Pergamon

Tetrahedron Vol. 50, No. 18, pp. 5323-5334, 1994

0040-4020/94 $6.00+0.00

0040-4020(94)E0195-Y

Lissoclinotoxins: Antibiotic Polysulfur Derivatives From The Tunicate *Lissoclinum perforatum*. Revised Structure of Lissoclinotoxin A

Marc Litaudon, François Trigalo, Marie-Thérèse Martin, François Frappier and Michèle Guyot*.

Laboratoire de Chimie, URA 401 C.N.R.S., Muséum National d'Histoire Naturelle, 63 rue Buffon, 75005 Paris, France.

Summary.- Lissoclinotoxin B, a new pentathiepin derivative, was isolated from the tunicate *Lissoclinum perforatum*. Structure elucidation of this compound is described based on spectroscopic data, together with a revised structure for lissoclinotoxin A. A synthesis of tetraacetyl isolissoclinotoxin A is described.

Within the family Didemnidae (Tunicates), species of the genus *Lissoclinum* appear to be a rich source of sulfur-containing compounds with promising biological activity. The following cytotoxic cyclic peptides can be mentioned: ulicyclamide, ulithiacyclamide[1,2,3], patellamide[4] and lissoclinamide[5] isolated from *L. patella*; the benzopentathiepin varacin[6], and the thioalkaloid varamines[7] from *L. varau*. The first non-sulfur compounds described from a *Lissoclinum* sp. were the polypropionate derived macrocycles, bistramides and bistratenes[8,9] which have demonstrated activity in a variety of systems, and cyclooxazoline[10], a cytotoxic cyclic hexapeptide, all isolated from *L. bistratum*. The structure of bistramide A (bistratene A) was recently revised by analysis of 2D INADEQUATE spectra [11].

We have found that the methanolic extract of *Lissoclinum perforatum* collected in northern Brittany, exhibited strong cytotoxic and antimicrobial potential, as well as modest antifungal activities. In a preliminary paper[12], we described the isolation of the bioactive lissoclinotoxin A **1a** from this tunicate. We hereby report on the structure of a second bioactive compound: lissoclinotoxin B **3a**, and a revised structure, **1b**, for lissoclinotoxin A, determined using reverse long-range heteronuclear correlations and additional mass spectrometric analyses. This new assignment was indirectly confirmed by synthesis of tetraacetyl isolissoclinotoxin A **2a**.

Lissoclinum perforatum (Verrill 1871), a thinly encrusting tunicate, was collected in shallow waters off Dinard harbour (northen Brittany). The freeze-dried animals were extracted with dichloromethane, then with methanol. The methanolic extract exhibited strong antibiotic activity towards *Staphylococcus aureus* and *Escherichia coli* with inhibition zones of 35 mm and 24 mm/300 µg, respectively, in a disk diffusion assay and cytotoxicity against L1210 leukemia cell line (ID_{50} 3.5 µg/ml).

Chromatographic separations were monitored by the antimicrobial assay. A silica gel column chromatography using a chloroform-methanol gradient elution, afforded a potent active fraction obtained at ratio

8/2. After low and high pressure chromatography on silicagel, using the same solvent ratio as eluent, a new antibacterial compound, lissoclinotoxin B **3a** was obtained, besides lissoclinotoxin A.

LISSOCLINOTOXIN B.

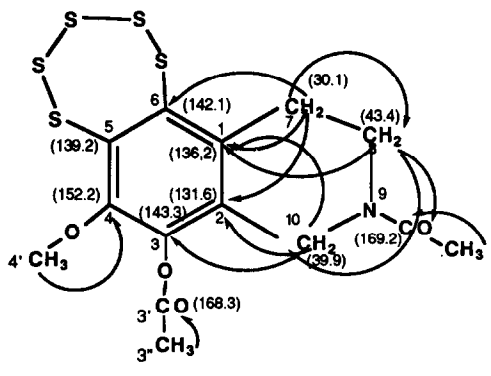
Lissoclinotoxin B **3a**, was a minor component of *Lissoclinum perforatum*, obtained as a yellow powder after extensive chromatographic separations on silicagel using chloroform-methanol 8/2 as eluent. EIMS exhibited a base peak at m/z 273 and a weak peak at m/z 337 (HREIMS 336.9386, calculated for $C_{10}H_{11}NO_2S_5$ 336.9388). Since lissoclinotoxin B, like lissoclinotoxin A, is weakly soluble in most organic solvents, we tried to obtain more soluble derivatives. Acetylation with acetic anhydride-pyridine led to the diacetyl derivative **3b**, CIMS : m/z 422 $(M+H)^+$.

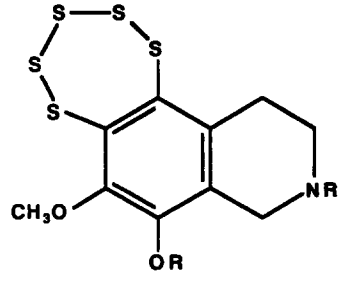
^1H NMR of **3a** and **3b** differ from the previously described lissoclinotoxin A and diacetyl lissoclinotoxin A **1a** and **1c** only in the absence of the aromatic proton signal and the presence of an additional deshielded methylene signal as an AB system (J =17.4 Hz) at δ 4.24, 4.21 for **3a** and 4.52, 4.61 ppm for **3b**. It was likely that lissoclinotoxin B was a cyclized derivative of lissoclinotoxin A.

