were steam distilled to give a blue essential oil (0.33%) which was distilled at reduced pressure (15 mmHg) to produce different fractions of clear oil and a residue (bp 260–265 °C). This residue (120 mL) was mixed with 300 g of silica gel 60 packed into a cylindrical decantation funnel and eluted with 1.5 L of hexane. The eluent was combined into 12 fractions based on TLC comparison. The column was then washed with EtOAc/MeOH (5%) to yield a brown, viscous oil. Fractions 1–4 (60 g) yielded a mixture of terpenes which was analyzed by the method of Craveiro et al.[3,14] The following terpenes were identified: α- and β-pinene, camphene, myrcene, limonene, γ-terpinene, camphor, terpinen-4-ol, copaene, β-elemene, α-gurjunene, cyperene, β-caryophyllene, thujopsene, *trans*-β-farnesene, γ- and δ-cadinene, γ-muurolene, and palustrol.

Fractions 5–9 (2.5 g) yielded a dark blue oil that was rechromatographed on a column of silica gel G (25 g) using hexane as eluent and nitrogen pressure to produce a convenient flow rate. Guayazulene (50 mg) and 240 mg of pure marmelerin (1), as a clear, colorless oil were obtained. An additional 140 mg of 1 were also obtained from the less polar fraction of the neutral part of the hexane extract of roots by elution with hexane of a similar type of silica gel column.

Marmelerin (1): colorless, mobil oil, positive Erlich test; $[\alpha]^{23}_D$ −63° (*c* 1.0, $CHCl_3$); CD (*c* 5.0, MeOH) $\Delta\epsilon_{292}$ −0.65; $\Delta\epsilon_{280}$ −0.78, $\Delta\epsilon_{270}$ −1.7, $\Delta\epsilon_{257}$ +10.1, $\Delta\epsilon_{225}$ +9.1; UV λ_{max}^{MeOH} (log ε) 289 (3.42), 278 (3.51), 255 (4.18), 222 (4.32); IR (neat, cm^{-1}) 3040–2850, 1658, 1626, 1585, 1453, 1298, 1216, 1146, 1040, 850; MS, *m/z* (relative intensity) 214 (M^+, 51), 199 (100), 184 (13), 171 (21), 153 (11), 143 (15), 141 (15), 129 (11), 128 (18), 115 (18), 105 (11), 91 (21), 77 (12).

To a solution of 50 mg of marmelerin in 1.0 mL of $CHCl_3$ were added 4 drops of a saturated solution of picric acid in ethanol that after refrigeration, filtration, and recrystallization attempts yielded 40 mg of a reddish solid material: mp 95–98 °C; ^1H NMR (90 MHz, $CHCl_3$), δ 9.75 (1 H, br s), 9.15 (2 H, s), 6.90 (1 H, s), 3.60 (1 H, m), 2.80 (2 H, m), 2.35 (3 H, s), 2.30 (3 H, s), 2.25 (3 H, s), 1.90 (1 H, m), 1.20 (3 H, d, *J* = 8.0 Hz).

Dihydromarmelerin (3). Marmelerin (50 mg) was hydrogenated over PtO_2 in MeOH during 56 h and gave the dihydro derivative **3** as a colorless, mobil oil: negative Erlich test; CD (*c*, 10% in MeOH) $\Delta\epsilon_{289}$ −0.46, $\Delta\epsilon_{237}$ −1.37, $\Delta\epsilon_{223}$ +3.40, $\Delta\epsilon_{219}$ +3.70; UV λ_{max}^{MeOH} (log ε) 286 (3.51), 235 sh (3.52), 221 sh (3.89), 211 (3.89); IR (neat, cm^{-1}) 3040–2850, 1616, 1605, 1460, 1330, 1260, 1188, 1087, 878, 850; MS, *m/z* (relative intensity) 216 (M^+, 74), 201 (79), 187 (17), 173 (29), 159 (100), 141 (19), 131 (12), 128 (26), 115 (28), 93 (15), 91 (22).

Acknowledgment. We are thankful to CAPES (Coordenacao de Aperfeicoamento do Pessoal de Nivel Superior–Brazil) for sponsoring the Ph.D. program of E. R. Silveira.

