LITUARINES, A NEW CLASS OF MARINE MACROCYCLIC LACTONES ISOLATED FROM THE NEW CALEDONIAN SEA PEN *LITUARIA AUSTRALASIAE*

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The Coelenterates have been investigated for biological activity, especially the order *Alcyonacea* (Soft corals) and *Gorgonacea*. Although many chemical studies on the Pennatulacea order (Sea pens) have been carried out, the *Veretillidae* family has been comparatively little investigated (1). The metabolites that have been isolated from Sea pens are all diterpenes (2).

In the course of our survey on physiologically active substances (3-4) in marine organisms, we found that certain members contain potentially important antineoplastic contituents.

We have now isolated three new 22-membered-ring lactones **1**, **2** and **3**, named **Lituarines A, B** and **C**, from extracts of the New Caledonian Sea pen : *Lituaria australasiae.*

The sea-pen *Lituaria australasiae* (Gray, 1970), a pennatulacean octocoral of the *Veretillidae* family, was collected at night by SCUBA diving near the "Baie de Saint Vincent" in the Western part of the New Celedonian lagoon, on a shallow sandy bottom, and immediately deep-frozen on board. Zoological sampling was made in the meantime.

The freeze-dried animal (fresh weight : 12,5 kg) was first extracted with a mixture of ethanol and water (80/20). The extract was then partitioned into a water soluble portion and a CH_2Cl_2 soluble one. The latter (96 g) was subjected to silica gel chromatography under vacuum (eluent : CH_2Cl_2 with increasing proportions from 0 to 3% of MeOH). The fractions that showed an antifungal activity were joined, then defatted with hexane, and purified by inverse phase column chromatography (silica gel Lichroprep RP8, 25-40mm) using a gradient elution of water (from 40 to 20%) in MeOH. Three fractions were obtained. Further putification by the HPLC way was necessary, using a Microporasil column (30x0,8). The 3 fractions were eluted with CH_2Cl_2 and the respective proportions of MeOH (1,0; 1,3; 2,5%). This last step yielded Lituarines A (20 mg), B (10 mg) and C (24 mg).

The CH_2Cl_2 soluble extract, submitted to biological assays proved to inhibit the growth of the following fungi : *Fusarium oxysporum.*, *Helminthosporium turscicum*, *Penicillium italicum*, *Phytophtora parasitica.*

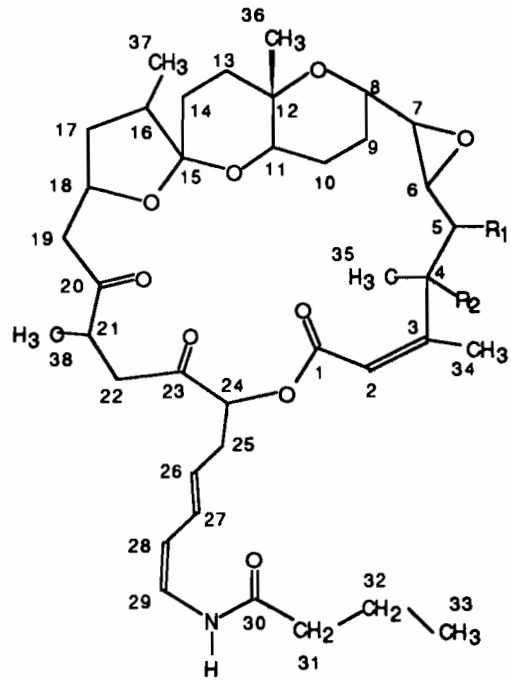
Further investigation with the KB cells test showed that partially purified Lituarines were cytotoxic (DI_{50} from 1 to 5.10-3µg/ml).

Antineoplastic activity (P-388 lymphocytic leukemia assay) was found when testing a mixture of the three substances. Because of this high toxicity, new assays with pure Lituarines will be made soon.

Structure elucidation of lituarines 1, 2 and 3 by NMR spectroscopy

As Lituarines could not be obtained in crystalline state suitable for X-Ray crystal determination, unequivocal assignment of structures **1**, **2** and **3** was performed by 1D and 2D ^{1}H and ^{13}C NMR studies. NMR multipulse sequences used were COSY, TOCSY (56ms), NOESY, ROESY (350ms) and DEPT (135).

Lituarines are the first macrocyclic lactones with two fised tetrahydropyran rings reported from marine sources.