Long-range heteronuclear correlation experiments (HMBC) of **3b** showed correlations between the methylene at δ 4.61, 4.52 and the carbon signals at δ 131.6, 143.3 and 136.2, respectively, assigned to C-2, C-3 and C-1. Other correlations shown in figure 1 and table 1 allowed the assignment of the substituents on the benzene ring, and suggested a methoxyl at position 4, contrary to the previously described lissoclinotoxin A structure[12].

Since the comparison of the sulfur content of both diacetylated and tetraacetylated derivatives of lissoclinotoxin A[12] established the existence of a sulfur bridge on the benzene ring, and owing to the presence of the ion at m/z 337 observed by MS, we conclude that lissoclinotoxin B does possess a pentathiepin moiety as it is the case for varacin[6]. Therefore, structure **3b** could be assigned to diacetyl lissoclinotoxin B and structure **3a** to lissoclinotoxin B.

The position of the methoxyl group we had located on C-3 in lissoclinotoxin A together with the MS data indicating the presence of a pentathiepin ring system, prompted us to reassess the structure of lissoclinotoxin A.





3a R = H

3b R = CO-CH_3

Fig.1.- Long-range ^{13}C-^1H correlations observed for diacetyl lissoclinotoxin B **3b**.

Table 1- ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR ($CDCl_3$) data for diacetyl lissoclinotoxin B **3b**

Assignment	^{13}C	^{1}H	HMBC
1	136.2		
2	131.6		
3	143.3		
3'	168.3		
3"	20.4	2.37 (s, 3H)	C3'
4	152.2		
4'	62.7	3.86 (s, 3H)	C_4
5	139.2		
6	142.1		
7	30.1	3.06 (dt, 1H) ; 3.21 (dt, 1H)	C_1, C_2, C_6, C_8
8	43.4	3.66 (m, 2H)	C_1, $C_{9'}$, C_{10}
9'	169.2		
9"	21.3	2.15 (s, 3H)	C9'
10	39.9	4.61 (d, 1H) ; 4.52 (d, 1H)	C_1, C_2, C_3,

LISSOCLINOTOXIN A, REVISED STRUCTURE.

Lissoclinotoxin A, the major active component, was isolated as a beige amorphous powder, poorly soluble in organic solvents. In EIMS the base peak was at m/z 261, for which a formula $C_9H_{11}NO_2S_3$ was provided by HREIMS. In CIMS a small peak (8%) at m/z 326 suggested the presence of a pentasulfur derivative by comparaison with lissoclinotoxin B. Furthermore, lissoclinotoxin A gives the characteristic UV spectrum (absorption at 246 nm)[13] of a pentathiepin derivative as observed for lissoclinotoxin B as well.

Structure elucidation was mainly based on NMR data of the tetraacetyl derivative obtained by treatment of lissoclinotoxin A with acetic anhydride in DMF, in the presence of DMAP. The formula $C_{17}H_{21}NO_6S_2$, gained from the highest ion (m/z 357, 100%, M^+-42), indicated the presence of only two sulfur atoms, in agreement with elemental analysis.

A strong nOe indicated that the aromatic proton was adjacent to the ethylamine side chain, but other nOe's could not be interpreted unambiguously. A weak nOe suggested however, that the aromatic proton was adjacent to the methoxyl group. This result, associated with the existence of the natural product, 3-methyldopamine, had previously led us to the conclusion that the structure of lissoclinotoxin A was **1a**, i.e. 3-methoxy-4-hydroxy-5,6-trithia-phenylethylamine, the diacetate **1c**, and the tetraacetate **2a**, respectively[12].

Additional long range heteronuclear correlations (HMBC) was performed on the tetraacetyl derivative. The aromatic proton displayed a large coupling (160 Hz) with the carbon C-2, a weak correlation with C-3 at δ 145.5 ppm and a coupling of 7~9 Hz with both signals at δ 152.2 and 131.2 ppm. The latter could therefore be assigned to C-4 and C-6, C-4 being correlated with the methoxyl. The protons at C-7 were coupled with C-2 and the quaternary carbon at 131.2 ppm we assigned at C-6. (Fig.2). The methoxyl group should be therefore located on the C-4 carbon.

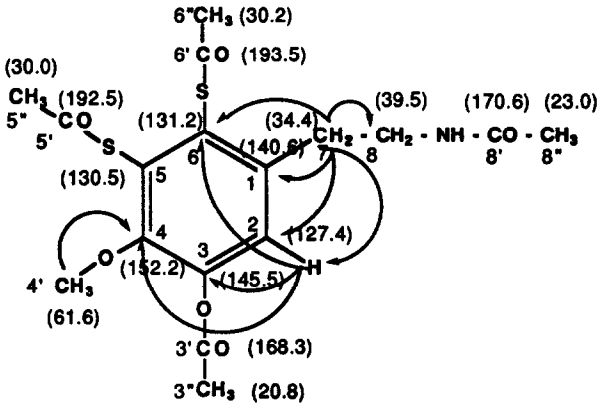
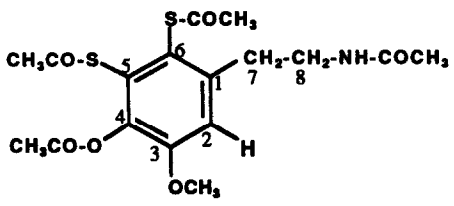


Fig. 2- Long-range C-H correlations for tetraacetyl lissoclinotoxin A **2b**.

