New Nitrogenous Bisabolene-Type Sesquiterpenes from a Micronesian Marine Sponge, *Axinyssa* Species

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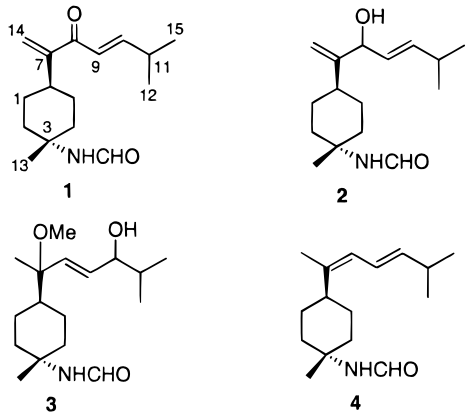
Chemical investigation of an *Axinyssa* species of sponge collected at Yap Island afforded three new nitrogenous sesquiterpenes, **1**−**3**, together with the known compound 3-formamidotheonellin (**4**). The structures of compounds **1**, **3**, and **4** were confirmed by spectroscopic methods, and compound **2** was tentatively identified by comparison of its ^{1}H and ^{13}C NMR data with those of **1**, **3**, and **4** and ^{1}H decoupling experiments.

In a continuing search for biologically active substances from marine invertebrates,[1] we found that the total extract of an *Axinyssa* sp. of sponge collected at Yap Island, Federated States of Micronesia, inhibited the growth of brine shrimp. This toxicity was traced to a CH_2Cl_2-soluble mixture, and from this three new formamido-substituted bisabolene sesquiterpenes, **1**−**3**, were isolated in addition to the known sesquiterpene 3-formamidotheonellin (**4**).[2] We describe here the isolation and structure determination of the new compounds.



1 **2** **3** **4**

Sponges of the genus *Axinyssa* have previously been reported to yield a variety of sesquiterpene isothiocyanates, formamides,[3] and a sesquiterpene isonitrile,[3d] but this is the first report of nitrogenous bisabolenes from sponges of this genus. Other nitrogenous bisabolenes have been obtained from sponges (*Theonella* cf. *swinhoei*,[2,4] *Halichondria* sp.,[5] and *Acanthella* cf. *cavernosa*[6]) and mollusks.[5b,7]

The identity of compound **4** was confirmed by comparison of its ^{1}H and ^{13}C NMR and MS data with those reported for 3-formamidotheonellin.[2] In addition to those ^{13}C NMR signals that had been reported earlier for **4**, we observed doubled carbon signals at δ 53.6 and 52.4, which were assigned to C-3 from HMBC data. The signals at δ 46.0 and 46.2 previously assigned[2] to C-3 are reassigned to C-6. The doubling of many of the NMR signals due to the occurrence of rotational isomers of the formamide group was observed as reported in the literature.[2,5b] This same phenomenon was observed in the NMR spectra of the new compounds **1**−**3**, indicating they all contained the formamide group.

Compound **1** was obtained as a colorless oil whose EIMS showed a molecular ion peak at *m*/*z* 263, indicating the presence of nitrogen in agreement with the doubling of most NMR peaks due to a formamide group as noted above. Taking into account the peak-doubling phenomenon, the NMR data combined with the observed M^+ led to a formula of $C_{16}H_{25}O_2N$. The formamide group N*H* signals were identified as broad, exchangeable (CD_3OD) proton resonances at δ 5.91 (d, 1.5 Hz) and 5.31 (br s), which were coupled (COSY), respectively, to downfield signals at δ 8.29 and 8.02 (each d, 12 Hz), which in turn were one-bond coupled to downfield carbon resonances at δ 162.6 and 160.4, respectively. The ^{13}C NMR spectrum also provided evidence for one ketone (α,β-unsaturated, δ 193.3, 193.7) and two double bonds. Thus, **1** was confirmed to have one carbocyclic ring.

