Metabolites of the Marine Sponge *Dendrilla* sp.

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Received March 9, 1984

The sponge *Dendrilla* sp. was collected from a marine lake in Palau, Western Caroline Islands. *Dendrilla* sp. contained the known diterpenes dehydroambliol A (1) and norrisolide (2), together with the novel metabolites 1-bromo-8-ketoambliol A acetate (3), dendrillolide A (4), dendrillolide B (5), and dendrillolide C (6). The structures of the new metabolites were determined by interpretation of spectral data, application of a biosynthetic hypothesis, and chemical interconversions.

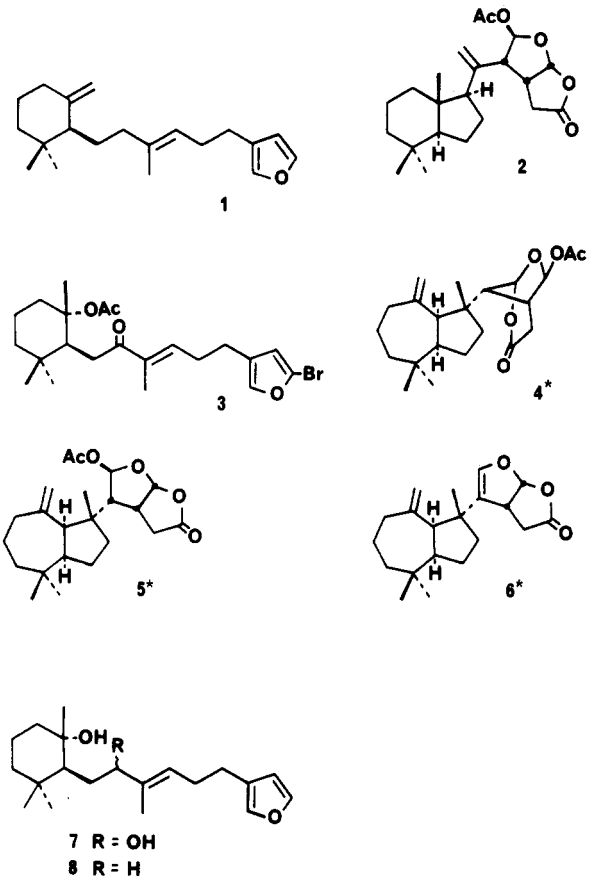
The marine lakes of Palau, Western Caroline Islands, are unique microenvironments with most of the lakes having different flora and fauna. The deep purple sponge *Dendrilla* sp.[1] was collected from a lake on an island in Iwayama Bay. This marine sponge contained a number of diterpenes that can be related biosynthetically to metabolites described previously. We have recently reported the structures of two of the diterpene constituents; dehydroambliol A (1) from the sponge *Dysidea amblia*[2] and norrisolide (2) from the spongivorous dorid nudibranch *Chromodoris norrisi*.[3] In this paper we propose structures for the new metabolites 1-bromo-8-ketoambliol A acetate (3), dendrillolide A (4), dendrillolide B (5), and dendrillolide C (6) (Chart I).

The ethyl acetate soluble material from a methanolic extract of *Dendrilla* sp. was chromatographed on Sephadex LH-20. The fractions that showed antimicrobial activity against *Bacillus subtilis* were further purified by flash chromatography on TLC-grade silica and by LC on μ-Partisil to obtain dehydroambliol A (1, 0.005% dry weight), 1-bromo-8-ketoambliol A acetate (3, 0.34% dry weight), dendrillolide A (4, 0.31% dry weight), dendrillolide B (5, 0.004% dry weight), dendrillolide C (6, 0.008% dry weight), and norrisolide (2, 0.01% dry weight). Dehydroambliol A (1) and norrisolide (2) were identified by comparison of spectral data with those of authentic samples.