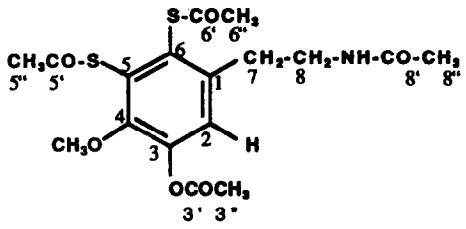
Table 2 : ^{1}H, ^{13}C NMR and HMBC data for lissoclinotoxin A tetraacetate **2b**
^{13}C NMR data for synthetic tetraacetyl isolissoclinotoxin **2a** ($CDCl_3$).

	2b			**2a**
Attribution	^{13}C	^{1}H	HMBC	^{13}C
1	140.6			143.4
2	127.4	7.09 (s, 1H)	C_3, C_4, C_6, C_7	115.4
3	145.5			153.3
3'	168.3			56.2
3"	20.8	2.27 (s, 3H)	$C_{3'}$	
4	152.2			140.9
4'	61.6	3.71 (s, 3H)	C_4	167.9
4"				23.1
5	130.5			128.8
5'	192.5			191.8
5"	30.0	2.34 (s, 3H)	$C_{5'}$	30.0
6	131.2			124.1
6'	193.5			194.3
6"	30.2	2.37 (s,3H)	$C_{6'}$	30.0
7	34.4	2.89 (t, 2H)	C_1, C_2, C_6, C_8	35.0
8	39.5	3.35 (dt, 2H)		39.7
8'	170.6			170.6
8"	23.0	1.84 (s, 3H)	$C_{8'}$	23.4
NH		5.23 (t, 1H)		

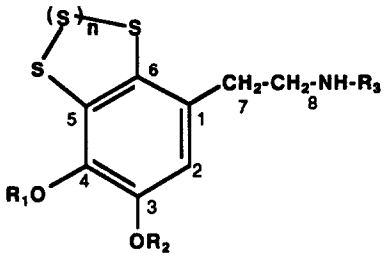
From these observations which are summarized in figure 2, the structure for tetraacetyl lissoclinotoxin A must be **2b** rather than **2a** as previously described. This structural reassessment was indirectly confirmed by comparison of the spectroscopic data of **2b** with those of the synthetic **2a** (*vide infra*).



2a



2b



1a : $R_1 = R_3 = H$; $R_2 = CH_3$, n=1
1b : $R_1 = CH_3$; $R_2 = R_3 = H$, n=3
1c : $R_1 = R_3 = COCH_3$; $R_2 = CH_3$, n=1
1d : $R_1 = CH_3$, $R_2 = R_3 = COCH_3$, n=3
1e : $R_1 = R_3 = COCH_3$; $R_2 = CH_3$, n=3
1f : $R_1 = CH_3$, $R_2 = R_3 = COCH_3$, n=1

Fig.3 -Structure of lissoclinotoxin A, isolissoclinotoxin A and derivatives.

***Synthesis of isolissoclinotoxin A tetraacetate* 2a.**

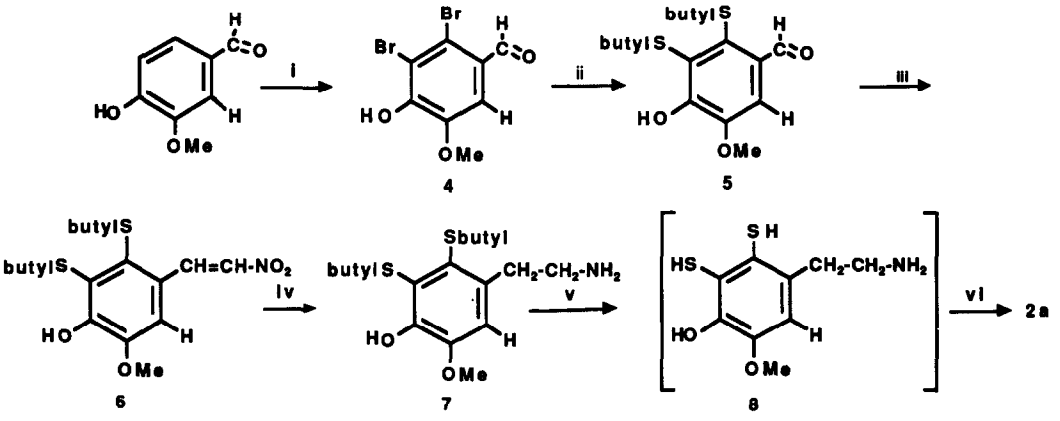
The need for reference compounds led us to synthesize lissoclinotoxins and analogues. Because the most efficient methods regarding the introduction of a trithiane or a pentathiepin rings described in the literature use benzodithiols as starting materials, we first focused our effort onto the synthesis of the two isomeric tetraacetylated derivatives **2a** and **2b**, starting respectively from vanillin and isovanillin. This synthesis, in which separate introduction of the ethylamine side chain and the thioacetates were accomplished, is outlined in scheme 1. In fact, the first step of this synthesis, i.e. the introduction of two bromine atoms on the aromatic ring appeared easy in the case of vanillin, but difficult in the case of isovanillin. We thus pursued our synthetic effort onto the synthesis of isolissoclinotoxin **2a**.

As this work was under submission, Ford and Davidson [14] published a synthesis of varacin starting also from vanillin and very recently, a total synthesis of the varacinium trifluoroacetate was described[15].

