Notes

New Carbonimidic Dichlorides from the Australian Sponge *Ulosa spongia* and Their Possible Taxonomic Significance

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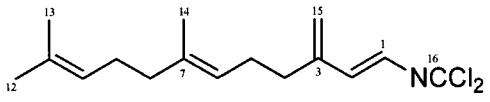
Received February 8, 2001

Five sesquiterpene carbonimide dichlorides (**1**−**5**) have been isolated from the dichloromethane extract of the Australian sponge *Ulosa spongia*. The structures of the two new compounds, ulosins A (**1**) and B (**2**), were elucidated by employing spectroscopic techniques (NMR, MS, UV, and IR). The chemotaxonomic significance of our data is discussed.

To date only 14 natural products, all of which are sesquiterpenes containing a carbonimidic dichloride functionality, have been isolated from several marine sponges, mainly those of the orders Halichondrida and Axinellida (the latter is now not widely recognized as a valid order, with its various families now assigned to the Halichondrida and Poecilosclerida), and a nudibranch. In 1977 Faulkner and Wratten published data for the first two carbonimidic dichlorides from the marine sponge *Pseudaxinyssa pitys*.[1] Another two publications followed, which described the isolation of an additional four carbonimidic dichlorides from the same sponge.[2,3] Hirota et al. reported the isolation of five new potent antifouling carbonimidic dichlorides from the sponges *Halichondria axinyssa*[4] and *Axinyssa* sp.[5] Recently, Tanaka and Higa described reticulidins A and B from the nudibranch *Reticulidia fungia* to be cytotoxic toward KB and L1210 cells.[6] Biosynthetic studies of compounds containing the carbonimidic dichloride ($N{=}CCl_2$) functionality were first undertaken by Garson et al.[7] Using ^{14}C-labeled cyanide and thiocyanate, they suggested isocyanide and isothiocyanate to be involved in the biosynthesis of the dichloroimine carbon. In the present study the isolation and structure elucidation of five $N{=}CCl_2$-containing secondary metabolites obtained from *Ulosa spongia* (de Laubenfels, 1954), belonging to the class Demospongiae, family Halichondriidae (order Halichondrida), are described. The higher taxonomic placement of this species is still in question, with possibilities for inclusion in the order Poecilosclerida (families Desmacellidae or Mycalidae) or the Halichondrida (families Halichondriidae or Dictyonellidae) (J. N. A. Hooper, R. W. M. van Soest, and E. Hajdu, personnel communications). Our chemical data support its inclusion in the Halichondrida, although its true family and genus assignment are still unresolved. Sponges of the families Desmacellidae or Mycalidae, in contrast to the family Halichondriidae, so far, are not known to produce carbonimidic dichlorides.

A sample of *U. spongia*, collected at Wistari Reef, was extracted with CH_2Cl_2. Chromatographic separation of this extract yielded five $N{=}CCl_2$-containing sesquiterpenes. The known compounds **3**−**5** were identified by comparing their spectral data with those reported in the literature.[1,3,6]



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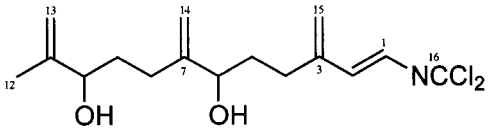

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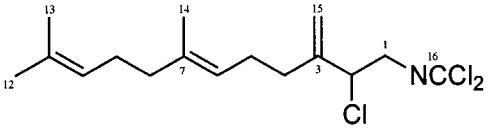

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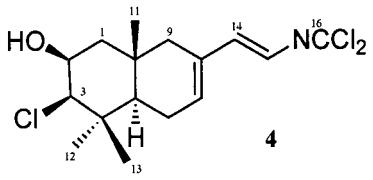

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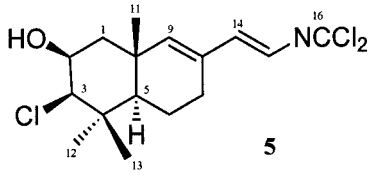

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Mass spectral analysis of compound **1** indicated it to have the molecular formula $C_{16}H_{23}Cl_2N$ and showed it to have five elements of unsaturation. It exhibited a strong IR absorption at 1639 cm^{-1} and a weak ^{13}C signal at δ 125.2, both characteristic of carbonimide dichloride derivates. In comparison with literature data the strong UV absorption at 284 nm indicated the $N{=}CCl_2$ group to be conjugated to

