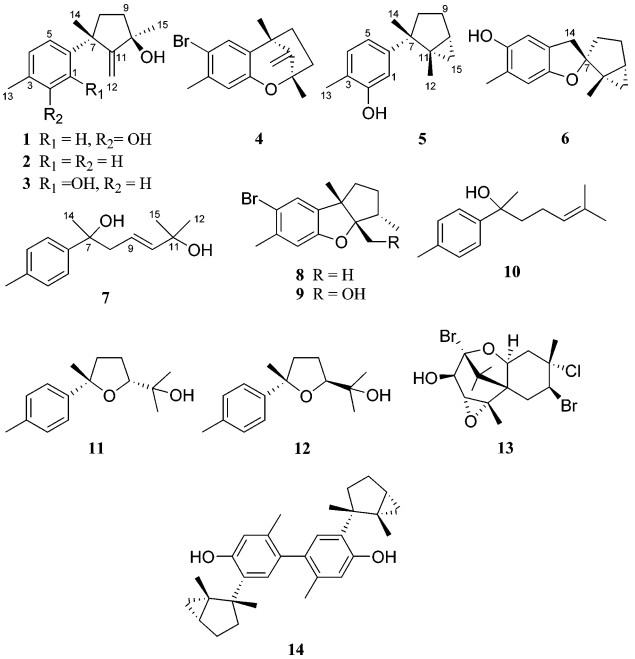
Seven new sesquiterpenes (**1**−**7**), together with seven known sesquiterpenes, aplysin (**8**), aplysinol (**9**), gossonorol (**10**), 7,10-epoxy-*ar*-bisabol-11-ol (**11**), 10-epi-7,10-epoxy-*ar*-bisabol-11-ol (**12**), johnstonol (**13**), and laurebiphenyl (**14**), have been isolated from the red alga *Laurencia tristicha*. The structures of new compounds were established as laur-11-en-2,10-diol (**1**), laur-11-en-10-ol (**2**), laur-11-en-1,10-diol (**3**), 4-bromo-1,10-epoxylaur-11-ene (**4**), cyclolauren-2-ol (**5**), laurentristich-4-ol (**6**), and *ar*-bisabol-9-en-7,11-diol (**7**) by means of spectroscopic methods including IR, HRMS, and 1D and 2D NMR techniques. Compound **6** possessed a novel rearranged skeleton. All compounds were tested against several human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), hepatoma (Bel 7402), colon cancer (HCT-8), and HELA cell lines. Laurebiphenyl (**14**) showed moderate cytotoxicity against all tested cell lines, with $IC_{50}$ values of 1.68, 1.22, 1.91, 1.77, and 1.61 $\mu$g/mL, respectively. Other compounds were inactive ($IC_{50}$ > 10 $\mu$g/mL).

Marine red algae of the genus *Laurencia* (Ceramiales, Rhodomelaceae) collected in various parts of the world have proved to produce a rich and diverse range of secondary metabolites including sesquiterpenes,[1−8] $C_{15}$ acetogenins,[9−11] and a few di- and triterpenes.[12] There are, however, very few reports concerning the secondary metabolites isolated from *Laurencia* species from Chinese waters. As part of our program to assess systematically the chemical and biological diversity of seaweeds widely distributed in the south Sea of China,[13−16] the red alga *Laurencia tristicha* Tseng, Chang, E. Z. et B. M. Xia was collected from the Naozhou Island, Zhanjiang City, China. The chemical investigation of this alga resulted in the isolation and structural elucidation of 14 sesquiterpenes including seven new compounds, laur-11-en-2,10-diol (**1**), laur-11-en-10-ol (**2**), laur-11-en-1,10-diol (**3**), 4-bromo-1,10-epoxylaur-11(15)-ene (**4**), cyclolauren-2-ol (**5**), laurentristich-4-ol (**6**), and *ar*-bisabol-9-en-7,11-diol (**7**), of which **6** possessed an unusual novel carbon skeleton not satisfying the isoprene rule and biogenetically proposed from rearrangement of a cyclolaurane skeleton, and **4** was synthetically reported as a racemate.[17] By comparing with corresponding literature data six known compounds were readily identified as aplysin (**8**),[18−20] aplysinol (**9**),[21−23] gossonorol (**10**),[24,25] 7,10-epoxy-*ar*-bisabol-11-ol (**11**), 10-epi-7,10-epoxy-*ar*-bisabol-11-ol (**12**),[26,27] and laurebiphenyl (**14**),[28] and the structure of compound **13** was mainly established by 2D NMR experiments and confirmed by X-ray crystallographic analysis to be identical with johnstonol.[29] Because some confusing trivial names have been given to aromatic sesquiterpenes with the bicyclic laurane and tricyclic cyclolaurane carbon skeletons from species of the genus *Laurencia* (for example, laurinterol is a compound with a tricyclic cyclolaurane skeleton, but isolaurinterol (4-bromolaur-11-en-1-ol) and allolaurinterol [4-bromolaur-10(15)en-1-ol] are compounds with bicyclic laurane skeletons),[23,30,31] the new compounds **1**−**5** were designated on the basis of the skeletons suggested by Crews.[2] We report here the isolation and structural elucidation of compounds **1**−**7**. In addition, NMR data of johnstonol were given in the Experimental Section due to absence of these data in the literature.[29] All compounds were tested against several human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), hepatoma (Bel 7402), colon cancer (HCT-8), and HELA cell lines. Laurebiphenyl (**14**) showed moderate cytotoxicity against all tested cell lines, with $IC_{50}$ values of 1.68, 1.22, 1.91, 1.77, and 1.61 $\mu$g/mL, respectively. Other compounds were inactive ($IC_{50}$ > 10 $\mu$g/mL).




