Bioactive Constituents from *Boswellia papyrifera*

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Phytochemical investigation of the stem bark extract of *Boswellia papyrifera* afforded two new stilbene glycosides, *trans*-4′,5-dihydroxy-3-methoxystilbene-5-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-*β*-D-glucopyranoside (**1**), *trans*-4′,5-dihydroxy-3-methoxystilbene-5-*O*-[α-L-rhamnopyranosyl-(1→6)]-*β*-D-glucopyranoside (**2**), and a new triterpene, 3α-acetoxy-27-hydroxylup-20(29)-en-24-oic acid (**3**), along with five known compounds, 11-keto-*β*-boswellic acid (**4**), *β*-elemonic acid (**7**), 3α-acetoxy-11-keto-*β*-boswellic acid (**8**), *β*-boswellic acid (**9**), and *β*-sitosterol (**10**). The stilbene glycosides exhibited significant inhibition of phosphodiesterase I and xanthine oxidase. The triterpenes (**3**−**9**) exhibited prolyl endopeptidase inhibitory activities.

The genus *Boswellia* (Burseraceae), consisting of 10 species of trees and shrubs, is distributed in the tropical parts of Asia and Africa.[1] *Boswellia papyrifera* (Del.) Hochst. is a deciduous, gum-producing, multipurpose perennial tree that grows in Sudanian and Sahelian regions. The tree is tapped on the stem for a kind of oleo-gum called "olibanum" (true frankincense). This gum resin is used in medicinal preparations for the treatment of amenorrhoea, menorrhagia, polyuria, rheumatism, ulcers, scrofulous affections, syphilis, sores, and nervous diseases. It is also used in diarrhea, asthma, and bronchitis.[2] The *Boswellia* plants are known to contain several acidic triterpenes, some of which show analgesic, immunosuppressant, antileukemic, hepatoprotective, and anti-inflammatory activities. Most of these activities are based on the inhibition of the enzyme 5-lipoxygenase.[3] Acetyl-11-keto-*β*-boswellic acid, a compound isolated from *Boswellia serrata*, exerts cytotoxic effects in in vitro human glioblastoma and leukemia cell lines.[4] Boswellic acids decrease the formation of leukotriene B_4 from endogenous arachidonic acid in rat peritoneal neutrophils in a dose-dependent manner with IC_{50} values from 1.5 to 7 μM and also inhibit the leukotriene synthesis via the inhibition of 5-lipoxygenase.[5]

Xanthine oxidase catalyzes the oxidative hydroxylation of hypoxanthine or xanthine using oxygen as a cofactor, and the resulting end products are superoxide anion ($O_2^{\bullet-}$) and uric acid. The inhibitors of xanthine oxidase enzyme can prevent the generation of excess superoxide anions.[6]

Phosphodiesterase I successively hydrolyzes 5′-mononucleotides from 3′-hydroxyl-terminated ribo- and deoxyribo-oligonucleotides. The enzyme has been widely utilized as a tool for structural and sequential studies of nucleic acids. The 5′-nucleotide phosphodiesterase isozyme-V test is useful in detecting liver metastatis in breast, gastrointestinal, lung, and various other forms of cancers.[7]

Prolyl endopeptidase catalyzes the hydrolysis of peptide bonds at the L-proline carboxy terminal and thus plays an important role in the biological regulation of proline-containing neuropeptides and peptide hormones, which are recognized to be involved in learning and memory.[8]

Results and Discussion

The MeOH extract of the stem bark of *B. papyrifera* was partitioned into hexane-, $CHCl_3$-, EtOAc-, and H_2O-soluble fractions. The H_2O-soluble extract was subjected to vacuum liquid chromatography (VLC, silica gel) and then column chromatography (Sephadex LH-20, silica gel) to obtain two new stilbenes, **1** and **2**.

The positive-ion HRFABMS of **1** exhibited the molecular ion at *m/z* 697.2719 $[M + H]^+$, corresponding to the formula $C_{33}H_{44}O_{16}$, which indicated 12 degrees of unsaturation. The fragment ions at *m/z* 551, 389, and 243 indicated the presence of one hexose and two deoxyhexose moieties. The presence of three sugars in **1** was also deduced from the negative-ion FABMS.

