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Highly Acylated Diterpenoids with a New 3,4-Secograyanane Skeleton from the Flower Buds of *Rhododendron molle*

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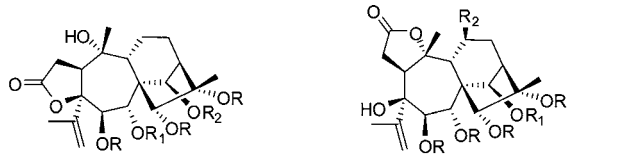
ABSTRACT



1 R = R_2 = Ac; R_1 = propionyl **3** R = Ac; R_1 = propionyl; R_2 = H
2 R = R_1 = Ac; R_2 = propionyl **4** R = Ac; R_1 = propionyl; R_2 = OAc

Four highly acylated diterpenoids with a new 3,4-secograyanane skeleton, secorhodomollolides A−D (1−4), have been isolated from the flower buds of *Rhododendron molle*. Their structures including absolute configurations were determined on the basis of spectroscopic data interpretation and single-crystal X-ray crystallography. Compound 4 exhibited significant analgesic and sedative effects at a dose of 5 mg/kg, and compound 2 showed selective cytotoxic activity against human hepatoma carcinoma cell line (Bel-7402) with IC_{50} 0.97 μM.

Species of the genus Rhododendron (Ericaceae) have long been used for the treatment of various diseases such as cough, bronchitis, osteomyelitis, pain, and injury in the People's Republic of China.[1] In particular, the extract of the flowers of *Rhododendron molle* G. Don, a well-known poisonous plant widely distributed in the southern Chinese Mainland, was used as a narcotic in clinical cases of surgery.[1] Chemical and biological investigation resulted in isolation and characterization of more than 20 diterpenoids with blood pressure and heart rate lowering, neurotoxic, cardiotoxic, cytotoxic, and insecticidal properties,[2] from different parts of this plant.[3] The diterpenoids are categorized into grayanane and kalmane

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(1) Jiangsu New Medical College *Dictionary of Traditional Chinese Medicine*; Shanghai Science and Technology Publishing House: Shanghai, 1977; pp 264−265, 300, 753, 1014−1015, 1156, 1415, 1447−1449, 1920, 2354−2355, 2489.

(2) For some examples, see: (a) Codding, P. W. *J. Am. Chem. Soc.* **1984**, *106*, 7905–7909. (b) Chen, G.; Gao, Y.; Mao, H. Y.; Li, Y. S. *Chin. Med. J.* **1985**, *98*, 325–328. (c) Yakehiro, M.; Yamamoto, S.; Baba, N.; Nakajima, S.; Iwasa, J.; Seyama, I. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 1328–1332. (d) Yakehiro, M.; Yuki, T.; Yamaoka, K.; Furue, T.; Mori, Y.; Imoto, K.; Seyama, I. *Mol. Pharmacol.* **2000**, *58*, 692–700. (e) Zhong, G. H.; Liu, J. X.; Weng, Q. F.; Hu, M. Y.; Luo, J. J. *Pest Manage. Sci.* **2006**, *62*, 976–981.

(3) For some examples, see: (a) Wuhan Medical College: *Yaoxue Tongbao* **1980**, *15*, 39−40. (b) Pu, Q. L. *Zhongcaoyao* **1983**, *14*, 293–294. (c) Liu, Z. G.; Pan, X. F.; Chen, C. Y.; Chen., J. S. *Acta Pharmacol. Sin.* **1990**, *25*, 830–833. (d) Li, C. J.; Wang, L. Q.; Chen, S. N.; Qin, G. W. *J. Nat. Prod.* **2000**, *63*, 1214–1217, and references cited therein. (e) Bao, G. H.; Wang, Li. Q.; Cheng, K. F.; Feng, Y. H.; Li, X. Y.; Qin, G. W. *Planta Med.* **2003**, *69*, 434–439. (f) Chen, S. N.; Zhang, H. P.; Wang, L. Q.; Bao, G. H.; Qin, G. W. *J. Nat. Prod.* **2004**, *67*, 1903–1906. (g) Zhong, G. H.; Hu, M. Y.; Wei, X. Y.; Weng, Q. F.; Xie, J. J.; Liu, J. X.; Wang, W. X. *J. Nat. Prod.* **2005**, *68*, 924–926.

