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Reticulatain-1 and -2 with reticulatamone : three new polyketides from the seeds of *Annona reticulata [1]*

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Summary – We have isolated four annonaceous acetogenins of identical molecular formula $C_{37}H_{68}O_5$ from *Annona reticulata* seeds. They all belong to the A1 type (one tetrahydrofuran ring). Two of them, reticulatain-1 and reticulatain-2, are new and possess the very rare *threo-trans-erythro* stereochemical relationship across the THF ring. The other two, reticulatacin and uvariamicin III, were also isolated and fully characterized since uvariamicin III was only reported as a component of a mixture. Furthermore, a new acetogenin precursor was extracted and characterized, possessing a single asymmetric center of (*S*) absolute configuration, as proved by its total synthesis.

annonaceous acetogenin / stereospecific synthesis / isolation

Introduction

Annonaceous acetogenins are new polyketides, which have been isolated, up to now, only from the tropical and subtropical plants of the Annonaceae family [2]. They are characterized by common structural features, *ie* a long alkyl chain of 35-37 carbon atoms substituted by one or two tetrahydrofuran (THF) rings, several oxygenated groups (hydroxyl, ketone), and a γ-methyl γ-lactone terminal group. In addition to these natural biologically active products, some natural precursors have been isolated which bear only γ-lactone and either hydroxyl groups and/or 1,2-epoxides with double bonds on the long alkyl chain [3]. Most of these compounds have interesting cytotoxic activity with promising antitumoral potential, and some have antiparasitic, insecticide and immunomodulating properties [4]. In our continuing investigations in the search of new natural products with cytotoxic activities and good therapeutic indexes, we isolated and characterized three new acetogenins from the seeds of *Annona reticulata*, reticulatain-1 **1**, reticulatain-2 **2** and reticulatamone **3** with two known ones, reticulatacin **4** [5] and uvariamicin III **5**, (compound **5** has only been isolated previously in a mixture of three components from *Uvaria narum* [6a] and in a mixture of four compounds from *A bullata* [6b]).

Results and discussion

Reticulatain-1 **1** and -2 **2** were isolated and separated from the methanolic extract of *A reticulata* seeds, after liquid-liquid separation, chromatography on silica gel and HPLC purification (see *Experimental section*). These two new annonaceous acetogenins belong to the A1 group, characterized by the presence of only one THF ring and an α,β-unsaturated γ-methyl γ-lactone [4]. Both products possess similar spectroscopic data (IR, UV, ^{1}H and ^{13}C NMR, CI-MS); only EI-MS, liquid-secondary ion mass spectroscopy (L-SIMS) and collision-induced dissociation (CID) at constant B/E of $[M + Li]^+$ ions allowed us to differentiate these naturally occurring compounds.

The IR spectrum shows a large band centered at 3 500 cm^{-1} for both **1** and **2**, which is typical for hydroxyl groups, and a peak at 1 750 cm^{-1} characteristic of an α,β-unsaturated γ-lactone. The ^{1}H and ^{13}C NMR spectra of both compounds **1** and **2** confirmed the presence of the lactone ring by the chemical shifts for the proton H-36 at δ (ppm) 4.98, H-35 at δ 6.98, and CH_3-37 at δ 1.40, and by the chemical shifts for the carbon atoms for **1** and **2** respectively, as follows : C-1 at δ (ppm) 173.76 and 173.75, C-2 at δ 134.22 and 134.42, C-35 at δ 148.72 and 148.71, C-36 at δ 77.29 and 77.30, and C-37 at δ 19.10 and 19.20. As regards the THF ring, close examination of the ^{1}H NMR signals at δ 3.40 and

