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Highly oxygenated sesquiterpenes from the liverwort *Trocholejeunea sandvicensis*

El Hassane Lahlou, Toshihiro Hashimoto, Yoshinori Asakawa*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, 770-8514 Tokushima, Japan

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Abstract

Four pinguisane type sesquiterpenes were isolated from the liverwort *Trocholejeunea scandvicensis*, together with three aromatic compounds. The structures of the cited compounds were established on the basis of spectroscopic means. The first two compounds were new sesquiterpenes, while the other compounds were previously isolated from other liverworts and lichen sp., respectively. The stereochemistry for lejeuneapinguisanolide was determined by X-ray analysis; a possible biosynthetic pathway to it was postulated. The other new sesquiterpene is lejeuneapinguisenone.

Keywords: *Trocholejeunea sandvicensis*; Liverwort; Hepaticae; Sesquiterpenes; Pinguisanes; Aromatic compounds

1. Introduction

The interest in the liverworts (Hepaticae) has dramatically increased since their constituents have shown high therapeutic effects as antitumor activity, antimicrobial and antifungal activity, superoxide release inhibitory activity, lipoxygenase, calmodulin, hyaluronidase, cyclooxygenase and thrombin inhibitory activity, neuritic sprouting activity, muscle relaxing activity and other important biological activities (Asakawa, 1995, 1998). It has been demonstrated that most of the Hepaticae contain mainly sesqui- and diterpenes and lipophilic aromatic compounds, and the biological activities of liverworts are due to these substances (Asakawa, 1995, 1998).

In this paper, we report the isolation and structure determination of two new pinguisane type sesquiterpenes named lejeuneapinguisanolide (**1**) which presented high degree of oxidation and lejeuneapinguisenone (**2**). Also, we propose a possible biosynthetic pathway for **1** in which furanopinguisanol (**4**) and a molecule of oxygen would play key roles.

2. Results and discussion

Previously, we have reported the isolation and structure determination of several pinguisane type sesquiterpenes from the liverwort *Trocholejeunea sandvicensis* (Tori, Arbiyanti, Taira & Asakawa, 1993). Reinvestigation of this plant led to the isolation of lejeuneapinguisanolide (**1**), lejeuneapinguisenone (**2**), dehydropinguisenol (**3**), furanopinguisanol (**4**), 3,4-methylenedioxy-3′-methoxybibenzyl (**6**), apigenin-7,4′-dimethyl ether (**7**) and atranorin (**8**), respectively. The structures of compounds **3**–**4** and **6**–**8** were established by comparing their spectroscopic data and physical constants with those reported in the literature (Tori et al., 1993; Asakawa, 1982; Markharm & Given, 1988; Huneck & Yoshimura, 1996). These compounds were previously isolated from other liverworts and lichen sp. Compounds **1** and **2** were new sesquiterpenes.

The ^{1}H-NMR spectrum of **1** (Table 1) displayed signals for two tertiary methyl groups and two secondary methyl groups characteristic of a pinguisane skeleton

* Corresponding author. Tel.: +81-88-622-9611; fax: +81-88-655-3051.

E-mail address: asakawa@ph.bunri-ac.jp (Y. Asakawa).

