matching spectra, etc., were obtained. $[\alpha]^{20}_D$ +38.7° (c = 0.0295 in $CHCl_3$). ^1H NMR: δ 3.89 (1 H, dd, J = 12.5, 2.4 Hz) and 3.61 (1 H, dd, J = 12.5, 4.2 Hz, CH_2OH), 2.93 (1 H, td, J = 5.6, 2.4 Hz, H_3), 2.89 (1 H, dt, J = 2.4, 4.2 Hz, H_2), 1.2-1.67 (m, 11 H, CH_2 and OH), 0.86 (3 H, t, J = 6.8 Hz, CH_3). (This ^1H spectrum matched those for the (±) and (-) epoxy alcohols.) **Red-Al reductions** of the (+) and (-) epoxy alcohols were performed as described for the (±) alcohol, the (-) epoxy alcohol provided (+)-nonane-1,3-diol (64%), and the (+) epoxy alcohol the (-)-nonane-1,3-diol (53%). **(+)-Nonane-1,3-diol**: $[\alpha]^{20}_D$ = +6.03 (c = 1.2 in ethanol). ^1H NMR: 3.8 (3 H, m, CH-O), 2.2 (2 H, br s, OH), 1.2-1.7 (12 H, m), 0.8 (3 H, t, J = 6.8 Hz, CH_3). The ^{13}C and MS data were essentially identical with those listed for the racemic compound. **(-)-Nonane-1,3-diol**: $[\alpha]^{20}_D$ = -5.4° (c = 1.40 in ethanol) with appropriate ^1H and ^{13}C NMR and MS.

Ozonolysis of (*R*,*Z*)-(+)-12-hydroxyoctadec-9-enoic acid was conducted in the standard way at -70 °C (methanol solvent; dimethyl sulfide present) to provide **(*R*)-3-hydroxynonanal** (Kugelrohr distillation: 120 °C, 1 mm). ^1H NMR: 4.1 (1 H, m, CH-OH), 2.6 (2 H, m, CH_2CO), 2.4 (1 H, br s, OH), 1.3 (11 H, m), 0.8 (3 H, t, CH_3). MS (m/z, rel intensity): (158, 0, M^+), (73, 28.9), (70, 36.6), (57, 24.4), (55, 66.7), (45, 54.7). **Reduction** of the above aldehyde was effected with $NaBH_4$ in methanol in the normal way to provide **(*R*)-(-)-nonane-1,3-diol** (Kugelrohr: 100 °C, 0.1 mm) (lit.[50] bp 97-99 °C, 0.1 mm)). $[\alpha]^{22}_D$ = -5.5° (c = 5.8 in ethanol) (lit.[50] $[\alpha]^{22}_D$ = -6.30° (c = 10 in ethanol)). MS (m/z, rel intensity): (113, 22.1), (97, 14.6), (75, 100), (70, 24.4), (57, 53.2), (55, 52.0), (45, 43.3).

Hydroxy-Substituted Spiroketals. The syntheses of some of the various hydroxy-substituted spiroketals have been described elsewhere[14] and will be reported in full in a separate publication.[30]

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Diterpenes from the Marine Sponge *Aplysilla polyrhaphis* and the Dorid Nudibranch *Chromodoris norrisi*[1]

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Nine diterpenes of the spongian class have been isolated from the marine sponge *Aplysilla polyrhaphis* collected in the Gulf of California. *A. polyrhaphis* contained the known compounds macfarlandin E (**2**), aplyviolene (**3**), norrisolide (**4**), shahamin C (**5**), the γ-lactone **6**, and four novel diterpenes, polyrhaphins A-D (**7-10**). The structures of the polyrhaphins were determined by interpretation of spectral data and chemical interconversions. Polyrhaphin D (**10**) is the first example of the isospongian carbon skeleton. Macfarlandin E (**2**), norrisolide (**4**), shahamin C (**5**), and polyrhaphin A (**7**) were also isolated from specimens of the dorid nudibranch *Chromodoris norrisi* that were collected in the same locality as *Aplysilla polyrhaphis*, which is the presumed dietary source. Shahamin C (**5**), the γ-lactone **6**, and polyrhaphin C (**9**) inhibit feeding by the Gulf of California rainbow wrasse *Thalossoma lucasanum*. Some of the diterpenes also exhibit antimicrobial activity.

We have recently undertaken an investigation of sponges within the order Dendroceratida to test the hypothesis that the absence of siliceous spicules which is characteristic of this order may be compensated by an increase in the variety and quantity of secondary metabolites produced by the animals. This hypothesis originated from the observation that the loss of a physical defense mechanism, such as spicules, may result in the development of an alternate chemical defense mechanism.[2] The purple dendroceratid sponge *Aplysilla polyrhaphis* was collected in a mangrove lagoon on Isla San Jose in the Gulf of California. This extremely fragile, leafy sponge was not overgrown by other invertebrates or algae in this highly diverse and productive environment.

Nine diterpenes derived by rearrangement of the spongian diterpene skeleton **1** have been isolated from *A. polyrhaphis*. The major metabolite, macfarlandin E (**2**), has been previously reported from the dorid nudibranch *Chromodoris macfarlandi*,[3,4] while the analogous monoacetate, aplyviolene (**3**), was isolated from the dendroceratid sponge *Chelonaplysilla violacea*.[5] Norrisolide (**4**) was originally found in the nudibranch *Chromodoris norrisi*,[6] while shahamin C (**5**) was recently reported from the sponge *Dysidea* sp.,[7] and the γ-lactone **6** was isolated from the sponge *Aplysilla rosea*.[8] Four new rearranged spongian diterpenes, polyrhaphins A (**7**), B (**8**), C (**9**), and D (**10**), have been isolated and their structures determined. Polyrhaphins A-C (**7-9**) are rearranged spongian diterpenes each consisting of a hydrocarbon portion derived from the A and B rings of the spongian diterpene skeleton **1** and an oxygenated portion derived from the C and D rings of the spongian skeleton. In determining the structures of these compounds the nature of each of these fragments was elucidated separately, and the two parts were then related to one another. Polyrhaphin D (**10**) is a novel isospongian diterpene in which a 2,3-fused tetrahydrofuran ring replaces the 3,4-fused tetrahydrofuran ring normally found in spongian diterpenes. Polyrhaphin D

