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J. Nat. Prod., **1994**, 57 (10), 1336-1345• DOI: 10.1021/np50112a002 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 10, pp. 1336-1345, October 1994

BISTRAMIDES A, B, C, D, AND K: A NEW CLASS OF BIOACTIVE CYCLIC POLYETHERS FROM *LISSOCLINUM BISTRATUM*

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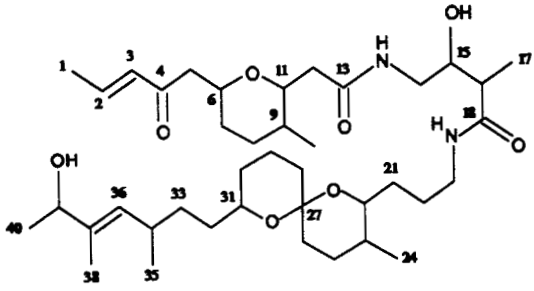
ABSTRACT.—The isolation and characterization is described of four novel cyclic polyethers, bistramides B [**2**], C [**3**], D [**4**], and K [**5**], which are closely related to the previously reported bistramide A [**1**] from the New Caledonian urochordata *Lissoclinum bistratum*. The structures of these metabolites were defined by spectroscopic methods. The four compounds exhibited in vitro cytotoxicity toward six tumor cell lines, including the human non-small cell lung carcinoma (NSCLC-N6) line. Cytofluorimetric analysis with bistramide K showed a complete block of NSCLC-N6 cells in the G_1 phase. Bistramide D and particularly bistramide K are less toxic than bistramides A, B, and C and are thereby effective in vivo against NSCLC-N6.

Among Didemnidae (Ascidiacea), *Lissoclinum* species are well known to contain cytotoxic compounds and continue to interest marine chemists. From *L. patella* collected in different geographical locations, a series of cyclic peptides, namely, ulicyclamide and ulithiacyclamide (1–5), ulithiacyclamide B (5,6), patellamides A–E (7,8), lissoclinamides 1–8 (9,10), prelissoclinamide (6), dihydrolissoclinamide (6), and preulicyclamide (11) have been identified. All of these contain oxazole and thiazole moieties. Lissoclinolide, an ethylenic γ-lactone (12), and patellazoles A and B (13,14), a series of thiazole-containing macrolides, have further been isolated from other specimens of *L. patella*. *Lissoclinum perforatum* and *L. vareau* have been shown to contain lissoclinotoxin A (15) and varacin (16), respectively, two compounds derived from phenylethylamine bearing a polysulfide ring. Furthermore, *L. vareau* also contains varamines A and B (17), two novel alkaloids. Specimens of *L. bistratum* (Sluiter) collected in the Philippines and the Great Barrier Reef have been shown to contain cyclic hexapeptides, namely, bistratamides A–D (18–20). An unidentified specimen of *Lissoclinum* sp. from the Fiji Islands and *L. bistratum* from New Caledonia contain bistramide A (bistratene A) (21) [**1**], a macrocyclic ether with potent cytotoxic, antiproliferative (22–24), and neurotoxic (25–27) activities. Extensive hplc experiments performed on active fractions from *L. bistratum* collected in New Caledonia showed that this organism contained up to twenty compounds closely related to bistramide A (BST-A). We report here the structures and pharmacological properties of four new bistramides, namely, bistramides B–D and K ([**2–5**], BST-B, BST-C, BST-D, and BST-K, respectively).

RESULTS AND DISCUSSION

Lissoclinum bistratum (ref. ORSTOM UA79) was collected near Nouméa, New Caledonia, in the spring of 1990 and freeze-dried shortly after collection. A specimen was



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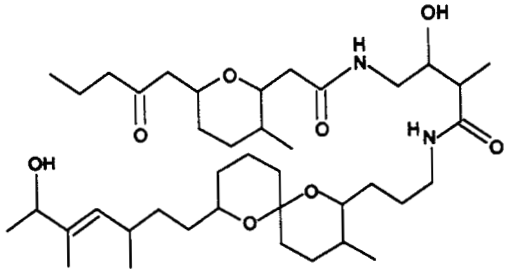

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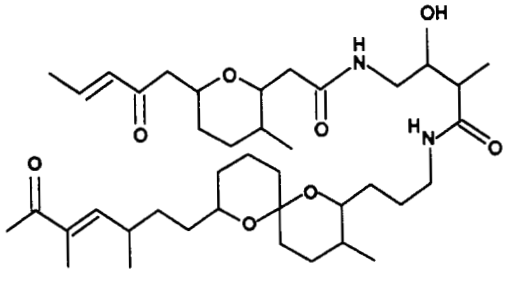

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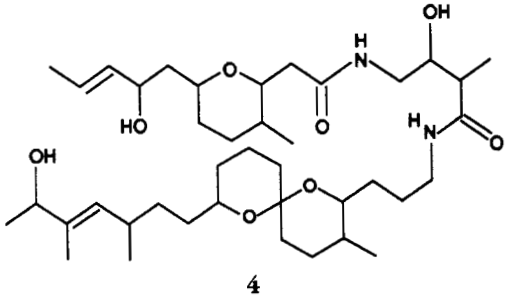

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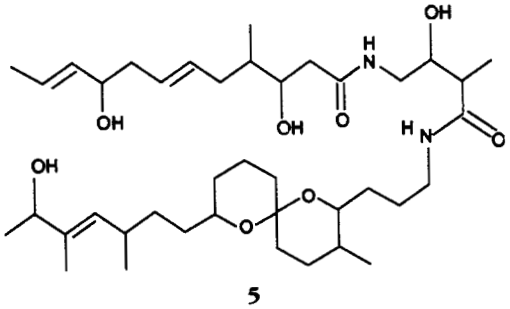

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TABLE 1. Electron-Impact High-Resolution Mass Spectrometric (hreims) Data for Bistramides A [**1**], B [**2**], C [**3**], D [**4**], and K [**5**].

