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Study of the structure–activity relationships of the acetogenin of annonaceae, muricatacin and analogues

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Summary — A study of the structure–cytotoxic activity of the acetogenin of Annonaceae, muricatacin **1**, is reported. Indeed, muricatacin **1** has shown promising antitumoral activity. Therefore several 5-hydroxy-4-alkanolides were prepared and then tested against KB and VERO cell lines. A few other analogues were synthesized and tested against both cell lines. Thus this work allowed us to better determine the pharmacophore of the molecule and to propose muricatacin **1** instead of a more complicated acetogenin of Annonaceae as a lead compound in the search for new antineoplastic agents.

butyrolactone / acetogenin of Annonaceae / cytotoxicity

Introduction

4-Alkanolides are naturally occurring products isolated from a large variety of plants which present different biological properties depending on the length of the alkyl chain, the relative and absolute configurations of the stereogenic centers, and on the presence of other functions (eg, hydroxyls, tetrahydrofurans, amino goups, etc). For instance, 4-nonanolide and 4-octanolide, which are used in perfumery have a strong smell of coconuts with a cumin flavor in the latter, and 4-undecanolide has a pronounced peach flavor [1]. The 5-hydroxy-4-hexanolides are constituents of several wines [2], Xeres [3] and are also present in tobacco fumes [4]. The whisky and cognac lactones are known for participating in the bouquets of such alcohols and have been synthesized [5]. The 5-hydroxy-6-phenyl-4-hexanolide was isolated from the bacteria *Erwinia quercina*, but is an artefact of isolation, since the biological activity is due to the corresponding hydroxy carboxylic acid [6]. The 5-hydroxy-4-decanolides have been described as growth factors in *Streptomyces griseus* via non-elucidated mechanisms [7]. A few years ago, muricatacin (5-hydroxy-4-heptadecanolide) **1** was isolated from the seeds of *Annona muricata* (Annonaceae) and showed cytotoxic activity on tumor cell lines (A-549 (lung carcinoma) with ED_{50} = 23.3 μg/mL, MCF-7 (breast carcinoma) with ED_{50} = 9.5 μg/mL, and HT-29 (colon carcinoma) with ED_{50} = 14.0 μg/mL) [8]. Stereospecific syntheses of (+)-muricatacin as well as (–)-muricatacin and epimers have been reported by several groups [9–13], and cytotoxicity of (+)-muricatacin against KB and VERO cells has been studied [14]. Taking in account these interesting biological activities, the aim of this study is to determine the influences of various parameters (eg, the configurations of the stereogenic centers, the length of the alkyl chain and structural modifications such as the introduction of unsaturation, ketones, nitrogen atoms) on cytotoxic activity.

Chemistry

Homochiral compounds **1–4** were synthesized through an acylation–reduction sequence from L-glutamic acid, using the approach described by Larchevêque [15], and which is shown in scheme 1. Nitrous deamination of L-glutamic acid (+)-**5** ($NaNO_2$, HCl) led to carboxylic acid (+)-**6**, which after treatment with oxalyl chloride in the presence of a catalytic amount

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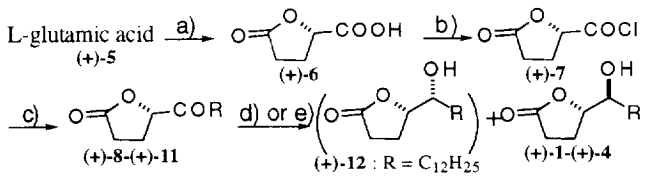
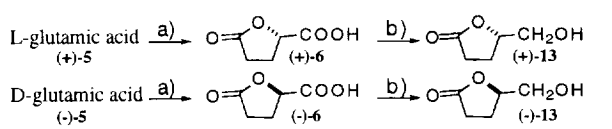


Scheme 1. a) $NaNO_2$, HCl; b) $(COCl)_2$, DMF cat, CH_2Cl_2; c) RMgBr, THF, –78 °C; d) L-Selectride®, THF, –78 °C; e) n-Bu_3SnH•SiO_2, CH_2Cl_2.

