of saturated $NaHCO_3$ solution. Extraction into CH_2Cl_2 followed by drying and concentration afforded a colorless syrup. Chromatography on silica gel (CH_2Cl_2), and recrystallization from EtOH gave 1.47 g (41%) of **8** as a colorless solid: mp 139–141 °C; 360-MHz ^1H NMR δ 3.48 (s, OCH_3), 4.33 (m, H-5), 4.67 (m, H-6 + H-6′, $J_{6,6'}$ = 12.8 Hz), 4.77 (dt, H-4, $J_{3,4}$ = 9.1 Hz, $J_{H\text{-}4,F\text{-}4}$ = 51.3 Hz, $J_{4,5}$ = 10 Hz), 5.18 (m, H-2), 5.22 (d, H-1, $J_{1,2}$ = 3.5 Hz), 6.12 (dt, H-3, $J_{H\text{-}3,F\text{-}4}$ = 14.7 Hz, $J_{2,3}$ = 9 Hz), 7.35–7.63 (m, 9 H), 7.96–8.13 (m, 6 H); ^{19}F NMR (^1H decoupled) ϕ −197.6 (s); $[\alpha]_D$ 119.1° (*c* 1.0, $CHCl_3$).

Anal. Calcd for $C_{28}H_{25}O_8F$: C, 66.14; H, 4.96; F, 3.74. Found: C, 65.99; H, 5.07; F, 3.73.

Acknowledgment. We thank Dr. G. S. Reddy of this department for obtaining the 360-MHz ^1H NMR spectra and for extensive decoupling experiments.

Registry No. **1**, 97-30-3; **3**, 4577-39-3; **4**, 617-04-9; **6**, 84073-36-9; **7**, 3601-36-3; **8**, 84065-98-5; DAST, 38078-09-0.

Srilankenyne, a New Metabolite from the Sea Hare *Aplysia oculifera*

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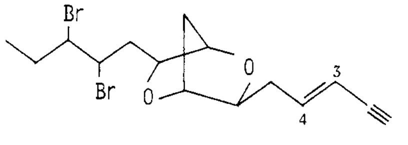
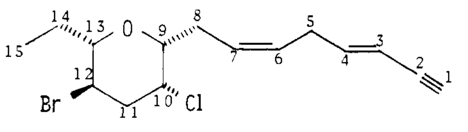
The structure of a C_{15}-tetrasubstituted tetrahydropyran, isolated from a sea hare, was elucidated by spectral analysis.

Sea hares that feed on the red algal genus *Laurencia* have been a convenient source of *Laurencia* metabolites, predominantly sesquiterpenes and derivatives of unbranched polyunsaturated C_{15} hydrocarbons bearing oxygen and halogen functions. More than 200 metabolites have been isolated to date from *Laurencia* spp. and from sea hares.[1] We recently reported the structures of the bicyclic ocellenynes (**1a**,**b**) from *Aplysia oculifera* collected in Hawaii.[2] The same animal from Duwa, Sri Lanka,[3] contained as its principal metabolite the monocyclic srilankenyne (**2**), which is the subject of this report. Srilankenyne (**2**) is a dialkyl tetrahydropyran derivative. Surprisingly, this type has been rather uncommon among *Laurencia* constituents.[1]



1 a, b (Z) 3,4



2

Table I. NMR Data of 2

C	chem shift, ppm, multiplicity (*J*, Hz) ^{13}C	^1H	effect of ^1H decoupling
1	76.32 d	2.81 d (2.2)	5.48[a]
2	82.8 s		
3	109.17 d	5.48 m	same as H-7
4	143.96 d	6.23 dt (15.8, 6.8)	2.9 dd (6.4, 2.2) 5.48 ch
5	30.87 t[b]	2.90 ddd (6.8, 6.4, 2.2)	5.48[a] 6.23 d (15.8)
6	126.04 d[b]	5.48 m	same as H-7
7	128.48 d[b]	5.48 m	2.36 ch 2.81 s 2.90 d (6.8) 6.23 ch
8	31.08 t[b]	2.36 complex AB pattern	3.57 d (1.7) 5.48 ch 2.72 ch 4.05 dd (3.0, 1.75) 4.14 ch
9	78.73 d[b]	3.57 dt (7.0, 1.75)	2.36 ch 4.05 dd (3.4, 3.0)
10	60.47 d	4.05 ddd (3.4, 3.0, 1.75)	2.4 ch 2.72 dd (14.0, 4.4) 3.57 t (7.0)
11	43.85 t	2.72 ddd (14.0, 3.0, 4.4) 2.40 ddd (14.0, 12.0, 3.4)	was not decoupled similar to irradiation of 2.36
12	46.54 d	4.14 ddd (12.0, 10.2, 4.4)	2.40 ch 2.72 dd (14.0, 3.0) 3.36 dd (8.4, 2.5)
13	83.83 d[b]	3.36 ddd (10.2, 8.4, 2.5)	1.54 qd (14.5, 7.2) 2.02 qd (14.5, 7.2) 4.14 dd (12.0, 4.4)
14	26.19 t	1.54 qdd (7.2, 8.4, 14.5) 2.02 qdd (7.2, 2.5, 14.5)	0.97 d (7.2) 2.02 ch[a] 3.36 dd (10.2, 2.5) 0.97 d 7.2 1.54 ch 3.36 dd (10.2, 8.4)
15	9.28 q	0.97 t (7.2)	1.54 dd (14.5, 8.4) 2.02 dd (14.5, 2.5)

[a] Changed pattern. [b] Interchangeable with closest value.