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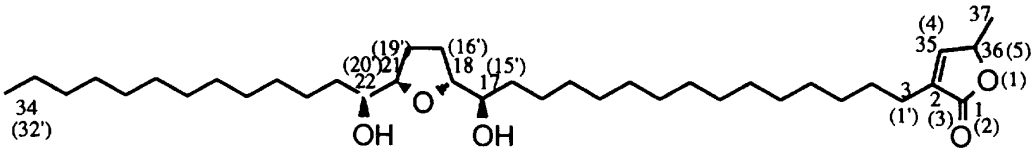
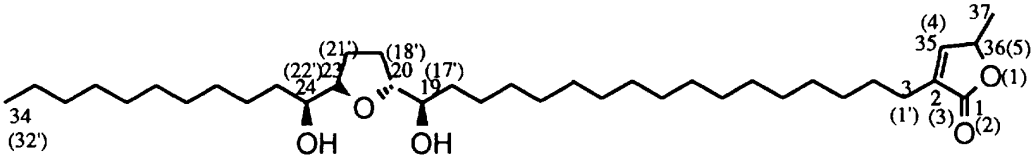
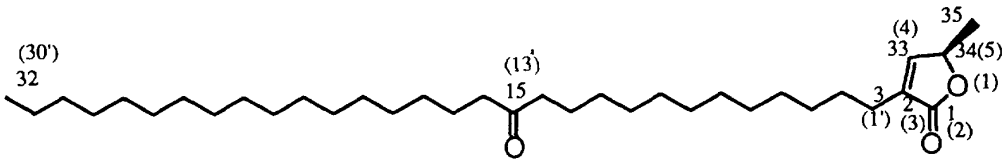


reticulatain-1 **1** (absolute configurations may be inverted).



reticulatain-2 **2** (absolute configurations may be inverted).



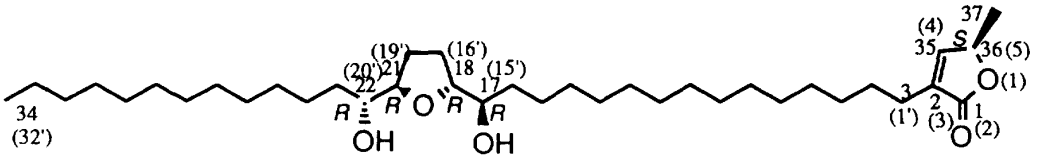
reticulatamone **3**.

3.84 integrating for one and three protons respectively, and comparison with models of known relative configuration [7], allowed us to characterize the presence of an α,α'-dihydroxylated THF ring with *threo-trans-erythro* relative configuration. This stereochemical relationship is rare in the annonaceous acetogenins of the A1 group (to date only annonacin-A [8] and jetein [9] have shown such a configuration) and was confirmed by ^{13}C NMR by the presence of four peaks at δ 71.45, 82.08, 83.16, and 74.24 for C-17, 18, 21, 22 for **1** and 71.74, 82.25, 83.25 and 74.32 for C-19, 20, 23, 24 for **2**. EI-MS of both products **1** and **2** shows a peak at $m/z = 592$ corresponding to the molecular ion $[M]^+$ for the molecular formula $C_{37}H_{68}O_5$. For the location of the THF ring, close examination of the spectra obtained by CID at constant B/E of lithium-cationized molecules of **1** and **2** allowed us to locate the THF rings of **1** between C-18 and C-21 and between C-20 and C-23 for **2** [10].

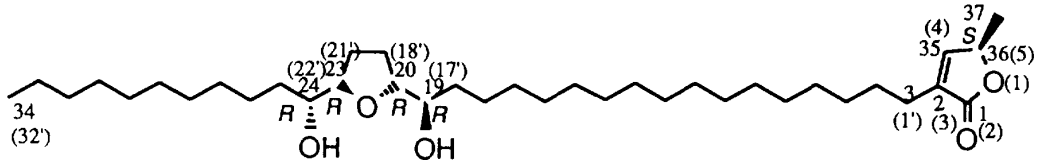
The new annonaceous acetogenin reticulatamone **3** ($[\alpha]_D = +12°$, c = 1, $CHCl_3$) was isolated from a more apolar fraction. Compound **3** possesses very unusual structural features for such compounds, since besides the terminal γ-lactone ring, an oxo-function at C-15 is the sole functional group present. This product is related to reticulatamol which possesses a hydroxyl group at C-15 in place of the oxo function. This was isolated from the same extract, and has also been synthesized previously [11].

