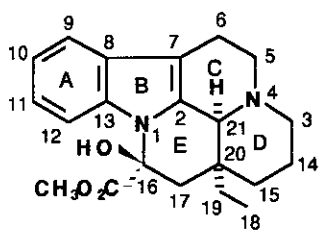
SYNTHESIS OF POTENTIAL CYTOTOXIC QUATERNARY AMMONIUMS BY OXIDATION OF EBURNANE ALKALOIDS

Guy Lewin*[a], Corinne Schaeffer [b], Alain Pierré [b], Nicolas Guilbaud [b], Ghanem Atassi [b], and Jacques Poisson [c]

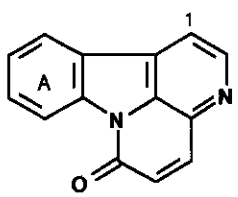
Laboratoire de Pharmacognosie, Faculté de Pharmacie, 1 rue Vaubénard, 14032 Caen Cedex France [a]. Institut de Recherches Servier, 11 rue des Moulineaux, 92150 Suresnes France [b]. Laboratoire de Chimie des Substances Thérapeutiques Naturelles, Faculté de Pharmacie, av. J.B. Clément, 92296 Châtenay-Malabry Cedex France [c]

Abstract- Oxidation of vincamine and substituted analogs on ring-A was carried out in two steps: benzylic oxidation by Jones reagent and aromatization of ring-C with $Hg(OAc)_2$ or $Tl(OCOCF_3)_3$ in trifluoroacetic acid. Final compounds are quaternary ammoniums which exhibited weak cytotoxicity or no cytotoxicity according to substituents on ring-A and nature of ring-E.

In the field of indole alkaloids, several compounds with an eburnane skeleton as vincamine (**1**), vinpocetine and vinburnine are used in medicine for their cerebrovascular activity. Otherwise, many alkaloids with a canthin-6-one structure (**2**) exhibit cytotoxicity and antileukemic activity.[1,2] It is known that this activity has relation to the planar structure and enhances with oxygenation at positions 1 and on the ring-A.[3]

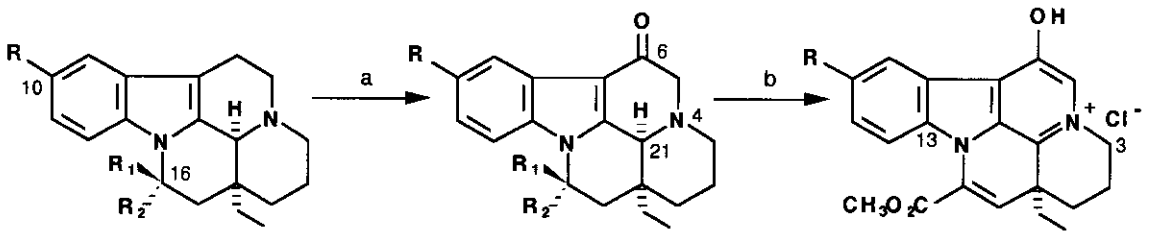


1 (biogenetic numerotation) [4]



2

Therefore, in search of new cytotoxic products, we synthesized new eburnane derivatives by oxidation of vincamine and some analogs on the cycle C. These new compounds own a phenolic group at position-6, a partial planar structure (on cycles A, B, C and, for some of them, part of cycle E) and various substitutions (chloro, bromo, nitro, methoxy) on the ring A. Oxidation of cycle C was carried out in two steps: a) benzylic oxidation on C-6 by Jones reagent (CrO_3/H_2SO_4/acetone);[5] b) oxidation of 4-21 bond by mercuric acetate or thallium tris(trifluoroacetate) (TTFA) with aromatization of the cycle C into hydroxypyridinium. In the second step, when trifluoroacetic acid was used as solvent instead of acetic acid, it was possible (according to the substituents on ring A) to effect aromatization then dehydration of the 16-17 bond. In that manner, vincamine **(1)**, 10-bromovincamine **(3)**[6] and 10-chloro-16-epivincamine **(4)**[7] respectively led *via* 6-oxo compounds to quaternary ammoniums **(8)**, **(9)** and **(10)** (Scheme 1).



a: Jones reagent; b: $Hg(OAc)_2$ or $Tl(OCOCF_3)_3$ in trifluoroacetic acid.

	R	R_1	R_2
1	H	OH	CO_2CH_3
3	Br	OH	CO_2CH_3
4	Cl	CO_2CH_3	OH

	R	R_1	R_2
5	H	OH	CO_2CH_3 (24%)
6	Br	OH	CO_2CH_3 (21%)
7	Cl	CO_2CH_3	OH (23%)

	R	
8	H	(55%)
9	Br	(50%)
10	Cl	(32%)