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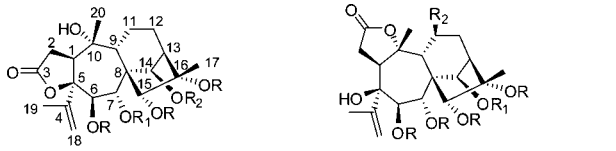
skeletons biogenetically derived from *ent*-kaurane derivatives.[4] In a preliminary pharmacological test, an EtOH extract of the flower buds of *R. molle* at a dose of 50 mg/kg (i.p.) showed significant sedative effect in a pentobarbital sodium-induced sleep test.[5] As part of a program to assess the chemical and biological diversity of traditional Chinese medicines,[6] we carried out the investigation of the extract.[7] From the EtOAc soluble portion of the extract, four highly acylated diterpenoids with a new 3,4-secograyanane skeleton, designated as secorhodomollolides A−D (**1**−**4**), have been characterized. We report herein the isolation, structure elucidation, postulated biogenetic formation, and biological activities of compounds **1**−**4**.[8]



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2 R = R_1 = Ac; R_2 = propionyl **4** R = Ac; R_1 = propionyl; R_2 = OAc
1a R = R_1 = R_2 = H **4a** R = R_1 = H; R_2 = OH

Figure 1. Structures of compounds **1**−**4**, **1a**, and **4a**.

Secorhodomollolide A (**1**, Figure 1) showed IR absorption bands for hydroxy (3359 cm^{-1}), five-membered lactone (1783 cm^{-1}), and ester carbonyl (1739 and 1711 cm^{-1}) functional groups. The (+)-ESIMS of compound **1** exhibited quasi-molecular ion peaks at *m*/*z* 661 $[M + K]^+$, 645 $[M + Na]^+$, and 640 $[M + NH_4]^+$. The molecular formula $C_{31}H_{42}O_{13}$ of **1** was indicated from HRESIMS at *m*/*z* 645.2531 (calcd for $C_{31}H_{42}O_{13}Na$, 645.2523). The ^1H NMR of **1** in $CDCl_3$[9] showed resonances attributed to a terminal double bond at δ 5.63 (br s, H-18a) and 5.20 (br s, H-18b), four acylated oxymethines at δ 6.20 (br d, J = 7.8 Hz, H-7), 6.01 (d, J = 7.8 Hz, H-6), 5.48 (br s, H-14), and 4.94 (s, H-15), an exchangeable hydroxy proton at δ 4.30 (s, *H*O-10), and four acetyl methyls at δ 2.12, 2.02, 1.93, and 1.90, as well as resonances due to a propionyl unit at δ 2.15 (2H, m) and 1.10 (3H, t, J = 7.5 Hz). In addition, it showed resonances due to three tertiary methyls at δ 1.87 (H_3-19), 1.81 (H_3-17), and 1.35 (H_3-20), together with partially overlapped resonances with complex coupling patterns attributed to aliphatic methylenes and methines between δ 1.68 and 3.47 (Table 1). Besides carbon resonances of the acyl units, the ^{13}C NMR and DEPT spectra of **1** showed 20 carbon resonances (Table 2) including 3 methyls, 4 methylenes (1 olefinic), 7 methines (4 oxygen-bearing), and 6 quaternary carbons (a carboxyl, an olefinic, and 3 oxygen-bearing). These spectroscopic data suggested that compound **1** was a highly acylated diterpenoid with a parent nucleus different from the grayanane or kalmane skeletons reported before,[2] which was confirmed by the spectroscopic data of the basic hydrolyzed product, compound **1a**.[10]

