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SOME DITERPENES FROM THE SEA PEN *STYLATULA* SP.

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Abstract—The sea pen *Stylatula* sp. from the Gulf of California contained one major and several minor metabolites. The structure of stylatulide (2), the major metabolite, has been reported previously. In this paper, the structural elucidations of four minor metabolites, 17-epi-stylatulide (13), the lactone 14, the primary alcohol 15 and related methyl ester 17 are described. We have described several reactions of stylatulide (2) and its derivatives which illustrate the complexity of reactions on these compounds.

The chlorinated diterpenes from the gorgonian coral *Briarium asbestinum* and from the sea pens *Ptilosarcus gurneyi* and *Stylatula* sp. are among the more complex marine natural products. The structures of briarein A (1) from *B. asbestinum*[1] and stylatulide (2) from *Stylatula* sp.[2] have been determined by X-ray diffraction analysis. The structural elucidation of ptilosarcone (3) and ptilosarcenone (4) depend on comparison of their spectral data with those of briarein A.[3] The minor metabolites of *B. asbestinum*[4] and *Stylatula* sp. have been difficult to characterize due to their complex spectral data and unpredictable reactivity toward standard reagents. In this paper we wish to describe some of the unusual reactions of stylatulide (2) and report the structures of four related minor constituents of the sea pen *Stylatula* sp.[5]

Florisil chromatography of the ethyl acetate soluble material from *Stylatula* resulted in the isolation of stylatulide (2) (0.8% of dry weight) and five minor metabolites, four of which have been identified. Stylatulide (2) crystallized from 1:1 hexane–dichloromethane, m.p. 179–180°, and the structure was elucidated by X-ray diffraction analysis. In the mass spectrum of stylatulide (2), the highest molecular weight ion observed corresponded to loss of acetic acid from the molecular formula $C_{26}H_{35}ClO_{10}$ which was determined by elemental analysis. We have assigned every signal in the richly detailed 220 MHz ^{1}H NMR spectrum using spin-decoupling techniques (Table 1). The protons at C-9 and C-10

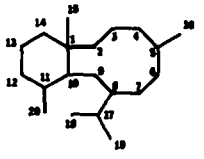


Table 1. ^{1}H NMR spectra ($CDCl_3$) of stylatulide (2), 17-epi-stylatulide (13), the lactone 14, the primary alcohol 15, and the methyl ester 17

H at C#	2	13	14	15	17
2	5.93(d, 9Hz)	5.61	5.03	4.92	5.00
3	1.70(m) 2.59(m)	--	--	--	--
4	∿2.4(m)	--	--	--	--
6	4.63(bs)	5.00	5.42	5.75	6.80
7	4.71(bs)	5.18	5.42	5.43	5.43
9	5.50(s)	5.23	5.99	6.02	6.07
10	3.04(s)	3.22	2.97	2.99	3.07
12	2.97(d, 4Hz)	2.92	5.42	5.41	5.45
13	2.10(d, 18Hz) 2.27(m)	--	--	--	--
14	4.90(d, 6.5Hz)	4.79	4.76	4.77	4.82
15	1.10(s)	1.10	0.98	0.95	0.98
16	5.79(bs) 6.00(bs)	5.59 5.92	2.00	4.06 4.32	
17	3.18(q, 7Hz)	3.21	--	--	--
18	1.31(d, 7Hz)	1.54	1.21	1.23	1.28
20	1.29(s)	1.31	2.00	1.91	1.98
-OAc	1.95 2.00 2.27	1.95 2.01 2.20	1.96 2.03 2.17	1.71 2.02 2.16	1.92 1.98 2.19
-OMe					3.79

Table 2. ^{13}C NMR spectra ($CDCl_3$) of stylatulide (2), lactone 14 and alcohol 15

C#	2	14	15
1	45.3	44.5	44.5
2	72.2*	73.5*	73.4*
3	28.3	28.5	26.6
4	27.6	26.7	25.5
5	146.0	134.7	135.0
6	54.4(b)	120.5†	120.1†
7	78.0(b)	70.4*	70.1*
8	81.6	81.8	81.8
9	81.3*	79.1*	78.9*
10	51.6	40.3	39.9
11	58.9	147.0	147.6
12	59.6	117.7†	118.4†
13	33.5	31.9	31.8
14	72.3*	74.8*	75.7*
15	13.9	14.5	14.2
16	121.6	27.5	67.4
17	43.1	43.8	44.0
18	6.6	7.0	7.0
19	174.7	176.6	176.8
20	22.1	24.3	24.3
$CH_3\underline{C}OO$	170.1 170.7 170.8	169.8 170.5 171.3	169.7 171.4 171.5
$\underline{C}H_3COO$	21.1(3)	21.2 21.4(2)	21.4(2) 21.5

*† These signals may be interchanged

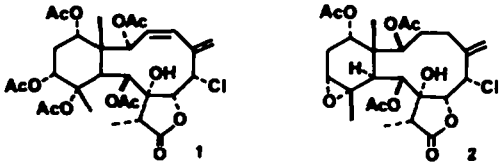
are approximately orthogonal, resulting in a very small ($J < 1$ Hz) coupling constant between the signals at δ 5.50 and 3.04. The signal at δ 2.10 due to the axial proton at C-13 is coupled only to the equatorial C-13 proton signal at 2.27, which is also coupled to the signals at 2.97 (d, $J = 4$ Hz, C-12) and 4.90 (d, $J = 6.5$ Hz, C-14). At 25°, in $CDCl_3$ solution, the signals at δ 4.63 (C-6) and 4.71 (C-7) appear as broad humps while at elevated temperatures (50–60°) in C_6D_6 solution the signals sharpen to doublets ($J = 3.7$ Hz). This observation requires that there be two conformations of the 10-membered ring having a slow rate of interconversion. The ^{13}C NMR spectrum has been assigned (Table 2) except for those signals due to the three acetate groups and the carbon atoms to which they are attached.[6] Again, the signals at δ 54.4 (C-6) and 78.0 (C-7) were exceptionally broad.