Scheme 1

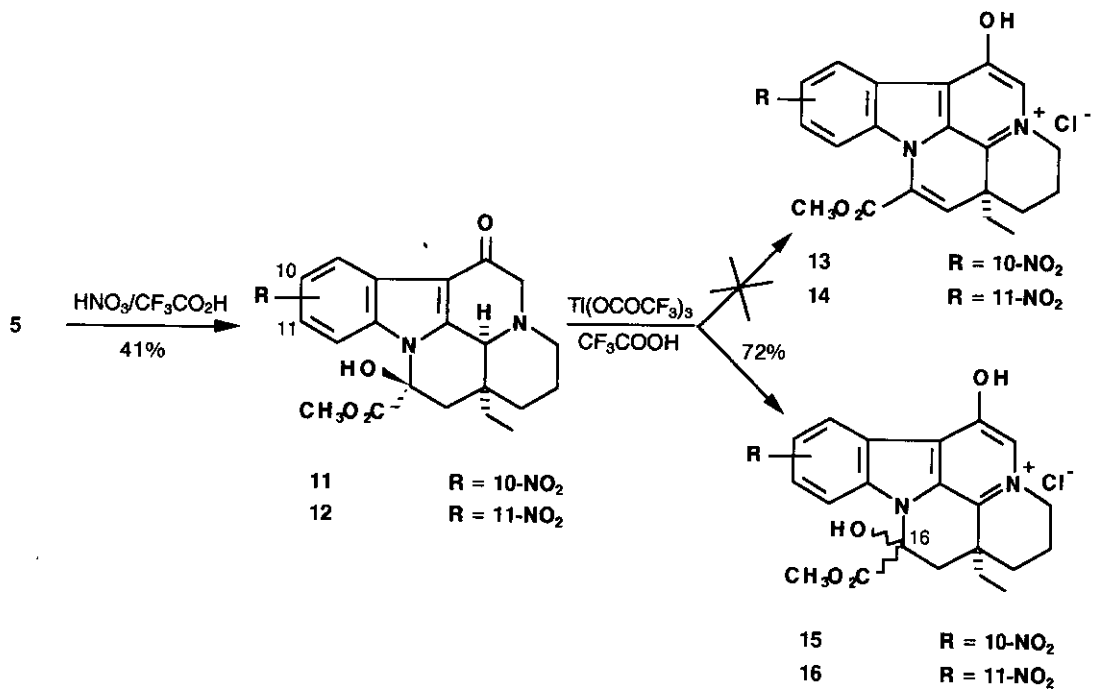
The structures of these compounds were deduced from spectral analysis, especially ^1H and ^{13}C nmr studies on the bromo derivative **(9)** (Table 1). Compound **(9)** is a monochloride (molecular formula $C_{21}H_{20}N_2O_3BrCl$ according to microanalysis), but ^1H nmr may not unambiguously choose between several possible resonant forms. 2 D ^{13}C-^1H ^1J and ^3J correlation experiments allowed all carbons assignments and confirmed the structure **(9)**. Especially significant was the chemical shift of C-6 near 150 ppm: the shielding of this carbon inferred the presence at C-6 of a phenol group and not a carbonyl or a phenolate,[8] clearly excluding a structure of zwitterion phenolate-ammonium on N-1 or N-4. Otherwise, chemical shifts of C-13 and C-3 near 138 and 53 ppm and

deshielded H-3 signals at 4.7-5.0 ppm located the ammonium function at N-4. The phenol group exhibited in ir a broad band around 2500 cm^{-1} [9] and in ^1H nmr a deshielded signal at 12.5-13.5 ppm, which suggested intermolecular hydrogen bonds.

Table 1: ^1H and ^{13}C Chemical Shifts of Compound **(9)**

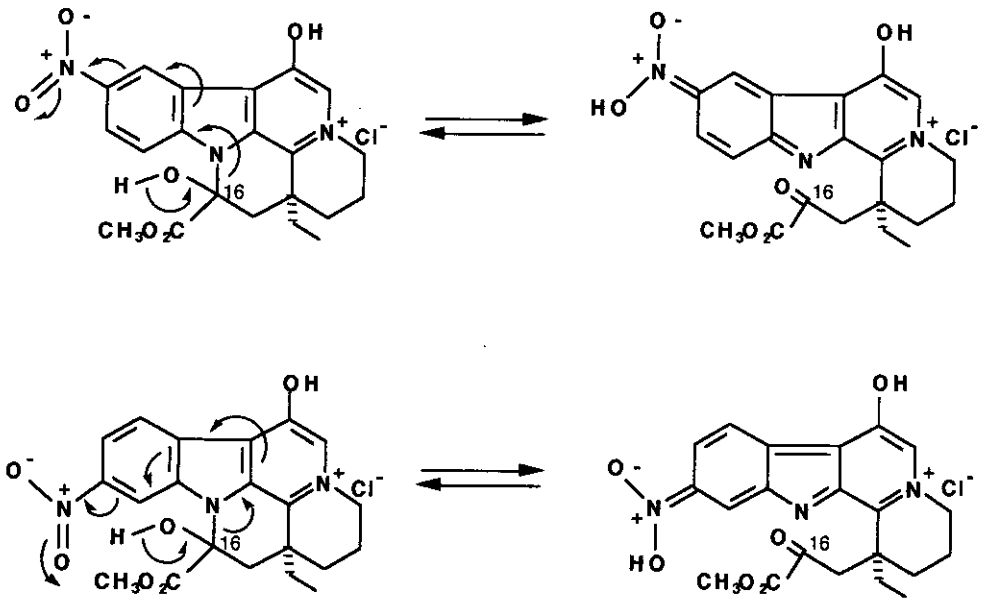
atom	DMSO *d6*		$CDCl_3$	
	^1H	^{13}C	^1H	^{13}C
2		134.2		134.5
3	4.70	52.4	4.7-5.0	53.4
5	8.35	124.6	8.80	124.8
6		149.6		151.2
7		118.4		120.0
8		115.0		116.4
9	8.30	126.0	7.80	127.1
10		122.5		123.3
11	7.88	132.4	7.45	133.1
12	7.75	117.6	7.55	116.6
13		137.9		138.6
14	2.1-2.4	18.2	2.2-2.6	19.0
15	2.1-2.3	28.2	2.1-2.4	28.7
16		127.2		128.3
17	6.30	125.2	6.20	123.3
18	0.65	8.2	0.97	8.5
19	1.8-2.0	33.4	2.0-2.1	34.9
20		40.0		39.6
21		133.0		133.0
$\underline{C}O_2CH_3$		161.5		161.8
$CO_2\underline{C}H_3$	3.95	53.2	4.02	53.5
OH	13.50		12.50	

Nitration of the ring A was achieved on 6-oxovincamine **(5)**[5] and mainly afforded a mixture 6/4 of 10-nitro-6-oxovincamine **(11)** and 11-nitro-6-oxovincamine **(12)** separated by tlc. Oxidation of **11** by TTFA led to **15** instead of the attempted product **13** (in FAB^+/ms main peak at m/z 412; in ^1H nmr absence of the olefinic signal of H-17). **15** was not a pure compound but an epimeric mixture in a ratio 1/1 measured in ^1H nmr owing to the splitting of signals into two equal parts. In the same way, **12** furnished the mixture 1/1 of epimers **(16)** (Scheme 2).

