MURISOLIN: A NEW CYTOTOXIC MONO-TETRAHYDROFURAN-γ-LACTONE FROM ANNONA MURICATA

Saw Hla Myint [a], Alain Laurens [a], Reynald Hocquemiller [a], André Cavé [a], Daniel Davoust, [b] and Diego Cortes [c*]

[a] Laboratoire de Pharmacognosie, URA 496 CNRS, Faculté de Pharmacie, Université Paris-Sud, 92290 Châtenay-Malabry, France

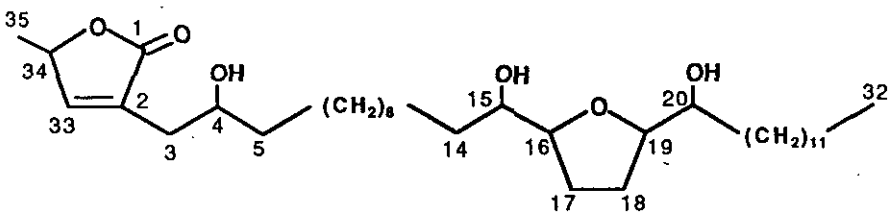
[b] Laboratoire de Chimie Organique, Service RMN, URA 464 CNRS, Université de Rouen, 76134 Mont Saint-Aignan, France

[c] Laboratoire de Pharmacognosie, Faculté de Médecine et Pharmacie, Université de Rouen, 76800 Saint Etienne du Rouvray, France

Abstract - Using cytotoxicity as a bioassay guide led to the isolation of a new active acetogenin from the seed of Annona muricata. Murisolin, 1, is the first example of a mono-tetrahydrofuran-γ-lactone acetogenin with only three hydroxyl groups. Its structure was characterised by mass spectrometry and 2D homonuclear and heteronuclear correlations nmr spectroscopy. The relative stereochemistry of four of its six chiral centers was established by ^{1}H-nmr comparative spectral studies between the murisolin triacetate and some bistetrahydrofuran acetogenin acetates.

Annona muricata (Annonaceae) is a fruit tree originating from central America. It is cultivated for its fruit (corossol, guanabana, soursop) in tropical and subtropical areas. The crude methanolic extract of seeds was biologically potent in brine shrimp test[1] and "crown gall tumor".[2] Through fractionating with solvents (petroleum ether, methylene chloride and methanol) monitored by bioassay, followed by several steps of purification of the active extract (petroleum ether) involving column and thin layer chromatographies, led to murisolin, 1, a new acetogenin. Murisolin is the third representative of mono-tetrahydrofuranic fatty γ-lactone after annonacin from Annona densicoma[3] and goniothalamicin from Goniothalamus giganteus.[4] Murisolin is cytotoxic to different cell culture systems (E.D.50 $< 10^{-1}$ µg/ml in VERO and E.D.50 $< 10^{-3}$ µg/ml in KB) and to brine shrimp (D.L.50= 2 ppm). Several bioactive bistetrahydrofuran-γ-lactone acetogenins[5-17] have also been isolated exclusively from the Annonaceae species and recently annonacinone, isolated from Annona densicoma,[18] and bullatalicin isolated from Annona bullata,[19] have been described.

Murisolin, 1, was isolated from petroleum ether extract of the seeds of Annona muricata. It has been obtained in an amorphous state. The molecular formula, $C_{35}H_{64}O_6$ has been determined by high resolution mass spectrometry (MH^+: 581.4702), and confirmed by mass spectrometry of its acetate derivative.



1, murisolin

Figure 1. ^{1}H-Nmr, 400 MHz Bruker ($CDCl_3$) of *murisolin*, **1**, R= H, and *murisolin triacetate*, **2**, R= Ac (*in* parenthesis). Coupling constants (J, Hz): J_{34-35}= 7; J_{33-34}= 1.6; J_{3a-33} <1; J_{3b-33} <1; J_{3a-3b}= 15; J_{3a-4}= 8; J_{3b-4}= 3; J_{32-31}= 7. (a) : multiplet signal for **2** at δ 1.48-1.65.

