

Limonoids from the stem bark of *Cedrela odorata*

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Abstract

Four nomilin/obacunol derivatives and a swietenolide derivative, together with seven known limonoids, were isolated from stem bark of *Cedrela odorata* and their structures established by spectroscopic methods. Antifeedant activity of the isolated compounds was also tested.

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Keywords: *Cedrela odorata* L.; Meliaceae; Limonoids; Rings A,D-seco limonoids; Nomilin/obacunone derivatives; Rings B,D-seco limonoid; 8 β ,14 α -dihydrosvietenolide

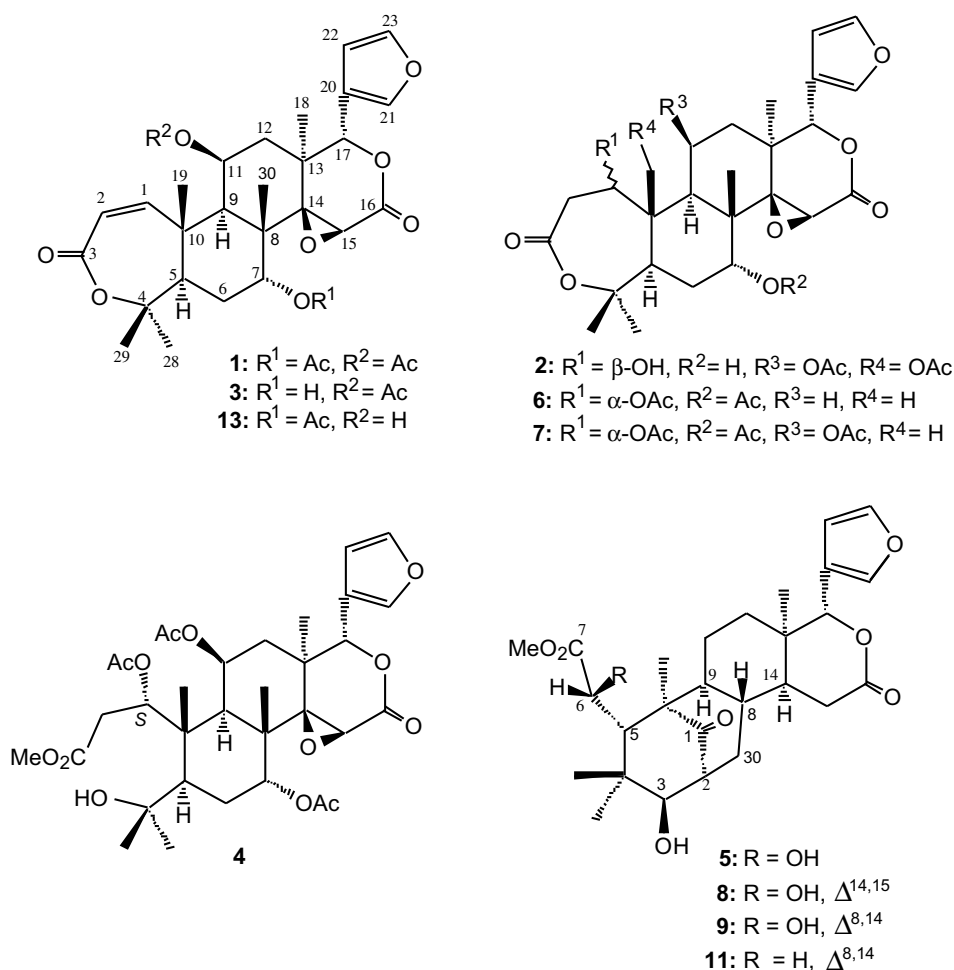
1. Introduction

Cedrela odorata L. is a valuable meliaceous timber tree native to the tropical region of America, but it has been introduced into cultivation in Africa and several South-East Asian and Pacific countries (Danida Forest Seed Center, 2000). The plant is used in folk medicine. In South America, an infusion of its bark is used as a remedy for diarrhea, fever, anti-inflammation, vomiting, hemorrhage and indigestion. In Africa, the decoction of the bark is used against malaria and/or fever (Madureira et al., 1950) and, recently, some studies on antimalarial activity of the extracts have been reported (Machinnon et al., 1997; Omer et al., 2003). Since 7-oxo-7-deacetoxygedunin (Bevan et al., 1963) in addition to sesque- and triterpenoids was isolated, many limonoids have been reported from *C. odorata*. A striking characteristic of the limonoids from this genus is the fact that two types of ring-opened limo-

