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New Friedelane Triterpenes from *Lepidobotrys staudtii*

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Abstract: Three new dihydroxy-3-friedelanone triterpenes were isolated from the leaves and the stem bark of the Cameroonian medicinal plant *Lepidobotrys staudtii*. Structure elucidation by spectroscopic techniques showed that they are 2α,29-dihydroxy-3-friedelanone (**1a**), 2β,21α-dihydroxy-3-friedelanone (**2a**) and 6β,21α-dihydroxy-3-friedelanone (**3**). In addition, the known monohydroxy-3-friedelanones **4**, **5**, **6** and **7** were obtained. Copyright © 1996 Elsevier Science Ltd

The friedelanes constitute an important class of the triterpenoids, and several friedelanes have been reported to possess biological activities. Among the 3-friedelanones, in which C-3 is oxidised to a keto function, an example is the inhibitory activity against P388 lymphocytic leukaemia *in vivo* of 28,30-dihydroxy-3-friedelanone.[1] *Lepidobotrys staudtii* Engl. (Lepidobotryaceae) is a medium-sized tree that occurs in the rain forests of East and South Cameroon.[2,3] It is known as "Masakwa seko" by the Baka pygmies of the Eastern Province, and is used frequently in the traditional medicine. A boiled decoction of the stem bark of *L. staudtii*, for example, is a popular remedy against malaria and burns.[4] In spite of these interesting properties, no phytochemical investigation has been performed with this plant. In continuation of our research on secondary metabolites from Cameroonian medicinal plants, possessing antiparasitic activity in particular,[5] the stem bark and leaves of *L. staudtii* were procured for antimalaria screening and chemical studies. From the dichloromethane extract of the leaves and the stem bark of *L staudtii*; three new friedelane triterpenes were isolated by chromatography, and shown to be the dihydroxy-3-friedelanones **1a**, **2a** and **3**. Four related monohydroxy-3-friedelanones, 21α-hydroxy-3-friedelanone (**4a**),[6] 30-hydroxy-3-friedelanone (**5a**)[7,8] 29-hydroxy-3-friedelanone (**6**)[7] and 28-hydroxy-3-friedelanone (**7**),[8] which previously have been reported from other sources, were also obtained. Due to separation difficulties, compounds **1a** and **2a** were obtained pure only as their acetates **1b** and **2b**, which could be separated by silica gel chromatography, and the structure elucidations were carried out with the acetates. However, mass and NMR spectra of the mixture of **1a** and **2a** were in complete agreement with the structures presented in Figure 1. The general appearance of the EI mass spectra and the ^{1}H NMR spectra of the compounds suggested that they possess a friedelane skeleton. The mass spectra of **1a/2a** and **3** showed typical[9] 3-friedelanone fragmantation patterns, with peaks

at *m/z* 273 and 177, and the ^{1}H NMR spectra contained signals of 7 or 8 methyl groups of which all are singlets except one doublet. The HMBC correlations observed from the methyl protons to the surrounding carbon atoms were also in agreement with the friedelane skeleton (see Table 2 for details).

