

New Dihydroalkylhexenones from *Lannea edulis*

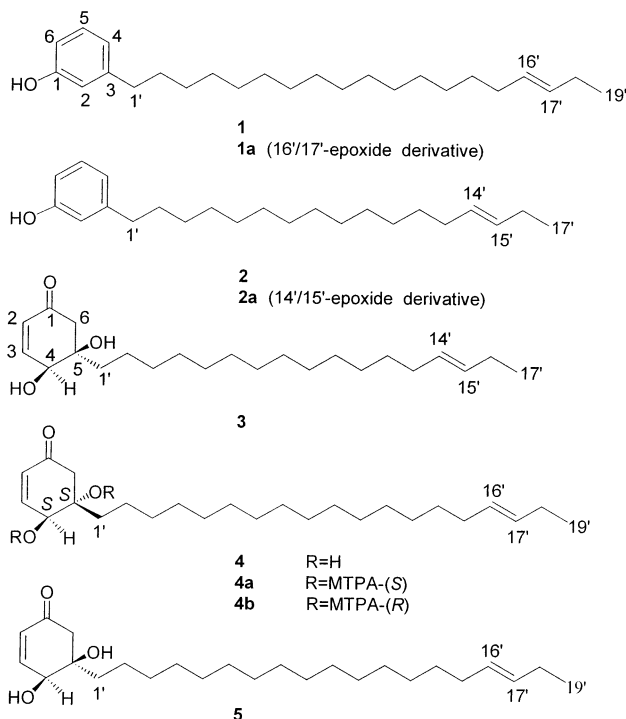
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Activity-guided isolation of radical-scavenging compounds from the dichloromethane extract of the root bark of *Lannea edulis* led to isolation of two known bioactive alkylphenols [cardonol 7 (**1**) and cardonol 13 (**2**)], and three new dihydroalkylhexenones were also isolated (**3–5**). Their structures were elucidated by spectroscopic and chemical methods. The absolute configuration of **4** was determined by the Mosher ester method.

In our ongoing search for new bioactive compounds from plants used in the traditional medicine of Zimbabwe,¹ the root bark of *Lannea edulis* Engl. (Anacardiaceae)² was investigated. This plant is used to treat various infectious diseases.¹ In a preliminary biological screening, the methanol and dichloromethane extracts of the root bark were tested for their antifungal and antibacterial activities, but no evidence of activity could be attributed to these extracts. Besides these assays, the same extracts were also tested in routine screening for radical-scavenging activity in a DPPH assay.³ The dichloromethane extract of *L. edulis* was active in this assay, and activity-guided isolation of this extract led to the purification of two non-isoprenoid long-chain phenols (**1** and **2**) and three new 5-alkyl-4,5-dihydroxy-2-cyclohexen-1-ones (**3–5**).



Compounds **1** and **2** were identified as 3-[14'-nonadecenyl]phenol (cardonol 7) and 3-[16'-heptadecenyl]phenol

(cardonol 13), respectively, by comparison of their data with those of published values.⁴

The ¹H and ¹³C NMR spectral data (Tables 1 and 2) of compound **3** showed signals characteristic of an α,β -unsaturated ketone⁵ (carbonyl at δ_C 201.9, two coupled olefinic protons at δ_H 6.90 and 6.05), a secondary hydroxyl group (δ_H 4.59, coupled in the HSQC experiment to the carbon at δ 66.1), and a tertiary alcohol (δ_C 74.4). Besides these functional groups, there was a methylene carbon at δ_C 44.5 coupled in the HSQC experiment with two protons at δ_H 1.96 and 2.65. The ¹H–¹H COSY experiment showed correlations between the olefinic proton at δ_H 6.90 and 4.59, suggesting the presence of a hydroxyl group at position C-4. The ¹³C NMR signals observed between δ_H 29.1–29.7 indicated the presence of a long side chain. The ¹³C NMR signal at δ_C 37.1 was assigned to C-1'. The HRESIMS of compound **3** showed a pseudomolecular ion at m/z 387.28679 (calcd for C₂₃H₄₀O₃Na, 387.28696), in agreement with an α,β -unsaturated dihydroxy ketone substituted with a 17-carbon alkyl chain. The ¹³C NMR spectrum (Table 2) showed two signals at δ_C 129.3 and 131.8, similar to those observed in compounds **1** and **2**, characteristic of the presence of a double bond in the alkyl chain. The correlations observed in the HMBC spectrum between the terminal methyl at δ_H 0.96 (CH₃-17') and the carbon at δ_C 131.8 (C-15') suggested the presence of a double bond between C-14'/C-15'. The relative configuration was established using NOESY experiments (Figure 1). The correlation between H-4 (δ_H 4.59) and one of the H-6 methylene protons at (δ_H 2.65) suggested the pseudoaxial disposition of H-4. Another correlation was observed between H-4 and one of the C-1' methylene protons at δ_H 1.40–1.80, indicating *cis*-oriented hydroxyl groups.⁵ Because of the small available amount of **3**, the absolute configuration could not be assigned. Compound **3** was identified as 5-[14-heptadecenyl]-4,5-dihydroxy-2-cyclohexenone.