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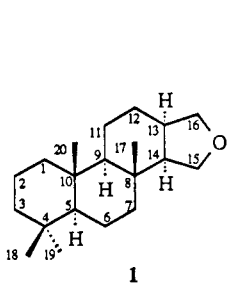

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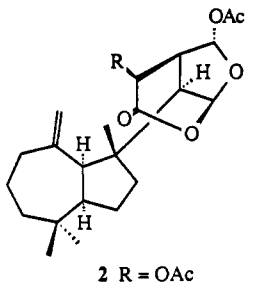

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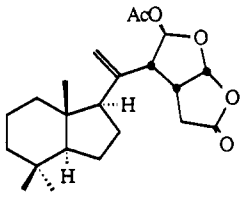
3 R = H



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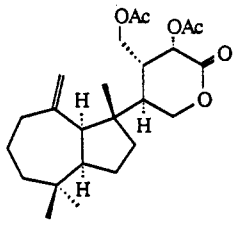

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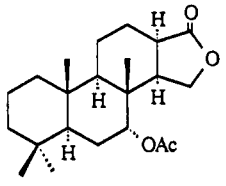

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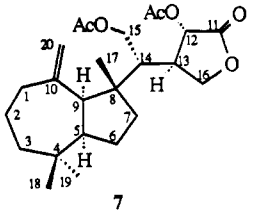

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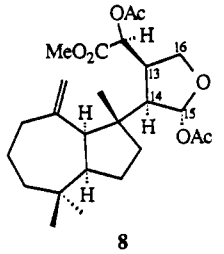

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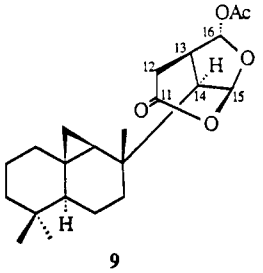

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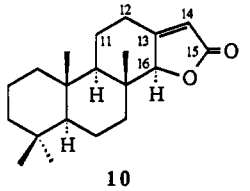

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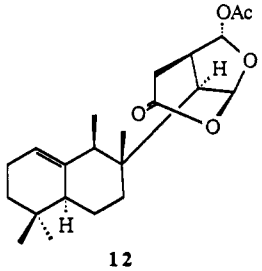

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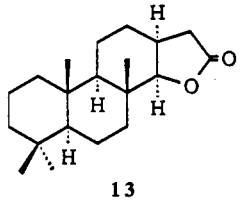


13

(**10**) is the first example of an isospongian diterpene.

The hexane extract of the lyophilized sponge tissue of *A. polyrhaphis* was fractionated by flash chromatography on silica. Fractions containing spongian diterpenes were further purified by flash chromatography on silica and by HPLC on Partisil to obtain, in order of increasing retention time, macfarlandin E (**2**), polyrhaphin B (**8**), polyrhaphin A (**7**), polyrhaphin D (**10**), polyrhaphin C (**9**), aplyviolene (**3**), norrisolide (**4**), the γ-lactone **6**, and shahamin C (**5**).

Polyrhaphin A (**7**) was isolated as a clear oil and was determined to have a molecular formula of $C_{24}H_{36}O_6$ by high-resolution mass measurement of the molecular ion (m/z = 420.2501). Infrared bands at 1795, 1745, and 1220 cm^{-1} indicated γ-lactone and ester moieties. The presence of one lactone unit and two acetates was verified by the observation of ^{13}C NMR signals at δ 172.4 (s), 170.3 (s), 169.3 (s), 70.7 (t), 69.3 (d), 63.6 (t), 20.9 (q), and 20.5 (q) and ^1H NMR signals at δ 2.14 (s, 3 H) and 2.08 (s, 3 H). Comparison of the ^{13}C NMR data of polyrhaphin A (**7**) with those of the known compounds macfarlandin E (**2**), aplyviolene (**3**), and shahamin C (**5**) (Table I) indicated the presence of a perhydroazulene subunit. In addition, ^1H NMR signals at δ 4.87 (d, 1 H, J = 1.4 Hz), 4.61 (d, 1 H, J = 1.4 Hz), and 2.54 (d, 1 H, J = 8.7 Hz) were characteristic of the exocyclic methylene protons and the H-9 ring junction proton in the perhydroazulene ring system, respectively. The C-11/C-16 subunit was derived by interpretation of ^1H NMR COSY data. The mutually coupled signals at δ 4.49 (dd, 1 H, J = 9.1, 8.8 Hz, H-16α) and 4.04 (dd, 1 H, J = 10.0, 9.1 Hz, H-16β) were both coupled to a methine proton signal at δ 3.19 (m, 1 H, J = 10.5, 10.0, 8.8, 1.8 Hz, H-13) that was in turn coupled to a downfield signal at δ 5.69 (d, 1 H, J = 10.5 Hz, H-12); these signals were assigned to the protons on an α-acetoxy-β-substituted γ-lactone ring. The methine signal at δ 3.19 was further coupled to a second methine signal at δ 1.79 (m, 1 H, H-14) that was in turn coupled to the acetoxymethylene proton signals at δ 4.56 (dd, 1 H, J = 11.8, 3.2 Hz, H-15) and 3.94 (dd, 1 H, J = 11.8, 9.6 Hz, H-15′). The C-11/C-16 subunit must be joined at C-14 to C-8 of the perhydroazulene subunit. The stereochemistry about the γ-lactone ring in polyrhaphin A was determined by nuclear Overhauser effects difference spectroscopy (NOEDS). Irradiation of H-12 caused a 4.4% enhancement of the resonance assigned to H-16β, while irradiation of H-13 resulted in a 5.3% enhancement of the H-16α signal. These results indicated the trans orientation of H-12 and H-13 as shown.

