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LISSOCLINOLIDE, THE FIRST NON-NITROGENOUS METABOLITE FROM A *LISSOCLINUM* TUNICATE

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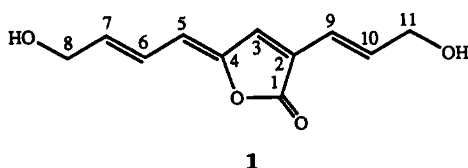
ABSTRACT.—The isolation and characterization of a new metabolite from the tunicate *Lissoclinum patella* is described. The structure of lissoclinolide [**1**] was solved using spectral and chemical methods, and it is shown to be the first non-nitrogenous, non-sulfur-containing compound to be isolated from the genus *Lissoclinum*.

Tunicates of the genus *Lissoclinum* have been an extremely rich source of novel secondary metabolites, many of which are biologically active. To date, three distinct classes of compounds have been reported. *Lissoclinum vareau*, a lavender species collected in the Fiji Islands, is the source of the heteroaromatic pigments varamine A and varamine B (1). *Lissoclinum patella* Gottschatt, 1898 (Didemnidae) has produced both cyclic peptides (2) and the polypropionate-derived macrocycles called the patellazoles (3). A common trademark of all *L. patella* metabolites previously isolated is the presence of a thiazole ring and often an oxazoline ring. In contrast, we now wish to report a new *L. patella* metabolite, lissoclinolide [**1**], which is the first non-nitrogenous, non-sulfur containing compound isolated from the genus *Lissoclinum*.

Lissoclinolide was obtained as a pale yellow glass after purification by solvent partition followed by Si gel flash chromatography [$CHCl_3$-MeOH (95:5)]. The



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molecular formula $C_{11}H_{12}O_4$ was assigned by ^{13}C nmr and an eims high resolution measurement on the $[M]^+$ ion of the corresponding diacetate derivative (292.0949, Δ −0.8 mmu). The ir spectrum suggested the presence of a hydroxyl group (3316 cm^{-1}) and an unsaturated lactone carbonyl positioned within a strained ring (1745 cm^{-1}). The uv spectrum (MeOH) showed absorptions at 340 (ϵ 54,000), 240 (ϵ 8500), and 204 (ϵ 17,000) nm, which are consistent with a highly conjugated system.

Complete analysis of the ^1H- and ^{13}C-nmr data (see Table 1), including both one bond (HETCOR, J = 140 Hz) and multiple bond (COLOC, J = 8 Hz) heteronuclear correlation experiments, allowed the molecular connectivities to be established. The configurations of both side chain double bonds were assigned as trans based on the observed coupling constants $J_{H6,H7}$ = 15.4 Hz, and $J_{H9,H10}$ = 16.0 Hz. The orientation of the double bond exo to the lactone ring ($\Delta^{4,5}$) was assigned using difference nOe spectroscopy. A 10% enhancement was observed between H-3 and H-5, indicating that the double bond is held in the *Z* configuration. In addition, nOe enhancements of H-9 (7%) and H-10 (3%), upon irradiation of H-3, indicate that the side chain containing these protons exists in both the *S* trans and *S* cis conformations. Alternatively, the irradiation of H-5 induces an nOe enhancement of H-7 (9%) but not H-6, indicating that this side chain lies preferentially in the *S* trans conformation.

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TABLE 1. Nmr Assignments for Lissoclinolide [1] δ CD_3OD (referenced to CHD_2OD).

Atom	^{1}H (400 MHz)	^{13}C (100 MHz)	LR ^{1}H to ^{13}C corr.
1		169.67	
2		128.59	
3	7.36 (s)	136.28	C-1, C-4, C-9
4		148.98	
5	6.01 (d, 11.4)	114.57	C-4, C-7
6	6.76 (ddt, 15.4, 11.4, 1.8)	123.71	
7	6.17 (dt, 15.4, 5.2)	140.37	C-5
8	4.20 (dd, 5.2, 1.8)	63.15[a]	C-7
9	6.45 (dt, 16.0, 1.8)	119.12	C-1
10	6.92 (dt, 16.0, 4.9)	138.55	C-2
11	4.22 (dd, 4.9, 1.8)	63.10[a]	C-9, C-10

[a]Assignments may be interchanged.

