Pergamon

Tetrahedron, Vol. 53, No. 6, pp. 2055-2060, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(96)01168-4

Alboinon, an Oxadiazinone Alkaloid from the Ascidian *Dendrodoa grossularia*

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Abstract: The ascidian *Dendrodoa grossularia*, collected in the Baltic Sea, contains the new 1,3,5-oxadiazin-2-one alkaloid alboinon (**1**).

INTRODUCTION

The ascidian *Dendrodoa grossularia* lives in the North Sea and Baltic Sea at a depth of between 8 and 20 m. The red globular organisms measure up to 1.5 cm and stay on stones or seaweed, either alone or in groups. Up to now, the α-carboline alkaloids grossularin-1 and -2 and the thiadiazole dendrodoin have been isolated from *D. grossularia*[1]. Studies of the biosynthesis of these alkaloids have led us to an interesting substance with an 1,3,5-oxadiazin-2-one framework, for which the name alboinon (**1**) is proposed. The oxadiazinone system was mentioned only a few times in the literature in mechanistic and preparative investigations[2] and was found in nature for the first time.

RESULTS AND DISCUSSION

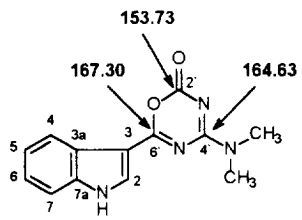
Extraction of the ascidian (260 g wet weight) with chloroform/methanol followed by solvent partition (toluene vs. water) and repeated gel permeation chromatography of the toluene layer (Sephadex LH-20, eluent: chloroform/methanol 1:1 v/v) yielded alboinon (**1**) as a faint yellow substance (0.4 mg). High resolution EI-MS gave the molecular formula $C_{13}H_{12}N_4O_2$ (M^+, *m/z* = 256). The molecular ion was confirmed in the CI-MS (reagent gas: isobutane). Fragments *m/z* 144 (C_9H_6NO), 116 (C_8H_6N) and 89 (C_7H_5) point to a carboxyindole unit, *m/z* 142 ($C_9H_6N_2$) furnished the corresponding cyano fragment. Splitting off of CO_2 from the molecular ion resulted in fragment *m/z* 212 ($C_{12}H_{12}N_4$).

The ^{1}H NMR spectrum shows a singlet at δ 8.28, signals of an 1,2-disubstituted aromatic system [8.27, 7.52, 7.32 (2H)] and a dimethylamino group at δ 3.29 and 3.49. Comparative values in the ^{13}C NMR spectrum agree with an indole system substituted in position 3. In the coupled ^{13}C NMR spectrum a signal at δ 153.73 appears as a sharp singlet. Signals at δ 164.63 und 167.30 show in the HMBC spectrum long-range couplings to the *N,N*-dimethylamino group and to 2-H of the indole system.

Table 1: ^{13}C NMR (150.9 MHz) and ^{1}H NMR (600.13 MHz) data for alboinon (**1**) in $CDCl_3/CD_3OD$ 1:1 v/v.

Position	^{13}C δ (ppm)	J $^{1}H^{13}C$ (Hz)	^{1}H δ (ppm)	J $^{1}H^{1}H$ (Hz)
2	134.89	D, 187.0	8.28	s, 1H
3	106.61	d, 4.7		
3a	125.96	m		
4	123.22	Dd, 159.5, 8.3	8.27	d, 1H, 7.9
5	124.24	Dd, 160.0, 8.3	7.32	dd, 1H, 8.0, 7.9
6	121.99	Dd, 163.0, 8.0	7.325	dd, 1H, 8.0, 7.9
7	113.17	Dd, 160.7, 8.3	7.52	d, 1H, 8.0
7a	138.09	dd, 8.0, 3.5		
2`	153.73	s		
4`	164.63	m		
6`	167.30	m		
$-N(CH_3)_2$	37.22, 37.98	Qd, 139.7, 3.5	3.49, 3.29	s, 3H; s, 3H