## Results and Discussion

Compound **1** was obtained as colorless needles (MeOH), mp 99−100 °C, $[\alpha]_D^{20}$ −70.5° (*c* 0.19, MeOH). The IR spectrum displayed absorption bands for hydroxy (3417 and 3168 $cm^{-1}$) and aromatic ring (1622, 1585, and 1514 $cm^{-1}$)

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**Table 1.** $^1$H NMR Data for Compounds **1**−**7**[a]

| no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1 | 6.84 s | 7.25 d (8.0) | | | 6.95 s | | 7.35 d (8.0) |
| 2 | | 7.07 d (8.0) | 6.67 s | 6.58 s | | 6.41 s | 7.10 d (8.0) |
| 4 | 6.96 d (7.5) | 7.07 d (8.0) | 6.54 d (8.0) | | 6.96 d (7.5) | | 7.10 d (8.0) |
| 5 | 6.75 d (7.5) | 7.25 d (8.0) | 7.13 d (8.0) | 7.21 s | 6.80 d (7.5) | 6.63 s | 7.35 d (8.0) |
| 8α | 1.97 ddd (13.0, 6.5, 6.5) | 2.01 ddd (13.0, 6.5, 6.5) | 2.37 ddd (13.0, 6.5, 6.5) | 2.04 ddd (12.0, 12.0, 3.0) | 1.37 m | 1.45 m | (a) 2.41 dd (13.5, 6.0) |
| 8*β* | 1.90 ddd (13.0, 7.0, 6.5) | 1.93 ddd (13.0, 7.0, 6.5) | 1.98 ddd (13.0, 7.0, 6.5) | 1.69 ddd (12.0, 12.0, 6.5) | 1.58 ddd (12.5, 7.5, 7.5) | 1.85 dd (14.0, 8.0) | (b) 2.45 dd (13.5, 6.0) |
| 9α | 1.58 ddd (13.0, 6.5, 7.0) | 1.58 ddd (13.0, 7.0, 6.5) | 1.59 ddd (13.0, 7.0, 6.5) | 1.88 ddd (12.0, 12.0, 3.0) | 1.58 ddd (12.5, 7.5, 7.5) | 1.64 dd (12.0, 7.5) | 5.54 ddd (15.5, 6.0, 6.0) |
| 9*β* | 1.77 ddd (13.0, 6.5, 6.5) | 1.81 ddd (13.0, 6.5, 6.5) | 1.77 ddd (13.0, 6.5, 6.5) | 2.19 ddd (12.0, 12.0, 6.5) | 1.92 m | 1.98 m | |
| 10 | | | | | 1.05 ddd (7.5, 4.0, 4.0) | 1.25 ddd (7.5, 4.0, 4.0) | 5.59 d (15.5) |
| 12a | 5.38 s | 5.40 s | 5.46 s | (a) 4.97 s | 1.20 s | 1.06 s | 1.17 s |
| 12b | 4.86 s | 4.88 s | 4.89 s | (b) 5.09 s | | | |
| 13 | 2.13 s | 2.26 s | 2.20 s | 2.22 s | 2.14 s | 2.12 s | 2.29 s |
| 14α | 1.41 s | 1.44 s | 1.57 s | 1.49 s | 1.29 s | 2.89 d (15.5) | 1.46 s |
| 14*β* | | | | | | 3.27 d (15.5) | |
| 15α | 1.28 s | 1.28 s | 1.32 s | 1.52 s | 0.58 dd (5.0, 4.0) | 0.45 dd (5.0, 4.0) | 1.17 s |
| 15*β* | | | | | 0.38 dd (7.5, 5.0) | 0.29 dd (7.5, 5.0) | |
| Ar*OH* | 7.94 brs | | 8.12 brs | | 7.85 brs | 7.41 brs | |
| *OH* | 3.59 brs | 3.59 brs | 3.51 brs | | | | 3.71 brs; 3.34 brs |

[a] $^1$H NMR data were measured in acetone-$d_6$ at 500 MHz. Proton coupling constants ($J$) in Hz are given in parentheses. The assignments were based on DEPT, $^1$H−$^1$H COSY, HSQCm and HMBC experiments.