1-Bromo-8-ketoambliol A acetate (3) was obtained as an unstable oil that decomposed autocatalytically in chloroform solution. The molecular formula, $C_{22}H_{31}BrO_4$, was determined from the ^{13}C NMR spectrum (22 signals) and high-resolution mass measurements of the M − AcOH ions at m/z 378.1213 and 380.1189 ($C_{20}H_{27}BrO_2$). The presence of an acetate group was indicated by an infrared band at 1740 cm^{-1}, a ^1H NMR signal at δ 1.78 (s, 3 H), and ^{13}C NMR signals at δ 169.7 (s) and 21.9 (q). The infrared band at 1680 cm^{-1} and ultraviolet absorption at 227 nm (ε 9000) were indicative of an α,β-unsaturated ketone group. The ^1H NMR spectrum contained a signal at δ 6.35 (t, 1 H, J = 7 Hz), due to the β-proton on an α-substituted α,β-unsaturated ketone, and two signals at δ 5.85 (s, 1 H) and 6.78 (s, 1 H) assigned to α and β protons on a 2,3- or 2,4-disubstituted furan ring. The ^{13}C NMR signal at δ 201.2 (s) was due to the ketone carbon while six signals at δ 140.5 (d), 139.5 (d), 137.7 (s), 126.8 (s), 122.1 (s), and 112.1 (d) were due to the conjugated olefin and the furan group.

Reduction of the bromofuran 3 with lithium aluminum hydride in dry ether gave both the diol 7 and ambliol A (8).[2] This transformation established the carbon skeleton of the bromofuran 3 and the position of the ketone at carbon 8. The position of the bromine atom on the furan ring was determined by repeating the reduction of the bromofuran 3 using lithium aluminum deuteride in ether as the reducing agent and quenching the reaction with deuterium oxide to obtain a deuterio derivative of ambliol A. The furan proton signal at δ 7.24 (br s, 1 H) was greatly reduced in intensity compared with those at δ 7.12 (br s, 1 H) and 6.17 (br s, 1 H), indicating deuterium substitution at C-1.[4]

Chart I[a]



[a] Asterisk mean that stereochemical assignments are based on a biosynthetic hypothesis.

Dendrillolides A and B (4 and 5) have the same molecular formula, $C_{22}H_{32}O_5$, as norrisolide (2), while dendrillolide C (6) has lost the elements of acetic acid. Certain features of the ^1H NMR spectra (Table I) indicated that the dendrillolides 4–6 all have the same carbon skeleton but that this carbon skeleton is different from that of norrisolide (2). Furthermore, the ^1H NMR spectra indicated that both dendrillolide B (5) and norrisolide (2) share

(1) For a description of *Dendrilla*, see: Bergquist, P. B. *N. Z. J. Zool.* **1980**, *7*, 486.
(2) Walker, R. P.; Faulkner, D. J. *J. Org. Chem.* **1981**, *46*, 1098.
(3) Hochlowski, J. E.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1983**, *48*, 1141.
(4) Jackman, L. M.; Sternhell, S. "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry"; Pergamon: Oxford, England, 1969; p 214.

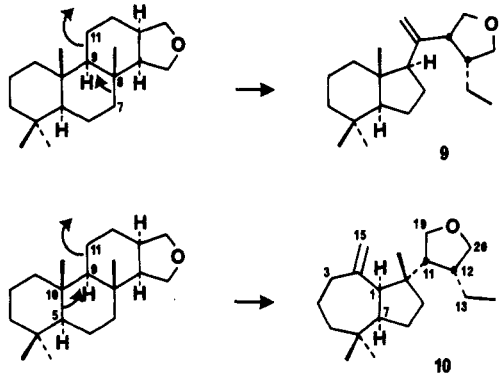
0022-3263/84/1949-3204$01.50/0

Table I. 360-MHz ^{1}H NMR Spectra of Norrisolide (2), Dendrillolide A (4), Dendrillolide B (5), and Dendrillolide C (6) [Chemical Shift, Multiplicity, Integration, Coupling Constants]