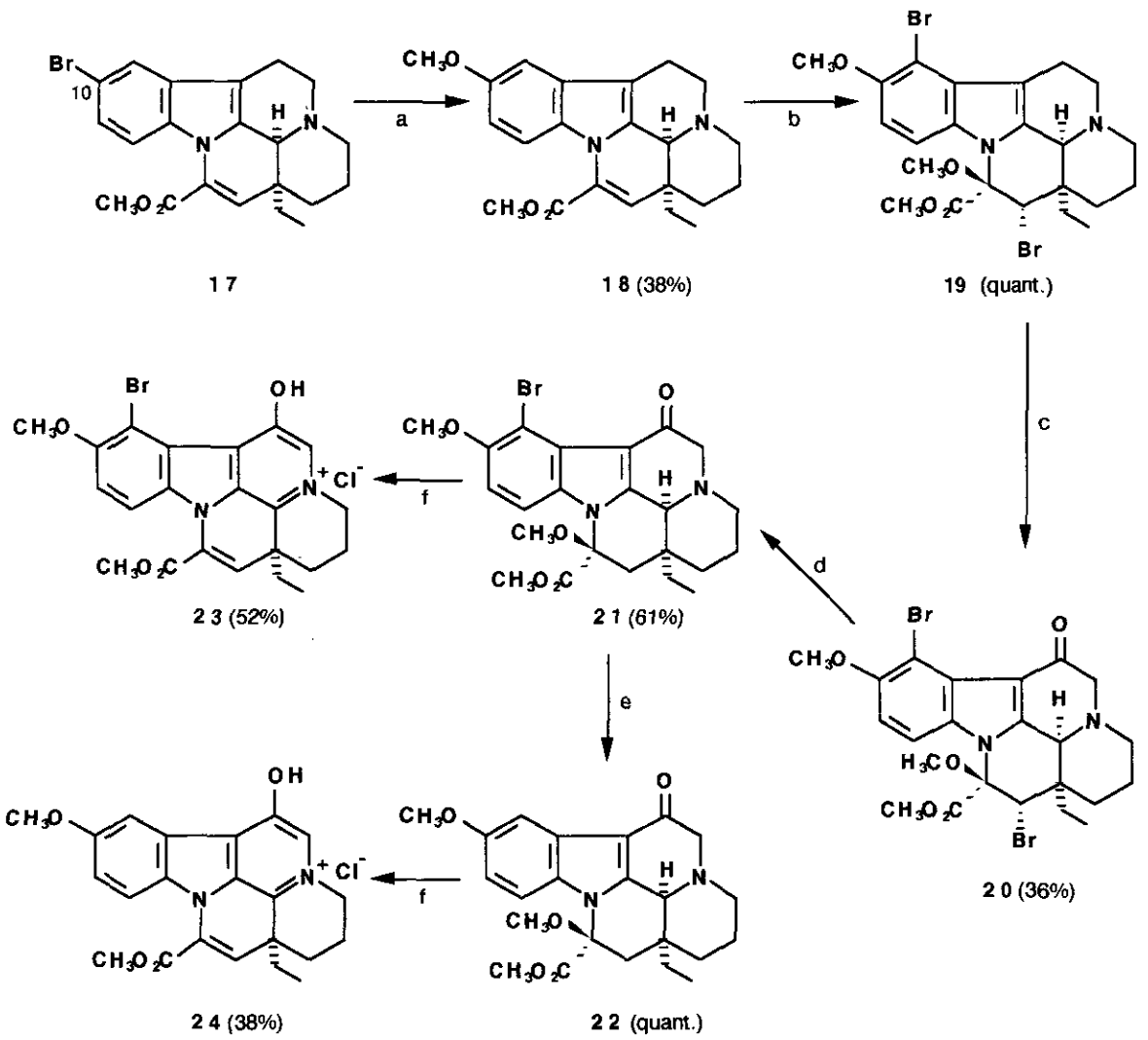


Scheme 2

Electroattractive effect of the nitro group at C-10 or C-11 prevented the dehydration of the 16-17 bond and explained the epimerization at C-16 by breaking of the C1-C16 bond (Scheme 3).



Scheme 3



a: CH_3ONa, CuI, collidine; CH_3OH, HCl; b: Br_2 in CH_3OH; c: Jones reagent; d: H_2, PtO_2 in DMF; e: H_2, Pd/C 5% in CH_3OH; f: $Hg(OAc)_2$ in trifluoroacetic acid.

Scheme 4

Oxygenation at position 10 was performed by nucleophilic substitution (MeONa, CuI, collidine)[10,11] on a 10-bromo derivative. 10-Bromovincamine (**3**) was unsuitable because it would lead to an eburnamonine type structure[12] whereas **6** or **9** gave dirty reactions. Therefore we started from 10-bromo-16-apovincamine (**17**)[13] which provided in two steps 10-methoxyapovincamine (**18**). Direct benzylic oxidation of **18** failed; so synthesis of the attempted quaternary ammonium (**24**) was accomplished from **18** in four steps (Scheme 4): **18** was reacted with bromine in methanol[14] and afforded the dibromo (**19**) which was oxidised by Jones reagent in **20**.

On hydrogenolysis the bromine at C-17 was removed to generate **21** then **22**. Finally, oxidation of **21** and **22** by mercuric acetate respectively furnished **24** and its 9-bromo analog (**23**).

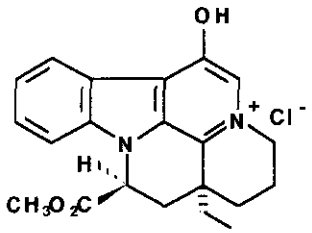
Biological results

The study of the biological properties of eight new derivatives (**8**, **9**, **10**, **15**, **16**, **23**, **24** and the last one **25** the 16-17 dihydroderivative of **8** stereoselectively obtained from **8** by catalytic hydrogenation) was carried out first in vitro on L1210 leukemia cells in culture. The most cytotoxic compound was **9**, which is however about 100 fold less potent than doxorubicin used as a reference molecule (Table 2). Compounds (**15**, **16**, and **25**) were totally inactive, the cellular viability being superior to 80 % at 50 μM. When tested in vivo against the P388 leukemia, compound (**9**) failed to increase the survival of leukemic mice at any dosage tested (50 to 600 mg/kg) while doxorubicin was very active at 10 mg/kg.