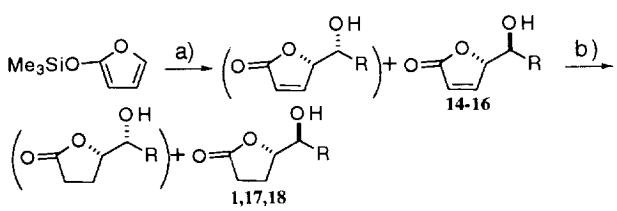
of DMF afforded the corresponding acid chloride (+)-**7** in 92% yield. Acylation of the desired Grignard reagents with (+)-**7** led to the expected butyrolactonic ketones ((+)-**8**: R = Et, 65%; (+)-**9** : R = $C_{10}H_{21}$, 92%; (+)-**10**: R = $C_{12}H_{25}$, 63%; (+)-**11** : R = $C_{14}H_{29}$, 52%). Then diastereoselective reductions of the ketones **8**–**11** were performed with L-Selectride®, leading to the major *threo* (*syn*) (4*S*, 5*S*) isomers with dr = 98:2 ((+)-**2**: R = Et, 85%; (+)-**3**: R = $C_{10}H_{21}$, 96%; (+)-**1**: R = $C_{12}H_{25}$, 90%; (+)-**4**: R = $C_{14}H_{29}$, 50%). The *erythro* (4*S*, 5*R*) isomer (+)-**12** (R = $C_{12}H_{25}$) was obtained in 64% yield by reduction of (+)-**10** with n-Bu_3SnH in the presence of SiO_2 (dr = 77:23) [16].

The (4*S*) and (4*R*) 5-hydroxy-4-pentanolides (+)-**13** and (–)-**13** were separately prepared from the corresponding carboxylic acids by reduction with BH_3•SMe_2, as depicted in scheme 2.



Scheme 2. a) $NaNO_2$, HCl; b) BH_3•SMe_2, THF.

Threo compounds **14**–**16** were prepared as racemic mixtures and with dr = 80:20 by addition at –78 °C of trimethylsilyloxyfuran (TMSOF) on the corresponding aldehydes in CH_2Cl_2, in the presence of $TiCl_4$ (**14**: R = C_5H_{11}, 77%; **15**: R = C_7H_{15}, 80%; **16**: R = $C_{12}H_{25}$, 40%), using the approach described by Jefford [17] and represented in scheme 3. The corresponding



Scheme 3. $TiCl_4$, CH_2Cl_2, –78 °C; b) H_2, Pd/C, toluene.

saturated racemic products **1** and **17**, **18** were quantitatively obtained by hydrogenation of **14**–**16** with H_2 in the presence of palladium on charcoal.

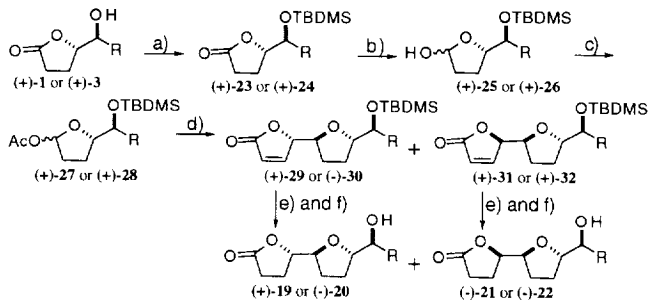
Compounds (+)-**19**-(–)-**22** were prepared in an enantiomerically pure form from the key intermediates **27** and **28**, respectively [18]. The latter were obtained from **1** or **3** after hydroxyl protection (TBDMSCl, imidazole, DMF, 94%), reduction of the lactones to the corresponding lactols **25** or **26** (DIBAL-H, toluene, –78 °C, 98%) and acetylation (Ac_2O, Et_3N, 92%), and then stereoselective C-glycosylations with TMSOF (dr = 40:60, overall yield = 55%) followed by quantitative hydrogenation and deprotection as described in scheme 4.

Aza-analogues (–)-**36**, (+)-**37** were prepared by an acylation–reduction sequence from L-pyroglutamic acid (–)-**33** after treatment with oxalyl chloride to afford the corresponding acid chloride **34**: acylation of dodecylmagnesium bromide with **34** led to the expected ketone (–)-**35** in moderate yield (40%). Then reduction of the latter with $NaBH_4$ in the presence of 1 equiv $MnCl_4Li_2$ in THF/MeOH (99:1) at –40 °C afforded the major *erythro* (4*S*, 5*R*) derivative (–)-**36** in 80% yield and dr = 82:18. However, when the ketone (-)-**35** was first protected as the *N*-Boc derivative (Boc_2O, DMAP, Et_3N, 44%) prior to the $NaBH_4$ reduction at –10 °C followed by removal of the *N*-Boc protecting group, the major *threo* (4*S*, 5*S*) isomer (+)-**37** (dr = 95:5) was then obtained in 65% yield for the last two steps [19], as shown in scheme 5.