Spectroscopic techniques failed to unequivocally define the stereochemistry about the C-8/C-14 bond and therefore the stereochemistry of polyrhaphin A (**7**) was determined by chemical correlation with the known compound macfarlandin E (**2**). Each compound was reduced with lithium aluminum hydride, and the product mixture from the reduction was acetylated with acetic anhydride (Scheme I). In each case the tetraacetate (**11**) was isolated as one of the major products. These transformations confirmed the structure and stereochemistry of polyrhaphin A (**7**).

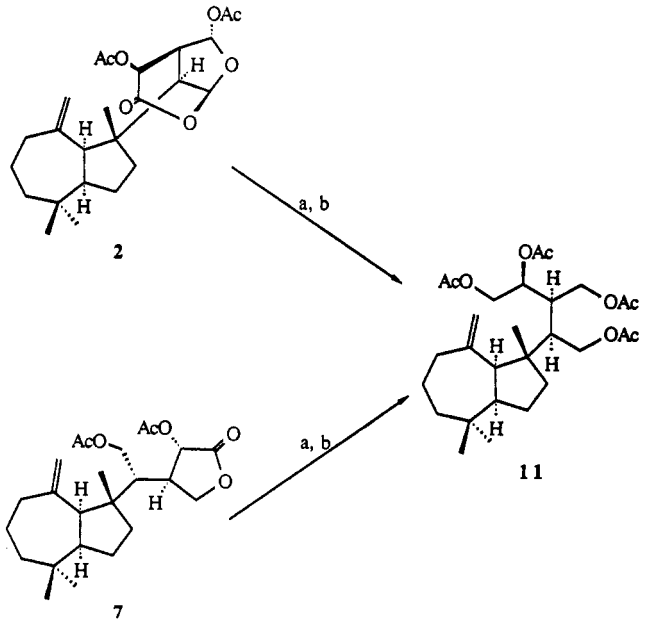
Polyrhaphin B (**8**) was isolated as a clear oil and was determined to have a molecular formula of $C_{25}H_{38}O_7$ from high-resolution mass measurement of the molecular ion (m/z = 450.2611). The ^{13}C NMR resonances at δ 170.2 (s, 2C), 169.8 (s), 52.5 (q), 21.4 (q), and 20.6 (q) along with infrared bands at 1745 and 1225 cm^{-1} indicated the presence of two acetates and one methyl ester. Comparison of ^{13}C NMR (Table I) and ^1H NMR data to known compounds suggested the same perhydroazulene subunit for the hydrocarbon portion of the molecule. The ^1H NMR COSY spectrum showed a continuous ^1H–^1H spin system from CH-12 through CH_2-16, which was assigned to a trisubstituted tetrahydrofuran unit. The acetal signal at δ 6.27 (d, 1 H, J = 3.7 Hz, H-15) was coupled to a methine signal at δ 2.56 (dd, 1 H, J = 6.8, 3.7 Hz, H-14), which was in turn coupled to a methine signal at δ 2.85 (m, 1 H, J = 6.8, 6.2, 6.2, 5.5 Hz, H-13). The H-13 signal was coupled to a pair of signals due to methylene protons on a carbon

Table I. ^{13}C NMR Data [50 MHz, $CDCl_3$, chemical shift (δ), multiplicity] for Macfarlandin E (2), Aplyviolene (3), Shahamin C (5), Polyrhaphin A (7), and Polyrhaphin B (8)

carbon no.[a]	2	3	5	7	8
1	38.3 (t)[b]	37.6 (t)[b]	37.1 (t)	37.0 (t)[b]	37.4 (t)[b]
2	28.3 (t)[c]	28.3 (t)[c]	28.8 (t)	28.9 (t)[c]	28.7 (t)[c]
3	37.5 (t)[b]	38.0 (t)[b]	37.6 (t)	37.9 (t)[b]	37.7 (t)[b]
4	30.1 (s)	36.1 (s)	36.1 (s)	36.1 (s)	36.2 (s)
5	54.2 (d)	54.2 (d)	54.6 (d)	54.7 (d)	55.5 (d)[d]
6	27.0 (t)[c]	26.9 (t)[c]	26.0 (t)	26.4 (t)[c]	26.1 (t)[c]
7	40.0 (t)[b]	38.6 (t)[b]	37.6 (t)	39.0 (t)[b]	38.0 (t)[b]
8	45.4 (s)	45.6 (s)	48.2 (s)	48.4 (s)	47.4 (s)
9	57.7 (d)	57.9 (d)	54.8 (d)	56.3 (d)	55.5 (d)[d]
10	152.5 (s)	153.1 (s)	153.2 (s)	153.9 (s)	153.3 (s)
11	165.8 (s)	167.5 (s)	168.8 (s)[b]	169.3 (s)[d]	169.8 (s)[e]
12	65.8 (d)	33.2 (t)	66.8 (d)	69.3 (d)	71.0 (d)
13	54.2 (d)	38.3 (d)	36.4 (d)	40.7 (d)[e]	44.0 (d)
14	51.8 (d)	49.3 (d)	45.2 (d)	48.6 (d)[e]	53.2 (d)[d]
15	101.2 (d)	100.8 (d)[d]	65.9 (t)	70.7 (t)	99.8 (d)
16	96.0 (d)	101.1 (d)[d]	64.2 (t)	63.6 (t)	69.0 (t)
17	24.5 (q)	23.9 (q)	20.7 (q)[c]	21.4 (q)[f]	23.4 (q)
18	26.0 (q)	26.0 (q)	25.5 (q)	25.5 (q)	25.8 (q)
19	34.2 (q)	34.2 (q)	34.4 (q)	34.4 (q)	34.3 (q)
20	115.2 (t)	114.8 (t)	115.2 (t)	115.0 (t)	114.7 (t)
$OCOCH_3$	169.3 (2 s)	167.5 (s)	169.4 (s)[b]	170.3 (s)[d]	170.2 (2 s)[e]
			170.6 (s)[b]	172.4 (s)[d]	
$OCOCH_3$	21.1 (q)	21.0 (q)	20.6 (q)[c]	20.9 (q)[f]	21.4 (q)
	20.6 (q)		20.4 (q)[c]	20.5 (q)[f]	20.6 (q)
OCH_3					52.5 (q)

[a] Assignments of 5 were made from the analysis of the ^1H–^{13}C 2D NMR shift correlation experiment. Exchange of assignments for C-1/C-2 and C-4/C-8 in 2 were based on analysis of the long-range (two and three bond) ^1H–^{13}C 2D NMR shift correlation experiment of a related perhydroazulene spongian diterpene.[14] The carbon numbering system is based on the assumption that these metabolites are derived from the spongian diterpene skeleton 1. Signals for 3, 7, and 8 were assigned by analogy to 5. [b–f] Assignments may be interchanged within a column.

