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Gracilipene: a heterocyclic *seco-trisnor*-oleanane from *Calophyllum gracilipes* (Guttiferae)

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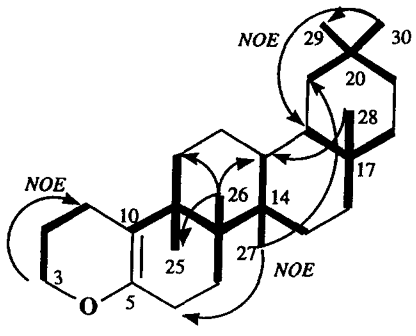
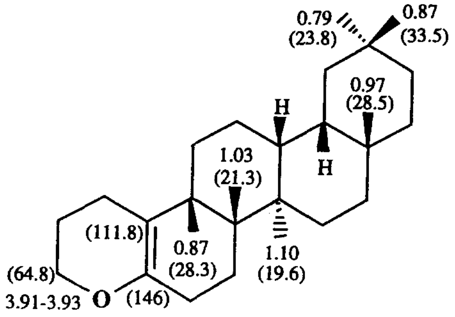
Abstract: Gracilipene, a novel heterocyclic *trisnor*-triterpene from the leaves of *Calophyllum gracilipes*, shows an unprecedented rearranged *seco-trisnor*-oleanane structure with a dihydropyran ring-A, as determined by NMR spectra and single crystal X-ray analysis. Other known triterpenes isolated include friedelin, lupeol, lupenone, β-sitosterol, stigmasterol, 3β-hydroxy-30-norlupan-20-one, lupane-3β,20-diol, (20*R*)-3β-hydroxylupan-29-oic acid, betulinic acid and squalene. © 1997 Elsevier Science Ltd.

Plants of the genus *Calophyllum* are now considered to have medicinal value as a potential source of anti-HIV compounds.[1] A study of the leaf extract of the Malaysian plant *Calophyllum gracilipes* has led to the isolation of a novel heterocyclic *seco-trisnor*-triterpene with a rearranged oleanane skeleton.

The CH_2Cl_2-CH_3OH (v/v, 1:10) extract of the leaves (1.5 kg) of *C. gracilipes* was re-extracted into hexane. The crude hexane extract (36 g) was repeatedly chromatographed on silica gel (Merck 9380) using a hexane-ethyl acetate gradient but did not yield the bioactive coumarin derivatives found in related species;[1] instead a relatively high yield of several triterpenes was obtained. Friedelin (0.02%) was an abundant triterpene and other known triterpenes (from the bark) include lupeol (0.04%), lupenone (0.008%), β-sitosterol (0.003%), stigmasterol (0.004%), 3β-hydroxy-30-norlupan-20-one (0.00066%), lupane-2β,20-diol (0.002%), (20*R*)-3β-hydroxylupan-29-oic acid (0.001%), betulinic acid (0.0033%) and squalene (0.00033%). A minor non-polar triterpene, gracilipene (**1**) (5 mg, 0.00033%) could be isolated as almost colourless plates (hexane), m.p. 199-201°C, $[\alpha]_D^{25} = -28.1°$ (c = 0.5, $CHCl_3$). High resolution EI-MS showed **1** had a molecular ion of 384 which corresponded to $C_{27}H_{44}O$ (found 384.3391; calcd. 384.339197). Compound **1** had an olefinic stretching band (medium-strong)[2] at 1650 cm^{-1}. The ^1H NMR spectrum (500 MHz, $CDCl_3$) of **1** gave signals for six methyl singlets at δ 0.79, 0.87 (2 x Me), 0.97, 1.03 and 1.10. The absence of signals for olefinic protons showed the olefin was tetrasubstituted as was also evident from the IR spectrum. The 2D HMQC spectrum provided correlations for all the protons and the carbon atoms which were also determined from DEPT data to comprise 6 Me, 12 CH_2, 2 CH and 7 quaternary carbons. Long-range coupling correlations to the six methyl groups in the HMBC spectrum allow for the assignments of structural units with connectivity as shown below.