Table 2

N°	Cytotoxicity[a] IC50 (μM)	Antitumor activity against P388 leukemia in mice[b]	
		Dosage (mg/kg)	% T/C (dose)
8	19.9		
9	4.8	50-600	102 (600 MK)
10	12.2		
15	>50		
16	>50		
23	21.4		
24	38.4		
25	>50		
Doxorubicine	0.030	10	>280 (10 MK)

[a]: Inhibition of L1210 cells proliferation measured by the microculture tetrazolium assay (means of 2-4 experiments); [b]: mice were inoculated with 10^6 P388 cells on day 0 by the IP route. Treatment with compound (**9**) or doxorubicine was given IP on day 1.



25

As expected, our oxidative method provided cytotoxic compounds but with a weak activity. Oxygenation on the ring-A did not enhance the cytotoxicity as with canthin-6-ones whereas halogenation on C-10 afforded the more active products (**9**, **10**). The 16-17 double bond appeared necessary for an activity (**15**, **16** and **25** were devoided of cytotoxicity), probably by increasing the planar character of the molecule.

EXPERIMENTAL

Chemistry

Uv spectra were acquired on a Unicam SP 1800, ir spectra taken on a Perkin-Elmer 457 spectrophotometer and optical rotations on a Schmidt-Haensch polarimeter. Eims were determined with a Nermag R10-10C. and FAB^+/ms with a Finnigan Mat 90 using a thioglycerol matrix. Nmr spectra were carried out on a Bruker AC-200 (200 MHz for ^1H, 50.3 MHz for ^{13}C) and on a Bruker AMX-500 (500.13 MHz for ^1H, 125.76 MHz for ^{13}C). 2 D ^1H-^{13}C ^1J and ^3J correlation experiments were obtained in inversed detection according to standard microprograms Bruker; delays were optimized for $^1J_{(CH)} = 125$ Hz and $^3J_{(CH)} = 5$ Hz and relaxation delay was 1 s.

Benzylic oxidation by Jones reagent:

In a typical procedure, a mixture of conc H_2SO_4 (4.5 ml) and H_2O (4.5 ml) was added to the indole alkaloid (10 mmol) in acetone (160 ml) at 0°C. After dissolving, chromic anhydride (5 g, 50 mmol) in acetone (25 ml) was added in 15 min with good stirring at 0°C and then the reaction was kept on at room temperature for 3 h. The reaction mixture was diluted with water, 30% aqueous NaOH was added until pH 4 then the solution was thoroughly extracted with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4 and evaporated. In that manner, **1** (8.85 g, 25 mmol), **3** (8.7 g, 20 mmol), **4** (777 mg, 2 mmol), **19** (584 mg, 1.05

mmol) respectively provided **5** (2.2 g, 24%) by crystallization in acetone, **6** (1.88 g, 21%) by crystallization in methanol, **7** (185 mg, 23%) after flash chromatography on silica gel (CH_2Cl_2-MeOH 94-6 v/v) and crystallization in methanol, **20** (215 mg, 36%) after flash chromatography on alumina (CH_2Cl_2).

Oxidation by mercuric acetate or thallium tris(trifluoroacetate) (TTFA):

Both reagents were used on with 6-oxovincamine (**5**) and gave the same compound with the same yield; furthermore reaction conditions being similar with both reagents, the following typical procedure is described. Mercuric acetate or TTFA (1 mmol) was added to a solution of the alkaloid (1 mmol) in trifluoroacetic acid (15 ml) and the reaction mixture was gently heated at reflux under nitrogen (1-5 h). The cooled solution was diluted with CH_2Cl_2 and the organic layer was washed with water until pH 6, then dried over Na_2SO_4, filtered and evaporated under vacuum. The dried residue was dissolved in methanolic 0.1N HCl (20 ml) and evaporated under vacuum to dryness. In that manner: **5** (1.47 g, 4 mmol) heated 4 h with mercuric acetate (1.28 g, 4 mmol) provided **8** (845 mg, 55%) by crystallization in acetone; **6** (1.788 g, 4 mmol) heated 3 h with TTFA (2.18 g, 4 mmol) provided **9** (930 mg, 50%) by crystallization in MeOH-EtOAc; **7** (80 mg, 0.2 mmol) heated 4 h with mercuric acetate (64 mg, 0.2 mmol) provided **10** (27 mg, 32%) by crystallization in acetone; a mixture 6/4 of **11** and **12** (103 mg, 0.25 mmol) heated 1 h with TTFA (136 mg, 0.25 mmol) provided the mixture 6/4 of **15** and **16** (81 mg, 72%) by crystallization in MeOH-EtOAc; a sample of the mixture **15** and **16** was heated 10 h at reflux in trifluoroacetic acid and was entirely recovered; the compounds **(15)** and **(16)** were finally separated by tlc on silica gel (CH_2Cl_2-EtOH 88-12 v/v several migrations); **21** (49 mg, 0.1 mmol) heated 5 h with mercuric acetate (32 mg, 0.1 mmol) provided **23** (25 mg, 52%) by crystallization in MeOH-EtOAc; **22** (41 mg, 0.1 mmol) heated 1.5 h with mercuric acetate (32 mg, 0.1 mmol) provided **24** (15 mg, 38%) by crystallization in acetone.

10-Chloro-16-epivincamine (4). A solution of 10-chloro-14-15-dehydro-16-epivincamine (1.55 g, 4 mmol) in 0.04N methanolic hydrochloric acid (150 ml) was hydrogenated under 1 atm pressure of hydrogen with PtO_2 catalyst (150 mg) for 1 h at room temperature. The catalyst was filtered, water then 0.5N aqueous NaOH added until pH 8 and the layer was extracted with CH_2Cl_2. After the usual work-up, crystallization of the dried residue in acetone yielded **4** (880 mg, 57%): white crystals mp 208-210°C; Anal. Calcd for $C_{21}H_{25}N_2O_3Cl$: C 64.86, H 6.48, N 7.20, Cl 9.12. Found: C 65.02, H 6.44, N 7.12, Cl 9.29; EIms m/z (% rel. int.) 390 and 388 (5 and

15) (M‡), 389 (6), 387 (9), 372 (14), 370 (32), 343 (34), 341 (100), 302 (33), 300 (95); uv (EtOH) λ_{max} nm (log ε) 233 (4.54), 282 (3.95), 289 (3.95), 299 (3.83); ^{1}H nmr ($CDCl_3$) δ 0.90 (t, J = 7.5 Hz, 3H, H-18), 3.73 (s, 3H, CO_2CH_3), 3.80 (s, 1H, H-21), 4.2 (br s, 1H, exch. by D_2O, OH), 7.06 (dd, J_{ortho} = 9 Hz, J_{meta} = 2 Hz, 1H, H-11), 7.25 (d, J_{ortho} = 9 Hz, 1H, H-12), 7.40 (d, J_{meta} = 2 Hz, 1H, H-9).

