Aromatic Secondary Metabolites from the Sponge Tedania ignis

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AROMATIC SECONDARY METABOLITES FROM THE SPONGE *TEDANIA IGNIS*[1]

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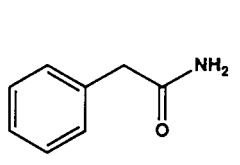
ABSTRACT.—In a continuing investigation of the minor constituents of extracts of the sponge *Tedania ignis*, eight aromatic compounds **1–8** have been isolated and identified. Included were four indoles, a carbazole, a β-carboline, and two substituted benzene derivatives. Three of the compounds were not known as natural products, and three others had not been isolated previously from the marine biosphere. Compounds **5** and **6** may be artifacts produced by condensation of the extraction solvent (Me_2CO) with simpler indole aldehydes.

The isolation and identification of tedanolide, a potent antineoplastic agent, from the sponge *Tedania ignis* Duchassaing and Michelotti (Demospongiae) by Schmitz's group (1) has prompted us to study the chemistry and ecology of the microbial communities associated with this sponge (2,3). As part of that study, we have also explored the minor metabolites of the sponge extracts and have found the first naturally occurring polybrominated benzo-*p*-dioxins (4). Herein, we report the isolation and characterization of eight aromatic compounds from Bermudian *T. ignis*.

RESULTS

A large collection of *T. ignis* was made in 1979 from a number of locations in Harrington Sound. The substantial organic soluble extracts (ca. 86 g) were initially bulk-separated by means of a solvent-solvent partitioning scheme (5); the CCl_4- and $CHCl_3$-soluble fractions were then separated via gel permeation chromatography (BioBeads S-X4 and Sephadex LH-20). Three compounds were obtained in pure form from the extracts during the Sephadex LH-20 step, while the remaining compounds required low pressure chromatography on silica or centrifugal countercurrent chromatography for purification.



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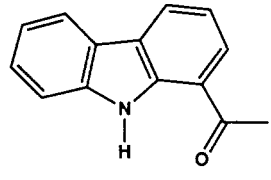

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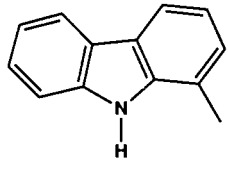

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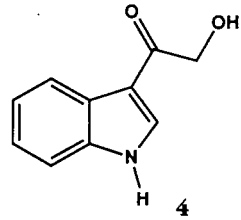

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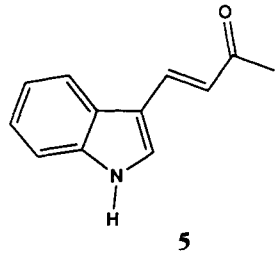
2-Phenylacetamide [**1**], 1-methyl-carbazole [**2**], and 1-acetyl-β-carboline [**3**] were readily identified by analysis of the ms, nmr, and ir data and comparison to literature reports. Compound **1** had been previously reported from terrestrial plants (6–8) and microorganisms (9,10); similarly, compounds **2** (11–13) and **3** (14) were previously known, but none of the three compounds had been isolated from the marine biosphere. Compound **4** proved to be 3-hydroxymethylketoindole, previously isolated by Gerwick's group from a red alga (15) and by our group from a *Micrococcus* sp. growing in association with *T. ignis* (2).

Compound **5** had two spin systems and three isolated one-proton signals. A four-spin system in the aromatic region represented four adjacent protons on a benzene ring, while the ir spectrum (3466 cm^{-1}) suggested indole or pyrrole *N*-H. The remaining spin system consisted of olefinic protons trans disposed on a polarized carbon-carbon double bond (δ 7.85 and 6.75, $J = 16$ Hz). A conjugated methyl ketone was evident from the nmr (δ 198.7 and 2.3, 3H, s), ir (1643 cm^{-1}), and ms ($[M-15]^+$, $[M-43]^+$) data. An indole was indicated by the characteristic ^{13}C-nmr shifts; substitution at C-3 was determined by an nOe between the N-H and H-2 protons, the downfield shift of H-2, and the ^{13}C-nmr shifts. Thus, **5** had to be **4**-(indol-3-yl)-but-(3*E*)-en-2-one, not previously known from nature but prepared synthetically (16).

