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Terminalin A, a novel triterpenoid from *Terminalia glaucescens*

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Abstract—Terminalin A **1**, a new A-*seco*-triterpene, was isolated from the stem bark of *Terminalia glaucescens* Planchon. This compound has an unprecedented rearranged *seco*-glutinane structure with a pyran ring-A and an isopropanol moiety, as determined by spectroscopic and single-crystal X-ray diffraction analysis. Other known triterpenes, friedelin, β-sitosterol, stigmasterol, lupeol, betulinic acid, β-amyrin and long chain fatty acids were also isolated.

The *Terminalia* species (Combretaceae) are extensively used in the indigenous medicines of Central African regions.[1] *Terminalia glaucescens* is prescribed as an anti-dysenteric and anti-microbial agent and is reportedly also useful in the last phase of AIDS.[2] The extract of the plant showed a wide spectrum of antibacterial activity against periodontopathic bacteria.[3] The ethanolic extract of *T. glaucescens* also exhibited antiplasmodial activity.[4] Cyotoxic effects[5] and aldose reductase inhibition activity of the methanolic extract of *T. glaucescens*[6] have also been reported. The current phytochemical study on the stem bark extract of *T. glaucescens* collected from Cameroon has led to the isolation of a novel triterpene compound, terminalin A **1** (Fig. 1) with a rearranged glutinane skeleton, along with a number of the other constituents. The compound exhibited inhibitory activity against prolyl endopeptidase (PEP).

The methanolic extract of the stem bark of the plant after column and thin-layer chromatography afforded, compound **1**. The HREI MS of compound **1** showed an M^+ at m/z 444.3123 in agreement with the molecular formula $C_{30}H_{52}O_2$ (calcd 444.3129) indicating the presence of five double bond equivalents, which accounted for five rings. A strong IR absorption at 3316 cm^{-1} indicated an -OH function. The ^1H NMR spectrum (400 MHz, $CDCl_3$) of compound **1** contains singlets for eight methyls at δ 0.94, 0.97 (6H), 1.07, 1.08, 1.14, 1.26 and 1.29. The signals resonating at δ 3.67 and 3.85 were assigned to the geminally coupled methylene protons α- to the oxygen of the pyran ring. Connectivities among the C-1, C-2 and C-3 protons were established from the ^1H–^1H COSY spectrum. The ^{13}C NMR spectrum of **1** showed resonances for all 30 carbons in the molecule. The DEPT spectrum showed the presence of eight methyl, twelve methylene, three methine and seven quaternary carbons.[7]

The HMBC spectrum supported the proposed structure for compound **1**. Single-crystal X-ray diffraction analy-

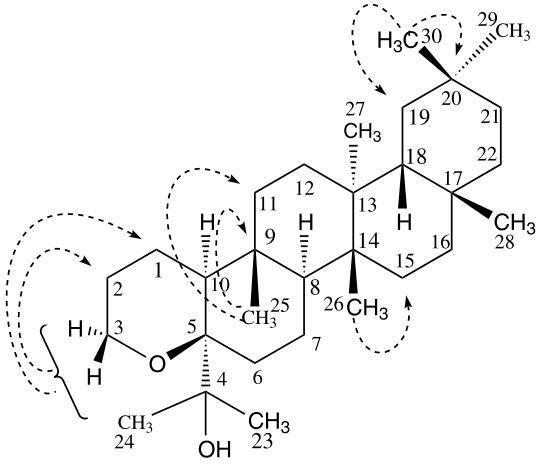

Figure 1. Important HMBC correlations in terminalin A **1**.

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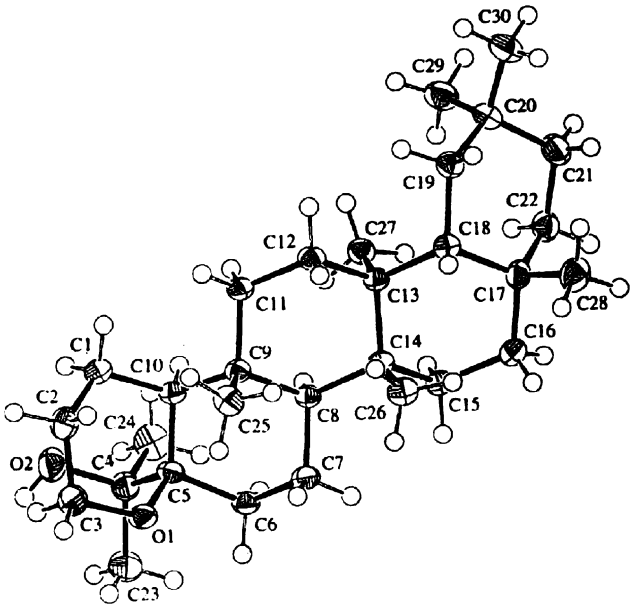