noids have been isolated together with intact apoeuphol compounds. This includes ring A,D-seco limonoids such as nomilin and obacunol (Mitsui et al., 2004), and other ring B,D-seco limonoids of angolensates and mexicanolides (Taylor, 1984; Sanni et al., 1987; Veitch et al., 1999), the latter of which have an unique bicyclo[3.3.1] ring system.

In our continuing search for limonoid antifeedants from the family Meliaceae (Nakatani et al., 2001; Abdelgaleil et al., 2006), we isolated four new nomilin/obacunol derivatives (1)–(4), and a new dihydrosvietenolide (5) from *C. odorata*. Seven known compounds of two nomilin derivatives, 7-acetyldihydronomilin (6) (Ahmed et al., 1978; Mondon et al., 1978) and 7-acetyl-11 β -acetoxydihydronomilin (7) (Marcella and Mootoo, 1981), and five mexicanolides, 3 β ,6-dihydroxydihydrocarapin (8) (Mootoo et al., 1999), swietenolide (9) (Connolly et al., 1968), xylocensin K (10) (Kokpol et al., 1996), 3 β -hydroxydihydrocarapin (11) (Adesida et al., 1971), and cedrodorin (12) (Veitch et al., 1999), were also isolated. We describe herein the isolation and structural elucidation of the new limonoids. Antifeedant activity of the isolated compounds is also reported.

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2. Results and discussion

After defatting with hexane, the methanol extract of the stem bark was partitioned successively with ether, acetone, and methanol. Each extract was subjected to on SiO_2 chromatography, followed by reversed HPLC separation of the resulted limonoid fractions to give four compounds, **1**, **2**, **6** and **7**, from the ether and acetone extracts, and eight compounds, **3–5**, and **8–12**, from the methanol extract, respectively.

Compound (**1**) was obtained as prisms. The molecular formula of $\text{C}_{30}\text{H}_{36}\text{O}_{10}$, (13 unsaturations) was determined by analysis of the HRFAB-MS (m/z : 557.2387 $[\text{M} + 1]^+$, Δ 0.0 μm) and ^{13}C NMR spectrum. The IR spectrum displayed absorption bands for saturated ($1740\text{--}1735\text{ cm}^{-1}$) and unsaturated carbonyl (1695 cm^{-1}) and olefinic (1637 cm^{-1}) groups. The UV spectrum also indicated the presence of an α,β -unsaturated ester group at 225 nm. From the ^1H and ^{13}C NMR spectra, it was evident that seven of elements of unsaturation were present as double bonds: three carbon–carbon double bonds (one furan ring) and four CO (as esters). Therefore, the molecule is hexacyclic.

All protons directly bonded with carbon atoms were assigned by analysis of the HMQC spectrum. From the subsequent 2D NMR studies using $^1\text{H}\text{--}^1\text{H}$ COSY, HMBC and NOESY spectra, it was strongly suggested that **1** was an obacunol derivative (Tables 1 and 2). Thus, the signals based on a conjugated ester group at δ_{H} 6.29 (H-1) and 5.94 (H-2) and δ_{C} 166.4 (C-3) were assigned to the seven-membered A ring. On the other hand, two characteristic singlets at δ 5.59 and 3.55 due to H-17 and H-15 showed the presence of an epoxidized δ -lactonic D ring. The positions of the two acetyl groups were readily elucidated by HMBC correlations of H-7 and H-11 with acetate carbonyl carbons.

