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A Novel Triterpene from the Roots of *Paullinia pinnata*: 6α-(3'-methoxy-4'-hydroxybenzoyl)-lup-20(29)-ene-3-one

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Paullinia pinnata L. (Sapindaceae) is an endemic West African plant that is extensively used in traditional medicine to treat various diseases. Previous phytochemical analysis by various groups led to the isolation of several novel lupene-based triterpenene derivatives along with other classes of compounds. As part of our continued phytochemical studies on the roots of this plant, we have now identified yet another novel triterpene, 6α-(3'-methoxy-4'-hydroxybenzoyl)-lup-20(29)-ene-3-one. The identification of the compound through comprehensive spectroscopic studies is discussed.

Keywords: *Paullinia pinnata*, Sapindaceae, Novel triterpene, Lupine.

Paullinia pinnata L. (Sapindaceae) is a woody vine plant endemic to tropical West Africa. The root preparations of the plant are used to treat dysentery, malaria and other infections; and as an aphrodisiac [1-3b]. In support of the traditional use of the plant as a wound healing agent, the methanol extract of *P. pinnata* roots demonstrated a strong stimulatory effect on the proliferation of a key cellular component of wound healing, skin fibroblasts [3c]. The use of the plant for treating infections was also validated by the work of Annan *et al.* [3d] that reported the antimicrobial and antibacterial-resistant modifying activity of the root extract. As part of studies on the search for active principles responsible for the traditional uses of the plant, Lasisi *et al.* [4] isolated two known (**1, 2**) and a novel 3-*O*-isovaniolloyl lupene (**3**) derivatives (Figure 1). Furthermore, the novel compound **3** was shown to have good antibacterial effect when tested against a range of Gram-positive and Gram-negative bacterial test species [4]. Another study by Annan *et al.* [5a] also examined the root extract of the plant and identified two novel 6-*O*-β-3-methoxy-4-hydroxy-benzoyl derivatives of lupene (**4**, **5**, Figure 1) that displayed significant fibroblast proliferative activities. In the present study, we re-examined the root extract of the plant and have now identified a new compound, a 6-*O*-α-3-methoxy-4-hydroxybenzoyl lupene derivative (**6**, Figure 1).

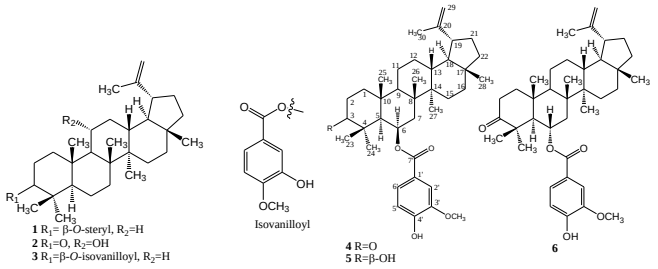

Figure 1: Lupene derivatives from the roots of *P. pinnata*.

Accurate mass analysis showed the $[M + H]^+$ ion, $C_{38}H_{54}O_5$ plus H, at m/z 591.4040 (expected/theoretical 591.4044); $[M + NH_4]^+$, $C_{38}H_{54}O_5$ plus NH_4 at m/z, 608.4306 (theoretical, 608.4310); $[M + Na]^+$ $C_{38}H_{54}O_5$ plus Na at 613.3856 (theoretical, 613.3863). The ^{13}C and DEPT-135 NMR spectra showed signals indicating the presence of a 30-carbon triterpene skeleton (Table 1). These data together with the ^1H NMR spectrum were in good agreement with the assignment of the lupane triterpene skeleton which was previously identified from the plant (**1-5**). In addition to the triterpene skeleton, the ^{13}C NMR data showed signals for seven deshielded (> δ 120) carbons (one carbonyl, 3 quaternary and 3 methine groups) and one methoxyl group (**Table 1**). The ^1H NMR signals of this structural moiety clearly indicate the 1,3,4- signals of a trisubstituted aromatic ring system which, together with the methoxyl (δ 3.94) and hydroxyl (δ 6.01) functional groups, suggest either a vanilloyl or a isovanilloyl structure. Interestingly, the lupene esters of both of these C6-C1 aromatic units (**3**-**5**) were previously isolated from the roots of *P. pinnata* [4,5a]. The downfield signal of the oxomethine together with the appearance of the free aromatic hydroxyl group in the ^1H NMR spectrum (Table 1) suggest esterification *via* the C-7' carbonyl center of the vanillate/isovanillate moiety. The placement of the oxomethine group at C-6 instead of other sites such as the previously reported C-11 position (**2**) was based on the ^2J and ^3J HMBC correlation study that revealed all the expected connectivities for **6** (Table 1). The assignment of the carbonyl functional group at C-3, the C20-C29 double bond and confirmation of the ester unit as vanilloyl were also substantiated by the HMBC data (Table 1), as reported for the similar lupene derivative, **4** [5a]. If one closely studies the ^1H NMR spectrum, however, the coupling constant for H-5 was 11.4 Hz, which was much higher than that expected for a β-oriented *O*-derivative (7.4 Hz, [5a]). This suggests that the vannilloyl attachment at **6** is α-oriented. Further evidence for the C-6 stereochemistry assignment came from the NOESY studies which unequivocally confirm the proposed structure as **6**. As H-6 in this proposed structure is β-oriented, it showed NOESY correlation with β-oriented methyls (H_3-23, H_3-25, H_3-26) and protons (H-7β). These observations were just the opposite to what was shown in the NOESY studies of the **6** epimer, **4** [5a]. The H-5α proton similarly

