A New Indole Alkaloid from the Marine Tunicate *Dendrodoa grossularia*

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A new indole alkaloid (**1**) was isolated from the tunicate *Dendrodoa grossularia*. The structure was determined by spectroscopic means, particularly extensive 1D and 2D NMR studies and chemical transformation. X-Ray crystallographic analysis of **2**, obtained from **1** by treatment with Ac_2O-pyridine, unambiguously provided the structure and the relative stereochemistry at C-5′ of compound **2**, and hence of **1**.

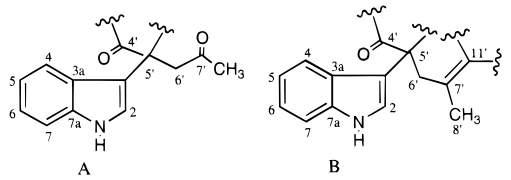
Previous studies on the constituents of *Dendrodoa grossularia* Beneden, 1846 (family Styelidies), have resulted in the isolation of four indole alkaloids: dendrodoine,[1] 3-indolylimidazol-4-one (**3**),[2] grossularine-1, and grossularine-2.[3,4] A continuing investigation of this tunicate to isolate bioactive compounds led to the isolation of the new indole alkaloid **1**. We report the structure elucidation of **1** and the formation of the tetrahydropyridinone (**2**), on treatment of **1** with Ac_2O-pyridine.

Specimens of *D. grossularia* were extracted successively with CH_2Cl_2 and a mixture of CH_2Cl_2−MeOH 1:1. After concentration, the combined extract was subjected to Si gel column chromatography (CH_2Cl_2−MeOH gradient system). Fractions eluted with 10−30% MeOH were chromatographed on another Si gel column (CH_2Cl_2-MeOH 95:5), followed by preparative TLC purification to yield **1**, (0.002%). On TLC, **1** exhibited a positive color reaction when sprayed with Dragendorff's reagent.

Compound **1** was obtained as a white powder, mp 265−266 °C, $[\alpha]_D$ −15 (*c* 0.14, MeOH). The IR spectrum (KBr) displayed an absorption at 3200 cm^{-1}, supporting the presence of NH groups, and bands at 1710 and 1640 cm^{-1} indicative of several carbonyl functions. The FABMS showed a pseudomolecular ion at *m/z* 299, and the molecular formula was shown to be $C_{16}H_{19}O_2N_4$ by HR FABMS analysis. The ^{1}H NMR spectrum in CD_3OD showed proton signals due to two *N*-methyl groups at δ 3.22 (3H, s) and 3.04 (3H, s), an acetyl group at δ 2.16 (3H, s), and a methylene group at δ 3.45 (2H, dd, J = 16.8 Hz); the splitting observed in the aromatic proton region indicated a monosubstituted indole: δ 6.9 (1H, t, J = 8 Hz), 7.1(1H, t, J = 8 Hz), 7.26 (1H, s), 7.33 (1H, d, J = 8 Hz), and 7.46 (1H, d, J = 8 Hz). The presence of an indole moiety was supported by the UV absorption maxima observed at 222, 282, and 290 nm. In addition, the ^{1}H NMR spectrum in DMSO-d_6 exhibited two D_2O-exchangeable singlets at δ 11.01 and 8.2, which were assigned to two NH functions.

The ^{13}C NMR spectrum (Table 1) of **1,** assigned with the assistance of a J_{mod} experiment, indicated the presence of 16 carbon signals corresponding to five aromatic methines, one methylene, three methyl groups, one quaternary sp carbon, two carbonyl carbons, three quaternary aromatic carbons, and an unpaired sp^2 carbon. The assignment of all the protonated carbons was established by ^{1}H-detected heteronuclear one-bond ^{1}H−^{13}C correlation experiments (HMQC).[5] The presence of the indole skeleton was further corroborated via 2D NMR spectroscopy, including HMBC [6] measurements (Table 1).

