Triterpenes from *Maesopsis eminii*

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Two pentacyclic triterpenes, 1α,3β-dihydroxybauer-7-en-28-oic acid (**1**) and 3β-hydroxybauer-7-en-28-oic acid (**2**), together with sitosterol-3-β-*O*-D-glucopyranoside and stigmasterol have been isolated from the bark of the plant *Maesopsis eminii.* Their structures have been elucidated by spectroscopic methods. One of the triterpenes (**1**) is new, and its structure was confirmed by X-ray crystallographic analysis. This new triterpene displayed moderate antibacterial activity against *Bacillus subtilis* ATCC 6633.

Plants of the family Rhamnaceae are known as rich sources of anthraquinones, steroids, and terpenoids.[1] The genus *Maesopsis eminii* (Engler) is monospecific and is widely distributed throughout the African continent, especially in tropical regions.[2] This plant has previously been studied with respect to its constituents, but only anthraquinones and some phenolic compounds[3−5] have been reported until now. This paper describes the first chemical study on *M. eminii* from central Africa, which is used there in traditional medicine thanks to the diuretic, purgative, emetic, and antidiarrhetic activity of its bark.[6] The bark is also used in the Sangmelima region of the Southern province in Cameroon as abortifacient.[7] As part of our contribution to the phytochemical study of Cameroonian medicinal plants, we examined the stem bark of *Maesopsis eminii*. We now report on the isolation and structure elucidation of two pentacyclic triterpenes, one of which is new, in addition to stigmasterol and sitosterol-3-β-*O*-D-glucopyranoside. The structures of these compounds were elucidated spectroscopically, and the structure of the new triterpene (**1**) was confirmed by X-ray diffraction analysis.

Air-dried stem bark of *M. eminii* was extracted with MeOH at room temperature. The extract was concentrated to dryness, and this residue was re-extracted successively with petroleum ether, $CHCl_3$, and ethyl acetate. $CHCl_3$ and ethyl acetate extracts were combined on the basis of their similar composition (TLC), and the mixture was submitted to column chromatography over silica gel. Triterpene (**1**) and sitosterol-3-β-*O*-D-glucopyranoside were isolated from this column. The known triterpene (**2**) and stigmasterol were isolated by column chromatography of the petroleum ether extract. Compound **2** was identified by comparison of its spectroscopic data with the literature values as 3β-hydroxybauer-7-en-28-oic acid.[8,9]

Compound **1** was obtained as colorless crystals, mp 302−304 °C, and reacted positively to the Liebermann-Burchard test for terpenoids. Its molecular formula, $C_{30}H_{48}O_4$, as established by ESI-FT-ICR mass spectrometry (*m/z* 471.34805; calcd for $C_{30}H_{47}O_4$, 471.34798), corresponded to seven double-bond equivalents.

The broad band decoupled ^{13}C NMR spectrum of compound **1** showed 30 carbon signals, which were assigned by DEPT and HMQC techniques as seven methyl groups, eight methylene groups, eight methine groups, and seven quaternary carbons, among them a carbonyl group (δ 181.5), two oxygenated sp^3 carbons, and two sp^2 carbons (see Table 1). These ^{13}C resonances were found to be close to those of a friedours-7-ene.[10]

The ^1H NMR spectrum of **1** also contained resonances corresponding to seven methyl groups in the region δ 0.66 to 1.02, two of which appeared as doublets at δ 0.82 (J = 4.5 Hz) and 1.00 (J = 6.0 Hz). These were assigned to Me-29 and Me-30, respectively. The acid proton and the ethylenic proton (H-7) were observed as singlets at δ 12.16 (broad) and 5.35, respectively. The ^1H NMR spectrum also contained signals of two exchangeable hydroxy protons at δ 4.35 and 4.19. The two hydroxy groups are attached to C-1 (δ 70.4) and C-3 (δ 70.8), respectively. All ^1H and ^{13}C signals were uniquely assigned by HH-COSY, HMQC, and HMBC experiments.

The CI mass spectrum of **1** exhibited peaks at *m/z* 490 $[M + NH_4^+]$, 473 $[MH^+]$, 455 $[M - OH]^+$, the base peak at *m/z* 437 $[MH^+ - 2H_2O]$, and 427 $[M - COOH]^+$. The signal at *m/z* 263 is an indication for an oxygenated bauerene skeleton of which the base peak generally appears at *m/z* 231.[11] This suggestion was further confirmed by the peak at *m/z* 227 corresponding to the loss of two water molecules from the fragment at *m/z* 263. The occurrence of these peaks can be explained by the fragmentation displayed in Scheme 2.