5,6-dibromovanillin **4** was prepared according to Raiford[16]. Treatment of **4** with n-butyl-S-Cu following the method of Adams and coll.[17] afforded the disulfur derivative **5**. Introduction of the ethylamine side-chain proved to be easy according to[18], by condensing dithiobutyl-5,6-vanillin **5** with nitromethane and subsequently reducing both the double bond and the nitro group by $AlLiH_4$. The total yield of this sequence reached 42 % .

Of the methods describing the reducion of thioethers into thiols, none proved efficient for reducing the di-S-butyl derivative **7**. In our hand, the dithiol derivative **8** was obtained after treatment of the di-S-butyl derivative **7** by means of Na in pyridine, but only in poor yield owing to the difficulty in isolating the dithiol. In order to optimize the yield of the recovering material, the crude reduced mixture was directly acetylated to give the tetraacetylated isolissoclinotoxin A **2a**, which was then obtained in a 14 % yield.

^{1}H NMR data of **2a** differ from those of **2b** chiefly in the chemical shifts of the aromatic H-2 (δ 6.99 and 7.09) and the methoxyl (δ 3.82 and 3.71). Long-range H-C heteronuclear correlations (HMBC) displayed a large coupling constant (160 Hz) between H-2 and carbon resonating at 115.4 ppm (C-2) and a 7-8 Hz coupling constant with the carbons at δ 140.9 and 124.1 ppm attributed respectively to C-4 and C-6, showing consequently the inversion of the two substituents borne by C-3 and C-4 in both compounds. These data indirectly corroborate the structure **2b** established for tetraacetyl lissoclinotoxin A.



i: Br_2, acet. acid; ii : but-S-Cu/quinoline, pyridine; iii: CH_3NO_2, ammonium acetate;

iv: $LiAlH_4$, diethyl ether ; v: Na, pyridine; vi: acetyl chloride.

Scheme 1- synthesis of tetraacetate **2a**.

Discussion.

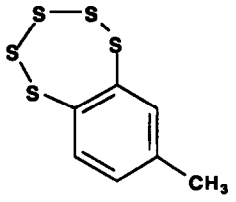
While we published the structure of lissoclinotoxin A[12], Ireland and co-workers described the structure of varacin,[6] a structurally close congener of lissoclinotoxin A. On the basis of a prominent molecular ion at m/z 435 observed by FAB MS of the trifluoroacetyl derivative, the authors concluded to a pentathiepin ring, but they did not exclude the possibility of a mixture of pentathiepin and trithiane.

Both lissoclinotoxins exhibited in CIMS, a small ion respectively at m/z 326 and 338 suggesting the presence of a pentasulfur containing compound, the base peak corresponding to the trithiane. The pentathiepin structure was ascertained by UV data.

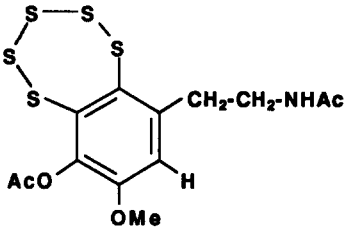
In EIMS only lissoclinotoxin B gave an ion (m/z 337) which provided the formula corresponding to a pentathiepin. This peak has only a low intensity (5-8%) compared to the base peak corresponding to the trithiane.

Diacetyl lissoclinotoxin B gave a weak peak $(M+H)^+$ at m/z 422 (10%) in CIMS, but only the FAB mass spectrum of diacetyl lissoclinotoxin A displayed a peak at m/z 410 (4%) corresponding to the pentathiepin..

To obtain further informations we looked at the spectral behaviour of synthetic analogues possessing pentathiepin rings. We synthesized, on one hand, the pentathiepin **9** derived from the commercially available toluene dithiol according to Chenard,[13] and on the other hand the pentathiepin **1e** isomer of diacetyl lissoclinotoxin A[19].



9



1e

In CIMS, the pentathiepin **9** exhibited only a minor molecular ion at m/z 251 $[M+H]^+$(18%) and a base peak at m/z 187 corresponding to the loss of two sulfur atoms.

Likewise, the pentathiepin **1e** displayed a weak molecular ion $[M+H]^+$(17%) at m/z 410 and a base peak at m/z 346. The ^1H NMR spectrum of this compound displayed two complex multiplets centered at δ 3.14 and 3.45 ppm for the methylenes 7 and 8, respectively.

If the purification of tetraacetyl lissoclinotoxin A was relatively easy to achieve, the diacetyl derivative was difficult to obtain. Several runs of acetylation (acetic anhydride-pyridine) of lissoclinotoxin A always led to a complex mixture of products. In ^1H NMR spectra of diacetyl derivative **1d**, we observed a singlet at δ 7.03 for the aromatic proton and two complex multiplets centered at δ 3.47 and 3.13 ppm for the methylene protons. These chemical shifts correspond to the pentathiepin derivative, according to the ^1H NMR data of the synthetic derivative **1e**, confirmed by the signal pattern, in favor of restricted rotation around the side chain bonds, as observed in the synthetic pentasulfur TEOC varacin [14].

In partially purified samples of diacetyl lissoclinotoxin A, additional signals (10 to 20% in intensity): δ 6.64 (s), 3.54 (dt), 2.83 ppm (t), which can be attributed to the trithian compound **1f**, were observed. These chemical shifts associated with the multiplicity are similar to those displayed in the tetraacetyl derivative **2b** ^1H NMR: δ 7.09 ppm, 3.35 ppm (dt), 2.89 ppm (t) in which free rotation of the side-chain is supposed to take place. From these data it appears that during acetylation (acet.anh -pyr) the pentathiepin ring can be partially converted into the trithiane. It is worth noting that the transposition of benzopentathiepins into trithianes can easily occur[13] even while standing in solvents such as methanol or hexane/ethylamine. Detailed analysis of the conversion of the pentathiepin into the trithiane which might occur during extraction, transformation and/or purification processes will be described later.

