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Muridienin-1 and -2 : The Missing Links in the Biogenetic Precursors of Acetogenins of Annonaceae

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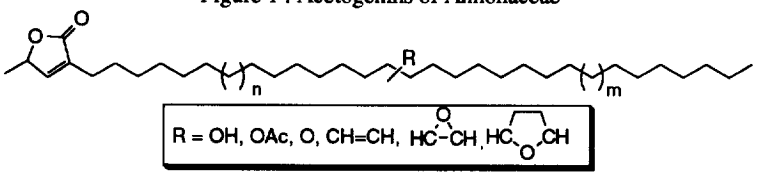
Abstract : Isolation of $\Delta^{n,n+4}$ diunsaturated γ-lactone derivatives of lacceroic and ghedoic acids, allows us to confirm the biogenetic pathway of the bioactive acetogenins of Annonaceae. Copyright © 1996 Elsevier Science Ltd

Acetogenins of Annonaceae are an important class of natural products which possess a large variety of biological properties such as cytotoxic, antitumoral, antiparasitic, insecticide, immunosuppressive activities[1a,b]. These compounds have several structural features in common such as a long alkyl chain (32 or 34 carbon atoms) ended by a γ-lactone ring, and substituted by oxygenated groups (e.g. tetrahydrofuran (s), epoxide (s), hydroxyl (s), etc...). Since about 220 acetogenins have been isolated from around 31 different species of Annonaceae (and only from Annonaceae), we have proposed a classification depending both on the presence and positions of the tetrahydrofuran (THF) rings, as well as on the nature of the lactone ring[1a]. Recently, in our laboratory, we have isolated the first acetogenins of Annonaceae which do not possess any THF ring[2-11], but all other functional groups such as those described above (hydroxyl(s), carbonyl, epoxide(s), double bond(s)). We have classified these compounds in the type E, and postulated that these compounds are the naturally occuring precursors of the THF derivatives (Fig. 1).

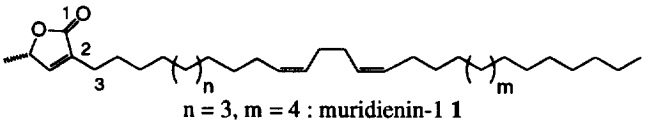
Figure 1 : Acetogenins of Annonaceae



Since then many non-THF acetogenins have been isolated by other[12-15] and chemical transformations have led to the THF parent compounds[2,4]. Isolation of these intermediates have confirmed that the saturated fatty acids in 32 or 34 carbon atoms (lacceroic and ghedoic acids, respectively) are first functionalized at one end to form the γ-lactone ring, and then enzymatic processes (such as dehydrogenation, hydroxylation) should occur[16]. Indeed, we succeeded in

isolating reticulatamol[8] (and its C-37 counterpart), derived from the fully saturated precursor by a single hydroxylation at C-15, confirming the occurRence of these enzymatic steps. However, the $\Delta^{n,n+4}$ diunsaturated precursors were still postulated and never isolated. Therefore, we wish to report in this letter the isolation and characterization of muridienin-1 **1** and muridienin-2 **2** (Fig. 2), the missing links in the biogenetic pathway postulated by us and other[17a,b].

Figure 2



n = 3, m = 4 : muridienin-1 **1**
n = 5, m = 4 : muridienin-2 **2**

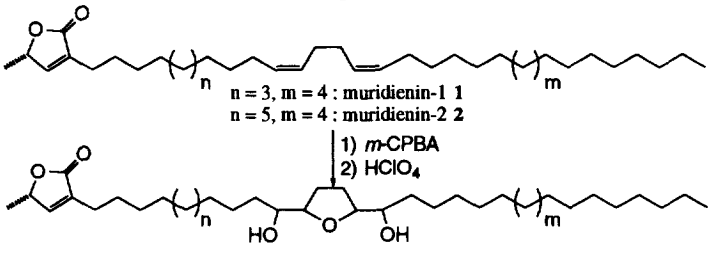
From the CH_2Cl_2 extract of the roots of *Annona muricata*, muridienin-1 **1**[18] (24 mg) and muridienin-2 **2**[18] (12 mg) were isolated after a chromatography on silica gel, followed by reverse phase HPLC (column : μBondapack C18; eluant : MeOH/H_2O (99/1); flow rate : 9 ml/min; Rt = 30.8 and Rt = 36.4 min, for **1** and **2**, respectively). It is worthnoting that both compounds displayed identical spectroscopic data (except mass spectrometry analyses). A positive Kedde reaction, conforted by ^1H and ^{13}C NMR spectra, allowed us to characterize for both compounds the presence of the terminal α,β-unsaturated γ-methyl-γ-lactone of subtype-1a (due to the peak resonances of the vinylic proton at δ (ppm) 6.99, carbinolic proton at δ 4.99, methyl protons at δ 1.41, and the carbonyl at δ (ppm) 173.80, the two ethylenic carbon atoms at δ 148.64 and 134.16, the carbinolic carbon at δ 77.22 and the methyl carbon at δ 19.04). The equivalence of both C-3 protons at δ (ppm) 2.26 indicates the lack of any substituent at C-4. Examination of the rest of the ^1H and ^{13}C NMR spectra, shows the absence of the typical pattern of the THF fragments. The presence of a multiplet at δ (ppm) 5.38-5.41 integrating for four hydrogen atoms, and three signals at δ (ppm) 130.20, 129.44, 128.96, clearly indicated the presence of two double bonds. A multiplet at δ (ppm) 2.08 integrating for 8 protons, correlated to the ethylenic protons (HOHAHA), and to the signal at δ (ppm) 27.25 (HMQC), corresponds to the allylic protons. Irradiation at δ (ppm) 2.08, and observation of the signal at δ 5.38-5.41, showed the coupling constant between the vinylic hydrogens (J = 10.6 Hz), and therefore allowed us to characterize a *cis* configuration for both double bonds (which is conforted by the chemical shifts at δ 27.25 for the allylic carbon atoms). The CIMS (CH_4) spectrum of muridienin-1 **1** shows a peak at m/z = 515 corresponding to [MH^+] ion, allowing us to propose the molecular mass of 514 and therefore to propose the molecular formula : $C_{35}H_{62}O_2$. CIMS (CH_4) spectrum of muridienin-2 **2** shows a peak at m/z = 543, in accord with a molecular formula : $C_{37}H_{66}O_2$.

