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Absolute configurations of two acyclic triterpenoids from *Ekebergia capensis*

Yumi Nishiyama[a,*], Masataka Moriyasu[a], Momoyo Ichimaru[a], Atsushi Kato[a], Simon G. Mathenge[b], Joseph N. Nganga[c], Francis D. Juma[c]

[a]*Department of Natural Medicinal Chemistry, Kobe Pharmaceutical University, 4-19-1, Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan*

[b]*Department of Botany, University of Nairobi, P.O. Box 30197, Nairobi, Kenya*

[c]*Department of Medicine, University of Nairobi, P.O. Box 30588, Nairobi, Kenya*

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Abstract

The absolute configurations of two acyclic triterpenoids **1** and **2**, previously isolated from the bark of *Ekebergia capensis* (Meliaceae) have been determined by the modified Mosher's method.

Keywords: *Ekebergia capensis*; Meliaceae; Acyclic triterpenoid; 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene; 1,2,3,22,23-pentahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene; Modified Mosher's method; Absolute configuration

1. Introduction

Two new acyclic triterpenoids were previously isolated from the bark of *Ekebergia capensis*, 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**1**) and 1,2,3,22,23-pentahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**2**)(Nishiyama et al., 1996). The configurations of the double bonds were determined to be all *E* from the ^{1}H and ^{13}C NMR data (Nishiyama et al., 1996). However, the absolute configuration at the stereogenic centers has not been established yet. Therefore, the main goal of this work was to determine the absolute configurations of the sterogenic carbinol centers of triterpenoids **1** and **2** using the Mosher ester methodology.

2. Results and discussion

In order to determine the absolute configurations of the sterogenic carbinol centers at C-3 and C-22 in compound **1**, we first tried the Horeau method (Horeau, 1961, 1962) without success. Therefore, we applied the modified Mosher ester methodology (Ohtani, Kusumi, Kashman & Kakisawa, 1991; Kusumi, Hamada, Ishitsuka, Ohtani & Kakisawa, 1992). The analysis of the $\Delta\delta H$ (S–R) data (Fig. 1) of the (S)- and (R)-MTPA Mosher ester derivatives of compound **1**, showed that the absolute configurations of the chiral centers at C-3 and C-22 were both *R*.

Compound **2** has three chiral centers at C-2, C-3 and C-22. By application of the modified Mosher's method, the configuration of C-22 is determined to be *R*. Because C-2 and C-3 are close to each other, the determinations of these absolute configurations is difficult. To solve this problem, we prepared the acetonide compounds of **2**. Namely, on treatment of **2** with 2,2-dimethoxypropane, *p*-toluenesulfonic acid in acetone (Ngnokam, Massiot, Bliard & Tsamo, 1995), **2** gave

* Corresponding author. Tel.: +81-78-441-7543; fax: +81-78-441-7544.

E-mail address: nisiyama@kobepharma-u.ac.jp (Y. Nishiyama).

