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Isolation and structure elucidation of sabadelin, an acetogenin from roots of *Annona muricata*☆

Christophe Gleye[a], Alain Laurens[a,*], Olivier Laprévote[b], Laurent Serani[b], Reynald Hocquemiller[a]

[a]*Laboratoire de Pharmacognosie, UPRES-A CNRS 8076 (BioCIS), Faculté de Pharmacie, Université Paris XI, 92296, Châtenay-Malabry cedex, France*

[b]*Laboratoire de Spectrométrie de Masse, Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette, France*

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Abstract

A new acetogenin, sabadelin, has been isolated from the roots of *Annona muricata* and was characterised using tandem mass spectrometry (MS/MS). The structure determination of this compound was strengthened by epoxidation and transformation leading to a known mono-tetrahydrofuran acetogenin. Sabadelin is probably an intermediate in the biosynthetic pathway of mono-THF acetogenins, and it is proposed as a biogenetic precursor of *cis*-panatellin. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Annona muricata*; Annonaceae; Polyketide; Acetogenins; Sabadelin

1. Introduction

Research in the field of Annonaceous acetogenins has evidenced a rapidly increased during the last years (Zafra-Polo, Figadère, Gallardo, Tormo & Cortes, 1998). Currently, more than 300 acetogenins have been isolated.

Annona muricata (Annonaceae), known as ''soursop'', ''sir sak'' or ''guanabana'', is a popular fruit tree cultivated throughout the tropical regions of the world (Kerharo, 1974). Intensive chemical investigations of the seeds and leaves led to the isolation of more than 50 mono-THF acetogenins (Cavé, Figadère, Laurens and Cortes, 1997). Recently, some key intermediate in the biosynthesis of these acetogenins has been isolated from this species, namely epomuricenins-A and -B, montecristin (Gleye, Laurens, Hocquemiller, Cavé, Laprévote & Serani, 1997a), cohibins-A and -B (Gleye, Laurens, Hocquemiller, Cavé, Laprévote & Serani, 1997b), muridienins-1 and -2 (Gleye, Laurens, Hocquemiller, Figadère & Cavé, 1996), muridienins-3 and -4, muricadienin and chatenaytrienins-1, -2 and -3 (Gleye et al., 1998a). The aim of this study is to describe a new product, sabadelin (**1**), which is probably a biogenetic precursor of *cis*-panatellin.

2. Results and discussion

Sabadelin (**1**) (Fig. 1) is a white solid wax obtained from a methanolic extraction of *Annona muricata* roots following by a partition between water and CH_2Cl_2. The CH_2Cl_2 extract was chromatographed on silica gel and Sabadelin (**1**) was isolated by preparative reversed-phase HPLC.

The molecular formula of (**1**) was established to be $C_{35}H_{62}O_3$ from the exact mass measurements of the $[M + H]^+$ ion in the HRCIMS (m/z 531.4695).

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* Corresponding author.

E-mail address: alain.laurens@cep.u-psud.fr (A. Laurens).

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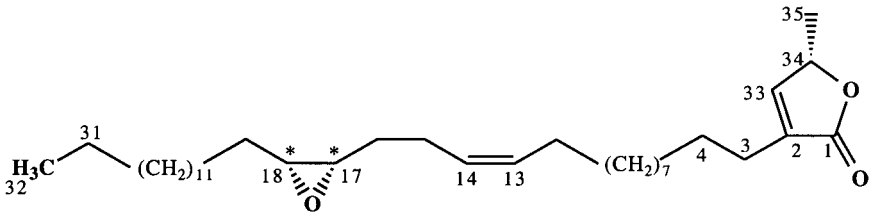

Fig. 1. Structure of sabadelin (**1**). (*) Absolute configurations may be inverted.