The frozen animals were blended with acetone. The filtrate from the acetone suspension was concentrated at reduced pressure to a dark brown syrup, which was partitioned between water and dichloromethane. The organic residue was chromatographed on Bio-Sil A and then by HPLC, yielding srilankenyne (**2**) as a colorless liquid, $[\alpha]_D$ +7.14°. A molecular ion cluster at *m/z* 334, 332, and 330 suggested a formula of $C_{15}H_{20}BrClO$, and IR bands at 3300 and 2100 cm^{-1} indicated a terminal enyne function. ^1H NMR signals at δ 2.81 (H-1), 5.48 (H-3), and 6.23 (H-4), with corresponding ^{13}C NMR shifts at δ 76.32 (d, C-1), 82.8 (s, C-2), 109.17 (d, C-3), and 143.96 (d, C-4) fully confirmed the enyne tail of the molecule. A coupling constant of 15.8 Hz for H-4 showed the trans geometry of the 3,4-olefin. The chemical shifts of the acetylenic ≡CH also are characteristic of a *trans*-enyne. Corresponding values for *cis*-enyne are found at lower field.[4] The ether nature of the sole oxygen atom was seen in the IR spectrum by 1100 and 1082 (sh) cm^{-1} bands and a lack of hydroxyl and carbonyl absorption. The ^{13}C NMR spectrum exhibited six additional low-field signals. Two of these, doublets at δ 83.83 and 78.73, were assigned to carbons bearing oxygen, two doublets at δ 60.47 and 46.54 to carbons bearing

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(1) Erickson, K. L. In "Marine Natural Products"; Scheuer, P. J., Ed.; Academic Press: New York, Vol. 5, in press.

(2) Schulte, G. R.; Chung, M. C. H.; Scheuer, P. J. *J. Org. Chem.* **1981**, *46*, 3870–3873.

(3) Collected by E. D. deS., Oct 1978, and identified by Professor E. Alison Kay.

(4) Howard, B. M.; Fenical, W.; Hirotsu, K.; Solheim, B.; Clardy, J. *Tetrahedron* **1980**, *36*, 171–176.

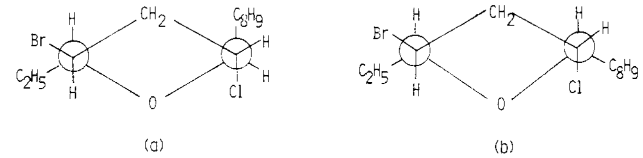
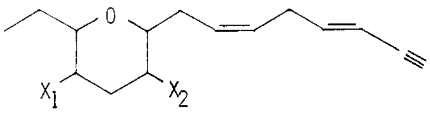


Figure 1. Newman projection of **2**: (a) C_8 side chain axial; (b) C_8 side chain equatorial.

halogen, and two doublets at δ 128.48 and 126.04 to an isolated olefin. The compound must therefore be monocylic. Analysis of the ^{1}H NMR spectrum (Table I) including decoupling experiments allowed unambiguous assignment of all protons and hence definition of a structural framework (**3**), which received support from mass spectral fragments corresponding to successive losses from the parent ion of C_2H_4, C_8H_9, HBr, and Cl. The proton data also placed the isolated double bond at C-6. Its cis stereochemistry was deduced from the ^{13}C chemical shift of the doubly allylic methylene at C-5, which resonates at δ 30.87 (or 31.08). Analogous methylenes in fatty acids are observed at δ 25.7 (cis–cis), 30.5 (cis–trans), or δ 35.7 (trans–trans).[5] Confirmation of the C-6,7 cis stereochemistry was provided by the 200-MHz ^{1}H NMR spectrum of **2** in deuteriobenzene, where the cis- and trans-olefin signals do not overlap. Computer simulation of the two cis protons using a chemical shift difference of 11.5 Hz and a J value of 11 Hz resulted in a pattern that fits the observed signals. Yet to be elucidated were the positions of the halogens and the stereochemistry at the four chiral centers.



3 X_1, X_2 = Br or Cl

A coupling constant of J = 10.2 Hz between H-12 and H-13 denotes a trans diaxial relationship, thus demanding diequatorial configuration for X_1 and the C_2 side chain. On the other hand, H-10 lacks large coupling (J = 3.4, 3.0, 1.75 Hz), thereby necessitating axial configuration for X_2. Orientation of the C_8 side chain remains to be settled, once the identities of X_1 and X_2 are known.