The ^{1}H NMR spectrum of **1** contained signals for an exocyclic methylene group, one deshielded quaternary methyl group and two secondary methyls. The α,β-unsaturated ketone sequence $(CH_3)_2CH-CH=CH-C(O)$ with a trans double bond was established from COSY correlations, a large olefinic proton coupling constant (J = 15.5 Hz), and lowfield olefinic proton absorption (ca. δ 6.80, 6.44). The exocyclic methylene group (COSY, HMQC) was positioned based on HMBC correlations from the exomethylene protons to the ketone carbon and a methine carbon (δ_C, 37.7, 37.8/δ_H 2.60, C-6). The proton signal for this methine group was identified as part of the sequence $-CH(CH_2-CH_2)_2-$ by COSY correlations, and this was expanded to $-CH(CH_2-CH_2)_2-C(CH_3)-NHCHO$ by the following HMBC correlations: H-13 to C-2/4, C-3 and CHO to C-3. Compound **1** thus was confirmed as having the same carbocyclic skeleton as **4**. The relative stereochemistry of **1** is proposed to be the same as in **4** because the ^{13}C NMR chemical shifts of the sp^3 carbons, including that of the methyl group at C-3,[2] in the two compounds are the same (δ 22.1, 24.6 for **1** *vs* δ 22.1, 24.3 for **4**), as are the shapes of the H-6 ^{1}H NMR signals. Compound **1** is thus 3-formamidobisabolane-14(7),9-dien-8-one.

The formula $C_{16}H_{27}O_2N$ was deduced for compound **2** from ^{1}H and ^{13}C NMR data combined with the highest mass ion observed in the LRFABMS, *m*/*z* 248, corresponding to

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$[M - 18 + H]^+$. Compound **2** showed ^1H and ^{13}C NMR features similar to those of compound **1**, indicating the presence of a formamide, an exocyclic methylene group, a trans disubstituted double bond, an isopropyl group, and a quaternary methyl. However, in place of the ketone ^{13}C NMR signal noted for **1**, compound **2** exhibited signals for an oxygenated methine (δ 4.5, d, 6.5 Hz/δ 75.6, 75.4) whose proton signal was coupled with one of the protons of the trans double bond. Decoupling of the isopropyl methine proton confirmed that this group was also adjacent to the trans double bond, thus confirming the partial structure $-CH(OH)-CH=CH-CH(CH_3)_2$. This sample decomposed in $CHCl_3$ solution before additional data could be acquired, but on the basis of the close similarity of the remainder of the ^1H and ^{13}C data of **2**, with that of **1** and **4**, compound **2** was assigned the structure shown, including the relative stereochemistry at C-3 and C-6 based in particular on the chemical shift (δ 24.7, 27.9) of the methyl group at C-3[2] and the shape of the H-6 peak compared to the ^1H NMR spectrum of **4** and **1**. The stereochemistry at C-8 was not determined.

Compound **3** showed NMR signals due to a formamide, one double bond, two quaternary methyls, and two secondary methyls. An oxygenated methine (δ 3.89/77.8, 77.9) and a methoxy group (δ 3.11/49.9, 50.0) were identified by HMQC data. A COSY experiment established the partial structure $-CH=CH-CH(OH)-CH(CH_3)_2$. That one quaternary methyl (δ 1.17, 1.18) and the methoxy group are attached to the same quaternary carbon (δ 78.6, 78.8) was evident from the HMBC experiment, and this quaternary methyl group signal also showed a correlation to the methine carbon signals at δ 46.96, 47.03 (C-6). The remaining ^{13}C NMR signals were very similar in chemical shift to those of carbons 1−5 and 13 of **1**, **2**, and **4**. HMBC correlations were observed between the methyl signal (H-13) at δ 1.28, 1.36 and the quaternary carbon signals at δ 52.8, 54.0 and the methylene signals at δ 37.2, 39.5. The data are all consistent with structure **3**, including the relative stereochemistry at C-3 and C-6 based on the chemical shift of the methyl group at C-3 (δ 22.0, 24.5). Compound **3** also decomposed before other spectra such as MS, IR, UV, and optical rotation could be obtained. Brine shrimp testing[9] was not carried out on pure **1**−**4** due to lack of sufficient material.