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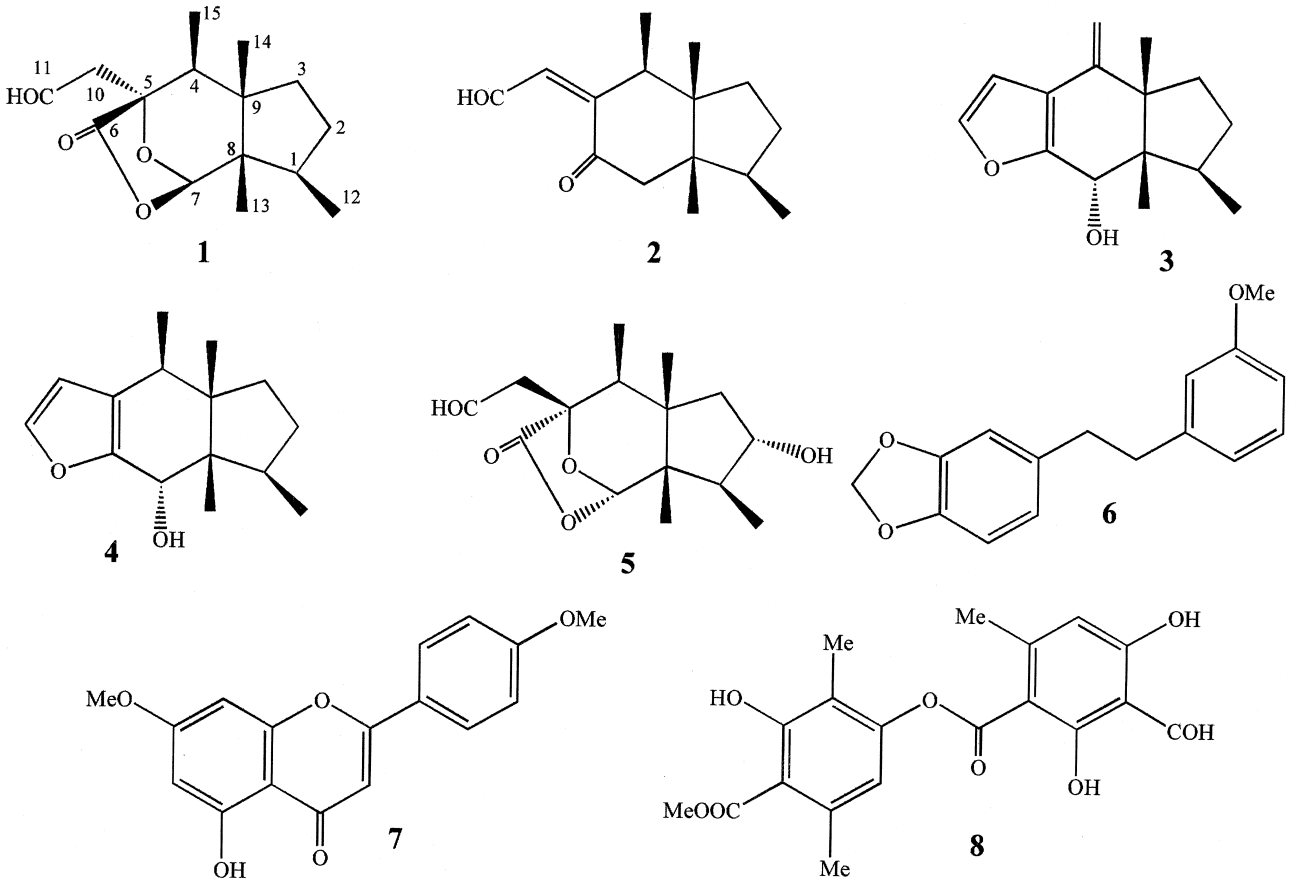
Table 1
^{1}H-NMR spectral data of compounds **1** and **2** (600 MHz, C_6D_6, TMS)

H	1	2
1	2.45 *m*	1.60 *m*
2a	1.01 *m*	1.11 *m*
2b	1.54 *m*	1.26 *m*
3a	1.17 *m*	0.93 *m*
3b	0.97 *m*	1.41 *m*
4	1.68 *q* (J = 6.1 Hz)	2.11 *q* (J = 2.4, 6.6 and 13.2 Hz)
7a	5.00 *s*	1.81 *d* (J = 15.0 Hz)
7b	–	2.21 *d* (J = 15.0 Hz)
10a	2.30 *dd* (J = 3.8 and 16.5 Hz)	5.77 *dd* (J = 2.4 and 7.8 Hz)
10b	2.43 *dd* (J = 1.2 and 16.5 Hz)	–
11	9.40 *dd* (J = 0.6 and 7.4 Hz)	10.17 *d* (J = 7.8 Hz)
12	0.42 *s*	0.39 *s*
13	0.54 *d* (J = 4.2 Hz)	0.40 *d* (J = 7.2 Hz)
14	0.80 *s*	0.41 *s*
15	0.53 *d* (J = 3.0 Hz)	1.56 *d* (J = 6.6 Hz)

Table 2
^{13}C-NMR spectral data for compounds **1** and **2** (150 MHz, C_6D_6, TMS)