H at C no.	2[a]	4[b]	5[c]	6[c]
1α		2.56, d, 1 H, 8.7	2.66, d, 1 H, 8.0	2.58, d, 1 H, 8.0
2β		1.77, m, 1 H	1.83, m, 1 H	1.72, m, 1 H
3α		2.32, m, 1 H	2.37, m, 1 H	2.34, m, 1 H
4β		1.70, m, 1 H	1.74, m, 1 H	1.77, m, 1 H
4α		1.35, m, 1 H	1.40, m, 1 H	1.40, m, 1 H
5β		1.55, m, 1 H	1.60, m, 1 H	1.64, m, 1 H
5α		1.24, m, 1 H	1.28, m, 1 H	1.28, m, 1 H
7α		1.69, m, 1 H	1.72, m, 1 H	2.00, m, 1 H
8		~1.54, m, 2 H	~1.68, m, 2 H	1.77, m, 2 H
9		~1.63, m, 2 H	1.98, m, 1 H	1.85, m, 1 H
11	3.07, dd, 1 H, 9.4, 3.3	2.48, dd, 1 H, 6.5, 6.2	2.27, dd, 1 H, 7.3, 3.8	
12	3.36, m, 1 H, 9.4, 7, 7, 5.9	2.80, m, 1 H, 10.1, 9.3, 6.5, 4.1	3.15, m, 1 H, 11.3, 7.3, 6.2, 6.0	3.57, m, 1 H, 9, 6.8, 4, 1
13	2.55, d, 2 H, 7	2.45, dd, 1 H, 17.5, 10.1	2.97, dd, 1 H, 18.3, 6.2	2.68, dd, 1 H, 17.4, 4
13		2.21, dd, 1 H, 17.5, 9.3	2.44, dd, 1 H, 18.3, 11.3	2.64, dde, 1 H, 17.4, 9
15	0.66, s, 3 H	4.49, d, 1 H, 2	4.57, d, 1 H, 2	4.58, d, 1 H, 2
15		4.76, d, 1 H, 2	4.82, d, 1 H, 2	4.88, d, 1 H, 2
16	0.86, s, 3 H	0.92, s, 3 H	0.94, s, 3 H	0.91, s, 3 H
17	0.84, s, 3 H	0.95, s, 3 H	0.97, s, 3 H	0.96, s, 3 H
18	5.09, s, 1 H, 1			
18	5.15, d, 1 H, 1	0.85, s, 3 H	1.00, s, 3 H	1.01, s, 3 H
19	6.44, d, 1 H, 3.4	6.39, d, 1 H, 6.2	6.40, d, 1 H, 3.8	6.14, d, 1 H, 1.0
20	6.14, d, 1 H, 6.0	5.82, d, 1 H, 4.1	5.97, d, 1 H, 6.0	6.24, d, 1 H, 6.8
OAc	2.07, s, 3 H	2.00, s, 3 H	2.00, s, 3 H	

[a] $CDCl_3$. [b] CCl_4 + 1% C_6D_6. [c] CCl_4.

Scheme I. Proposed Biosynthesis of "Norrisane" 9 and "Dendrillane" 10 from a "Spongian" Precursor



the same fused γ-lactone–tetrahydrofuran ring system but that in dendrillolide B (**5**), C-11 is adjacent to a quarternary aliphatic carbon atom. In considering possible $C_{14}H_{23}$ bicyclic fragments of the dendrillolide carbon skeleton, it seemed reasonable that the dendrillolides might be derived from the same biosynthetic precursor as norrisolide. We had proposed (Scheme I) that the norrisolide skeleton be derived from a "spongian" skeleton[5] by cleavage of the 9,11 bond with concomitant migration of the parallel 7,8 bond. Cleavage of the 9,11 bond with migration of the alternative parallel 5,10 bond gives a perhydroazulene skeleton that is completely compatible with the spectral data for the dendrillolides.