The IR spectrum of **3** shows a band at 1 710 cm^{-1} typical for the carbonyl group of a ketone and another band at 1 750 cm^{-1} characteristic of an α,β-unsaturated γ-lactone. The ^1H and ^{13}C NMR spectra show the pattern observed for the lactone ring described above. The ^1H NMR spectrum also shows a triplet at δ (ppm) 2.38 integrating for four hydrogen atoms corresponding to the methylenes at C-14 and C-16. A typical chemical shift at δ (ppm) 211.51 is observed in the ^{13}C NMR corresponding to the carbonyl group of a ketone. CI-MS of **3** shows a peak at $m/z = 533$ corresponding to the $[M + H]^+$ ion of the molecular formula $C_{35}H_{64}O_3$. This was confirmed by a peak at $m/z = 539$ ($[M + Li]^+$) obtained by L-SIMS in the presence of lithium [10]. EI-MS shows a now typical fragmentation at $m/z = 308$ (McLafferty) which allowed us to locate the carbonyl group at C-15. To further confirm the structure of **3**, spectroscopic data were compared with those obtained for a sample of compound **3** that we have prepared in our laboratories; all data (IR, UV, NMR, Mp, MS, $[\alpha]_D$) are identical. This confirms the location of the oxo function, and *the absolute configuration* of the only asymmetric center of the molecule at C-34 being (S), since the sign (+) and absolute value are identical to those observed for compound **3** prepared as its (S) enantiomer (see *Experimental section*). Furthermore **3** was reduced with the couple n-Bu_3SnH/SiO_2 in CH_2Cl_2 [11, 12] to produce reticulatamol, as expected, which showed spectroscopic data identical to the sample of reticulatamol that we had prepared earlier [11].

In addition to these three new compounds, two known annonaceous acetogenins were separated and fully characterized. Reticulatacin **4**, which has been also isolated from *A. reticulata* [5], and whose absolute configurations were determined recently as 17R, 18R, 21R, 22R, and 36S [13], and confirmed by its total synthesis [14a,b]. Furthermore, reticulatacin **4** is probably identical to the uvariamicin II isolated so far as a mixture of three or four products [6a,b]. Uvariamicin III **5**, which was previously obtained from *U. narum* as an insepa-



reticulatacin **4**.



uvariamicin III **5**.

rable mixture with uvariamicin I and II [6a] and from *A. bullata* as an inseparable mixture with uvariamicin I, II and IV [6b], was isolated and fully characterized (see *Experimental section*). Because of a very similar specific rotation with reticulatacin **4**, and because **4** and **5** possess the same relative stereochemical relationship across the THF skeleton (*threo-trans-threo*) (see *Experimental section*), the absolute configuration of uvariamicin III **5** can be assumed to be 19*R*, 20*R*, 23*R*, 24*R*, and 36*S*.

In conclusion, we have succeeded in isolating four annonaceous acetogenins of type A1 with identical molecular formula $C_{37}H_{68}O_5$ by using HPLC. Two of them, reticulatain-1 and -2, are new and have the very rare *threo-trans-erythro* stereochemical relationships across the THF ring. The other two, reticulatacin and uvariamicin III, were known but the latter was not fully characterized because it had only been isolated in a mixture of several components. Furthermore, we have isolated a new acetogenin precursor possessing only an oxo function, reticulatamone, with the unambiguously defined (*S*) absolute configuration at C-34. The biological properties of these new compounds will be reported in a forthcoming report.

Experimental section

General methods

Optical rotations were measured with a Perkin-Elmer 241 Polartronic at 25°C. UV spectra were obtained in MeOH on a Unicam 1800. IR spectra were recorded in $CHCl_3$ on a Nicolet 205 FTIR. CI mass spectra were recorded with a AEI-MS9 mass spectrometer. L-SIMS and CID-*B*/*E* linked scanning experiments were performed on a Kratos MS-80 mass spectrometer under the control of a DS90 data system (cesium ion energy : 20 keV, matrix : *m*-nitrobenzyl alcohol + LiCl; collision gas : argon; collision energy : 4 keV). ^1H and ^{13}C NMR spectra (in $CDCl_3$ with $CHCl_3$ as internal reference) were obtained with a Bruker AC-200 at 200 and 50 MHz, respectively. HPLC analytical analyses were performed with a pump (Waters 600) with UV detection (PDA 990 Waters, at λ 210 nm) and injector (Waters 715) on a Novapack C18 column (4 mm × 150), flow rate : 1.5 mL/min, 10 μg/injection, eluent : CH_3CN/H_2O (95:5). Preparative HPLC was performed with a pump (Waters 600), detector (Waters 484) and injector (Waters 46 K), on Radial Pack C18 (6 μm, 2.5 cm × 10), flow rate : 12 mL/min, 20 mg/injection, eluent : CH_3CN/H_2O (98:2).

Plant material

Seeds of *A. reticulata* were collected in Vietnam (Vinh Long area), and identified by Bui Chi Hieu of the CERMT in Ho Chi Minh Ville (Vietnam).