Registry No. 1, 93304-72-4; 3, 93304-73-5; α-pinene, 80-56-8; β-pinene, 127-91-3; camphene, 79-92-5; myrcene, 123-35-3; limonene, 138-86-3; γ-terpinene, 99-85-4; camphor, 76-22-2; terpinen-4-ol, 562-74-3; copaene, 3856-25-5; β-elemene, 515-13-9; α-gurjunene, 489-40-7; cyperene, 2387-78-2; β-caryophyllene, 87-44-5; thujopsene, 470-40-6; *trans*-β-farnesene, 18794-84-8; γ-cadinene, 39029-41-9; δ-cadinene, 483-76-1; γ-muurolene, 30021-74-0; palustrol, 5986-49-2.

(14) Craveiro, A. A.; Rodrigues, A. S.; Andrade, C. H. S.; Matos, F. J. A.; Alencar, J. W.; Machado, M. I. L. *J. Natl. Prod.* 1981, *44*, 602.

Two Steroidal Alkaloids from a Marine Sponge, *Plakina* sp.

Richard M. Rosser and D. John Faulkner*

Scripps Institution of Oceanography (A-012F), La Jolla, California 92093

Received June 18, 1984

The antimicrobial metabolites of *Plakina* sp., a marine sponge that overgrows coral heads, are the steroidal alkaloids plakinamine A (**1**) and plakinamine B (**2**). The structures were elucidated by interpretation of spectral data and by comparison of the ^{13}C NMR data with those of model compounds synthesized from ergosterol.

Steroidal alkaloids are well-known metabolites of certain terrestrial plants,[1] but they have not been reported previously from marine organisms. During a search for antibiotics from marine invertebrates, we encountered a sponge of the genus *Plakina* whose crude extracts showed antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*. Bioassay-directed fractionation of the crude extract led to the isolation of two steroidal alkaloids, plakinamine A (**1**) and plakinamine B (**2**) (Chart I). The structures of the two steroidal alkaloids were elucidated by interpretation of spectral data and comparison with model compounds synthesized from ergosterol.

The sponge, *Plakina* sp., was collected in shallow waters (−5 m) at Mant Island, Ponape, where it was observed to overgrow and kill corals. A methanolic extract of the freeze-dried sponge was found to possess antimicrobial activity. Solvent partition was followed by chromatography on Sephadex LH-20 (methanol) and Sephadex G-15 (water) to obtain an active fraction containing one major and one minor component. The minor metabolite, plakinamine B (**2**, 0.1% dry weight), was obtained as a hydrochloride salt by fractional crystallization from methanol. The major metabolite, plakinamine A (**1**, 0.3% dry weight), was separated from the residue by taking advantage of its solubility in aqueous acid.

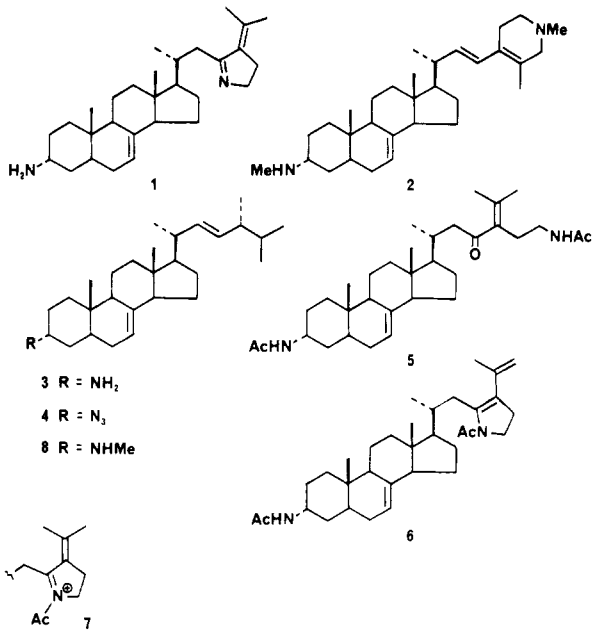
The steroidal nature of plakinamine A (**1**) was suggested by the molecular formula, $C_{29}H_{46}N_2$, and by ^1H NMR signals at δ 0.59 (s, 3 H) and 0.77 (s, 3 H) and two ^{13}C NMR signals at δ 12.0 (q) that could be assigned to the C-18 and C-19 methyl groups of a steroid. A striking feature of the ^{13}C NMR spectrum was the presence of five signals at δ >100; the signals at δ 117.7 (d) and 139.2 (s) were in good agreement with literature values for C-7 and C-8 of a Δ^7 sterol[2] while the signals at δ 173.2 (s), 137.1 (s), and 129.0 (s) could be assigned to a fully substituted double bond and an imine group. The ultraviolet chromophore at 246 nm (ε 7100) implied the presence of an α,β-unsaturated imine. A major fragmentation in the mass spectrum gave peaks at *m/z* 123.1052 ($C_8H_{13}N$, base peak), 299.2645 ($C_{21}H_{33}N$), and 300.2721 ($C_{21}H_{34}N$) that could be explained

(1) The steroidal alkaloids are regularly reviewed in "The Alkaloids", Specialist Periodical Reports, Royal Society of Chemistry, London, Vols. 1–13, 1971–1983.