Compound **4** showed signals in the ¹H and ¹³C NMR spectra (Tables 1 and 2) similar to those observed for compound **3**, indicating the presence of an α,β -unsaturated dihydroxyketone substituted by a long alkyl chain. The HRESIMS showed a pseudomolecular ion peak at m/z 451.31850 (calcd for C₂₅H₄₄O₃Na, 415.31826) and was in agreement with an α,β -unsaturated dihydroxyketone substituted by a 19-carbon alkyl chain. The ¹³C NMR spectrum showed two signals at δ_C 129.1 and 131.5 characteristic of the presence of a double bond in the alkyl chain. The correlations between the methyl at δ_H 0.95 (CH₃-19') and the carbon signal at δ_C 131.5 in the HMBC spectrum suggested the presence of a double bond between C-16'/C-

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Table 1. ^1H NMR (δ values CDCl_3 , 500 MHz) Data of Compounds **3–5** and the Mosher Ester Derivatives **4a** and **4b**

position	3	4	5	4a	4b
2	6.05, dd ^{a,b}	6.03, dd ^{a,b}	6.02, dd ^{a,b}	6.14, dd ^{a,b}	6.17, dd ^{a,b}
3	6.90, dd ^{a,b}	6.92, dd ^{a,b}	6.92, dd ^{a,b}	6.69, dd ^{a,b}	6.79, dd ^{a,b}
4	4.59, m	4.60, m	4.58, m	5.88, m	5.81, m
6	1.96/2.65, m	2.0/2.30, m	1.94/2.60, m	2.96/3.10, m	2.44/2.67, m
1'	1.40–1.80, m	1.40–1.79, m	1.41–1.79, m	1.93–2.0, m	1.93, m
2'–12'	1.25–1.30, m	1.25–1.30, m	1.25–1.30, m	1.25–1.30, m	1.25–1.30, m
13'	2.00, m	1.25–1.30, m	1.25–1.30, m	1.25–1.30, m	1.25–1.30, m
14'	5.40, m	1.25–1.30, m	1.25–1.30, m	1.25–1.30, m	1.25–1.30, m
15'	5.40, m	2.00, m	2.00, m	2.00, m	2.00, m
16'	2.00, m	5.40, m	5.40, m	5.40, m	5.40, m
17'	0.96, t ^c	5.40, m	5.40, m	5.40, m	5.40, m
18'		2.00, m	2.00, m	2.00, m	2.00, m
19'		0.95, t ^c	0.95, t ^c	0.95, t ^c	0.95, t ^c

^{a,b} $J = 1.95$, 10 Hz. ^c $J = 7.3$ Hz.