The relatively large chemical shift difference between the C_{14} protons (Δδ = 0.48) suggests interaction with a bulky group at C-12, most likely an equatorial bromine. This was proven by single-frequency on-resonance ^{1}H–^{13}C decoupling experiments in deuteriobenzene. In that solvent the H-10 and H-12 protons are separated by 60 Hz. Irradiation of CHX_1 at 79.542438 Hz collapsed the HC–Br doublet at δ 46.5 to a singlet. Conversely, the HC–Cl doublet at δ 60.5 collapsed to a singlet when it was irradiated at 79.542378 Hz, Hence X_1 = Br and X_2 = Cl.

Finally, the orientation of the C_8 side chain could be determined. In the fully coupled ^{13}C NMR spectrum of **2** the C-11 methylene appears as a doublet of doublets ($^1J_{CH}$ = 139, 128 Hz), further split by $^2J_{CCH}$ and $^3J_{CCCH}$ couplings of about 1 Hz. The maximum width of the pattern due to long-range coupling is about 4 Hz. Such a pattern can be obtained only if all geminal and vicinal couplings are equal to one another and are about 1 Hz. In Figure 1 it may be seen that both C-11–C-12–H-12 and C-11–C-10–H-10 angles are about 109°, which renders the $^2J_{CCH}$ coupling constants about equal and small, 1–2 Hz. Vicinal coupling ($^3J_{CCCH}$) between carbon and hydrogen depends on the dihedral angle between CCC and CCH bonds and is governed by a Karplus relationship.[6] If the C_8 side chain is axial (Figure 1a), this dihedral angle will be 180°, leading to an 8-Hz coupling. The resulting pattern is resolvable as a doublet. This is not observed. If, however, C_8 is equatorial (Figure 1b), a dihedral angle of 60° results, about equal to the situation with the C_2 side chain, which is known to be equatorial. Hence orientation of the C_8 side chain is equatorial.

Experimental Section

Mass spectra were obtained on a MAT 311 mass spectrometer. IR spectra were recorded on a Perkin-Elmer 467 spectrometer. A Beckman ACTA 111 spectrophotometer was used to measure UV spectra. ^{1}H NMR spectra were determined on a Varian XL-200 NMR spectrometer. Natural abundance ^{13}C NMR spectra (noise, off-resonance, and specific proton decoupled) were recorded on a Varian FT-80 spectrometer.

Isolation. *A. oculifera* were collected at Duwa, Sri Lanka, at low tide from a reef where the animals were browsing. The animals were about 30 mm long and exuded a purple pigment on contact. The animals (450 g) were frozen and later homogenized with acetone. Filtration and concentration at reduced pressure gave a dark brown residue, which was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 solubles (1.5 g) were chromatographed on Bio-Sil A (hexane/CH_2Cl_2, 65:35) followed by HPLC (Partisil, hexane/CH_2Cl_2, 80:20) to give **2** as a colorless liquid: $[\alpha]_D$ +7.14° (c 0.98, CH_2Cl_2); $\lambda_{max}^{(MeOH)}$ 227 nm (ε 13600); IR (CH_2Cl_2) 3300, 3020, 2960, 2850, 2100, 1100, 1082 (sh), 960, 818 cm^{-1}; HRMS, m/z 332.0364, calcd for $C_{15}H_{20}{}^{81}Br^{35}ClO$, 332.0366; MS, m/z 334, 332, 330 (M^+), 306, 304, 302 ($M^+ - C_2H_4$), 297, 295 ($M^+ - Cl$), 255, 253, 251 ($M^+ - C_6H_7$), 229, 227, 225 ($M^+ - C_8H_9$), 215 ($M^+ -$ HBrCl), 201, 199, 197 ($M^+ - C_{10}H_{13}$), 81 (C_8H_9, base peak); ^{13}C NMR ($CDCl_3$) and ^{1}H NMR ($CDCl_3$), see Table I; ^{1}H NMR (C_6D_6) δ 6.24 (1 H dt), 5.53 (1 H m), 5.34 (2 H m), 4.25 (1 H, ddd), 3.44 (1 H ddd), 3.22 (1 H ddd), 3.07 (1 H dt), 2.7–1.9 (10 H, complex), 1.67 (1 H qdd), 1.08 (1 H t).

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[5] Pfeffer, P. E.; Luddy, F. E.; Unruh, J.; Shoolery, J. N. *J. Am. Oil Chem. Soc.* **1977**, *54*, 380–386.

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Stereochemistry and Carbon-13 Nuclear Magnetic Resonance Spectroscopy of the Histamine-Liberating Sesquiterpene Lactone Thapsigargin. A Modification of Horeau's Method

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Recently, thapsigargin (**1**) and thapsigargicin (**2**), the two major skin irritants of *Thapsia garganica* (Apiaceae = Umbelliferae) have been isolated,[1] and the relative con-