We found few reactions of stylatulide which gave single products or even product mixtures with a major component. We attempted to remove the acetate groups by base catalyzed hydrolysis under a variety of conditions and by lithium aluminum hydride reduction but always obtained a complex mixture of products. The exocyclic olefinic bond could not be converted into an epoxide, even under forcing conditions.[8] The epoxide ring could not be cleaved using periodic acid.

Ozonolysis of stylatulide (2) in methanol solution at −78° gave ketone 5 in 90% yield. The ^1H NMR spectrum no longer showed any broadening of the signal for C-6 (δ 4.35). Treatment of stylatulide with boron trifluoride etherate in dry benzene resulted in ring contraction to an aldehyde 6 in 80% yield. The ^1H NMR spectrum of aldehyde 6 contained an aldehyde proton singlet at δ 9.39. The protons at C-9 (δ 5.61) and C-10 (δ 3.09) were no longer orthogonal and their signals were coupled ($J = 6$ Hz). As was the case with most compounds in this series, neither ketone 5 nor aldehyde 6 showed a parent ion in the mass spectrum but the $[M-60]^+$ ions, due to loss of acetic acid, were clearly visible.

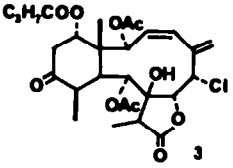
It was known from the research on briarein-A (1)[4] that reduction of the δ-chloro-γ-lactone portion of the molecules with zinc caused ring opening to give a γ,δ-unsaturated acid. The products from treatment of stylatulide (2) with zinc dust were highly dependent on the reaction conditions employed. Prolonged treatment of stylatulide with granular zinc in refluxing ethyl acetate gave the acid 7. The crude acid 7 was converted into the corresponding methyl ester 8 with diazomethane prior to characterization. In the UV spectrum of ester 8 the diene chromophore at 224 nm (ϵ 2900) was weaker than expected, suggesting that, as in briarein A (1), the olefinic

groups cannot attain planarity. The ^{1}H NMR spectrum of ester **8** contained the *trans*-olefinic proton signals at 5.26 (d, 1H, J = 13 Hz) and 6.00 (d, 1H, J = 13 Hz) and the methyl ester signal at 3.68 (s, 3H). The ^{1}H NMR spectrum also indicated that there had been no change in the substituents about the 6-membered ring; in particular, a signal due to the epoxide proton at δ 2.88 (d, 1H, J = 4Hz) was observed.



1

2



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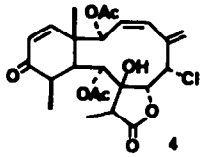

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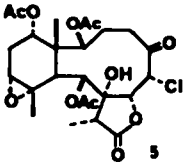

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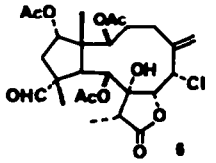

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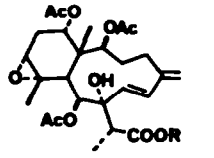
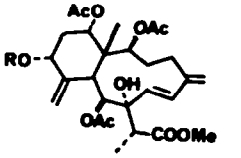


7 R = H

8 R = Me



9 R = H

10 R = Ac

In an attempt to increase the rate of reduction, the reaction was repeated with a small quantity of acetic acid added to the reaction mixture. After treatment of the product mixture with diazomethane, two methyl esters **9** and **12** were obtained. The ^{1}H NMR spectrum of the major methyl ester **9** contained the four olefinic signals of the diene system at δ 4.80 (bs, 1H), 4.98 (s, 1H), 5.25 (d, 1H, J = 13 Hz) and 6.00 (bd, 1H, J = 13 Hz) together with two additional olefinic signals at 4.98 (s, 1H) and 5.16 (s, 1H) and a signal at 4.20 (m, 1H) appropriate for the protons of the allylic alcohol moiety. The presence of a secondary alcohol functionality was confirmed by conversion of **9** into a tetra-acetate **10**, in which the signal due to the proton at C-12 was shifted to ~5.30 ppm. Oxidation of **9** with pyridinium chlorochromate in dichloromethane solution gave the cross-conjugated dienone **11** [UV; 232 nm (ϵ 16,000)] by elimination of acetic acid from the intermediate ketone. The ^{1}H NMR spectrum of dienone **11** contained signals at δ 6.87 (d, 1H, J = 11 Hz) and 5.99 (d, 1H, J = 11 Hz) due to the new endocyclic olefinic protons and 6.19 (s, 1H) and 5.46 (s, 1H) due to the exocyclic olefinic protons (C-20) as well as the familiar signals at 6.03 (bd, 1H, J = 13 Hz), 5.26 (d, 1H, J = 13 Hz), 5.00 (bs, 1H) and 4.82 (bs, 1H) due to the protons on the diene system.