The UV spectrum of **1** exhibited absorptions at 320, 306, and 218 nm, which indicated the presence of a conjugated aromatic system.[9] The ^{1}H NMR (400 MHz, CD_3OD) spectrum showed the presence of three anomeric protons, resonating at δ 4.71 (s), 4.94 (d, J = 7.4 Hz), and 5.28 (s), in addition to the signals for a 1-, 3-, 5-trisubstituted aromatic ring at δ 6.42 (br s), 6.62 (br s), and 6.69 (br s), one methoxy group at δ 3.79 (s), a *para*-disubstituted aromatic ring at δ 7.44 (2H, d, J = 8.5 Hz) and 6.89 (2H, d, J = 8.5 Hz), and two olefinic protons at δ 7.04 (d, J = 16.0 Hz) and 6.85 (d, J = 16.0 Hz).[10] The large coupling constant (16.0 Hz) indicated the presence of *trans*-olefinic coupling. These observations suggested that the compound could be a *trans*-stilbene.[11] The ^{13}C NMR spectrum exhibited three anomeric carbon signals at δ 100.5, 102.1, and 102.4. The presence of a downfield signal at δ 79.1 and a downfield CH_2 signal at δ 67.5 in the ^{13}C NMR spectrum indicated the attachment of α-L-rhamnose moieties at C-2″ and C-6″ of *β*-D-glucose. The presence of two methyl doublets in compound **1** was attributed to the presence of two rhamnopyranosyl units.

The anomeric proton at δ 4.94 (H-1″) showed HMBC interaction with δ 159.5 (C-5), indicating the attachment of *β*-D-glucose at C-5. The anomeric proton of the rhamnose moiety at δ 5.28 (H-1‴) exhibited long-range correlation with C-2″ of the glucose moiety at δ 79.1, while the anomeric proton at δ 4.71 (H-1‴) showed HMBC correlation with C-6″ of the glucose moiety at δ 67.5. The positions of the sugar residues were further deduced by 1-D TOCSY and HMBC experiments and by acid hydrolysis. Acid hydrolysis afforded aglycone, D-glucose, and L-rhamnose (1:

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Table 1. ^{1}H and ^{13}C NMR Assignments of Compounds **1** and **2** in CD_3OD[a]

