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Stereospecific Synthesis Of (+)-Muricatacin: A Biologically Active Acetogenin Derivative◊

B. Figadère*, J-C. Harmange, A. Laurens and A. Cavé

Laboratoire de Pharmacognosie, associé au C.N.R.S.
Faculté de Pharmacie 92296 Châtenay-Malabry (France)

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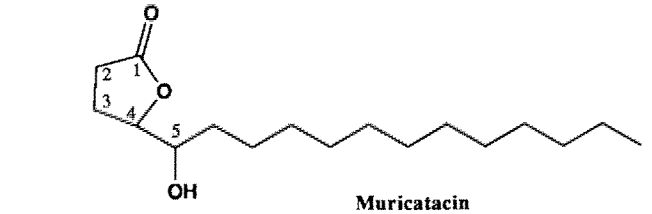
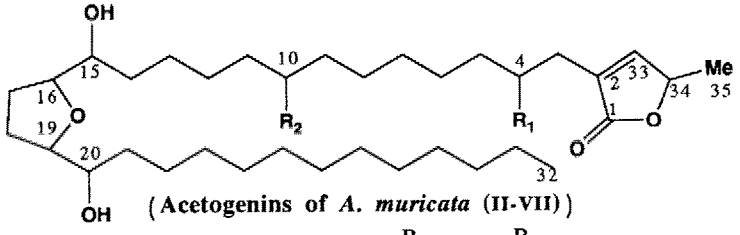
Abstract: *(+)-muricatacin and analogs have been synthesized without ambiguity about the absolute configuration at the C-4 and C-5 centres. The observed $[\alpha]_D$ are reported as well as the results obtained for the cytotoxicity assay with KB and VERO cell lines.*

Annonacae family has, these last years, afforded original acetogenins, biologically active as antitumorals, parasiticides and pesticides[1]. Recently, muricatacin, a simple active acetogenin derivative, has been isolated from the seeds of *Annona muricata*[2], and shows cytotoxic activity on tumor cell lines[2] (with A-549, lung carcinoma, ED_{50}= 23.3 µg/mL). Previously, six biologically active monotetrahydrofuranic acetogenins (annonacin, annonacinone, corossolin, corossolone, murisolin, solamin) have been isolated from the seeds of the same species[3-5].



Muricatacin



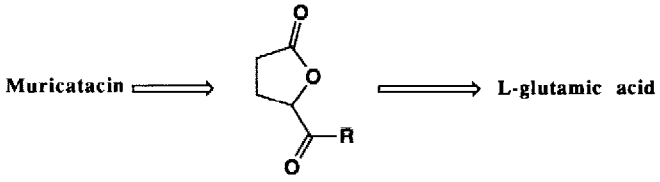
(Acetogenins of *A. muricata* (II-VII))

		R_1	R_2
- II	annonacin:	OH	OH
- III	annonacinone:	OH	O
- IV	corrosolin:	H	OH
- V	corrosolone:	H	O
- VI	murisolin:	OH	H
- VII	solamin:	H	H

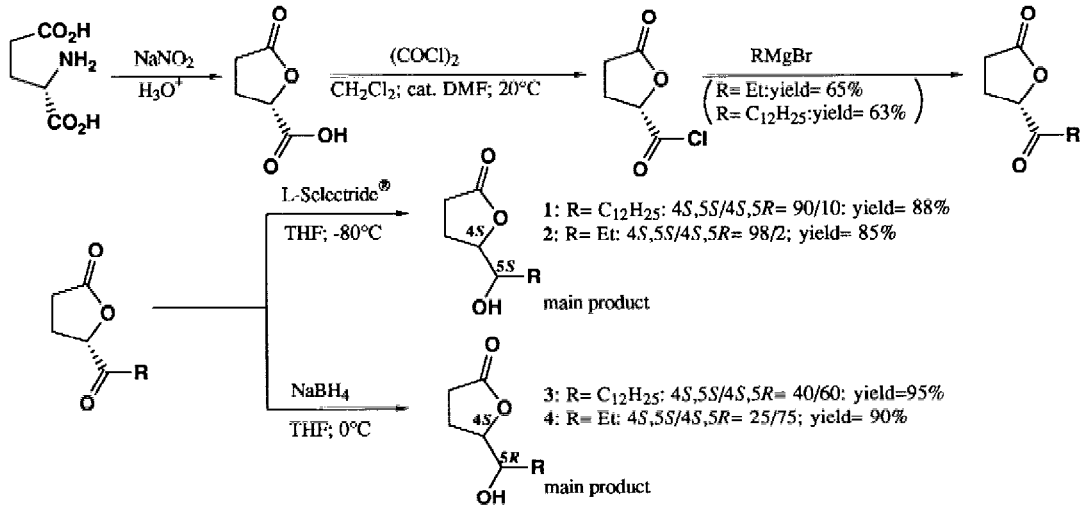
Muricatacin is probably a product of oxidative cleavage of monotetrahydrofuranic acetogenins and could be considered to represent a product of the catabolism in the plant. Therefore it is interesting to synthesize this product to 1) specify its absolute configuration at C-4 and C-5 positions, 2) determine wether it is a precursor or a metabolite of acetogenins, and 3) deduce the absolute configuration of the monotetrahydrofuranic acetogenins. It also allows us to compare its biological activity with isomers and analogs with KB and VERO cell lines in

order to establish a structure-activity relationship.