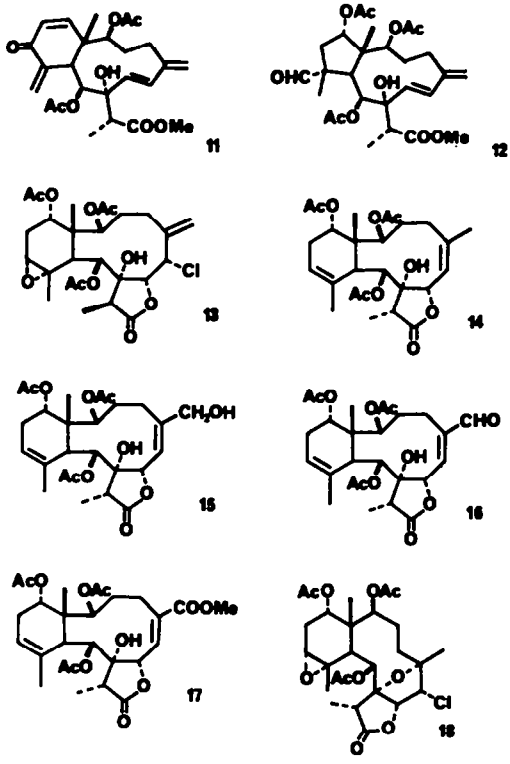
The minor methyl ester **12** contained an aldehyde signal at δ 9.31 (s, 1H) in the ^{1}H NMR spectrum. Comparison of the ^{1}H NMR spectrum of methyl ester **12** with that of the aldehyde **6** and the methyl ester **8** indicated that the aldehyde **12** contained both the diene system and the ring contracted aldehyde system. Both methyl esters **9** and **12** could be formed by acid catalyzed opening of the epoxide ring which accompanied the reductive opening of the δ-chloro-γ-lactone moiety. Since we have no evidence to the contrary, we have assumed that the proton at C-17 did not epimerize during these reactions (see following paragraph).

Treatment of stylatulide (**2**) with 1,-5-diazabicyclo[5.4.0]undecene-5 (DBU) in tetrahydrofuran solution at 25° resulted in the formation of 17-epi-stylatulide (**13**). The ^{1}H NMR spectrum of 17-epi-stylatulide (**13**) differed from that of stylatulide (**2**) at C-6 (δ 5.00 vs 4.63), C-7 (δ 5.18 vs 4.71), C-9 (δ 5.23 vs 5.50) and C-18 (δ 1.54 vs 1.31). Treatment of stylatulide (**2**) with DBU in tetrahydrofuran containing 10% deuterium oxide gave 17-epi-stylatulide (**13**) in which the C-17 proton signal at δ 3.21 was absent and the C-18 methyl signal at 1.54 was a singlet, indicating that only the C-17 proton was involved in the epimerization.

A small quantity of 17-epi-stylatulide (**13**) (0.01% dry weight) was isolated as a minor metabolite of *Stylatula* but it is not known whether this material results from epimerization of stylatulide (**2**) during the isolation procedures.

The minor metabolites of *Stylatula* sp. have been identified from their spectral properties. The most abundant metabolite after stylatulide was the lactone **14** (0.1% dry weight). The high resolution mass measurement indicated a molecular formula of $C_{26}H_{36}O_9$. The IR spectrum contained hydroxyl (3350 cm^{-1}), γ-lactone (1775 cm^{-1}) and acetate (1740 cm^{-1}) bands. The ^{13}C NMR spectrum contained four ester carbonyl signals at δ 176.6, 171.3, 170.5 and 169.8, four olefinic carbon signals at 147.0 (s), 134.7 (s), 120.5 (d) and 117.7 (d) assigned to two trisubstituted olefinic bonds and five C atoms bonded to oxygen at 81.8 (s), 79.1(d), 74.8 (d), 73.5 (d) and 70.4 (d). The seven Me carbons could all be assigned. The three acetate methyl signals appeared at δ 21.2 and 21.4 (2C), the C-18 methyl at 7.0, the C-15 methyl at 14.5, and the two vinyl Me signals at 24.3 and 27.5, indicating that both olefins had the Z-geometry. The ^{1}H NMR spectrum contained three acetate Me signals at δ 2.17, 2.03 and 1.96, two vinyl Me signals at 2.00 (bs, 6H) and two additional Me signals at 1.21 (d, 3H, J = 7 Hz) and 0.98 (s, 3H). The six low-field proton signals could not be assigned from the chloroform-d spectrum in which several signals overlapped. In the benzene-d_6 spectrum, the C-6 and C-7 protons gave rise to an AB pattern at δ 5.36 (d, 1H, J = 10 Hz) and 5.62 (d, 1H, J = 10 Hz) with the C-12 olefinic proton at 5.34 (bs, 1H) and the three α-acetoxy protons at 6.16 (s, 1H), 5.21 (d, 1H, J = 7 Hz) and 4.91 (bs, 1H). Assuming the same carbon skeleton as stylatulide, the structure of the lactone **14** can be assigned from these spectral data; in particular, the multiplicities of the α-acetoxy proton signals suggest that the configurations of these centers are the same as in stylatulide (**2**).

The more polar fractions from the chromatography of the acetone extracts contained a primary alcohol **15** (0.025% dry weight) which had the molecular formula $C_{28}H_{36}O_{10}$. The ^{13}C NMR spectrum was very similar to that of the lactone **14**, the major difference being the presence of a signal at δ 67.4 and the absence of one

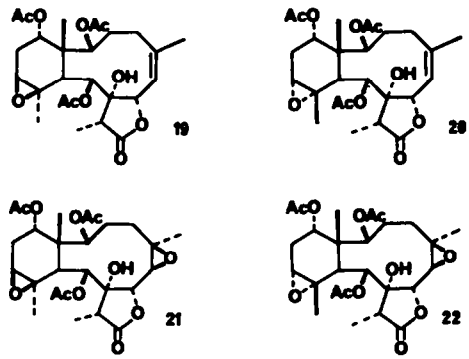


signal in the δ 25–30 region (Table 2). The ^{1}H NMR spectrum indicated that one of the vinyl Me groups in lactone **14** had become a hydroxymethylene group in the alcohol **15**. On acetylation, the hydroxymethylene protons at δ 4.06 (d, 1H, J = 15 Hz) and 4.32 (d, 1H, J = 15 Hz) were shifted downfield to 4.53 and 4.99. Oxidation of the alcohol **15** with manganese dioxide in dichloromethane gave the α,β-unsaturated aldehyde **16** [UV 228 (ϵ 8500)]. The ^{1}H NMR spectrum of the aldehyde **16** contained an aldehyde proton signal at δ 9.54 (s, 1H) and the β-proton of the α,β-unsaturated aldehyde at 6.53 (bd, 1H, J = 10 Hz) was coupled to a proton at 5.56 (d, 1H, J = 10 Hz) indicating that the aldehyde must be at C-16. Attempts to oxidize the lactone **14** to the aldehyde **16** with selenium dioxide in refluxing ethanol were unsuccessful.

