

P. 3  
p. 15

## Dichlorolissoclimide, a New Cytotoxic Labdane Derivative from Lissoclinum voeltzkowi Michaelson (Urochordata)

C. Malochet-Grivois<sup>a\*</sup>, P. Cotellet<sup>b</sup>, J.F. Biard<sup>a</sup>, J.P. Hénichart<sup>c</sup>, C. Debitus<sup>d</sup>,  
C. Roussakis<sup>a</sup> and J.F. Verbist<sup>a</sup>.

a-Substances Marines à Activité Biologique, Faculté de Pharmacie, BP 1024, 44035 Nantes, France.

b- URA 351 CNRS Université des Sciences et Techniques de Lille Flandres Artois, 59655 Villeneuve d'Ascq, France.

c- Unité 16 INSERM, Place de Verdun, 59045 Lille Cedex, France.

d- Laboratoire de Pharmacologie, ORSTOM, BP A5, Nouvelle Calédonie.

**Abstract :** Spectral methods were used to determine the structure of a new cytotoxic compound, dichlorolissoclimide 1, isolated from the New Caledonia ascidian Lissoclinum voeltzkowi Michaelson.

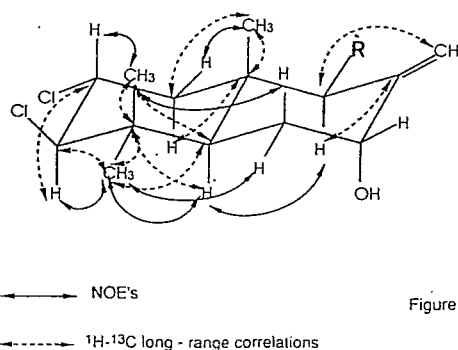
It is known that ascidians usually contain original nitrogenous compounds<sup>1,2</sup>. We isolated a new nitrogenous labdane cytotoxic substance, dichlorolissoclimide 1 (0.004%), from the EtOH extract of Lissoclinum voeltzkowi Michaelson (Urochordata, Didemnidae) gathered in 1988 on Platier du Mont Dore, New Caledonia. Isolation was performed by liquid/liquid purification and HPLC and monitored by cytotoxic bioassay using SESAME mathematical analysis<sup>3</sup>.

The molecular formula of compound 1, C<sub>20</sub>H<sub>29</sub>Cl<sub>2</sub>NO<sub>4</sub>,  $\alpha_D^{20} = -20$ , was determined by analysis of its spectral mass and NMR data (Table 1). The <sup>13</sup>C spectra revealed 20 carbons attached to a total of 26 hydrogen atoms. The highest FAB mass peaks at m/z 418, 420 and 422 (C<sub>20</sub>H<sub>30</sub>Cl<sub>2</sub>NO<sub>4</sub><sup>+</sup>) were thus attributed to the (M H<sup>+</sup>) ion. FAB mass spectra revealed other peaks at m/z 400, 402 and 404 (C<sub>20</sub>H<sub>28</sub>Cl<sub>2</sub>NO<sub>3</sub><sup>+</sup>) attributable to (M H<sup>+</sup>-H<sub>2</sub>O) fragmentation. The highest mass peak at m/z 399.1367 (C<sub>20</sub>H<sub>27</sub>NO<sub>3</sub><sup>35</sup>Cl<sub>2</sub>) in the EIHRMS confirmed the loss of a molecule of H<sub>2</sub>O (The 400/402/404 (100%, 87%, 37%) ratio is consistent with two chlorine atoms).

The deshielded <sup>13</sup>C NMR resonances of  $\delta$  184.8 (C) and 182.6 (C) indicated the presence of two ester and/or amide functional groups (IR :  $\nu$  1710 cm<sup>-1</sup>) and implied the existence of a methylenic double bond at  $\delta$  153.7 (C) and 108.6 (CH<sub>2</sub>). Compound 1 thus had to be tricyclic to account for its unsaturation number.