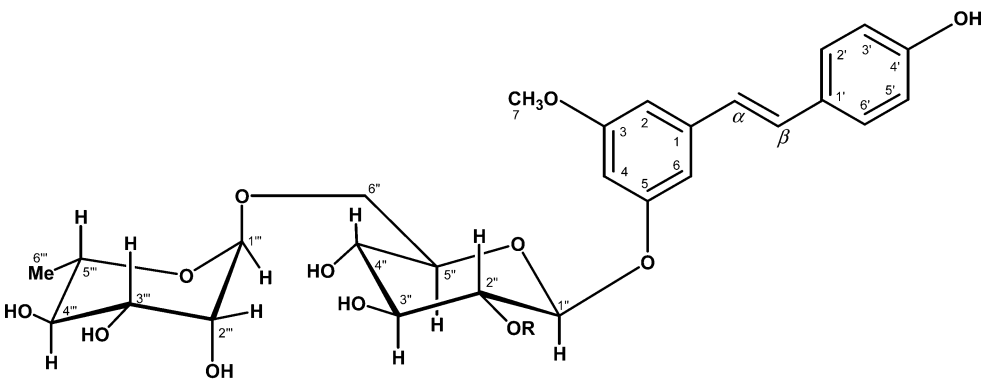
	1			**2**		
position	δ(C)	δ(H)	(HMBC) (H→C)	δ(C)	δ(H)	(HMBC) (H→C)
1	141.9 (C)			141.2 (C)		
2	107.2 (CH)	6.69 (br s)	3, 4, 6, α	107.8 (CH)	6.72 (br s)	3, 4, 6, α
3	160.3 (C)			160.3 (C)		
4	103.8 (CH)	6.42 (br s)	2, 3, 6	104.3 (CH)	6.41 (br s)	3, 5, 6
5	159.5 (C)			159.5 (C)		
6	108.3 (CH)	6.62 (br s)	2, 4, 5, α	108.3 (CH)	6.65 (br s)	2, 4, 5, α
7	55.7 (CH_3)	3.79 (s)	3	55.7 (CH_3)	3.79 (s)	3
1′	131.7 (C)			131.7 (C)		
2′	128.8 (CH)	7.44 (d, J = 8.5 Hz)	3′, β	128.8 (CH)	7.44 (d, J = 8.5 Hz)	3′, β
3′	115.1 (CH)	6.89 (d, J = 8.5 Hz)	1′, 4′	115.1 (CH)	6.88 (d, J = 8.5 Hz)	1′, 4′
4′	160.9 (C)			160.9 (C)		
5′	115.1 (CH)	6.89 (d, J = 8.5 Hz)	1′, 4′	115.1 (CH)	6.88 (d, J = 8.5 Hz)	1′, 4′
6′	128.8 (CH)	7.44 (d, J = 8.5 Hz)	3′, β	128.8 (CH)	7.44 (d, J = 8.5 Hz)	3′, β
α	127.4 (CH)	6.85 (d, J = 16.0 Hz)	6, β, 1′	127.4 (CH)	6.85 (d, J = 16.2 Hz)	1, 2, β
β	129.7 (CH)	7.04 (d, J = 16.0 Hz)	1, 2′, α	129.7 (CH)	6.99 (d, J = 16.2 Hz)	1, 2′, α
1″	100.5 (CH)	4.94 (d, J = 7.4 Hz)	5	102.3 (CH)	4.86 (d, J = 7.02 Hz)	5, 2″
2″	79.1 (CH)	3.63	1″, 4″	74.9 (CH)	3.46	
3″	79.0 (CH)	3.56		77.9 (CH)	3.41	
4″	72.2 (CH)	3.43		72.3 (CH)	3.70	
5″	76.7 (CH)	3.53		76.9 (CH)	3.55	4″
6″	67.5 (CH_2)	3.97, 4.01	1‴	67.6 (CH_2)	3.64, 4.02	1‴
1‴	102.1 (CH)	4.71 (s)	2‴, 5‴, 6″	102.1 (CH)	4.72 (s)	2‴, 5‴, 6″
2‴	72.2 (CH)	3.86		72.1 (CH)	3.85	
3‴	72.1 (CH)	3.68		71.3 (CH)	3.47	
4‴	74.0 (CH)	3.34		74.1 (CH)	3.39	
5‴	69.8 (CH)	3.65		69.8 (CH)	3.65	
6‴	17.9 (CH_3)	1.17 (d, J = 6.1 Hz)	5‴	17.9 (CH_3)	1.18 (d, J = 6.1 Hz)	4‴, 5‴
1⁗	102.4 (CH)	5.28 (s)	2″, 2⁗, 5⁗			
2⁗	72.3 (CH)	3.94				
3⁗	71.4 (CH)	3.62				
4⁗	74.1 (CH)	3.43				
5⁗	69.9 (CH)	4.08				
6⁗	18.2 (CH_3)	1.32 (d, J = 6.1 Hz)	5⁗			

[a] ^{1}H NMR and ^{13}C NMR recorded at 400 and 100 MHz, respectively.

2), which were identified by comparative TLC with standard sugars using the solvent system BuOH/EtOAc/2-propanol/HOAc/H_2O (7:20:12:7:6). On the basis of the above spectroscopic studies, the structure of compound **1** was determined as *trans*-4′,5-dihydroxy-3-methoxystilbene-5-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **2** was isolated as a light brown gummy material. The spectroscopic data of compound **2** were similar to that of **1** except one rhamnose, which was absent in **2** at the C-2″ position. The positive-ion HRFABMS of **2** exhibited the molecular ion at *m/z* 551.2140 ($C_{27}H_{34}O_{12}$), which indicated 11 degrees of unsaturation. The fragment ions at *m/z* 405 and 243 indicated the presence of two sugar units, hexose and a deoxyhexose.

The UV spectrum of **2** exhibited absorptions at 318, 304, and 216 nm. The ^{1}H NMR spectrum (400 MHz, CD_3OD) of **2**, in addition to other signals, showed two anomeric protons at δ 4.72 (s) and 4.86 (d, J = 7.0 Hz) and a methyl doublet at δ 1.18 (J = 6.1 Hz), suggesting the presence of two sugars, α-L-rhamnopyranose and β-D-glucopyranose. In the ^{13}C NMR spectrum (CD_3OD, 100 MHz), the anomeric carbon atoms appeared at δ 102.1 and 102.3. The anomeric proton at δ 4.86 (corresponding to δ_C 102.3) showed a coupling constant of 7.0 Hz, indicating the presence of a β-D-glucosidic linkage.[12] The positions of the sugar residues were further deduced by 1-D TOCSY and HMBC experiments and by acid hydrolysis. Acid hydrolysis afforded aglycone, D-glucose, and L-rhamnose (1:1), which were identified by comparative TLC with standard sugars using