Extraction and isolation

The toxicity of the crude extracts, as well as of the different fractions, were studied against *Artemia salina* (brine shrimp test) [15]. The dried and powdered seeds of *A. reticulata* (6 kg) were extracted with MeOH. The bioactive methanolic extract was partitioned between H_2O and hexane to yield 143 g of hexanic extract. A portion (14 g) of this extract was fractionated by flash chromatography (elution with CH_2Cl_2/EtOAc 98:2), which was further purified by HPLC to afford 32.3 mg of **1** with a retention time (R_T = 23 min, 46.2 mg of **2** with R_T = 24 min, 41 mg of **4** with R_T = 29 min, and 28 mg of **5** with R_T = 30 min. **3** (27 mg) was isolated by chromatography on silica gel eluted with heptane EtOAc (98:2).

rel-*(5S)*-rel-*(15'R,16'R,19'R,20'S)-3-(16',19'-Epoxy-15',20'-dihydroxydotriacontyl)-5-methylfuran-2(5H)-one* = reticulatain-1 **1**

Whitish amorphous solid, $C_{37}H_{68}O_5$, $[\alpha]_D$ = +22 (*c* = 1, $CHCl_3$).

UV λ_{max} = 207 nm.

IR ν_{max} film cm^{-1} : 3 500, 2 930, 2 850, 1 750.

^1H NMR δ 0.87 (3H, t, *J* = 6.8 Hz, CH_3-34), 1.20-1.50 (52H, m, H-4 to H-16, H-19 to H-20, H-23 to H-33), 1.40 (3H, d, *J* = 6.8 Hz, CH_3-37), 3.40 (1H, m, H-17), 3.84 (3H, m, H-18, 21, 22), 4.98 (1H, qd, *J* = 6.8, 1.5 Hz, H-36), 6.98 (1H, d, *J* = 1.5 Hz, H-35).

^{13}C NMR δ : 14.00 (CH_3-34), 19.10 (CH_3-37), 22.56 (CH_2-33), 25.06, 25.16, 25.47, 25.88, 27.29, 28.49, 29.07, 29.20, 29.52, 31.79, 32.47, 33.13, (CH_2-3 to 16, CH_2-19, CH_2-20, CH_2-23 to 32), 71.45 (CH-17), 74.24 (CH-22), 77.29 (CH-36), 82.08 (CH-18), 83.16 (CH-21), 134.22 (C-2), 148.72 (CH-35), 173.76 (C-1).

CI-MS (isobutane) *m/z* : 593 $[M + H]^+$; L-SIMS + LiCl *m/z* : 599 $[M + Li]^+$; EI-MS *m/z* : 592 $[M]^+$, 574 $[M\text{-}H_2O]^+$, 556 $[M\text{-}2H_2O]^+$, 403, 375, 351, 323, 295, 241;

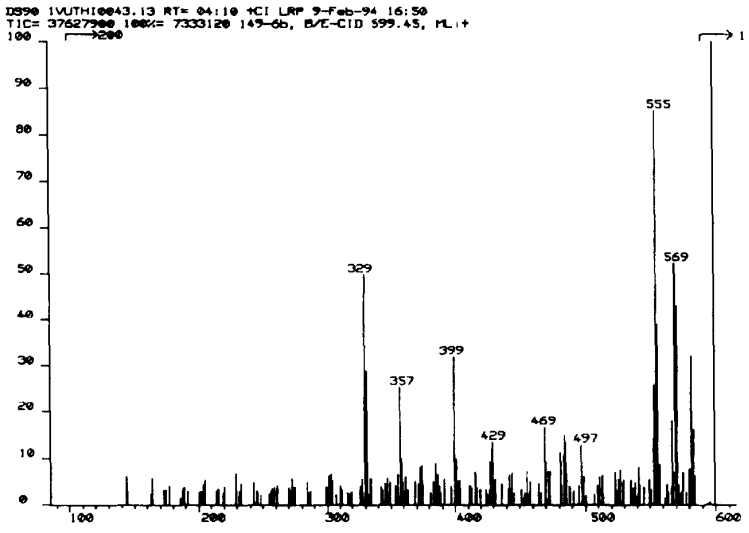


Fig 1. L-SIMS + LiCl (CID B/E) of **1**.