10-Bromo-6-oxovincamine (6): light pink crystals mp 255-257°C; Anal. Calcd for $C_{21}H_{23}N_2O_4Br$: C 56.38, H 5.18, N 6.26, Br 17.86. Found: C 56.09, H 5.40, N 6.19, Br 18.05; EIms m/z (% rel. int.) 448 and 446 (100 and 89) (M‡), 447 (61), 445 (40), 347 (32), 346 (83), 345 (38), 344 (75), 331 (49), 329 (43), 318 (66), 316 (73); uv (EtOH) λ_{max} nm (log ε) 224 (4.47), 247 (4.38), 270 (4.16), 291 (4.12), 300 (4.14); ir (KBr) 3290, 1740, 1635 cm^{-1}; ^{1}H nmr ($CDCl_3$-10 % CD_3OD) δ 0.90 (t, J = 7.5 Hz, 3H, H-18), 3.30 (d, J = 17 Hz, 1H, H-5), 3.70 (d, J = 17 Hz, 1H, H-5), 3.75 (s, 3H, CO_2CH_3), 4.10 (s, 1H, H-21), 7.05 (d, J_{ortho} = 9 Hz, 1H, H-12), 7.25 (dd, J_{ortho} = 9 Hz, J_{meta} = 2 Hz, 1H, H-11), 8.18 (d, J_{meta} = 2 Hz, 1H, H-9).

10-Chloro-6-oxo-16-epivincamine (7): white crystals mp 263-264°C; Anal. Calcd for $C_{21}H_{23}N_2O_4Cl$: C 62.61, H 5.75, N 6.95, Cl 8.80. Found: C 62.89, H 5.74, N 6.83, Cl 8.73; EIms m/z (% rel. int.) 404 and 402 (36 and 100) (M‡), 403 (41), 401 (45), 345 (11), 343 (21), 317 (7), 315 (21), 302 (17), 300 (43), 287 (10), 285 (27), 274 (16), 272 (44); uv (EtOH) λ_{max} nm (log ε) 224 (4.41), 245 (4.34), 269 (4.13), 297 (4.15); ir (KBr) 3290, 1740, 1635 cm^{-1}; ^{1}H nmr ($CDCl_3$-10 % CD_3OD) δ 0.95 (t, J = 7.5 Hz, 3H, H-18), 3.40 (d, J = 17 Hz, 1H, H-5), 3.75 (d, J = 17 Hz, 1H, H-5), 3.75 (s, 3H, CO_2CH_3), 4.18 (s, 1H, H-21), 7.20 (br s, 2H, H-11 and H-12), 8.10 (d, J_{meta} = 2 Hz, 1H, H-9).

6-Hydroxy-*py*-tetradehydroapovincamine chloride (8): bright yellow crystals mp 223-225°C; $[\alpha]_D$ = -524° (c = 0.5, MeOH); Anal. Calcd for $C_{21}H_{21}N_2O_3Cl$: C 65.54, H 5.50, N 7.28. Found: C 65.20, H 5.44, N 7.16; EIms m/z (% rel. int.) 348 (3), 319 (27), 290 (11), 261 (100); uv (EtOH) λ_{max} nm (log ε) 257 (4.48), 294 (3.84), 320 (4.00), 346 (3.49), 392 (3.81), 407 (3.91); ir (CH_2Cl_2) 2500, 1735 cm^{-1};^{1}H nmr (DMSO-d6) δ 0.60 (t, J = 7.5 Hz, 3H, H-18), 1.65-2.5 (m, 6H, H-14, H-15, H-19), 3.95 (s, 3H, CO_2CH_3), 4.67 (m, 2H, H-3), 6.35 (s, 1H, H-17), 7.47 (td, J_{ortho} = 9 Hz, J_{meta} = 2 Hz, 1H, H-10), 7.75 (m, 2H, H-11 and H-12), 8.30 (s, 1H, H-5), 8.35(*dd*, J_{ortho} = 9 Hz, J_{meta} = 2 Hz, 1H, H-9).

10-Bromo-6-hydroxy-*py*-tetradehydroapovincamine chloride (9): bright yellow crystals mp 238-240°C; $[\alpha]_D$ = -397° (c = 0.7, MeOH); Anal. Calcd for $C_{21}H_{20}N_2O_3BrCl$: C 54.39, H 4.35, N 6.04, Cl 7.64, Found: C 54.29, H 4.35, N 6.04, Cl 7.29; EIms m/z 428-426, 399-397, 370-368, 341-339 (100); uv (EtOH) λ_{max} nm (log ε) 261 (4.56), 300 (3.97), 319 (3.97), 346 (3.64), 398 (3.79), 417 (3.91); ir (CH_2Cl_2) 2500, 1735 cm^{-1}. ^1H and ^{13}C nmr: see Table 1 in the text.

10-Chloro-6-hydroxy-*py*-tetradehydroapovincamine chloride (10): bright yellow crystals mp 215-217°C; EIms m/z 384-382, 355-353, 326-324, 297-295 (100); uv (EtOH) λ_{max} nm (log ε) 259 (4.36), 299 (3.77), 317 (3.77), 346 (3.42), 396 (3.74), 414 (3.83); ir (CH_2Cl_2) 2500, 1735 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.90 (t, J = 7.5 Hz, 3H, H-18), 1.8-2.7 (m, 6H, H-14, H-15, H-19), 4.03 (s, 3H, CO_2CH_3), 4.7-4.95 (m, 2H, H-3), 6.25 (s, 1H, H-17), 7.35 (dd, J_{ortho} = 9 Hz, J_{meta} = 2 Hz, 1H, H-11), 7.62(d, J_{ortho} = 9 Hz, 1H, H-12), 7.67 (d, J_{meta} = 2 Hz, 1H, H-9), 8.80 (s, 1H, H-5), 13 (br s, 1H, exch. by D_2O, OH).