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10.1021/np0100587 CCC: $20.00
Published on Web 06/14/2001

Table 1. ^{1}H NMR Spectral Data for Compounds **1** and **2** (δ in ppm, J in Hz)[a]

proton	**1**[b]	**2**[c]
1	6.87 (1H, d, J = 13.2)	6.96 (1H, d, J = 13.2)
2	6.61 (1H, d, J = 13.2)	6.64 (1H, d, J = 13.2)
4	2.28 (2H, m)	2.34 (1H, m)
		2.59 (1H, ddd, J = 5.0, 9.5, 14.5)
5	2.23 (2H, m)	1.93 (2H, m)
6	5.15 (1H, brt, J = 7.0)	3.64 (1H, dd, J = 3.5, 9.0)
8	1.99 (2H, m)	2.03 (1H, m)
		2.22 (1H, m)
9	2.06 (2H, m)	1.50 (2H, m)
10	5.09 (1H, brt, J = 7.0)	3.68 (1H, dd, J = 2.2, 11.0)
12	1.68 (3H, s)	1.69 (3H, s)
13	1.61 (3H, s)	4.83 (1H, s)
		5.08 (1H, s)
14	1.61 (3H, s)	4.70 (1H, s)
		4.71 (1H, s)
15	5.19 (1H, s)	4.99 (1H, s)
	5.23 (1H, s)	5.04 (1H, s)

[a] All assignments are based on extensive 1D and 2D NMR measurements (HMBC, HMQC, COSY). [b] $CDCl_3$, 500 MHz. [c] Benzene-d_6, 500 MHz.

Table 2. ^{13}C NMR Spectral Data for Compounds **1** and **2** (δ in ppm)

	1[a,c]		**2**[b,c]	
carbon	^{13}C	HMBC	^{13}C	HMBC
1	130.8 (d)[d]	2	131.5 (d)	2
2	137.0 (d)	1, 4	136.9 (d)	1, 15
3	143.9 (s)	1, 2, 4	144.7 (s)	1, 4
4	32.1 (t)	2	28.2 (t)	5, 15
5	26.7 (t)	4	31.0 (t)	
6	123.4 (d)	4, 5, 14	77.3 (d)	5, 14
7	135.9 (s)	8, 14	147.0 (s)	8
8	39.7 (t)	14	33.6 (t)	9, 14
9	26.6 (t)		32.8 (t)	8
10	124.2 (d)	8, 12, 13	80.7 (d)	9, 12, 13
11	131.4 (s)	12, 13	146.1 (s)	10, 12
12	25.7 (q)	13	19.1 (q)	13
13	17.7 (q)	12	110.3 (t)	12
14	16.0 (q)	8	106.9 (t)	8
15	120.3 (t)	1, 2, 4	120.5 (t)	2
16	125.2 (s)	2	124.9 (s)	1

[a] $CDCl_3$, 125.75 MHz. [b] Benzene-d_6, 125.75 MHz. [c] Assignments are based on extensive 2D NMR measurements (HMBC, HMQC, COSY). [d] Implied multiplicities by DEPT (C = s, CH = d, CH_2 = t, CH_3 = q).