1 R = α-L- Rhamnose

2 R = H

Table 2. ^{1}H and ^{13}C NMR Assignments of Compound **3** in CD_3OD[a]

position	δ(C)	δ(H)	(HMBC) (H→C)
1	33.6 (CH_2)		
2	25.7 (CH_2)		
3	73.9 (CH)	5.23 (br s)	24, 5, 31
4	37.3 (C)		
5	47.5 (CH)	1.46	3
6	19.3 (CH_2)		
7	33.8 (CH_2)		
8	42.5 (C)		
9	49.4 (CH)		
10	40.6 (C)		
11	20.7 (CH_2)		
12	26.7 (CH_2)		
13	37.0 (CH)		
14	46.3 (C)		
15	29.4 (CH_2)		
16	34.2 (CH_2)		
17	46.3 (C)		
18	50.1 (CH)	1.71 (d, J = 11.2 Hz)	
19	48.9 (CH)	1.65 (d, J = 11.2 Hz)	
20	150.3 (C)		
21	28.9 (CH_2)		
22	37.0 (CH_2)		
23	23.4 (CH_3)	1.09 (s, 3H)	3, 5, 24
24	178.5 (C)		
25	14.5 (CH_3)	1.15 (s, 3H)	
26	15.5 (CH_3)	1.03 (s, 3H)	8, 10
27	59.6 (CH_2)	3.72 (1H, d, J = 11.1 Hz, 27a) 3.29 (1H, d, J = 11.1 Hz, 27b)	14
28	13.0 (CH_3)	0.79 (s, 3H)	16, 18, 22
29	109.2 (CH_2)	4.67 (1H, d, J = 2.0 Hz, 29a) 4.56 (1H, d, J = 2.0 Hz, 29b)	
30	18.7 (CH_3)	1.68 (s, 3H)	19, 20, 29
31	170.9 (C)		
32	21.0 (CH_3)	2.01 (s, 3H)	

[a] ^{1}H NMR and ^{13}C NMR recorded at 500 and 125 MHz, respectively.

the solvent system BuOH/EtOAc/2-propanol/HOAc/H_2O (7:20:12:7:6). The cross-peak due to long-range correlations between C-5 (δ 159.5) of the aglycone and H-1″ of β-glucose (δ 4.86) indicated that the glucose residue was linked to C-5 of the aglycone, while the downfield CH_2 signal at δ 67.6 in the ^{13}C NMR spectrum indicated the attachment of the α-L-rhamnose moiety with C-6″ of β-D-glucose unit. On the basis of these spectroscopic studies, the structure of compound **2** was determined to be *trans*-4′,5-dihydroxy-3-methoxystilbene-5-*O*-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **3** was obtained as a white amorphous powder with IR absorptions at 1721 and 3474 cm^{-1}, indicating the presence of carbonyl and hydroxyl groups, respectively. The molecular formula of compound **3** was deduced as $C_{32}H_{50}O_5$ (*m/z* 514.3721 in HREIMS) with eight degrees of unsaturation. The ^{13}C NMR spectrum of **3** revealed 32 carbon signals, which were deduced by DEPT ^{13}C NMR as six methyls, 10 methylenes, five methines, five quaternary carbons, one acetoxy methine, one secondary alcohol, one carboxylic acid, one acetoxy group, and two olefinic carbons (one methylene and one quaternary carbon).[13] The detailed analysis of the ^{1}H NMR spectrum showed the presence of a lup-20(29)-ene parent skeleton. The tertiary methyls appeared at δ 1.15, 1.09, 1.03, and 0.79 (3H each, s, CH_3-25, CH_3-23, CH_3-26, CH_3-28, respectively), one vinylic methyl at δ 1.68 (3H, s, CH_3-30), one acetoxy methyl at δ 2.01 (CO*CH*$_3$), two protons of an isoprenyl moiety at δ 4.67 and 4.56 (1H each, d, J = 2.0 Hz, H-29a and H-29b), one carbinol proton at δ 5.23 (1H, br s, H-3), two protons of a primary alcohol at δ 3.72 and 3.29 (1H each, d, J = 11.1 Hz, H-27a and H-27b), and a lupenyl H-19$_\beta$ proton at δ 1.65 (1H, d, J = 11.2 Hz). The equatorial disposition (β-orientation) of H-3 was deduced from $W_{1/2}$ = 8.0 Hz and also by the absence of interaction between H-3 and H-5 in the NOESY experiment. In the HMBC experiment, the oxymethine proton at δ 5.23 (H-3) showed $^3J_{CH}$ correlations with C-5 (δ 47.5), C-24 (δ 178.5), and C-31 (δ 170.9), while the alcoholic methine carbon at δ 73.9 showed $^3J_{CH}$ interactions with H-5 (δ 1.46) and H-23 (δ 1.09), supporting the presence of an equatorial H-3. The location of the carboxylic group[14] at C-24 was deduced by HMBC interactions, and the carboxylic carbon signal at δ 178.5 showed $^3J_{CH}$ interactions with H-3 (δ 5.23), H-23 (δ 1.09), and H-5 (δ 1.46). The position of the C-27 hydroxyl group was established from the HMBC spectrum, in which the H-27b proton at δ 3.29 showed $^2J_{CH}$ correlation with C-14 (δ 46.3), while the C-28 methyl protons at δ 0.79 showed $^3J_{CH}$ correlations with C-18 (δ 50.1). These correlations were in agreement with the proposed structure **3**. The ^{1}H and ^{13}C NMR chemical shift assignments of compound **3** are based on the ^{1}H−^{1}H-COSY, HMQC, and HMBC spectra. The structure was therefore assigned as 3α-acetoxy-27-hydroxylup-20(29)-en-24-oic acid.