C	1	2
1	37.4	40.7
2	30.4	35.1
3	38.6	30.0
4	41.4	42.7
5	79.7	161.3
6	172.7	202.1
7	107.2	49.7
8	48.1	50.1
9	47.7	48.7
10	45.4	129.7
11	196.3	191.1
12	13.2	18.6
13	14.3	14.5
14	19.3	15.6
15	9.6	11.4

type. A proton signal at δ 9.40 ppm corresponding to an aldehyde group was observed. Its IR spectrum showed bands for lactone (1794 cm^{-1}), aldehyde (1732 cm^{-1}) and acetal (928 cm^{-1}) groups. The ^{13}C-NMR spectrum (Table 2) accounts for the presence of 15 carbon atoms in the molecule of which those resonating at 196.3, 172.7 and 107.2 were assignable to aldehydic, lactonic and acetal groups, respectively. The mass spectrum of **1** showed a $[M + 1]^+$ at m/z 267 corresponding to a molecular formula $C_{15}H_{23}O_4$, confirmed by its high resolution mass spectrum. The position of the lactone group was determined by combined HMQC and HMBC spectra of **1**. In the HMBC spectrum, the proton signal at δ 1.68 ppm corresponding



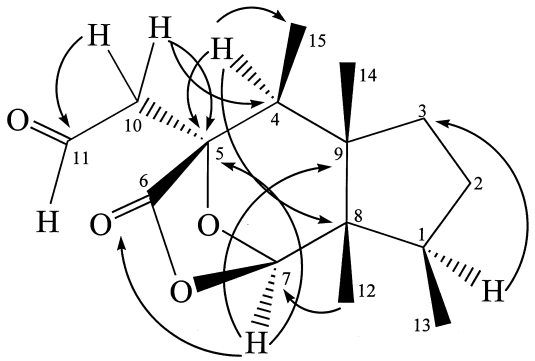


Fig. 1. Three-and two-bond correlations for compound **1** in the HBMC spectrum.

to the methine H-4 showed correlation at two bonds with a quaternary carbon signal at 79.7 ppm attributed to the (C-5). The C-5 was three bonds away from the methine proton signal at δ 5.00 ppm in the HMBC. This proton was linked to a carbon signal at 107.2 ppm in the HMQC which was assigned to C-7. The H-7 signal exhibited correlations at three bonds with C-9 and with a carbon signal at 172.7 ppm ascribed to the carbonyl group at (C-6) in the HMBC spectrum. These correlations indicated that the lactone group was located between C-5 and C-7. Other important correlations were depicted in Fig. 1. The relative stereochemistry around C-5 and C-7 was deduced from the NOESY spectrum of **1**, since weak NOE was observed between the proton H-7 and the methyl H-12, while the proton H-1 did not show NOE with H-7. A molecular mechanic calculation using the MNDO program, showed that the H-7 is far from the H-1. The distance is about 3.08 Å, which explained the absence of NOE between these two protons in the NOESY spectrum. These results suggested an α equatorial position for the H-7 and a β diaxial position for the lactone group. A good crystal was obtained and the stereostructure of lejeuneapinguisanolide for **1** was established by X-ray diffraction analysis as shown in Fig. 2.

The ^{1}H-NMR spectrum of **2** (Table 1) was similar to that of **1**, and presented two tertiary methyl groups and two secondary methyl groups of a pinguisane skeleton type. An olefinic proton as a double doublet at δ 5.77 ppm (J = 2.4, 7.8 Hz) and an aldehydic proton as a doublet at δ 10.17 ppm (J = 7.8 Hz) were also found in the same spectrum, suggesting an adjacent position for these protons. The presence of the aldehyde group and the double bond in the molecule was confirmed by the observation of the carbon signals at δ 191.1, 161.3 and 129.7 ppm in the ^{13}C-NMR spectrum of **2** (Table 2), while carbon signal at δ 202.1 ppm indicated the existence of a ketone group in the