Scheme I. Chemical Correlation of Polyrhaphin A (7) with Macfarlandin E (2)[a]



[a] (a) $LiAlH_4$, Et_2O; (b) Ac_2O, pyridine.

bearing oxygen [δ 4.02 (dd, 1 H, J = 8.8, 5.5 Hz, H-16β) and 3.88 (dd, 1 H, J = 8.8, 6.2 Hz, H-16α)] and to a downfield signal at δ 5.19 (d, 1 H, J = 6.2 Hz, H-12) assigned to the proton on a carbon atom bearing both acetoxy and carbomethoxy groups.

The relative stereochemistry of polyrhaphin B (8) was determined by NOEDS measurements. Irradiation of the signal at δ 2.74 (d, 1 H, J = 8.7 Hz, H-9) produced enhancements of the signals at δ 1.95 (m, 1 H, H-5, 25.4%) and 2.56 (dd, 1 H, J = 6.8, 3.7 Hz, H-14, 5.5%). These results confirmed the cis orientation of the perhydroazulene ring junction and indicated the proximity of H-9 and H-14. The stereochemistry about the tetrahydrofuran ring was deduced by NOEDS experiments in which irradiation of the H-14 proton produced enhancement of the resonances assigned to H-13 (9.7%) and H-16α (3.3%) while irradiation of the H-15 acetal signal resulted in enhancement of the H-16β proton signal (3.1%). These results indicate the stereochemistry shown for polyrhaphin B (8) but did not allow assignment of the relative stereochemistry about C-12. Due to their common biosynthetic ancestry it seems reasonable to assign to polyrhaphin B the same C-12 stereochemistry as that found in macfarlandin E (2) and polyrhaphin A (7).

Table II. Comparison of ^1H NMR Data [360 MHz, $CDCl_3$, chemical shift (δ), multiplicity, coupling constant (Hz)] for Polyrhaphin C (9) and Macfarlandin D (12)

^1H at carbon no.	polyrhaphin C (9)	macfarlandin D (12)
12α	3.03 (dd, 19.6, 5.8)	3.12 (dd, 20, 6.2)
12β	2.68 (d, 19.6)	2.64 (d, 20)
13	2.70 (m, 5.8, 2.8, 1.1)	2.62 (m, 6.2, 3.1, 1.2)
14	2.35 (dd, 2.7, 2.8)	2.45 (dd, 3.1, 3.1)
15	5.71 (dd, 2.7, 1.1)	5.75 (dd, 3.1, 1.2)
16	6.15 (s)	6.13 (s)

Polyrhaphin C (9) was isolated as a clear oil and was found to have a molecular formula of $C_{22}H_{32}O_5$ by analysis of the ^{13}C NMR spectrum and high-resolution mass measurement of the fragment ion at m/z = 316.2038 (M – AcOH)$^+$. Observation of infrared bands at 1755, 1750, and 1220 cm^{-1} along with ^{13}C NMR resonances at δ 169.4 (s), 167.8 (s), and 21.0 (q) and a ^1H NMR signal at δ 2.10 (s, 3 H) suggested the presence of a δ-lactone and an acetate unit. Comparison of the relevant ^1H NMR chemical shifts and coupling constants with those of macfarlandin D (12)[3] indicated the existence of a monoacetylated [3.2.1] oxabicyclo unit (Table II). This was also verified by ^{13}C NMR data and NOEDS experiments. The mo-

lecular formula and ^{13}C NMR data indicated that the hydrocarbon portion of polyrhaphin C (**9**) was tricyclic. The observation of virtually coupled proton signals at δ 0.53 (br s, 1 H, H-20), 0.52 (m, 1 H, H-9), and 0.25 (dd, 1 H, J = 10.1, 5.6 Hz, H-20′) and ^{13}C NMR signals at δ 12.9 (t, C-20), 22.9 (s, C-10), and 25.7 (d, C-9) indicated the presence of a cyclopropyl ring in this tricyclic skeleton.

Analysis of the ^1H NMR COSY spectrum indicated that the cyclopropyl proton signals constituted an isolated spin system. In addition, only three aliphatic methyl signals were observed in the ^1H NMR of polyrhaphin C (**9**), suggesting that the fourth methyl group from the spongian diterpene precursor had been incorporated into the cyclopropyl ring. Two possible tricyclic hydrocarbon skeletons can be proposed on the basis of biosynthetic considerations. A C-9 carbonium ion has been proposed as a precursor of the norrisolide, macfarlandin D, and macfarlandin E carbon skeletons. Cyclization between C-9 and either C-17 or C-20 produces two tricyclic carbon skeletons that contain the required cyclopropane ring.[9] Evidence for the C-9/C-20 bond formation came from a NOEDS experiment in which irradiation of the C-17 methyl signal at δ 1.07 (s) resulted in enhancements of the resonances at δ 3.03 (dd, 1 H, J = 19.6, 5.8 Hz, H-12α, 9.2%) and 0.53 (br s, 1 H, H-20, 6.0%). These results can only be explained if the structure of polyrhaphin C (**9**) is drawn as shown. The diterpene carbon skeleton of polyrhaphin C has not previously been reported, although similar cyclopropane-containing metabolites are common in the triterpene series (cf. cycloartenol).