Compound	M^+ (*m/z*) (Experimental)	Formula	M^+ (*m/z*) (theoretical)
[**1**]	704.4981	$C_{40}H_{68}N_2O_8$	704.4975
[**2**]	706.5143	$C_{40}H_{70}N_2O_8$	706.5131
[**3**]	702.4841	$C_{40}H_{66}N_2O_8$	702.4818
D [**4**]-H_2O	688.5006	$C_{40}H_{68}N_2O_7$	688.5026
K [**5**]-H_2O	688.5022	$C_{40}H_{68}N_2O_7$	688.5026

identified by Dr. F. Monniot (Museum d'Histoire Naturelle de Paris). The CH_2Cl_2 extract yielded bistramides B–D and K as non-crystalline solids by low-pressure and high-pressure liquid chromatography. BST-B [**2**], -C [**3**], -D [**4**], and -K [**5**] were identified by comparison of their respective ms, ^1H- and ^{13}C-nmr spectral data with those of BST-A [**1**] (28).

Ms (ei and fab) (Table 1) and nmr data (Table 2) showed that BST-B [**2**], -C [**3**], -D [**4**], and -K [**5**] are closely related to BST-A [**1**]. The highest ions observed for BST-D and BST-K in both eims and positive-ion fabms represented the molecular ion minus H_2O. However, $[M-H]^-$ ions were observed for both metabolites in the negative-ion fabms. All bistramides showed quite similar ir spectra.

Bistramide B [**2**] differed from BST-A [**1**] by having two additional mass units (Table 1). The ^{13}C-nmr spectra obtained for BST-B and BST-A were quite similar for all carbon atoms, but there was no double bond between C-2 (16.75 ppm) and C-3 (43.14 ppm) for BST-B. Thus, BST-B [**2**] was assigned as 2,3-dihydrobistramide A.

Bistramide C [**3**] differed from BST-A [**1**] by having two fewer mass units. The ^{13}C-nmr signal for C-39 at 200.17 ppm clearly revealed an additional carbonyl group at that position instead of the hydroxyl group of BST-A. These data all indicated that BST-C [**3**] was 39-oxobistramide A.

The hreims of bistramide D [**4**] gave an M^+-H_2O ion which indicated a molecular formula of $C_{40}H_{68}N_2O_8$. The fabms (negative-ion mode) gave an $[M-H]^+$ ion at *m/z* 705 suggesting that BST-D is a dihydro analog of BST-A [**1**]. This was confirmed by analysis of the ^{13}C-nmr spectrum of BST-D, which revealed that the carbonyl C-4 signal in BST-A (198.89 ppm), BST-B (204.57 ppm) and BST-C (198.39 ppm) was replaced by an sp^3 carbon bearing a hydroxy group (71.95 ppm).

From the ei-hrms and fabms, bistramide K [**5**] clearly appeared to be an isomer of BST-D [**4**]. This new analogue differs significantly from the others in that the tetrahydropyran moiety (C-6–C-11) has been replaced by an (*E*)-6,7-en-11-ol group, with the unsaturation index remaining unchanged. The stereochemistry of the new 6,7-double bond was assigned as (*E*) from the coupling constant of the two protons H-6 and H-7 (14.0 Hz), which is similar to those of H-2 and H-3 both in BST-A [**1**] (15.7 Hz) and BST-K [**5**] (15.3 Hz). As previously observed for BST-D, BST-K readily lost a molecule of H_2O in the eims but displayed an $[M-H]^-$ ion at *m/z* 705 in the negative-ion fabms.

The stereochemistries (both relative and absolute) of the chiral centers in compounds **2–5** were not determined.

Cytotoxicity was tested in vitro (Table 3) for six tumor cell lines, with five of them being widely available: KB, P388, P388/dox. (doxorubicin-resistant), B16, and HT29, and the last being human non-small-cell lung carcinoma cells, NSCLC-N6 (22, 23, 29, 30). The differential cytotoxicity against the P388 and P388/dox. cell lines was especially large for BST-D [**4**] and BST-K [**5**]. Therefore, these compounds are probably susceptible to the multidrug resistance (mdr) phenomenon.

TABLE 2. NMR Data for Bistramides A [**1**], B [**2**], C [**3**], D [**4**], and K [**5**].