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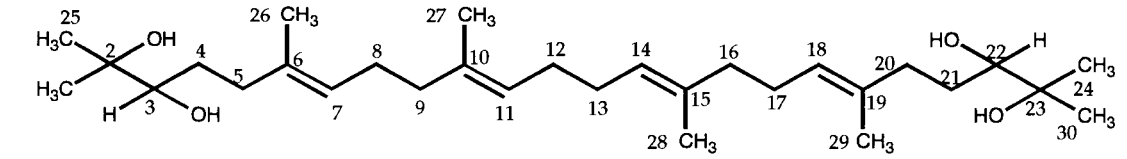
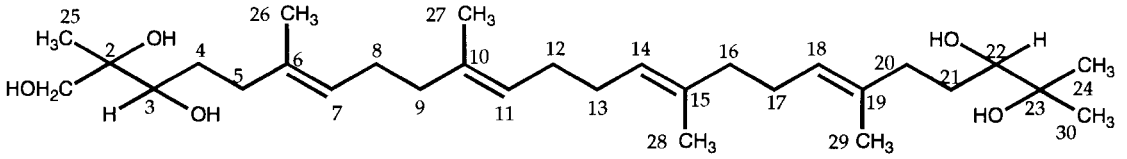
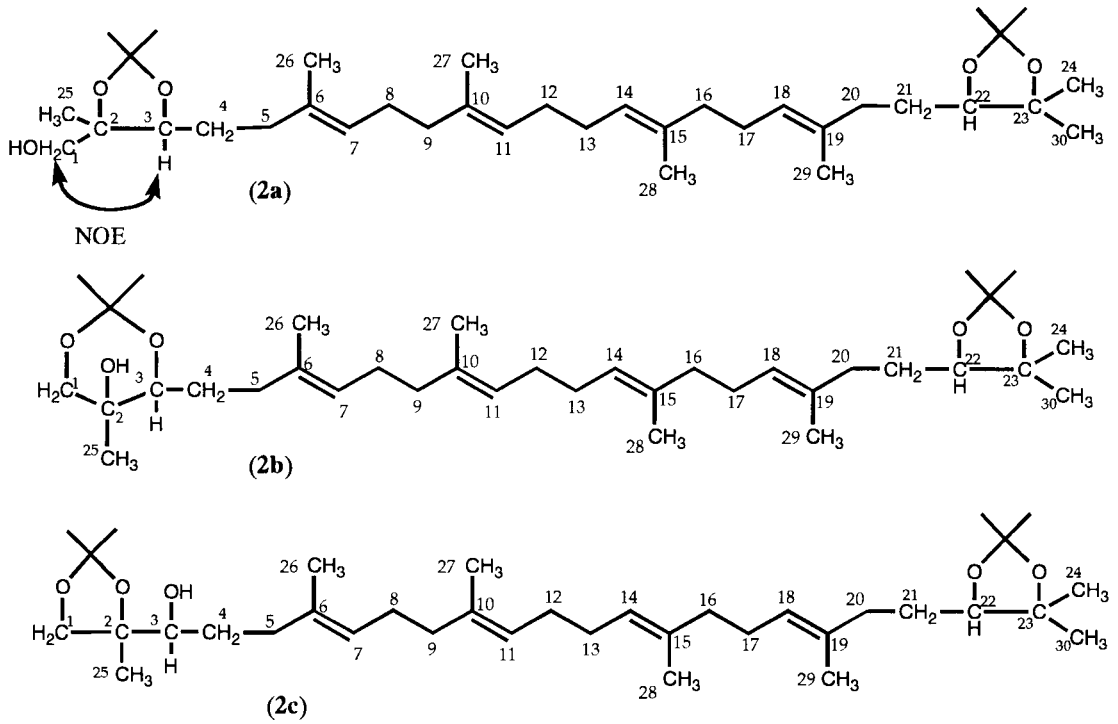


2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**1**)



1,2,3,22,23-pentahydroxy-2,6,10,15,19,23- hexamethyl-6,10,14,18-tetracosatetraene (**2**)



three acetonide compounds, **2a**, **2b** and **2c**. Because **2** contained a vicinal triol moiety (C-1, C-2 and C-3), three possible acetonides could be formed. The structures of each product were elucidated by MS spectrum, ^{1}H and ^{13}C NMR and especially 2D NMR spectral data. The NOESY experimental data on **2a** reveal that the relative configuration between C-2 and C-3 is *threo*, namely the cross peak between H-3 and H_2-1 is observed. In **2c**, because a secondary alcohol site (C-3) remained, the modified Mosher's method was applied.

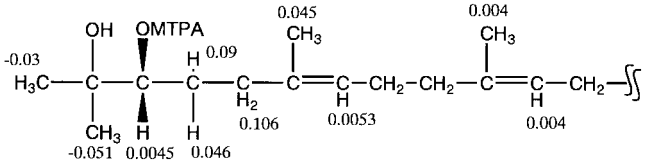


Fig. 1. $\Delta\delta$H values obtained for the MTPA ester of compound **1**.

From the results in Fig. 2, absolute configuration of C-3 is determined to be *R*, consequently C-2 is *R*.

3. Experimental

3.1. General

^{1}H NMR: 500 MHz; ^{13}C NMR: 125 MHz, with

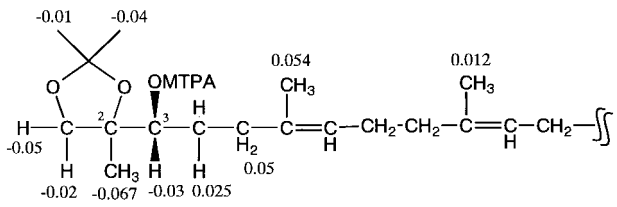


Fig. 2. $\Delta\delta$H values obtained for the MTPA ester of compound **2c**.