The fourth novel compound, polyrhaphin D (**10**), was isolated as a white solid which displayed a molecular ion at m/z = 302.2242 ($C_{20}H_{30}O_2$) in the high-resolution mass spectrum. The infrared bands at 1755, 1740, and 1650 cm^{-1} and the UV absorption at 217 nm (ϵ 6700) were assigned to an α,β-unsaturated γ-lactone. The ^{13}C NMR signals at δ 169.3 (s, C-13 and C-15), 112.8 (d, C-14), and 91.8 (d, C-16) and ^1H NMR signals at δ 5.68 (dd, 1 H, J = 1.8, 1.8 Hz, H-14) and 4.33 (br s, 1 H, $W_{1/2}$ = 2.9 Hz, H-16) indicated a β,γ-disubstituted α,β-unsaturated γ-lactone. Incorporation of the lactone ring as ring D of a tetracyclic diterpene is supported by comparison of the remaining signals in the ^{13}C NMR spectrum with the data for model compounds[10] that contain the same trans-anti-trans fused tricyclic ring system.

The α-orientation of the H-16 proton was determined by the observation of a NOE from H-16 to the axial proton at C-12 [δ 2.27 (m, 1 H, J = 14.0, 11.2, 6.8, 1.8 Hz, 7.9%)]. The structure was verified by diimide reduction of polyrhaphin D (**10**) to form the dihydro derivative **13**. The ^1H NMR spectrum of dihydropolyrhaphin D (**13**) included signals at δ 2.30 (dd, 1 H, J = 17.4, 12.0 Hz) and 2.40 (dd, 1 H, J = 17.4, 9.6 Hz) that were assigned to the α-methylene unit in the γ-lactone ring. These two signals were coupled to a broad signal at δ 2.67 (m, 1 H, H-13), which was also coupled to the H-16 signal at δ 4.10 (d, 1 H, J = 8.1 Hz), which had shifted upfield from its position in polyrhaphin D (**10**).

A collection of the brightly colored dorid nudibranch *C. norrisi* was obtained from the same location in the Gulf of California as *A. polyrhaphis*. *C. norrisi* had been previously reported to possess norrisolide (**4**)[6] and another rearranged spongian diterpene now identified as macfarlandin E (**2**).[11] Additional studies of other chromodorid nudibranches have led to the isolation of other rearranged spongian diterpene metabolites.[3,12] These observations prompted us to investigate *C. norrisi* in order to determine whether the nudibranch possessed other spongian diterpenes found in *A. polyrhaphis*.

The acetone extract of three specimens of *C. norrisi* was partitioned between dichloromethane and water. The dichloromethane-soluble material was separated by silica flash chromatography followed by HPLC on Partisil to yield four spongian diterpenes that were identified as macfarlandin E (**2**), polyrhaphin A (**7**), norrisolide (**4**), and shahamin C (**5**) by comparison with authentic materials from *A. polyrhaphis*. The four metabolites were obtained in approximately the same ratios in both *C. norrisi* and *A. polyrhaphis*, suggesting that the compounds in *C. norrisi* are of dietary origin and that there is no preferential concentration of any specific metabolite.

The observation that *C. norrisi* was sequestering some of the metabolites from *A. polyrhaphis* implies that they are of ecological significance to the nudibranch. In order to investigate one potentially beneficial property, fish feeding deterrence assays were carried out on each of the metabolites which had been obtained in sufficient quantity. The fish feeding bioassay[13] employed the rainbow wrasse *Thalassoma lucasunum*, which is an omnivorous fish commonly found in the Gulf of California. Polyrhaphin C (**9**), the γ-lactone **6**, and shahamin C (**5**) were found to deter feeding at concentrations of 100 μg metabolite/mg food but macfarlandin E (**2**), aplyviolene (**3**), norrisolide (**4**), and polyrhaphin A (**7**) were inactive in our assays at this concentration.

Some of the diterpenes have also been shown to exhibit other interesting biological activities. Polyrhaphin C (**9**) showed antimicrobial activity against *Staphylococcus aureus* at 100 μg/disk and *Bacillus subtilis* at 10 μg/disk while aplyviolene (**3**) was also active against *B. subtilis* at 10 μg/disk. With the exception of polyrhaphins B and D, which were not tested, the remaining metabolites were inactive in the antimicrobial screen.

Experimental Section

Extraction and Chromatography of *A. polyrhaphis*. The purple dendritic sponge *A. polyrhaphis* was collected by hand at a depth of 0–2 m in a mangrove lagoon on Isla San Jose in the Gulf of California (Lat. 24°52′ N, Long. 110°35′ W). The sponge was stored frozen for approximately 1 month and then freeze-dried. The lyophilized sponge tissue (158 g) was successively extracted with hexane (3 × 1 L), dichloromethane (4 × 1 L), ethyl acetate (2 × 1 L), and methanol (2 × 1 L). Approximately two-thirds of the crude hexane extract (4 g) was fractionated by silica flash chromatography (Kieselgel 60, 230–400 mesh, column size 40 × 4.5 cm) using an elution gradient from hexane to ethyl acetate to methanol. This produced two fractions that were judged by ^1H NMR spectroscopy to consist primarily of spongian diterpenes.

The first fraction (2.0 g) was separated by HPLC on Partisil using hexane/diethyl ether (2:8) as eluant to obtain macfarlandin E (**2**, 877 mg, 0.55% dry weight) and three fractions of impure spongian diterpenes. The least polar of these fractions (52 mg) was separated by HPLC on Partisil using hexane/diethyl ether (3:7) to yield polyrhaphin B (**8**, 3.0 mg, 0.002% dry weight). The second (44 mg) and third (45 mg) fractions were separated in the

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same manner to obtain polyrhaphin A (7, 21 mg, 0.01% dry weight) and polyrhaphin D (10, 4.5 mg, 0.003% dry weight), respectively.