Table 1. ^1H NMR Spectroscopic Data of Compounds **1**−**4**, **1a**, and **4a**[a]

no.	1	1a	2	3	4	4a
1	3.47 dd	3.84 dd	3.45 dd	2.89 dd	3.30 dd	3.86 dd
2a	2.66 dd	2.71 dd	2.66 dd	2.75 dd	2.75 dd	2.70 dd
2b	2.58 dd	2.40 dd	2.56 dd	2.20 m	2.06 dd	2.00 dd
6	6.01 d	4.68 d	5.99 d	5.18 d	5.26 d	4.14 d
7	6.20 br d	4.79 br d	6.12 br d	5.62 d	5.80 d	4.83 d
9	2.90 m	2.62 m	2.89 m	2.88 m	3.03 m	1.58 m
11a	1.68 m	1.68 m	1.68 m	1.85 m	5.38 m	4.14 m
11b	1.68 m	1.40 m	1.68 m	1.73 m		
12a	1.81 m	1.65 m	1.81 m	2.20 m	2.38 m	1.91 m
12b	1.81 m	1.49 m	1.81 m	1.62 m	1.90 m	1.85 m
				3.23	3.26	
13	3.12 m	1.91 m	3.10 m	br d	br d	2.07 d
14	5.48 br s	4.94 s	5.56 br s	5.73 s	6.59 s	5.50 s
15	4.94 s	3.74 s	4.91 s	5.09 s	4.92 s	3.88 s
17	1.81 s	1.19 s	1.80 s	1.57 s	1.58 s	1.32 s
18a	5.63 br s	4.91 br s	5.63 br s	5.09 s	5.10 s	5.10 b rs
18b	5.20 br s	4.77 s	5.22 br s	4.94 s	4.95 s	4.88 s
19	1.87 s	1.92 s	1.90 s	1.67 s	1.68 s	1.76 s
20	1.35 s	1.16 s	1.34 s	1.70 s	1.75 s	1.56 s

[a] Data were recorded at 500 or 600 MHz in $CDCl_3$ for **1**−**4** and DMSO-d_6 at 60 °C for **1a**, and MeOH-d_4 for **4a**. $J_{1,2a} \approx$ 11.0 Hz, $J_{1,2b} \approx$ 12.5 Hz, and $J_{2a,2b} \approx$ 17.5 Hz for **1**−**4**, **1a**, and **4a**; $J_{6,7} \approx$ 7.8 Hz for **1**, **1a**, and **2**; $J_{6,7} \approx$ 9.0 Hz for **3** and **4**; $J_{6,7} \approx$ 14.0 Hz for **4a**; $J_{12\alpha,13} \approx$ 7.8 Hz and $J_{12\beta,13} \approx J_{13,14} \approx$ 0 Hz for **3**, **4**, and **4a**. For data for acyl units, see the Supporting Information.

The structure of compound **1** was constructed by 2D NMR data analysis. The proton and protonated carbon resonances in the NMR spectra of **1** were assigned unambiguously by $^1H-^1H$ COSY and HMQC spectroscopic data interpretation. The HMBC spectrum of **1** showed a series of long-range heteronuclear correlations for establishing the carbon skeleton and locating the substituents (Figure S1, Supporting Information). HMBC correlations for H-1/C-3, C-6, and C-9, H-6/C-1, C-4, C-5, C-7, and C-8, H-7/C-5, C-8, and C-9, H-9/C-1, and C-8 indicated the presence of a seven-membered ring moiety consisting of C-1 and C-5 to C-10 in **1**. HMBC correlations for H-9/C-11, C-12, and C-14, H-13/C-11, C-15, and C-17, H-15/C-8, C-9, C-16, and C-17, H_3-17/C-13, C-15, and C-16, revealed that there was another seven-membered ring consisting of C-8, C-9, C-11 to C-13, C-15, and C-16 in **1**. HMBC correlations of C-14 with H-9, H-12, and H-15, and both C-15 and C-16 with H-14 indicated that C-8 and

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(5) At subhypnotic dosage of pentobarbital sodium (23 mg/kg, i.p.), the falling asleep rate was increased by 83%.

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(8) For plant material, experimental procedures, and physical-chemical properties for compounds **1**−**4**, see the Supporting Information.