Table 2. ^{13}C NMR (δ values CDCl_3 , 125 MHz) Data of Compounds **3–5**

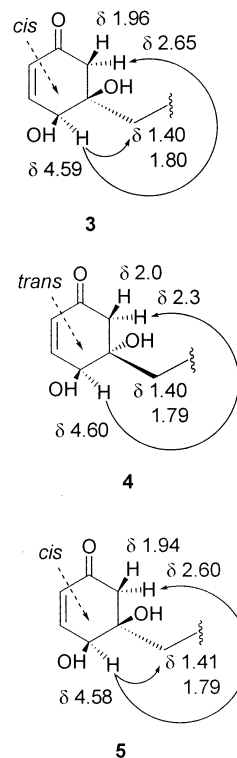
carbon	3	4	5
1	201.9	201.3	201.1
2	125.8	125.8	125.3
3	152.7	152.6	152.4
4	66.1	66.2	65.9
5	74.4	75.6	75.6
6	44.5	44.4	44.7
1'	37.1	37.1	37.5
2'–12'	29.1–29.7 ^a	29.1–29.8 ^b	29.1–29.8 ^b
13'	25.5	29.1–29.8 ^b	29.1–29.8 ^b
14'	129.3	29.1–29.8 ^b	29.1–29.8 ^b
15'	131.8	25.6	25.8
16'	32.5	129.1	129.5
17'	13.9	131.5	131.2
18'		32.6	32.4
19'		14.0	14.4

^a δ values 29.1, 29.4, 29.5, 29.6, 29.7. ^b δ values 29.1, 29.4, 29.5, 29.6, 29.7, 29.8.

17'. The relative configuration between C-4/C-5 was established by the NOE experiment (Figure 1). The correlation was observed between H-4 (δ_{H} 4.60) and one of the methylene protons at H-6 (δ_{H} 2.30), suggesting that the hydroxyl groups were *trans*-oriented in **4**.⁴ To determine the absolute configuration of the asymmetric centers C-4 and C-5, the di-(*S*)- and di-(*R*)-methoxytrifluoromethylphenylacetic acid (MTPA) esters (**4a** and **4b**) were prepared.⁶ However, the absolute stereochemistry of vicinal diols cannot be easily solved by a straightforward application of Mosher's method, partially because the $\Delta\delta_{\text{H}}$ ($\delta_{\text{H}} = \delta_{\text{S}} - \delta_{\text{R}}$) effects caused by the two vicinal MTPA groups may reinforce each other or cancel, and this could cause confusion. Despite this, Shi et al. demonstrated that the determination of the absolute configuration in the compounds with vicinal diols is feasible.⁷ Another important point consisted in the MTPA conformation: to invalidate Mosher's method, MTPA groups must be compelled to assume conformations that are different from the ideal one as originally proposed.⁶ The molecular model of **4** showed the two vicinal MTPA groups preserve their ideal conformation.

Finally, the $\Delta\delta_{\text{H}}$ ($\delta_{\text{H}} = \delta_{\text{S}} - \delta_{\text{R}}$) of **4a** and **4b** showed that the absolute configuration at C-4 is *S* (Table 1). As the relative configuration between C-4 and C-5 was determined as *trans*, the same *S* absolute configuration could be deduced for C-5.⁵ Compound **4** was identified as 5-[16'-nonadecenyl]-4*S*,5*S*-dihydroxy-2-cyclohexenone.

The ^1H and ^{13}C NMR data (Tables 1 and 2) of compound **5** were very similar to those observed for compound **4**. The HRESIMS data of **5** indicated a molecular formula of $\text{C}_{25}\text{H}_{44}\text{O}_3$, the same as that for **4**. The relative configuration of the cyclohexenone moiety was established by NOESY experiments (Figure 1). The correlations between H-4 (δ_{H}

**Figure 1.** Correlations observed in the NOESY spectrum of compounds **3–5**.

4.58) and one of the H-6 methylene protons (δ_{H} 2.60) suggested the pseudoaxial disposition of H-4. Another correlation was observed between H-4 and one of the C-1' methylene protons at δ_{C} 1.41–1.79, revealing *cis*-oriented hydroxyl groups.⁴ As for **3**, compound **5** was isolated in only a small amount, and therefore the absolute configuration has not been determined. Compound **5** was identified as 5-[16-nonadecenyl]-4,5-dihydroxy-2-cyclohexenone.

Radical-scavenging properties of compounds **1–5** were evaluated using the DPPH method, and quercetin and BHT [2,6-di(*tert*-butyl)-4-methylphenol] were used as reference compounds.⁸ When using DPPH as a TLC spray reagent, only compounds **1** and **2** (10 μg) were active and reduced the radical, while the same amounts of **3–5** were completely inactive.