**Table 2.** $^{13}$C NMR Data for Compounds **1**−**7**[a]

| no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1 | 113.9 CH | 127.0 CH | 155.9 qC | 153.6 qC | 113.9 CH | 153.5 qC | 125.8 CH |
| 2 | 155.7 qC | 129.4 CH | 118.0 CH | 118.5 CH | 156.2 qC | 111.1 CH | 129.1 CH |
| 3 | 122.0 qC | 135.6 qC | 137.4 qC | 137.3 qC | 121.8 qC | 123.8 qC | 135.9 qC |
| 4 | 131.0 CH | 129.4 CH | 120.2 CH | 114.7 qC | 131.0 CH | 149.4 qC | 129.1 CH |
| 5 | 118.1 CH | 127.0 CH | 128.9 CH | 127.3 CH | 118.5 CH | 112.1 CH | 125.8 CH |
| 6 | 148.6 qC | 146.7 qC | 131.7 qC | 134.4 qC | 148.5 qC | 125.6 qC | 147.0 qC |
| 7 | 50.8 qC | 50.8 qC | 50.0 qC | 46.0 qC | 48.6 qC | 98.2 qC | 73.9 qC |
| 8 | 40.0 $CH_2$ | 39.9 $CH_2$ | 37.2 $CH_2$ | 42.2 $CH_2$ | 38.7 $CH_2$ | 36.6 $CH_2$ | 48.2 $CH_2$ |
| 9 | 39.8 $CH_2$ | 39.8 $CH_2$ | 40.1 $CH_2$ | 37.8 $CH_2$ | 26.2 $CH_2$ | 26.2 $CH_2$ | 122.5 CH |
| 10 | 79.0 qC | 78.9 qC | 79.1 qC | 83.8 qC | 24.2 CH | 25.6 CH | 143.2 CH |
| 11 | 167.3 qC | 167.4 qC | 167.3 qC | 155.8 qC | 30.0 qC | 31.6 s qC | 70.1 qC |
| 12 | 108.1 $CH_2$ | 108.2 $CH_2$ | 108.1 $CH_2$ | 101.6 $CH_2$ | 18.5 $CH_3$ | 15.2 $CH_3$ | 30.4 $CH_3$ |
| 13 | 15.6 $CH_3$ | 20.8 $CH_3$ | 20.7 $CH_3$ | 22.4 $CH_3$ | 15.6 $CH_3$ | 16.5 $CH_3$ | 20.9 $CH_3$ |
| 14 | 30.7 $CH_3$ | 30.7 $CH_3$ | 28.1 $CH_3$ | 18.4 $CH_3$ | 26.5 $CH_3$ | 36.7 $CH_2$ | 29.6 $CH_3$ |
| 15 | 29.0 $CH_3$ | 29.0 $CH_3$ | 28.8 $CH_3$ | 21.0 $CH_3$ | 16.3 $CH_2$ | 14.5 $CH_2$ | 30.4 $CH_3$ |

[a] $^{13}$C NMR data were measured in acetone-$d_6$ at 125 MHz. The assignments were based on DEPT, $^1$H−$^1$H COSY, HSQC, and HMBC experiments.

functional groups. The EIMS exhibited a molecular ion at *m/z* 232 $[M]^+$, and the molecular formula was determined as $C_{15}H_{20}O_2$ by the HREIMS at *m/z* 232.1466 $[M]^+$. The $^1$H NMR spectrum ($Me_2CO$-$d_6$) of **1** showed three methyl singlets at $\delta$ 1.28 (3H, s, $H_3$-15), 1.41 (3H, s, $H_3$-14), and 2.13 (3H, s, $H_3$-13), a pair of singlets characteristic for an exocyclic methylene at $\delta$ 4.86 (1H, s, H-12b) and 5.38 (1H, s, H-12a), and four multiplets assignable to two mutually coupled methylenes at $\delta$ 1.58 (1H, ddd, $J$ = 13.0, 7.0, and 6.5 Hz, H-9α), 1.77 (1H, ddd, $J$ = 13.0, 6.5, and 6.5 Hz, H-9*β*), 1.90 (1H, ddd, $J$ = 13.0, 7.0, and 6.5 Hz, H-8*β*), and 1.97 (1H, ddd, $J$ = 13.0, 6.5, and 6.5 Hz, H-8α), in addition to signals attributed to a 1,2,4-trisubstituted (or 1,3,4-trisubstituted) benzene ring at $\delta$ 6.75 (1H, d, $J$ = 7.5 Hz, H-5), 6.84 (1H, s, H-1), and 6.96 (1H, d, $J$ = 7.5 Hz, H-4), a phenolic hydroxy at $\delta$ 7.94 (1H, brs, exchangeable), and an alcoholic hydroxy at $\delta$ 3.59 (1H, brs, exchangeable). These data suggested that **1** was laur-11-en-10-ol[23] or laur-10(15)-en-11-ol[30,32] with an additional hydroxy group substituting on the benzene ring moiety. The $^{13}$C NMR and DEPT spectral data (Table 2) confirmed this suggestion by showing 15 carbon signals consisting of three methyls, three methylenes (one $sp^2$ hybrid), three quaternary carbons (one $sp^2$ hybrid and one oxygenated), and the trisubstituted benzene ring. On the basis of 2D NMR spectroscopic analysis including $^1$H−$^1$H gCOSY, gHSQC, and gHMBC experiments of **1** the position of the double bond of the cyclopentane moiety and the hydroxy group on the benzene ring was established. The $^1$H−$^1$H gCOSY and gHSQC experiments resulted in the unambiguous assignment of the protonated carbons and their corresponding protons in the NMR spectra (Table 1). In the HMBC spectrum of **1** long-range correlations of C-11 with $H_2$-8, $H_2$-9, $H_3$-14, and $H_3$-15 and correlations of both C-7 and C-10 with $H_2$-12 unequivocally revealed that the double bond was between C-11 and C-12 of the cyclopentane moiety, while HMBC correlations from both H-1 and H-5 to C-7 and from $H_3$-13 to C-2, C-3, and C-4 demonstrated that the hydroxy group was at C-2 of the benzene ring moiety. The relative configuration of **1** was determined by an NOE difference experiment. Irradiation of $H_3$-15 gave NOE enhancements of H-1, H-5, H-8α, and H-12a, demonstrating that the methyl group at C-10 and the aromatic ring were on the same side of the cyclopentane ring. From the biogenetic point view,[33] the absolute configuration at C-7 of **1** was proposed to be identical to that of the co-occurring (−)-aplysin, of which the absolute configuration has been determined by the enantiocontrolled synthe-

sis.[19] Consequently, the structure of **1** was proposed as (1*R*,3*R*)-(−)-3-(3-hydroxy-4-methylphenyl)-1,3-dimethyl-2-methylidenecyclopentanol, designated as laur-11-en-2,10-diol.