(9) Though the NMR resonances of compound **1** in $CDCl_3$ were broadened due to thermal dynamic fluctuation of the molecule in the solution, this was improved by using DMSO-d_6 as the solvent, see the Supporting Information.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1**−**4**, **1a**, and **4a**[a]

no.	1	1a	2	3	4	4a
1	53.8 d	53.8 d	53.9 d	56.0 d	41.9 d	42.9 d
2	35.7 t	32.2 t	35.7 t	31.0 t	30.0 t	31.6 t
3	173.2 s	171.1 s	173.2 s	173.8 s	173.2 s	177.6 s
4	145.9 s	145.5 s	146.0 s	146.5 s	146.1 s	150.0 s
5	89.5 s	90.0 s	89.5 s	75.3 s	75.5 s	77.4 s
6	69.8 d	67.7 d	69.7 d	73.8 d	73.0 d	73.6 d
7	66.5 d	71.1 d	66.9 d	68.6 d	67.0 d	72.8 d
8	55.6 s	55.4 s	55.6 s	52.5 s	52.5 s	54.7 s
9	42.9 d	44.3 d	42.9 d	40.3 d	60.0 d	57.4 d
10	75.1 s	75.1 s	75.2 s	88.8 s	87.5 s	91.2 s
11	20.7 t	21.5 t	20.5 t	18.9 t	67.6 d	64.0 d
12	24.9 t	25.1 t	24.9 t	22.9 t	30.7 t	35.9 t
13	45.9 d	49.9d	46.0 d	42.5 d	41.5 d	51.3 d
14	81.7 d	78.5 d	81.2 d	76.3 d	75.0 d	79.9 d
15	86.2 d	85.1 d	86.4 d	89.9 d	91.3 d	91.4 d
16	88.6 s	78.4 s	88.6 s	87.8 s	87.7 s	83.0 s
17	21.0 q	22.1 q	20.9 q	18.9 q	19.5 q	25.9 q
18	116.6 t	106.9 t	116.7 t	113.3 t	113.7 t	112.4 t
19	20.8 q	19.4 q	20.4 q	18.6 q	18.6 q	19.5 q
20	31.6 q	32.8 q	31.7 q	28.6 q	29.2 q	26.7 q

[a] Data were recorded at 125 MHz in $CDCl_3$ for **1**−**4**, DMSO-d_6 at 60 °C for **1a**, and MeOH-d_4 for **4a**. The multiplicity was determined by the DEPT experiment. For data for acyl units, see the Supporting Information.

C-13 were bridged by C-14. HMBC correlations for H_3-20/C-1, C-10, and C-9 and H_3-17/C-13, C-14, C-15, and C-16 demonstrated that two tertiary methyl groups were located at C-10 and C-16, respectively. In addition, HMBC correlations for H-1/C-2, H_2-2/C-1, C-3, C-5, and C-10 indicated that C-1 was connected through C-2 to C-3 while correlations for H_3-19/C-4, C-5, and C-18 and H_2-18/C-5 and C-19 located an isopropenyl at C-5. Meanwhile, the HMBC spectrum showed that H-6, H-14, and H-15 correlated with three acetyl carbonyls, respectively, H-7 with the propionyl carbonyl, and *H*O with C-20. This located the three acetyloxyls at C-6, C-14, and C-15, the propionyloxyl at C-7, and the hydroxy at C-10 in **1**, respectively. Considering the oxygenated nature of C-3, C-5, and C-16 and the molecular formula of **1**, a lactone ring was proposed between C-3 and C-5 and the remaining acetyloxyl was located at C-16. Accordingly, the planar structure of compound **1** was elucidated as 6,14,15,16-tetraacetyloxy-10-hydroxy-7-propionyloxy-3,4-secograyan-4(18)-ene-3,5-lactone.

In the NOESY spectrum of **1**, cross-peaks between H-1 and H-18a, between both H-6 and H-18b with H_3-19, between *H*O-10 with H-14 and H-18a, and between H-13 and H-14 (Figure S2, Supporting Information) indicated that these protons oriented on the same side of the ring system. In addition, cross-peaks between H-15 with H-9 and H_3-17 demonstrated that they were oriented in another side of the ring system. H-7 was assigned to be opposite H-6 based on a coupling constant of 7.8 Hz for the vicinal protons and no NOESY cross-peak observed between them. To prove the above elucidation and to determine the absolute configuration of **1**, a single crystal X-ray diffraction by using an anomalous scattering of Cu Kα radiation[11] was carried out. An ORTEP drawing, with the atom-numbering scheme indicated, is shown in Figure 2, demonstrating a configuration of 1*S*,5*R*,6*R*,7*S*,8*S*,9*R*,10*R*,13*R*,14*R*,15*R*,16*S* for **1**. Therefore, the structure of **1** was determined and designated as secorhodomollolide A.