The NOESY spectrum showed that **1** possessed the same ring junctions as those of the basic limonoid skeleton. The stereochemistry of the two acetoxy groups was assigned to be 7α and 11β by NOE correlations of H-7 and H-11 with $8\beta\text{-Me}$ (Me-30) and $9\alpha\text{-H}$, respectively. The β configuration of 14,15-epoxide was confirmed by the presence of a strong NOE between $7\beta\text{-H}$ and $15\alpha\text{-H}$. Thus, compound **1** is 11β -acetoxyobacunyl acetate. The 11-deacetyl compound has been reported from *C. sinensis* (Mitsui et al., 2004).

Table 1
¹H NMR spectroscopic data for odoratins A–D (1–4) and odorlide (5)

No	1	2	3	4	5
1	6.29 <i>d</i> (12.6)	4.09 <i>dd</i> (3.7, 1.4)	6.35 <i>d</i> (12.5)	6.76 <i>br d</i> (9.6)	—
2(α) (<i>Pro-R</i>)	5.94 <i>d</i> (12.6)	2.92 <i>dd</i> (17.1, 3.7)	5.92 <i>d</i> (12.5)	2.55 <i>br d</i> (14.9)	2.97 <i>dt</i> (9.8, 3.8)
2 β (<i>Pro-S</i>)	—	2.33 <i>dd</i> (17.2, 1.4)	—	2.35 <i>dd</i> (14.9, 9.6)	—
3	—	—	—	—	3.61 <i>dd</i> (9.8, 4.1)
5	2.50 <i>dd</i> (13.1, 2.4)	2.21 <i>dd</i> (11.9, 4.8)	2.81 <i>dd</i> (13.3, 2.6)	1.78 <i>dd</i> (11.9, 3.3)	3.31 <i>br s</i>
6 α	2.12 <i>ddd</i> (15.4, 2.6, 2.4)	1.90 <i>m</i>	1.83 <i>dt</i> (14.4, 3.2)	1.93 <i>dt</i> (14.5, 3.5)	4.46 <i>br s</i>
β	1.94 <i>ddd</i> (15.4, 13, 1, 2.4)	1.89 <i>m</i>	2.02 <i>br t</i> (14.0)	1.89 <i>dt</i> (2.0, 13.9)	—
7	4.56 <i>dd</i> (2.6, 2.4)	4.60 <i>t</i> (2.3)	3.54 <i>br s</i>	4.50 <i>br t</i> (2.5)	—
8	—	—	—	—	2.43 <i>dq</i> (4.4, 11.7)
9	2.49 <i>d</i> (4.6)	2.67 <i>d</i> (4.6)	2.54 <i>d</i> (4.1)	2.49 <i>br d</i> (3.1)	1.24 <i>m</i>
11 α	5.60 <i>ddd</i> (9.8, 5.5, 4.6)	5.30 <i>ddd</i> (9.8, 5.1, 4.6)	5.61 <i>dt</i> (9.4, 4.6)	5.70 <i>dt</i> (9.8, 4.0)	1.60–1.66
12 α	2.33 <i>dd</i> (14.3, 9.8)	2.43 <i>dd</i> (14.6, 9.8)	2.27 <i>dd</i> (14.4, 9.6)	2.46 <i>dd</i> (14.9, 10.0)	1.20 <i>ddd</i> (16.0, 12.2, 6.2)
12 β	1.51 <i>dd</i> (14.3, 5.5)	1.41 <i>dd</i> (14.6, 5.1)	1.51 <i>dd</i> (14.4, 5.4)	1.47 <i>dd</i> (14.9, 4.0)	1.64 <i>m</i>
14	—	—	—	—	1.44 <i>ddd</i> (11.4, 7.2, 1.4)
15 α	3.55 <i>s</i>	3.56 <i>s</i>	3.77 <i>s</i>	3.58 <i>s</i>	2.84 <i>dd</i> (18.8, 7.2)
15 β	—	—	—	—	2.77 <i>dd</i> (18.7, 1.4)
17	5.59 <i>s</i>	5.58 <i>s</i>	5.58 <i>s</i>	5.57 <i>s</i>	5.80 <i>s</i>
18	1.21 <i>s</i>	1.29 <i>s</i>	1.22 <i>s</i>	1.29 <i>s</i>	0.96 <i>s</i>
19(<i>Pro-R</i>)	1.53 <i>s</i>	4.48 <i>d</i> (13.1)	1.49 <i>s</i>	1.42 <i>s</i>	1.33 <i>s</i>
19(<i>Pro-S</i>)	—	5.10 <i>d</i> (13.1)	—	—	—
21	7.40 <i>t</i> (1.6)	7.41 <i>t</i> (1.6)	7.39 <i>t</i> (1.7)	7.40 <i>s</i>	7.42 <i>s</i>
22	6.32 <i>br d</i> (1.0)	6.31 <i>d</i> (1.5)	6.32 <i>br s</i>	6.33 <i>s</i>	6.37 <i>s</i>
23	7.39 <i>br s</i>	7.40 <i>br s</i>	7.39 <i>br s</i>	7.40 <i>s</i>	7.42 <i>s</i>
28	1.34 <i>s</i>	1.25 <i>ss</i>	1.41 <i>s</i>	1.22 <i>s</i>	0.92 <i>s</i>
29	1.45 <i>s</i>	1.14 <i>s</i>	1.26 <i>s</i>	1.29 <i>s</i>	1.00 <i>s</i>
30 α	1.34 <i>s</i>	1.13 <i>s</i>	1.26 <i>s</i>	1.30 <i>s</i>	1.27 <i>dt</i> (4.5, 12.8)
30 β	—	—	—	—	2.65 <i>dt</i> (12.9, 4.1)
OH	—	—	1.87 <i>br d</i> (3.0)	—	—
1-OAc	—	—	3.55 <i>s</i>	1.95 <i>s</i>	3.53 <i>s</i>
7-OAc	2.13 <i>s</i>	—	—	2.11 <i>s</i>	3.57 <i>s</i>
11-OAc	2.15 <i>s</i>	2.13 <i>s</i>	2.13	2.10 <i>s</i>	2.80 <i>s</i>
19-OAc	—	2.13 <i>s</i>	—	—	—