Compound **2** was obtained as colorless needles (MeOH), mp 62−63 °C, $[\alpha]_D^{20}$ −32.7° (*c* 0.56, MeOH) and displayed absorption bands for a hydroxyl (3342 $cm^{-1}$) and an aromatic ring (1512 $cm^{-1}$) in the IR spectrum. The EIMS exhibited a molecular ion at *m/z* 216 $[M]^+$, and the molecular formula was determined as $C_{15}H_{20}O$ by the HREIMS at *m/z* 216.1507 $[M]^+$, which was one less oxygen atom than that of **1**. The $^1H$ NMR spectrum of **2** showed an aromatic methyl singlet at $\delta$ 2.26 (3H, s, $H_3$-13) and a characteristic $A_2B_2$ spin coupling system due to a 1,4-disubstituted benzene ring at $\delta$ 7.07 and 7.25 (each 2H, d, $J$ = 8.0 Hz). The remaining signals were almost identical to those of the cyclopentane moiety of **1**. These data revealed that **2** was an analogue of **1** deoxygenated at C-2 of the benzene moiety, which was confirmed by $^{13}C$ NMR data (Table 2), 2D NMR, and NOE difference experiments ($^1H-^1H$ COSY, HSQC, and HMBC) of **2**. Therefore, the structure of **2** was determined as (1*R*,3*R*)-(−)-3-(4-methylphenyl)-1,3-dimethyl-2-methylidenecyclopentanol, named laur-11-en-10-ol.

Compound **3** was obtained as colorless needles (MeOH), mp 95−97 °C, $[\alpha]_D^{20}$ −93.8° (*c* 0.08, MeOH), and showed IR, EIMS, and NMR spectral features similar to those of **1**, indicating **3** was an isomer of **1**. A comparison of the $^1H$ NMR data between **1** and **3** indicated that H-4 and H-5 of **3** were shifted by $\Delta\delta$ 0.42 (upfield) and $\Delta\delta$ 0.38 (downfield) ppm, respectively, demonstrating that the phenolic hydroxy group was at C-1 in **3** instead at C-2 of **1**. This was confirmed by the comparison of the $^{13}C$ NMR data between **1** and **3** (Table 2), as well as by gHSQC and gHMBC experiments of **3**. Although some of the NMR data for the cyclopentane moiety of **3** were significantly different from those of **1**, the NOE difference experiment of **3** showed enhancements of H-5, H-12a, and the phenolic hydroxy proton by irradiation of $H_3$-15, demonstrating that the configuration of the cyclopentane moiety of **3** was identical to that of **1**. Therefore, the structure of **3** was proposed as (1*R*,3*R*)-(−)-3-(2-hydroxy-4-methylphenyl)-1,3-dimethyl-2-methylidenecyclopentanol, designated as laur-11-en-1,10-diol. As an intermediate in the enantiocontrolled synthesis of (−)-debromoaplysin and (−)-aplysin, the epimer at C-10 of **3** has been reported.[19]

Compound **4** was obtained as colorless needles (MeOH), mp 74−76 °C, $[\alpha]_D^{20}$ +70.9° (*c* 0.16, MeOH). The IR spectrum displayed absorption bands for an aromatic ring (1604, 1554, and 1479 $cm^{-1}$). The EIMS exhibited characteristic monobrominated molecular ions at *m/z* 292/294 (1:1) $[M]^+$. The $^1H$ NMR spectrum of **4** (Table 1) was similar to those of compounds **1**−**3** except that the aromatic region displayed two uncoupled aromatic proton singlets due to a 1,2,4,5-tetrasubstituted benzene ring at $\delta$ 6.58 (1H, s, H-2) and 7.21(1H, s, H-5), indicating that **4** was a brominated analogue of **1** or **3** with the bromine atom substituted on the benzene ring moiety. The absence of the hydroxy absorption band in the IR spectrum of **4** and the two remaining oxygenated quaternary carbons at $\delta_C$ 153.6 and 83.8 in the $^{13}C$ NMR spectrum of **4** (Table 2) suggested that **4** was a 1,10-oxane derivative of **3**. This was further proved by the 2D NMR experiments of **4**. The relative stereochemistry of **4** was elucidated by the NOE difference experiment. $H_3$-14 ($\delta$ 1.49, s) was enhanced by irradiation of H-5 and H-12b ($\delta$ 5.09, s), while $H_3$-15 ($\delta$ 1.52, s) was enhanced by irradiation of H-12a ($\delta$ 4.97, s), but not enhanced by irradiation of H-2. Accordingly, the structure of **4** was determined as (+)-(1*R*,9*R*)-4-bromo-1,5,9-trimethyl-12-methylidene-8-oxa-tricyclo[7.2.1.$0^{2,7}$]dodeca-2,4,6-triene and named 4-bromo-1,10-epoxylaur-11-ene. As the key intermediate in the synthesis of (±)-aplysin and (±)-filiformin, **4** was synthesized as a racemate.[17]