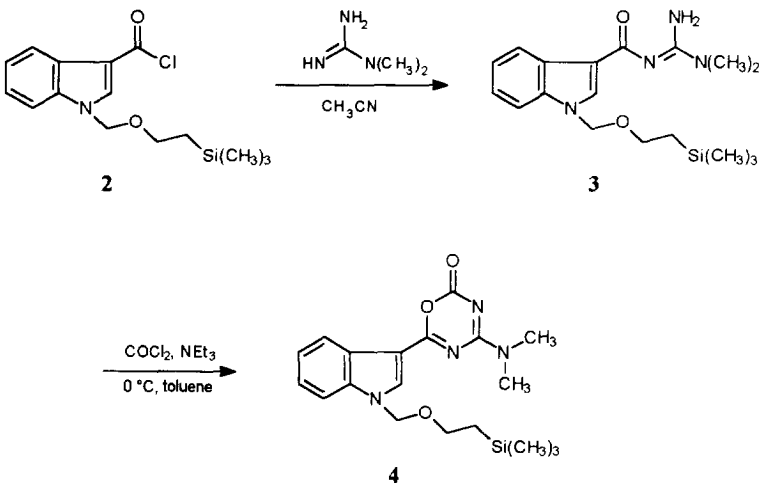
A band in the IR spectrum at 1750 cm^{-1} and the splitting off of CO_2 from the molecular ion *m/z* 256 points to a δ-lactone. Summarizing these arguments, it follows that alboinon has the structure **1**.



alboinon (**1**)

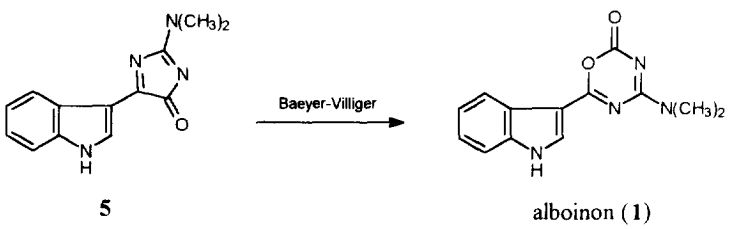
The proposed structure for **1** could be confirmed by a synthesis of the SEM protected compound **4** (see scheme 1). *N*-2-(Trimethylsilyl)ethoxymethylindole-3-carboxylic acid methylester was saponified with sodium hydroxide and transformed into the acid chloride **2**[3,4]. Treatment with *N,N*-dimethylguanidine[5] and subsequently

with phosgene[6] led to the protected oxadiazinone **4**. The ^{1}H and ^{13}C NMR values of **4** are in good agreement with the data of alboinon (**1**). When attempts are made to remove the protecting group, the oxadiazinone system is attacked, even under mild conditions.



Scheme 1

A better and easier access to alboinon (**1**) and to the oxadiazinone system is possible by Baeyer-Villiger rearrangement of the imidazole **5**[7] by treatment with *m*-chloroperbenzoic acid (see scheme 2)[8]. The imidazole **5** is also contained in the ascidian and could possibly be biosynthesized from 2-(3-indolyl)glycine[9] or indolylglyoxylic acid[10] which we were able to isolate for the first time from a natural source.



Scheme 2

EXPERIMENTAL

^{1}H NMR and **^{13}C NMR** spectra were recorded on a Bruker AMX2-600 spectrometer operating at 600.13 MHz and 150.9 MHz, respectively. Chemical shifts are given in ppm relative to residual solvent signal of methanol-d_4 at 3.35 ppm and 49.0 ppm respectively; solvent: methanol-d_4/chloroform-d_1, 1:1 v/v. **EI MS** were obtained (DI, 210°C, 35 eV) on a Finnigan MAT 95Q instrument. Analytical **HPLC** was performed at 20 °C on a Waters System equipped with two Waters 510 pumps, Waters 717 plus autosampler, photodiode array detector 996, Millennium 2010 software and a 4 x 250 mm column with Nucleosil 100, 5 C_{18} (Macherey-Nagel); **gradient 1**: linear gradient, 0 min: water/acetonitrile = 9:1, 0.1 % TFA, 45 min: 100 % acetonitrile, 0.1 % TFA, flowrate 1.0 ml/min, range of detection 200-797 nm; **gradient 2**: linear gradient, 0 min: 100 % water, 0.1 % TFA, 45 min: 100 % acetonitrile, 0.1 % TFA, flowrate 1.0 ml/min, range of detection 200-797 nm. **TLC** was carried out on Merck silica gel 60 F_{254} aluminium sheets in the system C_6H_6/HCO_2Et/HCO_2H (10:5:3, v/v). Column chromatography (**CC**) was done using Sephadex LH-20 (Pharmacia); eluent: methanol and methanol/chloroform 1:1 (v/v).

