Lanneanol: A new cytotoxic dihydroalkylcyclohexenol and phenolic compounds from *Lannea nigritana* (Sc. Ell.) Keay

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1. Subject and source

The genus *Lannea* belongs to the family Anacardiaceae and consists of 40 species. *Lannea nigritana* (Sc. Elliot) Keay is a small tree of 3–6 m of height found in the tropical rain forest (Berhaut, 1971; Letouzey, 1972). It is used in the traditional medicine for the treatment of various infectious diseases (Berhaut, 1971; Burkill, 1985). The stem bark of this plant was collected in *Makenene*, Centre Province of Cameroon in December 2003. The identification was done by Mr. Nana from the National Herbarium of Yaounde where a voucher specimen (N° 40408/HNC) documenting the collection is deposited.

2. Previous work

The chemical investigation of the genus *Lannea* has led to the isolation of various secondary metabolites, including alkylphenols, alkylhexenones, flavonoids, tannins, polysaccharides and several benzoic acid derivatives (Groweiss et al., 1997; Islam and Tahara, 2000; Ramachandran and Bhuwan, 1968; Sulochana et al., 1967; Sultana and Ilyas, 1986a,b; Queiroz et al., 2003). Cytotoxicity of some alkylated hydroquinones from the genus *Lannea* was reported (Groweiss et al., 1997). The extract from the stem bark of *Lannea coromandelica* showed significant zoosporicidal activity (Islam et al., 2002) and the bioassay-guided fractionation and chemical characterization of this extract revealed that the active constituents were angular type polyflavonoid tannins (Islam and Tahara, 2000). The different parts of *Lannea velutina* were evaluated for their antifungal, larvicidal, molluscicidal, antioxidant and radical scavenging properties (Diallo et al., 2001). The antibacterial activity of the roots of *Lannea acida* was also evaluated

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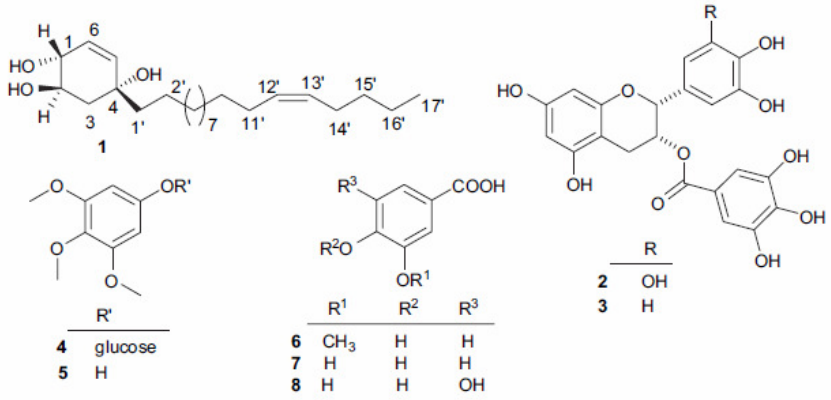
(Kone et al., 2004). Several other species from the genus *Lannea* have been studied. However, to the best of our knowledge no phytochemical and pharmacological studies were done on *L. nigritana* (Sc. Ell.) Keay.