Table 3. In Vitro Free Radical Scavenging and Xanthine Oxidase and Phosphodiesterase I Inhibitory Activities of Compounds **1** and **2**

	IC$_{50}$ (μM)[a]		
compound	DPPH scavenging activity	XO inhibition activity	phosphodiesterase I inhibition activity
1		178 ± 4.62	992 ± 17.00
2		129 ± 3	589 ± 17.00
PG[b]	30 ± 0.27	628 ± 5.0	
BHA[b]	44 ± 2.00	591 ± 8.0	
allopurinol[c]		7.45 ± 0.17	
cystein[d]			748 ± 15.00
EDTA[d]			274 ± 7.00

[a] IC$_{50}$ values are the mean ± standard mean (SEM) error of three assays. [b] Standard compounds for DPPH scavenging activity. [c] Standard inhibitor of xanthine oxidase. [d] Standard compounds for phosphodiesterase I inhibitory activity.

Compounds **1** and **2**, by virtue of their phenolic nature, were tested for antioxidant activities in a battery of assays. They were inactive in a DPPH radical scavenging assay, but significantly inhibited the xanthine oxidase enzyme (EC 1.1.3.22) with IC$_{50}$ values of 178 and 129 μM, respectively. The results are presented in Table 3.

Compounds **1** and **2** were screened against phosphodiesterase I (EC 3.1.4.1) enzyme with varying degrees of activity (Table 3). The results showed that compound **2** is more active than **1**, with IC$_{50}$ values of 589 (compound **2**) and 992 μM (compound **1**), respectively.

Compounds **3**−**9** were screened against PEP (EC 3.4.21.26). Their IC$_{50}$ values are shown in Table 4 along with the positive control (bacitracin). Among the known constituents, compounds **4**, **8**, and **9** have been reported as potential inhibitors of lipoxygenase enzyme.[15] These compounds have also been reported as inhibitors of growth of human leukemia in HL-60 cells, and DNA, RNA, and protein synthesis in HL-60 cells.[5] We report here, for the first time, the PEP inhibitory activity of different compounds isolated from *B. papyrifera*. Among the compounds **4**, **5**, **7**, **8**, and **9**, compound **8** (3α-acetoxy-11-keto-β-boswellic acid) showed the maximum inhibitory potential against prolyl endopeptidase (PEP) enzyme, as indicated by the lower IC$_{50}$ value of 7.89 μM. When the activities of these compounds were compared with their structures, it