HMBC (bold) and NOE Difference Correlations



Some Chemical Shift Data (δ)

The cross peaks of vicinally coupled protons in the H-H COSY spectrum indicated that rings C, D and E of **1** were similar to those of oleanane.[3] The relatively low field, coupled protons (δ 3.92 and 3.82 ppm) were two geminal protons (H-3) α to an oxygen atom in a dihydropyran ring, as is also evident from the chemical shift of one of the quaternary olefinic carbons (δ 146.6). Connectivity among the H-1, H-2, H-3 protons was established from the H-H COSY spectrum. The NOE-difference spectrum provided evidence for the stereochemical relationship of the methyl groups (arrowed as shown above), *cis* B/C ring junction, *trans* C/D and *cis* D/E junctions. Although NOE difference enhancement was observed for 28-Me and H-13, that of Me-28 and H-18 was too weak to be recorded so that the D/E junction was not established unambiguously even though it was expected to be an oleanane derivative from the carbon chemical shift data.[4] The assigned structure of **1** as a degraded and rearranged oleanane derivative was readily confirmed by single crystal X-ray analysis,[5] as illustrated in Figure 1.

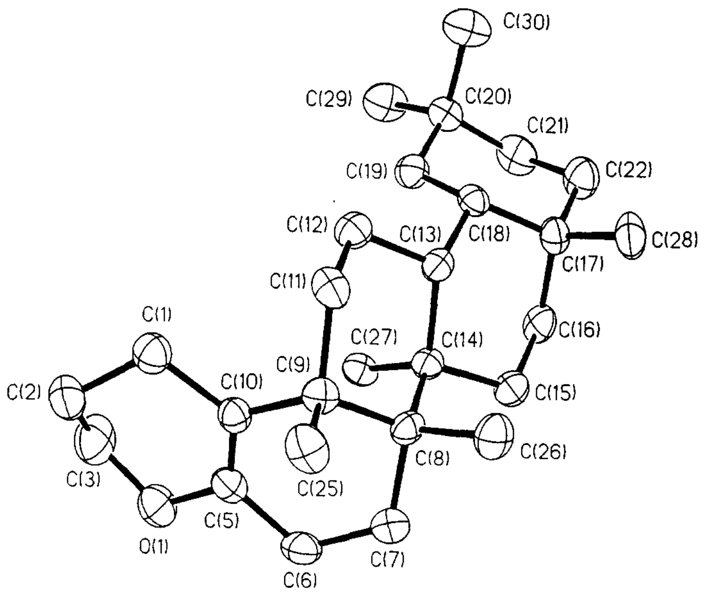
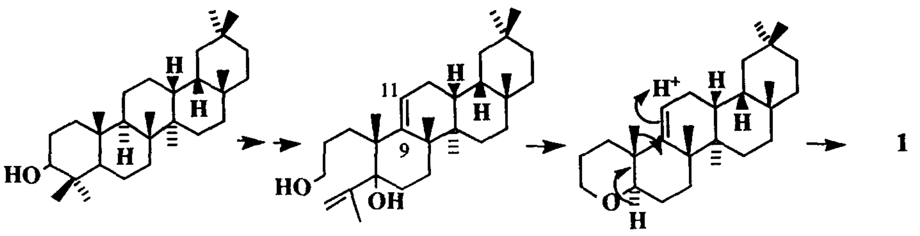


Figure 1. Molecular Structure of Gracilipene (**1**)

Although degraded triterpenes are well known natural products,[6-9] a rearranged, heterocyclic *seco-trisnor*-oleanane derivative such as **1** has not been found before. The degradation of ring A of triterpenes to open *seco-* and *des*-ring-A derivatives is however common from the action of microorganisms.[6-9] Examples of plant-derived, *seco*-A triterpene derivatives which have been encountered previously show that ring A can be degraded following extensive oxidation of the attached 23,24-methyl groups and normally leads to ring A opening.[6-9] Only one example of *seco*-triterpenoids containing a dihydropyran ring-A similar to **1** has been found (from *Euphorbia supina*[10]), but this has a different rearranged fernane skeleton. Additionally gracilipene (**1**) has a defined stereochemistry with *cisoid* Me-25/Me-26, *transoid* Me-26/Me-27, *transoid* Me-27/H-13, *cisoid* H-13/H-18 and *cisoid* H-18/Me-28 relationships representative of an oleanane precursor **1a**. A hypothetical biosynthetic route to **1** (Scheme 1) could be from a 9,11-dehydrooleanane derivative **1b**; some recent examples of related friedelane triterpenes are karounidiol derivatives from *Trichosanthes kirilowii*.[11] The heterocyclic ring-A may be considered to be derived by a series of biochemical oxidation and reduction steps, after which a stereospecific carbocationic 25-methyl shift of **1c** leads to the novel triterpene **1** having the observed stereochemistry. Instances of the opening of ring A are well documented and recent examples of triterpene analogues similar to the first postulated intermediate shown have been reported.[12,13] Several examples of triterpenes bearing unsaturation at the 9,11-positions have also been isolated, e.g. oleanane derivatives[11] and putrone.[14]