Compound **5** was obtained as a colorless gum (MeOH), $[\alpha]_D^{20}$ +2.1° (*c* 0.22, MeOH), and displayed absorption bands for a hydroxy group (3400 $cm^{-1}$) and an aromatic ring (1622 and 1501 $cm^{-1}$) in the IR spectrum. The EIMS exhibited a molecular ion at *m/z* 216 $[M]^+$, and the molecular formula was determined as $C_{15}H_{20}O$ by the HREIMS at *m/z* 216.1509 $[M]^+$. In the $^1H$ NMR spectrum signals at $\delta$ 6.96 (1H, d, $J$ = 7.5 Hz, H-4), 6.95 (1H, s, H-1), 6.80 (1H, d, $J$ = 7.5 Hz, H-5), 2.14 (3H, s, $H_3$-13), and 7.85 (1H, brs) indicated that **5** contained the aromatic structural unit 3-hydroxy-4-methylphenyl completely identical to that of **1**. In addition, the presence of two methyl singlets at $\delta$ 1.20 (3H, s, $H_3$-12) and 1.29 (3H, s, $H_3$-14) and signals attributed to a cyclopropyl at $\delta$ 0.38 (dd, $J$ = 7.5 and 5.0 Hz, H-15$\beta$), 0.58 (dd, $J$ = 5.0 and 4.0 Hz, H-15$\alpha$), and 1.05 (ddd, $J$ = 7.5, 4.0, and 4.0 Hz, H-10), as well as signals assignable to two methylenes at $\delta$ 1.37 (1H, m, H-8$\alpha$), 1.58 (2H, ddd, $J$ = 12.5, 7.5, and 7.5 Hz, overlapped H-8$\beta$ and H-9$\alpha$), and 1.92 (1H, m, H-9$\beta$), in combination with the molecular composition and six degrees of unsaturation of the molecule, revealed that the remaining moiety of **5** was a dimethyl[3.1.0]bicyclohexyl unit. Therefore, **5** was proposed to be a debrominated derivative of laurinterol[21] with the tricyclic cyclolaurane skeleton. This elucidation was further confirmed by the $^{13}C$ NMR data (Table 2) and 2D NMR experiments of **5**. The HMBC from $H_3$-12 to C-7, C-10, C-11, and C-15 and from $H_3$-14 to C-7, C-8, and C-11 verified the dimethyl[3.1.0]bicyclohexyl unit, while long-range correlations from $H_3$-13 to C-2, C-3, and C-4 and from both H-1 and H-5 to C-7 unequivocally confirmed the substitution relationship among the methyl, the hydroxy, and dimethyl[3.1.0]bicyclohexyl on the benzene ring. In the NOE difference experiment $H_3$-12 and H-10 were enhanced by irradiation of H-15$\beta$, while H-1, H-5, and H-8$\alpha$ were enhanced by irradiation of H-15$\alpha$, demonstrating that the two methyl groups and H-10 were on the same side of the [3.1.0]bicyclohexyl ring system. Therefore, the structure of **5** was proposed as (+)-(1*S*,2*R*)-2-(3-hydroxy-4-methylphenyl)-1,2-dimethyl-[3.1.0]bicyclohexane, named cyclolauren-2-ol.

Compound **6** was obtained as a colorless gum (MeOH), $[\alpha]_D^{20}$ −13.7° (*c* 0.005, MeOH), and displayed absorption bands for a hydroxyl (3559 $cm^{-1}$) and an aromatic ring (1602 and 1506 $cm^{-1}$) in the IR spectrum. The EIMS exhibited a molecular ion at *m/z* 230 $[M]^+$, and the molecular formula was determined as $C_{15}H_{18}O_2$ by the HREIMS at *m/z* 230.1304 $[M]^+$. The $^1H$ NMR spectrum of **6** showed two aromatic singlets at $\delta$ 6.41 (1H, s, H-2) and 6.63 (1H, s, H-5), an aromatic methyl singlet at $\delta$ 2.12 (3H, s, H-13), and a phenolic hydroxy group at $\delta$ 7.41 (1H, s), indicating the presence of a 1,2,4,5-tetrasubstituted benzene ring with a methyl and a hydroxy as two of the four substituents. The remaining $^1H$ NMR signals of **6** were similar to those of the [3.1.0]bicyclohexyl moiety of **5** except that one methyl singlet of **5** was replaced by an AB spin system characteristic for an isolated methylene group at $\delta$ 2.89 (1H, d, $J$ = 15.5 Hz, H-14$\alpha$) and 3.27 (1H, d, $J$ = 15.5 Hz, H-14$\beta$). The $^{13}C$ NMR and DEPT spectra of **6** showed that the isolated methylene carbon was at $\delta$ 36.7 (C-14) and that one of the two quaternary carbons of the 1,2,4,5-tetrasubstituted benzene ring appeared at $\delta$ 125.6 (C-6)