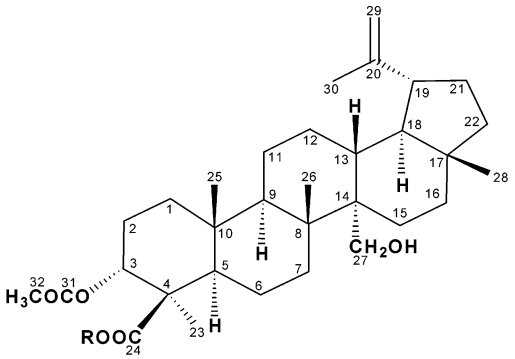
Table 4. In Vitro Quantitative Inhibition of Prolyl Endopeptidase

compound	IC_{50} (μM)[a]
trans-4′,5-dihydroxy-3-methoxystilbene-5-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (**1**)	NA
trans−4′,5-dihydroxy-3-methoxystilbene-5-*O*-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (**2**)	NA
3α-acetoxy-27-hydroxylup-20(29)-en-24-oic acid (**3**)	2.866 ± 0.064
11-keto-β-boswellic acid (**4**)	36.32 ± 0.772
methyl 3α-acetoxy-27-hydroxylup-20(29)-en-24-oate (**5**)	57.43 ± 4.01
methyl ester of 11-keto-β-boswellic acid (**6**)	114.75 ± 5.14
β-elemonic acid (**7**)	39.74 ± 1.614
3α-acetoxy-11-keto-β-boswellic acid (**8**)	7.89 ± 0.02
β-boswellic acid (**9**)	9.75 ± 0.521
bacitracin[b]	129.26 ± 3.28

[a] IC_{50} values are the mean ± standard mean error of three assays. [b] Standard compound for prolyl endopeptidase (PEP) inhibitory activity.

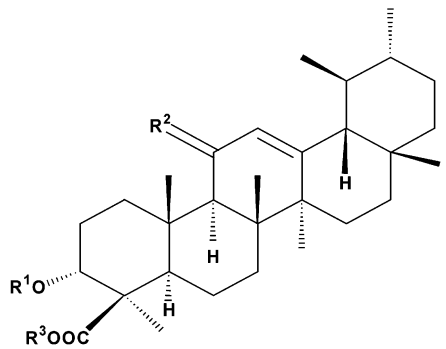
was found that the substituent at C-3, C-11, and C-24 might play an important role, as β-boswellic acid (**9**) showed a lower IC_{50} value (9.75 μM) as compared to 11-keto-β-boswellic acid (**4**) (IC_{50} = 36.32 μM). Acetylation of 11-keto-β-boswellic acid (**8**) enhanced the activity (IC_{50} = 7.89 μM), while methylation of 11-keto-β-boswellic acid (**6**) decreased the activity (IC_{50} = 114.75 μM). Compound **7**, which belongs to a tetracylic triterpene class, showed low inhibitory activity (IC_{50} = 39.74 μM) as compared to β-boswellic acid (**9**), which does not have a free carboxyl group at the C-24 position.

The new compound **3**, a lupane-type triterpene, was the most active PEP inhibitor among the tested compounds **4**, **5**, **6**, **7**, **8**, and **9**, as indicated by its low IC_{50} value (2.86 μM).

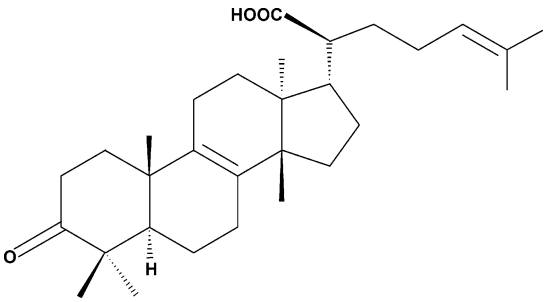


3 R = H 3α-Acetoxy-27-hydroxylup-20(29)-ene-24-oic acid

5 R = CH_3 Methyl 3α-acetoxy-27-hydroxylup-20(29)-ene-24-oate



	R^1	R^2	R^3	
4	H	O	H	11-Keto- β-boswellic acid
6	H	O	CH_3	Methyl ester of 11-keto-β-boswellic acid
8	-COCH3	O	H	3α-Acetoxy-11-keto-β-boswellic acid
9	H	H_2	H	β-Boswellic acid



7 β-Elemonic acid

Experimental Section

General Experimental Procedures. Melting points were measured on a YANACO apparatus. Optical rotations were measured on Schmidt + Haensch Polartronic D. UV and IR spectra were recorded on Hitachi UV 3200 and JASCO 302-A spectrophotometers. The ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H-COSY, TOCSY, HMQC, and HMBC spectra were recorded on Bruker AMX 400 and AMX 500 MHz NMR spectrometers; chemical shifts are in ppm (δ) relative to $SiMe_4$ as internal standard and coupling constants are in Hz. EI and HREI MS were measured on Varian MAT 311A and JEOL HX 110 mass spectrometers (*m/z*, rel int %). Column chromatography (CC) was carried out on silica gel (70−230 mesh). Thin-layer chromatography (TLC) was performed on precoated silica gel plates (DC-Alufolien 60 F_{254} of E. Merck), and spots were detected at 254 and 366 nm, by using ceric sulfate spraying reagent.