BIOLOGICAL ACTIVITY OF LISSOCLINOTOXINS.

Lissoclinotoxins are powerful antimicrobial agents. Activity towards *Staphylococcus aureus* was first evaluated by the petri disk bioassay in which **1b** and **3a** exhibited 35 and 30 mm/100 µg inhibition zone

respectively (31 mm/5 µg for oxacilline). The MIC values of Lissoclinotoxin A were then evaluated towards a number of strains: the results are summarized in table 3. Lissoclinotoxin A exhibited antimicrobial activities in the same range as those obtained with the reference antibiotic cefatoxim.

Table 3-MIC of lissoclinotoxin A compared to cefotaxim (µg/ml)

Strains	MIC µg/ml	
	Cefotaxim	Lissoclinotoxin A
Staphylococcus aureus	1.2 - 10	0.08 - 0.15
Streptococcus faecalis	0.6 - 2.5	0.3 - 0.6
Citrobacter spp	0.08 - 10	0.3 - 0.6
Klebsiella spp	0.01 - 0.15	0.3 - 0.6
Escherichia coli	0.005 - 5	0.15 - 0.6
Enterobacter spp	0.08 -40	0.3 - 0.6
Serratia spp	0.08 - 2.5	0.3 - 0.6
Salmonella spp	0.08 - 3	0.3 - 0.6
Pseudomonas aeruginosa	2.5 - 40	2.5 - 10
Acinetobacter	10	0.3
Proteus spp	0.005 - 0.3	0.15 - 0.6

Moreover, lissoclinotoxin A an B are active against ichthyopathogenic strains such as *Aeromonas salmonicida* (strain 643) and *Vibrio angularium* (strains 5536 and 408): inhibition zones compared to those of penicillin G and ampicillin are given in table 4.

Table 4.-Activity of lissoclinotoxin A and B toward ichthyopathogenic strains (Ø, mm).

Strains	Penicillin G (6 µg)	Ampicillin (5µg)	Lissoclinotoxin A (30 µg)	Lissoclinotoxin B (50µg)	(5µg)
Aeromonas salmonicida 643	0	40	40	16	8
Vibrio anguillarum 5536	12	30	35	15	12
Vibrio anguillarum 408	12	0	30	18	15

Lissoclinotoxin A showed also moderate activities against several strains of fungi (CMI > 40 µg/ml) and yeast with CMI of 20 and 40 µg/ml against *Trichosporon mentagrophytes* and *Candida albicans*, respectively.

Toxicity towards L1210 leukemia cell line was tested and resulted in an IC_{50} of 1µg/ml. However, *in vivo* bioassays showed only an increasing life time (T/C) of 125% at a dose of 3.125 mg/kg, with a toxic effect at higher dose (T/C of 25% at a dose of 12.5 mg/kg). In the sea-urchin test, lissoclinotoxin A caused the inhibition of cell division at a concentration of 16 µg/ml. This antimitotic activity was not correlated with the the polymerisation or depolymerisation processes in the tubulin assay.

Bioassay directed toward *Plasmodium falciparum* showed strong antimalarial activity of lissoclinotoxin A. The IC_{50} of this compound is 296 nM/l on a resistant strain where the usual antimalarials quinine, mefloquine, halofantrine and chloroquine exhibit IC_{50} of 350, 40, 2 and 580 nM/l, respectively.

Another trithiane derivative was isolated from a New Zealand tunicate *Aplydium* sp[20] derived from vanillin, for which cytotoxic and antibacterial activities against Gram (+) strains was demonstated. Varacin[6] exhibited potent antifungal and cytotoxic activity, but no antimicrobial activity was reported.

EXPERIMENTAL SECTION.

Extraction and purification.

Lissoclinum perforatum was collected in the harbour off Dinard (France) and freeze-dried or frozen until used. The freeze-dried animals were homogenized in a blender in dichloromethane and filtered, then extracted twice with methanol. The frozen animals were homogenized in methanol and further extracted with methanol and a methanol-chloroform 1/1 mixture. The combined extracts were partially evaporated under reduced pressure. The aqueous residue was extracted with dichloromethane, and the aqueous phase evaporated to dryness. Then the slurry residue was triturated three times with methanol, to yield the methanol extract.

In both cases, only the methanol extracts exhibited a strong activity in the antimicrobial disk assay performed on *Staphylococcus aureus* and *Escherichia coli* (inhibition zone of 35 mm and 24 mm/300 µg respectively), and showed cytotoxic activity against L1210 leukemia cell line (ID_{50} 3.5 µg/ml).

Flash chromatography of the methanolic extract on silica gel, using chloroform with increasing amounts of methanol as eluent, afforded an active fraction eluted with chloroform-methanol 8/2. This fraction was submitted to a low pressure silica gel column chromatography with chloroform-methanol 8/2 as eluent, followed by a preparative HPLC using the same solvent system. Finally a preparative TLC on silicagel afforded pure lissoclinotoxins A and B.