IR and UV spectra of sabadelin (**1**) along with a positive Kedde's reaction revealed the presence of an α,β-unsaturated γ-lactone, confirmed by ^{1}H-NMR spectroscopy (Table 1) (Cavé et al., 1997). In addition, the ^{1}H- and ^{13}C-NMR spectra revealed the lack of THF ring by the absence of its characteristic chemical shifts (Cavé et al., 1997). The existence of an epoxide group was suggested by a two-proton multiplet at δ 2.91–2.93 (H-17, H-18) and two carbon resonances at δ 56.8 (C-18) and δ 57.3 (C-17). The presence of an isolated double bond was evidenced by multiple resonances due to two olefinic protons at δ 5.39–5.41 (H-13, H-14) and by two carbon peaks at δ 128.3 (C-13) and δ 131.0 (C-14). The configuration of the double bond was assigned as *cis* by measurements of the vicinal coupling constants ($^3J = 10.7$ and 11.1 Hz, respectively) between the ethylenic protons after selective irradiation of the allylic methylene groups in the ^{1}H-NMR (Roblot, Laugel, Leboeuf, Cavé & Laprévote, 1993). A cross-peak in the double-relayed COSY between the methine protons at δ 5.39 (H-14) and 2.93 (H-17) allowed to place this double bond two methylene units away from the epoxide groups.

Nevertheless, these results do not allow the location of the epoxide-double bond system and their relative position in connection with the lactone ring or the terminal methyl. This structural problem was further solved by mass spectrometry.

The lack of any structurally significant fragment ion peaks in the EIMS spectrum made it necessary to use high-energy collision-induced dissociation (CID) of $[M + Li]^+$ complex (precursor ion obtained by fast cesium ion bombardment and selected at m/z 537).

The MS/MS spectrum of the $[M + Li]^+$ ion at m/z 537 displayed a number of fragment ion peaks among which two different ion series can be distinguished in the spectrum, depending on whether or not, they possess, the terminal lactone ring.

The first ion series (series A), containing the lactone moiety, was formed by remote charge fragmentations (Adams & Gross, 1987) of the whole aliphatic chain leading to the typical pattern of successive ion peaks separated by 14 mass units (Fig. 2). This ion series was interrupted at the substitution or unsaturation sites, thus allowing their location on the alkyl chain. Fragment ion peaks at m/z 229 and 283 were indicative of a double bond (C-13/C-14). The presence of the fragment ions at m/z 339 and 327 suggested the location of the epoxide group between C-17 and C-18 on the alkyl chain. It is also noteworthy that the low mass end of the A series consisted of a radical ion at m/z 118, formed by a cleavage β to the lithiated lactone.

The B series corresponded to ions containing the methyl-terminal side chain of the lithiated molecule. The loss of the lactone ring occurred by a β-cleavage, with loss of 112 daltons from the precursor $[M + Li]^+$ ion (fragment-ion peak at m/z 425) (Laprévote et al., 1992). The sequential remote charge site fragmentations of the aliphatic chain comprised between the lactone and the double bond led to successive ion peaks in the range m/z 425–259 (Fig. 3). The fragment ions at m/z 313 and 259 confirmed the location of the double bond at (C-13/C-14) in the aliphatic chain of (**1**).

In order to confirm the double bond location related

Table 1
^{1}H- and ^{13}C-NMR data ($CDCl_3$, δ) of (**1**)[a]

Atom no.	^{1}H-NMR	^{13}C-NMR
1	–	173.9
2	–	134.3
3	2.26 *t*	25.2
4	1.55 *m*	27.2[b]
5/10	1.25–1.29	26.6–29.7
11	1.32 *m*	26.6–29.7
12	2.05 *m*	27.4[b]
13	5.41 *m*	128.3
14	5.39 *m*	131.0
15	2.22 *m*	24.3
16	1.58 *m*	28.0[b]
17	2.93 *m*	57.3[c]
18	2.91 *m*	56.8[c]
19	1.50 *m*	27.9[b]
20/29	1.25–1.29	26.6–29.7
30	1.25–1.29	31.9
31	1.25–1.29	22.7
32	0.88 *t*	14.1
33	6.98 *d*	148.8
34	4.99 *dq*	77.4
35	1.41 *d*	19.2

[a] $J_{3-4} = 7.3$ Hz; $J_{12-13} = 11$ Hz; $J_{31-32} = 6.8$ Hz; $J_{33-34} = 1.6$ Hz; $J_{34-35} = 6.8$ Hz.
[b,c] interchangeables.