10-Nitro-6-oxovincamine (11) and **11-Nitro-6-oxovincamine (12)**. To a solution of 6-oxovincamine (**5**) (368 mg, 1 mmol) in trifluoroacetic acid (15 ml), HNO_3 (d = 1.33, 2.5 ml) was added and the solution was kept at room temperature for 20 h. The reaction mixture was diluted with CH_2Cl_2, and iced water then 5N aqueous NaOH carefully added until pH 4. The organic layer was washed with water, dried over Na_2SO_4, filtered and evaporated. Crystallization of the dried residue in methanol afforded the nitro compounds (**11**) and (**12**) in mixture (170 mg, 41%). A part of this mixture (50 mg) was purified by tlc on silica gel (CH_2Cl_2-MeOH 97-3 v/v, several migrations) and provided **11** (18 mg) and **12** (12 mg) recrystallized from methanol for analysis.
10-Nitro-6-oxovincamine (11): yellowish crystals mp 233-235°C; $[\alpha]_D$ = +38.6° (c = 0.5, $CHCl_3$); EIms m/z (% rel. int.) 413 (80) (M^+), 412 (35), 354 (31), 343 (20), 326 (29), 324 (26), 311 (100), 296 (55), 283 (62); uv (EtOH) λ_{max} nm (log ε) 240 (4.20), 261 (4.40), 308 (3.97); ir (KBr) 3390, 1740, 1645 cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.00 (t, J = 7.5 Hz, 3H, H-18), 3.45 (d, J = 17 Hz, 1H, H-5), 3.78 (d, J = 17 Hz, 1H, H-5), 3.85 (s, 3H, CO_2CH_3), 4.20 (s, 1H, H-21), 4.90 (br s, 1H, exch. by D_2O, OH), 7.22 (d, J_{ortho} = 9 Hz, 1H, H-12), 8.12 (dd, J_{ortho} = 9 Hz, J_{meta} = 2 Hz, 1H, H-11), 9.05 (d, J_{meta} = 2 Hz, 1H, H-9).
11-Nitro-6-oxovincamine (12): yellowish crystals mp 212-214°C; $[\alpha]_D$ = +38.4° (c = 0.6, $CHCl_3$); EIms m/z (% rel. int.) 413 (75) (M^+), 412 (28), 354 (24),353 (26), 326 (38), 311 (100), 296 (66), 283 (56); uv (EtOH) λ_{max} nm (log ε) 260 sh (4.03), 281 (4.28), 340 (3.88); ir (KBr) 3400, 1740, 1645 cm^{-1}; ^1H nmr

($CDCl_3$) same main signals as **11** except for aromatic protons at δ 8.10 (d, J_{meta} = 2 Hz, 1H, H-12), 8.25-8.35 (m, 2H, H-9 and H-10).

6-Hydroxy-10-nitro-*py*-tetradehydrovincamine chloride (15) (mixture 1/1 of 16R and 16S): FAB^+/ms m/z 412 (100); uv (EtOH) λ_{max} nm 223, 241, 289, 356, 372, 392; ^1H nmr (CD_3OD): the spectrum exhibited a splitting of signals in two equal parts and the 1/1 epimeric ratio was easily proved on signals of CO_2CH_3 (3.75 and 3.90 ppm), H-12 (7.70 and 8.05 ppm) and H-5 (7.90 and 7.95 ppm).

6-Hydroxy-11-nitro-*py*-tetradehydrovincamine chloride (16) (mixture 1/1 of 16R and 16S): FAB^+/ms m/z 412 (100); uv (EtOH) λ_{max} nm 236, 275 sh, 305, 328, 404, 430 sh; ^1H nmr (CD_3OD): same splitting as in **15** was observed and the 1/1 epimeric ratio proved on signals of CO_2CH_3 (3.75 and 3.95 ppm), H-5 (7.90 and 7.95 ppm) and H-12 (8.50 and 8.75 ppm).

10-Methoxyapovincamine (18). Sodium (207 mg, 9 matom) was dissolved in absolute methanol (5 ml) and the cooled solution was diluted with dry 2,4,6-collidine (20 ml). Copper (I) iodide (288 mg, 1.5 mmol) and 10-bromoapovincamine (**17**) (1.245 g, 3 mmol) were added and the mixture was stirred under nitrogen at 120°C. After 4 h, further copper iodide (144 mg, 0.75 mmol) was added to the cooled mixture and the reaction was stirred again for 2 h at 120°C. The reaction mixture was diluted with water, neutralized with 5% aqueous phosphoric acid until pH 6 and thoroughly extracted with n-butanol. The organic layer was filterd and evaporated under vacuum provided an oily residue which was taken up in dry methanol (50 ml), bubbled with hydrogen chloride, heated at reflux for 3 h, then evaporated to dryness.The residue was dissolved in trifluoroacetic acid (20 ml) and left at room temperature for 15 h. The mixture was diluted with cold water, 5N aqueous NaOH was added until pH 8 and the solution extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and evaporated. Crystallization of the dried residue in methanol yielded pure methoxyapovincamine (**18**) (414 mg, 38%): white yellowish crystals mp 150-151°C; $[\alpha]_D$ = +132° (c = 1, $CHCl_3$); HRms Calcd for $C_{22}H_{26}N_2O_3$: 366.1945. Found: 366.1953; Elms m/z (% rel. int.) 366 (42) ($M^{\cdot}$), 337 (100), 296 (98); uv (EtOH) λ_{max} nm (log ε) 230 (4.34), 278 (4.08), 325 (3.90); ir (KBr) 1725, 1610 cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.00 (t, J = 7.5 Hz, 3H, H-18), 3.88 and 3.95 (2s, 6H, CO_2CH_3 and OCH_3-10), 4.15 (s, 1H, H-21), 6.12 (s, 1H, H-17), 6.80 (dd, J_{ortho} = 9 Hz, J_{meta} = 2 Hz, 1H, H-11), 6.92 (d, J_{meta} = 2 Hz, 1H, H-9), 7.15 (d, J_{ortho} = 9 Hz, 1H,

H-12).