Experimental Section

General Experimental Procedures. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter (CHCl_3 , c in g/100 mL). UV spectra were measured on a Perkin-Elmer Lambda 20 spectrophotometer. IR spectra were obtained on a Perkin-Elmer FTIR instrument. ^1H and ^{13}C NMR spectra were

recorded on a Varian Unity Inova 500 spectrometer (500 and 125 MHz, respectively) in CDCl₃ or CD₃OD; chemical shifts are reported in ppm as δ values relative to Me₄Si (internal standard). HRESIMS were recorded on a Bruker FTMS 4.7 T mass spectrometer. EIMS and D/CIMS spectra were obtained on a Finnigan-MAT/TSQ-700 triple stage quadrupole instrument (EIMS: 70 eV; D/CI-MS: NH₃, positive-ion mode). TLC: silica gel 60 F₂₅₄ Al sheets (Merck), detection at 254 nm and with vanillin-sulfuric acid reagent.⁹ Open column chromatography was performed using silica gel 60 (40–63 and 63–200 μ m; Merck). Analytical HPLC was carried out on a HP 1100 system equipped with a photodiode array detector (Agilent Technologies). Extracts and fractions were analyzed on a Nova-Pak C₁₈ column (5 μ m, 4.6 \times 250 mm; Waters). Purification of Mosher esters was performed on a Nova-Pak C₁₈ column (RCM; 10 μ m, 8 \times 10 mm; Waters). Medium-pressure chromatography (MPLC) separation was carried out using a Buchi 681 pump equipped with a Knauer UV detector using a Lichroprep C₁₈ column (15–25 μ m, 40 \times 500 mm, Merck).

Plant Material. The roots of *Lannea edulis* were collected in Harare, Zimbabwe, in September 1999. A voucher specimen was deposited by S.M. at the Institut de Pharmacognosie et Phytochimie, Lausanne, Switzerland (no. 93073).

Extraction and Isolation. Root bark powder (3 kg) was exhaustively extracted with CH₂Cl₂ (3 \times 24 h), followed by MeOH, and concentrated in vacuo to give 17 g of CH₂Cl₂ extract and 10 g of MeOH extract. The CH₂Cl₂ extract (7 g) was fractionated by open column chromatography on silica gel (70 g), with a stepwise gradient of CH₂Cl₂–EtOAc (8:1 to 4:1) to give 16 fractions (F1 to F16). All fractions were evaluated against DPPH radicals,⁷ with only fraction F3 found to be active. F3 (800 mg) was filtered on Sephadex LH-20 gel (Pharmacia) with MeOH–CHCl₃ (1:1) and yielded five further fractions (BC₁–BC₅). Fraction BC₃ was purified by MPLC using a C₁₈ column packed with Lichroprep (15–25 μ m, 2 \times 80 mm; Merck) eluting with MeCN–H₂O (gradient: 70:30 to 100% in 6 h, UV 210 nm) to yield **1**⁴ (50 mg) and **2**⁴ (30 mg). Fraction 8 (230 mg) was purified by MPLC eluted with MeCN–H₂O (gradient: 60:40 to 100% in 7 h, UV 210 nm) and yielded **3** (6 mg). Fraction 10 (270 mg) was purified by MPLC eluting with MeCN–H₂O (gradient: 65:35 to 100% in 7 h, UV 210 nm) to afford **4** (30 mg) and **5** (5 mg).

5-[14-Heptadecenyl]-4,5-dihydroxy-2-cyclohexenone (3): amorphous white powder; $[\alpha]_D^{25} -23.5^\circ$ (*c* 0.46, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 219.9 (4.8) nm; IR (KBr) ν_{\max} 3500, 2915, 1684, 1470, 1051 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 364 [M]⁺ (22), 346 [M – H₂O]⁺ (45), 265 (21), 149 (19), 132 (23), 120 (58), 107 (95), 84 (100), 82 (34); HRESIMS *m/z* 387.28679 (calcd for C₂₃H₄₀O₃Na, 387.28696).

5-[16-Nonadecenyl]-4S,5S-dihydroxy-2-cyclohexenone (4): amorphous white powder; $[\alpha]_D^{25} +3.2^\circ$ (*c* 1.1, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 218.8 (4.3) nm; IR (KBr) ν_{\max} 3498, 2910, 1680, 1475, 1051, 1028 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 392 [M]⁺ (19), 374 [M – H₂O]⁺ (22), 203 (10), 149 (12), 96 (11), 84 (60), 57 (100); HRESIMS *m/z* 415.31850 (calcd for C₂₅H₄₄O₃Na, 415.31826).