Chemical shifts are expressed in ppm.

J-values in parentheses are in Hz.

All spectra were measured in CDCl₃ at 600 MHz.

Compound **2** was obtained as a white amorphous powder. The molecular formula (C₃₀H₃₈O₁₂) was determined by the HRFAB-MS (*m/z*: 591.2417 [M + 1]⁺, Δ −2.5 μ m)

and NMR data. The IR spectrum showed strong absorptions due to hydroxyl (3500–3200 cm^{−1}) and ester groups (1745–1735 cm^{−1}) different from that of **1**. The ¹H NMR

Table 2
¹³C NMR spectroscopic data for odoratins A–D (1–4) and odoraleide (5)

No	1	2	3	4	5
1	149.8	79.4	151.5	76.4	221.3
2	120.0	34.4	120.2	35.1	48.8
3	166.4	169.3	166.8	172.1	78.7
4	83.9	80.6	84.3	74.9	39.8
5	45.5	54.7	43.9	46.4	43.9
6	27.0	24.0	31.2	26.5	73.2
7	74.1	74.2	71.5	75.1	177.0
8	42.0	42.7	43.0	41.6	35.2
9	49.5	45.9	48.3	39.7	59.1
10	45.5	45.3	45.2	48.3	51.4
11	67.7	68.7	68.2	69.9	21.3
12	36.5	36.8	37.3	37.9	35.3
13	37.6	37.9	37.2	36.9	35.6
14	68.6	67.7	69.0	68.9	45.4
15	55.2	55.0	55.4	54.9	30.5
16	166.7	166.4	167.6	167.3	171.2
17	78.0	78.0	78.4	78.3	76.6
18	17.2	17.9	17.6	17.9	21.8
19	19.4	67.3	19.2	17.9	17.0
20	119.7	120.0	120.2	120.0	121.4
21	141.3	141.4	141.2	141.4	140.7
22	109.7	109.6	109.8	109.8	109.7
23	143.4	143.5	143.2	143.4	143.4
28	31.8	30.5	31.9	34.9	23.6
29	25.5	21.3	26.0	26.7	22.5
30	20.1	20.5	20.3	20.2	36.6
OAc	169.7	168.8	169.9	169.8	–
–	21.2	21.2	21.6	21.0	–
–	169.7	169.7	–	169.9	–
–	21.5	21.1	–	21.5	–
–	–	–	–	170.9	–
–	–	–	–	20.6	–
OMc	–	–	–	52.1	53.4