The IR spectrum showed a strong and broad band at $\tilde{\nu}$ 3423 cm^{-1} (OH). The medium peak at $\tilde{\nu}$ 1380 cm^{-1} is indicative for two geminal methyl groups. In addition, a very strong and sharp band was observed at $\tilde{\nu}$ 1693 cm^{-1} (carbonyl). The presence of the trisubstituted ethylene function (C=CH−) in **1** is supported by the multiple bands observed between $\tilde{\nu}$ 680 and 1000 cm^{-1}. All spectroscopic data were confirmed by the X-ray diffraction analysis of compound **1** (Figure 1, and Tables S1 and S2, Supporting Information).[12] The relative configuration of the asymmetric carbon atoms was deduced from the X-ray data (see Supporting Information). In particular, in ring A of **1**, the hydroxy group on C-1 is axial and oriented below the plane of the molecule (1α-hydroxy), whereas the one on C-3 is equatorial, oriented above the plane (3β-hydroxy). Compound **1** is a new pentacyclic triterpene, named 1α,3β-dihydroxybauer-7-en-28-oic acid.

Compound **2** was isolated as a white powder (mp 308−310 °C), and the molecular formula, $C_{30}H_{48}O_3$, was estab-

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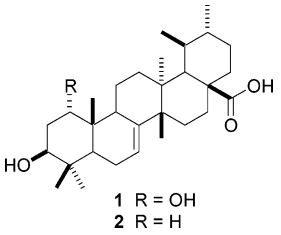
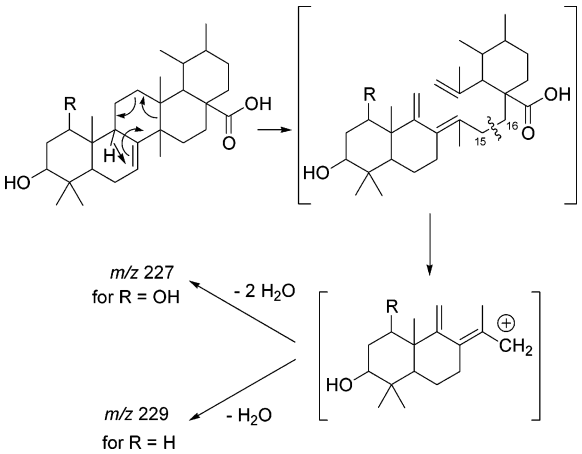
Table 1. NMR Data of **1** and **2**, Recorded in DMSO-D_6

	1		2
atom no.	^{1}H NMR	^{13}C NMR	^{13}C NMR
1	3.50; s	70.4; CH	36.3; CH_2
2	1.62; 1.67; m	35.0; CH_2	27.4; CH_2
3	3.59; d	70.8; CH	76.8; CH
4		38.6; C	38.4; C
5	1.57; s	43.2; CH	49.9; CH
6	1.92; m 2.08; m	23.8; CH_2	23.7; CH_2
7	5.35; s	115.3; CH	116.4; CH
8		144.9; C	144.2; C
9	1.60; m	43.2; CH	47.2; CH
10		38.5; C	34.7; C
11	1.50; m	27.8; CH_2	27.6; CH_2
12	1.52; m	32.1; CH_2	32.0; CH_2
13		36.6; C	36.6; C
14		40.6; C	40.5; C
15	1.43; m 1.73; m	15.0; CH_2	15.8; CH_2
16	1.30; m 1.75; m	32.1; CH_2	32.0; CH_2
17		43.7; C	43.7; C
18	2.31; s	47.6; CH	47.5; CH
19	1.05	31.6; CH	31.6; CH
20	1.10	36.2; CH	36.1; CH
21	1.07; m 1.54; m	28.8; CH_2	28.7; CH_2
22	1.60; m 2.15; m	25.5; CH_2	25.5; CH_2
23	0.75; s	14.8; CH_3	14.9; CH_3
24	0.87; s	27.8; CH_3	27.6; CH_3
25	0.66; s	13.1; CH_3	12.6; CH_3
26	1.02; s	23.1; CH_3	23.1; CH_3
27	0.98; s	21.2; CH_3	21.3; CH_3
28		181.5; C	181.4; C
29	0.82; d	21.1; CH_3	21.1; CH_3
30	1.00; d	23.3; CH_3	23.2; CH_3

Scheme 1. Structures of Compounds **1** and **2**



Scheme 2. Fragmentation Mechanism of Compounds **1** and **2**



lished by EIMS (*m/z* 456). NMR analysis and comparison of the spectroscopic data[10,13] proved that compound **2** is 3β-hydroxybauer-7-enoic acid (mp 305−308 °C). It is a known analogue of **1** with a hydroxy group attached to position 3.[9,14,15] This suggestion was also supported by the fragments at *m/z* 247 (63) and 229 (66) present in the mass spectrum, which were explained by the same fragmentation mechanism as for compound **1**. The new triterpene (**1**) displayed moderate activity against *Bacillus subtilis* ATCC 6633 in concentration > 50 μg/mL.