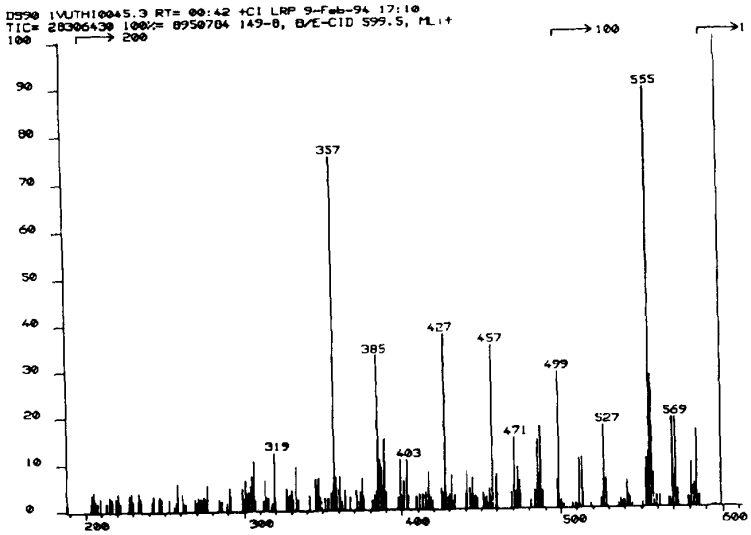


Fig 2. L-SIMS + LiCl (CID B/E) of **2**.

L-SIMS + LiCl (CID B/E) m/z : 599 $[M + Li]^+$, 555 $[M + Li\text{-}CO_2]^+$, 329/357, 399/429 (fig 1).

rel-*(5S)*-rel-*(17'R,18'R,21'R,22'S)-3-(18',21'-Epoxy-17',22'-dihydroxydotriacontyl)-5-methylfuran-2(5H)-one* = reticulatain-2 **2**

Whithish amorphous solid, $C_{37}H_{68}O_5$, $[\alpha]_D$ = +28 (c = 1, $CHCl_3$).

UV λ_{max} = 207 nm.

IR ν_{max} film cm^{-1} : 3 500, 2 930, 2 850, 1 750.

^1H NMR δ 0.87 (3H, t, J = 6.8 Hz, CH_3-34), 1.20-1.50 (52H, m, H-4 to H-18, H-21 to H-22, H-25 to H-33), 1.40 (3H, d, J = 6.8 Hz, CH_3-37), 3.40 (1H, m, H-19), 3.84 (3H, m, H-20, 23, 24), 4.98 (1H, qd, J = 6.8, 1.5 Hz, H-36), 6.98 (1H, d, J = 1.5 Hz, H-35).

^{13}C NMR δ : 14.05 (CH_3-34), 19.20 (CH_3-37), 22.65 (CH_2-33), 25.18, 25.40, 25.58, 25.96, 27.44, 28.60, 29.18, 29.30, 29.62, 31.90, 32.66, 33.34, (CH_2-3 to 18, CH_2-21, CH_2-22, CH_2-25 to 32), 71.74 (CH-19), 74.32 (CH-24), 77.30 (CH-36), 82.25 (CH-20), 83.25 (CH-23), 134.42 (C-2), 148.71 (CH-35), 173.75 (C-1).

CI-MS (isobutane) m/z : 593 $[M + H]^+$; L-SIMS + LiCl m/z : 599 $[M + Li]^+$; EI-MS m/z : 592 $[M]^+$, 574 $[M\text{-}H_2O]^+$, 556 $[M\text{-}2H_2O]^+$, 403, 385, 351, 323, 241; L-SIMS + LiCl (CID B/E) m/z : 599 $[M + Li]^+$, 555 $[M + Li\text{-}CO_2]^+$, 357/385, 427/457 (fig 2).

(5S)-3-(13'-Oxodotriacontyl)-5-methylfuran-2(5H)-one = reticulatamone **3**

It was isolated as a white solid, $C_{35}H_{64}O_3$, $[\alpha]_D$ = +12 (c = 1, $CHCl_3$).

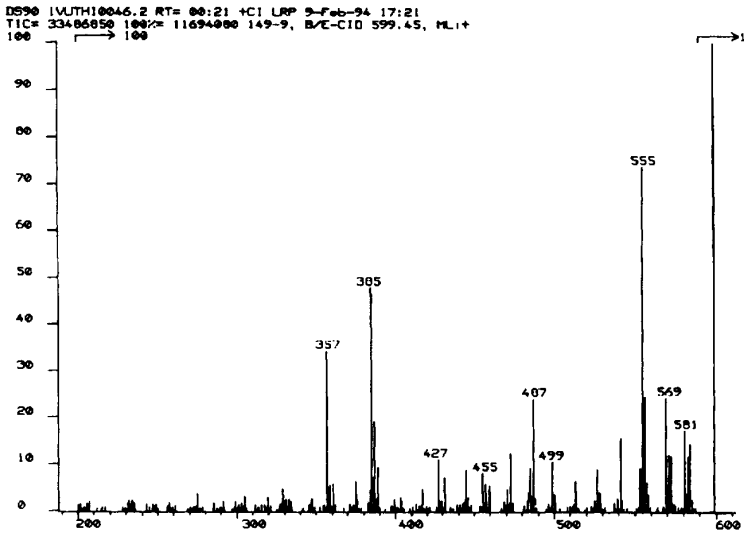


Fig 3. L-SIMS + LiCl (CID B/E) of **5**.

