Tetrahedron Vol. 47, No. 41, pp. 8729-8732, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00
Pergamon Press plc

LEPADIN A, A DECAHYDROQUINOLINE ALKALOID FROM THE TUNICATE *CLAVELINA LEPADIFORMIS*

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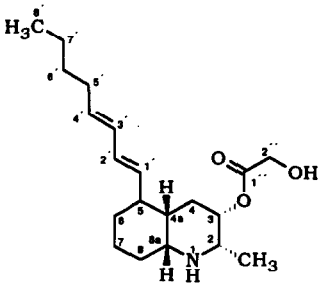
(*Received in Germany* 31 *July* 1991)

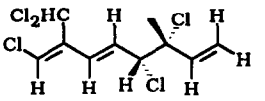
Summary: The tunicate *Clavelina lepadiformis*, which was collected in the North Sea, contains the new decahydroquinoline alkaloid lepadin A (**1**), in addition to the pentachlorooctatriene **2**. The structures have been determined on the basis of spectroscopic evidence and in comparison with published data for compound **2**.

Clavelina lepadiformis is a colonial ascidian living mainly at a depth between 4 and 25 m on light-exposed submarine rocks and rock soil. The zooids measure up to 2 cm, their tunic is absolutely transparent and allows a view on the intestine.

A collection near the island of Helgoland in the North Sea yielded the first decahydroquinoline alkaloid from a tunicate, for which the name lepadin A (**1**) is proposed. Surprisingly the known pentachlorooctatriene[1] **2** occurred as well.



1



2

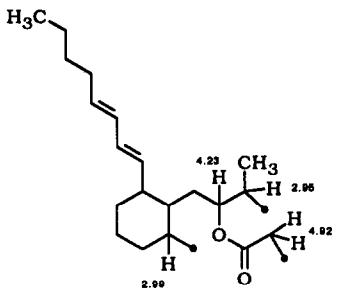
The pharmacological properties of lepadin A and additional decahydroquinoline alkaloids from *Clavelina lepadiformis* will be reported elsewhere.[2]

Results and Discussion

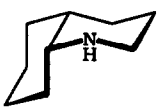
Extraction of the tunicates with chloroform/methanol followed by solvent partitioning (toluene vs. water) and repeated gel permeation chromatography of the toluene layer (Sephadex LH-20, eluant: chloroform/methanol 1:1 v/v) yielded lepadin A (**1**) as a colorless unstable varnish without defined melting point.

High resolution EI-MS gave the molecular formula $C_{20}H_{33}NO_3$ (M^+, m/z = 335) which corresponds to five unsaturation equivalents. Two are needed for a conjugated diene system (λ_{max} 235 nm, four protonated olefinic

carbons in the ^{13}C NMR spectrum), one can readily be attributed to an ester or lactone group (IR: 1735 cm^{-1}, ^{13}C NMR: δ = 172.90). The ^{1}H NMR spectrum (Table 1) looks inhomogenous: 23 protons, including two methyl groups, lead to strong signal overlap in the region between δ 0.9 and 2.2. The aforementioned butadienyl system shows couplings to the highfield part of the spectrum whereas the protons at δ 4.92, 4.23, 2.99 and 2.95 are in the vicinity of heteroatoms. Careful analysis of the COSY, HMQC and HMBC data then leads to partial structure ***A***.



A



B

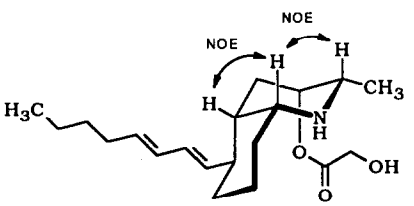
C

The position of the NH and OH groups was clarified by a deuterium-induced isotope shift experiment (DIS).[3] Because the signals of C-2 and C-8a both exhibit DIS values of 6 Hz in the ^{13}C NMR spectrum, the NH group forms part of a piperidine ring. Similarly, C-2´´ shows a DIS value of 8 Hz which indicates the presence of a hydroxyacetyl moiety. This leads to formula **1** for lepadin A.

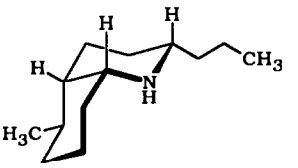
The ^{1}H and ^{13}C NMR shift values for the piperidine part of **1** are similar to those of juliprosopin which has been isolated by Hesse et al.[4] from the leguminous *Prosopis juliflora*.

The relative stereochemistry of alkaloid **1** was determined through NOE experiments and the proton coupling constants. In the ROESY[5] spectrum H-8a displays correlations to H-4a, H-$4_{(ax)}$ and H-2. Therefore, the decahydroquinoline unit has a *cisoid* ring fusion with both the methyl group and the octa-1,3-diene side-chain equatorially, and the hydroxyacetyl group axially situated. An additional NOE between H-2´´ and H-2´ shows that both side-chains are oriented nearly parallel to each other.

The *cis*-decahydroquinoline ring system can exist in two interconvertible conformations ***B*** and ***C***. From the size of the vicinal coupling constants, equatorial and axial positions can be assigned to H-8a (J = 3.4, 3.1 and 2.8 Hz) and H-4a/H-5, respectively. This is in agreement with conformation ***C*** for lepadin A which is confirmed by NOE experiments. The same conformation has been determined for pumiliotoxin C[6], a venom of neotropical frogs of the family *Dendrobatidae*.[7]



lepadin A $[\alpha]_D^{20}$ = -8.5°



2-(S)-pumiliotoxin C $[\alpha]_D^{20}$ = -13°

Table 1: ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) data for lepadin A (1) in $CDCl_3/CD_3OD$ 1:1 v/v. Chemical shifts are given in ppm relative to the solvent peak of CD_3OD at 49.0 and 3.35 ppm respectively.