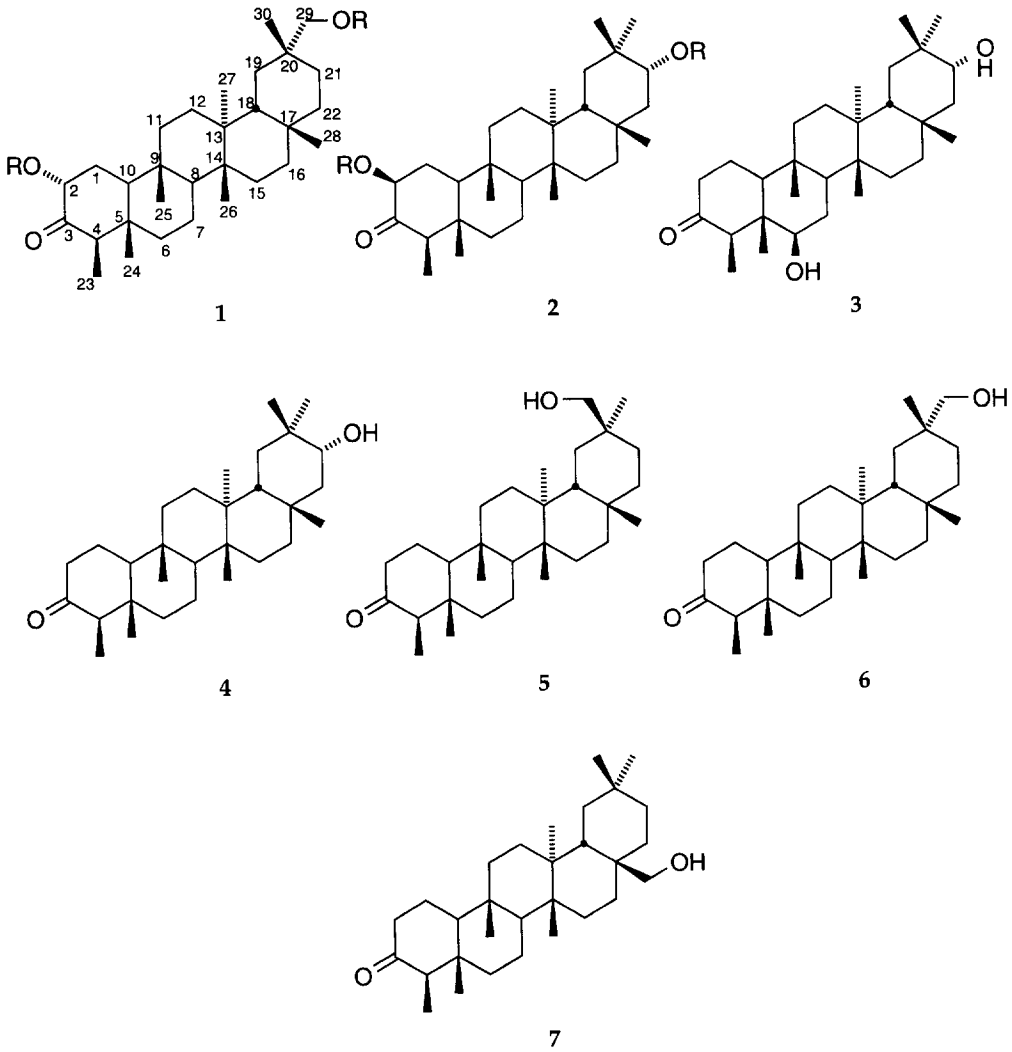

Figure 1. a: R = H; b: R = Ac

The high resolution EI mass spectrum of the diacetate **1b** showed that the exact mass of the molecular ion (*m/z* 542) corresponds to the elemental composition $C_{34}H_{54}O_5$. This was consistent with the ^{13}C NMR data (see Table 1), which indicated the presence of three carbonyl signals (at δ 208.2, 171.5 and 169.9) and two secondary alcohol carbons (at δ 76.5 and 72.8). The ^{1}H NMR spectrum contain nine methyl signals, two (at δ 2.11 and 2.06) were not present in the ^{1}H NMR spectrum of the mixture of **1a** and **2a** and were

attributed to the acetates. The structure of **1b** was determined by the analysis of the 2D NMR data, and the correlations observed in the HMBC spectrum (summarised in Table 2) led to the unambiguous assignment of all protons and carbons. The relative stereochemistry of **1b** was supported by its NOESY spectrum, correlations were observed between 1β-H and 2-H as well as 24-H_3, and between 24-H_3 and 25-H_3 establishing that the acetoxy group at C-2 is α. NOESY correlations were also observed between 29-H_2 and 27-H_3 as well as 21α-H, and between 21β-H and 28-H_3 as well as 30-H_3, showing that the oxymethylene group is α. In addition, comparison of the ^{13}C NMR data of **1b** with those of related compounds[7,8,10] supported the suggested structure.

The spectroscopic data of the diacetate **2b** were similar to those of **1b**, suggesting that the two compounds are constitutional isomers. The ^{1}H NMR spectrum of **2b** show that it contains an additional methyl group, while the oxymethylene group of **1b** has disappeared, suggesting that the position of the second acetoxy group has changed. HMQC and HMBC experiments showed that this new position is C-21 (see Table 2 for details). The NOESY spectrum of **2b** showed that the configuration of C-2 is inversed, correlations were observed between 2α-H and 4-H as well as 10-H. Further NOESY correlations were observed between 21β-H and 28-H_3 showing that the acetoxy group at C-21 is α, as previously has been observed with related friedelanes.[11,12]

Table 1. ^{13}C NMR data for compounds **1b**, **2b** and **3**. The spectra were recorded in $CDCl_3$, and the solvent signal (δ 77.0) was taken as reference.