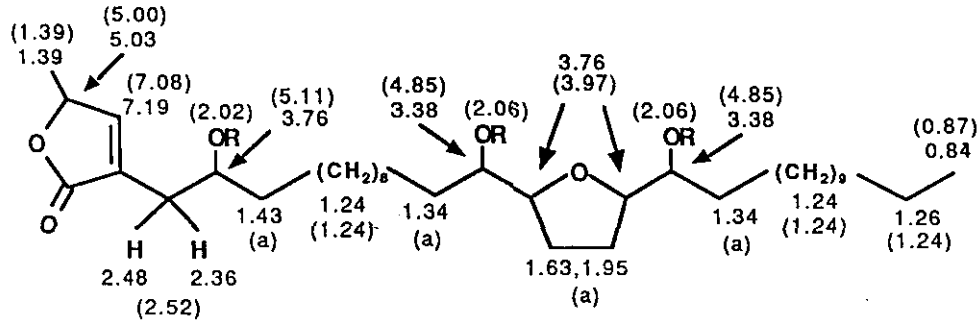
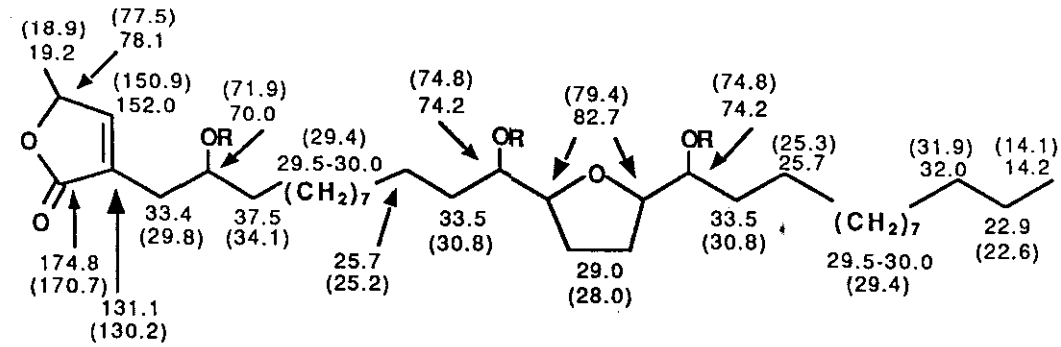


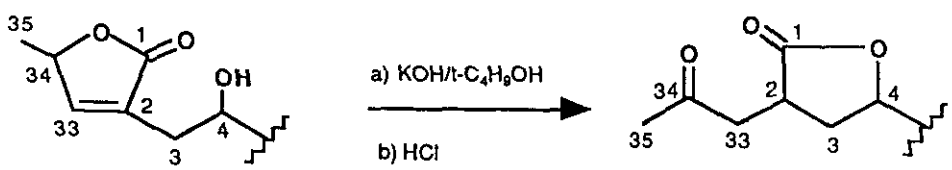
Figure 2. ^{13}C-Nmr, 100 MHz Bruker ($CDCl_3$) of murisolin, **1**, R= H, and murisolin triacetate, **2**, R= Ac (in parenthesis). R= $COCH_3$: (δ 170.9 and 21.1).