The second, more polar, fraction from the original flash chromatography (166 mg) was chromatographed on silica (Kieselgel 60, 230–400 mesh, column size 21.5 × 1.5 cm) using a hexane/ethyl acetate solvent gradient to remove the fats and sterols. The remaining material (121 mg) was separated by HPLC on Partisil in hexane/diethyl ether (4:6) to yield polyrhaphin C (9, 6.6 mg, 0.004% dry weight), aplyviolene (3, 8.8 mg, 0.006% dry weight), norrisolide (4, 31.1 mg, 0.02% dry weight), and a mixture of two more spongian diterpenes. This mixture (66 mg) was subjected to further HPLC on Partisil (3:7, hexane/ethyl acetate) to obtain the γ-lactone 6 (8.7 mg, 0.005% dry weight) and shahamin C (5, 32.8 mg, 0.02% dry weight).

Extraction and Chromatography of *C. norrisi*. Three specimens of the brightly colored dorid nudibranch *C. norrisi* were collected in the same area as *A. polyrhaphis*, and the animals were stored in acetone for approximately 2 months. The acetone extract of the animals was partitioned between dichloromethane and water. The organic soluble portion (109 mg) was flash chromatographed on silica (Kieselgel 60, 230–400 mesh, column size 20.0 × 1.2 cm) using a hexane to diethyl ether to ethyl acetate solvent gradient to elute the material. This yielded macfarlandin E (2, 36.3 mg, 12.1 mg/animal) and two fractions of impure spongian diterpenes. These two fractions were separated by HPLC on Partisil in hexane/diethyl ether (4:6) to obtain polyrhaphin A (7, 0.4 mg, 0.13 mg/animal), norrisolide (4, 0.5 mg, 0.17 mg/animal), and shahamin C (5, 3.6 mg, 1.2 mg/animal).

Polyrhaphin A (7): Clear oil; $[\alpha]_D$ −23.0° (c = 0.80, $CHCl_3$); IR ($CHCl_3$) 1795, 1745, 1220 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (s, 3 H), 0.95 (s, 3 H), 0.98 (s, 3 H), 2.08 (s, 3 H), 2.14 (s, 3 H), 2.35 (m, 1 H), 2.54 (d, 1 H, J = 8.7 Hz), 3.19 (m, 1 H, J = 10.5, 10.0, 8.8, 1.8 Hz), 3.94 (dd, 1 H, J = 11.8, 9.6 Hz), 4.04 (dd, 1 H, J = 10.0, 9.1 Hz), 4.49 (dd, 1 H, J = 9.1, 8.8 Hz), 4.56 (dd, 1 H, J = 11.8, 3.2 Hz), 4.61 (d, 1 H, J = 1.4 Hz), 4.87 (d, 1 H, J = 1.4 Hz), 5.69 (d, 1 H, J = 10.5 Hz); ^{13}C NMR, see Table I; HRMS (EI) m/z 420.2501 (M^+), $C_{24}H_{36}O_6$ requires 420.2512.

Polyrhaphin B (8): clear oil; $[\alpha]_D$ +54.2° (c = 0.40, $CHCl_3$); IR ($CHCl_3$) 1745, 1225 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (s, 3 H), 0.95 (s, 3 H), 1.02 (s, 3 H), 2.07 (s, 3 H), 2.15 (s, 3 H), 2.35 (m, 1 H), 2.56 (dd, 1 H, J = 6.8, 3.7 Hz), 2.74 (d, 1 H, J = 8.7 Hz), 2.85 (m, 1 H, J = 6.8, 6.2, 6.2, 5.5 Hz), 3.77 (s, 3 H), 3.88 (dd, 1 H, J = 8.8, 6.2 Hz), 4.02 (dd, 1 H, J = 8.8, 5.5 Hz), 4.57 (d, 1 H, J = 2.0 Hz), 4.81 (d, 1 H, J = 2.0 Hz), 5.19 (d, 1 H, J = 6.2 Hz), 6.27 (d, 1 H, J = 3.7 Hz); ^{13}C NMR, see Table I; HRMS (EI) m/z 450.2611 (M^+), $C_{25}H_{38}O_7$ requires 450.2618.

Polyrhaphin C (9): clear oil; $[\alpha]_D$ −25.3° (c = 0.30, $CHCl_3$); IR ($CHCl_3$) 1755, 1750, 1220 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.25 (dd, 1 H, J = 10.1, 5.6 Hz), 0.52 (m, 1 H), 0.53 (br s, 1 H), 0.85 (s, 3 H), 0.87 (s, 3 H), 1.07 (s, 3 H), 2.10 (s, 3 H), 2.35 (dd, 1 H, J = 2.7, 2.8 Hz), 2.68 (br d, 1 H, J = 19.6 Hz), 2.70 (m, 1 H, J = 5.8, 2.8, 1.1 Hz), 3.03 (dd, 1 H, J = 19.6, 5.8 Hz), 5.71 (dd, 1 H, J = 2.7, 1.1 Hz), 6.15 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 169.4 (s), 167.8 (s), 101.4 (d), 100.8 (d), 51.2 (d), 46.9 (d), 42.5 (t), 38.8 (t), 37.8 (d), 36.7 (t), 34.6 (s), 33.5 (t), 31.7 (s), 30.5 (q), 26.6 (q), 25.7 (d), 22.9 (s), 21.4 (t), 21.0 (q), 20.3 (q), 17.6 (t), 12.9 (t); HRMS (EI) m/z 316.2038 (M − AcOH)$^+$, $C_{20}H_{28}O_3$ requires 316.2038.