3. Present study

The air-dried and powdered stem bark of *L. nigritana* (5 kg) was soaked in methanol for 48 h, at room temperature. The methanolic extract was concentrated under reduced pressure to give a brown residue (206 g), which was re-extracted with EtOAc. Removal of the solvent on the EtOAc extract gave 53 g of residue which was subjected to vacuum column chromatography (VCC) over silica gel (60 GF_{254} Merck) and eluted with *n*-hexane, *n*-hexane/EtOAc, EtOAc, EtOAc/MeOH in order of increasing polarity. Fractions were monitored by TLC and ^{1}H NMR. Similar fractions were combined. Fractions 1–5 (5.5 g) contained mainly a mixture of hydrocarbons and were not investigated. Fractions 6–15 (10.0 g) obtained from the elution with *n*-hexane/EtOAc (90/10) were rechromatographed over silica gel using *n*-hexane/EtOAc (95/5) as eluant to yield compound **5** (He et al., 2005) (3.0 mg) and **6** (Hiroyuki et al., 2006) (40 mg). Fractions 16–25 (8 g) obtained with *n*-hexane/EtOAc (75/25) were subjected to column chromatography and the fractions obtained with *n*-hexane/EtOAc (70/30) were passed through Sephadex LH-20 column to yield **1** (10.0 mg) and **7** (Werner et al., 2004) (22.3 mg). Fractions 26–40 (20.0 g) obtained with *n*-hexane/EtOAc (50/50) were combined and diluted in acetone and after 1 day gave a precipitate. After filtration, compound **4** (Fang et al., 1993; Srivastava and Kulshreshtha, 1989) (35.5 mg) was obtained. The mother liquor after column chromatography over silica gel, gave compound **8** (Werner et al., 2004) (4.1 mg) as white needles in *n*-hexane/EtOAc (70/30) The fractions obtained with *n*-hexane/EtOAc (50/50) was passed through Sephadex LH-20, then purified by PTLC to give compounds **2** (15.2 mg) and **3** (Coxon et al., 1972; Tanaka et al., 1984) (10.7 mg).

The structures of the isolated compounds (**1**–**8**) were established by spectroscopic and spectrometric methods including NMR and mass techniques, and confirmed by comparison with the literature data.



Lanneanol (**1**) was obtained as pale yellow oil, $[\alpha]_D^{25} + 3.7$ (c 0.80, $CHCl_3$). Its molecular formula was determined to be $C_{23}H_{42}O_3$ on the basis of the HRESIMS which showed a pseudomolecular ion peak at *m/z* 389.30245 (calcd for $C_{23}H_{42}O_3Na$, 389.30315). The IR spectrum showed absorption bands at 3368, 3075, 3046 cm^{-1} suggesting the presence of three hydroxyl groups in the structure of **1**. The ^{1}H and ^{13}C NMR spectra (Table 1) showed signals characteristic of two oxymethine groups at δ_H 4.00/δ_C 75.2 and 4.38/δ_C 70.9 and a signal of a quaternary oxygenated carbon at δ_C 66.2. Four olefinic protons can also be deduced in the ^{1}H NMR spectrum by signals at δ_H 5.33 (3H) and 5.74 (1H). In the ^{13}C NMR, signals of the corresponding carbons were observed at δ_C 130.8 and 133.3, respectively. Furthermore, the ^{13}C NMR spectrum showed signal of a methylene group at δ_C 41.5. In the ^{1}H–^{1}H COSY experiment (Fig. 1), correlations between the olefinic proton (δ_H 5.74) and the proton signal at δ_H 4.00 (oxymethine proton) suggest an α,β-unsaturated oxygenated carbon. Further correlations were observed between the proton signal at δ_H 4.38 (oxymethine proton) and the proton signals at δ_H 4.00 (oxymethine proton), 2.15 and 1.42 (methylene protons). The HMBC spectrum (Table 1) exhibited ^2J and ^3J correlations between the methylene proton signals (δ_H 2.15

Table 1
^{1}H and ^{13}C NMR spectral data (δ and J values) and important HMBC correlations of compound **1** in MeOD