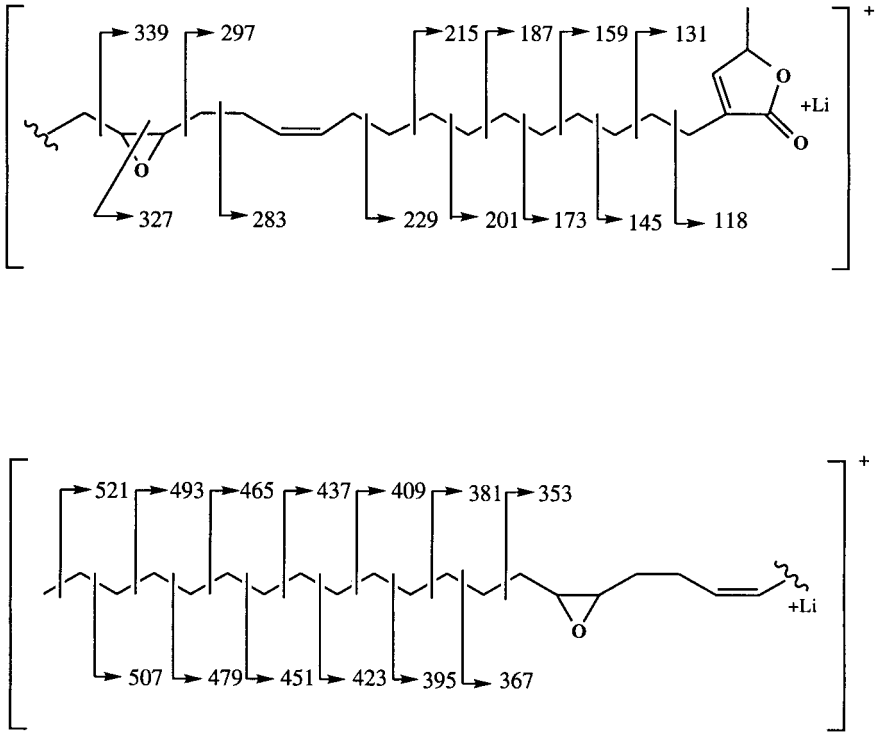

Fig. 2. Fragmentations MS/MS of sabadelin (**1**) corresponding to A series.

to the epoxide group, (**1**) was oxidized with *m*-chloroperbenzoic (*m*-CPBA) acid (Vu Thi et al., 1993) to give the bis-epoxide (**2**). Then, (**2**) was rearranged using perchloric acid ($HClO_4$) (Gromek, Figadère, Hocquemiller, Cavé & Cortes, 1993) into a mixture of stereoisomeric mono-tetrahydrofuran acetogenins (**3**) (Fig. 4). The ^{1}H and ^{13}C spectra of (**3**) displayed a mixture of the characteristic *threo*/*trans*/*threo* and *threo*/*cis*/*threo* relative configuration for the chiral centers of THF system (Fujimoto et al., 1994) which confirmed the *cis* relationship of the double bond and was consistent with a *cis* geometry of the epoxide group for (**1**). The CIMS spectral data were in agreement with those of *cis*-panatellin, reported by us in an earlier study from the roots of *Annona muricata* (Gleye, Duret, Laurens, Hocquemiller & Cavé, 1998b). *Cis*-panatellin was identified by HPLC analysis of the obtained mixture (**3**) by the method of internal standard.

In order to determine the stereoconfiguration of C-34, Sabadelin (**1**) was then subjected to oxidative degradation with ruthenium chloride in the presence of periodic acid prior to enzymatic incubation with L- and D-lactate deshydrogenase and nicotinamide–adenine–dinucleotide (NAD) (Duret et al., 1996). Since NADH was detected only in the L-LDH incubation medium, the presence of *L*-lactic acid could then be deduced, and thus the absolute configuration (*S*) for the stereogenic center C-34 of the α, β-unsaturated γ-lactone of (**1**).

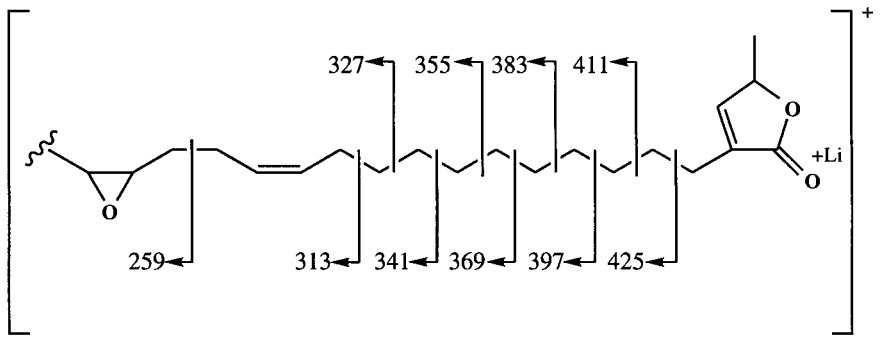

Fig. 3. Fragmentations MS/MS of sabadelin (**1**) corresponding to B series.