Carbon	Compound									
	1		**2**		**3**		**4**		**5**	
	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)
1	18.43 (q)	1.91 (dd, 6.8, 1.4)	13.66 (q)	0.89 (t, 7.5)	18.38 (q)	1.90 (dd, 6.8, 1.5)	17.59 (q)	1.65 (dd, 6.3, 1.4)	17.62 (q)	1.65 (dd, 6.4, 1.4)
2	144.50 (d)	6.90 (dq, 15.7, 6.8)	17.13 (t)	1.60 (m)	144.20 (d)	6.88 (dq, 15.9, 6.8)	134.02 (d)	5.72 (dqd, 15.7, 6.3, 1.0)	126.26 (d)	6.80 (dqd, 15.3, 6.4, 1.0)
3	132.07 (d)	6.15 (dq, 15.7, 1.5)	45.89 (t)	2.38 (t, 7.3)	132.16 (d)	6.12 (dq, 15.9, 1.7)	125.75 (d)	5.58 (ddq, 15.7, 6.3, 1.4)	133.53 (d)	5.46 (ddq, 15.3, 6.4, 1.4)
4	198.89 (s)	—	204.57 (s)	—	198.39 (s)	—	71.95 (d)	4.12 (t, 6.3)	71.90 (d)	4.04 (t, 6.4)
5	45.24 (t)	2.91 (dd, 17.0, 8.9) 2.53 (dd, 17.0, 3.0)	48.14 (t)	2.60 (dd, 2.5, 17.2) 2.81 (dd, 17.2, 9.9)	45.36 (t)	2.92 (dd, 16.9, 8.8) 2.58 (dd, 16.9, 3.1)	42.86 (t)	1.70 (m) 1.55 (m)	40.64 (t)	2.20 (m) 2.14 (m)
6	64.80 (d)	4.20 (m)	64.54 (d)	4.15 (m)	64.94 (t)	4.17 (m)	69.39 (d)	3.87 (m)	127.68 (d)	5.43 (dt, 14.0, 7.0)
7	30.78 (t)	1.69 (m) 1.41 (m)	30.68 (t)	1.66 (m) 1.14 (m)	30.82 (t)	1.68 (m) 1.37 (m)	31.07 (t)	1.63 (m) 1.30 (m)	131.73 (d)	5.47 (dt, 14.0, 7.0)
8	26.52 (t)	1.63 (m) 1.33 (m)	26.48 (t)	1.55 (m) 1.14 (m)	26.60 (t)	1.62 (m) 1.33 (m)	26.43 (t)	1.64 (m) 1.30 (m)	36.66 (t)	2.13 (dd, 13.5, 1.65) 1.94 (dd, 13.5, 1.65)
9	33.32 (d)	1.92 (m)	33.31 (d)	1.95 (m)	32.96 (d)	1.90 (m)	32.96 (d)	1.93 (m)	38.40 (d)	1.58 (m)
10	17.14 (q)	0.86 (d, 7.0)	17.13 (q)	0.84 (d, 7.0)	17.09 (q)	0.82 (d, 7.1)	16.85 (q)	0.83 (d, 7.0)	14.40 (q)	0.88 (d, 6.9)
11	74.82 (d)	4.06 (dd, 10.9, 4.6)	74.80 (d)	4.05 (dd, 10.6, 4.9)	74.80 (d)	4.06 (dd, 11.8, 4.8)	74.22 (d)	4.22 (m)	71.43 (d)	3.88 (dt, 8.8, 4.2)
12	32.33 (t)	2.75 (dd, 15.1, 11.7) 2.15 (dd, 14.9, 1.4)	32.33 (t)	2.72 (m) 2.12 (dd, 15.1, 1.5)	32.53 (t)	2.73 (dd, 15.4, 11.7) 2.15 (m)	33.12 (t)	2.68 (dd, 15.0, 11.8) 2.18 (dd, 15.0, 0.5)	36.66 (t)	2.31 (m) 2.30 (m)
13	173.42 (s)	—	173.45 (s)	—	173.40 (s)	—	172.33 (s)	—	173.64 (s)	—
14	44.85 (t)	3.50 (dt, 14.0, 5.8) 3.24 (dt, 14.0, 5.7)	44.68 (t)	3.51 (m) 3.29 (m)	44.73 (t)	3.48 (m) 3.22 (m)	43.80 (t)	3.48 (m) 3.29 (m)	43.39 (t)	3.32 (t, 5.6)
15	73.81 (d)	3.72 (dt, 10.3, 5.1)	73.86 (d)	3.69 (m)	73.80 (d)	3.69 (m)	73.11 (d, 1.9)	3.75 (m)	72.57 (d)	3.68 (dt, 5.4, 5.6)
16	43.36 (d)	2.38 (dq, 5.0, 7.0)	43.37 (d)	2.41 (m)	43.37 (d)	2.38 (m)	43.19 (d)	2.35 (m)	43.31 (d)	2.33 (m)

TABLE 2. Continued.