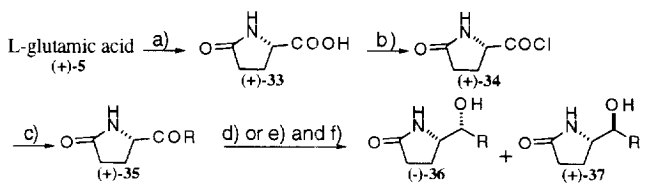
Biological results and discussion

Evaluation of the cytotoxicity of all compounds was performed against KB and VERO cell lines, and the results have been reported in table I. Cytotoxicity was expressed as those concentrations of product which caused 50% loss of cells (EC_{50}) (see *Experimental protocols*).

It is noteworthy that the cytotoxicity is dependent on the length of the alkyl chain, and muricatacin (with R = $C_{12}H_{25}$) is the most active (entry 1). A shorter chain dramatically decreases the activity (entries 5–8, 10, 12), whereas a longer chain (entry 13) does not increase the activity. However, unsaturation in the lactone ring improves the activity of the compounds with a short chain (entries 9 and 11), but has no effect on the parent molecule (entry 4). It is very important to note that (+)- and (–)-muricatacin have the same activity toward both cell lines (entries 1 and 2). However, it seems that *epi*-muricatacin (*erythro* butanolide) is somewhat less active that the parent *threo* compound (entries 1 and 3). When other functionalities are present, such as an oxo function (entry 14), the activity is about the same as for muricatacin.



Scheme 4. a) TBDMSCl, DMF, imidazole; b) DIBAL-H, toluene, –78 °C; c) Ac_2O, Et_3N; d) TMSOF, $TrClO_4$, Et_2O, 0 °C (**29/31** or **30/32** = 40:60); e) H_2, Pd/C, EtOAc; f) HF, THF.



Scheme 5. a) Δ; b) $(COCl)_2$; c) $C_{12}H_{25}MgBr$, THF, –78 °C; d) $NaBH_4$•$MnCl_4Li_2$, THF/MeOH (99:1), –40 °C; e) Boc_2O, DMAP, Et_3N; f) $NaBH_4$, THF/MeOH (99:1), –10 °C, then HCl.

Addition of a tetrahydrofuran ring does not modify the activity as long as the length of the alkyl chain is not shortened (entries 15–17). In the cases of the pyrrolidones, the aza-analogues of muricatacin, the activity is either identical to the activity of the parent molecule or even better, whatever the relative or absolute configurations (entries 18–20).

In conclusion, the structural modifications of muricatacin allowed us to determine the pharmacophore of the molecule. The length of the alkyl chain is crucial for the activity. Interestingly, both enantiomers have the same activity, and aza-analogues retain the activity with a slight improvement. However, the mechanism of action is still not understood. Indeed, it has been postulated that acetogenins of Annonaceae may have ionophoric properties due to their complexation properties [20]. However, some NMR experiments have shown that muricatacin does not bind cations such as Ca^{2+}, Ba^{2+} and K^+ with high efficiency. Since it has been proposed that acetogenins of Annonaceae act as an inhibitor of complex I in the mitochondrial respiratory system [21], it is possible that muricatacin acts via an identical mechanism. However, more experiments are needed before any conclusion can be made.

Experimental protocols

General approach

For each compound, ^1H- and ^{13}C-NMR spectra were recorded employing a Bruker AR200P spectrometer and are consistent with the proposed structures. Mass spectra were recorded with a Nermag 1010. Specific rotations were measured with a Perkin–Elmer 241 Polartronic polarimeter. All chemicals were purchased from Aldrich Chemical Co and used without any purification; solvents came from OSI, and were distilled prior to use.

*Acyl-butyrolactones **8–11***

A solution of acid chloride **7** (3 g, 0.02 mol), prepared as in ref [15] in 34 mL anhydrous THF was cooled to –78 °C, and 1 equiv of Grignard solution (0.3 M) was slowly added (2 h). The mixture was then stirred for 2 h at –78 °C before hydrolysis with a saturated solution of amonium chloride (20 mL). The organic layer was then extracted with ethyl acetate (3 × 20 mL), dried over magnesium sulfate, filtered, and the solvent evaporated under reduced pressure. The off-white crystalline precipitate so obtained was purified by flash chromatography to yield the desired butyrolactonic ketones **8–11** (see text for yields).