<sup>1</sup>H COSY, NOE (one and two dimensional experiments) and single-bond HECTOR NMR experiments routinely elaborated the spin systems for H-1 to H-14. The relative configurations of C-2, C-3, C-5, C-7, C-9 and C-10 were determined on the basis of the coupling constants and NOEs (fig.1).

Long range <sup>1</sup>H-<sup>13</sup>C correlations were observed using ordinary <sup>1</sup>H-<sup>13</sup>C shift correlation pulse sequence<sup>4</sup> rather than COLOC pulse sequence<sup>5</sup>, with delay times optimized for long-range couplings (D 1 and D 2 set at 50 ms and 25 ms).



4 JUL. 1992

ORSTOM Fonds Documentaire

N° 35 693, ex 1

Cote B

Carbon	<sup>13</sup> C	<sup>1</sup> H	Carbon	<sup>13</sup> C	<sup>1</sup> H
1	52.1	2.23 (dd, 12.8, 4.3) 1.57 (dd, 12.8, 11.7)	12	70.6	3.99 (bs)
2	65.7	4.45 (dt, 11.7, 4.3)	13	49	2.82 (bs)
3	81.3	3.91 (d, 11.7)	14	33.6	2.55 (dd, 17.6, 4.9) 2.47 (dd, 17.6, 9.0)
4	45.4		15	184.8*	
5	54.9	1.49 (dd, 12.1, 1.8)	16	182.6*	
6	37.9	1.92 (bs) 1.21 (q, 12.1)	17	108.6	5.24 (d, 1.65) 4.82 (d, 1.65)
7	75.4	3.80 (bs)	18	33.4	1.09 (s)
8	153.7		19	21.5	0.86 (s)
9	52.8	1.55 (dd, 10.1, 3.5)	20	18.2	0.66 (s)
10	44.0		NH		11.57 (bs)
11	32.8	1.67 (dd, 13.2, 3.5) 1.37 (dd, 13.2, 10.1)	OH(C7)		5.02 (d, 4.9)
			OH(C12)		4.94 (d, 4.85)

Table 1. <sup>1</sup>H and <sup>13</sup>C NMR Data of Compound 1 (DMSO-D6)

These correlations (fig. 1) enable us to propose structure 1 for dichlorolissoclimide.

Naturally occurring succinimides are very rare, and only one other succinimide compound has been isolated from a marine organism: isosegoline A from another ascidian, *Eudistoma* sp.<sup>6</sup> Compound 1 is the first labdane and the first chlorinated substance isolated from Urochordata.

Its strong cytotoxic activity has been determined on human carcinoma KB cells (IC<sub>50</sub>: 14 ng/ml) and P388 leukemia cells (IC<sub>50</sub>: 1 ng/ml).

Acknowledgement: The authors wish to thank Mrs F. Monnot for identification of the ascidian and Mr J. Le Botterff for cytotoxic tests.

#### REFERENCES

- Ireland C.M., Roll D.M., Molinski T.F., McKee T.C., Zabriskie T.H. and Swersey J.C., "Biomedical Importance of Marine Organisms" D. G. Fautin ed., California Acad. Sci., San Francisco, 1988, 13, 41-57.
- Gouiffès D., Moreau S., Helbecque N., Bernier J.L., Hénichart J.P., Barbin Y., Laurent D., Verbist J.F., *Tetrahedron*, 1988, 44, 451-459.
- Pouchus Y.F., Benslimane A.F. and Verbist J.F., *Tetrahedron Computer Methodology* 1989, 2, 55-64.
- Morris G.A., Hall L.D., *J. Am. Chem. Soc.*, 1981, 103, 4703-4711.
- Kessler H., Griesinger C., Zarbock J., Loosli H.R., *J. Magn. Reson.*, 1984, 57, 331-336.
- Rudi A., Benayahu Y., Goldberg I. and Kashman Y., *Tetrahedron Lett.*, 1988, 29, 3861-3862.

(Received in France 13 June 1991)