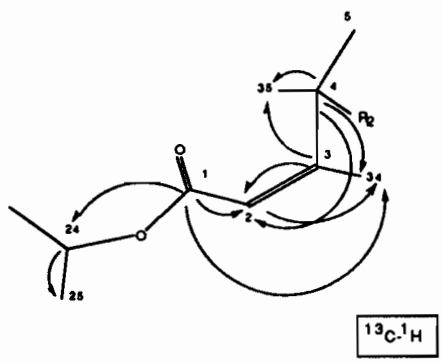


Lituarines

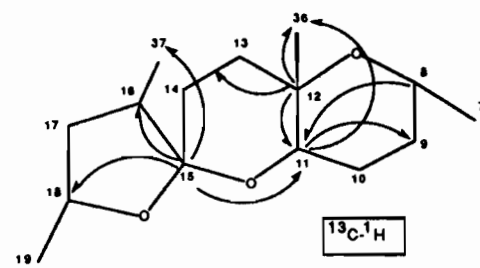
	R_1	R_2
1	H	H
2	$OCOCH_3$	OH
3	OH	OH

The E and Z configurations of the $\Delta^{26(27)}$ and $\Delta^{28(29)}$ double bonds and the trans configuration of the 6,7-oxirane ring were suggested by the coupling constants ($J_{26,27}$= 15Hz, $J_{28,29}$ = 9,7Hz and $J_{6,7}$ = 2,2Hz) and confirmed by the ROESY spectrum (cross-peak H_{26} and H_{29}).

The connection of the ester oxygen on C-1 witch the C-24 and the trans configuration of the tetrahydropyran rings junction were established by the ^{1}H-^{13}C bidimensional heterocorreled experiments HMQC (5) and HMBC (6) (Scheme 1 and 2).

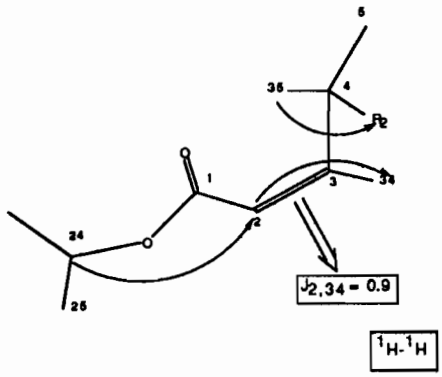


Scheme 1

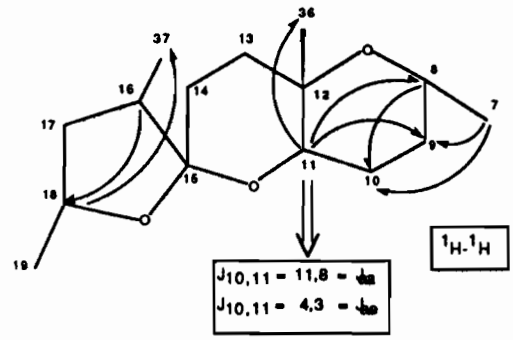


Scheme 2

The linkages were established and confirmed by the long-range ^{1}H-^{1}H (TOCSY) correlation spectrum (Scheme 3 and 4).



Scheme 4



Scheme 5

Supplementary material available :

FABMS, ^{1}H NMR, ^{13}C NMR, DEPT (135), ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC spectra of Lituarines A, B and C, and ^{1}H-^{1}H TOCSY (56ms), ROESY (350ms), NOESY, ^{1}H-^{13}C HMBC spectra of Lituarine C.

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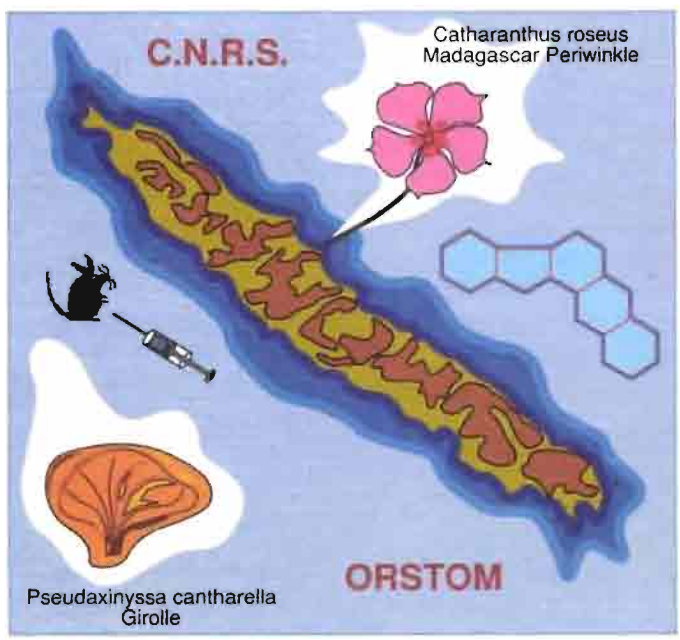

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