Experimental Section

General Experimental Procedures. The UV spectrum was obtained on a Hewlett−Packard spectrometer. NMR spectra were measured on a Varian VXR-500 instrument at 500 MHz (^1H) and 125 MHz (^{13}C), using $CDCl_3$ as solvent and reference. EIMS were recorded on a Hewlett−Packard 5985 mass spectrometer and FABMS on a VG ZAB-E instrument. Preparative HPLC was performed using a Phenomenex RP-18 column with a refractive index detector. Flash chromatography was carried out on Si gel 60-H (230−400 mesh).

Animal Material. The sponge was collected from a depth of 12 m in Goofnuw Channel on the island of Yap, Federated States of Micronesia, in August 1995. The sponge forms a dense anastomosing mat of short branches, 10−15 mm in diameter, each branch with blunt, rounded ends. The texture is compressible and flexible, the surface forms soft, relatively long conules. The color in life is greenish yellow, the surface usually completely obscured by silt. The skeleton consists of large randomly oriented oxeas that form short tracts at the surface resulting in a conulose surface. The sponge is an undescribed species of *Axinyssa* (order Halichondrida, family Halichondriidae). A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 1998.8.10.1), and one is retained at the University of Oklahoma (13-YA-95).

Extraction and Isolation. Freshly collected specimens were frozen for transportation and then put in 95% EtOH and stored at 5 °C. The extraction and solvent partitioning were conducted as described previously.[8] The CH_2Cl_2-soluble fraction of the sponge (76 g dry wt after extraction) was chromatographed on a Si gel column using a hexane−EtOAc step gradient elution. The eluate from hexane−EtOAc (3:1) elution was further fractionated by a gravity flow C_{18} column, and eluted with MeOH−H_2O mixtures. The eluate from 80% MeOH was repeatedly purified on a C_{18} reversed-phase HPLC column (MeOH−H_2O,3:2) and afforded compounds **1** (2.7 mg), **2** (1.1 mg), **3** (1.5 mg), and **4** (13.5 mg).

3-Formamidobisabolane-14(7),9-dien-8-one (1): $[\alpha]_D$ +28° (*c* 0.25, MeOH); IR (film) ν_{max} 3500−3100 (br), 1672 (br) cm^{-1}; UV (MeOH) λ_{max} 234 nm; ^1H NMR ($CDCl_3$) δ 8.29 (1/2H, d, J = 12.0 Hz, CHO), 8.02 (1/2H, d, J = 12.0 Hz, CHO), 6.83 (1/2H, dd, J = 15.5, 7.0 Hz, H-10), 6.80 (1/2H, dd, J = 15.5, 7.0 Hz, H-10), 6.46 (1/2H, d, J = 15.5 Hz, H-9), 6.44 (1/2H, d, J = 15.5 Hz, H-9), 5.91 (1/2H, br d, J = 11.5 Hz, NH) 5.84 and 5.63 (each 1/2H, s, H-14), 5.81 and 5.62 (each 1/2H, s, H-14), 5.31 (1/2H, br s, NH), 2.60 (1H, m, H-6), 2.46 (1H, m, H-11), 2.04 (1H, br d, J = 12.0 Hz, H-2 and H-4), 1.80 (1H, br d, J = 12.5 Hz, 1/2H-2 and 1/2H-4), 1.72−1.60 (5H, m, H-2, H-4, H-1, and H-5), 1.41−1.30 (1H, m, H-1 and H-5), 1.43 and 1.35 (each 1.5 H, s, H-13), 1.05 (6H, d, J = 7.0 Hz, H-12 and H-15); ^{13}C NMR ($CDCl_3$) δ 27.8, 27.6 (each 1C, t, C-1 and C-5), 37.0, 39.2 (each 1C, t, C-2 and C-4), 37.7, 37.8 (each 1/2C, d, C-6), 52.5, 53.6 (each 1/2C, s, C-3), 153.1, 153.5 (each 1/2C, s, C-7), 193.3, 193.7 (each 1/2C, s, C-8), 123.6, 123.8 (each 1/2C, d, C-9), 155.2, 155.3 (each 1/2C, d, C-10), 31.29, 31.31 (each 1/2C, d, C-11), 21.3 (1C, q, C-12), 22.1, 24.6 (each 1/2C, q, C-13), 12.8, 121.2 (each 1/2C, t, C-14), 21.3 (1C, q, C-15), 160.4, 162.6 (each 1/2C, d, CHO); multiplicities were assigned from a HMQC experiment; EIMS *m*/*z* 263 $[M]^+$, 175 (100).