Polyrhaphin D (10): white solid; $[\alpha]_D$ +6.7° (c = 0.12, $CHCl_3$); IR ($CHCl_3$) 1755, 1740, 1650 cm^{-1}; UV (MeOH) λ_{max} 217.1 nm (ε 6700); ^1H NMR ($CDCl_3$) δ 0.71 (s, 3 H), 0.83 (s, 3 H), 0.86 (s, 3 H), 0.89 (s, 3 H), 1.87 (m, 1 H, J = 13.2, 6.8, 2.6, 1.1 Hz), 2.05 (m, 2 H), 2.27 (m, 1 H, J = 14.0, 11.2, 6.8, 1.8 Hz), 2.82 (m, 1 H, J = 14.0, 4.2, 1.1, 1.1 Hz), 4.33 (br s, 1 H, $W_{1/2}$ = 2.9 Hz), 5.68 (dd, 1 H, J = 1.8, 1.8 Hz); ^{13}C NMR ($CDCl_3$) δ 169.3 (s, 2 C), 112.8 (d), 91.8 (d), 56.6 (d), 54.7 (d), 42.0 (s), 41.9 (t), 40.5 (t), 40.4 (t), 37.8 (s), 33.4 (s), 33.3 (q), 27.2 (t), 21.5 (q), 21.0 (t), 18.6 (t), 18.2 (t), 16.2 (q), 12.2 (q); HRMS (EI) m/z = 302.2242 (M^+), $C_{20}H_{30}O_2$ requires 302.2246.

Aplyviolene (3): clear oil; $[\alpha]_D$ −17.8° (c = 0.36, $CHCl_3$); IR ($CHCl_3$) 1755, 1230 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 (s, 3 H), 0.99 (s, 3 H), 1.03 (s, 3 H), 2.10 (s, 3 H), 2.38 (m, 1 H), 2.42 (dd, 1 H, J = 2.8, 2.6 Hz), 2.62 (m, 1 H, J = 5.3, 2.8, 1.1 Hz), 2.67 (br d, 1 H, J = 19.5 Hz), 2.69 (d, 1 H, J = 8.9 Hz), 2.87 (dd, 1 H, J = 19.5, 5.3 Hz), 4.60 (d, 1 H, J = 2.0 Hz), 4.86 (d, 1 H, J = 2.0 Hz), 5.69 (dd, 1 H, J = 2.6, 1.1 Hz), 6.13 (s, 1 H); ^{13}C NMR, see Table I; HRMS (EI) m/z 316.2038 (M − AcOH)$^+$, $C_{20}H_{28}O_3$ requires 316.2038.

Reduction and Acetylation of Macfarlandin E (2). A solution of macfarlandin E (2, 24.6 mg) in dry diethyl ether (2 mL) was added to a stirred solution of lithium aluminum hydride (48.4 mg) in dry diethyl ether (3 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature after 0.5 h and then refluxed for 1 h. Excess LAH was destroyed by addition of ethyl acetate (2 mL) and then 0.5 N hydrochloric acid (5 mL). The reaction mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic extracts were dried over anhydrous sodium sulfate. The solvent was removed to yield a polar oil (18.5 mg), which remained on the base line on silica TLC using ethyl acetate/methanol (1:1) as the developing solvent. Acetic anhydride (1 mL) was added to a solution of the oil in pyridine (3 mL), and the reaction mixture was stirred at room temperature for 19 h. This reaction was worked up by addition of ethyl acetate (5 mL) and water (5 mL) followed by extraction of the aqueous portion with ethyl acetate (2 × 10 mL). The combined organic extracts were dried over sodium sulfate, and the solvent was removed to yield a crude product (25.6 mg). This mixture was separated by silica flash chromatography (Kieselgel 60, 230–400 mesh, column size 6.5 × 0.7 cm) using hexane/diethyl ether (6:4) as eluant to obtain the tetraacetate 11 (13.8 mg, 48% yield).

Tetraacetate (11): clear oil; $[\alpha]_D$ +20.1 (c = 0.49, $CHCl_3$); IR ($CHCl_3$) 1740, 1225 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (s, 3 H), 0.94 (s, 3 H), 0.99 (s, 3 H), 1.88 (ddd, 1 H, J = 10.0, 6.4, 3.4 Hz), 2.03 (s, 3 H), 2.04 (s, 3 H), 2.07 (s, 3 H), 2.10 (s, 3 H), 2.34 (m, 1 H), 2.70 (m, 1 H, J = 6.8, 6.4, 5.6 Hz), 2.80 (d, 1 H, J = 8.7 Hz), 3.98 (dd, 1 H, J = 12.3, 6.9 Hz), 4.03 (dd, 1 H, J = 11.8, 10.0 Hz), 4.20 (d, 2 H, J = 6.8 Hz), 4.48 (dd, 1 H, J = 11.8, 3.4 Hz), 4.57 (dd, 1 H, J = 12.3, 2.0 Hz), 4.67 (d, 1 H, J = 2.0 Hz), 4.82 (d, 1 H, J = 2.0 Hz), 5.36 (ddd, 1 H, J = 6.9, 5.6, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 170.7 (s, 2 C), 170.6 (s), 169.8 (s), 154.5 (s), 114.5 (t), 71.0 (d), 65.3 (t), 64.0 (t), 63.7 (t), 55.7 (d), 54.4 (d), 49.4 (s), 48.2 (d), 39.8 (t), 39.5 (d), 38.0 (t) 37.4 (t), 36.1 (s), 34.5 (q), 29.0 (t), 26.8 (t), 25.6 (q), 21.7 (t), 21.1 (q), 20.9 (q, 2 C), 20.7 (q); HRMS (EI) m/z 508.3050 (M^+), $C_{28}H_{44}O_8$ requires 508.3036.

Reduction/Acetylation of Polyrhaphin A (7). Polyrhaphin A (7, 4.7 mg) was dissolved in dry diethyl ether (1 mL) and added to a suspension of lithium aluminum hydride (9.5 mg) in dry diethyl ether (1 mL) at 0 °C. After 0.5 h the reaction mixture was allowed to come to room temperature and then refluxed for 1.5 h. The reaction was quenched by addition of ethyl acetate (3 mL) and 0.5 N hydrochloric acid (3 mL). The solution was extracted with ethyl acetate (3 × 7 mL) and dried over anhydrous sodium sulfate, and the solvent was removed to obtain a clear oil (4.8 mg). This oil was immediately dissolved in pyridine (1 mL) and acetylated with acetic anhydride (0.5 mL). After 14 h at room temperature the reaction mixture was worked up by addition of ethyl acetate (3 mL) and water (3 mL). The aqueous layer was extracted with ethyl acetate (3 × 4 mL), the combined organic extracts were dried over anhydrous sodium sulfate, and the solvents were removed to yield a crude product (5.1 mg). The tetraacetate 11 (1.4 mg, 24% yield) was isolated from this product mixture by the same procedure described for the reaction of macfarlandin E (2).