Although we were unable to determine all the coupling constants, spin-decoupling experiments revealed the ^{1}H NMR chemical shift values for every proton in dendrillolides A–C (**4**–**6**). Because the solvent systems were chosen to minimize overlap of signals, the ^{1}H NMR data for the $C_{14}H_{23}$ portions of dendrillolides A–C were not identical but were sufficiently similar to permit the assumption of a common bicyclic ring system. Considering now the data for the major isomer, dendrillolide A (**4**), ^{13}C NMR signals at δ 153.7 (s) and 113.3 (t) together with ^{1}H NMR signals at δ 4.49 (d, 1 H, J = 2 Hz) and 4.76 (d, 1 H, J = 2 Hz) indicated a 1,1-disubstituted olefin. There were two ^{1}H NMR signals at δ 2.56 (d, 1 H, J = 8.7 Hz) and 2.32 (m, 1 H) that were not coupled to one another and had chemical shifts typical of allylic protons. Furthermore, each of these allylic protons showed a nuclear Overhauser enhancement on irradiation of one of the olefinic protons (4.49 → 2.56 and 4.76 → 2.32). Spin-decoupling experiments indicated that the δ 2.56 signal was at one end of a -CHCHCH$_2$CH$_2$- sequence of protons while the δ 2.32 signal was part of a -CH$_2$CH$_2$CH$_2$- chain (see Table I). Three methyl groups with ^{1}H NMR signals at δ 0.85 (s, 3 H), 0.92 (s, 3 H), and 0.95 (s, 3 H) were attached to two fully substituted carbon atoms having ^{13}C NMR signals at δ 36.0 (s) and 46.7 (s); the remaining carbon chain was also attached at one of the fully substituted carbons. These data were fully compatible with the proposed perhydroazulene moiety. Furthermore, the 8.7-Hz coupling constant between protons at C-1 and C-7 was in the range expected for a perhydroazulene with a cis ring junction; the cis ring junction was also expected from the biosynthetic hypothesis. Signals in the ^{1}H NMR spectra of dendrillolides B (**5**) and C (**6**) could be assigned to the same perhydroazulene skeleton (Table I).

Comparison of spectral data revealed that the $C_8H_9O_5$ residue of dendrillolide B (**5**) was identical with the lactone–acetal portion of norrisolide (**2**). In particular, the infrared bands at 1790 and 1760 cm^{-1} were assigned to a γ-lactone and an acetate attached to a hemiacetal group as found in norrisolide (**2**). The ^{1}H NMR spectrum contained signals at δ 2.00 (s, 3 H, -OAc), 6.40 (d, 1 H, J = 3.8 Hz, C-19), 2.27 (dd, 1 H, J = 7.3, 3.8 Hz, C-11), 3.15 (m, 1 H, J = 11.3, 7.3, 6.2, 6.0 Hz, C-12), 5.97 (d, 1 H, J = 6.0 Hz, C-20), 2.97 (dd, 1 H, J = 18.3, 6.2 Hz), and 2.44 (dd, 1 H, J = 18.3, 11.3 Hz, C-13). One would not expect the ^{1}H NMR data for this segment of dendrillolide B to be identical with the corresponding data for norrisolide since C-11 is not allylic but the small differences in coupling constants were cause for concern. However, the observation of a nuclear Overhauser effect between the protons at C-11 and C-12 together with almost identical coupling constants for the acetal protons in both molecules established the stereochemistry of this segment of den-

(5) Kazlasukas, R.; Murphy, P. T.; Wells, R. J.; Noack, K.; Oberhänsli, W. E.; Schonhölzer, P. *Aust. J. Chem.* **1979**, *32*, 867.

drillolide B (5). The stereochemical relationship between the two segments could not be reliably determined so we have selected the relative stereochemistry that is consistent with the biosynthetic hypothesis.

Dendrillolide C (6), molecular formula $C_{20}H_{23}O_8$, is the product formed by elimination of acetic acid from dendrillolide B (5). The infrared bands at 1800 and 1640 cm^{-1} were assigned to a γ-lactone and an enol ether, respectively. In the ^1H NMR spectrum the C-19 proton signal at δ 6.14 (d, 1 H, J = 1 Hz) was coupled to an allylic proton signal at δ 3.57 (m, 1 H, J = 9, 6.8, 4, 1 Hz) that was in turn coupled to an acetal proton signal at δ 6.24 (d, 1 H, J = 6.8 Hz) and two mutually coupled methylene proton signals at δ 2.64 and 2.68. The remaining proton signals (Table I) were almost identical with those of dendrillolide B (5).