Compound **6** was a homologue of **5**, displaying the same aromatic systems but containing a methylene group interspersed between the ring and the conjugated ketone. This was indicated by a higher frequency carbonyl stretch (1661 cm^{-1}), less polarization and deshielding of the olefinic protons (δ 7.0, 6.1), and coupling of the methylene to the olefinic proton β to the carbonyl.

Of the four indoles, compound **7**, $C_{16}H_{17}NO_7$, was the most unusual. As in **5** and **6**, a C-3 substituted indole was evident, as was the conjugated methyl ketone. The difference lay in the presence of two vinyl methyl groups and two additional fully substituted sp^2 carbons. The chemical shifts of the olefinic protons suggested placement next to the ketone, although the coupling constant indicated a trans configuration. Irradiation of the vinyl methyl signals revealed nOe relationships between the olefinic proton at δ 5.6 and the methyl at δ 1.73 and between the other olefinic proton (δ 8.0) and the methyl at δ 2.16. These facts were accommodated by structure **7**.

The last compound, $C_{13}H_{16}O$, also contained a conjugated methyl ketone, with



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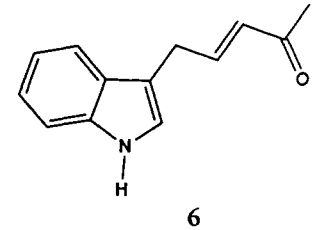

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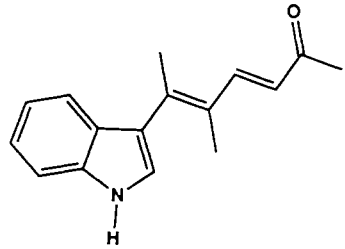

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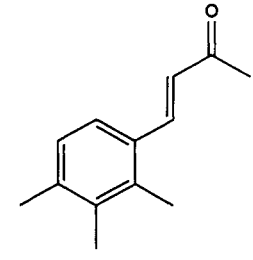

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evidentiary data from the ir (1673 cm^{-1}), ^{13}C nmr (δ 198.44), and ^{1}H nmr [δ 2.20 (3H, s), δ 7.9 and 6.57 (ea 1H, d, $J = 16$)]. A 1,2,3,4-tetrasubstituted phenyl ring was indicated by a pair of *ortho*-disposed aromatic protons and three aryl methyl groups in the ^{1}H nmr. The substitution pattern was determined as follows. The different chemical shifts of the aromatic protons suggested that one (δ 7.37) was adjacent to the unsaturated sidechain; the other one had to be next to a methyl group. A pair of nOe experiments confirmed structure **8** for this compound; nOe's were observed between the olefinic proton α to the ketone and the aryl proton at δ 7.37 and between the other aryl proton and a methyl group (δ 2.28).

DISCUSSION

2-Phenylacetamide [**1**] has long been known as a metabolic product of phenylalanine (17). Compound **2**, 1-methylcarbazole, has not previously been found in a marine invertebrate; it has been reported to have insecticidal and antimicrobial activity (18) but was shown to be non-mutagenic (19). In our hands, **2** was cytotoxic (Table 1) but only moderately antimicrobial. 1-Acetyl-β-carboline [**3**] was also previously known from the plant *Ailanthus malabarica* (14) but had not been isolated from a marine source. The most closely related marine compounds are the simple monosubstituted β-carbolines from the bryozoan *Castaticella hastata* (20). Cytotoxicity (Table 1) and marginal antimicrobial activity were associated with **3**.

TABLE 1. Cytotoxicity Screening Results for Compounds **2**, **3**, **5–8**.[a]

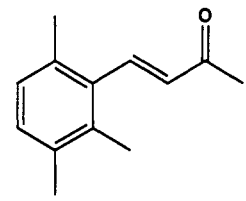
Compound	Mortality (%)			
	4 h	6 h	8 h	24 h
2	82	100		
3	14	21	43	100
5	0	0	8	39
6	0	0	27	91
7	20	20	20	70
8	0	50	90	100

[a]Brine shrimp cytotoxicity (32); all compounds tested at 25 ppm; compounds **1** and **4** were inactive at the dose tested.