(2) Blunt, J. W.; Stothers, J. B. *Org. Magn. Reson.* 1977, *9*, 439.

0022-3263/84/1949-5157$01.50/0

Chart I



as resulting from cleavage of the C-20 to C-22 bond in a steroid that contained one nitrogen attached to the steroid nucleus and one nitrogen in the side chain.

The position of the amino group on the steroid nucleus was determined by comparison of ^{13}C NMR data with those of a model compound, 3α-amino-5α-ergosta-7,22-diene (**3**). Our choice of model compound for synthesis was based on the assumption that the amino group was on ring A, since we could find a good correlation between the ^{13}C NMR signals assigned to the carbons in rings B–D of ergosta-7,22-dien-3-β-ol[3] and signals in the spectrum of plakinamine A (**1**). Furthermore, the ^1H NMR signal at δ 3.19 (br s, 1 H) could be assigned to the 3β-proton of a 3α-aminosterane.[4] 3α-Amino-5α-ergosta-7,22-diene (**3**) was synthesized in a routine manner. Selective hydrogenation of ergosterol using Raney nickel as catalyst gave ergosta-7,22-dien-3β-ol[5] which was converted via the corresponding mesylate into 3α-azido-5α-ergosta-7,22-diene (**4**). Reduction of the azide **4** with lithium aluminum hydride in THF gave the amine **3**. Comparison of the ^{13}C NMR data of plakinamine A (**1**) with those of amine **3** revealed an excellent correlation for carbons 1–16, 18, and 19 (Table I).

The side chain was defined by interpretation of the ^1H NMR data. Irradiation at 2.04 ppm caused decoupling to be observed in two mutually coupled signals at δ 2.73 (dd, 1 H, J = 15, 2 Hz) and 2.28 (dd, 1 H, J = 15, 11 Hz) and also in the methyl signal at δ 0.95 (d, 3 H, J = 7 Hz). These signals were assigned to the protons at C-20, C-22, and C-21, respectively. The signals at δ 1.79 (s, 3 H) and 1.99 (s, 3 H) were assigned to the vinyl methyl groups at C-26 and C-27. On addition of trifluoroacetic acid, the methyl signals shifted to δ 2.02 and 2.28 as might be expected for methyl groups on an olefin conjugated to an imine. One can deduce from the molecular formula that the side chain contains a ring. The mutually coupled signals at δ 3.77 (br t, 2 H) and 2.53 (br s, 2 H) were assigned to two methylene groups in a ring formed between the imine nitrogen and the tetrasubstituted olefin. Although biosynthetic considerations as well as the spectral data clearly favored a five-membered ring, the alternative 2-alkyl-3,4-dimethyl-5,6-dihydropyridine ring system could not be immediately discarded.[6]

Table I. ^{13}C NMR Data for Plakinamine A (**1**), Plakinamine B (**2**), 3α-Amino-5α-ergosta-7,22-diene (**3**), Ketone **5**, and 3α-(*N*-Methylamino)-5α-ergosta-7,22-diene (**8**)