besides two quaternary carbons connecting with the methyl ($\delta$ 123.8, C-3) and hydroxy ($\delta$ 149.4, C-4) groups. These data indicated that the isolated methylene directly connected with the tetrasubstituted benzene ring to give a 2,4,5-trisubstituted benzyl moiety. In addition, in the $^{13}$C NMR and DEPT spectra the quaternary carbon of the [3.1.0]-bicyclohexyl moiety at $\delta$ 98.2 (C-7) and the remaining quaternary carbon of the 2,4,5-trisubstituted benzyl moiety at $\delta$ 153.5 (C-1) in combination with the molecular composition and seven degrees of unsaturation of **6** revealed a unique spiro structure for **6**. To confirm the above elucidation and to determine positions of the substituents, 2D NMR experiments were carried out for **6**. The proton and corresponding carbon signals in the NMR spectra of **6** were unambiguously assigned by $^1$H−$^1$H gCOSY and gHSQC experiments (Tables 1 and 2). In the HMBC spectrum long-range correlations from H-5 to C-1, C-3, and C-14, from $H_3$-12 to C-7, C-10, C-11, and C-15, from $H_2$-14 to C-1, C-5, C-6, C-7, C-8, and C-11, and from $H_2$-15 to C-7, C-9, C-10, C-11, and C-12 confirmed that the tetrasubstituted benzene ring (C-6) connected through the isolated methylene (C-14) with the methyl[3.1.0]bicyclohexyl moiety (C-7). Meanwhile, HMBC correlations from $H_3$-13 to C-2, C-3, and C-4 and from the phenolic hydroxy proton to C-3, C-4, and C-5 unambiguously established that the methyl and hydroxy groups on the benzene ring were at C-3 and C-4, respectively. The relative configuration of **6** was determined by the NOE difference experiment of **6**. Irradiation of $H_3$-12 enhanced H-10, H-14$\beta$, and H-15$\beta$, and irradiation of H-8$\alpha$ enhanced H-14$\alpha$ and H-15$\alpha$. These enhancements revealed that the methyl and isolated methylene groups on the [3.1.0]bicyclohexyl moiety were *cis* oriented. Therefore, the structure of **6** was proposed as (−)-(1′*S*,2′*R*)-5-hydroxy-6-methyl-spiro-dihydrobenzofuran-2(3*H*),2′-{1′-methyl-[3.1.0]-bicyclohexane}, named laurentristich-4-ol, which possessed an unusual rearranged skeleton and biogenetically may be synthesized from the possible precursor **5** by an oxidation rearrangement procedure.

Compound **7** was obtained as colorless needles (MeOH), mp 77−79 °C, $[\alpha]_D^{20}$ +3.6° (*c* 0.09, MeOH). The IR spectrum displayed absorption bands for hydroxy groups (3379 cm$^{-1}$) and an aromatic ring (1514 cm$^{-1}$). The positive FABMS exhibited a quasimolecular ion at *m/z* 235 [M + H]$^+$, and the molecular formula was determined as $C_{15}H_{22}O_2$ by the HRFABMS at *m/z* 235.1712 [M + H]$^+$. In the $^1$H NMR spectrum of **7** signals at $\delta$ 2.29 (3H, s, $H_3$-13), 7.10 and 7.35 (each 2H, d, $J$ = 8.0 Hz, H-2, H-4, and H-1 and H-5) indicated the presence of a *p*-tolyl group, and signals at $\delta$ 2.41 (1H, dd, $J$ = 13.5, 6.0 Hz, H-8a), 2.45 (1H, dd, $J$ = 13.5, 6.0 Hz, H-8b), 5.54 (1H, ddd, $J$ = 15.5, 6.0, and 6.0 Hz, H-9), and 5.59 (1H, d, $J$ = 15.5 Hz, H-10) revealed the presence of a *trans* propenyl unit, in addition to three methyl singlets at $\delta$ 1.17 (6H, s, $H_3$-12 and $H_3$-15) and 1.46 (3H, s, $H_3$-14) and two exchangeable hydroxy protons at $\delta$ 3.34 (1H, brs) and 3.71 (1H, brs). The $^{13}$C NMR and DEPT spectra showed two oxygenated quaternary carbons at $\delta$ 70.1 (C-11) and 73.9 (C-7) besides protonated carbons corresponding to the above units. Considering five degrees of unsaturation, the NMR data suggested that **7** was an aromatic bisabolene derivative with two hydroxy groups at C-7 and C-11, respectively. This was confirmed by 2D NMR experiments of **7**. The double bond in the side chain was assigned between C-9 and C-10 on the basis of long-range correlations from $H_3$-12 and $H_3$-15 to C-10 and C-11 and from $H_3$-14 to C-6, C-7, and C-8 in the HMBC spectrum of **7**. Therefore, the structure of **7** was determined as (+)-6-methyl-2-(*p*-tolyl)hept-4-en-2,6-diol and named *ar*-bisabol-9-en-7,11-diol. The stereochemistry of **7** has not been determined yet.

By using the MTT method[34,35] compounds **1**−**14** were tested against several human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), hepatoma (Bel 7402), colon cancer (HCT-8), and HELA cell lines. Laurebiphenyl (**14**) showed moderate cytotoxicity, with $IC_{50}$ values of 1.68, 1.22, 1.91, 1.77, and 1.61 $\mu$g/mL, respectively. Other compounds were inactive ($IC_{50}$ > 10 $\mu$g/mL).

## Experimental Section

**General Experimental Procedures.** Melting points were determined on an XT-4 micro melting point apparatus and are uncorrected. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR spectrophotometer. 1D and 2D NMR spectra were obtained at 500 and 125 MHz for $^1$H and $^{13}$C, respectively, on an Inova 500 MHz spectrometer in acetone-$d_6$ with solvent peaks as references. EIMS, HREIMS, FABMS, and HRFABMS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. Column chromatography was performed with silica gel (200−300 mesh) and Sephadex LH-20. TLC was carried out with glass precoated silica gel $GF_{254}$ plates. Spots were visualized under UV light or by spraying with 7% sulfuric acid in EtOH followed by heating. HPLC was performed using an Alltima C18 10 $\mu$m preparative column (22 × 250 mm).