***Lissoclinotoxin A* 1b.**

A beige amorphous solid, m.p. 240-242°C. MS (CI, CH_4): m/z 326 (8), 262 (100); HREIMS: found 260.9945, calculated 260.9951 for $C_9H_{11}NO_2S_3$. MS (EI): m/z: 261(100)(M^+-2S), 244, 232, 229 (M^+-3S), 197 (M^+-4S), 136, 121, 105, 97. ^1H NMR (δ ppm, DMSO-d_6) : 6.95 (s, 1H), 3.74 (s,3H), 3.08 (m, 2H), 2.92 (t,2H). UV (ethanol) λmax: 215 (ε 10440), 246 (ε 8874).

***Diacetyl derivative* 1d.**

To a solution of **1b** (5 mg) in pyridine (0.2 ml) were added acetic anhydride (0.2 ml). The mixture was left 24h at room temperature, then ice was added and the solution extracted with chloroform. Chromatographic separation on silica gel, using a mixture chloroform-acetone (7/3) furnished the diacetyl derivative **1d** in a 30% yield. ^1H NMR, δ (ppm, $CDCl_3$) : 7.03 (s, 1H), 3.74 (s, 3H), 3.47 (m, 2H), 3.13 (m, 2H), 2.34 (s, 3H), 1.96 (s,3H). FAB MS : m/z 410 (4)$(M+H)^+$, 346 (M+H-2S) (8). EIMS : 345 (M^+-2S), 303 (100) (M^+-2S-42).

***Tetraacetyl derivative* 2b.**

To a solution of crude lissoclinotoxin A (9.5 mg) in DMF (1mL) was added DMAP (0.5 mg) and acetic anhydride (1mL). After 2 h at room temperature the mixture was evaporated *in vacuo*. The residue was extracted with dichloromethane to yield a yellow powder (2.5 mg) after a preparative TLC on silicagel (chloroform-acetone 7/3). (NMR see table 2). HREIMS: m/z 357 (M^+-42); observed 357.0706, calculated for $C_{15}H_{17}NO_5S_2$: 357.0704. Elemental analysis S: 16.25 % calculated for two sulfur atoms 16.12 %.

***Lissoclinotoxin B* 3a.**

A pale yellow powder, m.p.310-313°C. CIMS m/z 338 $(M+H)^+$, 274. HREIMS, found 336.9386, calculated for $C_{10}H_{11}NO_2S_5$: 336.9388; 272.9946, calculated for $C_{10}H_{13}NO_2S_3$, 272.9951. U.V. (methanol): λ max 215 (ε 20000) and 246 (ε 6500). IR (KBr disk): ν (cm^{-1}) : 1560, 1730 (w), 2920, 3400. ^1H NMR (d-4 methanol): 4.24 (1H, d, *J*=17.4 Hz) 4.21 (1H, d, *J*=17.4 Hz) AB system, 3.79 (s, 3H), 3.43 (t, 2H), 3.25 (m, 2H).

***Diacetyl lissoclinotoxin B* 3b.**

To a solution of **3a** (4.5 mg) in pyridine (0.2 ml) were added acetic anhydride (0.2 ml). The mixture was left 24h at room temperature, then ice was added and the solution extracted with chloroform. Chromatographic separation by preparative TLC on silicagel, using a mixture chloroform-acetone (7/3) mixture, furnished the diacetyl derivative **3b**, yellow glassy material, in a 30% yield. (NMR see table 1). CIMS : m/z 422 $(M+H)^+$, 379 (M-42), 357 (M-64) 315 (M-42-64).

Synthesis of tetraacetyl isolissoclinotoxin A 2a.

***2,3-dibutylthio-4-hydroxy-5-methoxybenzaldehyde* 5.**

To a solution of cuprous n-butyl-mercaptide (13.8 g, 90 mmol) in a quinoline-pyridine mixture (250 ml, 4/1), maintained at 110° was added 5,6-dibromovanillin **4** (prepared according to[16], 9.30 g, 30 mmol) while stirring. The resulting solution was stirred for 1 hour at 110°. The warm solution was poured onto crushed ice containing an excess of hydrochloric acid, under magnetic stirring. The acidic solution was extracted with ether (3x500 ml) and the combined extracts washed twice with water (250 ml), dried over $MgSO_4$ and concentrated to a black oil This material was chromatographed on 250 g silica gel with EtOAc/cylohexane (2/8) to afford **2** (Rf 0.42) (2.92 g, 38%) as a crystalline product; m.p. 59°C; ^1H NMR. ($CDCl_3$) δ ppm, 10.70 (s, 1H, ArC<u>H</u>O), 7.58 (s, 1H, ArO<u>H</u>), 7.45 (s, 1H, Ar<u>H</u>), 3.90 (s, 3H, ArOC<u>H</u>$_3$), 2.85-2.75 (m, 4H, 2 x SC<u>H</u>$_2$), 1.50-130 (m, 8H, 2 x SCH$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 0.98-0.92 (m, 6H, 2 C<u>H</u>$_3$). ^{13}C NMR ($CDCl_3$): δ ppm, 192.0 , 152.7, 147.8, 137.9, 132.8, 126.2, 110.6, 56.2, 38.7, 36.2, 31.6, 31.2, 21.9, 21.8, 13.5. Anal. Calcd for $C_{16}H_{24}O_3S_2$: C, 58.54; H, 7.32; S, 19.51. Found C, 58.08; H, 7.20; S, 19.35.