Spectroscopic data of (+)-**10** are given as a representative example: $[\alpha]_D^{20}$ = +15.7 (*c* 0.35, MeOH); mp = 59–60 °C, ^1H-NMR ($CDCl_3$, 200 MHz) δ (ppm): 0.86 (t, *J* = 6.4 Hz, 3H), 1.24 (m, 18H), 1.49–1.69 (m, 2H), 2.16–2.32 (m, 1H), 2.42–2.62 (m, 5H), 4.77–4.85 (m, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ (ppm): 14.0, 22.6, 22.8, 24.5, 27.2, 29.0, 29.2, 29.3, 29.5, 31.8, 38.7, 81.6, 175.9, 207.5; MS-CI (CH_4) *m/z*: 283 (MH^+, 18), 197 (100).

*5-Hydroxy-4-alkanolides **1–4***

L-Selectride® reductions. A solution of ketone **8–11** (0.012 mol) in 128 mL THF was cooled to –78 °C, and 13.7 mL THF solution (1 N) of L-Selectride® diluted in 30 mL THF was slowly added (1 h). The reaction mixture was then stirred for 2 h at –78 °C prior to the addition of 40 mL HCl (1 N). The temperature was raised to 20 °C and the organic layer was then extracted with ethyl acetate (3 × 40 mL), dried over magnesium sulfate, filtered, and the solvent evaporated under reduced pressure. The off-white crystalline precipitate so obtained was purified by flash chromatography to yield the desired *threo* (4*S*, 5*S*) 5-hydroxy-4-alkanolide **1–4** (see text for yields).

Table I. Evaluation of cytotoxicity of all compounds studied utilizing KB and VERO cell lines.

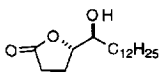
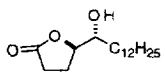
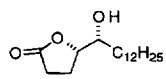
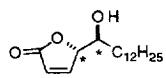
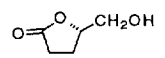
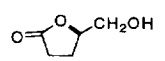
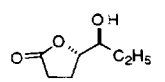
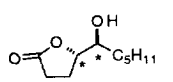
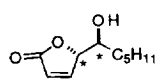
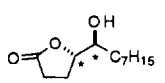
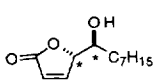
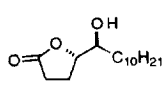
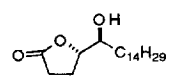
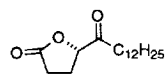
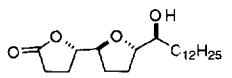
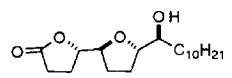
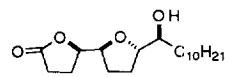
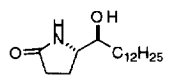
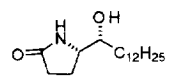
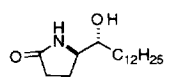
Entry	*Compounds*		*KB EC_{50} (μg/mL)*	*VERO EC_{50} (μg/mL)*
1		(+)-**1**	5	11
2		(−)-**1**	5	10
3		(+)-**12**	7.5	14
4		(+/−)-**16**	7	8
5		(+)-**13**	>100	>100
6		(−)-**13**	>100	>100
7		(+)-**2**	>100	>100
8		(+/−)-**17**	50	60
9		(+/−)-**14**	5	10
10		(+/−)-**18**	25	30
11		(+/−)-**15**	5	10
12		(+)-**3**	10	15
13		(+)-**4**	5	10
14		(+)-**10**	6	>10

Table I. *(Continued.)*

Entry	*Compounds*		*KB EC_{50} (μg/mL)*	*VERO EC_{50} (μg/mL)*
15		(+)-**19**	5	7
16		(−)-**20**	10	16
17		(−)-**21**	35	20
18		(+)-**37**	1.5	7
19		(−)-**36**	2	12
20		(−)-**37**	3.5	7

Spectroscopic data of (+)-**4** are given as a representative example: $[\alpha]_D^{20}$ = +17.5 (*c* 0.8, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ (ppm): 0.87 (t, *J* = 6.5 Hz, 3H), 1.10–1.42 (m, 24H), 1.43–1.60 (m, 2H), 1.85 (d, *J* = 6 Hz, OH), 2.00–2.35 (m, 1H), 2.43–2.70 (m, 3H), 3.55 (m, 1H), 4.40 (td, *J* = 7.5, 5.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ (ppm): 14.1, 22.7, 24.1, 25.4, 28.7, 29.3, 29.5, 29.6, 31.9, 32.9, 73.6, 82.9, 177.1; MS-CI (NH_3) *m/z*: 330 (M + NH_4^+, 100).