**Material.** The red alga *Laurencia tristicha* was collected at the coast of Naozhou Island, Zhanjiang City, China, in April 2003, and identified by Dr. Lan-Ping Ding (Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, China). A voucher specimen (No. 2003052) was deposited at the Herbarium of the Institute of Oceanology.

**Extraction and Isolation.** The air-dried algae *Laurencia tristicha* (10.5 kg) were extracted with EtOH at room temperature for 3 × 72 h. After the solvent was removed under reduced pressure at <40 °C, a dark residue was obtained. The residue was suspended in water and then partitioned with EtOAc. The EtOAc fraction (550 g) was chromatographed over silica gel (1200 g), eluting with a gradient increasing of EtOAc (0−100%) in light petroleum, and separated into 16 fractions (I−XVI) on the basis of TLC analysis. Fraction I eluted by pure light petroleum was recrystallized with the same solvent to give a large amount of **8** (45.7 g, 0.44%). Fraction II eluted by 2% EtOAc in light petroleum was recrystallized with light petroleum to yield **9** (8.3 g, 0.079%), and the residue in the parent solution was subjected to chromatography over Sephadex LH-20 with petroleum ether−$CH_3Cl$−MeOH (5:5:1) as eluent to give three subfractions. The third subfraction was further purified by preparative HPLC with 90% MeOH in $H_2O$ as mobile phase to give compounds **5** (36 mg, 0.00034%), **6** (8 mg, 0.000076%), and **10** (42 mg, 0.0095%). Fraction III eluted by 3% EtOAc in light petroleum was separated by column chromatography over Sephadex LH-20 with petroleum ether−$CH_3Cl$−MeOH (5:5:1) as eluent to give three subfractions, and the last subfraction was further purified by preparative HPLC with 90% MeOH in $H_2O$ as mobile phase to give compounds **2** (68 mg, 0.00064%), **11** (23 mg, 0.00022%), **12** (2 mg, 0.000019%), **13** (48 mg, 0.00046%), and **14** (96 mg, 0.00091%). Fraction IV eluted by 5% EtOAc in light petroleum was separated by column chromatography over Sephadex LH-20 with petroleum ether−$CH_3Cl$−MeOH (5:5:1) as eluent to give two subfractions. The second subfraction was purified by preparative HPLC with 85% MeOH in $H_2O$ as mobile phase to give compounds **3** (72 mg, 0.00069%) and **4** (88 mg, 0.00084%). Fraction V eluted by 10% EtOAc in light petroleum was separated by column chromatography over Sephadex LH-20 with petroleum ether−$CH_3Cl$−MeOH (5:5:1) as eluent to give two subfractions. The second subfraction was purified by preparative HPLC with 80% MeOH in $H_2O$ as mobile phase to give compounds **1** (56 mg, 0.00053%) and **7** (126 mg, 0.0012%).

**Laur-11-en-2,10-diol (1):** colorless needles (MeOH); mp 99−100 °C; $[\alpha]_D^{20}$ −70.5° (*c* 0.19, MeOH); IR (KBr) $\nu_{max}$ 3417, 3168, 2962, 2870, 1622, 1585, 1514, 1458, 1417, 1377, 1277, 1255, 1215, 1132, 1092, 1076, 1049, 991, 933, 914, 879 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) and $^{13}C$ NMR (acetone-$d_6$, 125 MHz) data, see Tables 1 and 2; EIMS *m/z* (%) 232 (100) $[M]^+$, 217 (63) $[M - Me]^+$, 214 (21) $[M - H_2O]^+$, 199 (70), 189 (24), 174 (72), 159 (91), 148 (66), 121 (34), 109 (37), 91 (20), 81(15), 77 (17), 55 (12); HREIMS *m/z* 232.1466 (calcd for $C_{15}H_{20}O_2$, 232.1463).

**Laur-11-en-10-ol (2):** colorless needles (MeOH); mp 62−63 °C; $[\alpha]_D^{20}$ −32.7° (*c* 0.56, MeOH); IR (KBr) $\nu_{max}$ 3342, 2962, 2868, 1716, 1655, 1512, 1444, 1371, 1308, 1190, 1151, 1099, 1018, 951, 937, 901, 812 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) and $^{13}C$ NMR (acetone-$d_6$, 125 MHz) data, see Tables 1 and 2; EIMS *m/z* (%) 216 (43) $[M]^+$, 201 (58) $[M - Me]^+$, 198 (33) $[M - H_2O]^+$, 183 (78), 173 (15), 168 (12), 158 (76), 149 (69), 143 (100), 132 (41), 128 (30), 115 (29), 109 (22), 105 (28), 91 (26), 77(16), 71 (18), 69 (16); HREIMS *m/z* 216.1507 (calcd for $C_{15}H_{20}O$, 216.1514).