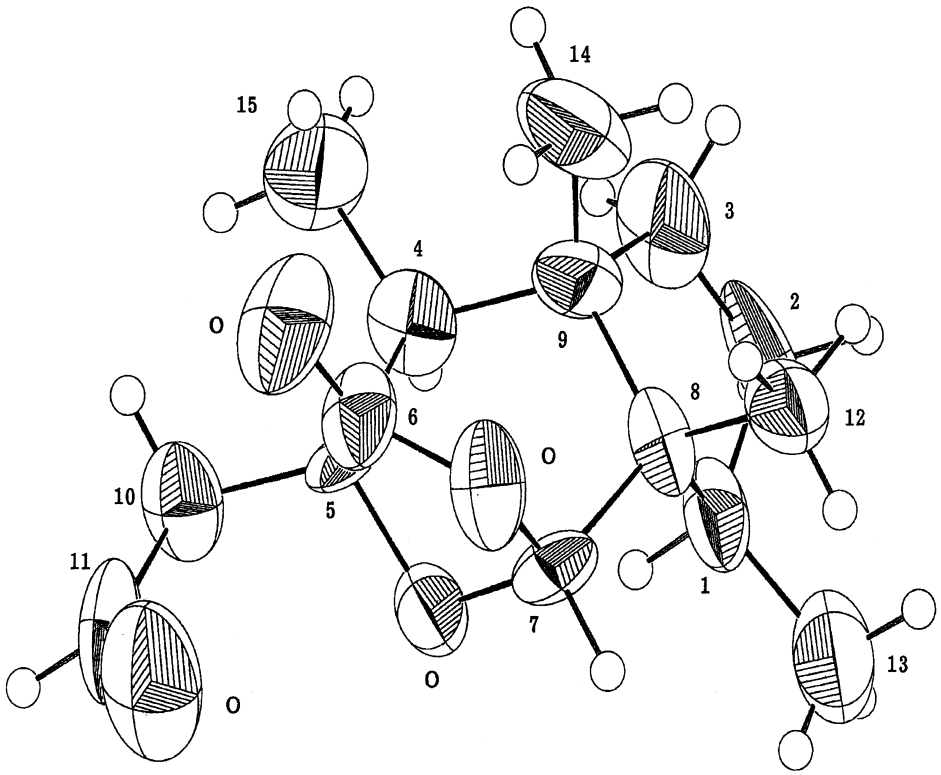


Fig. 2. An ORTEP view of compound **1**.

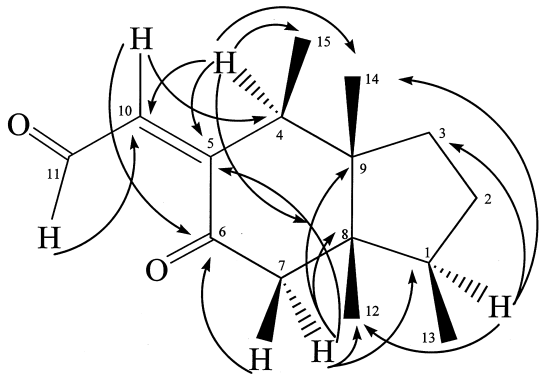


Fig. 3. Three-and two-bond correlations for compound **2** in the HBMC spectrum.

molecule. The mass spectrum of **2** exhibited a molecular ion at m/z 234, and its high resolution mass spectrum established a molecular formula $C_{15}H_{22}O_2$. In the HMBC spectrum of **2**, the olefinic proton H-10 displayed correlation at three bonds with the methine carbon C-4 and with the carbon signal at δ 202.1 assignable to the ketone group at (C-6). This latter correlation indicated that the ketone group was α,β unsaturated. Other important correlations were shown in Fig. 3. The *cis* position for the substitution group of the double bond in **2** was established by NOESY spectrum, since NOE was observed between the olefinic proton H-10 and the methyl proton H-15, while the aldehydic proton did not show NOE with this latter methyl. The data cited above was in accordance with the structure lejeuneapinguisenone **2** proposed for this new compound.

The absolute configuration of **1** and **2** might be the same as those found in the other liverworts, such as *Anuera pinguis* and *Porella retrospetans* (Asakawa, 1982, 1995).

Previously, we have described the isolation and the structure determination of porellapinguisanolide (**5**), a sesquiterpene with a similar structure to that of **1** with an α position for the lactone group and with one hydroxyl group attached to the C-2 from the liverwort *P. cordaena* (Toyota, Nagashima & Asakawa, 1989). Sesquiterpenes with high degree of oxygenation like **1** are rare in the Hepaticae family. The formation of **1** could be explained through a pathway in which the addition of a molecule of oxygen to the furan system of furanopinguisanol **4**, followed by intramolecular condensation could account for the formation of **1** as shown in Fig. 4. Compound **2** could be obtained by a cleavage of the epidioxide of **1**.