The presence of three hydroxyl groups in murisolin, **1**, was suggested by the formation of a triacetate, **2**. This was supported in the infrared spectrum of **1** by a prominent hydroxyl absorption at 3425 cm^{-1}; and the carbonyl absorption at 1740 cm^{-1} suggested an α,β-unsaturated γ-lactone, which was confirmed by the positive response to Kedde's reagent.[5-10] The initial ^1H and ^{13}C-nmr spectra of **1** (Figures 1 and 2) were similar to previous mono-tetrahydrofuran acetogenins.[3,4] The assignment of the ^1H-nmr spectrum of **1** was based on the ^1H-^1H COSY . The ^{13}C-nmr spectrum was assigned through the SPIN ECHO[20] experiment and 2D ^1H-^{13}C correlation. The 400 MHz ^1H-nmr spectrum of **1** (analysis of the ^1H-^1H COSY spectrum), showed resonances at δ 7.19 (d, 1H), 5.03 (dq, 1H) and 1.39 (d, 3H) attributed to H-33, H-34 and CH_3-35 of an α,β-unsaturated γ–lactone;[3-19] five protons on carbons bearing oxygens at δ 3.76 (m, 3H) and 3.38 (m, 2H); an ABX system at δ 3.76, 2.48 and 2.36, characteristic of the presence of an hydroxyl group at C-4 as in asimicin[10] and other acetogenins.[3,4,12,14-19] The position of this hydroxyl group is confirmed by the presence of an allylic coupling, in ^1H-^1H correlation, between H-33 (δ 7,19) and H-3a,3b (δ 2,48 and 2,36). A long alkyl chain was indicated by a triplet signal at δ 0.84 (CH_3-32) coupled with a broad intense signal at δ 1.24 which itself was also coupled with multiplet signals at δ 1.26-1.45. Finally, two multiplet resonances at δ 1.63 and 1.95 (CH_2-17 and CH_2-18) coupled with the multiplet at δ 3.76 (Figure 1). These assignments were confirmed in the ^{13}C-nmr spectrum (Figure 2). The SPIN ECHO[20] spectrum distinguished four methine signals at δ 82.7 (2C), 78.1 (1C), 74.2 (2C) and 70.0 (1C) associated with oxygenated carbons. The analysis of the 2D ^1H-^{13}C heteronuclear correlation spectra further indicated that the proton-34 at δ 5.03 is coupled to lactone methine carbon at δ 78.1; the signal at δ 70.0 coupled to multiplet at δ 3.76, is attributed to the oxygen-bearing carbon C-4;[10] and the resonances at δ 82.7 and 74.2 coupled to multiplets at δ 3.76 and 3.38, respectively, indicated that the hydroxyl groups on C-15 and C-20 and the tetrahydrofuran oxygen bearing carbons C-16 and C-19 are equivalent sets (Figure 2). The downfield shift, in the ^1H-nmr of the murisolin triacetate, **2** (Figure 1) of the signals for H-4 (δ 3.76 to δ 5.11) and H-15,20 (δ 3.38 to δ 4.85), supported these assignments.

The presence of a hydroxyl group in position 4 was definitely confirmed by the conversion of murisolin **1** to the isomer 34-oxo saturated γ-lactone, isomurisolin, **3** (see Figure 3).[16,18]

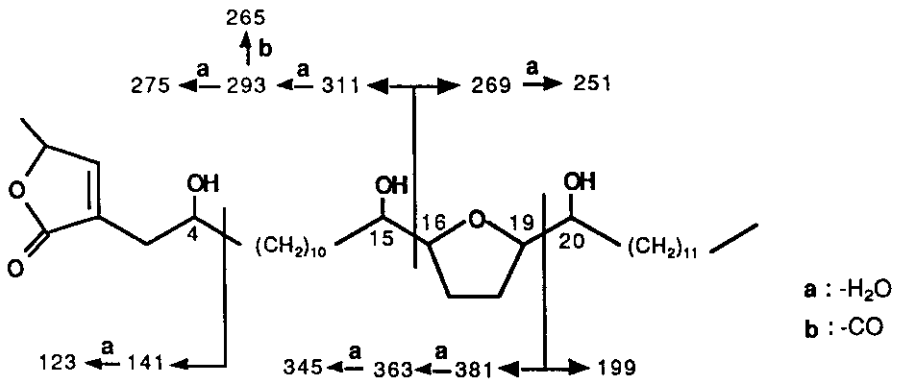
Figure 3. Conversion of murisolin, **1**, to isomurisolin, **3**.



1 **3**

The ms fragmentation pattern of **1** (Figure 4), supported the presence of a single tetrahydrofuran moiety and three hydroxyl groups. The positions of the hydroxyl groups beta to the γ-lactone (C-4) and alpha to the tetrahydrofuran ring (C-15 and C-20) were confirmed by the cleavage of the C-4/C-5, C-15/C-16 and C-19/C-20 bonds.

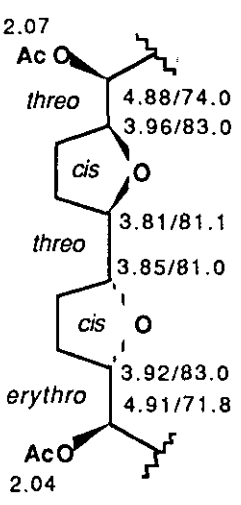
Figure 4. Ms fragment ions of **1**.