Dendrillolide A (4), the major diterpene of *Dendrilla* sp., was an isomer of dendrillolide B (5). Comparison of the ^1H NMR spectrum with that of dendrillolide B (5) indicated the presence of the same perhydroazulene segment. The infrared spectrum again indicated the presence of lactone (1785 cm^{-1}) and acetate (1740 cm^{-1}) groups. Two acetal groups gave rise to ^{13}C NMR signals at δ 105.1 (d) and 97.1 (d), compared with δ 107.1 (d) and 101.8 (d) for norrisolide (2), and ^1H NMR signals at δ 5.82 (d, 1 H, J = 4.3 Hz) and 6.39 (d, 1 H, J = 6.2 Hz). A signal at δ 2.48 (dd, 1 H, J = 6.5, 6.2 Hz) was coupled to the δ 6.39 acetal signal and to a signal at δ 2.80 (m, 1 H, J = 10, 9, 6.5, 4.1 Hz) that was in turn coupled to the acetal proton signal at δ 5.82 and to two methylene proton signals at δ 2.45 and 2.21. These data suggested that dendrillolides A and B had the same carbon skeleton and the same oxidation state at each carbon. It was not possible for dendrillolides A and B to be simple stereoisomers at C-11 or C-19 since the coupling constant between protons at C-12 and C-20 was 4.1 Hz rather than the 6–7 Hz required for the cis-fused bicyclo[3.3.0]lactone–acetal ring system. We therefore proposed that dendrillolide A (4) had the alternative bicyclo[3.2.1] ring system. The stereochemistry drawn was based on the alternative cyclization of the presumed dialdehyde–carboxylic acid intermediate.

Experimental Section[6]

Extraction and Chromatography. The deep purple sponge, *Dendrilla* sp. (Lendenfeld, 1883) was collected by hand, using SCUBA, from a marine lake on an island in Iwayama Bay, Palau, in March 1981. After being stored in methanol for ~1 year at 0 °C, the sponge (118 g dry weight) was placed in fresh methanol for 2 weeks at 25 °C. The sponge was removed and dried. The combined methanol extracts were evaporated under reduced pressure and the residue was partitioned between water (500 mL) and ethyl acetate (4 × 500 mL). The ethyl acetate extracts were dried over sodium sulfate and evaporated to obtain a black gum (2.92 g, 2.45% dry weight).

The crude extract was chromatographed on Sephadex LH-20 using methanol as eluant. Fractions that showed antimicrobial activity against *B. subtilis* were combined and further purified by flash chromatography using solvents of increasing polarity from hexane through ether to ethyl acetate. A fraction eluted with hexane gave dehydroambliol A (1, 6 mg, 0.005% dry weight). A fraction eluted with 20% ether in hexane was rechromatographed by LC on μ-Partisil using 40% ethyl acetate in hexane to obtain the bromofuran 3 (400 mg, 0.34% dry weight), dendrillolide A (4, 360 mg, 0.31% dry weight), dendrillolide C (6, 9 mg, 0.008% dry weight), dendrillolide B (5, 5 mg, 0.004% dry weight), and norrisolide (2, 12 mg, 0.01% dry weight).

1-Bromo-8-ketoambliol A acetate (3): oil; $[\alpha]^{20}_D$ +18.20° (c 1.2, $CHCl_3$); IR ($CHCl_3$) 1740, 1680, 1360, 1250, 1100 cm^{-1}; UV (MeOH) 227 (ϵ 9000); ^1H NMR (C_6D_6) δ 0.67 (s, 3 H), 0.83 (s, 3 H), 1.54 (s, 3 H), 1.64 (s, 3 H), 1.78 (s, 3 H), 2.02 (m, 4 H), 2.48 (dd, 1 H, J = 17, 5 Hz), 2.62 (dd, 1 H, J = 5, 6 Hz), 2.72 (m, 2 H), 5.85 (s, 1 H), 6.35 (t, 1 H, J = 7 Hz), 6.78 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 201.2 (s), 169.7 (s), 140.5 (d), 139.5 (d), 137.7 (s), 126.8 (s), 122.1 (s), 112.1 (d), 86.2 (s), 50.4 (d), 40.4 (t), 37.4 (t), 35.0 (s), 33.9 (t), 32.4 (q), 28.8 (t), 23.9 (t), 22.5 (q), 21.9 (q), 19.7 (q), 19.5 (t), 11.8 (q); high-resolution mass spectrum, obsd m/e 378.1213, 380.1189, $C_{20}H_{27}O_2Br$ (M – AcOH) requires 378.1194, 380.1174.