TMS as int. standard; HR-SIMS: 3-nitrobenzyl alcohol matrix + Na_2CO_3; TLC: silica gel.

3.2. (R)- or (S)-di-MTPA ester of 1

1 (20 mg) was dissolved in dichlromethane (0.5 ml) and DCC (about 15 mg), (*R*)- or (*S*)-MTPA acid (about 15 mg) and DMAP (about 10 mg) were added. The mixture was kept at room temperature for 6 h, then was submitted to pTLC (benzene:diethylether = 4:1) and the main ester compound was obtained (*R*-di-MTPA ester: 23 mg, *S*-di-MTPA ester: 18 mg).

3.3. Bis-acetonide of 2

2 (52 mg) was dissolved in acetone (10 ml) and 2,2-dimethoxypropane (1 ml) and *p*-toluenesulfonic acid (5 mg) were added. The reaction was completed within 20 min. at room temperature. Hexane was added and the organic phase was washed with saturated $NaHCO_3$ and dried over Na_2SO_4 and concentrated. The extract was subjected to pTLC (benzene:diethylether = 2:1) and three main products were obtained (**2a**: 23.6 mg, **2b**: 11.3 mg, **2c**: 17.2 mg).

2a: colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, s, CH_3-25), 1.10, 1.24 (each 3H, s, CH_3-24, 30), 1.33, 1.36, 1.42, 1.46 (each 3H, s, $CH_3 \times 4$), 1.45 ∽ 1.50 (m), 1.59, 1.60, 1.61, 1.62 (each 3H, bs, CH_3-26, 27, 28, 29), 1.63 (2H, m), 2.00 (m), 2.09 (4H, m), 2.20 (2H, m), 3.38 (1H, dd, J = 9.5, 11.5 Hz, H_A-1), 3.54 (1H, dd, J = 3.0, 11.5 Hz, H_B-1), 3.66 (1H, dd, J = 3.5, 9.5 Hz, H-22), 4.01 (1H, dd, J = 3.5, 9.5 Hz, H-3), 5.15 (4H, m, H-7, 11, 14, 18). ^{13}C NMR ($CDCl_3$) δ 16.02, 16.06 (C-26, 27, 28, 29), 18.86 (C-25), 22.94, 26.08 (C-24, 30), 26.69, 26.70, 26.77, 26.87, 28.58, 28.76 ($CH_3 \times 4$), 27.80, 27.97, 28.30, 36.69, 39.71, 39.72, 65.54 (C-1), 77.55 (C-3), 80.10 (C-23), 82.60 (C-2), 82.85 (C-22), 106.43, 107.06, 124.32, 124.34, 124.79 (C-7, 11, 14, 18), 134.22, 134.23, 135.04, 135.06 (C-6, 10, 15, 19). SIMS m/z 597$[M+Na]^+$, 559. HR-SIMS Found: 597.4502 $[M+Na]^+$; $C_{36}H_{62}O_5Na$ requires 597.4492.

2b: colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, s, CH_3-25), 1.10, 1.24 (each 3H, s, CH_3-24, 30), 1.33, 1.41, 1.42, 1.43 (each 3H, s, $CH_3 \times 4$), 1.47 (2H, m), 1.60, 1.61, 1.62 (each bs, CH_3-26, 27, 28, 29), 1.61 (m), 2.01 (m), 2.09 (4H, m), 2.20 (2H, m), 3.45, 3.73 (each 1H, d, J = 12.0 Hz, H_2-1), 3.56 (1H, dd, J = 4.5, 8.0 Hz, H-3), 3.68 (1H, dd, J = 3.5, 9.5 Hz, H-22), 5.15 (4H, m, H-7, 11, 14, 18). ^{13}C NMR ($CDCl_3$) δ 15.85, 16.00, 16.05 (C-26, 27, 28, 29), 18.28, 26.84, 28.56, 29.71 ($CH_3 \times 4$), 18.92 (C-25), 22.91, 26.05 (C-24, 30), 26.03, 26.66, 26.70, 27.76, 28.27, 35.15, 36.67, 39.70, 39.79, 66.89 (C-2), 70.49 (C-1), 75.13 (C-3), 80.07 (C-23), 82.82 (C-22), 98.91, 106.40, 124.23, 124.28, 124.74, 124.89 (C-7, 11, 14, 18), 134.22, 134.36, 135.04, 135.09 (C-6, 10, 15, 19). SIMS m/z 597$[M+Na]^+$, 559. HR-SIMS Found: 597.4481 $[M+Na]^+$; $C_{36}H_{62}O_5Na$ requires 597.4492.