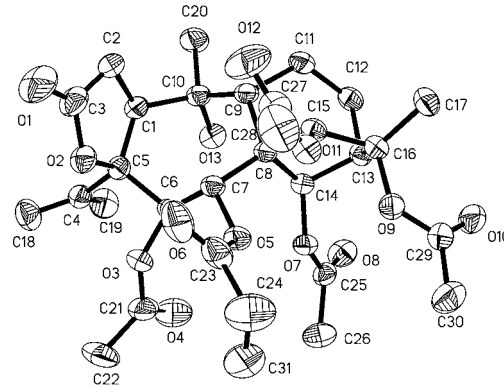


Figure 2. ORTEP diagram of secorhodomollolide A (**1**).

The spectroscopic data of secorhodomollolide B (**2**) were almost identical with those of compound **1**. However, the NMR resonances for H-7 and C-14 and the propionyl carbonyl of compound **2** were shifted by $\Delta\delta_H$ −0.08 and $\Delta\delta_C$ −0.5 and +1.6 ppm, respectively, as compared with those of **1**, whereas the resonances of H-14 and C-7 and one acetyl carbonyl were shifted in turn by $\Delta\delta_H$ +0.08 and $\Delta\delta_C$ +0.4 and −1.7 ppm. This suggested that the propionyloxyl at C-7 and the acetyloxyl at C-14 in **1** were exchanged mutually in **2**. The suggestion was verified unequivocally by the 2D NMR experiments of **2**, in particular, by the HMBC correlations from H-14 to the propionyl carbonyl (δ_C 172.7) and from H-7 to the acetyl carbonyl (δ_C 167.5). This was further confirmed by basic hydrolysis of **2** that generated **1a**. Therefore, the structure of compound **2** was assigned for secorhodomollolide B.

Secorhodomollolide C (**3**) displayed IR, ESIMS, and NMR spectroscopic features similar to those of **2**. However, in the NMR spectra of compound **3**, many resonances were shifted significantly (Tables 1 and 2). Detailed 2D NMR data analysis of **3** revealed that it possessed the carbon skeleton and acylated pattern completely identical with those of **2**. Therefore, the *cis*-fused 3,5-lactone in **2** was proposed to be changed into a *trans*-fused 3,10-lactone in **3**. This was supported by significant shifts of C-5 ($\Delta\delta_C$ −14.2 ppm) and C-10 ($\Delta\delta_C$ +13.6 ppm) of **3**, as compared with those of **2**. In the NOESY spectrum of **3**, cross-peaks between H-1 with H-6, H-14, and H_3-19, between H-7 and H_3-20, and between H-9 with H-15 and H_3-20 verified the presence of the

(10) For basic hydrolysis of compound **1** and physical-chemical properties of compound **1a**, see the Supporting Information.

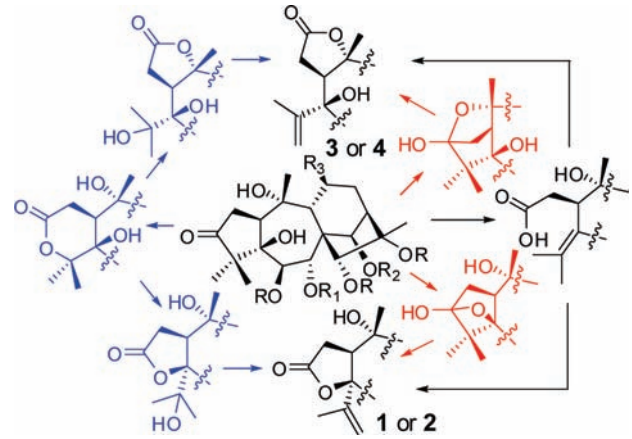
(11) For crystallographic data of **1**, see the Supporting Information.

trans-fused 3,10-lactone in **3**, and the identity of the stereochemistry of **3** and **2**. Accordingly, the structure of compound **3** was assigned for secorhodomollolide C.

