Tetrahedron Letters 54 (2013) 6023–6028

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Novel ring A rearranged isomers with γ-lactone from the fruits of *Aphanamixis grandifolia*

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ARTICLE INFO

Article history:
Received 11 March 2013
Revised 6 August 2013
Accepted 20 August 2013
Available online 31 August 2013

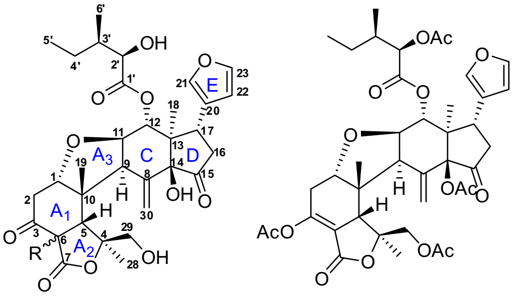
Keywords:
Limonoids
Isomers
Acetylated derivative
Density functional theory (DFT) calculation
Interconverting mechanism
Meliaceae

ABSTRACT

Aphagranols A (**1**) and B (**2**), a pair of isomers with a novel limonoid carbon skeleton, were isolated from the fruits of *Aphanamixis grandifolia*. Their structures were determined by spectroscopic analyses and further confirmed by their unique acetylated derivative aphagranol C (**3**). The possible equilibrium system of the enol interconversion was presented using the polarizable continuum model (PCM). The absolute configuration of **3** was established via experimental and calculated ECD spectra comparison. The γ-lactone moiety in ring A system was first found in this class of limonoids.

The structurally diverse and biologically significant limonoids from plants of the family Meliaceae have prompted continuous investigations on this kind of metabolites.[1–4] The genus *Aphanamixis* (Meliaceae) comprising ca. 4 species grows mainly in the tropical and subtropical regions of China.[5] The root bark of *Aphanamixis grandifolia* has been used as Chinese folk medicine to treat colds, numbness of limbs, and difficulty in both extending and flexing joints. Previous chemical investigations on *A. grandifolia* led to the identification of an array of structurally interesting compounds, including the modified triterpenoids (tirucallane-type[6–14] and cycloartane-type[15,16]), various limonoids,[17–19] and sesquiterpenoids.[20] In particular, the limonoid derivatives exhibited a significant biological activity to the murine P388 lymphocytic leukemia.[17,18] In continuation of our research, aphagranols A (**1**) and B (**2**) existing in solution as a mixture of interconverting C-6 regioisomers in a ratio of 2:1, with an unprecedented carbon skeleton, were isolated from the fruits of *A. grandifolia*. Their fully unique acetylated derivative aphagranol C (**3**) was further used for the study of structural interconverting mechanism of **1** and **2**. We present herein the isolation and structural elucidation of compounds **1**–**3** (Fig. 1).

The air-dried and powdered fruits of *A. grandifolia* (2.8 kg) were extracted with 95% aq. EtOH (3 × 10 L) under reflux. After the removal of the solvent under reduced pressure, the crude extract (405.3 g) was suspended in H_2O (1 L) and successively partitioned with petroleum ether (3 × 1 L) and EtOAc (3 × 1 L). The EtOAc extract (110.2 g) was subjected to a silica gel column, eluted with a gradient of CH_2Cl_2/acetone (20:1 to 0:1), to give seven fractions (A–G). Fraction F (12.5 g) was extensively separated over columns of silica gel and RP-C_{18} silica gel to obtain **1**/**2** (200.5 mg, a pair of isomers **1**:**2** = 2:1). Part of **1**/**2** (180.0 mg, 0.3 mM) and acetic anhydride (3.0 mM) were added into the solvent of pyridine (2.0 mL), then the mixture was stirred for 4 h at room temperature. After the removal of the solvents under reduced pressure, the residue



1 R=β-H
2 R=α-H

3

Figure 1. Structures of aphagranols A–C (**1**–**3**).

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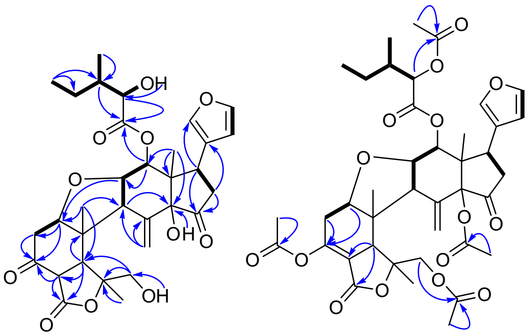
http://dx.doi.org/10.1016/j.tetlet.2013.08.075

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) data of **1** and **2** (DMSO-d_6)