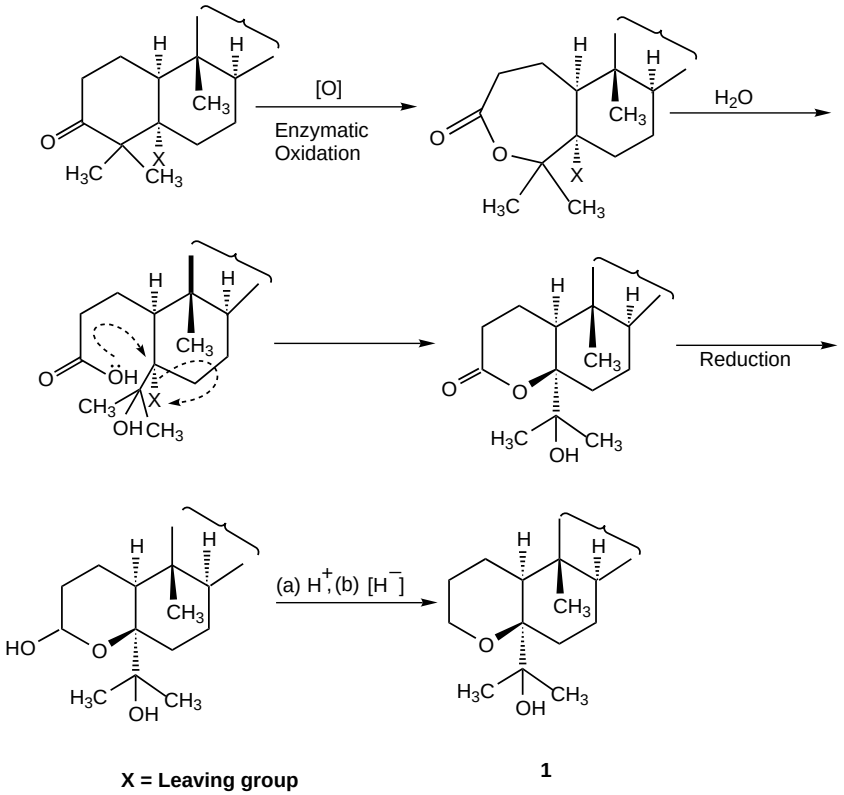
Figure 2. The ORTEP representation of the X-ray structure of terminalin A **1**.

sis was carried out to confirm unambiguously the structure **1** for this novel compound. The ORTEP representation is presented in Fig. 2.[8]

A number of ring A degraded triterpenes have been isolated earlier from sediments and petroleum, except for 3,4-*seco*-3-nor-olean-12-en-1-ol,[9] which has been isolated only from the leaf wax of some *Hoya* species (Asclepiadaceae). The biosynthesis of ring A degraded triterpenes have also been discussed on the basis of geochemical, photochemical and biochemical aspects.[10] Although degraded triterpenes are well known as natural products,[11–14] a rearranged heterocyclic *seco*-glutinane derivative such as **1** has not been found earlier. A related ring A degraded glutinane, dischidiol has been isolated from *Dischidia formosana*.[15] The degradation of ring A of triterpenes to open *seco* and *des*-ring-A derivatives is also common in microbially transformed products.[11–14] A proposed biosynthesis of terminalin A is given in Scheme 1.

Compound **1** showed inhibitory activity ($IC_{50}=73.23$ μM±1.467) against prolyl endopeptidase (PEP, EC 3.4.21.26).[16] PEP is a serine—peptidase that hydrolyzes peptide bonds at the *L*-proline carboxy terminal.[17] PEP also plays an important role in the metabolism of proline-containing neuropeptides, and it is recognized to be involved in learning and memory.[18] PEP has recently gained interest since its specific inhibitors can relieve scopolamine induced amnesia.[19,20] *Z*-Pro-prolinal was used as a standard inhibitor and its $IC_{50}=1.27$ nM±0.01, was approximately the same as mentioned by Tanaka et al.[21] in the assay.

Some other known compounds such as friedelin, β-sitosterol, stigmasterol, lupeol, betulinic acid, β-amyrin and long chain fatty acids have also been isolated for the first time from this plant species.



Scheme 1. A proposed biogenetic pathway to terminalin A **1**.