C	**1b**	**2b**	**3**	C	**1b**	**2b**	**3**
1	28.2	28.2	21.9	18	43.2	42.5	44.3
2	76.4	76.5	41.2	19	36.4	30.1	36.0
3	208.0	208.2	212.5	20	33.5	31.8	34.4
4	54.4	54.3	58.3	21	76.5	28.1	74.3
5	43.4	43.2	47.6	22	43.7	37.9	47.0
6	41.0	40.9	79.4	23	6.5	6.5	10.4
7	18.2	18.2	29.3	24	14.1	14.0	9.1
8	52.2	52.9	48.3	25	18.0	17.9	17.8
9	36.9	36.8	37.5	26	18.8	19.9	17.7
10	53.3	53.2	58.5	27	19.0	18.5	19.2
11	35.8	35.4	35.2	28	32.7	32.0	33.1
12	30.2	29.9	30.1	29	26.2	72.8	31.9
13	39.3	39.7	39.1	30	30.8	29.4	24.9
14	38.6	38.3	38.6	$\underline{C}H_3CO$	21.2	21.2	-
15	31.1	32.1	30.4	$\underline{C}H_3CO$	21.1	21.0	-
16	35.3	35.8	36.0	$CH_3\underline{C}O$	171.0	171.5	-
17	31.9	30.2	32.5	$CH_3\underline{C}O$	169.7	169.9	-

The comparison of the spectroscopic data of **3** indicate that this is a very similar metabolite, and the MS data indicated that it has the same elemental composition as **1a** and **2a**. Again, a combination of 2D

NMR experiments permitted the assignment of all signals and the determination of the structure (HMBC correlations are summarised in Table 2). The 1D NMR data are very similar to those of zeylandiol,[12,13] but the optical rotations are different ($[\alpha]_D$ + 11 ° for zeylandiol). In the NOESY spectrum, correlations were observed between 24-H_3 and 23-H_3 as well as 1β-H, between 1β-H and 26-H_3, between 2β-H and 23-H_3, and between 6α-H and 4α-H. Furthermore, correlations were observed between 21β-H and 28-H_3 as well as 29-H_3 and between 18-H and 28H_3 suggesting that the hydroxyl group at C-21 is α, and **3** is consequently 21-*epi*-zeylandiol.

Table 2. Pertinent ^1H-^{13}C long-range correlations observed with **1b**, **2b**, and **3**.

	1b	**2b**	**3**
^1H	^{13}C	^{13}C	^{13}C
2	1, 3, 10	1, 3, 4	1, 3
4	3, 5, 6, 10, 23, 24	3, 5, 23, 24	3, 5, 23, 24
6	4, 5, 24	5	4, 5, 7, 24
10	2, 4, 5, 8, 9, 24, 25	5, 9, 11, 24, 25	4, 6
21	20	30	20, 22, 29, 30
23	3, 4, 5	3, 4, 5	3, 4, 5
24	4, 5, 6, 10	4, 5, 6, 10	4, 5, 6, 10
25	8, 9, 10, 11	8, 9, 10, 11	8, 9, 10, 11
26	8, 13, 14, 15	8, 13, 14, 15	8, 13, 14, 15
27	12, 13, 14, 18	12, 13, 14, 18	12, 13, 14, 18
28	16, 17, 18, 22	16, 17, 18, 22	16, 17, 18, 22
29	19, 20, 21, 30	19, 20, 21, 30	19, 20, 21, 30
30	19, 20, 21, 29	19, 20, 21, 29	19, 20, 21, 30

EXPERIMENTAL

General: ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded in $CDCl_3$ with a Bruker ARX500 spectrometer with an inverse 5 mm probe equipped with a shielded gradient coil, and the solvent signals (7.26 and 77.0 ppm, respectively) were used as reference. COSY, HMQC and HMBC experiments were performed with gradient enhancements using shaped gradient pulses, and for the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH}$=145 Hz and $^2J_{CH}$=10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). Chemical shifts were given in ppm with solvent signal as reference. EIMS recorded by Jeol SX102 spectrometer at 70 eV. CC on Merk silica gel 60 and TLC on silica gel GF -254 precoated plates and detection accomplished by spraying with 50% H_2SO_4 followed by heating.

Plant material: Fresh stem bark and leaves of *L. staudtii* were collected at Lomie, Eastern Province of Cameroon in March 1996. Voucher specimens are kept in the Faculty of Science, University of Dschang, and at the National Herbarium, Yaounde.