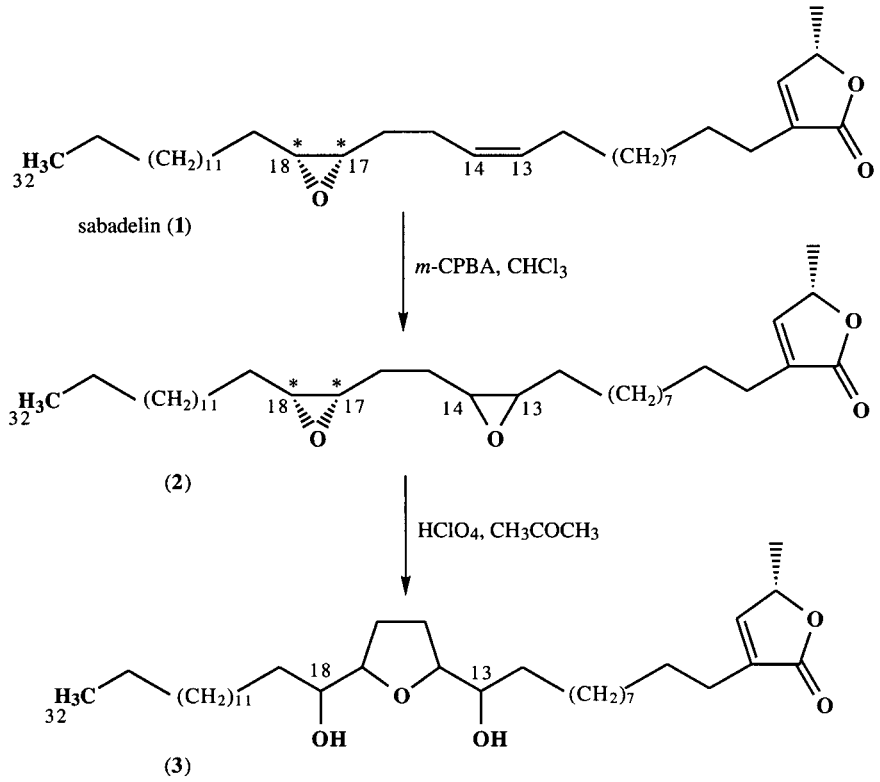

Fig. 4. Oxidation of (**1**) leading to corresponding acetogenins (**3**) via a mixture of diastereoisomeric epoxides (**2**). (*) Absolute configurations may be inverted.

Mono-THF acetogenins are proposed to be biosynthetized from double bonds of the parent fatty acids through epoxide intermediates (Roblot et al., 1993). On the basis of this hypothetic biogenetic pathway, sabadelin (**1**) must be derived from a $\Delta^{13,18}$ diunsaturated precursor, named muridienin-1, reported by us in an earlier study (Gleye et al., 1996). An oxidation step led to the unsaturated epoxide, sabadelin (**1**) or to the regioisomer epomuricenin-B with the double bond at C-17/C-18 and the epoxide group at C-13/C-14. In this way, we can postulate that oxidation of double bond is not regiospecific. An oxidation of the double bond (C-13/C-14 for sabadelin, C-17/C-18 for epomuricenin-B) could lead to a hypothetical *bis*-epoxide *anti* which rearranges to the dihydroxylated *cis*-panatellin.

Consequently, the isolation of sabadelin (**1**) is of particular interest regarding the previously suggested biogenetic pathway leading to tetrahydrofuran acetogenins of the Annonaceae.

Sabadelin (**1**) is the second example of acetogenin with a double bond between the lactone and an epoxide group after a regioisomer, epoxymurin B, isolated from stem bark of *Annona muricata* (Hisham, Sreekala, Pieters, de Bruyne, van den Heuvel & Claeys, 1993).