Tri-n-butyltin hydride/silica gel (n-Bu_3SnH/SiO_2) reduction. Ketone (+)-**10** (100 mg) were dissolved in 5 mL CH_2Cl_2, and 2 g SiO_2 were added; 0.19 mL *n*-Bu_3SnH was then added at room temperature. The reaction mixture was stirred for 24 h at 20 °C, and then filtered through a pad of silica gel, washed with pentane and then with CH_2Cl_2. Sixty mg (60%) of *erythro* (4*S*, 5*R*) 5-hydroxy-4-heptadecanolide (+)-**12** were obtained after purification by flash chromatography.

Spectroscopic data of (+)-**12**: $[\alpha]_D^{20}$ = + 32 (*c* 2.0, MeOH); mp = 67 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 0.87 (t, *J* = 6.4 Hz, 3H), 1.10–1.68 (m, 22H), 2.01–2.71 (m, 5H), 3.81–4.02 (m, 1H), 4.43 (td, *J* = 7.2, 3.2 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ (ppm): 14.1, 21.0, 22.6, 25.6, 28.7, 29.3, 29.5, 29.6, 31.9, 71.3, 82.9, 177.5; MS-CI (CH_4) *m/z*: 285 (MH^+, 31).

4S or 4R 5-Hydroxy-4-pentanolides (+)-13 and (−)-13
The corresponding carboxylic acids **5**, prepared as in ref [15] (20 g, 0.154 mol) were dissolved in 200 mL anhydrous THF, and 18.5 mL (0.185 mol, 1. 2 equiv of a 10 M solution in THF) of borane dimethylsulfide complex in 200 mL THF were added at 20 °C. After stirring at room temperature for 4 h, 100 mL methanol were added. The volatiles were then evaporated at reduced pressure, and the crude materials were distilled (bp = 140 °C, 0.6 mm Hg) to yield 13.8 g (77%) of the expected alcohols (+)-**13** or (−)-**13**.

Spectroscopic data of (+)-**13** are given as a representative example: $[\alpha]_D^{20}$ = + 28.0 (*c* 5.8, EtOH); bp = 130–140 °C/ 0.7 mmHg; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.10–2.71 (m, 4H), 3.55–3.93 (m, 3H), 4.52–4.70 (m, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ (ppm): 23.0, 28.5, 63.6, 80.9, 178.1.

Racemic 5-hydroxy-2-alken-4-olides 14–16
The desired aldehyde (1.1 mmol) was dissolved in 15 mL CH_2Cl_2 and the temperature was brought to −78 °C. Then TMSOF (0.18 mL, 1.1 mmol) was added, followed by $TiCl_4$ (1.1 mL, 1 N solution in toluene, 1.1 mmol). After stirring for 30 min, 10 mL 1 N solution of HCl were added, and the organic layer was then extracted with ethyl acetate (3 × 10 mL), dried over magnesium sulfate, filtered, and the solvent evaporated under reduced pressure. The off-white crystalline precipitate so obtained was purified by flash chromatography to yield the major racemic *threo* butenolides **14–16** with dr = 80:20 (see text for yields).

Spectroscopic data of (+/−)-**16** are given as a representative example: ^{1}H-NMR ($CDCl_3$, 200 MHz) δ (ppm): 0.87 (t, *J* = 6.5 Hz, 3H), 1.25 (m, 20H), 1.57 (m, 2H), 2.32 (m, OH), 3.75 (q, *J* = 5.5 Hz, 0.8H), 3.84 (m, 0.2H), 4.98 (m, 1H), 6.17 (dd, *J* = 1.6, 5.7 Hz, 1H), 7.46 (dd, *J* = 0.9, 5.7 Hz, 0.8H), 7.54 (dd, *J* = 0.9, 5.8 Hz, 0.2H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ (ppm): 14.1, 22.6, 24.7, 25.5, 29.3, 29.6, 31.9, 33.2, 33.8, 71.5, 71.7, 86.2, 94.4, 122.6, 122.8, 153.6, 153.8, 173.0.