***Alboinon* (1).** 10 mg (0.041 mmol) of 3-indolyl-imidazol-4-one (**5**)[7] were dissolved in 10 ml of chloroform and 9 mg (0.050 mmol) of *m*-chloroperbenzoic acid[8] were added. The mixture was stirred at room temperature in the dark for one hour. The organic layer was washed three times with 20 ml of $NaHCO_3$, three times with 20 ml of water, dried over $MgSO_4$, filtered and evaporated. The product was purified by chromatography on Sephadex LH-20 with methanol/chloroform (1:1 v/v); yield: 8.4 mg (80 %). The synthetic product is in every respect identical with the natural product. TLC: R_f = 0.26; HPLC: t_R = 21.45 min (gradient 1); UV (methanol): λ_{max} = 209.8, 252.0, 269.8, 334.2 nm; IR (KBr): ν_{max} 3177 (w), 2929 (w), 1750 (m, sh), 1735 (m), 1600 (s), 1580 (s, sh) 1535 (w), 1435 (s), 1408 (s), 1387 (s), 1298 (w), 1243 (m), 1218 (m), 1204 (m), 1162 (w), 735 (s), 771 (m), 747 (s) cm^{-1}; ^{1}H NMR and ^{13}C NMR (see table 1); MS: *m/z* (%) = 256,0951 (41, M^+, calc. for $C_{13}H_{12}N_4O_2$: 256.0960), 239 (10, $C_{13}H_{11}N_4O$), 228 (2, $C_{12}H_{12}N_4O$), 212 (1, $C_{12}H_{12}N_4$), 186 (5), 157 (4, $C_{10}H_7NO$), 144 (100, C_9H_6NO), 142 (5, $C_9H_6N_2$), 140 (2, $C_5H_6N_3O_2$), 116 (20, C_8H_6N), 113 (10, $C_4H_7N_3O$), 89 (9, C_7H_5).

***N*-2-(Trimethylsilyl)ethoxymethyl-indole-3-carboxylic acid**[3,4]. ^{1}H NMR: δ = 8.16 (d, 1H, J = 8.0 Hz), 7.97 (s, 1H), 7.54 (d, 1H, 8.0), 7.27 (dd, 8.0), 7.28 (dd, 8.0), 5.53 (s, 2H), 3.52 (dd, 2H, 8.1), 0.88 (dd, 2H, 8.1), – 0.06 (s, 9H);^{13}C NMR: δ = 168.10, 137.40, 135.64 (CH), 127.79, 123.69 (CH), 122.83 (CH), 122.07 (CH), 111.29 (CH), 108.65, 76.68 (CH_2), 66.78 (CH_2), 18.11 (CH_2), –1.37 (CH_3); MS: *m/z* (%) = 291 (20, M^+, calc. for $C_{15}H_{21}NO_3Si$: 291.1290), 218 (42, $C_{12}H_{12}NO_3$), 174 (48), 161 (27, $C_9H_7NO_2$), 144 (100, C_9H_6NO), 116 (17, C_8H_6N), 73 (38, C_3H_9Si). – 0.06

***N,N*-Dimethyl-*N'*[*N*-(2-trimethylsilyl)ethoxymethyl]-3-indolylguanidine (3)** [5]. ^{1}H NMR: δ = 8.39 (d, 1H, J = 7.9 Hz), 7.94 (s, 1H), 7.52 (d, 1H, 8.0), 7.24 (dd, 7.9, 8.0), 7.23 (dd, 7.9, 8.0); 5.55 (s, 2H), 3.53 (dd, 2H, 8.1), 3.19 (s, 6H), 0.89 (dd, 2H, 8.1), −0.055 (s, 9H); ^{13}C NMR: δ = 176.54, 161.93, 137.73, 134.22 (CH), 128.06, 123.04 (CH), 122.78 (CH), 122.10 (CH), 117.83, 110.92 (CH), 76.58 (CH_2), 66.61 (CH_2), 38.16 (2 CH_3), 18.21 (CH_2), −1.34 (CH_3); MS: *m/z* (%) = 360.1968 (100, M^+, calc. for $C_{18}H_{28}N_4O_2Si$: 360.1981), 343 (32), 244 (46, $C_{13}H_{16}N_4O$), 243 (63, $C_{13}H_{15}N_4O$), 216 (57), 198 (30), 144 (18, C_9H_6NO), 116 (19, C_8H_6N), 73 (59, C_3H_9Si).