Oxidation of the alcohol **15** with Jones' reagent gave an acid, which was treated with diazomethane to obtain the corresponding methyl ester **17**. An identical methyl ester **17** had been isolated from the sea pen as a minor product (0.02% dry weight).

During some unsuccessful attempts to interconvert compounds of the stylatulide group, we encountered several interesting reactions. Since the minor metabolites lacked chlorine, we attempted to remove the chlorine atom from stylatulide (**2**) using silver acetate in acetic acid. Treatment of stylatulide (**2**) with silver acetate and acetic acid in refluxing aqueous acetonitrile gave a good yield of a single product which still contained chlorine. The ^{1}H NMR spectrum of the product did not contain signals for exocyclic methylene protons but a signal for a Me group at δ 1.31 was observed together with a signal at δ 4.14 which was assigned to the α-chloro proton that was no longer allylic. The IR spectrum did not contain a OH band suggesting that the product was the cyclic ether **18**. The remaining spectral data did not contradict this assignment.

Treatment of the lactone **14** with excess *m*-chloroperbenzoic acid in dichloromethane solution gave a mixture of four products, two epoxides and two diepoxides, each pair being formed in a 2:1 ratio. The spectroscopic evidence showed that the $^{11}\Delta$ olefin was epoxidized preferentially to give a 2:1 ratio of 11β:11α epoxides (**19** and **20**) and that the $^5\Delta$ olefin was then epoxidized stereospecifically to obtain a 2:1 ratio 5β,11β and 5β,11α diepoxides (**21** and **22**). In both epoxides **19** and **20** the C-6 proton signal was at ~5.3 ppm while in the diepoxides **21** and **22** the C-6 proton signal was at ~3.3 ppm, both signals being coupled to the C-7 proton with a 10 Hz coupling constant. The regioselectivity of epoxide formation at the $^{11}\Delta$ olefin can be determined by comparison of the chemical shifts of the C-10 proton signal with that of stylatulide (**2**) at δ 3.04. The chemical shifts of the C-10 protons in **20** and **22** (the minor isomers) at δ 2.80 and 2.91 were much closer than those of **19** and **21** (the major isomers) at δ 2.41 and 2.65. Examination of a Dreiding model of lactone **14** revealed that the β face of the $^{11}\Delta$ olefin was slightly less hindered than the α face and also showed that only the β face of the $^5\Delta$ olefinic bond could be approached by reagent. Although we were unable to accomplish an interrelationship between either of the minor epoxides and stylatulide (**2**), the formation of the epoxides confirmed the locations of the two trisubstituted olefins in the lactone **16**.



The chlorinated diterpenes from *Briareum asbestinum* were first described above a decade ago. Structural elucidation of these compounds by chemical degradation gave inconclusive results due mainly to the high degree of functionality in the molecules. The X-ray diffraction analyses of briarein-A (**1**) and stylatulide (**2**) set the stage for the structural elucidations of related compounds by analysis of spectral and chemical data, but, as this study of stylatulide has shown, the products from chemical reactions on this group of diterpenes are often unpredictable.

Stylatulide (**2**) was found to be toxic to copepodite larvae of *Tisbe furcata johnsonii* at concentration greater than 0.5 ppm. These data suggest that stylatulide (**2**) and possibly the related diterpenes may serve to protect the coelenterates from larval settling. Although it has been suggested that this class of compounds might deter other predators, we have not found stylatulide to be a particularly toxic compound.[9]

EXPERIMENTAL[10]

Collection and extraction of Stylatula sp. The sea pens *Stylatula sp.* were collected at low tide from a sand flat between Isla Partida and Isla Espiritu Santo, Baja California, Mexico in April

1976. The sea pens (370 g dry weight) were preserved in acetone (1 L) for 1 month, homogenized in EtOH (1 L) and extracted in a soxhlet apparatus with 14% EtOH in CH_2Cl_2 (2 L). The combined extracts were concentrated and partitioned between water (500 ml) and EtOAc (3 × 500 ml). Evaporation of the EtOAc gave a brown gum (11.5 g).

Chromatography. The crude extract was introduced onto a column (80 × 5 cm dia.) of Florisil (420 g) in CH_2Cl_2. The materials were eluted with solvents of increasing polarity from CH_2Cl_2 to 15% acetonitrile in EtOAc and collected in 240 fractions (25 ml).

Fractions 85–105 gave a semi-solid mass (4.9 g) which was recrystalized from 1:1 hexane–CH_2Cl_2 to obtain white crystals of 2 (2.1 g). An additional quantity of 2 was obtained by rechromatography of the mother liquor (see below) to yield a total of 2.8 g (0.8% dry weight).

Stylatulide (2). M.p. 179–180°; $[\alpha]_D^{20}+65°$ (*c* 1.8, $CHCl_3$); IR (KBr) 3485, 1795, 1740, 1720, 1640 cm^{-1}; ^1H NMR see Table 1; ^{13}C NMR see Table 2; mass spectrum, *m/e* 482 and 484 (3:1, M-AcOH), 464, 448, 422 and 424 (3:1, M-2 AcOH); (Found: C, 57.32; H, 6.54; Cl, 6.72. $C_{26}H_{35}O_{10}Cl$ requires: C, 57.51; H, 6.50; Cl, 6.53%).