3-Formamidobisabolane-14(7),9-dien-8-ol (2): colorless oil; ^1H NMR δ 8.28 (1/2H, d, J = 13.0 Hz, CHO), 8.02 (1/2H, s, CHO), 5.81 (1/2H, br, s, NH), 5.68 (1/2H, dd, J = 15.0, 6.0 Hz, H-10), 5.65 (1/2H, dd, J = 15.0, 6.0 Hz, H-10), 5.35 (1H, dd, J = 15.0, 7.0 Hz, H-9), 5.25 (1/2H, br, s, NH), 5.13, 5.12, 4.90, 4.89 (each 1/2H, s, H-14), 4.50 (1H, d, J = 6.5 Hz, H-8), 2.29 (1H, m, H-11), 1.42, 1.36 (each 1.5H, s, H-13), 0.97 (6H, d, J = 6.0 Hz, H-12 and H-15); ^{13}C NMR δ 28.65, 28.74, 29.25, 29.27 (each 1/2C, C-1 and C-5), 39.3, 39.45, 39.50, 39.57 (each 1/2C, C-2 and C-4), 52.6, 53.8 (each 1/2C, C-3), 37.1 (C-6), 155.0, 155.5 (C-7), 75.4 (C-8), 128.1, 128.2 (C-9), 140.1, 140.2 (C-10), 30.7 (C-11), 22.2, 22.3 (C-12 and C-15), 108.3, 108.6 (each 1/2C, C-14), 24.7, 27.9 (each 1/2C, C-13), 160.4, 162.7 (each 1/2C, CHO); LRFABMS *m*/*z* 248.2 $[M - 18 + H]^+$.

3-Formamido-8-methoxybisabolan-9-en-10-ol (3): colorless oil; ^1H NMR δ 8.27 (1/2H, d, J = 12.0 Hz, CHO), 8.00 (1/2H, s, CHO), 5.56−5.48 (2H, m, H-8 and H-9), 3.89 (1H, m, H-10), 3.11 (3H, s, MeO), 1.36, 1.28 (each 1.5 H, H-13), 1.18, 1.17 (each 1.5 H, s, H-14), 0.92 (3H, d, J = 7.0 Hz, H-12 or H-15), 0.89 (3H, d, J = 6.5 Hz, H-12 or H-15); ^{13}C NMR δ 23.0, 23.1, 23.25, 23.34 (each 1/2C, C-1 and C-5), 37.2, 39.5 (C-2 and C-4), 52.8, 54.0 (each 1/2C, C-3), 46.96, 47.03 (each 1/2C, C-6), 78.6, 78.8 (each 1/2C, C-7), 132.73, 132.93 (each 1/2C, C-8), 134.73, 134.93 (each 1/2C, C-9), 77.82, 77.85 (each 1/2C, C-10), 33.95, 33.97 (each 1/2C, C-11), 17.9, 18.3 (each 1C, C-12 and C-15), 22.0, 24.5 (each 1/2C, C-13), 18.1 (C-14), 160.3, 162.5 (each 1/2C, CHO).

3-Formamidotheonellin (4): ^1H NMR;[2] ^{13}C NMR ($CDCl_3$) (DEPT) ($CDCl_3$) δ 162.9, 160.5 (each 1/2C, d, CHO), 140.3, 140.1 (each 1/2C, d, C-10), 139.9, 139.3 (each 1/2C, s, C-7), 123.35, 123.37 (each 1/2C, C-9), 123.1, 123.3 (each 1/2C, d, C-8), 53.6, 52.4 (each 1/2C, s, C-3), 46.2, 46.0 (each 1/2C, d, C-6), 39.0, 36.7 (each 1C, t, C-2 and C-4), 31.2 (1C, d, C-11), 27.07, 27.14 (each 1C, t, C-1 and C-5), 24.3, 22.1 (each 1C, q, C-13), 22.41, 22.39 (each 1/2C, q, C-12 and C-15), 14.9 (1C, q, C-14).

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