spectrum showed the presence of two acetyl groups and the 14/15-epoxide. Although the NMR spectrum was similar to that of **1**, the absence of one tertiary methyl and olefinic protons in the ring A and the presence of geminal protons (δ 4.48 and 5.10) assigned to a 19-methylene group by the HMBC correlations with C-5 and C-9 methine carbons, strongly suggested that **2** was 19-acetoxidihydronomilin. The HMBC correlations of two methine protons at δ 4.09 and 4.60 with C-3 and C-19, and C-5 and C-9, respectively, assigned the position of two hydroxyl groups to C-1 and C-7.

The same α -configuration of 7-OH in **2** as that in dihydronomilin derivatives was readily assigned by a NOE correlation between H-7 and 8 β -Me. However, H-1 showed NOEs with H-5, H-9 and H-11, not with any H₂-19, which suggested that the 1-OH group was β orientated. The large chemical shift difference of 0.62 ppm observed between the 19-methylene protons and a W-type long range coupling between the higher field one (H_{pro-R}) and 1 α -H also implied the stereochemistry around the rings A and B. Thus compound **2** is 11 β ,19-diacetoxy-1-deacetyl-1-epidihydronomilin.

The molecular formula of compound **3** was determined as C₂₈H₃₄O₉ by the accurate HRFAB-MS (m/z : 515.2281 [$M + 1$]⁺, Δ 0.0 μ m) and NMR spectroscopic data. The ¹H and ¹³C NMR data (Tables 1 and 2) were extremely

similar to those of **1** except for the absence of the 7-acetate group. Thus compound **3** is 11 β -acetoxycacunol.

Compound **4**, named odoraleide, was shown to have the molecular formula C₃₃H₄₄O₁₃ by analysis of the HRFAB-MS (m/z 649.2858 [$M + 1$], Δ –0.3 μ m) and NMR spectroscopic data. Although the NMR spectra of **4** resembled that of 7-acetyl-11 β -acetoxidihydronomilin (**7**) (Marcella and Mootoo, 1981), the presence of one methoxy group was observed. The HMBC and NOE data showed that **4** was identical to 7-*O*-acetyl-11 β -acetoxidihydronomilin **7** apart from the opening of the ring A lactone to a methyl ester. The *S* configuration at C-1 inferred from that of **7** was elucidated in a following manner by considering the low field shift of H-1 and NOE observations: H-1 (δ 6.76, *br d*, $J = 9.6$ Hz), highly deshielded by the closely spaced 4-OH group, showed NOE correlations to H-5 and Me-19; One (*Pro-S*) of H₂-2 at δ 2.35 showed strong NOEs with H-9 and H-11, and the other (*Pro-R*) at δ 2.87 was strongly correlated with H-5 and H-9. These findings suggested that the opened ring A had the preferred conformation shown in Fig. 1. The possibility of **4** to be produced by methanolysis of lactone **7** at extraction is possible; however, such an opening of the ring A has not been reported in nomilin derivatives.