3. Experimental

3.1. Apparatus

Optical rotations were measured with a Schmidt–Haensch polartronic E. UV spectra were determined in MeOH on a Philips PU 8720 spectrophotometer. IR spectra were recorded on a Perkin–Elmer 257 spectrophotometer. The ^{1}H-NMR spectra were obtained with a Bruker AC-400 at 400 MHz. The ^{13}C-NMR spectra were obtained with a Bruker AC-200 at 50 MHz. EIMS (48 eV) and CIMS (CH_4) were registered with a Nermag spectrometer R10-10C. MS/MS spectra were obtained using a ZabSpec-T five-sector tandem mass spectrometer (Micromass, Manchester, UK). The first analyzer (MS1) comprises a Zabspec triple sector ($E_1B_1E_2$) instrument and the second mass spectrometer (MS2) consists of a double sector instrument (B_2E_3) of reverse Mattauch–Herzog geometry focusing the ion beam on a focal plane. $[M + Li]^+$ precursor ions were generated by cesium ion bombardment at 30 keV (matrix: *m*-NBA + LiCl). The precursor ions submitted to MS/MS experiments were selected by MS1 set at appropriate E and B values and then focused in a collision cell located in the fourth field-free region

(between E_2 and B_2). Helium was introduced at a pressure leading to an attenuation of the precursor ion beam of almost 70%. The collision cell was floated at 4 kV so as attain a collision energy of 4 keV. Fragment ions detection was achieved by use of the MCAD detector operating with a mass ratio of 1.225 : 1.0 at an angle of 30° with regard on the ion beam (Ballard, Gaskell, Jennings, Scrivens & Vickers, 1992; Scrivens, Rollins, Jennings, Bordoli & Bateman, 1992; Bordoli & Bateman, 1992). For each MS/MS acquisition, the mass scale comprised between the precursor ion peak and the lowest mass end (m/z 50) was covered by successive overlapping exposures of 0.5 *s*. HPLC was performed with a pump (Waters 590), detector UV (Waters 84) and injector (Waters SSV).

3.2. Plant material

Roots of *Annona muricata* (Annonaceae) were collected in Guinea (Conakry) in October 1993. A voucher specimen (AL 380) has been deposited at the Faculty of Medecine and Pharmacy of Conakry.

3.3. Extraction and isolation

The dried and powdered roots (600 g) were extracted with MeOH to give a brown extract (60 g). The concentrated MeOH extract was partitioned between H_2O and CH_2Cl_2 to yield 45 g of CH_2Cl_2 extract. This extract was subjected to silica gel column chromatography (silica gel Merck 70–230 mesh) and eluted with hexane containing increasing amount of AcOEt. The Fractions collected were analyzed by TLC (silica gel Merck 60F254), on which basis they were grouped into 17 sets.

The solvent of the fraction number two was evaporated off. The resulting residue (3.6 g) was subjected successively to exclusion chromatography (Sephadex LH-20 Pharmacia–Biotech) and HPLC using reversed phase Waters preparative on μ Bondapak C_{18} 10 μm (250 × 20 mm) cartridge column, flow rate 9 ml/mn, 20 mg/injection and eluent CH_3OH/H_2O (97/3). A 7 mg amount of (**1**) was obtained.

3.4. Sabadelin (*1*)

White waxy solid (7 mg). $[\alpha]_D + 12°$ (*c* 0.19, MeOH). IR KBr cm^{-1} (2900, 2830, 1740, 1460, 1310, 1200, 1110, 1060, 1010). UV λ_{max} (MeOH) nm (log ε) 213.4 (3.63). HRCIMS (CH_4) m/z 531.4695 $[MH]^+$ calc. 531.4780 for $[C_{35}H_{63}O_3 + H]^+$. CIMS (CH_4): m/z 531 $[MH]^+$ (100%), 513 $[MH–H_2O]^+$. EIMS (40 eV): m/z 347, 181, 81, 55, 43. MS/MS: (Figs. 2 and 3). ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 50 MHz): Table 1.