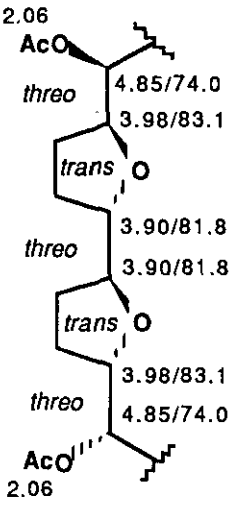
Annonacin,[3] goniothalamicin[4] and murisolin, **1**, were found to possess a single tetrahydrofuran ring and contain only 35 carbons (bistetrahydrofuran γ-lactones contain characteristically 37 carbons[5-17,19]). However, annonacin and goniothalamicin are tetrahydroxy isomer structures (molecular weight 596),[3,4] while murisolin (molecular weight 580) is the first trihydroxylated acetogenin with single tetrahydrofuran system.

Recently, Hoye deduced the relative configuration of bistetrahydrofuran-γ-lactone by comparing the ^{1}H-nmr signals of acetate derivatives with those of bis-tetrahydrofuran "dibutylated diacetate" models.[21,22] Although murisolin, **1**, is mono-tetrahydrofuranic, the chemical shifts in ^{1}H-nmr of murisolin triacetate, **2**, of the chiral centers on the tetrahydrofuran system and its two adjacent acetoxy bearing carbons showed similar shifts as dibutylated diacetate bis-tetrahydrofuran *threo, trans, erythro, trans, threo* or *threo, trans, threo, trans, threo*,[22] as well as asimicin acetate (*threo, trans, threo, trans, threo*).[21] This suggested that the configuration of **2** is probably *threo, trans, threo* (Figure 5). The ^{1}H and ^{13}C-nmr shifts for murisolin triacetate, **2** and murisolin, **1**, are very similar to those of asimicin triacetate and asimicin, but are different to those of rolliniastatin 1 triacetate and rolliniastatin 1; these observations suggested that **1** is also pseudosymmetrical about the midpoint of the tetrahydrofuran system.[12] In addition, the identical shifts of C-15/C-20 and C-16/C-19 for murisolin and the corresponding carbon pairs for asimicin supported this configuration (Figure 5).

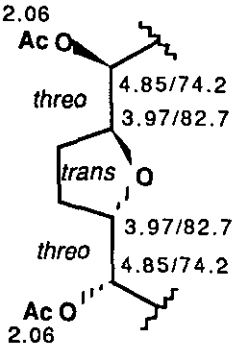
Figure 5. ^{1}H and ^{13}C Nmr assignments and relative stereochemistry of rolliniastatin 1,[12,21] asimicine[10] and murisolin, 1.



rolliniastatin 1 triacetate (^{1}H)
rolliniastatin 1 (^{13}C)



asimicin triacetate (^{1}H)
asimicin (^{13}C)



murisolin triacetate, 2 (^{1}H)
murisolin, 1 (^{13}C)

EXPERIMENTAL

General Experimental Procedures. Optical rotation determinations were made on a Schmidt-Haensch Polartronic I. Uv spectra were recorded using a Unicam 1800 spectrophotometer. Ir spectra were obtained using a Perkin-Elmer 257. The ^{1}H and ^{13}C-nmr spectra (tetramethylsilane as reference standard in deuteriochloroform solution) were obtained with a Bruker AM-400 spectrophotometer. Eims and cims (CH_4 and NH_3) were performed on a Nermag-Sidar.

Plant material. Seeds of A. muricata were collected in Cayenne in French Guiana. A voucher specimen is deposited in the herbaria of the Centre ORSTOM of Cayenne under reference HJ 2395.

Bioassays. The extracts were evaluated for lethality to brine shrimp larvae Artemia salina (1) and for inhibition of "crown gall tumor" on potato discs (2). Cytotoxicity was evaluated at the Laboratoire de Virologie-Immunologie, Châtenay-Malabry using standard protocols for KB (human nasopharyngeal carcinoma cell) and VERO (monkey epithelialoid renal cell).