The mother liquor from fractions 85–105 was rechromatographed on silica gel (100 g) and 130 fractions (10 ml) were eluted using solvents of gradually increasing polarity from 20% ether in hexane to EtOAc. Fractions 53–65 (450 mg) were purified by HPLC on μ-porasil using 1% EtOAc in $CHCl_3$ to obtain 14 (320 mg, 0.1% dry weight) as an oil: $[\alpha]_D^{20}-7°$ (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3350, 1775, 1740, 1730, 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.99 (s, 1H), 5.42 (m, 3H), 5.03 (d, 1H, J = 7 Hz), 4.76 (bs, 1H), 3.67 (OH), 2.97 (bs, 1H), 2.56 (m, 3H), 2.17 (s, 3H), 2.03 (s, 3H), 2.00 (bs, 6H), 1.96 (s, 3H), 1.66 (m, 1H), 1.21 (d, 3H, J = 7 Hz), 0.98 (s, 3H); ^{13}C NMR see Table 2; mass spectrum, *m/e* 492 (M^+), 432 (M-AcOH), 404, 390, 372 (M-2 AcOH); HRMS, Obs: 492.2373, $C_{26}H_{36}O_9$ requires: 492.2359.

Fractions 106–130 (265 mg) were combined and rechromatographed on preparative silica gel plates using 5% EtOAc in ether as eluant to obtain two fractions. The less polar material (150 mg) was purified by HPLC on μ-porasil using CH_2Cl_2 to obtain 17 (60 mg, 0.02% dry weight) as an oil. The more polar fraction (80 mg) was purified by HPLC on μ-porasil using CH_2Cl_2 methane to obtain 13 (22 mg, 0.007% dry weight).

Methyl ester 17. $[\alpha]_D^{20}-31°$ (*c* 1.0, $CHCl_3$); UV (MeOH) 218 nm (ε 7500); IR (film) 3350, 1775, 1730, 1710, 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.80 (d, 1H, J = 10 Hz), 6.07 (bs, 1H), 5.45 (bs, 1H), 5.43 (d, 1H, J = 10 Hz), 5.00 (d, 1H, J = 7 Hz), 4.82 (bs, 1H), 3.79 (s, 3H), 3.07 (bs, 1H), 2.19 (s, 3H), 1.98 (s, 6H), 1.92 (s, 3H), 1.28 (d, 3H, J = 7 Hz), 0.98 (s, 3H); mass spectrum, *m/e*, 505 (M-OMe), 476 (M-AcOH), 462, 416 (M-2 AcOH); HRMS, Obs: 505.2073, $C_{26}H_{33}O_{11}$ requires: 505.2073.

17-*Epi-stylatulide* (13). M.p. 205–206°; $[\alpha]_D^{20}-18°$ (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) 3550, 1800, 1740, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.92 (s, 1H), 5.61 (d, 1H, J = 9 Hz), 5.59 (s, 1H), 5.23 (d, 1H, J = 4 Hz), 5.18 (d, 1H, J = 4 Hz), 5.00 (bs, 1H), 4.79 (d, 1H, J = 5 Hz), 3.22 (s, 1H), 3.21 (q, 1H, J = 7 Hz), 2.92 (d, 1H, J = 4 Hz), 2.20 (s, 3H), 2.01 (s, 3H), 1.95 (s, 3H), 1.54 (d, 3H, J = 7 Hz), 1.31 (s, 3H), 1.11 (s, 3H); mass spectrum, *m/e* 464 and 466 (3:1 M-AcOH–H_2O), 422 and 424 (M-2AcOH); HRMS, Obs: 464.1596. $C_{24}H_{29}O_7Cl$ requires: 464.1602.

Fractions 131–150 (185 mg) were purified by HPLC on μ-porasil using ether as eluant to obtain 15 (105 mg, 0.03% dry weight) as an oil: $[\alpha]_D^{20}-13°$ (*c* 1.0, $CHCl_3$); IR (film) 3400, 1780, 1735, 1380 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.02 (bs, 1H), 5.75 (d, 1H, J = 10 Hz), 5.43 (d, 1H, J = 10 Hz), 5.41 (s, 1H), 4.92 (d, 1H, J = 7 Hz), 4.77 (bs, 1H), 4.32 (d, 1H, J = 15 Hz), 4.06 (d, 1H, J = 15 Hz), 2.99 (bs, 1H), 2.47 (m, 2H), 2.16 (s, 3H), 2.02 (s, 3H), 1.91 (bs, 3H), 1.71 (s, 3H), 1.23 (d, 3H, J = 7 Hz), 0.95 (s, 3H); ^{13}C NMR see Table 2; mass spectrum, *m/e*, 508 (M^+), 490, 448, 430, 388; HRMS, Obs: 508.2303. $C_{26}H_{36}O_{10}$ requires: 508.2308.

Ozonolysis of stylatulide (2). O_3 was bubbled through a soln of 2 (100 mg, 0.18 mmol) in MeOH (25 ml) at −78° until a blue color persisted (~15 min). Excess O_3 was removed in a stream of N_2 and MeS (1 ml) was added. The soln was allowed to warm to room temp. and the volatile materials were removed *in vacuo*. The residue was dissolved in CH_2Cl_2 and filtered through silica gel (1 g) to obtain 5 as an oil, yield, 90 mg (90% theoretical); IR ($CHCl_3$) 3400, 1790, 1740, 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.02 (s, 1H), 5.60 (d, 1H, J = 3 Hz), 4.96 (m, 2H), 4.35 (d, 1H, J = 4 Hz), 3.20 (q, 1H, J = 7 Hz), 2.97 (m, 2H), 2.27 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.49 (d, 3H, J = 7 Hz), 1.32 (s, 3H), 1.12 (s, 3H); mass spectrum, *m/e*, 484 and 486 (3:1, M-AcOH), 424 and 426 (M-2AcOH); HRMS, Obs: 484.1488. $C_{23}H_{29}O_9Cl$ requires: 484.1499.