The indole **4** was recently found in the culture extracts of the bacterium *Micrococcus* sp., obtained from tissues of *T. ignis* (2). Therefore, **4** would seem to represent the second chemotype of "sponge metabolite" actually produced by an associated bacterium (3). Compound **4**, as noted above, has also been found in extracts of a red alga (15); the 6-bromo analogue has been isolated from the sponge *Pleroma menoui* (21).

The indoles **5** and **6** were previously unknown as natural products, although **5** has been prepared synthetically (16). The extraction of the sponge with Me_2CO could have provided **5** and **6** as artifacts from indole-3-acetaldehyde and indole-3-propionaldehyde, respectively, via aldol condensation reactions. Indole-3-acetaldehyde and **4** are known microbial degradation products of tryptophan (22), but indole-3-propionaldehyde is not known from that pathway. Indole **7** was also previously unknown and cannot be derived by condensation of Me_2CO with a simple, known indole aldehyde.

Indole **4** was inactive in phytotoxicity and cytotoxicity screens, but indoles **5–7** are moderately cytotoxic (Table 1). Compound **6** proved phytotoxic to the rangeland weed pest leafy spurge (*Euphorbia esula*); necrotic lesions (2–4 mm) were produced by applied doses of 5 μg in a nicked leaf assay (23).



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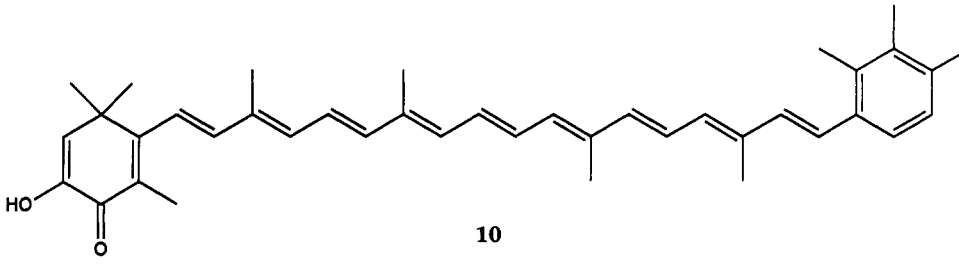

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Compound **8** was moderately active in the cytotoxicity screen. While a novel compound, it resembles **9**, a microbial transformation product of β-ionone (24) and a constituent of the volatiles from grape brandies (25), preserved prunes (26) and other fruit (27). The aromatic substitution pattern of **8** is found in at least three carotenoids of *Tedania digitata* (28–31), including tedanin [**10**] (28,29). Mikami's microbial conversion of β-ionone to 9 (24) suggests a similar biosynthetic process in the *Tedania* carotenoids; compound **8** is very likely a degradation product of carotenoids.

The very small quantities of **1–8** obtained from the extracts and their identity or similarity to known microbiol metabolites suggest that they are likely produced by microbes associated with *T. ignis*, are the results of microbial degradation of sponge compounds (e.g., **8**), or are obtained by the filter-feeding sponge from its diet.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were obtained using a Varian G34 spectrophotometer. Ir spectra were obtained with a Nicolet 5DX FT-ir spectrophotometer. Mass spectral data were determined using VG-MM16F and VG-7070EHF mass spectrometers. Nmr spectra were recorded using Bruker AC-300 and Bruker AM-500 spectrometers; chemical shifts are reported in ppm (δ units) relative to TMS (δ = 0). Solvents used were $CDCl_3$, CD_3OD, and Me_2CO-d_6, as indicated.

EXTRACTION OF *T. IGNIS*.—*T. ignis* was collected in the shallow waters of Bermuda, most notably Harrington Sound, in the fall of 1979 and stored in Me_2CO at −5°. A voucher specimen has been retained at the Center of Marine Biotechnology, University of Maryland. The Me_2CO/H_2O was collected by filtration and the Me_2CO evaporated in vacuo; the solids were then ground in MeOH, which was removed by filtration. The MeOH was evaporated, leaving an aqueous residue which was combined with similar material from the first filtration. The sponge solids were twice soaked overnight in CH_2Cl_2. Each time, the CH_2Cl_2 was removed by filtration. The resulting aqueous suspension was equilibrated with the CH_2Cl_2 phase. The aqueous phase was washed with fresh CH_2Cl_2 and lyophilized (506.5 g). The combined CH_2Cl_2 extracts were evaporated (86.36 g) and subjected to a solvent-solvent partitioning scheme (5).