Extraction and isolation of murisolin, 1. The pulverized seeds of A. muricata (860 g) were extracted in Soxhlet (24 h) successively with petroleum ether (3 l), dichloromethane (3 l) and methanol (3 l). The active petroleum ether extract was partially evaporated. The concentrated extract was filtrated to give 2.1 g of a white precipitate (A) and a filtrate; this filtrate was evaporated to give 175 g of oil, which was extracted with methanol. The methanolic solution

was concentrated to give 8.3 g of solid extract (B). A and B give positive response to α,β–unsaturated γ-lactone reagents. 1.5 g of A were further separated by column chromatography on tlc silica gel (60H, Merck 7736). Elution with CH_2Cl_2/AcOEt (4:6) gave murisolin, **1**, (80 mg) in the first fractions. **1** was obtained in amorphous state. Rf 0.38 (CH_2Cl_2/AcOEt/MeOH 20:70:5); $[\alpha]_D$ + 14.8° (c= 0.1, MeOH); uv λ max, EtOH, nm (log ε): 212 (3.87); ir ν max (film): 3425, 2910, 2840, 1740, 1470, 1315, 1195, 1115, 1070, 1025, 955, 845, 730, 715 cm^{-1}; hrms, m/z: (MH^+): 581.4702 (found), 581.4780 (Calcd.for $C_{35}H_{64}O_6$); cims (NH_3), m/z: 598 $(M + NH_4)^+$, 581 (MH^+); cims (CH_4), m/z: 581 (MH^+), 563 $(MH - H_2O)^+$, 545 $(MH - 2H_2O)^+$, 527 $(MH - 3H_2O)^+$, see Figure 4; ^{1}H-nmr (400 MHz) and ^{13}C-nmr (100 MHz), see Figures 1 and 2.

Murisolin triacetate, 2. 20 mg of **1** was dissolved in 0.1 ml of anhydrous pyridine and the mixture was stirred overnight with 0.4 ml of acetic anhydride at room temperature. **2** was quantitatively obtained as an oil. $C_{41}H_{70}O_9$; ir ν max (film): 2920, 2850, 1740, 1730, 1455, 1370, 1240, 1070, 1025, 720 cm^{-1}; cims (CH_4), m/z: 707 (MH^+), 647 $(MH - AcOH)^+$, 587 $(MH - 2\ AcOH)^+$, 527 $(MH - 3\ AcOH)^+$, 465 (C-19/C-20 cleavage), 405, 345, 311 (C-15/C-16 cleavage), 269, 251, 141; ^{1}H-nmr (400 MHz) and ^{13}C-nmr (100 MHz), see Figures 1 and 2.

Isomurisolin, 3. Murisolin, **1** (19 mg) was treated with 2% KOH in t-BuOH (1.8 ml) at room temperature for 24 h; the solution was acidified with 10 % HCl, and partitioned between CH_2Cl_2/H_2O. The major product (45 %) in organic extract is the 34-oxo saturated γ-lactone, **3**; $C_{35}H_{64}O_6$; ir ν max (film): 3450, 2910, 2840, 1765, 1715, 1470, 1415, 1355, 1305, 1270, 1190, 1070, 965, 755, 720 cm^{-1}; cims (CH_4), m/z: 581 (MH^+), 563 $(MH - H_2O)^+$, 545 $(MH - 2H_2O)^+$, 381 (C-19/C-20 cleavage), 363, 345, 311 (C-15/C-16 cleavage), 293, 199, 141; ^{1}H-nmr, $CDCl_3$, δ: 0.85 (3H, t, J= 7 Hz, CH_3- 32), 1.25-1.45 (42H, m, H- 5-14 and H- 21-31), 1.98, 1.66 (4H, 2m, H- 17 and H- 18), 1.98 (1H, m, H- 3b), 2.20 (3H, s, CH_3- 35), 2.52 (1H, m, H- 3a), 2.62 (1H, m, H- 33a), 3.03 (2H, m, H- 2 and H- 33b), 3.39 (2H, m, H- 15 and H- 20), 3.78 (2H, m, H- 16 and H- 19), 4.37 (1H, m, H- 4); ^{13}C-nmr, $CDCl_3$, δ: 205.6 (C-34), 178.3 (C-1), 82.6 (C- 16 and C- 19), 79.3 (C- 4), 74.0 (C- 15 and C- 20), 43.8 (C-33), 36.6 (C-2), 35.5-25.2 (C-3, C- 5-14 and C- 21-29), 31.9 (C-30), 30.0 (C-35), 22.6 (C-31), 14.1 (C-32).

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