Carbon	Compound									
	1		2		3		4		5	
	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)
17	15.57 (q)	1.26 (d, 7.0)	15.54 (q)	1.25 (d, 7.1)	15.55 (q)	1.22 (d, 7.1)	15.76 (d, 8.0)	1.21 (d, 7.0)	15.45 (q)	1.18 (d, 7.0)
18	175.14 (s)	—	175.19 (s)	—	175.10 (s)	—	175.60 (s)	—	175.72 (s)	—
19	39.49 (t)	3.30 (dt, 12.7, 6.6)	39.52 (t)	3.28 (q, 6.6)	39.58 (t)	3.30 (m)	39.36 (t)	3.25 (m) 3.15 (m)	39.40 (t)	3.24 (m) 3.19 (m)
20	25.86 (t)	1.82 (m) 1.55 (m)	25.81 (t)	1.80 (m) 1.55 (m)	29.89 (t)	1.83 (m) 1.55 (m)	25.59 (t)	1.70 (m) 1.50 (m)	25.56 (t)	1.78 (m) 1.50 (m)
21	30.44 (t)	1.73 (m) 1.36 (m)	30.43 (t)	1.72 (m) 1.34 (m)	30.56 (t)	1.74 (m) 1.36 (m)	30.24 (t)	1.64 (m) 1.27 (m)	30.22 (t)	1.65 (m) 1.31 (m)
22	74.26 (d)	3.15 (dt, 9.6, 1.8)	74.27 (d)	3.13 (dt, 1.8, 9.7)	74.30 (d)	3.15 (dt, 2.2, 9.4)	74.17 (d)	3.13 (dt, 6.9, 2.0)	74.16 (d)	3.12 (dt, 9.6, 2.1)
23	34.89 (d)	1.29 (m)	34.86 (d)	1.27 (m)	34.86 (d)	1.26 (m)	34.84 (d)	1.20 (m)	34.76 (d)	1.26 (m)
24	17.94 (q)	0.76 (d, 6.6)	17.98 (q)	0.79 (d, 6.45)	18.05 (q)	0.77 (d, 6.3)	17.95 (q)	0.78 (d, 6.5)	17.91 (q)	0.78 (d, 6.5)
25	27.87 (t)	1.58 (m) 1.46 (m)	27.91 (t)	1.60 (m) 1.43 (m)	28.00 (t)	1.58 (m) 1.44 (m)	27.85 (t)	1.58 (m) 1.45 (m)	27.75 (t)	1.56 (m) 1.42 (m)
26	36.10 (t)	1.61 (m) 1.45 (m)	36.11 (t)	1.60 (m) 1.46 (m)	36.16 (t)	1.58 (m) 1.42 (m)	36.09 (t)	1.61 (m) 1.47 (m)	35.98 (t)	1.60 (m) 1.43 (m)
27	95.44 (s)	—	95.49 (s)	—	95.20 (s)	—	95.50 (s)	—	95.48 (s)	—
28	35.47 (t)	1.56 (m) 1.38 (m)	35.49 (t)	1.48 (m) 1.39 (m)	35.53 (t)	1.52 (m) 1.38 (m)	35.41 (t)	1.53 (m) 1.31 (m)	35.30 (t)	1.53 (m) 1.35 (m)
29	19.23 (t)	1.83 (m) 1.54 (m)	19.23 (t)	1.53 (m) 1.52 (m)	19.20 (t)	1.83 (m) 1.56 (m)	19.22 (t)	1.75 (m) 1.50 (m)	19.14 (t)	1.76 (m) 1.52 (m)
30	31.34 (t)	1.52 (m) 1.13 (m)	31.34 (t)	1.51 (m) 1.16 (m)	31.41 (t)	1.40 (m) 1.14 (m)	31.24 (d)	1.55 (m) 1.12 (m)	31.17 (t)	1.49 (m) 1.22 (m)
31	69.07 (d)	3.45 (m)	69.09 (d)	3.42 (m)	68.90 (d)	3.44 (m)	69.13 (d)	3.35 (m)	68.98 (d)	3.40 (br t)
32	34.09 (t)	1.42 (m) 1.29 (m)	34.09 (t)	1.39 (m) 1.30 (m)	34.84 (t)	1.34 (m) 1.34 (m)	34.01 (t)	1.29 (m) 1.42 (m)	33.87 (t)	1.40 (m) 1.25 (m)

TABLE 2. Continued.