Rearrangement of stylatulide (2) *with boron trifluoride.* BF_3 etherate (100 μl, redistilled) was added to a soln of 2 (100 mg, 0.18 mmol) in benzene (5 ml) and the mixture was stirred under N_2 for 30 min at room temp. The reaction was quenched with sat $NaHCO_3$ aq (2 ml) and the product partitioned between water (10 ml) and CH_2Cl_2 (3 × 10 ml). The combined organic extracts were dried over $MgSO_4$ and the solvent evaporated to obtain an oil which was purified on a silica gel plate using CH_2Cl_2 as eluant to yield 6, yield 80 mg (80% theoretical); IR ($CHCl_3$) 3450, 1780, 1740, 1725, 1360 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.39 (s, 1H), 6.11 (bs, 1H), 5.77 (bs, 1H), 5.61 (d, 1H, J = 6 Hz), 5.40 (bt, 1H, J = 3 Hz), 5.09 (bs, 1H), 5.05 (d, 1H, J = 3 Hz), 4.84 (d, 1H, J = 3 Hz), 3.81 (s, 1H), 3.09 (d, 1H, J = 6 Hz), 2.46 (q, 1H, J = 7 Hz), 2.26 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.32 (s, 3H), 1.17 (d, 3H, J = 7 Hz), 1.08 (s, 3H); mass spectrum, *m/e* 482 and 484 (3:1, M-AcOH); HRMS, Obs: 482.1704. $C_{24}H_{31}O_8Cl$ requires: 482.1707.

Reduction of stylatulide with zinc

Procedure A. Activated granular Zn (325 mg) was added to a soln of 2 (100 mg, 0.18 mmol) in EtOAc (10 ml) and the mixture was boiled under reflux for 4 hr. The cooled soln was filtered to remove solids and the filtrate was concentrated *in vacuo* to obtain a crude product (100 mg). The product was dissolved in CH_2Cl_2 (9 ml) and ether (1 ml) and treated with excess ethereal diazomethane soln for 30 min. The product mixture was separated on a thick layer silica gel plate to obtain 2 (80 mg) and 8 (20 mg, 20% conversion): UV (MeOH) 224 nm (ε 2900); IR ($CHCl_3$), 3400, 1740, 1610 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.11 (d, 1H, J = 9 Hz), 6.00 (bd, 1H, J = 13 Hz), 5.60 (bs, 1H), 5.26 (d, 1H, J = 13 Hz), 4.97 (bs, 1H), 4.82 (d, 1H, J = 6 Hz), 4.77 (bs, 1H), 3.68 (s, 3H), 3.50 (OH), 3.14 (q, 1H, J = 7 Hz), 3.13 (s, 1H), 2.88 (d, 1H, J = 4 Hz), 2.11 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.29 (s, 3H), 1.27 (d, 3H, J = 7 Hz), 1.06 (s, 3H). Mass spectrum, *m/e*, 522 (M^+), 480, 462, 402; HRMS, Obs: 522.2477. $C_{27}H_{38}O_{10}$ requires: 522.2465.

Procedure B. Activated granular Zn (100 mg) was added to a soln of 2 (20 mg, 0.04 mmol) in EtOAc (10 ml) containing AcOH (10 μl, 0.17 mmol) and the mixture was boiled under reflux for 2 hr then stirred at room temp. for 16 hr. The mixture was treated as in Procedure A to obtain 9 (10 mg, 50% theoretical and 12 (5 mg, 25% theoretical).

Methyl ester 12 (*minor product*). UV (MeOH) 227 nm (ε 10,000); IR ($CHCl_3$) 3400, 1740, 1430, 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.31 (s, 1H), 5.93 (bd, 1H, J = 13 Hz), 5.72 (d, 1H, J = 9 Hz), 5.20 (d, 1H, J = 13 Hz), 5.09 (bs, 2H), 4.96 (d, 1H, J = 4 Hz), 4.85 (bs, 1H), 3.78 (d, 1H, J = 4 Hz), 3.65 (s, 3H), 3.52 (OH), 3.05 (q, 1H, J = 7 Hz), 2.10 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H), 1.27 (s, 3H), 1.15 (s, 3H), 1.05 (d, 3H, J = 7 Hz); mass spectrum, *m/e* 522 (M^+), 462, 448, 402, 342; HRMS, Obs: 522.2477. $C_{27}H_{38}O_{10}$ requires: 522.2465.

Methyl ester 9 (*major product*). UV (MeOH) 224 nm (ε 3600); IR ($CHCl_3$) 3500, 1730, 1410, 1350 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.14 (d, 1H, J = 9 Hz), 6.00 (bd, 1H, J = 13 Hz), 5.42 (bs, 1H), 5.25 (d, 1H, J = 13 Hz), 5.16 (bs, 1H), 4.98 (bs, 2H), 4.80 (bs, 1H), 4.62 (s, 1H), 4.20 (m, 1H), 4.02 (bs, 1H), 3.68 (s, 3H), 3.02 (q, 1H, J = 7 Hz), 2.11 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H), 1.16 (d, 3H, J = 7 Hz), 0.99 (s, 3H); mass spectrum, *m/e* 522 (M^+), 504, 462, 402, 342; HRMS, Obs: 522.2477. $C_{27}H_{38}O_{10}$ requires: 522.2465.