The molecular formula of compound **5** was determined to be C₂₇H₃₆O₈ by analysis of the HRFAB-MS (m/z 489.2327 [$M + 1$]⁺, Δ –0.5 μ m) and NMR spectroscopic data. The IR spectrum indicated absorption bands for hydroxyl (3600–3200 cm^{–1}), ester (1740 and 1735 cm^{–1}) and keto carbonyl (1710 cm^{–1}) groups. The UV spectrum showed only the presence of a furan ring at 215 nm. The CD spectrum exhibited the presence of a keto carbonyl group at 293 nm ($\Delta\epsilon$ –3.6: $n \rightarrow \pi^*$ of C=O). From the ¹H and ¹³C NMR spectra, it was clear that five of the elements of unsaturation were present as double bonds: one furan ring and three CO. Therefore, the molecule is pentacyclic. The NMR spectroscopic data strongly suggested that **5** was a swietenolide derivative. Thus, the H-6 methine

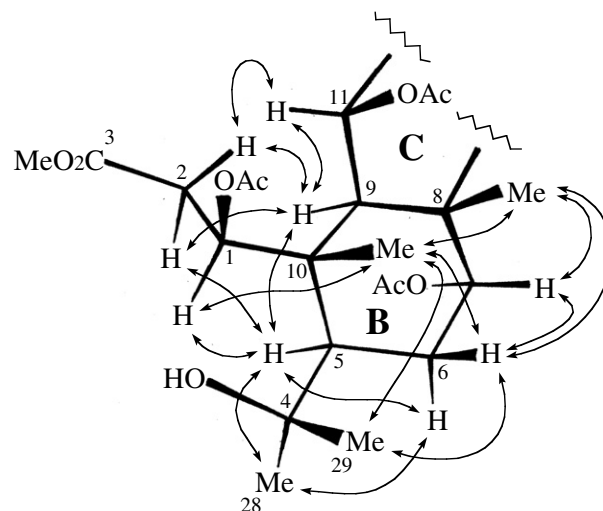


Fig. 1. Selected NOE correlations in **4**.

proton at δ 4.46 weakly coupling with the H-5 broad singlet showed an HMBC correlation with the C-7 ester carbonyl. In addition to this ester moiety, the presence of a characteristic low-field singlet at δ 5.80 due to H-17 and the absence of one tertiary methyl signal corresponding to Me-30 strongly suggested that **5** was a mexicanolide-type rings B,D- seco limonoid.

The structure of **5** was readily deduced from the NMR spectroscopic data, because they were very similar to those of swietenolide (**9**) (Chakraborty et al., 1968) and 3 β ,6-dihydroxydihydrocarapin (**8**) (Mootoo et al., 1999) isolated from *S. macrophylla* and *C. odorata*, except for the lack of an olefinic double bond in **5**. The stereochemistry of the ring junctions was elucidated by NOE correlations, in which strong NOEs of H-8 with H-5, H-17 and H-30 β , not with H-9 and H-14, showed that H-8 was on the β face of the molecule. H-14 had NOE correlations with Me-18, H-9 and H-15 α . The *R* configuration at C-6 was inferred from that of some known mexicanolides isolated in this study. This was also supported by strong NOE correlations of H-6 with Me-19 and H₂-11, because the 7-CO₂Me showing correlations with the H-5 and 4 β -Me (Me-29) should be oriented on the β face of the molecule. Therefore, this compound was proved to be 8 β ,14 α -dihydroswietenolide (**5**). Finally, the absolute stereochemistry was concluded to be the same as that of many swietenine derivatives having the same bicyclo[3.3.1]nonane ring system by the negative Cotton effect at 293 nm in the CD spectrum (McPhail and Sim, 1964; Kadota et al., 1990; Jimenez et al., 1998).

The antifeedant activity of the isolated compounds (**1**–**12**) against the third-instar larvae of *Spodoptera littoralis* (Boisd.) was briefly tested by a conventional leaf disk method (Wada and Munakata, 1968). The nomilin/obacunol derivatives were weakly active at 1000 ppm, corresponding to a concentration of ca. 20 μ g/leaf-cm². On the other hand, the mexicanolides showed moderate activities at the same concentration, the most potent is **5**, which was active at 500 ppm.

3. Experimental

3.1. General

¹H and ¹³C NMR spectra were measured at 600 and 150 MHz in CDCl₃ on JEOL FX-600 spectrophotometer. IR (KBr) and UV (MeOH) spectra were recorded on JASCO FT/IR 5300 and Shimadzu UV-210A spectrophotometers. Optical and CD spectra were measured in MeOH at 22° using JASCO DIP-370 S and JASCO J-720 spectropolarimeters. HPLC were performed on Waters μ Bondapak C₁₈ column by using 35–65% H₂O/MeOH as solvent.