In accord with biogenetic hypothesis, it can be postulated that the two double bonds are separated by two methylenic groups. In order to confirm this pattern and to locate it on the alkyl chain in both compounds, we decided to separately submit **1** and **2** to *m*-CPBA treatment (1 eq. in THF at room temperature), and then to $HClO_4$ (in THF at r.t.) to form the expected acetogenins

through epoxidation and rings opening and re-closure (Fig. 3). Indeed, the spectroscopic data (^{1}H NMR, MS) of the mixture of products so obtained from muridienin-1 **1** are in accord with a α,α'-dihydroxylated 2,5-disubstituted tetrahydrofuran (unknown mono THF acetogenins hydroxylated at C-13 and C-18), as well as the mixture of products obtained from muridienin-2 **2** (mixture of diastereomers containing uvariamicin I[19]). This confirms the $\Delta^{n,n+4}$ pattern for both dienes (Fig. 3). Furthermore, MS-EI fragmentations of the compounds obtained from **1** proved the location of the THF pattern between C-13 and C-18. Consequently, the location of the double bonds separated by two methylenic groups in the parent molecule, muridienin-1 **1** is at $\Delta^{13,17}$. In the same way, the two double bonds in **2** have been placed at $\Delta^{15,19}$.

Since both muricadienin-1 and -2 possess only one stereogenic center located on the γ-methyl-γ-lactone, we propose that absolute configurations at C-34 and C-36 are (*S*), because of the similar sign of the specific rotation[18] with reticulatamone[9], the only known acetogenin with only one stereogenic center in its γ-methyl-γ-lactone. Furthermore, $RuCl_3$-H_5IO_6 oxidation of muridienin-1 **1**, followed by stereospecific enzymatic oxidation of the so formed L-lactic acid led us to confirm the (*S*) absolute configuration at C-34 of muridienin-1[20], and in turn the (*S*) configuration at C-36 of muridienin-2.

Figure 3



n = 3, m = 4 : mixture of unknown acetogenins
n = 5, m = 4 : mixture containing uvariamicin-I

In conclusion, we have isolated the $\Delta^{13,17}$- and $\Delta^{15,19}$-diunsaturated precursors, muridienin-1 and muridienin-2, conforting therefore the biogenetic pathway of natural acetogenins of Annonaceae (only two other functionalized dienic acetogenins of Annonaceae were already isolated[21a,b]). In fact the $\Delta^{n,n+4}$ pattern has also been found in very long chain fatty acids isolated from marine organisms, principally in marine sponges[22] and sea anemones[23]. These common biogenetic intermediates with very primitive living organisms such as these invertebrates, led us to confirm the archaism of the Annonaceae family.

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18 muridienin-1 (oil): $C_{35}H_{62}O_2$; $[\alpha]_D$ = +11, c= 0.12, MeOH; UV λ : 210 nm (logε : 3.60); IR (ν_{max} cm^{-1}): 2930, 2860, 1755; ^1H NMR (200 MHz, in $CDCl_3$, ref. to $CHCl_3$, δ ppm): 0.90 (t, J= 6.99 Hz, 3H), 1.25-1.42 (brs, 40H), 1.41 (d, J= 6.80 Hz, 3H), 2.08 (m, 8H), 2.26 (t, J= 7.1 Hz, 2H), 4.99 (dq, J= 6.80, 1.73 Hz, 1H), 5.38-5.41 (m, 4H), 6.99 (d, J= 1.73 Hz, 1H); ^{13}C NMR (50 MHz, in $CDCl_3$, ref. to $CHCl_3$, δ ppm): 13.95, 19.04, 22.52, 25.01, 27.25, 30.56, 77.22, 128.96, 129.44, 130.20, 134.16, 148.64, 173.80; CIMS (CH_4) : 515 (MH^+).
muridienin-2 (oil): $C_{37}H_{66}O_2$; $[\alpha]_D$ = +10, c= 0.10, MeOH; UV λ : 215 nm (logε : 3.65); IR, ^1H, ^{13}C NMR data identical to those of muridienin-1; CIMS (CH_4) : 543 (MH^+).

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