Carbon	Compound									
	1		**2**		**3**		**4**		**5**	
	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)	^{13}C (mult.)	^{1}H (mult., J_{HH}, Hz)
33	33.48 (t)	1.41 (m) 1.30 (m)	33.48 (t)	1.32 (m) 1.28 (m)	33.38 (t)	1.49 (m) 1.55 (m)	33.48 (t)	1.40 (m) 1.30 (m)	33.35 (t)	1.35 (m) 1.30 (m)
34	31.88 (d)	2.36 (m)	31.88 (d)	2.38 (m)	31.80 (d)	2.58 (m)	31.82 (d)	2.32 (m)	31.65 (d)	2.31 (m)
35	20.97 (q)	0.96 (d, 6.8)	20.94 (q)	0.96 (d, 6.75)	20.10 (q)	1.01 (d, 7.1)	20.89 (q)	0.95 (d, 6.8)	20.83 (q)	0.91 (d, 4.7)
36	131.32 (d)	5.20 (d, 9.2)	131.40 (d)	5.16 (d, 9.5)	149.26 (d)	6.37 (dq, 9.8, 1.2)	131.14 (d)	5.20 (d, 9.3)	131.13 (d)	5.15 (d, 9.40)
37	137.16 (s)	—	137.22 (s)	—	136.29 (s)	—	137.15 (s)	—	137.04 (s)	—
38	11.82 (q)	1.62 (fd, 1.3)	11.81 (q)	1.60 (d, 1.20)	11.46 (q)	1.73 (fd, 1.2)	11.95 (q)	1.57 (fd, 1.3)	11.81 (q)	1.58 (fd, 1.3)
39	73.26 (d)	4.19 (m)	73.29 (d)	4.24 (m)	200.17 (s)	—	73.22 (d)	4.16 (m)	73.03 (d)	4.15 (q, 6.4)
40	21.75 (q)	1.25 (d, 6.3)	21.75 (q)	1.28 (d, 7.00)	25.54 (q)	2.28 (s)	21.78 (q)	1.25 (d, 6.3)	21.74 (q)	1.21 (d, 6.3)
NH		7.30 (br t, 5.8)		7.27 (br t, 6.0)		7.31 (br t, 5.9)		7.05 (br t, 5.9)		7.12 (br t, 6.5)
13/14 NH		6.95		6.92		6.97		6.75		6.80
18/19 OH-4...		(br t, 5.5) —		(br t, 6.9) —		(br t, 5.7) —		(br t, 5.5) n.o.[a]		(br t, 5.6) n.o.[a]
OH-11..		—		—		—		—		n.o.[a]
OH-15..		4.61 (d, 5.3)		4.61 (d, 4.9)		4.59 (br s)		4.62		4.88
OH-39..		3.70 (b)		n.o.		—		n.o.[a]		2.85

[a]n.o.=not observed.

TABLE 3. Cytotoxic Activity of Bistramides A [**1**], B [**2**], C [**3**], D [**4**], and K [**5**] (IC_{50} in μg/ml).[a]

Compound	KB	P388	P388/dox.	B16	HT29	NSCLC-N6
1	0.53	0.20	0.05	0.10	0.32	0.03
2	2.10	0.20	1.16	1.20	0.71	0.32
3	0.65	0.02	0.05	0.06	0.50	0.05
4	10.00	0.36	5.82	0.10	2.76	3.43
5	>10.00	0.57	>10.00	1.90	5.60	3.23
6-MP[b]	0.55	0.70	0.26	0.80	0.87	0.79

[a]Mean value for 3 experiments.
[b]6-Mercaptopurine as control.

Cytofluorimetric analysis (Figure 1a) was used to study the mechanism of action of the bistramides on the cell cycle. We observed a complete block of NSCLC-N6 cells in the G_1 phase after 48 h of growth with BST-K [**5**], and a significant decrease of the S phase

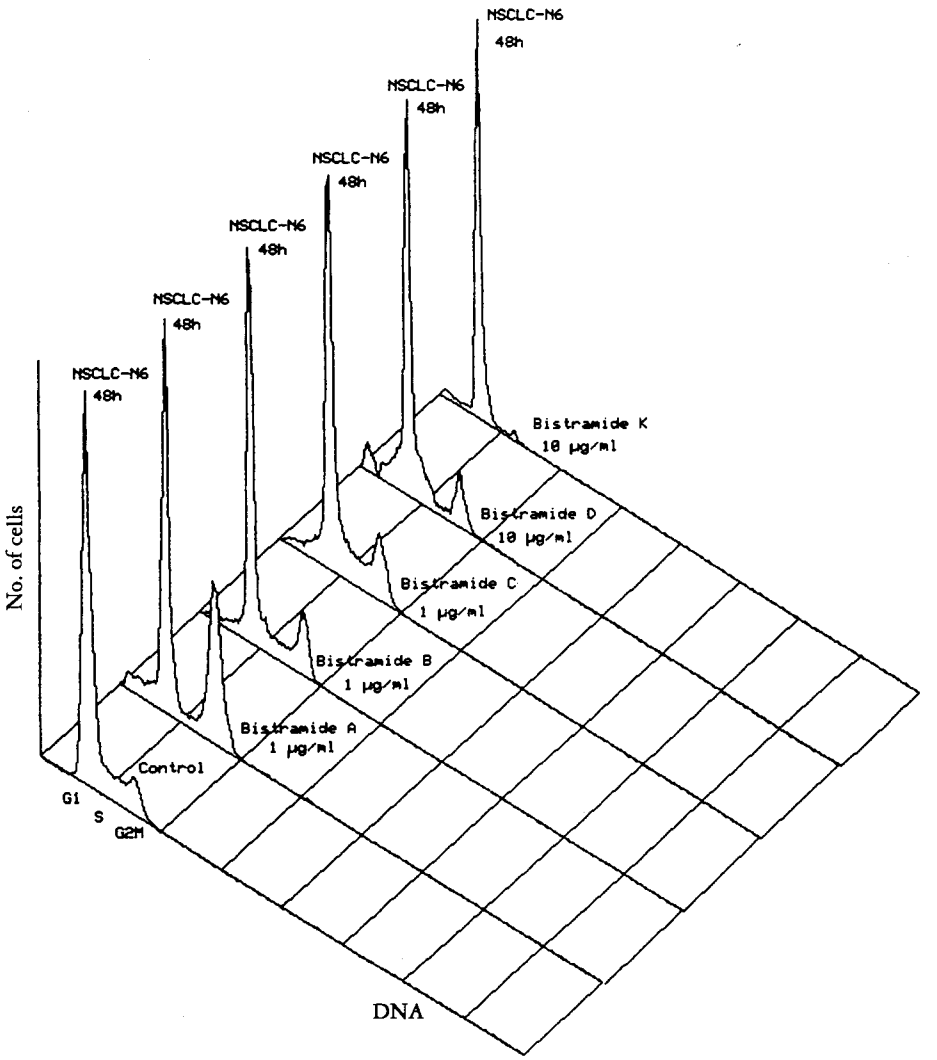

FIGURE 1a. DNA histogram of NSCLN-N6 cells cultured in the presence of different bistramides for 48 h.