Extraction and isolation: Dried powdered stem bark of *L. staudtii* (2.0 kg) was extracted sequentially

with dichloromethane (15 l), acetone (15 l) and methanol (15 l) for 24 hours. Each of the extracts was concentrated under vacuum to yield a CH_2Cl_2 extract (110 g) and an acetone extract (120 g). The powdered leaves (2.0 kg) were also subjected to the same extraction protocol, to give a CH_2Cl_2 extract (95 g) and an acetone extract (150 g).

Isolation of the triterpenes: The CH_2Cl_2 extracts of the leaves were subjected to flash chromatography using mixtures of hexane and ethylacetate as eluent. Fractions of 500 ml were grouped on the basis of their TLC profile. The portion eluted with 30 % ethylacetate in hexane was rechromatographed on Sephadex LH-20 to give five fractions containing essentially; a mixture of **1** and **2** (100 mg); *epi*-zeylandiol (**3**) (53 mg); a mixture of **4** and **5** (130 mg); **6** (12 mg), and **7** (15 mg). *Epi*-zeylandiol (**3**), 29-hydroxy-3-friedelanone (**6**) and canophyllol (**7**) were further purified by silica gel column chromatography and recrystallisation in methanol. The CH_2Cl_2 extract of the stem bark was chromatographed on silica gel eluted with hexane, hexane-EtOAc (9:1), hexane-EtOAc (4:1), hexane-EtOAc (1:1), and finally with pure EtOAc. 500 ml fractions were collected and combined on the basis of their TLC profiles. The fractions eluted with hexane-EtOAc (9:1) (5 g) were further purified by chromatography on silica gel (toluene:EtOAc 19:1), and on Sephadex LH-20 (solvent), yielding an unseparable mixture of **1** and **2**. The fractions contaning the mixtures of **1a** and **2a**, as well as **4a** and **5a**, were acetylated (acetic anhydride:pyridine 1:1) and the diacetates **1b** and **2b**, as well as the monoacetates **4b** and **5b**, were isolated by silica gel column chromatography.

*2α,29-dihydroxy-3-friedelanone diacetate (**1b**).* White crystals from methanol, m.p. 188-190 °C. $[\alpha]_D$ -25 ° ($CHCl_3$ *c* 0.1). $IR_{max}(cm^{-1})$ 2930, 1745, 1720, 1390, 1230, 1020. ^1H NMR ($CDCl_3$) 4.93 (dd, *J* 3.2 and 2.7 Hz, $H_{2\beta}$), 3.88 (d, *J* 10.6 Hz, H_{29a}), 3.81 (d, *J* 10.6 Hz, H_{29b}), 2.65 (d, J 6.6 Hz, $H_{4\alpha}$), 2.11(s, AcO), 2.07 (m, H_{1a}), 2.06 (s, AcO), 1.80(m, H_{1b}), 1.76 (m, $H_{10\alpha}$), 1.74 (m, H_{22a}), 1.74 (m, $H_{6\beta}$), 1.62 (m, H_{16a}), 1.52 (m, H_{21a}), 1.51(m, $H_{18\beta}$), 1.50 (m, H_{15a}), 1.49 (m, H_{7a}), 1.42 (m, H_{19a}), 1.38 (m, $H_{8\alpha}$), 1.37 (m, H_{7b}), 1.37 (m, H_{16b}), 1.35 (m, H_{11a}), 1.32 (m, $H_{6\alpha}$), 1.28 (m, H_{12a}), 1.28 (m, H_{15b}), 1.25 (m, H_{19b}), 1.19 (m, H_{21b}), 1.18 (m, H_{12b}), 1.14 (m, H_{11b}), 1.14 (s, H_{28}), 1.06 (s, H_{27}), 0.99 (s, H_{30}), 0.96 (s, H_{26}), 0.95 (m, H_{22b}), 0.88 (d, J 6.6 Hz, H_{23}), 0.82 (s, H_{25}), 0.69 (s, H_{24}). ^{13}C NMR (see Table 1). MS *m/z* 542.3962 (M^+, $C_{34}H_{54}O_5$ requires 542.3971, 33 %), 482 (54 %), 442 (23 %), 399 (17 %), 271 (28%), 245 (26 %), 203 (52 %), 196 (45 %), 177 (48 %), 135 (53 %), 123 (100 %), 104 (56 %), 95 (54 %).