A soln of 9 (8 mg, 0.015 mmol) in Ac_2O (0.3 ml) and pyridine (0.2 ml) was stirred at 60° for 4 hr. The reagents were removed *in vacuo* to obtain 10: IR ($CHCl_3$) 3400, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.21 (d, 1H, J = 9 Hz), 6.00 (bd, 1H, J = 13 Hz), 5.30 (m, 4M), 5.00 (bs, 1H), 4.80 (m, 2H), 4.73 (s, 1H), 3.89 (s, 1H), 3.66 (s, 3H), 3.01 (q, 1H, J = 7 Hz), 2.10 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.12 (d, 3H, J = 7 Hz), 1.00 (s, 3H); mass spectrum, *m/e*, 504 (m-AcOH), 444, 384, 324.

Oxidation of methyl ester **9** *with pyridinium chlorochromate.* Pyridinium chlorochromate (5 mg, 0.03 mmol) was added to a soln of **9** (7 mg, 0.015 mmol) in dry CH_2Cl_2 (2 ml) and the soln was stirred at 25° for 6 hr. After addition of PrOH (1 ml), the mixture was partitioned between water (5 ml) and CH_2Cl_2 (3 × 10 ml). The organic extract was dried over Na_2SO_4 and concentrated *in vacuo* to obtain an oil which was purified on a silica gel plate to obtain **11** (3 mg, 50% theoretical); UV (MeOH) 232 nm (ϵ 16,000); IR ($CHCl_3$) 3400, 1735, 1665, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.87 (d, 1H, J = 11 Hz), 6.19 (bs, 1H), 6.03 (bd, 1H, J = 13 Hz), 5.99 (d, 1H, J = 11 Hz), 5.90 (d, 1H, J = 9 Hz), 5.46 (bs, 1H), 5.26 (d, 1H, J = 13 Hz), 5.24 (bs, 1H), 5.00 (bs, 1H), 4.82 (bs, 1H), 3.75 (s, 1H), 3.67 (s, 3H), 2.97 (q, 1H, J = 7 Hz), 2.17 (s, 3H), 2.12 (s, 3H), 1.17 (s, 3H), 1.05 (d, 3H, J = 7 Hz); mass spectrum, *m/e*, 460 (M^+), 418, 400, 385, 358, 340; HRMS, Obs: 460.2099. $C_{25}H_{32}O_8$ requires: 460.2097.

Reaction of stylatulide **(2)** *with 1,5-diazabicyclo[5.4.0] undecene-5 (DBU).* A soln of **2** (32 mg, 0.06 mmol) in dry THF (2 ml) containing DBU (100 μl) was stirred at 25° for 16 hr. The mixture was partitioned between 3 N HCl (10 ml) and EtOAc (3 × 15 ml) and the organic extracts dried over Na_2SO_4 and evaporated to obtain **13** (31 mg, 97% theoretical) identical in all respects to the natural material.

Acetylation of alcohol **15.** A soln of **15** (7 mg, 0.02 mmol) in Ac_2O (0.25 ml) and pyridine (0.25 ml) was stirred at 25° for 4 hr. The excess reagents were evaporated *in vacuo* to obtain the corresponding acetate (7 mg, quantitative) as an oil: IR ($CHCl_3$) 3350, 1780, 1735, 1475 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.03 (bs, 1H), 5.44 (m, 3H), 4.99 (d, 1H, J = 16 Hz), 4.97 (d, 1H, J = 7 Hz), 4.79 (bs, 1H), 4.53 (d, 1H, J = 16 Hz), 2.19 (s, 3H), 2.13 (s, 3H), 2.02 (s, 3H), 1.99 (bs, 3H), 1.97 (s, 3H), 1.26 (d, 3H, J = 7 Hz), 0.97 (bs, 3H); mass spectrum, *m/e*, 490 (M-AcOH), 430, 402, 388, 370.

Oxidation of alcohol **15** *with manganese dioxide.* MnO_2 (60 mg) was added to a soln of **15** (7 mg, 0.02 mmol) in CH_2Cl_2 (5 ml) and the mixture was stirred vigorously at 25° for 20 hr. The soln was filtered through silica gel (0.5 g) using acetone, concentrated *in vacuo* and the resulting oil purified on a silica gel plate using ether as eluant to obtain **16** (4 mg, 55% theoretical): UV (MeOH) 228 nm (ϵ 8500); IR ($CHCl_3$) 3400, 1790, 1740, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.54 (s, 1H), 6.53 (bd, 1H, J = 10 Hz), 6.08 (bs, 1H), 5.56 (d, 1H, J = 10 Hz), 5.43 (bs, 1H), 4.89 (d, 1H, J = 9 Hz), 4.80 (bs, 1H), 3.00 (bs, 1H), 2.64 (m, 3H), 2.21 (s, 3H), 1.98 (bs, 6H), 1.90 (s, 3H), 1.30 (d, 3H, J = 7 Hz), 1.01 (bs, 3H); mass spectrum, *m/e*, 446 (M-AcOH), 404, 386, 358, 344, 326; HRMS, Obs: 446.1940. $C_{26}H_{34}O_{10}$ requires: 446.1940.

Oxidation of alcohol **15** *with Jones' reagent.* Jones' reagent[11] (50 μl, 0.56 mmol) was added dropwise to a stirred soln of the **15** (12 mg, 0.024 mmol) in acetone at 0°. The mixture was allowed to warm to 25° and stirring was continued for 2 hr. After addition of i-PrOH (1 ml), the mixture was partitioned between 1 N HCl (5 ml) and CH_2Cl_2 (3 × 10 ml). The combined organic extracts were dried over Na_2SO_4 and the solvent evaporated to yield an oil. Excess ethereal diazomethane soln was added to a soln of the oil in MeOH (1 ml). After 15 min the volatile material was evaporated and the resulting product purified on a silica gel plate to obtain **17** (3 mg, 30% theoretical), identical in all respects to the natural material.