a diene system.[3,6] ^{13}C NMR data showed the presence of 4 × C, 4 × CH, 5 × CH_2, and 3 × CH_3 groups for a total of 16 carbon resonances. These data also revealed the presence of five double bonds (4 × C=C; 1 × C=N) and thus accounted for all of the unsaturation within the molecule; **1** was thus acyclic. The ^{1}H NMR spectrum of **1** (see Table 1) contained three singlet methyl signals at δ 1.61 (2 × CH_3) and 1.68 and two singlet resonances at 5.19 and 5.23 due to an exo-methylene group. From the HMBC data of **1** (see Table 2) it was possible to establish the basic structure of **1**. Thus, long-range CH correlations observed between C-3 and H-1, H-2, and H_2-4, between C-16 and H-2, and between C-2 and H_2-4 clearly delineated connection of the exo-methylene group to the quarternary carbon C-3 and C-1 to the nitrogen atom. The ^{13}C NMR chemical shifts of the resonances for the C-14 methyl group (δ 16.0) revealed the double bond $\Delta^{6,7}$ to have the E configuration.[1] The coupling constant $J_{H-1,H-2}$ = 13.2 Hz established the geometry of $\Delta^{1,2}$ as trans. Further, long-range CH correlations between C-11 and H_3-12 and H_3-13 and between C-7 and H_3-14 together with the ^{1}H NMR chemical shifts of H_3-13 and H_3-14 indicated C-12 and C-13 to both bond to C-11, and C-14 to C-7. Cross-peaks in the ^{1}H−^{1}H COSY spectrum between H_2-4 and H_2-5, H_2-5 and H-6, H_2-8 and H_2-9, and H_2-9 and H-10 together with long-range CH correlations observed between C-3 and H_2-4, between C-6 and C-8 and H_3-14, and between C-10 and H_3-13 enabled the structure of compound **1** to be established. The new natural product is best described as [(1*E*,6*E*)-7,11-dimethyl-3-methylene-1,6,10-dodecatrienyl]carbonimidic dichloride, for which the trivial name ulosin A is proposed.

Compound **2** analyzed for $C_{16}H_{23}Cl_2NO_2$ by MS and showed a strong IR absorption at 1653 cm^{-1}, and a low-intensity ^{13}C NMR signal at δ 124.9 for a carbonimide dichloride function. Its ^{1}H NMR spectra contained resonances attributable to an allylic methyl group (δ 1.69, CH_3-12) and three exo-methylene groups (δ 4.83, 5.08, C-11/CH_2-13; 4.70, 4.71, C-7/CH_2-14; 4.99, 5.04, C-3/CH_2-15). The ^{13}C NMR data contained a total of 16 resonances for a methyl, seven methylene, four methine groups, and four quarternary carbons. The ^{13}C NMR resonances for C-6 and C-10 (δ 77.3, C-6; 80.7, C-10) showed both C atoms to have an oxygen atom as a direct neighbor. Taking the IR and MS data into account, those oxygen atoms must be present in the form of hydroxyl groups. The highest mass ion in the MS thus corresponds to $[M - H_2O]^+$. Interpretation of the HMBC data showed the exo-methylene groups to be C-11/CH_2-13, C-7/CH_2-14, and C-3/CH_2-15. These data also showed the methyl group (CH_3-12) to bond to C-11. ^{1}H−^{1}H COSY correlations between H_2-9 and H_2-8 and H-10, and between H_2-5 and H_2-4 and H-6, together with ^{1}H−^{13}C HMBC correlations between C-11 and H-10, C-7 and H_2-8, C-6 and H_2-14, and C-3 and H_2-4, allowed assignment of the C-3 to C-12 part of the molecule. Comparison of the ^{1}H NMR data, the ^{13}C NMR data, and the UV data of **2** with those of compound **1** indicated the N=CCl_2 group to also be connected to a conjugated diene system. This deduction was supported by the long-range CH correlations between C-16 and H-1 and between C-2 and H_2-15. The configuration of the $\Delta^{1,2}$ double bond was trans (J = 13.2 Hz), as in compound **1**. Due to the instability of compound **2**, the configurations at C-6 and C-10 were not resolved. For **2**, the trivial name ulosin B is proposed.

Experimental Section

General Experimental Procedures. HPLC was carried out using a Merck-Hitachi system consisting of a L-6200A pump, a L-4500 A photodiode array detector, and a D-6000 A interface together with a Knauer K-2300 differential refractometer as detector. ^{1}H (1D, 2D COSY) and ^{13}C (1D, DEPT 135, 2D HMQC, 2D HMBC) NMR spectra were recorded on Bruker Avance 500 DMX and Bruker Avance 300 DPX spectrometers in $CDCl_3$ and C_6D_6. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 7.26/77.0 ($CDCl_3$) and 7.16/128.0 (C_6D_6), respectively. UV and IR spectra were obtained employing Perkin-Elmer Lambda 40 and Perkin-Elmer Spectrum BX instruments, respectively. Optical rotations were measured on a Jasco DIP 140 polarimeter. HREIMS were recorded on a Kratos MS 50 spectrometer. All other experimental details were as previously reported.[8]

Animal Material. The sponge sample was collected in July 1998 at Wistari Reef, the Great Barrier Reef, Australia, from a depth of 20 m and stored in ethanol at −20 °C until workup. The sponge was identified by Dr. J. N. A. Hooper, Queensland Museum, South Brisbane, Australia. A voucher specimen has been deposited at the Queensland Museum, voucher no. QM G318527.