Secorhodomollolide D (**4**) had the molecular formula $C_{33}H_{44}O_{15}$ indicated from (+)-HRESIMS at *m*/*z* 703.2579 $[M + Na]^+$ (calcd $C_{33}H_{44}O_{15}Na$ 703.2578). The IR and NMR spectroscopic data of compound **4** resembled those of **3** (Tables 1 and 2). However, the NMR spectra of **4** exhibited resonances due to an additional acetyl unit at δ_H 2.07 and δ_C 168.5 and 21.3, in addition to the resonances attributed to an oxymethine at δ_H 5.38 (m) and δ_C 67.6, replacing those for the methylene (H_2-11 and C-11) of **3**. Meanwhile, the resonances for H-9, H-12a, and H-12b, and C-9 and C-12 of **4** were deshielded by $\Delta\delta_H$ +0.15, +0.18, and +0.28, and $\Delta\delta_C$ +19.7 and +7.8 ppm, respectively, as compared with those of **3**. These data indicated that **4** was an 11-acetyloxyl derivative of **3**, which was confirmed by 2D NMR data analysis of **4** and its basic hydrolysate (compound **4a**).[12] In particular, in the HMBC spectrum of **4** (Figure S1, Supporting Information), correlations from H-11 to the additional acetyl carbonyl and from H-14 to the propionyl carbonyl proved the location of the acyl units in **4**. In the NOESY spectrum of **4**, cross-peaks between H-1 with H-6, H-11, H-14, and H_3-19, between H-7 with *HO*-5, H-9, H-15, and H_3-20, and between H-15 and H_3-17 demonstrated that **4** was an 11β-acetyloxyl derivative of **3**. Thus, the structure of compound **4** was assigned and designated as secorhodomollolide D.

The plausible biosynthetic pathways of compounds **1**−**4** are postulated in Scheme 1. The biosynthetic precursors of **1**−**4** are proposed to be grayanane derivatives with a 5,10-dihydroxy-3-oxo substitution pattern. The precursors would be transformed into key lactonized intermediates by an enzymatic Baeyer−Villiger oxidation[13] followed by a sequentially or simultaneously intramolecular ester exchange and dehydration to yield **1**−**4** (blue part). A Grob fragmentation with a subsequent double bond migration via a radical intermediate in an allylic oxidation reaction, followed by esterification, would be an alternative (black part). It would be postulated also that intramolecular additions of the precursors would generate semiacetal intermediates, which would then undergo a simultaneously enzymatic catalyzed 1,4-dehydrogenation and carbon bond cleavage to generate **1**−**4** (red part). From a biogenetic point of view, the absolute configurations of **1**−**4** are consistent with those of grayanotoxin III, whose absolute configuration has been unambiguously determined.[2a]

Scheme 1. The Plausible Biosynthetic Pathways of **1**−**4**



Analgesic and sedative effects of compounds **1**−**4**, **1a**, and **4a** were tested with use of acetic acid-induced writhing and pentobarbital sodium induced-sleep models,[14] respectively. At a dose of 5 mg/kg (i.p.), in the acetic acid-induced writhing test, **4** exhibited an analgesic effect with 35% reduction of writhes, as compared with the blank control, while others less than 10% reduction of writhes. In the pentobarbital sodium induced-sleep test at a subhypnotic dosage of pentobarbital sodium (23 mg/kg, i.p.), **2** and **4** increased falling asleep rates by 66.7% and 100% (others less than 30%), respectively. In addition, with use of the MTT method,[15] cytotoxicities of these compounds were assayed against five human cancer cell lines including colon cancer (HCT-8), hepatoma (Bel 7402), stomach cancer (BGC-823), lung adenocarcinoma (A549), and human ovarian cancer (A2780). Only compound **2** showed a selective activity against the Bel-7402 cell line with IC_{50} values of 0.97 μM, but others were inactive (IC_{50} values >5 μM).

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Supporting Information Available: Plant material, experimental procedures, and physical-chemical properties for compounds **1**−**4**, **1a**, and **4a**; Table S1, ^1H and ^{13}C NMR spectroscopic data of acyl units of **1**−**4**; Figure S1, main $^1H-^1H$ COSY and HMBC correlations of **1**−**4**, **1a**, and **4a**; Figure S2, main NOESY correlations of **1**−**4**; copies of IR, MS, and 1D and 2D NMR spectra of **1**−**4**, **1a**, and **4a**; and X-ray crystallographic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) For basic hydrolysis of compound **4** and physical-chemical properties of compound **4a**, see the Supporting Information.

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