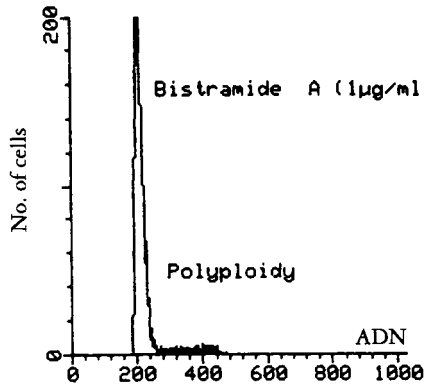

FIGURE 1b. By means of an additional gate on the DNA cytogram, the most fluorescent cells corresponding to the "4C" chromosome peaks (polyploidy).

by BST-A [**1**], -B [**2**], -C [**3**], and -D [**4**] (with partial block in the G_1 phase) as compared with control cells. In the case of BST-A [**1**], the observed increase of the G_2 M peak is probably due to the presence of cells with 4n chromosomes, a result of the inhibition in cytodieresis for those cells, hence the observed polyploidy (Figure 1b). A consequence of cell blockage in G_1 is cell death, hence the observed cellular debris in all curves before the G_1 peak. BST-D [**4**] and -K [**5**] were tested for their in vivo (iv and ip) antitumor activity in nude mice engrafted sc with NSCLC-N6. T/C values of 53% for BST-D, 49% for BST-K were obtained at day 30. BST-A [**1**] was tested in the PS model, with the observed T/C value being 118%; this compound was deemed too toxic for a significant antitumor effect to be observed (22, 23, 29, 30).

From the pharmacological point of view, these molecules exhibit an in vitro cytotoxic activity (that can be linked to the presence of a carbonyl group in C-4, especially when it is α,β-unsaturated as for BST-A [**1**] and -C [**3**]) and an antiproliferative activity as shown by the irreversible blocking of cells in the G_1 DT phase (24). These two effects, when associated in vitro, lead to interesting values of IC_{50} (<1 µg/ml) according to the National Cancer Institute norms for BST-A [**1**], -B [**2**], and -C [**3**], but less interesting values were observed for BST-D [**4**] and -K [**5**] (>1 µg/ml). However, the absence of toxicity in vivo, particularly for BST-K [**5**], allows a therapeutic plateau for daily treatment (for 16 days ip), and hence an antitumor activity in the case of slowly evolving tumors, such as non-small cell pulmonary carcinoma (T/C 49%), in spite of the mdr observed in vitro.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hreims were recorded on a Varian MAT 311 spectrometer at 70 keV and a resolving power of 1500. Fabms were obtained on a Kratos Concept II HH. The samples were dissolved in thioglycerol and a small drop of the sample solution was placed on the copper target of the fab direct-insertion probe. The sample was bombarded with 7 keV xenon atoms and the ions produced were accelerated through 8 kV and negative ions were detected. Nmr spectra were obtained using a C-5 dual ^1H, ^{13}C probe in a Bruker AM 400 WB spectrometer. Compounds were dissolved in $CDCl_3$ (Aldrich) and chemical shifts were derived relative to tetramethylsilane (TMS).

Low-pressure and high-pressure liquid chromatography (lplc, hplc) were performed on LDC, Kontron, and Cedi chromatographs. All solvents used in the extraction and separation were dehydrated and distilled prior to use.

EXTRACTION AND ISOLATION.—Substantial collections of *Lissoclinum bistratum* (13 kg, wet wt) were

made in the spring of 1990 near Ua and N'Do Islands, on the south coast of New Caledonia and immediately freeze-dried. A voucher specimen (No. UA 79) has been deposited at the Centre ORSTOM at Nouméa, New Caledonia. The dried powder (5900 g) cleaned of debris, but with symbiotic *Prochloron*, was extracted four times with CH_2Cl_2 (24 liters each). The organic solution was evaporated under reduced pressure yielding a crude extract (44.2 g, 0.75% of dried material), which was then dissolved in CH_2Cl_2 and separated on a lplc column (glass column, 100×5 cm; 900 g SDS Si gel 60–200 μm; isocratic EtOAc-MeOH, 93:7, 6.2 ml/min) yielding numerous bistramide-containing fractions. Two of these fractions were then rechromatographed by hplc (Cedi column, 30×5.2 cm; Si gel 8 μm; isocratic CH_2Cl_2-MeOH, 95:5, 90 ml/min) affording four main fractions: 1 (1004 mg), 2 (5950 mg), 3 (2992 mg), and 4 (532 mg). Fraction 1 was further purified by hplc (Interchim-column, 25×2.2 cm; Si gel 10 μm; isocratic CH_2Cl_2-MeOH-H_2O, 91.2:3.8:5.0, 20 ml/min) and gave fractions 1.1 and 1.2. Bistramide B ([**2**], 67 mg, 1.1×10^{-3}% of dry material) was obtained from fraction 1.1 by hplc (Biochrom-column, 25×1.0 cm; C-18 5 μm; CH_3CN-H_2O, 85:15, 5 ml/min). Fraction 1.2 was pure bistramide C ([**3**], 125 mg, 2.0×10^{-3}%). Fraction 2 was shown to be pure bistramide A ([**1**], 5950 mg, 0.1%). Fraction 3 was further rechromatographed by hplc (Cedi-column, 30×5.2 cm; C-18 15–25 μm; isocratic MeOH-H_2O, 85:15, 85 ml/min) and afforded bistramide D ([**4**], 1171 mg, 19.8×10^{-3}%). Finally, fraction 4 rechromatographed by hplc (Interchim-column, 25×2.0 cm; C-18 10 μm; MeOH-H_2O, 85:15, 10 ml/min) afforded pure bistramide K ([**5**], 213 mg, 3.6×10^{-3}%).