Diimide Reduction of Polyrhaphin D (10). Polyrhaphin D (10, 2.2 mg) was dissolved in dry methanol (2 mL) and placed in a flask which allowed air to be bubbled through the solution. A few crystals of copper acetate (<1 mg) followed by anhydrous hydrazine (10 μL) was added to the stirred solution. After 11 h ethyl acetate, methanol, and water (5 mL each) were added to the cloudy yellow solution. The mixture was filtered, and the organic solvents were removed to yield an aqueous suspension, which was extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with 1.0 N hydrochloric acid (10 mL) and water (10 mL) and dried over sodium sulfate, and the solvents were removed to yield a crude reaction mixture (2.6 mg). This mixture was filtered through silica (Kieselgel 60, 230–400 mesh, column size 6.5 × 0.6 cm) in hexane/diethyl ether (4:6) and chromatographed on Partisil in hexane/diethyl ether (1:1) to obtain dihydropolyrhaphin D (13, 0.3 mg, 12% yield) and unreacted starting material (0.3 mg).

Dihydropolyrhaphin D (13): clear oil; ^1H NMR ($CDCl_3$) δ 0.82 (s, 3 H), 0.86 (s, 3 H), 0.88 (s, 3 H), 0.96 (s, 3 H), 1.79 (m,

1 H), 2.02 (ddd, 1 H, J = 13.0, 3.6, 2.7 Hz), 2.30 (dd, 1 H, J = 17.4, 12.0 Hz), 2.40 (dd, 1 H, J = 17.4, 9.6 Hz), 2.67 (m, 1 H), 4.10 (d, 1 H, J = 8.1 Hz).

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A Versatile Synthesis of 1,1-Dioxo 7-Substituted Cephems: Preparation of the Human Leukocyte Elastase (HLE) Inhibitor 1,1-Dioxo-*trans*-7-methoxycephalosporanic Acid *tert*-Butyl Ester

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A four-step synthesis of the human leukocyte elastase (HLE) inhibitor 1,1-dioxo-*trans*-7-methoxycephalosporanic acid *tert*-butyl ester in 44% isolated yield from 7-aminocephalosporanic acid (7-ACA) is described. A variety of 7-substituents have been introduced via metal-catalyzed diazo insertion reactions, and the use of a flow reactor for chemodiscriminate control of particularly rapid reactions is presented. A chemoselective oxidation of 7-ACA *tert*-butyl ester to the corresponding 1,1-dioxide without formal N-protection is introduced.

Introduction

Neutral proteolytic enzymes, specifically human leukocyte elastase (HLE) released from human polymorphonuclear (PMN) leukocytes, have been implicated in the pathogenesis of adult respiratory distress syndrome (ARDS), emphysema, and rheumatoid arthritis.[1] Inhibition of HLE, therefore, may attenuate the onset of such diseases. Several laboratories, including our own, have instituted a search for compounds which show promise as in vivo inhibitors of HLE. At least four general classes of compounds have been identified which exhibit in vitro inhibition of HLE models.[2] These classes include 2-furoylsaccharin-, isotoic anhydride-, cephalosporin-,[3-5] and peptide-based compounds. In evaluating modified cephalosporin-based HLE inhibitors, we were presented with the opportunity to devise an adaptable and high-yielding general synthesis for the preparation of 1,1-dioxo 7-substituted cephems from the relatively inexpensive starting material 7-aminocephalosporanic acid (7-ACA). Our initial target was 1,1-dioxo-*trans*-7-methoxycephalosporanic acid *tert*-butyl ester (8), which has been established by Doherty et al.[6] as a compound showing strong in vitro inhibition of HLE.

With 7-ACA as starting material, it was obvious that any synthesis of 8 must effect two key chemical transformations, i.e. conversion of the *cis*-7-amino group to the *trans*-7-methoxy group and oxidation of the 1-sulfide to the corresponding 1-sulfone. The original procedure of Doherty accomplished these transformations via diazotization of 7-ACA-O-*t*-Bu followed by rhodium-catalyzed insertion into methanol and subsequent oxidation of the 7-methoxy sulfide with *m*-chloroperbenzoic acid (*m*-CPBA).[7] Due to the inherent problems associated with carbene (carbenoid) interaction with sulfide,[8] the insertion reaction on a relatively unstable diazo compound gave low yields of a key intermediate. During our investigation we discovered that if we transposed the reaction sequence so that S-oxidation occurred first, subsequent diazotization/insertion on the resulting sulfone improved dramatically to >90%. Furthermore, the intermediate diazo sulfone proved to be particularly useful for the preparation of other 7-substituted sulfone cephems which were either heretofore unknown or obtained only with great difficulty.

Our approach to an adaptable synthesis which would serve to supply the required 8 and yet be easily modified to supply other 7-substituted cephems from a common intermediate is presented below.

Discussion

1. 7-Aminocephalosporanic Acid *tert*-Butyl Ester (2). Our preparation of 7-ACA *tert*-butyl ester was based on the procedure described by Stedman[9] (52%). Existing reports in the literature for the preparation of this compound were sketchy, and further optimization of this reaction proved quite instructive. Superficially, this reaction can be viewed as a simple equilibrium between 7-ACA and and 7-ACA-O-*t*-Bu mediated by isobutylene. However, the reaction is actually comprised of a complex series of equilibria involving protonated 7-ACA and protonated isobutylene. The role of the acid catalyst is manifold. The acid catalyst must: (1) protonate the amino group of 7-

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