Plant Material. Stem barks of *B. papyrifera* (Del.) Hochst. were collected in December 1998 and identified by Dr. Achoundong from Garoua, Benove Region, Cameroon. A voucher specimen (#64941/HNC) was deposited at the National Herbarium (Yaounde, Cameroon).

Extraction and Isolation. The air-dried and pulverized stem barks of *B. papyrifera* (1.5 kg) were soaked in MeOH (7 days), and then the concentrated MeOH extract was partitioned with *n*-hexane, CH_2Cl_2, and EtOAc. The CH_2Cl_2 extract (22.29 gm) was subjected to vacuum liquid chromatography (VLC) (silica gel, 400 gm) using a gradient solvent system of *n*-hexane/EtOAc and EtOAc/MeOH, to give nine subfractions (F_1−F_9). Fraction F_1 (*n*-hexane/EtOAc, 19:1, 4.19 g) after repeated column chromatography (silica gel, eluent *n*-hexane/CH_2Cl_2) yielded β-sitosterol (10.2 mg) and β-elemonic acid[16] (**7**) (56.3 mg). Fraction F_3 (*n*-hexane/EtOAc, 4:1, 832 mg) was first subjected to column chromatography (silica gel, eluent *n*-hexane/EtOAc, 3:17) and then to thin-layer chromatography (silica gel, MeOH/$CHCl_3$/*n*-hexane, 1:6:13) to obtain β-boswellic acid (**9**) (13.2 mg) and 3α-acetoxy-11-keto-β-boswellic acid[17] (**8**) (5.4 mg). Fraction F_4 (*n*-hexane/EtOAc, 1:1, 2.2 gm) was first subjected to column chromatography on Sephadex LH-20 (50 gm) using H_2O/MeOH as eluent and then to thin-layer chromatography (silica gel, eluent acetone/*n*-hexane, 1:4, 466 mg) to yield 3α-acetoxy-27-hydroxylup-20(29)-en-24-oic acid (**3**) (16.7 mg) and 11-keto-β-boswellic acid (**4**) (10.4 mg).

The MeOH extract (342.7 gm) was subjected to VLC (gradient $CHCl_3$/MeOH), yielding four fractions (F_1−F_4). Fraction F_3 ($CHCl_3$/MeOH, 11:9, 76.45 gm) was first subjected to VLC (gradient $CHCl_3$/MeOH) and then to repeated column chromatography on Sephadex LH-20 (MeOH/H_2O, 1:9) and silica gel ($CHCl_3$/MeOH, 17:3), yielding two pure compounds, **1** (36 mg) and **2** (137.1 mg).

Compound 1: white crystalline solid; mp 166−170 °C; $[\alpha]^{25}_D$ −94.9° (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 320 (4.49), 306 (4.49), 218 (4.42) nm; IR (KBr) ν_{max} 3358, 1602, 715 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz), see Table 1; ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; FABMS (pos.) *m/z* 551 $(M + H - 146)^+$, 389 $(M + H - 146 - 162)^+$, 243 $(M + H - 146 - 162 - 146)^+$; HRFABMS (pos.) *m/z* 697.2719 $[M + H]^+$ (calcd for $C_{33}H_{44}O_{16}$, 697.2708).

Compound 2: light brown gummy material; $[\alpha]^{25}_D$ −89.8° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 318 (4.33), 304 (4.35), 216 (4.37) nm; IR (KBr) ν_{max} 3389, 1600, 1512, 1449 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz), see Table 1; ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; FABMS (pos.) *m/z* 405 $(M + H - 146)^+$, 243 $(M + H - 162 - 146)^+$; HRFABMS (pos.) *m/z* 551.2140 $[M + H]^+$ (calcd for $C_{27}H_{34}O_{12}$, 551.2128).