2c: colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.10, 1.24 (each 3H, s, CH_3-24, 30), 1.25 (3H, s, CH_3-25), 1.33, 1.407, 1.414, 1.42 (each 3H, s, $CH_3 \times 4$), 1.43 (m), 1.47 (m), 1.599, 1.602, 1.61, 1.62 (each 3H, bs, CH_3-26, 27, 28, 29), 1.63 (m), 2.01 (m), 2.08 (m), 2.20 (m), 2.26 (m), 3.48 (1H, td, J = 3.0, 9.5 Hz, H-3), 3.66 (1H, dd, J = 3.5, 9.5 Hz, H-22), 3.69, 3.87 (each 1H, d, J = 8.5 Hz, H_2-1), 5.16 (4H, m, H-7, 11, 14, 18). ^{13}C NMR ($CDCl_3$) δ 16.00, 16.04 (C-26, 27, 28, 29), 19.68 (C-25), 22.91, 26.06 (C-24, 30), 26.67, 26.85, 26.93, 27.28, 28.56 ($CH_3 \times 4$), 27.77, 28.27, 30.46, 36.52, 36.66, 39.70, 71.73 (C-1), 75.52 (C-3), 80.08 (C-23), 82.82 (C-22), 83.83 (C-2), 106.40, 109.60, 124.29, 124.76, 124.84 (C-7, 11, 14, 18), 134.21, 134.54, 135.01, 135.03 (C-6, 10, 15, 19). SIMS m/z 597$[M+Na]^+$, 559. HR-SIMS Found: 597.4501 $[M+Na]^+$; $C_{36}H_{62}O_5Na$ requires 597.4492.

3.4. (R)- or (S)-mono-MTPA ester of 2c

2c (15 mg) was dissolved in dichloromethane (2 ml) and DCC (about 15 mg), (*R*)- or (*S*)-MTPA acid (about 15 mg) and DMAP (about 10 mg) were added. The mixture was kept for 6 h, then was submitted to pTLC (benzene:diethylether = 8:1) and main ester compound was obtained (*R*-mono-MTPA ester: 12.6 mg, *S*-mono-MTPA ester: 13.7 mg).

3.5. (R)- or (S)-tri-MTPA ester of 2

2 (20 mg) was dissolved in dichloromethane (2 ml) and DCC (about 20 mg), (*R*)- or (*S*)-MTPA acid (about 20 mg) and DMAP (about 15 mg) were added. The mixture was kept for 12 h, then was submitted to pTLC (benzene:diethylether = 4:1) and the ester compound was obtained (*R*-tri-MTPA ester: 15 mg, *S*-tri-MTPA ester: 9 mg).

The structures of compounds mentioned above were determined by ^{1}H and ^{13}C NMR and various two dimentional 2D NMR data.

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References

Horeau, A. (1961). *Tetrahedron Lett.*, *15*, 506.

Horeau, A. (1962). *Tetrahedron Lett*., *21*, 965.
Kusumi, T., Hamada, T., Ishitsuka, M. O., Ohtani, I., & Kakisawa, H. (1992). *J. Org. Chem*., *57*, 1033.
Ngnokam, D., Massiot, G., Bliard, C., & Tsamo, E. (1995). *Nat. Prod. Lett*., *5*, 289.
Nishiyama, Y., Moriyasu, M., Ichimaru, M., Tachibana, Y., Kato, A., Mathenge, S. G., Nganga, J. N., & Juma, F. D. (1996). *Phytochemistry*, *42*, 803.
Ohtani, I., Kusumi, T., Kashman, Y., & Kakisawa, H. (1991). *J. Am. Chem. Soc*., *113*, 4092.