2β,21α-dihydroxy-3-friedelanone diacetate (**2b**). White powder from methanol, m.p. 234-236 °C. $[\alpha]_D$ -38 ° ($CHCl_3$ c 0.08). $IR_{max}(cm^{-1})$ 2900, 1735, 1710, 1240, 1220, 1010. ^1H NMR ($CDCl_3$) 4.98 (m, $H_{2\alpha}$), 4.92 (dd, *J* 13.5 and 4.6 Hz, $H_{21\beta}$), 2.66 (d, *J* 6.6 Hz, $H_{4\alpha}$), 2.13 (s, AcO), 2.09 (s, AcO), 2.09 (m, H_{1a}), 1.83 (m, H_{1b}), 1.78 (m, $H_{10\alpha}$), 1.75 (m, $H_{6\beta}$), 1.71 (m, H_{22a}), 1.64 (m, H_{19a}), 1.59 (m, H_{11a}), 1.56(m, H_{18}), 1.49 (m, H_{7a}), 1.49 (m, H_{11b}),1.49 (m, H_{15a}), 1.44 (m, $H_{8\alpha}$), 1.43 (m, H_{19b}), 1.38 (m, H_{16a}), 1.37 (m, H_{7b}), 1.34 (m, $H_{6\alpha}$), 1.34 (m, H_{12a}), 1.32 (m, H_{12b}), 1.31 (m, H_{15b}), 1.27 (s, H_{28}), 1.23 (m, H_{22b}), 1.22 (m, H_{16b}), 1.12 (s, H_{27}), 1.08 (s, H_{30}), 0.95 (s, H_{29}), o.94 (s, H_{26}), 0.88 (d, *J* 6.6 Hz, H_{23}), 0.84 (s, H_{25}), 0.71 (s, H_{24}). ^{13}C NMR (see Table 1). MS *m/z* 542.3988 (M^+, $C_{34}H_{54}O_5$ requires 542.3971, 7 %), 482 (30 %), 422 (52 %), 407 (25 %), 271 (33 %), 203 (43 %), 177 (50 %), 135 (47 %), 123 (100 %), 95 (45 %).

6β,21α-dihydroxy-3-friedelanone (**3**). White crystals from methanol m.p. 281-283 °C. $[\alpha]_D$ -8 °($CHCl_3$ c 0.1). $IR_{max}(cm^{-1})$ 3500, 2950, 1715, 1460, 1390, 1110, 1050. ^1H NMR 3.69 (m, $H_{21\beta}$), 3.66 (dd, *J* 10.7 and 4.7, $H_{6\alpha}$), 2.41 (q, *J* 6.7 Hz, $H_{4\alpha}$), 2.39 (m, $H_{2\beta}$), 2.27 (dd, *J* 13.2 Hz, $H_{2\alpha}$), 2.00 (m, $H_{1\alpha}$), 1.76 (m, $H_{1\beta}$),

1.70 (m, H_{22a}), 1.66 (m, $H_{7\beta}$), 1.63 (dd, *J* 14.4 and 4.2 Hz, $H_{19\alpha}$), 1.56 (m, $H_{19\beta}$), 1.56 (m, H_{16a}), 1.50 (m, $H_{8\alpha}$), 1.50 (m, $H_{10\alpha}$), 1.58 (m, $H_{18\beta}$), 1.43 (m, $H_{7\alpha}$), 1.43 (m, $H_{11\alpha}$), 1.43 (m, H_{15a}), 1.43 (m, H_{16b}), 1.38 (m, H_{12a}), 1.32 (m, H_{12b}), 1.32 (m, H_{15b}), 1.26 (m, $H_{11\beta}$), 1.23 (m, H_{22b}), 1.19 (s, H_{28}), 1.11 (d, *J* 6.7 Hz, H_{23}), 1.10 (s, H_{27}), 1.06 (s, H_{29}), 0.98 (s, H_{30}), 0.90 (s, H_{26}), 0.86 (s, H_{25}), 0.75 (s, H_{24}). ^{13}C NMR (see Table 1). MS *m/z* 458.3749 (M^+, $C_{30}H_{50}O_3$ requires 458.3760, 5 %), 440 (35 %), 424 (12 %), 411 (13 %), 302 (23 %), 273 (45 %), 246 (22 %), 231 (24 %), 218 (23 %), 177 (33 %), 161 (30 %), 123 (100 %), 105 (75 %), 95 (65 %), 81 (60 %).

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