Position	**1**		**2**	
	δ_C, Type	δ_H (Multi, *J* in Hz)	δ_C, Type	δ_H (Multi, *J* in Hz)
1	83.3, CH	4.03 (d, 5.5)	83.0, CH	3.81 (d, 3.0)
2α	40.6, CH_2	2.34 (d, 16.0)	40.8, CH_2	2.98 (d, 17.0)
2β		3.07 (dd, 16.0, 5.5)		2.29 (dd, 17.0, 3.0)
3	203.8, C		200.3, C	
4	90.1, C		87.1, C	
5	51.1, CH	3.36 (d, 9.5)	47.9, CH	2.92 (d, 15.5)
6	52.7, CH	4.01 (d, 9.5)	46.6, CH	3.67 (d, 15.5)
7	169.6, C		168.1, C	
8	141.0, C		141.8, C	
9	49.9, CH	3.38 (d, 10.0)	45.6, CH	3.85 (d, 10.0)
10	43.9, C		46.8, C	
11	80.5, CH	4.05 (dd, 10.0, 7.5)	79.5, CH	4.24 (t, 9.0)
12	74.4, CH	5.77 (d, 7.5)	74.7, CH	5.78 (d, 8.0)
13	47.4, C		47.4, C	
14	78.0, C		78.3, C	
15	209.3, C		209.1, C	
16α	41.1, CH_2	2.48 (dd, 19.5, 10.0)	41.3, CH_2	2.46 (dd, 19.0, 10.0)
16β		2.75 (dd, 19.5, 8.5)		2.76 (dd, 19.0, 8.5)
17	34.5, CH	3.62 (t, 9.0)	34.2, CH	3.58 (dd, 10.0, 8.5)
18	12.0, CH_3	0.74 (s)	12.2, CH_3	0.74 (s)
19	24.5, CH_3	1.32 (s)	23.8, CH_3	1.19 (s)
20	123.4, C		123.4, C	
21	140.2, CH	7.37 (s)	140.2, CH	7.37 (s)
22	111.4, CH	6.41 (s)	111.3, CH	6.39 (s)
23	142.5, CH	7.52 (s)	142.5, CH	7.52 (s)
28	19.0, CH_3	1.09 (s)	19.2, CH_3	1.49 (s)
29a	66.3, CH_2	3.66 (dd, 12.0, 6.0)	66.1, CH_2	3.59 (dd, 12.5, 5.5)
29b		3.62 (dd, 12.0, 6.0)		3.48 (dd, 12.5, 5.5)
30a	118.4, CH_2	6.10 (s)	118.0, CH_2	6.17 (s)
30b		5.13 (s)		5.22 (s)
1′	173.7, C		173.8, C	
2′	73.7, CH	3.40 (dd, 5.5, 3.5)	73.6, CH	3.40 (dd, 5.5, 3.5)
3′	38.7, CH	1.53 (m)	38.6, CH	1.53 (m)
4′a	23.5, CH_2	1.27 (m)	23.6, CH_2	1.27 (m)
4′b		1.03 (m)		1.03 (m)
5′	11.6, CH_3	0.68 (d, 7.5)	11.5, CH_3	0.71 (d, 7.5)
6′	15.1, CH_3	0.81 (d, 7.0)	15.1, CH_3	0.80 (d, 7.0)
14-OH		6.46 (s)		6.45 (s)
29-OH		5.35 (d, 6.0)		5.25 (d, 5.5)
2′-OH		5.00 (d, 5.5)		4.99 (d, 5.5)

was purified by a silica gel column eluted with petroleum ether/EtOAc (3:1) to give **3** (125.5 mg).

The mixture of **1**/**2**[21] was isolated as white optically active amorphous powder with $[\alpha]_D^{27}$ −38.4 (*c* 0.35, MeOH). Negative HR-ESI-MS measurement of the ion peak $[M-H]^-$ at *m*/*z* 599.2508 established a molecular formula of $C_{32}H_{40}O_{11}$ for **1**/**2**, indicating 13° of unsaturation. The IR spectrum of **1**/**2** had absorption bands at 3458 and 1770 cm^{-1} suggesting the presence of hydroxyl and ester carbonyl functionalities. A prominent feature of the ^{1}H NMR (measured in DMSO-d_6) spectrum of **1**/**2** (Table 1) was the presence of multiple pairs of signals each possessing the same splitting pattern. This suggested that the sample was a mixture of two similar compounds. Chemical exchange correlations were observed between these paired signals in a ROESY spectrum. Comparison of the integrals for each of the signals within a pair signified that a pair of isomers were present in equilibrium with a relative ratio of about 2:1 as measured by ^{1}H NMR. Each isomer (Table 1) in the detailed analyses of the 1D NMR spectra revealed a 2-hydroxy-3-methylpentanoyl moiety, a typical β-substituted furan, an exocyclic double bond, three tertiary methyls and four carbonyls as well as three exchangeable protons that were distinguished from the others by the HSQC spectrum. The above observations accounted for eight degrees of unsaturation and required that **1**/**2** possessed five additional rings.



1/2 **3**

Figure 2. ^{1}H–^{1}H COSY (bold lines) and the crucial HMBC (arrows) correlations of **1**–**3**.

Comprehensive analyses of the 2D NMR spectra, particularly by the HMBC spectrum, indicated the two isomers of **1**/**2** possessed the same planar structure. The complex ring system of the major isomer (**1**) was constructed by the HMBC correlations as follows (Fig. 2). The characteristic proton signals appearing as an ABX spin system at δ_H 4.03 (d, *J* = 5.5 Hz), 3.07 (dd, *J* = 16.0, 5.5 Hz), and 2.34 (d, *J* = 16.0 Hz) were assigned to H-1, H-2β, and H-2α, and an AX spin system at δ_H 3.36 (d, *J* = 9.5 Hz) and 4.01 (d, *J* = 9.5 Hz) was assigned to H-5 and H-6, respectively. The existence of a cyclohexanone group (ring A_1) was established by the ^{1}H–^{1}H COSY correlation of H-1/H_2-2 and H-5/H-6, together with their HMBC