3.2. Plant material

The stem bark of *Cedrela odorata* was collected in July 2002 at Pare de Kisantu in the Democratic Republic of

Congo. The plant material was identified by a junior lecturer Paul Malumba of Kinshasa University and a voucher specimen was deposited in the herbarium of Institut National Pour l'Etude et la Recherche Agronomiques of University of Kinshasa.

3.3. Extraction and isolation of compounds **1**–**6**

After defatting with hexane (7 l), the dried stem bark (1 kg) was extracted with MeOH (7 l) at room temperature for two weeks to give an extract (45.3 g). The extract was partitioned by successive extraction with Et₂O, acetone and MeOH (5 l each). The ether extract (5.4 g) was subjected to SiO₂ CC (200 g) with an 0–50% ether/CH₂Cl₂ gradient eluent. Four limonoid fractions eluted with 5–20% ether/CH₂Cl₂, and were purified through HPLC with a 30–40% H₂O/MeOH solvent system to give four compounds: **1** (6.7 mg), **2** (1.4 mg), **6** (4.4 mg) and **7** (16.2 mg). The same compounds were also obtained from the acetone extract (3.7 g): **1** (6.9 mg), **2** (4.3 mg), **6** (3.5 mg) and **7** (17.8 mg). The MeOH extract (18.9 g) was also roughly separated by chromatography on SiO₂, followed by HPLC purification of the resulted limonoid fractions with 5–30% H₂O/MeOH to give **3** (3.1 mg), **4** (2.5 mg), **5** (6.5 mg), **8** (1.5 mg), **9** (1.4 mg), **10** (3.2 mg), **11** (1.5 mg) and **12** (34 mg).

3.3.1. 11 β -Acetoxycobacunyl acetate (**1**)

Colorless prisms (MeOH); mp 249–252 °C; C₃₀H₃₆O₁₀; HRFAB-MS *m/z*: 557.2387 [*M* + 1]⁺ (calc. 557.2387); [α]_D +28.9 (c 0.35, CHCl₃); UV λ_{\max} nm (ϵ): 225 (6000); IR ν_{\max} cm^{−1}: 1740–1735, 1695, 1637; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2.

3.3.2. 11 β ,19-Diacetoxy-*l*-deacetyi-*l*-epidihydronomilin (**2**)

White amorphous powder; C₃₀H₃₈O₁₂; HRFAB-MS *m/z*: 591.2417 [*M* + 1]⁺ (calc. 591.2442); [α]_D −29.7 (c 0.89, CHCl₃); UV λ_{\max} nm (ϵ): 215 (14,000); IR ν_{\max} cm^{−1}: 3500–3200, 1740, 1700, 1635; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2.

3.3.3. 11 β -Acetoxycobacunol (**3**)

White amorphous powder; C₂₈H₃₄O₉; HRFAB-MS *m/z*: 515.2281 [*M* + 1]⁺ (calc. 515.2281); UV λ_{\max} nm (ϵ): 207 (6000); IR ν_{\max} cm^{−1}: 3500–3250, 1740; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2.

3.3.4. Odoralide (**4**)

White amorphous powder; C₃₃H₄₄O₁₃; HRFAB-MS *m/z*: 649.2858 [*M* + 1]⁺ (calc. 669.2861); [α]_D +29.7; UV λ_{\max} nm (ϵ): 225 (7000); IR ν_{\max} cm^{−1}: 3500–3200, 1740–1735; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2.

3.3.5. 8 β ,14 α -Dihydroswietenolide (**5**)

White amorphous powder; C₃₇H₄₆O₁₃; HRFAB-MS *m/z*: 699.3021 [*M* + 1]⁺ (calc. 699.3015); UV λ_{\max} nm (ϵ): 215

(6000); IR ν_{\max} cm^{-1} : 3600–3200, 1740, 1735, 1710; CD $\Delta\epsilon$ (nm): -3.6 (293); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

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