carbon	1	3	5	2	8
1	31.8	31.9	32.3	32.4	32.5
2	29.0	29.0	25.7	25.4	25.4
3	45.6	45.7	44.7	54.2	54.1
4	35.7	35.8	33.2	32.4	32.5
5	34.5	34.5	36.2	35.1	35.1
6	29.6	29.6	29.4	29.7	29.7
7	117.7	117.6	117.8	117.6	117.6
8	139.2	139.3	139.0	139.4	139.5
9	49.6	49.6	49.7	49.6	49.7
10	34.9	34.9	34.7	34.8	34.8
11	21.2	21.2	21.2	21.2	21.2
12	39.5	39.4	39.4	39.5	39.5
13	43.6	43.3	43.6	43.4	43.3
14	55.0	55.1	55.1	55.1	55.1
15	22.8	22.8	22.9	22.9	22.9
16	27.8	28.1	28.1	28.1	28.1
17	57.1	55.8	55.8	55.9	55.9
18	12.0	12.1	12.3	12.2	12.1
19	12.0	12.0	11.9	12.4	12.4
20	34.5	40.4	33.1	41.0	40.5
21	19.3	19.6	20.1[c]	16.5[e]	19.7
22	31.4	131.7	39.0	124.7[f]	131.7
23	173.2	135.6	210.9	134.7[f]	135.7
24	137.1[a]	42.7	134.8[d]	125.9[g]	42.8
25	129.0[a]	33.0	137.0[d]	127.6[g]	33.1
26	21.4[b]	19.9	20.9[c]	60.5	20.0
27	25.4[b]	21.1	22.5[c]	21.0[e]	21.1
28	41.1	17.6	29.0	26.5	17.6
29	56.1			49.9	52.4
NMe(3)				34.1	34.1
NMe(29)				45.7	
NAc			23.3, 23.7 169.1, 170.3		

[a-g] Values may be interchanged.

Acetylation of plakinamine A (**1**) with acetic anhydride in pyridine gave two products, the ketone **5** and the *N*-acetylenamine **6**. The molecular formula of the ketone **5**, $C_{33}H_{52}N_2O_3$, differed from that of plakinamine A (**1**) by addition of the elements of acetic anhydride. The ^{13}C NMR spectrum (Table I) contained signals at δ 210.9 (s), 170.3 (s), and 169.1 (s) that were assigned to a ketone and two amide carbons. Four ^1H NMR signals at δ 1.78 (s, 3 H), 1.79 (s, 3 H), 1.96 (s, 3 H), and 1.97 (s, 3 H) were assigned to two vinyl methyl groups and two *N*-acetyl groups: the amide N–H proton signals were observed at δ 5.69 (br s, 1 H) and 6.26 (br s, 1 H). Deuterium exchange of the NH protons sharpened a signal at δ 3.26 (m, 2 H) that was coupled to a signal at δ 2.39 (t, 2 H). The UV absorption at 244 nm (ε 5500) is indicative of an α,β-unsaturated ketone with a fully substituted olefinic bond. The ketone must arise by hydrolysis of an intermediate *N*-acetyl iminium salt **7**, presumably during workup.

Elimination of a proton from the same iminium salt provides a route to the *N*-acetylenamine **6**, $C_{33}H_{50}N_2O_2$. The ^1H NMR spectrum of **6** contained signals at δ 4.82 (s, 1 H) and 4.96 (s, 1 H) assigned to a olefinic methylene group. Irradiation of a vinyl methyl signal at δ 1.89 (s, 3 H) resulted in sharpening of both olefinic methylene protons: this confirms the presence of an isopropenyl

(3) Abraham, R. J.; Monasterios, J. R. *J. Chem. Soc., Perkin Trans. 2* **1974**, 662.
(4) Dadoun, H.; Conreur, C.; Cavé, A. *Phytochem.* **1973**, *12*, 923.
(5) Tadros, W.; Boulos, A. L. *Helv. Chim. Acta* **1975**, *58*, 668.

(6) Shortly after completion of this research, the spectral data for 3-isopropylidene-2-methyl-Δ¹-pyrroline was reported as follows: ^1H NMR ($CDCl_3$) δ 1.79 (s, 3 H), 1.99 (s, 3 H), 2.27 (t, 3 H, J = 1.8 Hz), 2.52–2.56 (m, 2 H), 3.73–3.74 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 20.7 (q), 21.8 (q), 24.9 (q), 30.9 (t), 56.1 (t), 130.2 (s), 136.9 (s), 170.8 (s); IR 1655 cm^{-1}. Gawley, R. E.; Termine, E. J. *J. Org. Chem.* **1984**, *49*, 1946.

group and thus eliminates the possibility of a dihydropyridine ring in plakinamine A (1). The remaining signals in the ^1H NMR spectrum of **6** are all consistent with the proposed structure as is a strong UV absorption at 274 nm (ϵ 9200). One unusual feature of the ^1H NMR spectrum of **6** is that the methylene protons adjacent to the amide nitrogen in the five-membered ring appear as two signals at δ 3.77 (m, 1 H) and 3.74 (m, 1 H).[7]

The minor metabolite plakinamine B (**2**) crystallized from methanol as the dihydrochloride salt, but we preferred to convert the salt to the free base since the salt gave erratic ^1H NMR spectra depending on concentration. The molecular formula of plakinamine B (**2**), $C_{31}H_{50}N_2$, indicated that this metabolite might be closely related to plakinamine A (1). The ^1H NMR spectrum contained two *N*-methyl signals at δ 2.33 (s, 3 H) and 2.38 (s, 3 H), indicating that the two additional carbons were present as *N*-methyl groups. However, the spectral data of **2** were not consistent with those expected for a simple methylated derivative of **1**.