ISOLATION.—The CCl_4 fraction (5.6 g) was applied to BioBeads S-X4 (4.5 × 94 cm) using hexane-CH_2Cl_2-EtOAc (4:3:1); seven fractions were obtained. The fourth and sixth fractions were applied separately to Sephadex LH-20 (2.5 × 180 cm) using MeOH-CH_2Cl_2 (1:1); six fractions were obtained in each case. The second and third fractions from BioBeads fraction four yielded **8** and **1**, respectively, after purification on silica. The fourth and sixth fractions from BioBeads fraction six were **6** and **5**, respectively.

The $CHCl_3$ crude extract was applied to BioBeads S-X4 as described above, and six fractions resulted. The fifth fraction was permeated through Sephadex LH-20 as above to give six fractions. Sephadex fractions five and six were fractionated via centrifugal countercurrent chromatography using $CHCl_3$-MeOH-H_2O (25:34:20) (lower phase mobile). Sephadex fraction five gave **3**, **2**, and **7**, while Sephadex fraction six provided **4**.

2-Phenylacetamide [**1**].—Compound **1** (5.8 mg): ir ν max ($CDCl_3$) 3519, 3412, 3036, 1687, 1586 cm^{-1}; hrms *m/z* (%) $[M]^+$ 135.0706 (19) (C_9H_9NO requires 135.0684), 92 (100); ^1H nmr (Me_2CO-d_6) δ 7.3–7.4 (5H, m), 5.85 (1H, br s), 5.50 (1H, br s), 3.6 (2H, s); ^{13}C nmr ($CDCl_3$) δ 173.5, 134.8, 129.4 (2C), 129.0 (2C), 127.4, 43.3.

1-Methyl carbazole [**2**].—Compound **2** (2.3 mg): hrms *m/z* (%) $[M]^+$ 181.0871 (100) ($C_{13}H_{11}N$ requires 181.0892); ^1H nmr (Me_2CO-d_6) δ 8.08 (1H, d), 7.93 (1H, d), 7.50 (1H, d), 7.35 (1H, t), 7.19 (1H, d), 7.16 (1H, t), 7.08 (1H, t), 2.56 (3H, s); ^{13}C nmr ($CDCl_3$) δ 139.4, 138.9, 126.4, 125.7, 123.9, 120.9, 119.7, 119.6, 119.5, 117.9, 110.7, 16.9.

1-Acetyl-β-carboline [**3**].—Compound **3** (4.5 mg): ir ν max ($CDCl_3$) 3444, 1671 cm^{-1}; hrms *m/z* (%) $[M]^+$ 210.0756 (90) ($C_{13}H_{10}NO_2$ requires 210.0793), 182 (37), 168 (100), 140 (34); ^1H nmr (Me_2CO-d_6) δ 8.5 (1H, d, *J* = 5 Hz), 8.4 (1H, d, 5), 8.3 (1H, d, 8), 7.8 (1H, d, 8), 7.6 (1H, dd, 7.3, 8), 7.3 (1H, dd, 7.3, 8), 2.8 (3H, s); ^{13}C nmr ($CDCl_3$) δ 203.3, 141.1, 138.2, 136.1, 135.4, 131.5, 129.3, 121.8, 120.7, 120.6, 119.1, 112.0, 25.9.

3-(Hydroxylacetyl)-indole [**4**].—Compound **4** (7.8 mg): uv λ max (EtOH) 295 nm (ε = 3500), 260 (2800), 239 (3700), 220 (4000); hrms *m/z* (%) $[M]^+$ 175.0651 (3) ($C_{10}H_9NO_2$ requires 175.0663), 144 (100), 130 (21), 116 (20), 89 (19); ^1H nmr (CD_3OD) δ 8.2 (1H, dd), 8.1 (1H, s), 7.4 (1H, dd), 7.15–7.25 (2H, m), 4.69 (2H, s); ^{13}C nmr (CD_3OD) δ 196.0, 138.2, 126.9, 124.4, 123.3, 122.7, 114.8, 112.9, 66.3.