The ^{1}H−^{13}C long-range spectrum of **1** enabled the assignment of partial structure A to be etablished. Of particular help were the correlations of the methylene protons CH_2-6′ at δ_H 3.45, (δ_C 48 ppm), with carbons resonating at δ 67.0, 207.3, 91.7, and 114.6 ppm assigned to carbons 5′, 7′, 4′ and 3, respectively. This interpretation was also supported by a correlation between the methyl group CH_3-8′ and the carbonyl carbon C-7′; however, HMBC experiments of **1** recorded in DMSO-d_6 failed to furnish correlations between the two D_2O-exchangeable protons and neighboring carbon atoms. The structure of **1** could be deduced from HRMS and comparison with the other indole derivatives isolated from this tunicate.



Because it was not possible to establish the groups linked to the partial structure A by long-range correlations, compound **1** was acetylated in the hope of obtaining additional information for completing the structure. Acetylation of **1** with Ac_2O−pyridine formed the synthetic diacetate derivate **2**. ^{1}H NMR spectrum run in $CDCl_3$ showed similar signals as depicted in the spectrum of compound **1**, except for the appearance of two singlets at δ 2.41 and 2.6 ppm for acetyl groups, and the conspicuous absence of the two NH protons. The ^{13}C NMR spectrum of **2** exhibited expected acetate carbonyl and methyl signals at δ 168.1 and 198.1 and δ 24.1 and 31.29 ppm, respectively, and two unexpected quaternary sp^2 carbons at δ 132.65 and 153.46 ppm.

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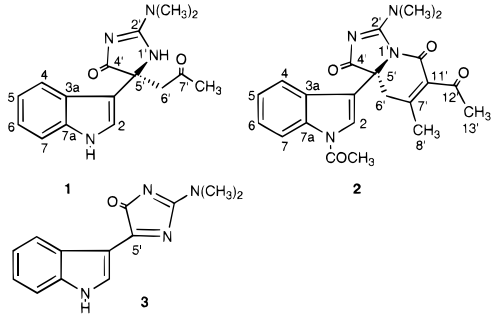
Table 1. ^{13}C and ^{1}H NMR Data (δ ppm, DMSO-d_6) and H−C Long-Range Correlations of **1** and **2**

	compound **1**			compound **2**		
position	δ ^{1}H (m, *J* in Hz)	^{13}C	HMBC ^{13}C	δ ^{1}H (m, *J* in Hz)	^{13}C	HMBC ^{13}C
1[b]	11.01 (s, 1H)					
2	7.26 (s, 1H)	124.1	3, 7a, 3a	7.43 (s, 1H)	122.9	3, 3a, 7a, 5′
3		114.6			119.8	
3a		125.8			126.9	
4	7.46 (d, 1H, 8)	120.2	6, 3a, 7a, 3	7.86 (d, 1H, 8)	120.8	7a, 6
5	6.9 (t, 1H, 8)	120.3	7, 3a	7.28 (t, 1H, 8)	124.2	7, 3a
6	7.1 (t, 1H, 8)	122.7	4, 7a	7.35 (t, 1H, 8)	125.8	4, 7a
7	7.33 (d, 1H, 8)	112.6	5, 3a	8.4 (d, 1H, 8)	116.7	
7a		138.6			136.7	
8					168.1	
9				2.6 (s, 3H)	24.1	8
1′[b]	8.2 (s, 1H)					
2′		170.8			168.6	
4′		191.7			183.8	
5′		67			67.5	
6′	3.45 (dd, 2H, 16, 8)	48	5′, 7′, 4′, 3	3.16 (dd, 2H, 16, 8)	39.1	3, 5′, 7′, 11′
7′		207.3			153.5	
8′	2.16 (s, 3H)	31	7′	1.99 (s, 3H)	21.8	6′, 11′, 7′
9′	3.22 (s, 3H)	38.8	2′	3.31 (s, 3H)	41.1[a]	
10′	3.04 (s, 3H)	37	2′	3.31 (s, 3H)	43.2[a]	2′, 9′, 10′
11′					132.6	
12′					198.1	
13′				2.41 (s, 3H)	31.9	12′
14′					159.9	

[a] Assignments may be reversed. [b] The signals disappeared after D_2O exchange. The $^{1}H-^{13}C$ correlations were based on a HMQC experiment.