Racemic 5-hydroxy-4-alkanolides 1, 17, 18
The butenolides **14–16** were quantitatively hydrogenated over palladium on charcoal in toluene at room temperature for 12 h. Filtration followed by flash chromatography purification on

silica gel led to the desired racemic butanolides **1**, **17**, **18**. Spectroscopic data (^{1}H- and ^{13}C-NMR) of these compounds are very close to those of (+)-**4** (see above).

*5,8-Epoxy-9-hydroxy-4-alkanolides **19–22***

The desired 5-hydroxy-4-alkanolide **1** or **3** (1.34 mmol) were first protected as the *tert*-butyldimethylsilyl ethers **23** or **24** (3.35 mmol, 2.5 equiv *tert*-butyldimethylsilyl chloride, 3.35 mmol, 2.5 equiv imidazole, in 10 mL DMF at room temperature for 48 h, followed by addition of 6 mL of water and extraction with ethyl acetate and usual workup). After purification of the corresponding silyl ethers by flash chromatography on silica gel (96%), compounds **23** or **24** (1.34 mmol) were dissolved in 3 mL toluene and the solution cooled to –78 °C. Then 1.6 mL Dibal-H (1.6 mol 1 N solution in toluene) were slowly added at –78 °C (30 min) and stirring was maintained for another 15 min before the addition of 4 mL saturated solution of amonium chloride. The reaction mixture was then poured into ethyl ether cooled at –78 °C and stirred for 15 h, letting the temperature rise to 20 °C. The solution was then filtered and the filtrate dried over magnesium sulfate, filtered and evaporated under reduced pressure. After purification of the corresponding lactols by flash chromatography on silica gel (98%), compounds **25** or **26** (1.34 mmol) were dissolved in 8 mL dry triethyl amine. Then 1.16 mL acetic anhydride were added with a catalytic amount of dimethylamino pyridine at 20 °C. After stirring for 12 h, solvents were evaporated and 8 mL water added. The organic layer was then extracted with ethyl acetate and washed with 12 mL 1 N solution of HCl and then with 12 mL 1 N solution of NaOH. The organic layer was then dried over magnesium sulfate, filtered, and evaporated under reduced pressure. The anomeric acetates were purified by flash chromatography on silica gel (96%). Compounds **27** or **28** (1.34 mmol) were then dissolved in 12 mL anhydrous ethyl ether and a catalytic amount of trityl perchlorate added at 0 °C, after which 0.45 mL TMSOF (2.68 mmol, 2 equiv) were added and the solution stirred for 30 min. Then 6 mL saturated solution of $NaHCO_3$ were added at 0 °C, and the organic layer was extracted with ethyl acetate and dried over magnesium sulfate, filtered, and evaporated under reduced pressure. The *erythro/trans/threo* and *threo/trans/threo* compounds **29–32** were separated by flash chromatography on silica gel to yield the expected products **29**, **31** or **30**, **32** in 80% combined yield from the 5-hydroxy-4-alkanolide, and in a ratio of 60:40. Compounds **29–32** were then separately hydrogenated and treated with a solution of HF in THF to yield, after purification by flash chromatography on silica gel, products (+)-**19**–(–)-**22**.

Spectroscopic data of (–)-**20** are given as a representative example: $[\alpha]_D^{20}$ = –4.5 (*c* 0.56, CH_3OH); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ (ppm): 0.88 (t, *J* = 7.2 Hz, 3H), 1.1–1.60 (m, 18H), 1.56–2.40 (m, 7H), 2.41–2.70 (m, 2H), 3.30–3.42 (m, 1H), 3.68–3.85 (dt, *J* = 7.2, 2.4 Hz, 2H), 3.85 (dt, *J* = 7.2, 2.4 Hz, 1H), 4.49 (m, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ (ppm): 14.1, 22.7, 24.6, 25.6, 28.2, 29.3, 29.6, 31.9, 33.8, 73.7, 80.8, 81.2, 82.5, 177.1; MS-CI (NH_3) *m/z*: 344 ($M + NH_4^+$, 100).