The structure of the steroid nucleus of plakinamine B (**2**) was easily established by comparison of its ^{13}C NMR data with that of 3α-(*N*-methylamino)ergosta-7,22-diene (**8**) (Table I), prepared in two steps from **3**. The chemical shifts for carbons 1–20 were almost identical in both spectra. The structure of the side chain was established from ^1H NMR data. Irradiation of the C-20 proton signal at δ 2.13 (m, 1 H) decoupled a C-21 methyl signal at δ 1.05 (d, 3 H, J = 7 Hz) and a C-22 olefinic proton signal at δ 5.42 (dd, 1 H, J = 15, 9 Hz) that was in turn coupled to an olefinic proton signal at δ 6.34 (d, 1 H, J = 15 Hz). The ^{13}C NMR spectrum indicated the presence of an additional tetrasubstituted olefinic bond and both the UV absorption at 241 nm (ϵ 2700) and the chemical shift of the C-23 olefinic proton signal required the presence of a conjugated diene between C-22 and C-25. A single vinyl methyl signal at δ 1.71 was assigned to the C-26 methyl group while the presence of a broad singlet at δ 2.86 suggested nitrogen substitution at C-27. Mutually coupled signals at δ 2.54 (dt, 1 H, J = 12, 6 Hz) and 2.51 (dt, 1 H, J = 12, 6 Hz) that were also coupled to a signal at δ 2.25 (m, 2 H) were assigned to two methylene groups in a six-membered ring. The structure proposed for plakinamine B (**2**) has the same carbon skeleton as that of plakinamine A (**1**). The ^{13}C NMR signals assigned to carbons 24–29 in plakinamine B (**2**) compare reasonably well with the signals of *N*-methyl-Δ^3-piperideine,[8] the closest simple model.

Plakinamine A (**1**) inhibited the growth of *S. aureus* and *C. albicans* at concentrations of 25 μg/disk and 10 μg/disk, respectively. The hydrochloride salt of plakinamine B (**2**) was active at concentrations of 10 μg/disk and 2 μg/disk against *S. aureus* and *C. albicans*.

Experimental Section

The freeze-dried sample (32.2 g) of *Plakina* sp. was sequentially extracted with dichloromethane, ethyl acetate, and methanol (2.5 L, 48 h each). The methanol extract was most active against *Candida albicans*, which was then used as bioassay organism during the separation. The chloroform-soluble material (1.7 g) from the methanol extract was partitioned against 40% aqueous methanol and the active material was transferred to the aqueous methanol phase which was evaporated and then lyophilized. The residue (660 mg) was chromatographed on Sephadex LH-20 in methanol and then Sephadex G-15 in water to obtain an active fraction (267 mg). The minor component, plakinamine B (**2**, 30 mg, 0.1% dry weight) crystallized from a methanolic solution of the active material. The residue was dissolved in chloroform (50 mL), and the solution was extracted with 1 N hydrochloric acid (50 mL). The aqueous phase was neutralized with saturated sodium bicarbonate solution and the amine was extracted into chloroform (2 × 50 mL). Evaporation of the dried, filtered extract gave plakinamine A (**1**, 96 mg, 0.3% dry weight) that was essentially pure.

Plakinamine A (1): mp 120–130 °C dec; $[\alpha]_D$ +16° (*c* 1.02, $CHCl_3$); IR ($CHCl_3$) 1660, 1580 cm^{-1}; UV (MeOH) 246 nm (ϵ 7100), ^1H NMR ($CDCl_3$) δ 0.59 (s, 3 H), 0.77 (s, 3 H), 0.95 (d, 3 H, J = 7 Hz), 1.79 (s, 3 H), 1.99 (s, 3 H), 2.04 (m, 1 H), 2.28 (dd, 1 H, J = 15, 11 Hz), 2.53 (br s, 2 H), 2.73 (dd, 1 H, J = 15, 2 Hz), 3.19 (br s, 1 H), 3.77 (br t, 2 H), 5.19 (br s, 1 H); ^{13}C NMR (CD_3OD) (see Table I); HRMS, m/z 422.3666, $C_{29}H_{46}N_2$ requires 422.3661.