**Laur-11-en-1,10-diol (3):** colorless needles (MeOH); mp 95−97 °C; $[\alpha]_D^{20}$ −93.8° (*c* 0.08, MeOH); IR (KBr) $\nu_{max}$ 3421, 2964, 2925, 2871, 1616, 1520, 1456, 1415, 1370, 1296, 1184, 1093, 1039, 949, 910, 810 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) and $^{13}C$ NMR (acetone-$d_6$, 125 MHz) data, see Tables 1 and 2; EIMS *m/z* (%) 232 (12) $[M]^+$, 214 (6) $[M - H_2O]^+$, 199 (19), 189 (3), 173 (2), 161 (100), 159 (35), 148 (7), 135 (8), 133 (10), 121 (10), 115 (8), 105 (6), 91 (9), 82 (5), 77 (8), 58 (16); HREIMS *m/z* 232.1471 (calcd for $C_{15}H_{20}O_2$, 232.1463).

**4-Bromo-1,10-epoxylaur-11-ene (4):** colorless needles (MeOH); mp 74−76 °C; $[\alpha]_D^{20}$ +70.9° (*c* 0.16, MeOH); IR (KBr) $\nu_{max}$ 2968, 2933, 2858, 1604, 1554, 1479, 1458, 1371, 1309, 1275, 1227, 1165, 1122, 1082, 1059, 906, 876, 858 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) and $^{13}C$ NMR (acetone-$d_6$, 125 MHz) data, see Tables 1 and 2; EIMS *m/z* (%) 294/292 (99:100) $[M]^+$, 279/277 (97:99) $[M - Me]^+$, 266/264 (8:8), 253/251 (7:8), 241/239 (10:10), 213 (12) $[M - Br]^+$, 198 (52) $[M - Me - Br]^+$, 183 (26), 159 (12), 149 (15), 115 (18), 91 (26), 71 (22); HREIMS *m/z* 292.0468 (calcd for $C_{15}H_{17}OBr$ 292.0463).

**Cyclolauren-2-ol (5):** colorless gum; $[\alpha]_D^{20}$ +2.1° (*c* 0.22, MeOH); IR (KBr) $\nu_{max}$ 3400, 2956, 2866, 1622, 1501, 1454, 1410, 1304, 1242, 1120, 995, 939, 870, 810 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) and $^{13}C$ NMR (acetone-$d_6$, 125 MHz) data, see Tables 1 and 2; EIMS *m/z* (%) 216 (53) $[M]^+$, 201 (20) $[M - Me]^+$, 187 (8), 175 (56), 173 (16), 159 (20), 148 (100), 133 (13), 121 (21), 108 (10), 93 (16), 93 (15), 91 (12), 77(12); HREIMS *m/z* 216.1509 (calcd for $C_{15}H_{20}O$ 216.1514).

**Laurentristich-4-ol (6):** colorless gum; $[\alpha]_D^{20}$ −13.7° (*c* 0.005, MeOH); IR (KBr) $\nu_{max}$ 3359, 2927, 2866, 1602, 1506, 1462, 1423, 1377, 1331, 1267, 1165, 1074, 1028, 999, 930, 858 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) and $^{13}C$ NMR (acetone-$d_6$, 125 MHz) data, see Tables 1 and 2; EIMS *m/z* (%) 230 (100) $[M]^+$, 215 (40) $[M - CH_3]^+$, 201 (7), 191 (10), 187 (22), 175 (12), 161 (16), 149 (20), 138 (28), 137 (90), 111 (14), 93 (32), 85 (26), 71 (38), 69 (42), 57 (45); HREIMS *m/z* 230.1304 (calcd for $C_{15}H_{18}O_2$ 230.1307).

***ar*-Bisabol-9-en-7,11-diol (7):** colorless needles (MeOH); mp 77−79 °C; $[\alpha]_D^{20}$ +3.6° (*c* 0.09, MeOH); IR (KBr) $\nu_{max}$ 3379, 2972, 2929, 2886, 1514, 1456, 1371, 1284, 1151, 1078, 978, 818 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) and $^{13}C$ NMR (acetone-$d_6$, 125 MHz) data, see Tables 1 and 2; FABMS *m/z* (%) 235 (34) $[M + H]^+$, 217 (26) $[M - H_2O + H]^+$, 199 (16), 145 (26), 135 (100), 119 (10); HRFABMS *m/z* 235.1721 (calcd for $C_{15}H_{23}O_2$ 235.1698).

**NMR data of johnstonol (13):** $^1H$ NMR (acetone-$d_6$, 500 MHz) $\delta$ 1.21 (3H, s, H-13), 1.32 (3H, s, H-14), 1.48 (3H, s, H-15), 1.79 (3H, s, H-7), 2.33 (2H, m, H-6), 2.47 (2H, m, H-3), 3.2 (1H, s, H-10), 3.97 (1H, d, $J$ = 5.5 Hz, H-9), 4.32 (1H, dd, $J$ = 11.0 Hz, 4.5 Hz, H-1), 4.59 (1H, dd, $J$ = 13.0 Hz, 5.0 Hz, H-4), 5.02 (1H, d, $J$ = 5.0 Hz, H−OH); $^{13}C$ NMR (acetone-$d_6$, 125 MHz) $\delta$ 60.1 (C-1), 70.3 (C-2), 46.1 (C-3), 75.2 (C-4), 51.7 (C-5), 34.5 (C-6), 31.4 (C-7), 113.0 (C-8), 75.3 (C-9), 62.0 (C-10), 62.1 (C-11), 50.3 (C-12), 18.7 (C-13), 25.2 (C-14), 21.8 (C-15).

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**Supporting Information Available:** $^1H$, $^{13}C$ NMR and DEPT spectra of compounds **1**−**7** and **13** and 2D NMR spectra of compound **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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