Spectroscopic data of (–)-**22** are given as a representative example: $[\alpha]_D^{20}$ = –16 (*c* 0.25, CH_3OH); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ (ppm): 0.82 (t, *J* = 6.8 Hz, 3H), 1.25 (m, 18H), 1.56–1.83 (m, 2H), 1.87–2.38 (m, 2H), 2.39–2.55 (m, 2H), 2.43 (m, 2H), 3.28–3.45 (m, 1H), 3.68–3.85 (dt, *J* = 7.2, 6.5 Hz, 2H), 3.85–4.12 (dt, *J* = 7.2, 6.5 Hz, 1H), 4.32–4.49 (dt, *J* = 6.6, 5.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ (ppm): 14.1, 22.6, 23.6, 25.5, 28.1, 28.5, 29.3, 29.6, 31.9, 33.5, 74.0, 79.9, 81.6, 82.5, 177.0; MS-CI (NH_3) *m/z*: 344 ($M + NH_4^+$, 100).

*Pyrrolidones **36–37***

Pyroglutamic acid (–)-**33** (2.5 g, 0.020 mol) and oxalyl chloride (9.2 mL, 0.16 mol, 5 equiv) were heated to reflux for 2 h. Then the excess oxalyl chloride was distilled and the crude acyl chloride thus obtained was dissolved in 100 mL anhydrous THF and cooled to –78 °C. Then 255 mL dodecylmagnesium bromide (0.61 mol, 3 equiv) were slowly added. The mixture was then stirred for 2 h at –78 °C before hydrolysis with a saturated solution of amonium chloride (100 mL). The organic layer was then extracted with ethyl acetate (3 × 100 mL), dried over magnesium sulfate, filtered, and the solvent evaporated under reduced pressure. The off-white crystalline precipitate so obtained was purified by flash chromatography to yield 2.17 g (40%) of the desired ketone (–)-**35**.

*$NaBH_4$: $MnCl_4Li_2$ reduction of (–)-**35**.* Ketone (–)-**35** (0.062 g, 0.22 mmol) was dissolved in 3 mL THF/MeOH (99:1) and cooled to –40 °C. Then 0.167 g $NaBH_4$ (0.44 mmol, 2 equiv) and 0.0465 g $MnCl_4Li_2$ (0.22 mmol, 1 equiv) were added. The reaction mixture was stirred for 2 h at this temperature before hydrolysis with 1 mL 1 M solution of HCl. Organic layer was then extracted with ethyl acetate (3 × 1 mL), dried with magnesium sulfate, filtered and evaporated. The crude mixture was then purified by flash chromatography to yield 0.0493 g (80%) of the inseparable products (–)-**36**:(+)-**37** (dr = 82:18 in favor of (–)-**36**).

*$NaBH_4$: reduction of the N-Boc derivative of (–)-**35**.* Ketone (–)-**35** (0.065 g, 0.23 mmol) was dissolved in 2 mL CH_2Cl_2 and Boc_2O (0.1 g, 0.46 mmol, 2 equiv), dimethylamino pyridine (DMAP) (0.028 g, 0.46 mmol, 2 equiv) and 0.03 mL triethyl amine (0.23 mmol, 1 equiv) were added. After stirring at 20 °C for 48 h, 1 mL water was added, and organic layer extracted with ethyl acetate (3 × 1 mL), dried over magnesium sulfate, filtered, and evaporated to dryness. The *N*-Boc derivative 0.039 g (44%) was purified by flash chromatography on silica gel. The latter (0.035 g, 0.09 mmol) was dissolved in 1 mL THF/MeOH (99:1) and cooled to –10 °C. $NaBH_4$ (0.0063 g, 0.18 mmol, 2 equiv) was then added, and the reaction mixture stirred for 1 h at this temperature before hydrolysis with 1 mL 1 M solution of HCl. Organic layer was then extracted with ethyl acetate (3 × 1 mL), dried with magnesium sulfate, filtered and evaporated to dryness. The crude mixture was then pourred into 5 mL ethyl acetate and 1 mL 3 M solution of HCl added. After 1 h of stirring, organic layer was then extracted with ethyl acetate (3 × 1 mL), dried with magnesium sulfate, filtered and evaporated under reduced pressure. The crude mixture was then purified by flash chromatography on silica gel to yield 0.020 g (57% for the last two steps) of the inseparable products (–)-**36**:(+)-**37** (dr = 5:95 in favor of (+)-**37**).