Plakinamine B (2): dihydrochloride; mp 180–200 °C dec; $[\alpha]_D$ +29° (*c* 1.19, MeOH); IR ($CHCl_3$) 2700, 2400 cm^{-1}; UV (MeOH) 241 nm (ϵ 2700) free base; ^1H NMR ($CDCl_3$) δ 0.56 (s, 3 H), 0.79 (s, 3 H), 1.05 (d, 3 H, J = 7 Hz), 1.71 (s, 3 H), 2.13 (m, 1 H), 2.25 (m, 2 H), 2.33 (s, 3 H), 2.38 (s, 3 H), 2.51 (dt, 1 H, J = 12, 6 Hz), 2.54 (dt, 1 H, J = 12, 6 Hz), 2.73 (s, 1 H, C-3), 2.86 (br s, 2 H), 5.16 (br s, 1 H, C-7), 5.42 (dd, 1 H, J = 15, 9 Hz), 6.34 (d, 1 H, J = 15 Hz); ^{13}C NMR (CD_3OD) (see Table I); HRMS, m/z 450.3973, $C_{31}H_{50}N_2$ requires 450.3974.

Acetylation of Plakinamine A (1). A solution of plakinamine A (**1**, 12 mg, 0.3 mmol) in pyridine (2 mL) and acetic anhydride (200 μL) was maintained at 0–4 °C for 14 h. The solvents were removed in vacuo and the residue was partitioned between dichloromethane and water. The dichloromethane layer was dried over sodium sulfate and the solvent evaporated to obtain an oil (~15 mg) that was chromatographed by LC on μ-Porasil, using 5% methanol in dichloromethane as eluant to obtain ketone **5** (8 mg, 54% theoretical) and the *N*-acetylenamine **6** (3 mg, 21% theoretical).

Ketone 5: IR ($CHCl_3$) 3450, 1660 cm^{-1}; UV (MeOH) 244 nm (ϵ 5500); ^1H NMR ($CDCl_3$) δ 0.59 (s, 3 H), 0.80 (s, 3 H), 0.96 (d, 3 H, J = 7 Hz), 1.78 (s, 3 H), 1.79 (s, 3 H), 1.96 (s, 3 H), 1.97 (s, 3 H), 2.02 (m, 1 H), 2.39 (dd, 1 H, J = 17, 9 Hz), 2.39 (t, 2 H, J = 6 Hz), 2.61 (dd, 1 H, J = 17, 2 Hz), 3.26 (m, 2 H), 4.15 (s, 1 H), 5.19 (br s, 1 H), 5.69 (br s, 1 H), 6.26 (br s, 1 H), ^{13}C NMR ($CDCl_3$) (see Table I); HRMS, m/z 524.3978, $C_{33}H_{52}N_2O_3$ requires 524.3978.

***N*-Acetylenamine 6**: IR ($CHCl_3$) 3450, 1660 cm^{-1}; UV (MeOH) 274 nm (ϵ 9200); ^1H NMR ($CDCl_3$) δ 0.54 (s, 3 H), 0.79 (s, 3 H), 0.81 (d, 3 H, J = 7 Hz), 1.89 (s, 3 H), 1.96 (s, 3 H), 2.11 (br s, 3 H), 2.39 (dd, 1 H, J = 12, 11 Hz), 2.63 (br t, 2 H, J = 8 Hz), 3.13 (br d, 1 H, J = 11 Hz), 3.74 (m, 1 H), 3.77 (m, 1 H), 4.15 (s, 1 H), 4.82 (s, 1 H), 4.96 (s, 1 H), 5.19 (br s, 1 H, C-7), 5.69 (br s, 1 H, NH); HRMS, m/z 506.3855, $C_{33}H_{50}N_2O_2$ requires 506.3872.

3α-Azido-5α-ergosta-7,22-diene (4). Methanesulfonyl chloride (0.5 mL) was added to a stirred solution of 5,6-dihydroergosterol (312 mg, 0.8 mmol), prepared by the method of Tadros and Boulos,[5] in anhydrous pyridine at 0 °C. After 2 h at 0 °C, the solution was poured into cold 1 N hydrochloric acid (50 mL) and the aqueous phase was extracted with dichloromethane (2 × 50 mL). The dichloromethane extract was dried over sodium sulfate and filtered, and the solvent was evaporated to obtain the mesylate (355 mg, 95% theoretical) that was used without further purification.