9,17-Dibromo-10-methoxyvincamine methyl ether (19). To a stirred solution of **18** (402 mg, 1.1 mmol) and 65% aqueous $HClO_4$ (1.1 mmol) in methanol (20 ml), a solution (10.5 ml, 2.2 mmol) of bromine in methanol (prepared by dilution of 1 ml of bromine with 90.5 ml of methanol) was added dropwise for 15 min at room temperature, then the reaction was kept on 1.5 h. The mixture was diluted with cold water, saturated $NaHCO_3$ solution added until pH 7 and the layer was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and evaporated afforded an amorphous residue of pure dibromo compound **(19)** (605 mg, quant. yield): amorphous; $[\alpha]_D$ = +104° (c = 0.6, $CHCl_3$); EIms m/z (% rel. int.) 558, 556 and 554 (15, 35, 14) ($M^{\ddagger}$), 499 (9), 497 (20), 495 (16), 477 (71), 475 (71), 417 (80), 415 (75), 376 (66), 374 (72); uv (EtOH) λ_{max} nm (log ε) 228 (4.32), 283 (3.86), 300 (3.74), 310 sh (3.50); ir ($CHCl_3$) 1760, 1740 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3H, H-18), 3.60 (s, 3H, OCH_3-16), 3.88 and 3.90 (2s, 6H, CO_2CH_3 and OCH_3-10), 4.10 (s, 1H, H-21), 4.75 (s, 1H, H-17), 6.80 (d, J_{ortho} = 9 Hz, 1H, H-11), 7.10 (d, J_{ortho} = 9 Hz, 1H, H-12).

9,17-Dibromo-10-methoxy-6-oxovincamine methyl ether (20): amorphous; EIms m/z (% rel. int.) 572, 570 and 568 (10, 31, 14) ($M^{\ddagger}$), 571 (37), 569 (52), 567 (36), 491 (88), 489 (88), 388 (76), 360 (100); uv (EtOH) λ_{max} nm (log ε) 221 (4.45), 257 (4.31), 298 (4.05); ir ($CHCl_3$) 1760, 1740, 1670 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3H, H-18), 3.78 and 3.82 (2s, 6H, CO_2CH_3 and OCH_3-16), 3.90 (s, 3H, OCH_3-10), 4.38 (s, 1H, H-21), 4.90 (s, 1H, H-17), 6.95 (d, J_{ortho} = 9 Hz, 1H, H-11), 7.10 (d, J_{ortho} = 9 Hz, 1H, H-12).

9-Bromo-10-methoxy-6-oxovincamine methyl ether (21). A solution of **20** (205 mg, 0.36 mmol) in dimethylformamide (15 ml) was hydrogenated under 1 atm pressure of hydrogen with PtO_2 catalyst (22 mg) at room temperature till end of the absorption. The catalyst was separated and the filtrate was diluted with water and extracted with Et_2O. The organic layer was dried over Na_2SO_4, filtered and afforded after evaporation under vacuum a dry residue (162 mg). This residue was purified by tlc on silica gel (CH_2Cl_2-MeOH 99-1 v/v, several migrations) and yielded **21** as a pure amorphous compound (108 mg, 61%) amorphous; $[\alpha]_D$ = +20° (c = 0.6, $CHCl_3$); EIms m/z (% rel. int.) 492 and 490 (35 and 31) (M^+), 433 (100), 431 (90), 167 (70), 149 (77); uv (EtOH) λ_{max} nm (log ε) 222 (4.43), 260 (4.31), 298 (4.02); ir ($CHCl_3$) 1750, 1670 cm^{-1}; ^1H nmr ($CDCl_3$) δ

1.00 (t, J = 7.5 Hz, 3H, H-18), 2.10 and 2.95 (2d, J = 15 Hz, 2H, H-17), 3.45 and 3.70 (2d, J = 17 Hz, 2H, H-5), 3.68 (s, 6H, CO_2CH_3 and OCH_3-16), 3.95 (s, 3H, OCH_3-10), 4.10 (s, 1H, H-21), 6.90 (d, J_{ortho} = 9 Hz, 1H, H-11), 7.35 (d, J_{ortho} = 9 Hz, 1H, H-12).

10-Methoxy-6-oxovincamine methyl ether (22). A solution of **21** (55 mg, 0.11 mmol) in methanol (10 ml) was hydrogenated under 1 atm pressure of hydrogen with Pd-C 5% catalyst (50 mg) at room temperature till end of the absorption. The catalyst was separated and the filtrate was diluted with water neutralised with 10% aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and provided after evaporation under vacuum pure compound **(22)** crystallizing with methanol (45 mg, quant. yield): white crystals mp 214-215°C; EIms m/z (% rel. int.) 412 (59) (M^+) 353 (100); uv (EtOH) λ_{max} nm (log ε) 219 (4.23), 252 (4.10), 278 (3.88), 298 (3.87); ^1H nmr ($CDCl_3$) δ 1.00 (t, J = 7.5 Hz, 3H, H-18), 2.15 and 2.63 (2d, J = 15 Hz, 2H, H-17), 3.40 and 3.78 (2d, J = 17 Hz, 2H, H-5), 3.65 and 3.75 (2s, 6H, CO_2CH_3 and OCH_3-16), 3.90 (s, 3H, OCH_3-10), 4.20 (s, 1H, H-21), 6.85 (dd, J_{ortho} = 9 Hz, J_{meta} = 2 Hz, 1H, H-11), 7.30 (d, J_{ortho} = 9 Hz, 1H, H-12), 7.65 (d, J_{meta} = 9 Hz, 1H, H-9).

9-Bromo-6-hydroxy-10-methoxy-*py*-tetradehydroapovincamine chloride (23): bright yellow crystals mp 237-239°C; FAB$^+$/ms m/z 457-459 (100); uv (EtOH) λ_{max} nm 249, 264, 302, 410 sh, 430; ir (KBr) 2500, 1735 cm^{-1}; ^1H nmr (CD_3OD-10 % $CDCl_3$) δ 0.80 (t, J = 7.5 Hz, 3H, H-18), 1.6-2.7 (m, 6H, H-14, H-15, H-19), 4.00 (s, 6H, CO_2CH_3 and OCH_3-10), 6.38 (s, 1H, H-17), 7.50 (d, J_{ortho} = 9 Hz, 1H, H-11), 7.65 (d, J_{ortho} = 9 Hz, 1H, H-12), 8.00 (s, 1H, H-5).