Mp = 83-85°C.

UV λ_{max} = 208 nm.

IR ν_{max} film cm^{-1} : 2 914, 2 848, 1 750, 1 710, 1 470.

^1H NMR δ : 0.89 (3H, t, J = 6.8 Hz, CH_3-32), 1.20-1.50 (50H, m, H-4 to H-13, H-17 to H-31), 1.42 (3H, d, J = 6.8 Hz, CH_3-35), 2.26 (2H, t, J = 6.8 Hz, CH_2-3), 2.38 (4H, t, J = 7 Hz, CH_2-14, CH_2-16), 4.99 (1H, qd, J = 6.8, 1.5 Hz, H-34), 7.00 (1H, d, J = 1.5 Hz, H-33).

^{13}C NMR δ : 14.05 (CH_3-32), 19.19 (CH_3-35), 22.65 (CH_2-31), 23.88, 25.15, 27.40, 29.15, 29.26, 29.40, 29.65, 31.90 (CH_2-3 to 13, CH_2-17 to 30), 42.79 (CH_2-14, CH_2-16), 77.31 (CH-34), 134.33 (C-2), 148.78 (CH-33), 173.77 (C-1), 211.51 (C-15).

CI-MS (isobutane) m/z : 533 $[M + H]^+$, 505 $[M + H\text{-}CO]^+$; L-SIMS + LiCl m/z : 539 $[M + Li]^+$; EI-MS m/z : 532 $[M]^+$, 514 $[M\text{-}H_2O]^+$, 308 (100), 251.

Synthesis of reticulatamone **3**

3 was prepared as described in reference [11]; $[\alpha]_D$ = +12 (c = 0.4, $CHCl_3$). Mp = 83-84°C; IR, UV, ^1H and ^{13}C NMR spectra, MS data are identical to the data reported above.

(5S)-(17'R,18'R,21'R,22'R)-3-(18',21'-Epoxy-17',22'-dihydroxydotriacontyl)-5-methylfuran-2(5H)-one = uvariamicin III **5**

It was isolated as a whitish amorphous solid, $C_{37}H_{68}O_5$, $[\alpha]_D$ = +19 (c = 1, $CHCl_3$).

UV λ_{max} = 207 nm.

IR ν_{max} film cm^{-1} : 3 500, 2 930, 2 850, 1 750.

^1H NMR δ : 0.89 (3H, t, J = 6.8 Hz, CH_3-34), 1.20-1.50 (52H, m, H-4 to H-18, H-21 to H-22, H-25 to H-33), 1.40 (3H, d, J = 6.8 Hz, CH_3-37), 3.40 (2H, m, H-19, 24), 3.84 (2H, m, H-20, 23), 4.98 (1H, qd, J = 6.8, 1.5 Hz, H-36), 6.98 (1H, d, J = 1.5 Hz, H-35).

^{13}C NMR δ : 14.10 (CH_3-34), 19.22 (CH_3-37), 22.68 (CH_2-33), 25.18, 25.60, 27.40, 28.75, 29.18, 29.32, 29.63, 31.91, 33.48, (CH_2-3 to 18, CH_2-21, CH_2-22, CH_2-25 to 32), 74.05 (CH-19, 24), 77.40 (CH-36), 82.63 (CH-20, 23), 134.37 (C-2), 148.80 (CH-35), 173.86 (C-1).

CI-MS (isobutane) m/z : 593 $[M + H]^+$; L-SIMS + LiCl m/z : 599 $[M + Li]^+$; EI-MS m/z : 592 $[M]^+$, 574 $[M\text{-}H_2O]^+$, 556 $[M\text{-}2H_2O]^+$, 403, 375, 351, 323, 295, 267, 241, 141; L-SIMS + LiCl (CID B/E) m/z : 599 $[M + Li]^+$, 555 $[M + Li\text{-}CO_2]^+$, 357/385, 427/457 (fig 3).

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