The synthesis of (+)-muricatacin and analogs is straightforward and uses a strategy developped by Larchevêque[6]. The retrosynthetic pathway is based on the preparation of the acyl-butanolides, followed by a stereoselective reduction :

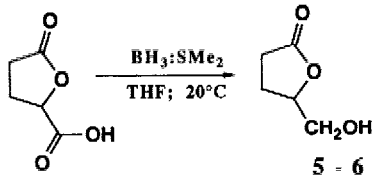


The acyl-butanolides were prepared by condensation of the carboxylic acid chloride, derived from L-glutamic acid after nitrous acid deamination, and the corresponding Grignard reagents[7]. The reductions of the acyl-lactones obtained were performed with high diastereoselectivity with L-Selectride® in order to get the 4*S*,5*S*-muricatacin **1**[8] and analog **2**, or with $NaBH_4$ for the preparation of the diastereoisomers 4*S*,5*R* **3**[8] and **4**.



The relative stereochemistry of the alcohols obtained either by reduction with L-Selectride®, or with $NaBH_4$, was determined by ^1H NMR[9], and the diastereomeric alcohols separated by flash chromatography.

The 4(*R*) and 4(*S*) hydroxymethyl-γ-lactones (**5** and **6** respectively) were obtained after reduction with $BH_3.SMe_2$ of the corresponding carboxylic acids in quantitative yields.



5 - 6

The ^{1}H and ^{13}C NMR data of (+)-muricatacin **1** are identical with the described values for the natural compound[2]. For the $[\alpha]_D$ it is worthnoting that the published value differs from ours[8] : an $[\alpha]_D$= -5.8 was reported for muricatacin isolated from *A. muricata*[2] whereas our measured value for the synthetic compound is +25. But in the case of the natural product, the absolute value is weak, and given without any experimental details about neither the solvent nor the concentration used. Assuming the solvent effects could be negligible, it means that natural muricatacin is present as an enantiomeric mixture with a slight excess of the 4*R*,5*R* isomer, based on our observed $[\alpha]_D$ value for (4*S*,5*S*) muricatacin **1**. With these results in hands, we can confirm the hypothesis advanced about the absolute configuration 4*R*,5*R* of natural muricatacin. In the case of muricatacin obtained after oxidation of annonacin[2], the $[\alpha]_D$ value is still weak but with negative sign ($[\alpha]_D$=-16). Therefore we can conclude for the absolute configuration of the four asymmetric centres of annonacin, based on its known relative configuration[1] *threo-trans-threo,* to be: 15*R*, 16*R*, 19*R*, 20*R*. We now can assume muricatacin isolated in the plant as an enantiomeric mixture is not a precursor of monotetrahydrofuranic acetogenins, but a degradation product of the acetogenins of *A. muricata.* Because all of the isolated monotetrahydrofuranic acetogenins from *A. muricata* have the relative configuration *threo-trans-threo*, and present identical signs for $[\alpha]_D$ than annonacin, we can deduce the same absolute configuration for the identical asymmetric centres in this series: 15*R*, 16*R*, 19*R*, 20*R*.

We have studied the cytotoxicity of (+)-muricatacin **1** and analogs; the observed results of bioassays with the synthetic products for KB and VERO cell lines are reported in table1, and compared with the reported data with isolated acetogenins from *A. muricata* [3-5].

Table 1

Product	Configuration	KB (ED_{50} µg/mL)	VERO (ED_{50} µg/mL)
5	4*R*	$>10^2$	$>10^2$
6	4*S*	$>10^2$	$>10^2$
2	4*S*,5*S*	$>10^2$	$>10^2$
1	4*S*,5*S*	5	11
3	4*S*,5*R*	7.5	14
solamin (VII)		$3\ 10^{-1}$	1
murisolin (VI)		10^{-3}	10^{-1}
corossolin (IV)		$1/3\ 10^{-2}$	$1/3\ 10^{-1}$
corossolone (V)		10^{-1}	310^{-1}
annonacinone (III)		10^{-2}	10^{-1}
annonacin (II)		10^{-4}	10^{-2}
vinblastine		10^{-3}	>3

There is no cytotoxicity till 100 µg/mL for both (*R*) or (*S*) hydroxymethyl-lactones **5** and **6** respectively. Hydroxypropyl-lactone **2** is slightly cytotoxic on KB cell line (30% at 100 µg/mL). Cytotoxicity appears with an increase of the alkyl chain length (R= $C_{12}H_{25}$) with KB (ED_{50}= 5 µg/mL and 7.5 µg/mL for **1** and **3** respectively) and with VERO (ED_{50}= 11 µg/mL and 14 µg/mL for the same products). For both cell lines the *threo* isomer **1** is more toxic than the *erythro* compound **3**. Nevertheless the cytotoxicity of both diastereoisomers (4*S*,5*S*;4*S*,5*R*, respectively **1** and **3**) is still weaker than that obtained for the monotetrahydrofuranic acetogenins isolated from *A. muricata* .