1a **1b** **1c**

Scheme 1. Plausible biosynthesis of gracilipene (**1**)

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References and Notes

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4. Selected spectroscopic data for **1**: ^{1}H NMR (500 MHz, CDCl3): δ 0.79 (3 H, s, Me-29), 0.87 (2 x 3 H, s, Me-30 and Me-25), 0.97 (3 H, 2, Me-28), 1.03(3 H, s, Me-26), 1.10 (3 H, s, Me-27), 2.00 (1 H, m, H-13), 2.02 (2 H, m, H-1a and H-1b), 2.04 (2 H, m, H-6a and H-6b), 3.91 and 3.93 (2 H, m, H-3a and H-3b); ^{13}C NMR (75 MHz, CDCl3): δ 19.6 (q, Me-27), 19.9 (t, C1), 21.3 (q, Me-26), 23.8 (q, Me-29; t, C2), 26.2 (t, C12), 26.4 (t, C7), 26.8 (t, C6), 28.1 (t, C16), 28.3 (q, Me-25), 28.48 (t, C15), 28.51 (q, Me-28), 30.8 (s, C20), 32.95 (s, C17), 33.5 (q, Me-30), 33.7 (t, C11), 34.79 (t, C21), 36.4 (d, C13), 37.5 (t, C22), 37.8 (t, C19), 40.7 (s, C9), 42.1 (s, C14), 43.2 (d, C18), 43.3 (s, C8), 64.8 (t, C3), 111.8 (s, C10) and 146.6 (s, C5). MS m/z (rel. int.): 384 (18%), 369 (100), 274 (4), 259(3), 231(6), 205(62), 163(74), 149(46), 135(32), 123(30), 109(67) and 95(66).

5. Crystal data for **1**: $C_{27}H_{44}O$, M = 384.62, monoclinic, $P2_1$, $a = 7.644(2)$, $b = 8.878(2)$, $c = 16.800(3)$Å, $\beta = 98.37(3)°$, $V = 1128.0(6)$Å^3 ($\lambda = 0.71073$Å), $Z = 2$, $D_{\text{calc}} = 1.132$ mg/m^3, $F(000) = 428$, $\mu = 0.066$ mm^{-1}, crystal size 0.08 x 0.18 x 0.20 mm^3. Data were collected at 294K in the range of 4.9 ~ 52°(2θ) ($0 \leq h \leq 9$; $0 \leq k \leq 10$; $-20 \leq l \leq 21$) using variable ω scan on a Rigaku AFC7R four-circle diffractometer with MoK$_\alpha$ radiation from a RU-200 rotating anode generator powered at 50 kV and 90 mA. Following application of an absorption correction based on background scan measurements, the structure was solved by direct methods. All non-hydrogen atoms were subjected to anisotropic refinement using SHELXL-93. The hydrogen atoms were placed in their calculated positions and allowed to ride on the carbon atoms to which they are attached. At convergence $R_1 = 4.53\%$, $wR_2 = 10.15\%$ [$I > 2\sigma(I)$] and $R_1 = 16.07\%$, $wR_2 = 12.60\%$ for all 2365 reflections, the largest difference peak was 0.19 and the largest difference hole was -0.14 eÅ^{-3}. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center and are also available upon request from The Chinese University of Hong Kong (CUHK). Any request to CUHK for this material should quote the full literature citation and reference number CGHTD.

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