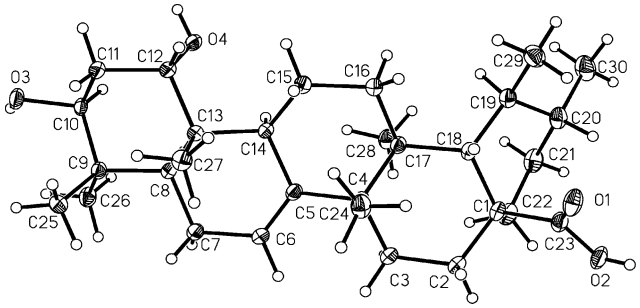


Figure 1. Molecular structure (relative configuration) of compound **1** according to X-ray analysis.

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi B-540 melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded at 250 or 500 MHz, and ^{13}C NMR spectra were recorded at 63 and 125 MHz, respectively, with TMS as an internal standard. IR spectra (KBr) were recorded on a Jasco FT/IR-410 spectrometer.

Plant Material. The stem bark of *Maesopsis eminii* was collected in February 2001 at Mfou in the Centre province of Cameroon. The sample was identified at the Cameroon National Herbarium in Yaounde, where a voucher specimen is on deposit under the references RL5561; f 234/SRF/Cam.

Extraction and Isolation. Air-dried, powdered stem bark of *M. eminii* (3 kg) was extracted at room temperature with MeOH and concentrated to dryness to afford a viscous residue (70 g). This residue was then re-extracted with petroleum ether, $CHCl_3$, and EtOAc, respectively. The petroleum ether extract, an oily mixture (4 g), was subjected to column chromatography over silica gel (0.04−0.063 mm) and eluted with a petroleum ether/EtOAc gradient of increasing polarity, resulting in 110 fractions of 150 mL each, which were combined on the basis of TLC analysis. Fractions 61−63, eluted with a mixture of petroleum ether/EtOAc, 4:1, were evaporated to afford compound **2**, which crystallized on standing. Fractions 92−110, eluted successively with the mixture of petroleum ether/EtOAc, 1:4, and EtOAc, respectively, were concentrated and chromatographed a second time over silica gel, eluted with a mixture of $CHCl_3$/MeOH, 9:1, to afford sitosterol 3-*O*-β-D-glucopyranoside (27 mg). The mixture of the $CHCl_3$ and EtOAc extracts (8 g) was subjected to column chromatography over silica gel (0.04−0.063 mm) and eluted with a gradient of increasing polarity with CH_2Cl_2/MeOH, resulting in 140 fractions of 20 mL each, which were combined on the basis of TLC analysis. Fractions 1−80 were eluted with a mixture of CH_2Cl_2/MeOH, 97:3. Fractions 72−92, eluted with a mixture of CH_2Cl_2/MeOH, 97:3, and CH_2Cl_2/MeOH, 93:7, respectively, were combined to afford 250 mg of a product, which, after purification by isocratic elution over small column chromatography using a mixture of $CHCl_3$/MeOH, 19:1, gave compound **1** (100 mg).

1α,3β-Dihydroxybauer-7-en-28-oic acid (1): colorless crystals; mp 302−304 °C; IR ν_{max} (KBr) cm^{-1} 3423, 2960, 2930, 2871, 1693, 1457, 1380, 1207, 1041, 790, 651; ^{1}H NMR (500 MHz, DMSO-D_6), see Table 1; ^{13}C NMR (125 MHz, DMSO-D_6), see Table 1; CI-MS *m/z* (int) 490 (66.46), 472 (16.33), 456 (25.49), 455 (77.34), 437 (100), 427 (41.40), 391 (23.17), 263 (3.39), 227 (6.24), 207 (16.25), 189 (5.52), 173 (5.86); ESI-FT-ICR MS *m/z* 471.34805, calcd for $C_{30}H_{47}O_4$ 471.34798.

3β-Hydroxybauer-7-en-28-oic acid (2): white powder; mp 308−310 °C (lit.[8] 305−308 °C); ^{13}C NMR (500 MHz, DMSO-D_6), see Table 1; EIMS *m/z* (%) 456 (69), 441 (100), 438 (6), 423 (54).

The complete crystallographic data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html. Entry CCDC-230712 contains the supplementary crystallographic data for this paper.

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Supporting Information Available: Information on the X-ray analysis (crystal data, structure refinement, atomic coordinates, bond distances, bond angles, and isotopic displacement parameters). This material is available free of charge via the Internet at http//pubs.acs.org.

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(12) Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: int+44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
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