***N*-(2-Trimethylsilylethoxymethyl)alboinon (4)** [6]. ^{1}H NMR: δ = 8.26 (s, 1H), 8.23 (d, 1H, J = 7.9 Hz), 7.61 (d, 1H, 8.1), 7.36 (dd, 7.9, 8.1), 7.37 (dd, 7.9, 8.1), 5.57 (s, 2H), 3.55 (dd, 2H, 8.0), 3.42 (s, 3H), 3.25 (s, 3H), 0.90 (dd, 2H, 8.0), −0.057 (s, 9H); ^{13}C NMR: δ = 166.86, 164.33, 153.24, 137.94, 137.18 (CH), 126.79, 124.74 (CH), 123.95 (CH), 122.40 (CH), 112.13 (CH), 106.85, 72.22 (CH_2), 67.19 (CH_2), 38.24 (NCH_3), 37.24 (NCH_3), 18.20 (CH_2), −1.32 ($SiCH_3$); MS: *m/z* (%) = 386.1742 (24, M^+, calc. for $C_{19}H_{26}N_4O_3Si$: 386.1774), 270 (18, $C_{14}H_{14}N_4O_2$), 269 (20, $C_{14}H_{13}N_4O_2$), 216 (19), 144 (10, C_9H_6NO), 142 (2, $C_9H_6N_2$), 73 (37, C_3H_9Si).

2-(3-Indolyl)glycine[9]**.** Indole and methyl oxalyl chloride were condensed to 2-(3-indolyl)oxoacetic acid. Reaction with hydroxylammonium chloride gave the oxime. Hydrogenation with palladium on charcoal (10 %) and saponification with potassium hydroxide in water/tetrahydrofuran yielded 2-(3-indolyl)glycine. HPLC (gradient 1): t_R = 6.34 min; TLC: R_f = 0.227, eluent: n-butanol/acetic acid/water (5:1:4, v/v); UV (methanol): λ_{max} = 214.6, 269.4 nm; ^{1}H NMR: δ = 7.76 (d, 1H, d, 7.9 Hz), 7.47 (s, 1H), 7.45 (d, 1H, d, 7.9 Hz), 7.22 (dd, 1H, 7.7, 7.9), 7.15 (dd, 1H, 7.7, 7.9), 5.28 (s, 1H); ^{13}C NMR: 170.88, 137.26, 126.17 (CH), 125.57, 123.08 (CH), 120.68 (CH), 118.53 (CH), 106.29, 50.38.

3-Indolylglyoxylic acid[10]**.** Condensation of oxalyl chloride with indole and subsequent hydrolysis of the resulting indolyl glyoxyl chloride gave 3-indolylglyoxylic acid. HPLC (gradient 2): t_R 24.87 min; UV (methanol) λ_{max} = 209.4, 255.4, 266.0, 324.6 nm; ^{1}H NMR: δ = 8.57 (s, 1H), 8.35 (d, 1H, d, 7.9 Hz), 7.49 (d, 1H, d, 7.9 Hz), 7.30 (m, 2H); ^{13}C NMR: δ = 179.85, 165.02, 139.06 (CH), 137.39, 126.86, 124.47 (CH), 123.50 (CH), 122.47 (CH), 113.80, 112.66 (CH); MS: *m/z* (%) = 189.0432 (39, M^+, calc. for $C_{10}H_7O_3N$: 189.0425), 161 (7, $C_9H_7NO_2$), 144 (100, C_9H_6NO), 116 (20, C_8H_6N), 89 (9, C_7H_5).

Acknowledgement. We acknowledge with gratitude the help and support we have received from the Biologische Anstalt Helgoland and Bruker Analytik GmbH, Karlsruhe.

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(*Received in Germany* 10 *October* 1996; *accepted* 9 *December* 1996)