3.5. Oxidation and rearrangement of sabadelin (*1*)

To 5 mg of (**1**) dissolved in 0.5 ml of $CHCl_3$ were added 1 eq. of *m*-CPBA. The mixture was stirred for 3 h at room temperature, washed with 1% $NaHCO_3$, extracted with CH_2Cl_2, evaporated in vacuo and purified by HPLC procedure using reversed phase Waters preparative on μ Bondapak C_{18} 10 μm (250 × 20 mm) cartridge column, flow rate 9 ml/mn, eluent CH_3OH/H_2O (95/5). (**2**) (5 mg) was isolated as a mixture of stereoisomers. (**2**) was dissolved in acetone (0.5 ml), treated with 70% perchloric acid (10 μl), stirred 8 h at room temperature, and then evaporated to dryness under vacuum. Purification by HPLC using reversed phase Waters preparative on μ Bondapak C_{18} 10 μm (250 × 20 mm) cartridge column, flow rate 9 ml/mn, eluent CH_3OH/H_2O/THF (90/10/5), give (**3**) (3.5 mg).

3.6. Bis-epoxides of sabadelin (*2*)

CIMS (CH_4): m/z 547 $[MH]^+$, 529 $[MH–H_2O]^+$, 511 $[MH–2H_2O]^+$, 267, 111, 97, 83. EIMS (40 eV): m/z 528, 267, 111, 109, 97, 95, 81, 55. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ 0.88 (3H, *t*, J = 7.1 Hz, H-32), 1.22–1.37 (38H, *m*, H-5 to H-11, H-20 to H-31), 1.41 (3H, *d*, J = 6.8 Hz, H-35), 1.51 (4H, *m*, H-12, H-19), 1.55 (2H, *m*, H-4), 1.68 (4H, *m*, H-15, H-16), 2.26 (2H, *t*, J = 7.0 Hz, H-3), 2.93–2.99 (4H, *m*, H-13, H-14, H-17, H-18), 4.99 (1H, *dq*, J = 1.5, 6.7 Hz, H-32), 6.98 (1H, *d*, J = 1.5 Hz, H-33). ^{13}C-NMR spectrum (50 MHz, $CDCl_3$): δ 14.1 (C-32), 19.0 (C-35), 22.5 (C-31), 25.0 (C-15, C-16), 25.2 (C-3), 26.3–29.9 (C-5 to C-11, C-20 to C-29), 27.4 (C-4, C-12, C-19), 31.8 (C-30), 56.5 and 57.0 (C-13, C-14, C-17, C-18), 77.5 (C-34), 134.2 (C-2), 148.8 (C-33), 173.9 (C-1).

3.7. Mono-tetrahydrofuran acetogenins (*3*)

CIMS (CH_4): m/z 565 $[MH]^+$ (100%), 547 $[MH–H_2O]+$, 529 $[MH–2H_2O]^+$, 346, 267, 229, 97. EIMS (40 eV): m/z 347, 267, 227, 199, 181, 121, 111. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ 0.89 (3H, *t*, J = 6.6 Hz, H-32), 1.27–1.36 (38H, *m*, H-5 to H-11, H-20 to H-31), 1.39–1.49 (4H, *m*, H-12, H-19), 1.41 (3H, *d*, J = 6.8 Hz, H-35), 1.55 (2H, *m*, H-4), 1.63–1.77 (2H, *m*, $H\text{-}15_a$, $H\text{-}16_a$), 1.95–2.00 (2H, *m*, $H\text{-}15_b$, $H\text{-}16_b$), 2.28 (2H, *t*, J = 7.9 Hz, H-3), 3.40–3.43 (2H, *m*, H-13, H-18), 3.79–3.83 (2H, *m*, H-14, H-17), 4.99 (1H, *dq*, J = 1.6, 6.7 Hz, H-34), 6.99 (1H, *d*, J = 1.6 Hz, H-33). ^{13}C-NMR spectrum (50 MHz, $CDCl_3$): δ 14.0 (C-32), 19.2 (C-35), 22.3 (C-31), 25.2 (C-3), 25.5–29.7 (C-5 to C-11, C-20 to C-29), 27.3 (C-4), 28.1 and 28.8 (C-15, C-16), 31.7 (C-30), 33.6 and 34.1 (C-12, C-19), 74.0 and 74.3 (C-13, C-18), 77.4 (C-34), 82.7–82.8 (C-14, C-17), 134.2 (C-2), 148.7 (C-33), 173.3 (C-1).

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