The values obtained for the cytotoxicity of the acetogenins compounds show that activity dramatically increases in the presence and with the number of hydroxyl groups. For instance, with the KB cell line, the ED_{50} for solamin (two hydroxyl groups present in the molecule) is 3.10^{-1} µg/mL, for annonacin (four hydroxyl groups present) 10^{-4} µg/mL.

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References and notes :

◊. Part 14: for part 13 see: Cortes D.; Myint S.H.; Leboeuf M.; Cavé A., *Tetrahedron Lett.* (in press)

1. Rupprecht J.K.; Hui Y.H.; McLaughlin J.L., *J. Nat. Prod.*, **1990**, *53*, 237.

2. Rieser M.J.; Koslowski J.F.; Wood K.V.; McLaughlin J.L., *Tetrahedron Lett.*, **1991**, *32*, 1137.

3. Myint S.H.; Laurens A.; Hocquemiller R.; Cavé A.; Davoust D.; Cortes D., *Heterocycles*, **1990**, *31*, 861.

4. Cortes D.; Myint S.H.; Laurens A.; Hocquemiller R.; Leboeuf M.; Cavé A., *Can. J. Chem.*, **1991**, *69*, 8.

5. Myint S.H.; Cortes D.; Laurens A.; Hocquemiller R.; Leboeuf M.; Cavé A.; Cotte J.; Quero A.M., *Phytochemistry*, **1991**, *30*, 3335.

6. Lalande J.; Larchevêque M., *Bull. Soc. Chim. Fr.*, **1987**, 116. When writing this communication, the synthesis of (-)-muricatacin with an $[\alpha]^{25}_D$ =-22.9, in 16 steps and one resolution of racemic mixture has been published (without any conclusions about the absolute configurations of acetogenins) by Scholtz G. and Tochterman W., *Tetrahedron Letters*, **1991**, *40*, 5535.

7. The use of organomanganese compounds can improve the yields up to 92% in the case of RMnCl (where R= $C_{12}H_{25}$): G. Cahiez, personal communication.

8. All new compounds were fully characterized by elemental analysis (C, H), ^{1}H-NMR, ^{13}C-NMR, MS, IR: (+)-muricatacin **1:** Mp= 65°C; ^{1}H-NMR (200 MHz, $CDCl_3$, ref. to $CHCl_3$) δ ppm; 0.85(t, J= 7.5Hz, CH_3), 1.20-1.40(m, $(CH_2)_{10}$), 1.55(m, CH_2CHOH), 2.10-2.20(m, CH_2-CH_2CO), 2.55(m, CH_2CO), 3.55(m, CH-OH), 4.40(m, CH-O). ^{13}C-NMR (50MHz, $CDCl_3$) δ ppm; 14.0, 22.6, 24.0, 25.4, 28.6, 29.3, 29.5, 29.6, 31.6, 32.9, 73.5, 83.0, 177.4. IR(solution in $CHCl_3$) cm^{-1}: 3580, 3440, 2920, 2840, 1770, 1460, 1375, 1260, 1170, 980, 910. $[\alpha]^{25}_D$=+25 (c=1.7, CH_3OH). MS-ei-20ev(%): 199(<1), 97(7), 87(15), 86(Base); MS-ci-CH_4(%): 285(MH^+, 31), 268(23), 267(Base), 265(17), 239(32), 199(25), 130(23), 125(36), 123(11), 115(21), 113(26), 112(26), 111(49), 109(16).

epi-muricatacin **3:** Mp= 67°C; ^{1}H-NMR (200 MHz, $CDCl_3$, ref. to $CHCl_3$) δ ppm; 0.85(t, J= 7.5Hz, CH_3), 1.20-1.70(m, $(CH_2)_{10}$), 2.10-2.40(m, CH_2-CH_2CO), 2.60(m, CH_2CO), 3.95(m, CH-OH), 4.45(m, CH-O). IR(solution $CHCl_3$) cm^{-1}: 3580, 3440, 2920, 2840, 1770, 1460, 1375, 1260, 1170, 980, 910. $[\alpha]^{25}_D$=+32 (c=2, $CHCl_3$)

9. Dana G.; Chuche J.; Monot J. R., *Bull. Soc. Chim. Fr.*, **1967**, 3308.

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