Bistramide A [**1**].—Amorphous solid; $[\alpha]^{20}D$ +10° (*c*=0.05, CH_2Cl_2); uv λ max 240 (ϵ_o 3140) nm; ir ν max 3300, 2900, 1640, 1550, 1440, 1380, 1230, 1070, and 980 cm^{-1}; ms, see Table 1; ^1H- and ^{13}C-nmr spectra, see Table 2.

Bistramide B [**2**].—Amorphous solid; $[\alpha]^{20}D$ +10° (*c*=0.01, CH_2Cl_2); uv λ max 233 (ϵ_o 2090) nm; ir ν max 3300, 2910, 1640, 1540, 1450, 1380, 1220, 1090, and 980 cm^{-1}; ms, see Table 1; ^1H- and ^{13}C-nmr spectra, see Table 2.

Bistramide C [**3**].—Amorphous solid; $[\alpha]^{20}D$ +10° (*c*=0.05, CH_2Cl_2); uv λ max 242 (ϵ_o 31700) nm; ir ν max 3300, 2900, 1650, 1540, 1440, 1390, 1230, 1070, and 985 cm^{-1}; ms, see Table 1; ^1H- and ^{13}C-nmr spectra, see Table 2.

Bistramide D [**4**].—Amorphous solid; $[\alpha]^{20}D$ +8° (*c*=0.04, CH_2Cl_2); uv λ max 232 (ϵ_o 490) nm; ir ν max 3350, 2950, 1650, 1550, 1450, 1380, 1230, 1080, and 985 cm^{-1}; ms, see Table 1; ^1H- and ^{13}C-nmr spectra, see Table 2.

Bistramide K [**5**].—Amorphous solid; $[\alpha]^{20}D$ +20° (*c*=0.02, CH_2Cl_2); uv λ max 230 (ϵ_o 130) nm; ir ν max 3300, 2940, 1650, 1550, 1450, 1380, 1230, 1090, and 990 cm^{-1}; ms, see Table 1; ^1H- and ^{13}C-nmr spectra, see Table 2.

IN VITRO BIOASSAYS.—*NSCLC-N6 cell line*.—This line was used for all determinations and derives from a human non-small-cell bronchopulmonary carcinoma (22), moderately differentiated, rarely keratinizing, classified as T2N0M0. The line was grafted into a nude mouse and then cultured in RPMI 1640 medium (Intermed) with 5% fetal calf serum, to which were added 100 i.u. penicillin/ml, 100 μg streptomycin/ml, and 2 mM glutamine.

Cytotoxicity determinations.—Experiments were performed in microtiter plates (0.1×10^5 cells/ml). Cell growth was estimated by a colorimetric assay based on conversion of tetrazolium dye (MTT) to a blue formozan product using live mitochondria at 72 h (31).

Flow cytometric assay.—For DNA staining, 0.8×10^5 cells were cultured in 25-ml flasks in the presence or absence of test compound. Cells were stained directly in the flasks by 1 ml Vindelov solution (32) after removal of culture medium. The DNA content of 2000 naked nuclei was measured using a Becton-Dickinson Fascan flow cytometer.

IN VIVO BIOASSAYS.—The NSCLC-N6 line was regularly transplanted sc into nude mice, with each mouse receiving 0.2 ml of a cell solution obtained by mechanical dispersion of about 1 g of excised mouse tumor in 4.8 ml of sterile saline solution. The mice were allocated in groups of six animals each for testing when tumor size reached 50–200 mm^3. Solutions of bistramides A [**1**], D [**4**], and K [**5**] were prepared in physiological salt solution supplemented with 2% DMSO (Sigma). Injections were made at days 1, 5, 9, 11 (iv) for BST-D (4×20 mg/kg), daily (days 1 to 16) ip for BST-K (10 mg/kg each), and at days 1, 5, 9 ip for BST-A (0.4 mg/kg).

LITERATURE CITED

1. C.M. Ireland and P.J. Scheuer, *J. Am. Chem. Soc.*, **102**, 5688 (1980).
2. J.M. Wasylyk, J.E. Biskupiak, C.E. Costello, and C.M. Ireland, *J. Org. Chem.*, **48**, 4445 (1983).