***2,3-dibutylthio-4-(2-nitroethylene)-phenol* 6.**

To a solution of 2,3-dibutylthio-4-hydroxy 5-methoxy-benzaldehyde **5** (3 g, 9.15 mmol) in nitromethane (40ml) was added ammonium acetate (0.8g). The solution was stirred for 3 hours at 100° after which the nitromethane was removed *in vacuo* to afford a yellow red oil. Purification by column chromatrography (silica gel 250 g, EtOAc-cyclohexane 2/8), afforded 3.0 g (88%) of a crystalline product (Rf 0.33); a sample was crystallized in ether, m.p. 81°C. ^1H NMR. ($CDCl_3$) δ ppm, 8.9 (d, 1H, *J*= 13.5 Hz, =C<u>H</u>NO$_2$), 7.50 (d, 1H, *J*= 13.5 Hz, C<u>H</u>=CHNO$_2$), 7.45 (br s, 1H, ArO<u>H</u>), 7.1 (s, 1H, Ar<u>H</u>), 3.9 (s, 3H, ArOC<u>H</u>$_3$), 2.85-2.75 (m, 4H, 2 x SC<u>H</u>$_2$), 1.50-130 (m, 8H, 2 x SCH$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 0.98-0.92 (m, 6H, 2 C<u>H</u>$_3$). ^{13}C NMR ($CDCl_3$) δ ppm 150.9, 147.7, 136.5, 127.2, 126.9, 138.9, 136.5, 110.1, 56.1, 38.2, 36.2, 31.6, 31.2, 21.9, 21.8, 13.5. Anal. Calcd for $C_{17}H_{25}NO_4S_2$: C, 54.99; H, 6.74; N, 3.77; S, 17.25. Found C, 55.01; H, 6.66; N, 3.81; S, 17.08.

***3-methoxy-4-hydroxy-5,6-dibutylthio-phenylethylamine* 7.**

A solution of compound **3** (2.5 g, 6.7 mmol) in absolute ether (200ml) was added dropwise to a suspension of $LiAlH_4$ (742 mg, 19 mmol) in absolute ether (200ml) and refluxed 3 hours with stirring. After cooling, the reaction was quenched by addition of an excess of ethyl acetate. The organic heterogenous solution was filtered through silica gel to remove the precipitate and eluted with 1l dichloromethane-methanol-ammonia,

65/25/4. The solvents were removed and the residue purified by column chromatography (eluent dichloromethane-methanol-ammonia, 90/10/1) yielding 1.1 g of amino product **7** (Rf 0.35) (48%) m.p. 65°C. ^{1}H NMR ($CDCl_3$) δ ppm, 7.25 (br s, 1H, ArOH), 6.8 (s, 1H, ArH), 5.30 (br s, 2H, NH_2), 3.84 (s, 3H, $ArOCH_3$), 3.12-3.0 (m, 4H, CH_2 CH_2NH_2) 2.83-2.70 (m, 4H, 2xSCH_2), 1.53-1.28 (m, 8H, 2 x $SCH_2CH_2CH_2$), 0.80-0.75 (m, 6H, 2 CH_3). ^{13}C NMR ($CDCl_3$) δ: 147.4, 146.6, 134.8, 130.1, 126.0 (aromatic carbons), 113.7 (CH arom.), 56.1, 42.3, 37.6, 36.6, 36.2, 31.6, 31.2, 21.9, 18.2, 13.5. Anal. Calcd for $C_{17}H_{29}NO_2S_2,H_2O$; C, 56.51; H, 8.58; N, 3.88; S, 17.73. Found C, 56.63; H, 8.28; N, 4.04; S, 17.62.

***3-methoxy-4-acetoxy-5,6-diacetylthio-N-acetylphenylethylamine* 2a.**

To a solution of the amino compound **7** (180 mg, 0.5 mmol) in anhydrous pyridine (5ml) was added sodium (130 mg, 5.65 mmol). The solution was heated for 4 hours at 120°C. After cooling at room temperature acetic anhydride (0.5 ml) and pyridine (1 ml) were added to the black solution. The reaction was allowed to proceed for 16 hours and the pyridine solution was poured onto an ice-water mixture (50 ml); the aqueous solution was extracted with ethyl acetate (3x50 ml). The organic layer was washed with a diluted HCl solution, water, $HNaCO_3$ solution, water and dried over sodium sulfate; the solvent removed under reduced pressure to yield 166 mg of crude product. Purification by chromatrography column (silica gel with dichloromethane-acetone (7/3) as eluent) yielded 29 mg of tetraacetate **2a** (14%) as an yellow oil. ^{1}H NMR. ($CDCl_3$) δ ppm, 6.99 (s, 1H, ArH), 5.75 (br s, 1H, NH), 3.82 (s, 3H, $ArOCH_3$), 3.45 (dt, 2H, *J*=7 Hz, CH_2 CH_2NH-) 2.98 (t, 2H, *J* =7Hz), CH_2 CH_2NH-) 2.38 (s, 3H, S-CO-CH_3), 2.35 (s, 3H, S-CO-CH_3), 2.26 (s, 3H, O-CO-CH_3), 1.90 (s, 3H, NH-CO-CH_3). ^{13}C NMR see table 2. CIMS: m/z 400 $(M+H)^+$, 358 (M+H-42), 279, 223, 198.

Antimalarial activity.

A strain of *Plasmodium falciparum* was isolated from a patient and maintained in erythrocytes as described in[21]. Antiplasmodium activity was evaluated by measuring incorporation of ^{3}H hypoxanthine according to[22].

Acknowledgments.