Detailed examination of the HMBC spectrum of **2** revealed long-range couplings between the C-6′ methylene protons at δ 3.16 and the methyl carbon at δ 21.8. Additional correlations between the C-8′ methyl protons and the two olefinic carbons δ 153.5 and 132.6 (7′ and 11′) were also observed. The HMBC spectrum of **2**, however, did not give enough correlations to solve the structure and only allowed expansion of the partial structure to B. Table 1 summarizes the ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC spectral assignments for **2**.



Fortunately, suitable crystals of **2** for a single-crystal X-ray diffraction study were obtained by slow crystallization from Me_2CO at 0 °C for one week. A crystal of **2** has the space group $P\bar{1}$, and is thus racemic. Likely, the optical rotation observed for compounds **1** and **2** is due to the presence of an excess of one enantiomer, and, in this case, it happens that the racemate crystallized.[7,8] The molecule is presented in relative configuration at carbon atom C-5′ in Figure 1. The atomic coordinates for the nonhydrogen atoms are listed in Table 2.

The structure of **1** was deduced from that of **2**. Acetylation of the two NH protons of compound **1** (1 and 1′) was followed by an intramolecular aldol condensation between the C-7′ carbonyl and 11′-CH3, followed by a C-alkylation at the 11′ position, thus affording the

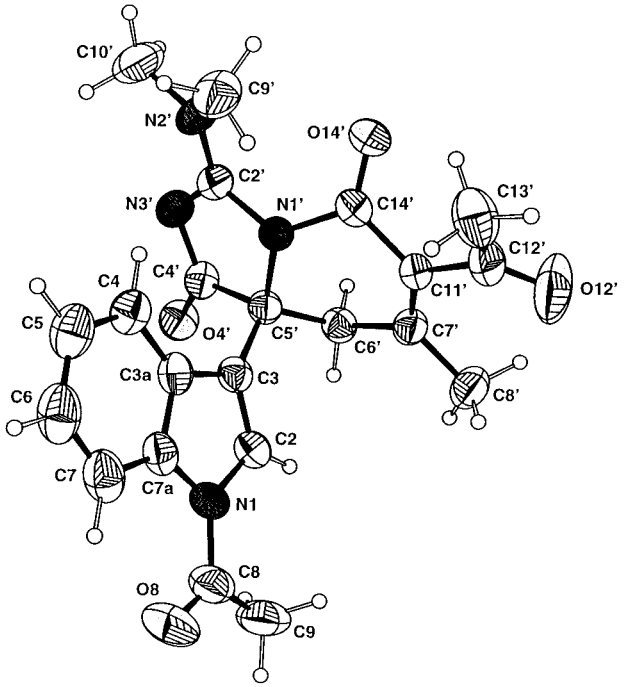


Figure 1. The molecular structure of **2** with atomic labeling. Displacement ellipsoides are drawn at the 50% probability level.

tetrahydropyridinone **2**. A mechanism is proposed in Figure 2 to explain this unusual rearrangement.

Indole derivative **1** can be considered to derive from the previously isolated 3-indolyl-imidazol-4-one (**3**),[2] by addition of a propan-2-one at the 5′ position.

Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AC 300 spectrometer with standard pulse sequences operating at 300.13 and 75.47 MHz, respectively. The chemical