3. D.F. Sesin, S.J. Gaskell, and C.M. Ireland, *Bull. Soc. Chim. Belg.*, **95**, 853 (1986).
4. K. Kohda, Y. Ohta, Y. Kawazoe, T. Kato, Y. Suzumura, Y. Hamada, and T. Shiori, *Biochem. Pharmacol.*, **38**, 4500 (1989).
5. D.E. Williams, R.E. Moore, and V.J. Paul, *J. Nat. Prod.*, **52**, 732 (1989).
6. C.M. Ireland, A.R. Durso, Jr., R.A. Newman, and M.P. Hacker, *J. Org. Chem.*, **47**, 1807 (1982).
7. F.J. Schmitz, M.B. Ksebati, J.S. Chang, J.L. Wang, H. Bilayet, and D. Van der Helm, *J. Org. Chem.*, **54**, 3463 (1989).
8. L.A. McDonald and C.M. Ireland, *J. Nat. Prod.*, **55**, 376 (1992).
9. C.J. Hawkins, M.F. Lavin, K.A. Marshall, A.L. Van den Brenk, and D.J. Watters, *J. Med. Chem.*, **33**, 1634 (1990).
10. C.J. Hawkins, J. Clifford, D.J. Watters, M.F. Lavin, D.L. Parry, and E.J. McCaffrey, PCT Int. Appl. WO 90 05,731 (1990).
11. A.R. Carroll, B.F. Bowden, and J.C. Coll, *Proc. 7th Int. Symp. Mar. Nat. Prod.*, Capri, Italy (1992).
12. B.S. Davidson and C.M. Ireland, *J. Nat. Prod.*, **53**, 1036 (1990).
13. T.M. Zabriskie, C.L. Mayne, and C.M. Ireland, *J. Am. Chem. Soc.*, **110**, 7919 (1988).
14. D.G. Corley, R.E. Moore, and V.J. Paul, *J. Am. Chem. Soc.*, **110**, 7920 (1988).
15. M. Litaudon and M. Guyot, *Tetrahedron Lett.*, **32**, 911 (1991).
16. B.S. Davidson, T.F. Molinski, L.R. Barrows, and C.M. Ireland, *J. Am. Chem. Soc.*, **113**, 4709 (1991).
17. T.F. Molinski and C.M. Ireland, *J. Org. Chem.*, **54**, 4256 (1989).
18. B.M. Degnan, C.J. Hawkins, M.F. Lavin, E.J. McCaffrey, D.L. Parry, and D.J. Watters, *J. Med. Chem.*, **32**, 1349 (1989).
19. B.M. Degnan, C.J. Hawkins, M.F. Lavin, E.J. McCaffrey, D.L. Parry, and D.J. Watters, *J. Med. Chem.*, **32**, 1354 (1989).
20. M.P. Foster, G.P. Concepcion, G.B. Caraan, and C.M. Ireland, *J. Org. Chem.*, **57**, 6671 (1992).
21. M.P. Foster, C.L. Mayne, R. Dunkel, R.J. Pugmire, D.M. Grant, J.M. Kornprobst, J.F. Verbist, J.F. Biard, and C.M. Ireland, *J. Am. Chem. Soc.*, **114**, 1110 (1992).
22. C. Roussakis, C. Gratas, A.F. Audouin, J. Le Boterff, C. Dabouis, M.J. Andre, E. Moyon, N.H. Vo, and J.F. Verbist, *Anticancer Res.*, **11**, 2239 (1991).
23. C. Roussakis, N. Robillard, D. Riou, J.F. Biard, G. Pradal, P. Piloquet, C. Debitus, and J.F. Verbist, *Cancer Chemother. Pharmacol.*, **28**, 283 (1991).
24. D. Riou, C. Roussakis, N. Robillard, J.F. Biard, and J.F. Verbist, *Biol. Cell*, **77**, 261 (1993).
25. D. Gouiffes, M. Juge, N. Grimaud, L. Welin, M.P. Sauviat, Y. Barbin, D. Laurent, C. Roussakis, J.P. Henichart, and J.F. Verbist, *Toxicon*, **26**, 1129 (1988).
26. M.P. Sauviat, D. Gouiffes-Barbin, E. Ecault, and J.F. Verbist, *Biochem. Biophys. Acta*, **1103**, 109 (1992).
27. M.P. Sauviat and J.F. Verbist, *Gen. Physiol. Biophys.*, **12**, 465 (1993).
28. D. Gouiffes, S. Moreau, N. Helbecque, J.L. Bernier, J.P. Henichart, Y. Barbin, D. Laurent, and J.F. Verbist, *Tetrahedron*, **44**, 451 (1988).
29. D. Riou, C. Roussakis, J.F. Biard, and J.F. Verbist, *Anticancer Res.*, **13**, 6 (1993).
30. C. Roussakis, J. Charrier, D. Riou, J.F. Biard, C. Malochet-Grivois, K. Meflah, and J.F. Verbist, *Anti-Cancer Drug Des.*, **9**, 119 (1994).
31. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).
32. L.L. Vindelov, *Virchows Arch. B*, **24**, 227 (1977).

Received 6 December 1993