6-Hydroxy-10-methoxy-*py*-tetradehydroapovincamine chloride (24): bright yellow crystals mp 215-218°C; FAB$^+$/ms m/z 379 (100); uv (EtOH) λ_{max} nm 261, 307,318, 344, 406, 426; ir (KBr) 2500, 1735 cm^{-1}; ^1H nmr (CD_3OD) δ 0.78 (t, J = 7.5 Hz, 3H, H-18), 1.7-2.6 (m, 6H, H-14, H-15, H-19), 3.95 and 4.00 (2s, 6H, CO_2CH_3 and OCH_3-10), 6.30 (s, 1H, H-17), 7.35 (dd, J_{ortho} = 9 Hz, J_{meta} = 2 Hz, 1H, H-11), 7.75 (d, J_{ortho} = 9 Hz, 1H, H-12), 7.80 (d, J_{meta} = 2 Hz, 1H, H-9), 8.00 (s, 1H, H-5).

6-Hydroxy-*py*-tetradehydro-16-deoxyepivincamine chloride (25). A solution of **8** (38 mg, 0.1 mmol) in ethanol (10 ml) was hydrogenated under 1 atm pressure of hydrogen with Pd-C 5% catalyst (35 mg) at room temperature for 2 h. After separation of the catalyst, the filtrate was evaporated under vacuum and yielded pure compound **(25)** by crystallization with EtOAc (26 mg, 68%): pale yellow crystals mp 232-234°C; FAB^+/ms m/z 351 (100); uv (EtOH) λ_{max} nm (log ε) 254 (4.49), 289 (3.79), 312 (3.95), 382 (3.92); ir (KBr) 2500, 1750 cm^{-1}; ^1H nmr (DMSO-d6) δ 0.85 (t, J = 7.5 Hz, 3H, H-18), 1.5-2.4 (m, 7H, H-14, H-15, H-19 and 1H-17), 3.00 (d, J = 15 Hz, 1H, H-17), 3.75 (s, 3H, CO_2CH_3), 4.55 and 4.75 (2m, 2H, H-3), 5.72 (d, J = 7 Hz, 1H, H-16), 7.48 (t, J_{ortho} = 9 Hz, 1H, H-10), 7.78 (m, 2H, H-11 and H-12), 8.03 (s, 1H, H-5), 8.30 (d, J_{ortho} = 9 Hz, 1H, H-9).

Biology

Cytotoxicity: L1210 cells were cultivated in a RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco), 2 mmol L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 10 mM HEPES buffer, (pH = 7.4). The cytotoxicity was measured by the microculture tetrazolium assay essentially as described previously.[15] Cells were exposed for 2 days to the drugs (nine graded concentrations in triplicate). Results are expressed as IC_{50}, the drug concentration inhibiting by 50% the absorbance with respect to untreated cells.
Antitumor activity: the antitumor activities were evaluated on the experimental P388 leukemia. Murine P388 leukemic cells were given by Mario Negri Institute (Milano, Italy) and were maintened by ip inoculation into DBA_2 mice (Iffa Credo, France). For the chemotherapeutic assays, the cells were inoculated ip (10^6 cells) into DBA_2 mice on day 0. The drugs were dissolved in saline and injected ip on day 1. The results are expressed in term of T/C in % median survival time of treated animals divided by median survival of controls.

ACKNOWLEDGEMENTS

The authors gratefully thank Mr. Yves Rolland (Lab. Servier) for its interest in this work, Mr. Jean-Paul Volland (Lab. Servier) and his whole laboratory (especially Michel Amm and Pierre-Hervé Lambert), Patrick Le Ménez and Jacqueline Mahuteau (Faculté de Pharmacie, Châtenay-Malabry) for nmr measurements and mass spectra recording. They are also grateful to the Deshors Company for a generous gift of vincamin.

REFERENCES

1. L.A. Anderson, A. Harris, and J.D. Phillipson, *J. Nat. Prod.*, 1983, **46**, 374.

2. N. Fukamiya, M. Okano, T. Aratani, K. Negoro, A.T. McPhail, M. Ju-Ichi, and K-H. Lee, *J. Nat. Prod.*,1986, **49**, 428.

3. K. Narayanan and J.M. Cook, *Heterocycles*, 1991, **32**, 2005.

4. J. Le Men and W.I. Taylor, *Experientia*, 1965, **21**, 508.

5. E. Corvi Mora, German Patent 2 725 248 (1976) (*Chem. Abstr.*, 1978, **88**, 105636 g).

6. G. Lewin, Y. Rolland, and J. Poisson, *Heterocycles*, 1980, **14**, 1915.

7. Compound **(4)** was prepared by catalytic hydrogenation of 10-chloro-14-15-dehydro-16-epivincamine.[6]

8. M. Shamma and D.M. Hindenlang, "Carbon-13 NMR Shift Assignments of Amines and Alkaloids", Plenum Press, New-York and London, 1979, pp. 12-13.

9. L.J. Bellamy, "The Infra-Red Spectra of Complex Molecules", 2nd ed., John Wiley and Sons, 1958, p. 105.

10. R.G.R. Bacon and S.C. Rennison, *J. Chem. Soc. C.*, 1969, 312.

11. B. Danieli, G. Lesma, G. Palmisano, and R. Riva, *J. Chem. Soc., Perkin Trans. 1*, 1987, 155.

12. Omnium Chimique S.A., French Patent 2 268 016 (1974) (*Chem. Abstr.*, 1976, **85**, 33247 u).

13. G. Lewin and J. Poisson, *Tetrahedron Lett.*, 1984, **25**, 3813.

14. G. Lewin and J. Poisson, *Bull. Soc. Chim. France*, 1984, 435.

15. M.C. Alley, D.A. Scuderio, A. Monks, M.L. Hursey, M.J. Czerwinski, D.L. Fine, B.J. Abbott, J.G. Mayo, R.H. Shoemaker, and M.R. Boyd, *Cancer Res.*, 1988, **48**, 589.

Received, 11th June, 1993