Sodium azide (660 mg, excess) was added to a solution of the mesylate (180 mg, 0.38 mmol) in dry dimethylformamide (5 mL). The solution was stirred under an atmosphere of dry nitrogen for 12 h at 100 °C. After cooling, the reaction mixture was diluted with water (50 mL) and extracted with dichloromethane (2 × 50 mL). The combined dichloromethane extracts were washed with water (3 × 50 mL), dried over sodium sulfate, and filtered. The orange-brown solution was shaken vigorously with active charcoal for 0.5 min and again filtered to obtain a pale yellow solution, which on concentration gave the azide **4** (148 mg, 91% theoretical): ^1H NMR ($CDCl_3$) δ 0.54 (s, 3 H), 0.79 (s, 3 H), 3.91 (br s, 1 H), 5.16 (br s, 1 H), 5.19 (m, 2 H).

3α-Amino-5α-ergosta-7,22-diene (3). Lithium aluminum hydride (100 mg, excess) was added to a solution of the azide **4** (148 mg, 0.35 mmol) in anhydrous tetrahydrofuran (10 mL). The reaction mixture was stirred under reflux for 6 h, cooled, and

(7) For a similar observation, see: Greger, H.; Grenz, M; Bohlmann, F. *Phytochem.* **1981**, *20*, 2579.

(8) Wenkert, E.; Cochran, D. W.; Hagaman, W. W.; Schell F. M.; Neuss, N.; Katner, A. S.; Potier, P.; Kan, C.; Plat, M.; Koch, M.; Mehri, H.; Poisson, J.; Kunesch, N.; Rolland, Y. *J. Am. Chem. Soc.* **1973**, *45*, 4990.

quenched by dropwise addition of water. The product was diluted with 2 M aqueous sodium hydroxide solution (50 mL) and extracted with dichloromethane (2 × 50 mL). The combined dichloromethane extracts were washed with water (50 mL), dried over sodium sulfate, and filtered, and the solvent was evaporated to obtain the amine **3** (140 mg, quantitative) that was essentially pure by TLC: ^{1}H NMR ($CDCl_3$) δ 0.55 (s, 3 H), 0.78 (s, 3 H), 3.19 (br s, 1 H), 5.17 (br s, 1 H), 5.19 (m, 2 H); ^{13}C NMR ($CDCl_3$) (see Table I).

3α-(*N*-Methylamino)-5α-ergosta-7,22-diene (8). Formic anhydride was prepared by addition of formic acid (440 mg) in cold chloroform (5 mL) to a cold solution of dicyclohexyl carbodiimide (700 mg) in chloroform (5 mL). The mixture was stirred vigorously at 0 °C for 5 min as dicyclohexylurea precipitated. Approximately half of the solution was added dropwise to a stirred solution of the amine (49 mg, 0.12 mmol) in dry pyridine (5 mL) at 0 °C. The mixture was stirred at 0 °C for 6 h. The solvents were removed in vacuo, and the residue taken up in dichloromethane and filtered to remove the urea. The solution was washed with water, dried over sodium sulfate, filtered, and concentrated to obtain the formamide, still slightly contaminated with dicyclohexyl urea.

Lithium aluminum hydride (60 mg, excess) was added to a solution of the formamide in dry tetrahydrofuran (10 mL), and the mixture was stirred under dry nitrogen for 6 h at 25 °C. The excess reagent was quenched by careful addition of water and the product partitioned between 0.5 M sodium hydroxide solution (50 mL) and dichloromethane (2 × 50 mL). The combined dichloromethane extracts were dried over sodium sulfate, and filtered, and the solvent evaporated to obtain a mixture of the *N*-methylamine 8 (19 mg, 37.5% theoretical) and unreacted formamide (21 mg, 40% theoretical) that were easily separated by silica gel chromatography.

3α-(*N*-Methylamino)-5α-ergosta-7,22-diene: ^{1}H NMR ($CDCl_3$) δ 0.54 (s, 3 H), 0.79 (s, 3 H), 2.36 (s, 3 H), 2.73 (br s, 1 H), 5.16 (br s, 1 H), 5.19 (m, 2 H); ^{13}C NMR ($CDCl_3$) (see Table I).