Acknowledgements

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7. Isolation procedure and selected spectroscopic data for **1**: The stem bark of *Terminalia glaucescens* Planchon. was collected at Mount Bankolo near Yaounde, Cameroon. A voucher specimen (# 9468 SRFCAM) was deposited at the National Herbarium (Yaounde, Cameroon). Air-dried stem barks (7.5 kg) of *T. glaucescens* were cut into pieces, dried, pulverized and soaked in a mixture of $MeOH/CH_2Cl_2$ (1:1) at room temperature for 24 h, then filtered and the solvent was evaporated under reduced pressure. The methanolic extract (611.5 g) was suspended in dist. water and defatted with pet. ether. The defatted extract was further extracted by chloroform, ethyl acetate and butanol. The pet. ether extract was repeatedly chromatographed on a silica gel column (18.3 g) using various polarities of solvent mixtures of pet. ether:ethyl acetate. After that preparative TLC was carried out on pre-coated plates (DC-Alufolien 60 F_{254} of E. Merck) and spots were detected at 254 and 366 nm, and also by using ceric sulphate spraying reagent. Purified compound **1** (11.25 mg, 1.5×10^{-4}% yield) was isolated as colorless prismatic crystals, mp 202–205°C (decomp.). $[\alpha]_D^{25}=-9.1$ (*c* 0.11, $CHCl_3$). UV λ_{max} (MeOH) (log ε) nm: 202 (3.22), 193 (4.14). IR data ν_{max} ($CDCl_3$) cm^{-1}: 3316 (OH), 2918 and 2849 (CH), and 1063 (C–O). ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.94 (3H, s, C-28), 0.97 (6H, s, C-29 and C-30), 1.07 (3H, s, C-26), 1.08 (3H, s, C-25), 1.14 (3H, s, C-27), 1.26 (3H, s, C-23), 1.29 (3H, s, C-24)., 3.85 (1H, ddd, $J_1=11.9$ Hz, $J_2=7.6$ Hz, $J_3=4.8$ Hz, H_{ax}-3), 3.67 (1H, ddd, $J_1=11.8$ Hz, $J_2=7.9$ Hz, $J_3=6.4$ Hz, H_{eq}-3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 16.8 (C-1), 18.3 (C-6), 18.5 (C-26), 19.2 (C-25), 19.4 (C-27), 21.1 (C-7), 24.6 (C-23), 25.7 (C-2), 26.5 (C-24), 35.1 (C-19), 35.9 (C-16), 33.1 (C-11), 38.7 (C-14), 32.1(C-28), 32.3 (C-30), 45.9 (C-10), 34.5 (C-29), 43.2 (C-18), 31.8 (C-15), 36.9 (C-21), 37.8 (C-9), 30.4 (C-12), 39.2 (C-13), 38.9 (C-22), 30.0 (C-17), 28.2 (C-20), 47.4 (C-8), 58.8 (C-3), 78.5 (C-4), 79.9 (C-5). HREI MS *m/z*: 444.3123, $C_{30}H_{52}O_2$ (calcd 444.3129). EI MS *m/z* (rel. int.%): 429 $[M-CH_3]^+$, 385 $[M-C_3H_7O]^+$, 205 $[C_{15}H_{25}]^+$, 111 $[C_7H_{11}O]^+$ and 95 $[C_7H_{11}]^+$. CI MS *m/z* (rel. int.%): 445 $[M+1]^+$, 427 $[M-OH]^+$, 385 $[M-C_3H_7O]^+$ and 204 $[M-C_{15}H_{24}]^+$.
8. Crystal data for **1**: Compound **1** was recrystallized from petroleum ether and $CHCl_3$, as colorless prismatic crystals. A crystal with dimensions $0.18\times0.15\times0.15$ mm^3 was selected for the crystallographic measurements. Mol. formula $=C_{30}H_{52}O_2$, molecular mass $=444.72$ amu, crystal system = orthorhombic, space group $=P2_12_12_1$, unit cell dimensions, $a=7.86450(10)$, $b=8.22300(10)$, $c=39.5500(6)$ Å, volume $=2557.69(6)$ $Å^3$, $D_{calcd}=1.155$ mg/m^3, $F(000)=992$; (Mo K) $=0.71069$ Å. Unit cell dimensions were determined by least squares fit of 3,765 reflections measured at 173°(2) K using Mo Kα radiation on a Nonius kappa CCD diffractometer. The intensity data within (θ) range of 3.6–30.0° were collected[22] at 173°(2) K. A total of 7,023 reflections were collected, of which 5,251 reflections were judged observed on the basis of $I>2\sigma(I)$. The structure was solved by direct methods[23] and expanded using Fourier techniques.[24] The structure was refined by the full matrix least-square calculations on F^2 with the aid of program SHELXL-97.[25] The final *R* and *Rw* factors were 0.051 and 0.113, respectively. Full details have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 186974.
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16. PEP inhibition activity for compound **1**: Prolyl endopeptidase (*Flavobacterium meningosepticum* origin) was purchased from Seikagaku Corporation (Tokyo, Japan) and *N*-benzyloxycarbonyl-Gly-Pro-*p*NA was obtained from Bachem Fine Chemicals Co. A specific inhibitor of PEP, *N*-benzyloxycarbonyl-pro-prolinal, was kindly donated by Dr. Hideaki Shimizu, Yakult Central Institute for Microbiological Research, Tokyo, Japan. The PEP inhibition activity was assayed by a modification of the method of Yoshimoto et al.[26] 100 mM Tris(hydroxymethyl)-aminomethane–HCl buffer containing 1 mM EDTA, pH 7.0, 247 μL, PEP (0.02 unit/300 μL) 15 μL and test sample in 8 μL MeOH, were mixed in a 96-well microplate and preincubated for 10 minutes at 30°C. The reaction was initiated by adding 30 μL of a 0.2 mM solution of *N*-benzyloxycarbonyl-Gly-Pro-*p*NA (in 40% 1,4-dioxane) as the substrate. The amount of released *p*-nitroaniline was determined spectrophotometrically, as an increase in absorption at 410 nm, with a 96-well microplate reader (Molecular Devices, Spectramax 340 USA). The IC_{50} values were the average of at least three determinations performed in triplicate.
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