4-(Indol-3-yl)-(3E)-buten-2-one [**5**].—Compound **5** (3.3 mg): uv λ max (EtOH) 353 nm (ε = 17800), 276 (9400), 223 (19000); ir ν max ($CDCl_3$) 3466, 1643, 1616, 1588 cm^{-1}; hrms *m/z* (%) $[M]^+$ 185.0838 (26) ($C_{12}H_{11}NO$ requires 185.0841), 170 (40), 144 (100), 115 (15), 89 (12); ^1H nmr (Me_2CO-d_6) δ 8.6 (1H, br s), 7.95 (1H, dd), 7.88 (1H, d), 7.85 (1H, d, *J* = 16 Hz), 7.5 (1H, dd), 7.2 (2H, m), 6.75 (1H, d, 16), 2.3 (3H, s); ^{13}C nmr ($CDCl_3$) δ 198.7, 137.3, 137.2, 129.2, 125.3, 123.5, 123.4, 121.7, 120.5, 113.7, 111.9, 27.4.

5-(Indol-3-yl)-pent-(3E)-en-2-one [**6**].—Compound **6** (2.8 mg): uv λ max (EtOH) 290 nm (ε = 5200), 282 (5900), 224 (20700); ir ν max ($CDCl_3$) 3481, 2282, 1661 cm^{-1}; hrms *m/z* (%) $[M]^+$ 199.1005 (79) ($C_{13}H_{13}NO$ requires 199.0997) 184 (19), 156 (100), 130 (47), 117 (13), 89 (7); ^1H nmr (Me_2CO-d_6) δ 10.1 (1H, br s), 7.5 (1H, d), 7.3 (1H, d), 7.19 (1H, s), 7.10 (1H, t), 7.0 (2H, m), 6.1 (1H, d, *J* = 16 Hz), 3.72 (1H, dd, 23, 6), 3.63 (1H, dd, 23, 6), 2.16 (3H, s); ^{13}C nmr (Me_2CO-d_6) δ 198.1, 147.5, 137.8, 132.1, 128.3, 124.5, 123.8, 122.3, 119.6, 119.3, 112.3, 112.1, 29.0, 26.8.

6-(Indol-3-yl)-5-methylhepta-(3E,5E)-dien-2-one [**7**].—Compound **7** (8.3 mg): uv λ max (EtOH) 290 nm (ε = 8300), 224 (12800); ir ν max ($CDCl_3$) 3475, 1653 cm^{-1}; hrms *m/z* (%) $[M]^+$ 239.1289 (80) ($C_{16}H_{17}NO$ requires 239.1310), 224 (73), 196 (100), 181 (43), 167 (13), 154 (16), 130 (16), 117 (16), 89 (3); ^1H nmr (Me_2CO-d_6) δ 10.3 (1H, br s), 8.0 (1H, d, *J* = 15.5 Hz), 7.4 (1H, d, 8), 7.20 (1H, d, 8), 7.13 (1H, s), 7.10 (1H, t, 8), 6.90 (1H, t, 8), 5.6 (1H, d, 15.5), 2.19 (3H, s), 2.16 (3H, s), 1.73 (3H, s); ^{13}C nmr ($CDCl_3$) δ 199.4, 146.8, 142.2, 135.9, 127.7, 127.3, 126.7, 123.2, 122.1, 119.7, 119.7, 113.8, 111.1, 28.0, 24.4, 20.8.

4-(2',3',4'-Trimethylphenyl)-but-(3E)-en-2-one [**8**].—Compound **8** (2.0 mg): ir ν max 1673 cm^{-1}; hrms *m/z* (%) $[M]^+$ 188 (8), $[M-15]^+$ 173.0975 (100), ($C_{12}H_{13}O$ requires 173.0966), 129 (15); ^1H nmr (Me_2CO-d_6) δ 7.9 (1H, d, *J* = 16 Hz), 7.37 (1H, d, 8), 7.04 (1H, d, 8), 6.57 (1H, d, 16), 2.34 (3H, s), 2.32 (3H, s), 2.28 (3H, s), 2.20 (3H, s); ^{13}C nmr ($CDCl_3$) δ 198.4, 142.4, 139.1, 136.2, 135.9, 131.6, 127.9, 127.8, 124.0, 27.8, 21.2, 16.0, 15.9.

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