Extraction and Isolation. After removal of the EtOH preservative, the sponge (wet wt 0.37 kg) was extracted with MeOH (3 × 0.5 L), followed by CH_2Cl_2 (3 × 0.5 L). The MeOH extract and the EtOH solution were evaporated to dryness,

combined, and partioned between MeOH/H_2O (1:1; 0.2 L) and CH_2Cl_2 (0.2 L). The combined CH_2Cl_2 extracts were evaporated to yield 3.1 g of a brown oil. This material was fractionated by vacuum liquid chromatography (VLC) over Si gel (5−40 μm), using gradient elution from petroleum ether containing increasing proportions of CH_2Cl_2, followed by MeOH, to yield eight fractions. ^{1}H NMR investigations of these fractions indicated VLC fractions 1 (100 mg), 2 (130 mg), 3 (530 mg), and 5 (340 mg) to be of further interest. All these fractions were purified by normal and reversed-phase (RP) HPLC. Fraction 1 (RP-C_{18}; 250 × 8 mm, 5 μm; MeOH/CH_2Cl_2 gradient, 0−10% CH_2Cl_2, 1.5 mL/min) gave 8.0 mg of compound **1** as a colorless oil. HPLC separation of fraction 2 (RP-C_{18}; 250 × 8 mm, 5 μm; MeOH/CH_2Cl_2 gradient, 0−10% CH_2Cl_2; 1.5 mL/min) yielded semipure compound **3**. Further purification by normal-phase HPLC (Si; 250 × 8 mm, 5 μm; 100% petroleum ether; 1.5 mL/min) afforded compound **3** (4.3 mg) as a colorless oil. Fraction 3 was rechromatographed over C_{18} RP HPLC (250 × 8 mm, 5 μm; MeOH/H_2O gradient, 0−20% H_2O, 1.5 mL/min) to yield semipure compound **2**. After purification by isocratic RP HPLC (C_{18}; 125 × 4 mm, 5 μm; MeOH/H_2O (4:1), 1.5 mL/min), 2.3 mg of a colorless oil was obtained. HPLC separation (RP-C_8; 250 × 8 mm, 5 μm; MeOH/H_2O gradient, 0−15% H_2O, 2.0 mL/min) of fraction 5 gave a mixture of compounds **4** and **5**. This mixture was further fractionated by RP HPLC (C_{18}; 125 × 4 mm, 5 μm; MeOH/H_2O gradient, 0−40% H_2O, 1.0 mL/min) to yield compounds **4** (4.0 mg) and **5** (5.3 mg).

Ulosin A, [(1*E*,6*E*)-7,11-dimethyl-3-methylene-1,6,10-dodecatrienyl]carbonimidic dichloride (1): colorless oil (8.0 mg, 0.003%); UV (MeOH) λ_{max} (log ϵ) 284 nm (4.5); IR (ATR) ν_{max} 2917, 1639, 1601, 1580, 899 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m*/*z* 299 (4), 264 (7), 228 (8), 69 (100); HREIMS *m*/*z* 299.1208 (calcd for $C_{16}H_{23}{}^{35}Cl_2N$, 299.1208).

Ulosin B, [(1*E*)-6,10-dihydroxy-11-methyl-3,7-dimethylene-1,11-dodecadienyl]carbonimidic dichloride (2): colorless oil (2.3 mg, 0.001%); $[\alpha]_D{}^{23}$ +20.9° (*c* 0.07 $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 283 nm (4.4); IR (ATR) γ_{max} 3445, 2926, 1653, 1602, 904 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m*/*z* 313 (2), 278 (51), 242 (41), 135 (100), 67 (91); HREIMS *m*/*z* 313.1014 (calcd for $C_{16}H_{21}{}^{35}Cl_2NO$ [M − H_2O], 313.1000).

Acknowledgment. We thank Dr. J. N. A. Hooper, Queensland Museum Brisbane, Queensland, Australia, for sponge taxonomy, and Dr. G. Eckhardt, Institute for Organic Chemistry, University of Bonn, for making all MS measurements.

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NP0100587