5-[16-Nonadecenyl]-4,5-dihydroxy-2-cyclohexenone (5): amorphous white powder; $[\alpha]_D^{25} -13.9^\circ$ (*c* 0.24, CHCl₃); UV

(CHCl₃) λ_{\max} (log ϵ) 218.4 (4.2) nm; IR (KBr) ν_{\max} 3500, 2935, 2912, 1684, 1470, 1050 cm⁻¹; ¹H and ¹³C NMR data see Tables 1 and 2, respectively; EIMS *m/z* 392 [M]⁺ (23), 374 [M – H₂O]⁺ (27), 203 (15), 149 (15), 95 (11), 83 (62), 57 (100); HRESIMS *m/z* 415.31782 (calcd for C₂₅H₄₄O₃Na, 415.31826).

Determination of Absolute Configuration of 4. Compound **4** (5 mg in 2 mL of CH₂Cl₂) was sequentially treated with pyridine (0.2 mL) and 100 mg of (*R*)-(-)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride (MTPA-chloride). The mixture was stirred at room temperature under a N₂ atmosphere for 5 h, and the reaction was monitored by HPLC. The reaction mixture was concentrated and dried, and the residue was dissolved in CH₂Cl₂, washed with a 1% NaHCO₃ solution, and evaporated in vacuo. The organic layer was purified by analytical HPLC using a Nova-Pak C₁₈ column (RCM; 10 μ m, 8 \times 10 mm; Waters), with MeCN–H₂O (60:40), affording the *per*-(*S*)-Mosher ester derivative **4a** (6.2 mg, 59.6%). The *per*-(*R*)-Mosher ester derivative **4b** (7.5 mg, 72.1%) was prepared by using 100 mg of the (*S*)-(-)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride reagent as described above. Compound **4a**: ¹H NMR (CDCl₃, 500 MHz), see Table 1; DCIMS *m/z* 841 [M + NH₄]⁺ (10), 815 (100), 392 (12), 328 (17), 376 (27), 338 (12), 285 (11), 280 (26), 252 (7), 100 (22), 77 (12). Compound **4b**: ¹H NMR (CDCl₃, 500 MHz), see Table 2; DCIMS *m/z* 841 [M + NH₄]⁺ (24), 815 (100), 789 (19), 781 (20), 599 (22), 580 (38), 476 (10), 391 (40), 376 (35), 338 (10), 285 (12), 277 (19), 196 (20), 100 (18).

DPPH Assay.¹⁰ After developing and drying, TLC plates were sprayed with a 0.2% diphenylpicrylhydrazole (DPPH) solution in MeOH. The plates were examined 30 min after spraying. Active compounds appeared as yellow spots against a purple background.

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Supporting Information Available: Spectral data for the known compounds **1**, **1a**, **2**, and **2a** are available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- Gelfand, M.; Mavi, S.; Drummond, R. B.; Ndemera, B. In *Traditional Medicinal Practitioner in Zimbabwe*; Mambo Press: Gweru, Zimbabwe, 1985.
- Troupin, V. In *Flore du Rwanda*; Institut National de Recherche Scientifique; Liège, Belgium, 1983; Vol. II.
- Cavin, A.; Hostettmann, K.; Dyatmyko, W.; Potterat, O. *Planta Med.* **1998**, *64*, 393–396.
- Franke, K.; Masaoud, M.; Schmidt, J. *Planta Med.* **2001**, *67*, 477–479.
- Groweiss, A.; Cardellina, J. H., II; Pannell, L. K.; Uyakul, D.; Kashman, Y.; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 116–112.
- Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
- Shi, G.; Gu, M.; He, K.; Wood, K. V.; Zeng, L.; Ye, Q.; MacDougal, J. M.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1996**, *4*, 1281–1286.
- Borns, W.; Saran, M.; Eltsner, E. F. In *Screening for Plant Antioxidants*. Linskens, H. F., Jackson, J. F., Eds.; Modern Methods of Plant Analysis. New Series; Springer: Berlin, 1992; Vol. 13, p 277.
- Godin, P. *Nature* **1954**, *174*, 134.
- Cuendet, M.; Hostettmann, K.; Potterat, O.; Dyatmiko, W. *Helv. Chim. Acta* **1997**, *80*, 1144–1152.

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