Position	δ_H	δ_C	HMBC
1	4.00 (m)	75.2	C-5, C-6
2	4.38 (m)	70.9	–
3	1.42 (dd, J = 5.5,13,1)	41.5	C-4, C-1′
	2.15 (dd, J = 1.5, 13.1)	–	C-1, C-2, C-4, C-1′
4	–	66.2	–
5	5.53 (m)	130.8	C-1, C-4, C-6
6	5.74 (dd, J = 2.1, 10.3)	133.3	C-2, C-3, C-5
1′	1.60 (m)	40.4	–
2′–10′	1.29–1.33 (br s)	24.1–31.5	–
11′	2.03 (m)	28.1	C-12′, C-13′
12′, 13′	5.33 (m)	130.8	C-11′, C15′
14′	2.03 (m)	28.1	C-12′, C-13′
15′	1.30 (m)	32.9	–
16′	1.30 (m)	23.7	C-17′
17′	0.90 (t, J = 7.0)	14.5	C-15′, C16′

and 1.42), the sp^3 oxygenated carbon signal (δ_C 66.2) and the sp^2 carbon signal (δ_C 130.8). The molecular formula ($C_{23}H_{42}O_3$) of **1** indicated three double bond equivalents. Considering the substraction of the two double bonds, one ring must exist in the structure of **1**. These observations were conclusive for the deduction of the partial structure of compound **1** as 1,2,4-trihydroxycyclohex-5-ene. Further analysis of the ^{1}H NMR spectrum of lanneanol (**1**) indicated a very strong signal at δ_H 1.29 and a triplet of three protons corresponding to the methyl group at δ_H 0.90 suggesting a long alkyl chain in its structure. The ^{13}C NMR spectrum showed a set of signals between δ_C 24.6 and 31.5 confirming the presence of a long side chain in the molecule. The electron ionization mass spectrum (EIMS) of lanneanol revealed the important peaks (Fig. 1) at 366 $[M]^+$, 236 $[M - CH_2(CHOH)_2(CH)_2COH - H]^+$, 111 $[M^+ - CH_3\ (CH_2)_{14}(CH)_2 - H_2O]^+$ which was evident for the deduction of the length ($C_{17}H_{33}$) of the long chain (Eyong et al., 2005; Konga et al., 2001; Mukhtar et al., 2002; Pettit et al., 2005; Raith and Neubert, 1998). This also confirmed the presence of a double bond in the alkyl chain. Furthermore, the characteristic ions (Fig. 1) at *m/z* 53 $[C_4H_7]^+$, 83 $[CH_3(CH_2)_3(CH)_2]^+$, 265 $[M - H_2O - 83]^+$ were important for the suggestion of the double bond at C-12′ and C-13′ (Eyong et al., 2005; Konga et al., 2001; Mukhtar et al., 2002). Moreover, the typical fragment ion at *m/z* 288, formed by elimination of propene from the ion at *m/z* 330 $[M - 2H_2O]$ through McLafferty rearrangement (Konga et al., 2001; Miemanang et al., 2006; Pettit et al., 2005; Raith and Neubert, 1998), further confirmed the position of the double bond in the long chain. Thus, the structure of lanneanol was determined as 4-(heptadec-12-enyl)cyclohex-5-ene-1,2,4-triol). The proposed structure was supported by HMBC experiment (Table 1), mass spectrum and by comparison of the NMR data with those for the related compounds published in the literature (Groweiss et al., 1997; Queiroz et al., 2003). The Z geometry of the double bond in the alkyl side chain was established on the basis of the chemical shift at δ_C 28.1 for the allylic carbons C-11′ and C-14′ (Deng et al., 1999; Horgen et al., 1997) and the relative configuration of C-1, C-2 and C-4 was assigned by the NOESY experiment (Fig. 1). Correlations were

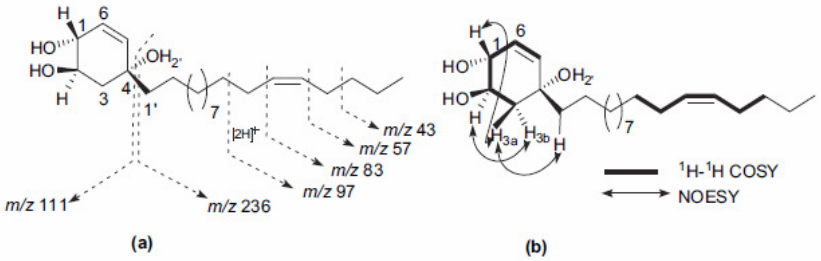


Fig. 1. (a) Mass fragmentation pattern and (b) important correlations observed in the ^{1}H–^{1}H COSY and NOESY spectrum of **1**.