Acknowledgment. We thank Dr. N. Boury-Esnault for identifying the sponge sample and Mary Kay Harper for antimicrobial analyses. This research was supported by grants from the National Institutes of Health (AI-11969) and the Sea Grant College Program (NA80AA-D-00120).

Two New Metabolites of the Sponge *Dysidea amblia* and Revision of the Structure of Ambliol B

Roger P. Walker, Richard M. Rosser, and D. John Faulkner*

Scripps Institution of Oceanography, La Jolla, California 92093

Lawrence S. Bass, He Cun-heng, and Jon Clardy*

Cornell University, Ithaca, New York 14850

Received July 3, 1984

Ambliol C (**6**), ambliofuran (**4**), pallescensin A (**7**), and pallescensolide (**8**) were isolated from a sample of *Dysidea amblia* collected at Pt. Loma, San Diego, CA. The structure of ambliol C (**6**) was determined from a single-crystal X-ray diffraction study performed on the ester **13** and that of pallescensolide (**8**) from ^{1}H NMR and other spectral data. The structure of ambliol B (**9**), one of five metabolites obtained from *D. amblia* collected at Scripps Canyon, La Jolla, CA, was reassigned. X-ray diffraction analysis of the acid **14** showed that ambliol B (**9**) contained a trans-fused decalin ring system rather than the cis ring junction proposed previously.

We previously reported the structural elucidation of five diterpenes (**1**–**5**, Chart I) from a sample of *Dysidea amblia* collected at Scripps Canyon, CA.[1] A second collection of *D. amblia* from Pt. Loma, CA, contained a different array of metabolites that included the known diterpene ambliofuran (**4**) and a known sesquiterpene pallescensin A (**7**).[3] Two new metabolites, ambliol C (**6**) and pallescensolide (**8**), related to pallescensin A (**7**), were isolated. Comparison of the spectral data and chemical reactions of ambliol B (**5**) and ambliol C (**6**) led us to review the previously proposed structure for ambliol B (**5**). In this paper we report the structural determination of ambliol C (**6**), the revised structural assignment for ambliol B (**9**), and the structural elucidation of pallescensolide (**8**).

Dysidea amblia (de Laubenfels)[2] was collected by hand using SCUBA (−20 m). The hexane-soluble material from a methanolic extract of the homogenized sponge was chromatographed on silica gel to obtain pallescensin A (**7**, 0.4% dry weight),[3] ambliofuran (**4**, 0.6% dry weight),[1] ambliol C (**6**, 1.0% dry weight), and pallescensolide (**8**, 0.03% dry weight).

Ambliol C (**6**), $[\alpha]_D$ −37.8° (*c* 2.0 $CHCl_3$), had the molecular formula $C_{20}H_{32}O_2$, isomeric with ambliols A and B. The infrared band at 3500 cm^{-1} indicated that ambliol C was an alcohol. The ^{13}C NMR spectrum contained signals at δ 142.8 (d), 137.8 (d), 126.2 (s), and 111.3 (d) due to a β-substituted furan and at δ 76.0 (s) due to a tertiary carbinol. Both the ^{13}C and ^{1}H NMR spectra of ambliol C (**6**) were sufficiently similar to those of ambliol B (**5**) to suggest that the molecules were stereoisomers.

The same chemical reactions that had been used in the prior structural assignment of ambliol B (**5**) were now performed on ambliol C (**6**). Dehydration of ambliol B (**5**) with phosphorus oxychloride in pyridine gave trisubstituted olefin **10** while acid-catalyzed dehydration produced tetrasubstituted olefin **11** (Chart II). Dehydration of ambliol C (**6**) with either phosphorus oxychloride in pyridine or *p*-toluenesulfonic acid in benzene gave a trisubstituted olefin, dehydroambliol C (**12**). Dehydroambliol C (**12**) was not identical with the trisubstituted olefin **10** from ambliol B (**5**), although it was subsequently determined that the olefinic bond was in the same position in both molecules.[4] These results were perplexing particu-

(1) Walker, R. P.; Faulkner, D. J. *J. Org. Chem.* **1981**, *46*, 1098.
(2) De Laubenfels, M. W. *Proc. U.S. Nat. Mus.* **1982**, *81*, 123.
(3) Cimino, G.; De Stefano, S.; Guerriero, A.; Minale, L. *Tetrahedron Lett.* **1975**, 1425.
(4) Walker, R. P. Ph.D. Thesis, University of California, San Diego, CA, 1981.

0022-3263/84/1949-5160$01.50/0