Hydrolysis of Compounds 1 and 2. Compounds **1** and **2** (each 15 mg) were dissolved in MeOH (10 mL) and 5 N HCl (10 mL). The mixtures were refluxed for 3 h. The solutions were neutralized with NaOH and extracted with EtOAc. The sugars in aqueous phase were identified as glucose and rhamnose by comparative TLC with standard sugars using the solvent system BuOH/EtOAc/2-propanol/HOAc/H_2O (7:20:12:7:6).

Compound 3: white amorphous powder; $[\alpha]^{27}_D$ +24.2° (*c* 0.066, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.98) nm; IR (KBr) ν_{max} 1721, 3474 cm^{-1};^{1}H NMR (CD_3OD, 500 MHz), see Table 2; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; EIMS *m/z* 410 $[M - CO_2 + CH_3COOH]^+$ (5), 379 $[M - CO_2 + CH_3COOH + CH_2OH]^+$ (8), 353 (2), 205 (7), 203 (27), 189 (38), 175 (69), 95 (100); HREIMS *m/z* 514.3721 (calcd for $C_{32}H_{50}O_5$, 514.3658).

Esterification of Compound 3. A 10 mg sample of compound **3** was dissolved in 5 mL of MeOH and treated with freshly prepared diazomethane (CH_2N_2). After comparative TLC, the resulting product was dissolved in distilled H_2O and extracted with $CHCl_3$. The $CHCl_3$-soluble extract was subjected to thin-layer chromatography (*n*-hexane/acetone, 90:10) to afford the methyl ester of compound **3**.

Methyl 3α-acetoxy-27-hydroxylup-20(29)-en-24-oate (5): white amorphous powder; IR ($CHCl_3$) ν_{max} 1735, 3143 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.32 (s, 1H, H-3), 4.65 (s, 1H, H-29a), 4.56 (s, 1H, H-29b), 3.34 (s, 3H, OMe); HREIMS *m/z* 528.3801 (calcd for $C_{33}H_{52}O_5$, 528.3814).

DPPH Radical Scavenging Assay. Compounds (1000 μM) were reacted with DPPH radicals (300 μM) at 37 °C for 30 min. The ratio of sample solution, prepared in DMSO to DPPH radical solution in EtOH was 5:95. The reaction was carried out in a 96-well microtiter plate reader (Molecular Devices, Spectramax 340). Finally the absorbance was measured at 515 nm.[18]

Xanthine Oxidase Inhibitory Assay. Xanthine oxidase (XO) (EC 1.1.3.22) inhibition activity was assayed in phosphate buffer (0.1 M, pH 7.5, 250 μL) and XO (0.003 unit/well, 20 μL), and the test sample in 10 μL of DMSO was diluted to the desired range of concentrations, mixed in a 96-well microplate, and preincubated for 10 min at room temperature. The reaction was initiated by adding 20 μL of 0.1 mM xanthine. The uric acid formation was measured spectrophotometrically at 295 nm by using a microtiter plate reader (Molecular Devices, Spectramax 384).[18]

Phosphodiesterase I Inhibitory Assay. Activity against phosphodiesterase I (Sigma P 4631) (EC 3.1.4.1) was assayed by using the reported method[19] with the following modifications: 33 mM tris-HCl buffer pH 8.8, 30 mM $Mg(C_2H_3O_2)_2 \cdot 4H_2O$ with 0.000742 U/well final concentration using microtiter plate assay, and 0.33 mM bis(*p*-nitrophenyl) phosphate (Sigma N-3002) as a substrate. Cystein and EDTA (E. Merck) were used as positive controls[20] (IC_{50} = 748 ± 15.00 and 274 ± 7.00 μM, respectively). After 30 min of incubation, the enzyme activity was monitored spectrophotometrically at 37 °C on a microtiter plate reader (Spectra Max, Molecular Devices) by following the release of *p*-nitrophenyl phosphate at 410 nm. Assays were conducted in triplicate.

PEP Inhibitory Activity. Prolyl endopeptidase (EC 3.4.21.26) was purchased from Seikagaku Corporation (Tokyo, Japan). *N*-Benzyloxycarbonyl-Gly-Pro-*p*NA and bacitracin were purchased from BACHEM Fine Chemicals Co. and Sigma Co., Ltd., respectively. PEP inhibitory activities were measured by a method developed by Yoshimoto[21] et al. and described in our previous publications.[22]

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