Spectroscopic data of (–)-**36**: $[\alpha]_D^{20}$ = –3.3 (*c* 1.33, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.88 (t, *J*= 7 Hz, 3H), 1.26 (m, 20H), 1.47 (m, 2H), 1.65 (m, 1H), 2.07 (m, 1H), 2.22 (m, 1H), 2.37 (m, 1H), 3.67 (m, 2H), 6.67 (bs, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ (ppm): 14.1, 20.3, 22.9, 25.7, 26.8, 29.6, 32.4, 59.1, 72.3, 177.7, 179.5; MS-CI (NH_3) *m/z*: 284 (MH^+).

Spectroscopic data of (+)-**37**: $[\alpha]_D^{20}$ = +5.9 (*c* 2.6, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 0.9 (t, *J* = 7 Hz, 3H), 1.3 (m, 22H), 1.8 (m, 1H), 2.1 (m, 1H), 2.35 (m, 2H), 3.33 (bs, 1H), 3.52 (dd, *J* = *J'* = 6.7 Hz, 1H), 4.35 (bs, 1H), 7.50 (bs, 1H); ^{13}C-NMR ($CDCl_3$, 62.5 MHz) δ (ppm): 14.4, 23.6, 24.5, 26.6, 30.3, 30.6 (several C), 31.3, 32.9, 34.1, 60.8, 75.4, 180.9.

Biological screening

Assays were performed in 96-wall culture plates. Serial threefold dilutions of compounds were added to a 24-h old mono-

layer of KB (human epidermoid carcinoma) or VERO (African green monkey kidney cells) and the plates incubated for three days at 37 °C in a humidified 5% CO_2 atmosphere. Cytotoxicity was evaluated by microscopic examination of the cells cultures and by a photometric method [22]. Briefly, plates were washed with buffered solution (PBS) to remove the dead cells, adherent cells were fixed in formol, stained with methylene blue, the dye eluted with HCl (0.1 M) and the plates were read at 650 nm using a multichannel spectrophotometer. Cytotoxicity was expressed as concentrations of product which caused 50% of loss of cells (EC_{50}).

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References

1 Müller J (1993) In: *Le Livre H&R du Parfum.* Glöss Verlag, Hamburg
2 Muller CJ, Kepner RE, Webb AD (1973) *Am J Enol Viticult* 24, 5
3 Muller CJ, Maggiora L, Kepner RE, Webb AD (1969) *J Agric Food Chem* 17, 1373
4 Schumacher JN, Green CR, Best FW, Newell MP (1977) *J Agric Food Chem* 25, 310
5 Ebata T, Matsumoto K, Yoshikoshi H et al (1993) *Heterocycles* 36, 1017
6 Wright AE, Schäfer M, Midland S, Munnecke DE (1989) *Tetrahedron Lett* 30, 5699–5702
7 Gräfe U, Reinhardt G, Schade W et al (1982) *J Antibiot* 35, 609
8 Rieser MJ, Kozlowski JF, Wood KV, McLaughlin JL (1991) *Tetrahedron Lett* 32, 1137–1140
9 Figadère B (1995) *Acc Chem Res* 28, 359–366
10 Somfai P (1995) *J Chem Soc Perkin Trans I* 817–819
11 Bonini C, Federici C, Rossi L, Righi G (1995) *J Org Chem* 60, 4803–4812
12 van Aar MPM, Thijs L, Zwanenburg B (1995) *Tetrahedron* 51, 11223–11234
13 Quayle P, Rahman S (1995) *Tetrahedron Lett* 36, 8037–8038
14 Figadère B, Harmange JC, Laurens A, Cavé A (1991) *Tetrahedron Lett* 32, 7539–7542
15 Larchevêque M, Lalande J (1987) *Bull Soc Chim Fr* 116
16 Figadère B, Chaboche C, Franck X, Peyrat JF, Cavé A (1994) *J Org Chem* 59, 7138–7141
17 Jefford CW, Jaggi D, Boukouvalas J (1987) *Tetrahedron Lett* 28, 4037–4038
18 Figadère B, Chaboche C, Peyrat JF, Cavé A (1993) *Tetrahedron Lett* 34, 8093–8096
19 Baussanne I, Schwardt O, Royer J, Pichon M, Figadère B, Cavé A (1997) *Tetrahedron Lett* 38, 2259–2262
20 Peyrat JF, Figadère B, Cavé A, Mahuteau J (1995) *Tetrahedron Lett* 36, 7653–7656.
21 Esposti MD, Ghelli A, Batta M, Cortes D, Estornell E (1994) *Biochem J* 301, 161–167
22 Fleury C, Cotte J, Quéro AM (1984) *Pathol Biol* 32, 628–630