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

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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\textsuperscript{1,2}. 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\textsuperscript{3}. The molecular formula of compound 1, C\textsubscript{20}H\textsubscript{29}Cl\textsubscript{2}NO\textsubscript{4}, \( \delta \text{C} = -20 \), was determined by analysis of its spectral mass and NMR data(Table 1). The \( ^{13}\text{C} \) 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\textsubscript{20}H\textsubscript{29}Cl\textsubscript{2}NO\textsubscript{4}) were thus attributed to the (M+H\textsuperscript{+}) ion. FAB mass spectra revealed other peaks at m/z 400, 402 and 404 (C\textsubscript{20}H\textsubscript{28}Cl\textsubscript{2}NO\textsubscript{3}) attributable to (M+H\textsuperscript{+}-H\textsubscript{2}O) fragmentation. The highest mass peak at m/z 399.1367 (C\textsubscript{20}H\textsubscript{27}N\textsubscript{O}\textsubscript{3}Cl\textsubscript{2}) in the EIHRMS confirmed the loss of a molecule of H\textsubscript{2}O (The 400/402/404 (100%, 87%, 37%) ratio is consistent with two chlorine atoms).

The deshielded \( ^{13}\text{C} \) 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\textsuperscript{-1}) and implied the existence of a methylenic double bond at \( \delta \) 153.7(C) and 108.6 (CH\textsubscript{2}). Compound 1 thus had to be tricyclic to account for its unsaturation number.

\( ^{1}\text{H} \) 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 \( ^{1}\text{H}^{-^{13}\text{C}} \) correlations were observed using ordinary \( ^{1}\text{H}^{-^{13}\text{C}} \) shift correlation pulse sequence\textsuperscript{4} rather than COLOC pulse sequence\textsuperscript{5}, with delay times optimized for long-range couplings (D 1 and D 2 set at 50 ms and 25 ms).

\[ \text{Figure 1} \]
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. 6. 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 (IC50: 14 ng/ml) and P388 leukemia cells (IC50: 1 ng/ml).

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REFERENCES


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