observed between H-3a (δ_H 2.15), H-1 (δ_H 4.00) and H-1′ (δ_H 1.60) and between H-3b (δ_H 1.42) and H-2 (δ_H 4.38). However, the absolute configuration of these three chiral centers (C-1, C-2 and C-4) could not yet be established.

Cytotoxic activity of compound **1** was evaluated against a panel of tumor cell lines using the monolayer assay method ($n = 38$) (Fiebig et al., 1999). The activity was observed in the test range from 0.001 μg/ml to 10.0 μg/ml. Concentration dependency was very steep between 1.0 μg/ml and 10.0 μg/ml in the majority of cell lines tested. Regarding inhibition of growth by more than 70% (T/C < 30%), no activity was observed up to 1.0 μg/ml, and 24 out of 38 (63%) cell lines were inhibited at 10.0 μg/ml. Cell lines (29/38) were inhibited in growth by more than 50%. Antitumor potency was moderately pronounced with mean $IC_{50} = 5.091$ μg/ml and mean $IC_{70} = 8.117$ μg/ml.

The panel of tumor cell lines used was: BXF Bladder, CEXF Cervix, CXF Colorectal, GXF Gastric, LXF Lung A adeno, MAXF Breast, MEXF Melanoma Xenograft, OVXF Ovarian Cancer Xenograft, PRXF Prostate, PXF Pleuramesothelioma, RXF Renal, UXF Uterus Body, XF Miscellaneous Cancer Xenograft.

4. Chemotaxonomic significance

Alkylhexenones, tannins, and some phenolic compounds are widely distributed in the Anacardiaceae family. The alkylhexenones appear to be biogenetic precursors of the alkyl phenols that are also quite common in the family Anacardiaceae. The alkylhexenones have been reported in *Lannea welwitschii* and in *Tapirira*, a genus from the family Anacardiaceae (Groweiss et al., 1997; David et al., 1998; Correia et al., 2001; Queiroz et al., 2003). Several alkyl phenols were isolated from *Mangifera*, *Melanorrhoea*, *Panopsis*, other genus of Anacardiaceae (Deng et al., 1999; Du et al., 1986; Kubo et al., 1994; Masuda et al., 2002; Spencer et al., 1980) and the occurrence of lanneanol from *L. nigritana* is not then surprisingly. According to the already classical definition of Freudenberg (1920), Anacardiaceae is a family of plant tannins. The tannin **3** and the two acids **7** and **8**, have been already reported in the genus *Mangifera* and *Rhus* from the Anacardiaceae family (Tanaka et al., 1984; Werner et al., 2004) and their presence in *L. nigritana* together with compound **2** confirms that the family Anacardiaceae is a rich source of tannins. To our knowledge, this is the first report of compounds **2**, **4**, **5** and **6** in the family Anacardiaceae. However, compound **2** was reported together with **3** in the green tea (*Camellia sinensis*) leaf from the family Theaceae (Coxon et al., 1972), compounds **5** and **6** in the genus *Naucleas* and *Citrus* from the family Rubiaceae (He et al., 2005; Hiroyuki et al., 2006) and compound **4** in the genus *Cotoneaster* and *Rosa* from the family Rosaceae (Fang et al., 1993; Srivastava and Kulshreshtha, 1989). These three families are also known to be the rich source of tannins and phenolic compounds and the isolation of the four compounds (**2**, **4**, **5**, **6**) in the family Anacardiaceae is particularly interesting since this strengthens the chemotaxonomic relationship of these four families.

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