Position	^{13}C δ (ppm)	(mult.)	^{1}H δ (ppm)	(mult.	int.	J in Hz)			
2	55.34	d	2.95	dq	1H	6.5	2.0		
3	71.73	d	4.92	ddd	1H	3.2	2.8	2.0	
4	31.64	t	1.66	ddd	1H	15.0	6.0	3.2	
			2.17	ddd	1H	15.0	2.8	1.2	
4a	38.63	d	1.37	m	1H				
5	40.33	d	2.50	dddd	1H	12.0	11.6	9.0	3.5
6	34.58	t	1.14	dddd	1H	13.8	13.6	12.0	3.5
			1.69	m	1H				
7	20.92	t	1.58	m	2H				
8	32.50	t	1.67	m	1H				
			1.81	ddm	1H	12.5	2.8		
8a	55.73	d	2.99	dddm	1H	3.4	3.1	2.8	
1′	136.65	d	5.30	dd	1H	14.5	9.0		
2′	131.68	d	5.98	dd	1H	14.5	10.5		
3′	130.90	d	5.96	dd	1H	14.2	10.5		
4′	133.38	d	5.57	dtr	1H	14.2	7.2		
5′	32.83	t	2.05	ddm	2H	7.2	7.0		
6′	22.80	t	1.35	m	2H				
7′	32.12	t	1.37	m	2H				
8′	14.17	q	0.90	tr	3H	7.0			
1″	172.90	s							
2″	61.25	t	4.23	AB system	2H	16.5			
2-Me	17.65	q	1.09	d	3H	6.5			

Surprisingly, the tunicate *C. lepadiformis* contained the pentachlorooctatriene **2**. Compounds of this type have so far only been detected in red alga (genus *Plocamium*) and in the seahare (genus *Aplysia*) which feed on *Plocamium*.[1] It is quite possible that it is not the red alga that produces these halogenated compounds but epiphytic monocellulars. Algae of the genus *Prochloron* have been proven as symbionts in tunicates.[8]

Interestingly, Cardellina et. al.[9] recently found the isomeric quinolizidine derivatives clavepectin A and B in *C. picta* which points to a close biogenetic relationship for both types of alkaloids.

Experimental

Specimens of *C. lepadiformis* (60 g, dry wt.) were collected using SCUBA (-6 m). The sample was ground in a turbomixer with methanol/chloroform (2:1) and repeatedly extracted with the same solvent. The organic layer was evaporated to dryness and subjected to solvent partitioning (toluene vs water). Repeated chromatography of the evaporated toluene layer on a Sephadex LH-20 column (eluant: chloroform/methanol. 1:1) yielded pure **2**, which was identified according to the literature.[1] **1** was contaminated with substantial amounts of fatty acids. Further chromatography on Sephadex LH-20 (eluant: methanol) gave pure **1** (4 mg) as an unstable colorless varnish.

Thin layer chromatography was performed on aluminia foils, silica gel 60 F_{254} (Merck. Darmstadt) in chloroform/ethanol/25% ammonia (9:1:0.5, v/v). IR spectra were recorded on a Perkin Elmer 1420 instrument. The UV spectra were measured with a Varian Cary 17 spectrometer. High resolution mass spectra were obtained on an AEI MS-50 instrument. ^1H NMR spectra (500 MHz) and ^{13}C NMR spectra (125 MHz) were recorded in $CDCl_3/CD_3OD$ (1:1) on a Bruker AMX 500 spectrometer using the solvent peaks of CD_3OD at 3.35 or 49.0 ppm respectively as internal reference. Rotating frame Overhauser effect spectra (ROESY) were accumulated in 512 increments with 16 transients for each value of t_1. The mixing time was 200 msec and the relaxation delay was 3 sec.

The deuterium-induced differential isotope shift (DIS) experiment was performed in a 5 mm coaxial dual NMR cell from Spintec. Inner and outer tube contained about the same concentration of **1** (0.6 mg) dissolved in absolutely dry acetone-d_6/benzene-d_6 (9:1), but the material of the outer tube was exchanged with CD_3OD and evaporated to dryness (three times) prior to dissolution in acetone-d_6/benzene-d_6. Although **1** was barely soluble, the combination of both solvents gave reliable results. In $CDCl_3$ as solvent the DIS values of **1** were not sufficiently reproducible.

Lepadin A (1). Colorless varnish, R_F 0.46; $[\alpha]_D$ -8.5° (c 0.002. $CHCl_3$); UV (MeOH): λ_{max} = 235 nm; IR ($CHCl_3$): 3680 (m), 3380 (m. br), 2930 (s), 2850 (m), 1735 (s), 1600 (m), 1450 (w), 1260 (s), 1090 (s), 990 cm^{-1} (s); MS: *m/z* 335.2465 (M^+, 100%, calculated for $C_{20}H_{33}NO_3$ 335.2460), 292 (22), 260 (36), 216 (19), 158 (80).

Acknowledgement. Support of this work by the Biologische Anstalt Helgoland and Fa. Bruker, Analytische Meßtechnik GmbH, Karlsruhe is gratefully acknowledged.

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