Table 1: ^{1}H (400 MHz), ^{13}C (100 MHz) and HMBC NMR assignments of compound **6**.

C	Type	δ_C	δ_H	Major ^{2}J and ^{3}J HMBC Correlations
1	CH_2	39.8	1.72 (H-1α) m, 1.91 (H-1β) m	C2,
2	CH_2	33.0	2.72 (H-2α) ddd (15.5, 12.0, 6.8) 2.30 (H-2β) ddd (15.2, 9.9, 3.6)	C1, C3
3	C	218.4	-	
4	C	46.7	-	
5	CH	55.9	2.13 d (11.4)	C4, C6, C10, C23, C24, C25
6	CH	72.4	5.35 dt (11.1, 4.0)	
7	CH_2	39.9	1.54 (H-7α), 1.88 (H-7β) m	C6, C8, C26
8	C	41.5	-	
9	CH	49.0	1.52 m	C7, C8
10	C	38.5	-	
11	CH_2	21.9	1.45, 1.48 m	
12	CH_2	25.1	1.10, 1.75 m	
13	CH	37.8	1.69 m	
14	C	43.0	-	
15	CH_2	27.5	1.00, 1.70 m	
16	CH_2	35.3	1.46, 1.32 m	
17	C	43.0	-	
18	CH	48.2	1.39 m	C12, C13, C14/C17, C16, C20, C28
19	CH	47.9	2.39 dt (11.3, 5.9)	C18, C20
20	C	150.8	-	
21	CH_2	29.8	1.41 m	C17, C19, C20, C22
22	CH_2	39.5	1.68 m	
23	CH_3	19.7	1.09 s	C3, C4, C5, C24
24	CH_3	31.3	1.33 s	C3, C4, C5, C23
25	CH_3	17.6	0.85 s	C1, C5, C10
26	CH_3	16.1	1.22 s	C9
27	CH_3	14.5	1.00 s	C15
28	CH_3	18.0	0.79 s	C16, C19
29	CH_2	109.5	4.69 (H-29a) br d (2.1), 4.58 (H-29b) br s	C19, C30
30	CH_3	19.3	1.69	C19, C20, C29
1'	C	122.8	-	
2'	CH	111.8	7.53 d (1.9)	C4', C6', C7'
3'	C	146.3	-	
4'	C	150.1	-	
5'	CH	114.1	6.95 d (8.4)	C3', C4', C6'
6'	CH	124.0	7.62 dd (8.4, 1.9)	C2', C4', C7'
7'	C	165.5	-	
	OCH_3	56.1	3.94 s	C3'
	OH	-	6.01	C3', C4', C5'

*Assignment was based on COSY, HSQC, HMBC and NOESY data, but coupling constants could not be accurately calculated due to overlapping signals.

showed NOESY interaction with α-oriented H-1 and H-7 protons. The NOESY correlation between the methoxyl group and H-2' also confirms the assignment of the ester subunit as vanilloyl. Finally, the optical activity of **6** ($[\alpha]_D$ +119) was found to be lower than that reported for its epimer, **4** (+152.2, [5a]). On the basis of all these data, the compound was identified as a novel lupene derivative 6α-(3'-methoxy-4'-hydroxybenzoyl)-lup-20(29)-ene-3-one (**6**).

In addition to the lupene derivatives, previous studies on *P. pinnata* have indicated the presence of alkaloid [5b], triterpene saponins [5c], flavone glycoside [5d], triterpenes and steroids [5e]. The leaves of the plant have also yielded 5α-portiferastane-3β,6α-diol and 2-(4-hydroxyl-3,5-dimethoxyl phenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-trioxaphenanthrene-6-one [6a], flavone glycosides [6b], ceramides, β-sitosterol and β-amyrin [5e]. A number of other pharmacological investigations have also been reported on the plant [6b].

Experimental

General: NMR, JEOL 500 MHz instrument; Optical rotations, CETI WZZ-2S Automatic Polarimeter; IR, Bruker ALPHA FT-IR; UV, and T90+ UV/VIS spectrometer; HRMS, Thermofisher LTQ Orbitrap XL spectrometer.

Plant material: The roots of *P. pinnata* were collected from Ejura, Ashanti region, Ghana in September 2013. A voucher specimen (KNUST/HM/2013/S025) has been deposited at the Department of Herbal Medicine Herbarium.