Dendrillolide A (4): oil; $[\alpha]^{20}_D$ +83.51° (c 3.8, $CHCl_3$); IR ($CHCl_3$) 1785, 1740, 1360, 1200, 985 cm^{-1}; ^1H NMR (see Table I); ^{13}C NMR (C_6D_6) δ 175.2 (s), 169.1 (s), 153.7 (s), 113.3 (t), 105.0 (d), 97.1 (d), 55.7 (d), 54.9 (d), 54.5 (d), 46.7 (s), 41.9 (d), 38.8 (t), 37.7 (t × 2), 36.0 (s), 34.5 (q), 28.8 (t), 28.7 (t), 27.1 (t), 25.8 (q), 24.1 (q), 20.7 (q); high-resolution mass spectrum, obsd m/e 376.2211, $C_{22}H_{32}O_5$ requires 376.2250.

Dendrillolide B (5): oil; IR 1790, 1740 cm^{-1}; ^1H NMR (see Table I); high-resolution mass spectrum, obsd m/e 376.2241, $C_{22}H_{32}O_5$ requires 376.2250.

Dendrillolide C (6): oil; $[\alpha]^{20}_D$ +130.90 (c 0.3, $CHCl_3$); IR (CCl_4) 1800, 1640 cm^{-1}; ^1H NMR (see Table I); high-resolution mass spectrum, obsd m/e 316.2038, $C_{20}H_{28}O_3$ requires 316.2031.

Reduction of Bromofuran 3 with Lithium Aluminum Hydride. A solution of bromofuran 3 (9.5 mg, 0.02 mmol) in dry ether (5 mL) was added to a suspension of lithium aluminum hydride (~20 mg) in dry ether (5 mL), and the mixture was stirred for 30 min at room temperature. Excess reagent was destroyed by addition of ethyl acetate (3 drops) followed by dropwise addition of 0.1 N hydrochloric acid. The ether layer was separated and dried over sodium sulfate, and the solvent was removed to obtain a mixture of products. The reaction products were separated by LC on μ-Partisil using 40% ethyl acetate–hexane as eluant to obtain ambliol A (8; 1.5 mg, 23% theoretical) and the diol (7; 4.0 mg, 58% theoretical). The sample of ambliol A (8) was identical in all respects with an authentic sample from *Dysidea amblia*.[2]

Diol 7: ^1H NMR (CCl_4) δ 0.78 (s, 3 H), 0.93 (s, 3 H), 1.13 (s, 3 H), 1.59 (s, 3 H), 1.95 (d, 1 H, J = 11.7 Hz), 2.25 (q, 2 H, J = 7 Hz), 2.46 (t, 2 H, J = 7 Hz), 3.78 (d, 1 H, J = 11.7 Hz), 3.80 (br s, 1 H), 5.38 (t, 1 H, J = 7 Hz), 6.17 (br s, 1 H), 7.12 (br s, 1 H), 7.24 (br s, 1 H); mass spectrum m/e 302 ($M^+ - H_2O$).

Acknowledgment. We thank Dr. Janice E. Thompson for identifying the sponge sample. High-resolution mass measurements were provided by the Bio-organic, Biomedical Mass Spectrometry Resource (A. L. Burlingame, Director) supported by NIH Research Grant No. RR00719. This research was funded by grants from the California Sea Grant Program and by the National Institutes of Health (AI-11969).

(6) For general procedures, see: Cartê, B.; Faulkner, D. J. *J. Org. Chem.* **1983**, *48*, 2314.