Table 2. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for **2**

	x	*y*	*z*	U(eq)[a]
N(1)	5486(3)	1612(3)	10842(3)	46(1)
C(2)	4966(4)	2588(4)	10109(3)	43(1)
C(3)	5554(4)	2720(3)	9106(3)	38(1)
C(3a)	6524(4)	1802(3)	9190(4)	42(1)
C(4)	7466(4)	1523(4)	8461(4)	51(1)
C(5)	8266(5)	573(4)	8810(5)	62(1)
C(6)	8146(5)	−103(4)	9874(5)	69(1)
C(7)	7240(5)	152(4)	10615(4)	62(1)
C(7a)	6452(4)	1114(4)	10268(4)	45(1)
C(8)	5127(5)	1225(5)	11962(4)	60(1)
O(8)	5640(4)	371(3)	12512(3)	86(1)
C(9)	4122(6)	1935(5)	12419(4)	72(2)
N(1′)	5382(3)	3321(3)	6975(2)	38(1)
C(2′)	6655(4)	3942(4)	6615(3)	42(1)
N(3′)	7327(3)	4814(3)	7313(3)	44(1)
C(4′)	6592(4)	4789(3)	8235(3)	41(1)
C(5′)	5322(4)	3745(3)	8154(3)	36(1)
C(6′)	3947(4)	4278(3)	8153(3)	40(1)
C(7′)	2695(4)	3361(3)	7686(3)	41(1)
C(8′)	1398(4)	3279(4)	8288(4)	57(1)
C(9′)	6823(5)	2395(4)	5206(4)	68(1)
C(10′)	8433(5)	4394(5)	5341(4)	76(2)
C(11′)	2830(4)	2731(3)	6771(3)	40(1)
C(12′)	1688(5)	1805(4)	6187(4)	57(1)
C(13′)	2098(5)	820(4)	5521(4)	71(1)
C(14′)	4134(4)	3037(4)	6206(4)	44(1)
N(2′)	7176(3)	3618(3)	5710(3)	56(1)
O(4′)	6895(3)	5452(2)	9064(2)	51(1)
O(12′)	465(3)	1849(4)	6253(3)	102(1)
O(14′)	4127(3)	3091(3)	5160(2)	66(1)
C(23)	9082(5)	3071(4)	2121(4)	57(1)
C(24)	9417(5)	3463(5)	936(4)	72(1)
C(25)	10020(6)	2283(5)	2870(5)	95(2)
O(23)	8086(4)	3406(4)	2487(3)	98(1)

[a] U(eq) is defined as one-third of the trace of the orthogonalized $\mathbf{U}_{ij}$ tensor.

shift values are reported as ppm units, and the coupling constants are in Hz. The programs used for J_{mod}, HMQC, and HMBC ($J = 7$ Hz) experiments were those furnished in the Bruker manual. HRFABMS (positive mode) of compound **1** was measured on a ZAB−SEQ spectrometer in a thioglycerine matrix at the Service central d'analyses du CNRS (Lyon); and EIMS of compound **2** on a Kratos MS 50 at 70 eV. UV spectra were obtained in MeOH, using a Kontron-type Uvikon 930 spectrophotometer, and IR spectra were obtained as KBr pellets on a Nicolet (Impact 400D) FT IR spectrophotometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter with a sodium lamp operating at $\lambda = 589$ nm in a 10-cm microcell. Si gel column chromatographies were carried out using Kieselgel 60 (230−400 mesh, E. Merck). Fractionations were monitored by TLC using aluminum-backed TLC sheets (Si gel 60 F_{254}, 0.25-mm thick) with visualization under UV (254 and 365 nm) and Dragendorff spray reagent. Preparative TLC was carried out on Merck Si gel F_{254} plates (0.5-mm thick).

Collection, Extraction, and Isolation. Specimens of the tunicate *D. grossularia* were collected in Brittany during low tides (Ile Callot, France) and stored in MeOH until used. The voucher specimens are stored in the Museum National d'Histoire Naturelle collection under the reference name S1 Den 17. The animal material (500 g, wet wt) was extracted with CH_2Cl_2 (1 L × 2) and then with MeOH−CH_2Cl_2 (1:1) (1L × 3) at room temperature. After filtration, the combined extracts were concentrated under vacuum at 40 °C to obtain 8 g of crude extract. The concentrated extract was partitioned between H_2O and CH_2Cl_2. The organic extract was subjected to Si gel column chromatography with CH_2Cl_2 containing increasing concentrations of MeOH. The fractions eluted with CH_2Cl_2−MeOH (9/1, to 7/3) were further purified by column chromatography on Si gel (eluent CH_2Cl_2−MeOH, 95/5) and finally by preparative TLC (CH_2Cl_2−MeOH, 9/1) to afford **1**, 10 mg (0.002% yield); FABMS $[M + H]^+$ *m/z* 299; HRFABMS *m/z* 299.1483, calcd for $C_{16}H_{19}O_2N_4$, 299.1507.