A bacterial metabolite named tetrenolin has been previously reported as possessing the same general structure as lissoclinolide (4,5). The earlier reports, however, fail to assign the configuration of the $\Delta^{4,5}$ double bond. Tetrenolin was reported to be active against a variety of Gram positive bacteria but inactive against Gram negative bacteria. In contrast, lissoclinolide exhibits slight activity against the Gram negative bacterium *Escherichia coli* but was inactive against Gram positive bacteria. Such differences in biological activity suggest that these compounds may differ in the orientation of the $\Delta^{4,5}$ double bond. We have clearly shown this bond to exist in the *Z* configuration in lissoclinolide.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on a Varian XL-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Difference nOe, HETCOR, and COLOC experiments were performed on an IBM AF200 spectrometer operating at 200 MHz for ^{1}H and 50 MHz for ^{13}C. ^{1}H-nmr spectra are referenced to the center signal of residual CHD_2OD (3.30 ppm), and the ^{13}C-nmr spectra are referenced to internal MeOH-d_4 (49.0 ppm). The ir spectrum was recorded on a Perkin-Elmer 1600 Series Ft-ir and the uv spectrum was obtained using a Beckman DU-8 photospectrometer. The eims data was obtained on a VG Micromass 7050E double focussing high resolution mass spectrometer with a VG data system 2000.

TUNICATE COLLECTION.—The fleshy green tunicate (1.06 kg) was collected using SCUBA on coral reef tops at depths of 1–5 m in the Yasawa island group, Fiji. The organism was identified by Dr. Francoise Monniot. A voucher sample is stored at the Museum National d'Histoire Naturelle, Paris, France. The animals were immediately frozen at −20° and remained so until extraction.

EXTRACTION, SEPARATION, AND PURIFICATION OF LISSOCLINOLIDE.—The lyophilized tunicates (218 g) were homogenized and repeatedly extracted with MeOH. The crude MeOH extract was then filtered and concentrated to 300 ml. H_2O (60 ml) was added and the solution was extracted with hexane (2 × 500 ml). The MeOH/H_2O fraction was further diluted with H_2O (60 ml) and extracted with CCl_4 (3 × 500 ml). Additional H_2O (120 ml) was then added and the solution was partitioned with $CHCl_3$ (3 × 500 ml). The latter two $CHCl_3$ fractions contained lissoclinolide and were combined and concentrated, yielding 138 mg of a yellow oil, which was then chromatographed over Si gel [$CHCl_3$-MeOH (95:5)] giving pure **1** (20 mg) as a pale yellow glass. Uv and ir data are presented in the text; ^{1}H- and ^{13}C-nmr data are compiled in Table 1.

ACETYLATION OF **1**.—To a solution of lissoclinolide (1.8 mg) in pyridine (0.5 ml) was added an excess of Ac_2O (200 μl). After stirring overnight at room temperature, the reaction was diluted with CH_2Cl_2 (5 ml), washed with H_2O (2 × 5 ml), dried ($MgSO_4$), and concentrated, yielding lissoclinolide diacetate (1.2 mg): ir (film) ν max 2935, 1765, 1741, 1367, 1231, 1049, 974 cm^{-1}; ^{1}H nmr (200 MHz, CD_3OD) δ 7.44 (1H, s), 6.90 (1H, m), 6.77 (1H, m), 6.48 (1H, br d, J = 16.0 Hz), 6.13 (1H, dt, J = 15.4, 5.2 Hz), 6.02 (1H, d, J = 11.4 Hz), 4.71 (4H,

m), 2.08 (3H, s), 2.07 (3H, m); eims *m/z* (rel. int.) [M]$^+$ 292 (4), 232 (4), 206 (4), 191 (11), 190 (100); hreims data is presented in the text.

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