Extraction and isolation: The air-dried roots (800 g) were powdered and Soxhlet-extracted with 1.5 L of $CHCl_3$. The extract was evaporated under reduced pressure to yield 6.4 g of crude extract. A portion of the $CHCl_3$ extract (6.00 g) was subjected to CC on SiO_2 eluting with light petroleum and gradually increasing the polarity of the solvent by 10% increments of EtOAc. TLC profiles of the aliquots (1 L) allowed the bulking of 5 fractions; F1 (1.22 g), F2 (0.87 g), F3 (0.74 g), F4 (1.48 g) and F5 (0.75 g). Fraction F3, which was collected from the light petroleum: EtOAc (60:40) fraction, was further subjected to repetitive CC on silica gel eluting with light petroleum with gradual increments (1.0%) of polarity using EtOAc to afford compound **6** **[**Rf: 0.7 (Light petroleum - EtOAc, 60:40)] (18.0 mg).

6α-(3'-Methoxy-4'-hydroxybenzoyl)-lup-20(29)-ene-3-one (**6**)
MP: 267-270°C
$[\alpha]_D$: +119 (*c* 0.1, $CHCl_3$).
IR (KBr): 3533 and 1702 cm^{-1}.
UV/Vis λ_{max} (MeOH) nm (log ε): 293 (1.795), 265 (2.025).
^{1}H and ^{13}C NMR ($CDCl_3$): Table 1.
ESI-MS *m/z*: [M + H^+] calcd for $C_{38}H_{54}O_5$ plus H,: 591.4044; found: 591.4040.

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References

[1] Abbiw D. (**1990**) *Useful plants of Ghana*. Intermediate Technology Publication Ltd, London.
[2] Irvine ER. (**1991**) In *Woody plants of Ghana with special reference to their use*, vol. 446. Oxford University Press, London, pp. 547–549.
[3] (a) Zanble AMS, Carpentier M, Kandoussi A, Schpaz, S, Petrault O, Ouk T, Hennuyer N, Fruchert JC, Staeles B, Bodret R, Duriez P, Baillent FJ, (**2006**) *P. pinnata* extracts rich in polyphenols, promote vascular relaxation via endothelium-dependent mechanism. *Cardiovascular Pharmacolology*, ***47***, 559–608; (b) Jimoh FO, Sofidiya MO, Afolayan A. (**2007**) Antioxidant properties of the methanol extracts from the leaves of *Paullinia pinnata*. *Journal of Medicinal Food*, ***15***, 215–219; (c) Annan K, Houghton PJ. (**2008**) Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of *Ficus asperifoli*a Miq. and *Gossypium arboreum* L., wound-healing plants of Ghana. *Journal of Ethnopharmacology*, ***119***, 141–144; (d) Annan K, Houghton PJ, Hylands PJ, Gibbons S. (**2005**) Antibacterial and resistant modifying activity of *Paullinia pinnata*. *Journal of Pharmacy and Pharmacology*, ***57***, S74 –S74.
[4] Lasisi AA, Ayinde BW, Adeleye AO, Onocha PA, Oladosu IA, Idowu PA. (**2014**) New triterpene isovanniloyl and antibacterial activity of constituents from the roots of *Paullinia pinnata* Linn (Sapindaceae). *Journal of Saudi Chemical Society*, *In Press.*
[5] (a) Annan K, Houghton PJ. (**2010**) Two novel lupane triterpenoids from *Paullinia pinnata* L. with fibroblast stimulatory activity. *Journal of Pharmacy and Pharmacology*, ***62***, 663-668; (b) Martins S. (**1877**) An alkaloid timbonine from *Paullinia pinnata*. *The Pharmaceutical Journal & Transactions*, ***VII***, 1020–1023; (c) Bowden K. (**1962**) Triterpene saponins from *P. pinnata*. *Journal of Pharmacology*, ***18***, 173–174; (d) Abouraseed EA, Toyang NT, Choinski J. Khan IA. (**1999**) Two new flavone glycosides from *Paullinia pinnata*. *Journal of Natural Products*, **62**, 1179–1181; (e) Miemanang RS, Krohn K, Hussain H, Dongo E. (**2006**) Paullinoside A and paullinomide A: a new cerebroside and a new ceremide from leaves of *Paullinia pinnata*. *Zeitscrift fuer Naturforschung*, ***61b***: 1123–1127.
[6] (a) Dongo E, Hussain H, Miemanang RS, Tazoo D, Schulz B, Krohn K. (**2009**) Chemical constituents of *Klainedoxa gabonensis* and *Paullinia pinnata*. *Records of Natural Products*, ***3***, 165–169; (b) Annanil K, Hudson JB, Dasouzal C, Akpaguanal K, Tower GHN, Ananson J, Gbeassori M. (**2000**) Investigating medicinal plants of Togo for antiviral and antimicrobial activities. *Pharmaceutical Biology*, ***38***, 40-45.