3. Experimental

3.1. General

^{1}H- and ^{13}C-NMR, and HMQC, HMBC and NOESY: TMS as internal standard using JEOL UNITY 600 (600 MHz); MS: JEOL JMS-AX 500; IR: JASCO FT/IR-5300; UV: HITACHI U-3000 spectrophotometer; Optical Rotation: JASCO DIP-1000 polarimeter; mp: YANACO MP.

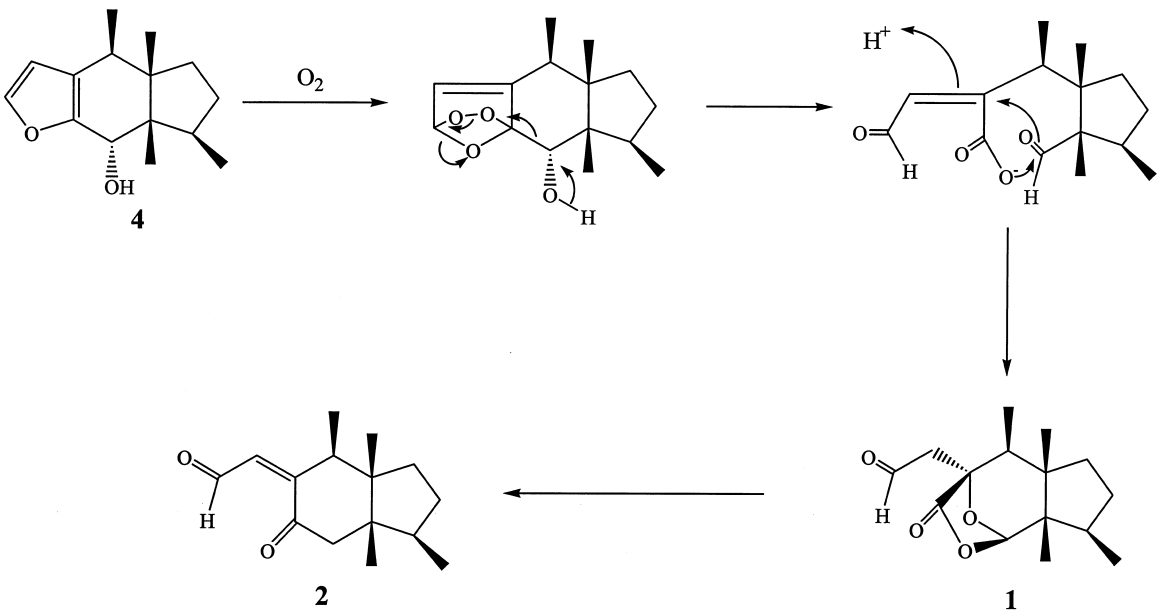


Fig. 4. A possible biosynthetic pathway for compound **1**.

3.2. Plant material

Trocholejeunea sandvicensis (Gott.) Mizut. was collected on the rock in Omichi, Tokushima, Japan, in March 1999, and identified by Yoshinori Asakawa.

3.3. Extraction and isolation

The plant material (1406 g) was extracted with Et_2O for one month. The crude extract was filtered and evaporated in vacuum to give a residue (20.51 g) which was subjected to a silica gel column (300 g). Elution was carried out using gradients of *n*-hexane–EtOAc, EtOAc and MeOH.

The material eluted with 100% *n*-hexane was subjected to a Sephadex LH-20 (100 g) column, previously equilibrated with *n*-hexane–$CHCl_3$–MeOH (2:1:1), and eluted with the same mixture, to give 3,4-methylenedioxy-3′-methoxybibenzyl (**6**) as a yellow solid (341 mg) (Asakawa, 1982).

Two fractions were obtained from the material eluted with 10% EtOAc. The first fraction was submitted to a Sephadex LH-20 (100 g) column previously equilibrated, and eluted with the same mixture of solvent mentioned above, to give dehydropinguisenol **3** (1.80 g) and furanopinguisanol **4** (520 mg) (Tori et al., 1993). The second fraction was applied to a Sephadex LH-20 (100 g) column, fraction containing (**2**) was subjected to a prep. TLC developed with benzene–EtOAc (95:5). The material at an R_f 0.7 was recovered and purified by HPLC in an CHEMCOSORB 5Si-U column (10 × 250 (w) C/N. 02511), eluting with *n*-hexane–EtOAc (8:2) at a flow rate of 3.0 ml min^{-1} with detection at 254 nm. The material eluted at R_t 10.4 min was collected, and concentrated to afford **2** (7.4 mg) as a colorless oil. The fraction containing (**8**) was submitted to a prep. TLC developed with benzene–EtOAc (95:5) to give atranorin **8** (5 mg) as a white powder (Huneck & Yoshimura, 1996).