Acetylation of 1. Compound **1** (2 mg) dissolved in a mixture of Ac_2O (0.4 mL) and pyridine (0.4 mL) was left overnight at room temperature. After addition of MeOH, the solvents were removed under reduced pressure and the residue purified by column chromatography (CH_2Cl_2) to give the diacetate **2** (90%): $[\alpha]_D$ −12 (*c* 0.09, $CHCl_3$); UV λ_{max} 220, 278 and 290 nm; IR ν_{max} (KBr) 1750, 1690, 1675, 1670, and 1650 cm^{-1}; EIMS *m/z* 406, 391, 362, 363, 248; NMR data, see Table 1.

X-ray Crystallographic Analysis of Compound 2. Crystal data: $C_{22}H_{22}N_4O_4$, C_3H_6O, $M_w = 464.52$, small crystal of 0.13 × 0.17 × 0.35 mm, triclinic, space group $P\bar{1}$, $Z = 2$, $a = 9.652$ (6), $b = 10.809$ (9), $c = 11.583$ (11) Å, $\alpha = 85.37$ (3), $\beta = 98.00$ (3), $\gamma = 98.24$ (3)°, $V = 1182$ Å^3, $d_{calc} = 1.31$ g cm^{-3}, F(000) = 492, λ (Mo Kα) = 0.7107 Å, $\mu = 0.08$ mm^{-1}.

Intensity data were measured on a Philips PW1100 diffractometer using graphite monochromated Mo Kα radiation and the (θ-2θ) scan technique up to $\theta = 25°$. Of the 6350 collected reflections ($-11 \le h \le 11$, $-12 \le k \le 12$, $l \le 13$), 4154 were unique ($R_{int} = 0.051$). Cell parameters were refined from 25 well-centered reflections with $5.8 \le \theta \le 9.7°$. The structure was solved by direct methods using SHELXS-86[9] and refined by full-matrix least-squares based upon Fo^2 using SHELXL-93.[10] All the hydrogen atoms were located in difference Fourier maps, those of the two methyl groups C8′ and C10′ were found disordered, occupying two staggered positions of equal weight. All of them were fitted at theoretical positions [d(C−H) = 1.00 Å] and assigned an isotropic thermal factor equivalent to that of the bonded carbon atom, plus 10%. In addition, a molecule of Me_2CO for each molecule of compound **2**, was found as residual solvent. Thus, refinement of 307 parameters converged to $R_1(Fo) = 0.0645$ [for 1931 Fo with $Fo > 4\sigma(Fo)$] and $wR_2(Fo^2) = 0.1846$ (for all the 4154 data with goodness-of-fit S = 1.066). The weights of the structure factors were assumed to be $w = 1/[\sigma^2(Fo^2) +$

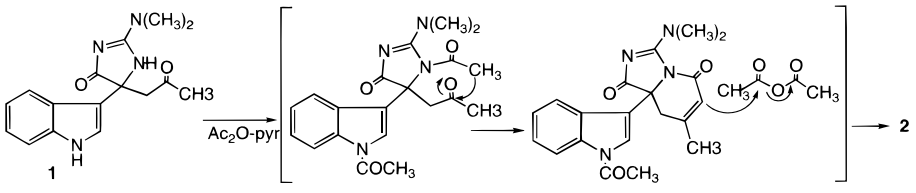


Figure 2. Proposed mechanism for the formation of compound **2**.

$(0.0692\ P)^2]$ where $P = (Fo^2 + 2\ Fc^2)/3$. The residual electron density was found between -0.27 and $0.21\ eÅ^{-3}$ in the final difference map. Only normal van der Waals contacts are observed in the crystal packing.

Atomic coordinates, thermal parameters, bond lengths, bond and torsion angles have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

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