Reaction of stylatulide **(2)** *with silver acetate.* A soln of **2** (65 mg, 0.13 mmol) and AgOAc (60 mg, 0.36 mmol) in aqueous acetonitrile (1:1, 6 ml) containing AcOH (100 μl) was boiled under reflux in the dark for 16 hr. After addition of 5% $NaHCO_3$ aq, the organic material was extracted with CH_2Cl_2 (3 × 20 ml) and the combined extracts dried over Na_2SO_4 and the solvent evaporated to obtain **18** (55 mg, 84% theoretical): IR ($CHCl_3$) 1790, 1740, 1730, 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.94 (bs, 1H), 5.56 (bd, 1H, J = 4 Hz), 4.87 (bt, 1H, J = 2 Hz), 4.70 (d, 1H, J = 4 Hz), 4.14 (d, 1H, J = 4 Hz), 2.94 (m, 3H), 2.26 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H), 1.47 (d, 3H, J = 7 Hz), 1.33 (s, 3H), 1.31 (s, 3H), 1.12 (s, 3H); mass spectrum, *m/e* 506 (M-HCl), 482 and 484 (3:1, M-AcOH), 442, 440, 382, 380; HRMS, Obs: 482.1695. $C_{24}H_{31}O_9Cl$ requires: 482.1707.

Reaction of lactone **14** *with m-chloroperbenzoic acid. m*-Chloreperbenzoic acid (34 mg, 0.17 mmol) was added to a soln of **14** (40 mg, 0.08 mmol) in CH_2Cl_2 (2 ml) and the soln was stirred at 25° for 3 hr. CH_2Cl_2 (10 ml) and 5% $NaHSO_3$ aq (5 ml) were added and the mixture was stirred vigorously for 15 min. The organic layer was separated, dried over Na_2SO_4, and the solvent evaporated to give an oil (39 mg). The product was chromatographed on a silica gel plate to obtain two fractions. The less-polar fraction (17 mg) was separated using HPLC on μ-porasil into two compounds, the 11β-epoxide **19** (10 mg, 24% theoretical) and the 11α-epoxide **20** (5 mg, 12% theoretical). The more polar fraction (12 mg) was separated using HPLC into two compounds, the 11β-diepoxide **21** (7 mg, 16% theoretical) and the 11α-diepoxide **22** (3 mg, 7% theoretical).

11β-Epoxide **19.** IR ($CHCl_3$) 3400, 1775, 1730, 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.00 (d, 1H, J = 3 Hz), 5.39 (d, 1H, J = 10 Hz), 5.34 (d, 1H, J = 10 Hz), 4.86 (d, 1H, J = 7 Hz), 4.74 (bt, 1H, J = 3 Hz), 2.95 (bd, 1H, J = 3 Hz), 2.49 (m, 3H), 2.41 (d, 1H, J = 3 Hz), 2.25 (s, 3H), 2.00 (bs, 9H), 1.45 (s, 3H), 1.32 (d, 3H, J = 7 Hz), 1.01 (s, 3H); mass spectrum, *m/e*, 508 (M^+), 448, 420, 405, 388; HRMS, Obs: 508.2317. $C_{28}H_{36}O_{10}$ requires: 508.2308.

11α-Epoxide **20.** ^1H NMR ($CDCl_3$) δ 6.12 (d, 1H, J = 3 Hz), 5.40 (d, 1H, J = 10 Hz), 5.30 (d, 1H, J = 10 Hz), 4.98 (bd, 1H), 4.68 (bt, 1H, J = 3 Hz), 3.00 (bt, 1H, J = 3 Hz), 2.80 (d, 1H, J = 3 Hz), 2.22 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.66 (s, 3H), 1.29 (d, 3H, J = 7 Hz), 0.98 (s, 3H).

11β-Diepoxide **21.** M.p. 270–271°; IR ($CHCl_3$) 3400, 1775, 1730, 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.02 (d, 1H, J = 3 Hz), 5.07 (d, 1H, J = 7 Hz), 4.71 (bt, 1H, J = 3 Hz), 4.48 (d, 1H, J = 10 Hz), 3.34 (d, 1H, J = 10 Hz), 3.02 (bt, 1H, J = 3 Hz), 2.65 (d, 1H, J = 3 Hz), 2.59 (q, 1H, J = 7 Hz), 2.17 (s, 3H), 2.04 (s, 6H), 1.72 (s, 3H), 1.51 (s, 3H), 1.36 (d, 3H, J = 7 Hz), 1.04 (s, 3H); mass spectrum, *m/e*, 524 (M^+), 481, 465, 464, 421, 404; HRMS, Obs: 524.2265. $C_{26}H_{36}O_{11}$ requires: 524.2257.

11α-Diepoxide **22.** ^1H NMR ($CDCl_3$) δ 5.84 (d, 1H, J = 3 Hz), 5.53 (bs, 1H), 5.46 (d, 1H, J = 7 Hz), 4.60 (d, 1H, J = 10 Hz), 3.26 (d, 1H, J = 10 Hz), 3.02 (bt, 1H, J = 3 Hz), 2.93 (q, 1H, J = 7 Hz), 2.91 (d, 1H, J = 3 Hz), 2.21 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.57 (s, 3H), 1.49 (s, 3H), 1.25 (d, 3H, J = 7 Hz), 1.17 (s, 3H).

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