The material eluted with 50% EtOAc was subjected to a Sephadex LH-20 (100 g) column, fractions that showed the existence of (**7**) were combined and submitted to a silica gel column (33 g) eluted with *n*-hexane–EtOAc (9:1) to give apigenin-7,4′-dimethyl ether **7** (10 mg) as a yellow solid (Markharm & Given, 1988).

The material eluted with 100% EtOAc was applied to a Sephadex LH-20 (100 g) column, fraction containing **1** was subjected to a prep. TLC developed with *n*-hexane–EtOAc (6:4). The band with an R_f 0.6 was cut off and injected to a HPLC column (CHEMCOSORB 5Si-U, 10 × 250 (w) C/N. 02511), eluting with *n*-hexane–EtOAc (6:2) at a flow rate of 3.0 ml min^{-1} with detection at 254 nm. The material eluted at R_t 8.25 min was collected, and concentrated to afford **1** (3.0 mg) as a colorless solid.

3.4. Lejeuneapinguisanolide (*1*)

Mp 180–197°C; $\{\alpha\}_D^{25}$ +23° (*c* 0.1, $CHCl_3$); FTIR ν_{max} cm^{-1}: 1794, 1732, 928; UV λ_{ma} nm (log ε): 236 (2.04), 233 (2.03) (*c* 0.38 × 10^{-3}, EtOH); ^{1}H- and ^{13}C-NMR: see (Tables 1 and 2); HREIMS: found 267.1592 $C_{15}H_{22}O_4$ requires 267.692; EIMS *m/z* (rel. int.): 267 $[M + 1]^+$ (52), 223 (100), 221 (89), 176 (41), 137 (45), 123 (46), 95 (42).

3.5. X-ray crystallographic analysis of *1*

Compound **1** was recrystallized from *n*-hexane–EtOAc. X-ray crystallographic analysis was carried out on a Mac Science MXC 18 diffractometer with Cu K_α radiation. The structure of **1** was solved by direct method using CRYSTAN SIR92 and refined by full-matrix least squares using CRYSTAN; $M_f = C_{15}H_{22}O_4$, $M_r = 268.00$, monoclinic, $P2_1$, $a = 11.324$ (6) Å, $b = 6.400$ (4) Å, $c = 10.190$ (7) Å, $\beta = 103.74$ (5)°, $V = 717.4$ (8) Å^3, $Z = 2$, $D_x = 1.240$ Mg m^{-3}, $D_m = 1.300$ Mg m^{-3}, λ (Cu K_α) = 0.71073 Å, $\mu = 0.828$ mm^{-1}, cell parameters from 20 reflections, $\theta = 1$–15°, $\mu = 0.828$ mm^{-1}, T = 298 K, absorption correction: none, $R_{int} = 0.092$, $\theta_{max} = 25.06°$, 1929 measured reflections, 1337 independent reflections, 503 observed reflections, refinement on F, $R = 0.060$, $wR = 0.073$, $S = 1.748$, 503 reflections, 171 parameters, only coordinates of H atoms refined, $(\Delta/\sigma)max = 0.3468$, $\Delta\rho_{max} = 0.18$ Å^{-3}, $\Delta\rho_{max} = -0.23$ Å^{-3}, extinction correction: none.

3.6. Lejeuneapinguisenone (*2*)

HREIMS: found 234.1587 $C_{15}H_{22}O_2$ requires 230.8587; EIMS *m/z* (rel. int.): 234 $[M]^+$ (6), 232 (20), 216 (73), 201 (38), 175 (34), 109 (100), 95 (81); ^{1}H- and ^{13}C-NMR: see Tables 1 and 2). IR and $[\alpha]_D$ for **2** were not recorded, because the compound was unstable and decomposed when it was stored in the refrigerator.

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