We thank Françoise Monniot (B.I.M., Muséum National d'Histoire Naturelle, Paris, France) for repeated collections of *Lissoclinum perforatum*, Centre National de la Recherche Scientifique and EEC (contract n° MAST-2-CT 91-0004) for financial support, Jean-Paul Brouard and Centre de Spectroscopie of CNRS (Lyon-Solaize) for mass spectral analysis, Dr D.J. Faulkner (Scripps Institution of Oceanography, La Jolla, California, USA) for helpful suggestions and D. Bustos for antimalarial bioassays.

REFERENCES.

1. Ireland, C.; Scheuer, P.J., *J. Am. Chem. Soc.* **1980**, *102*, 5688-5691.
2. Sesin, D.F.; Gagkell, S.J.; Ireland, C.M., *Bull. Soc. Chim. Belg.* **1986**, *95*, 853-867.
3. Ishida, T.; Ohishi, H.; Inoue, M.; Kamigauchi, M.; Sugiura, M.; Takao, N.; Kato, S.; Hamada, Y.; Shiori, T., *J. Org. Chem.* **1989**, *54*, 5337-5343.
4. Ireland, C.M.; Durso, A.R.; Newman, R.A.; Hacker, M.P., *J. Org. Chem.* **1982**, *47*, 1807-1811.
5. Degnan, B.M.; Hawkins, C.J.; Lavin, M.F.; McCaffrey, E.J.; Parry, D.M.; Van den Brenk, A.L.; Waters, D.J., *J. Med. Chem.* **1989**, *32*, 1349-1354.

6. Davidson, B.S.; Molinski, T.F.; Barrows, L.R.; Ireland, C.M., *J. Am. Chem. Soc.* **1991**, *113*, 4709-4710.
7. Molinski, T.F.; Ireland, C.M., *J.Org.Chem.* **1989**, *54*, 4256-4259.
8. Gouiffès, D.; Moreau, S.; Helbèque, N.; Bernier, J.L.; Hénichard, J.P.; Barbin, Y.; Laurent, D.; Verbist, J.F.,*Tetrahedron.* **1988**, *44*, 451-459.
9. Degnan, B.M.; Hawkins, C.J.; Lavin, M.F.; MacCaffrey, E.J.; Parry, D.L.; Waters, D.J., *J. Med. Chem.*, **1989**, *32*, 1354-1359.
10. Hambley, T.W.; Hawkins, C.J.; Lavin, M.F.; Van den Brek, A.; Waters, D.J., *Tetrahedron.* **1992**, *48*, 341-348.
11. Foster, M.P.; Mayne, C.L.; Dunkel, R.; Pugmire, R.J.; Grant, D.M.; Kornprobst, J.M.; Verbist, J.F.; Biard, J.F.; Ireland, C.M., *J. Am. Chem. Soc.* **1992**, *114*, 1110-1111.
12. Litaudon, M.; Guyot, M., *Tetrahedron Lett.* **1991**, *32*, 911-914.
13. Chenard, B.L.; Harlow, R.L.; Johnson, A.L.; Vladuchick, S.A., *J. Am. Chem. Soc.* **1985**,*107*, 3871-3879.
14. Ford , P.W.; Davidson, B.S., *J. Org. Chem.* **1993**, *28*, 4522.
15. Behar, V.; Danishefsky, S.J., *J. Am. Chem. Soc.* **1993**, *115*, 7017.
16. Raiford, L.C.; Hilman, G.C., *J. Am. Chem. Soc.* **1927**, *49*, 1571-1577.
17. Adams, R.; Reifschneider, W.; Ferreti, A., *Organ. Synth.* **1962**, *42*, 22-25.
18. Sekine, Y.; Creveling, C.; Bell, M.; Brossi, A., *Helv. Chim. Acta,*. **1990**, *73*, 426-432.
19. Trigalo, F.; Bijamane, A.; Guyot, M.; Frappier, F., *Natural prod. lett.*, in press.
    Compound **1e**: m.p. 160-162 °C, CIMS (rel intensity) m/z = 410 $(M+H)^+$ (17), 346 (100), 345 (77), 303 (26), 244 (23), 231 (8), 210 (12), 192 (8). ^1H NMR, δ in ppm ($CDCl_3$) 1.95 (s, 3H), 2.32 (s, 3H), 3.14 (m, 2H), 3.45 (m, 2H), 3.83 (s, 3H), 5.74 (m, 1H), 6.83 (s, 1H). ^{13}C NMR, (δ in ppm, $CDCl_3$) 20.29, 23.29, 35.13, 36.45, 56.45, 115.26, 134.86, 140.21, 143.61, 153.07. Anal calcd for $C_{13}H_{15}NO_4S_5$: C, 38.14; H, 3.69; N, 3.42; S, 39.14. Anal calcd for $C_{13}H_{15}NO_4S_5$: C, 38.14; H, 3.69; N, 3.42; S, 39.14. Found C, 37.75; H, 3.67; N, 3.59; S, 38.73.
20. Copp, B.R.; Blunt, J.W.; Munro, M.H.G.; Pannell, L.K., *Tetrahedron,*.**1989**, *30*, 3703-3706.
21. Druilhe, P.; Gentilini, M., *Methodologie Trop.* **1882**, *42*, 437-455.
22. Desjardins, R.E.; Canfield, C.J.; Haynes, J.D.; Chulay, J.D., *Antimicrobial Agents and Chemotherapy.* **1979**, *16*, 710-717

(*Received in Belgium* 7 *September* 1993; *accepted* 31 *January* 1994)