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# Cleistenolide and Cleistodienol: Novel Bioactive Constituents of *Cleistochlamys kirkii*

**Stephen Samwel[a], Stephen J.M. Mdachi[a], Mayunga H.H. Nkunya[a,*], Beatrice N. Irungu[a], Mainen J. Moshi[b], Brian Moulton[c] and Brian S. Luisi[c]**

[a]*Department of Chemistry, University of Dar es Salaam, P.O. Box 35061, Dar es Salaam, Tanzania*

[b]*Institute of Traditional Medicine, Muhimbili University College of Health Sciences, P.O. Box 65001, Dar es Salaam, Tanzania*

[c]*Department of Chemistry, Brown University, Box H, 324 Brook Street, Providence, RI 02912, USA*

*nkunya@chem.udsm.ac.tz*

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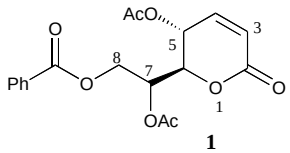
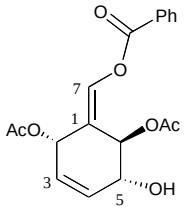
(-)-5-Acetoxy-6-(1-benzoyloxy-2-acetoxyethyl)-pyr-3-en-2-one (cleistenolide) and (-)-2,6-diacetoxy-5-hydroxy-cyclohex-3-enylidenemethyl benzoate (cleistodienol) were isolated as novel antimicrobial and cytotoxic constituents of *Cleistochlamys kirkii* (Annonaceae), together with (*Z*)-(+)-5-(2,3-dihydroxy-propylidene)-5*H*-furan-2-one and its acetyl and benzoyl derivatives, (-)-1,6-desoxy-β-senepoxide, pinocembrin and polycarpol. Structural determination was achieved based on spectroscopic and other physical data. The structure of cleistenolide was confirmed by single crystal X-ray crystallographic analysis.

**Keywords:** *Cleistochlamys kirkii,* Annonaceae, Heptenolides, Cleistenolide, Cleistodienol, antimicrobial, cytotoxicity.

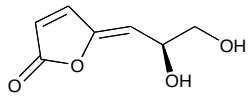
It has now been established that Annonaceae species are rich sources of structurally diverse natural products, some of which have wide spectra of biological activities. Thus, during our continuing investigations of some of the around 84 Annonaceae species occurring in Tanzania, several such natural products have been isolated [1]. While quite a number of the Annonaceae species have continued to be threatened with extinction, even before their constituents are established, our recent investigations of some of the newly described Annonaceae taxa have yielded compounds having interesting chemical structures and/or biological activities [2-5]. This has inspired us to continue investigating Tanzanian Annonaceae species for biologically active compounds, such as those with antimalarial, antitrypanosomal, mosquitocidal, antibacterial, antifungal and cytotoxic properties [3,6-9]. As part of these investigations, we have now analyzed cytotoxic extracts from *Cleistochlamys kirkii* Oliver, a plant species that grows in Tanzania and Mozambique [10], the extract of which is used as a traditional remedy for haemorrhoid wounds, rheumatism and tuberculosis [11].



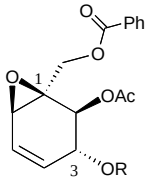

**1**




**2**




**3**




**4**: R = Ac
**5**: R = Me

Repeated chromatography of the cytotoxic dichloromethane and methanol extracts of dried fruits, leaves, stem and root barks yielded cleistenolide (**1**) and cleistodienol (**2**) as new antimicrobial and cytotoxic constituents, together

with (*Z*)-(+)-5-(2,3-dihydroxy-propylidene)-5*H*-furan-2-one (**3**), and its derivatives (*Z*)-(+)-2-hydroxy-3-(5-oxo-5*H*-furan-2-ylidene)-propyl benzoate (melodorinol), (*Z*)-2-acetoxy-3-(5-oxo-5*H*-furan-2-ylidene)-propyl benzoate (acetyl melodorinol), (*E*)-2-acetoxy-3-(5-oxo-5*H*-furan-2-ylidene)-propyl benzoate (*iso*-acetyl melodorinol) and (*Z*)-2-benzoyloxy-3-(5-oxo-5*H*-furan-2-ylidene)-propyl benzoate (benzoyl melodorinol) [12-16], (-)-1,6-desoxy-β-senepoxide [17], pinocembrin [12,14,18,19] and polycarpol [12].

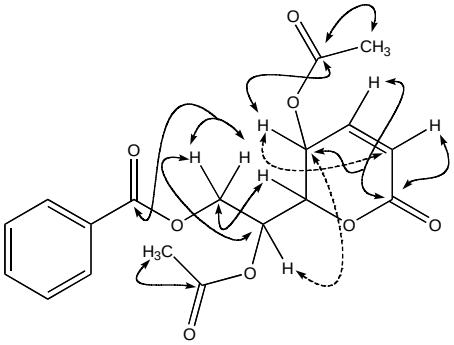


**Figure 1**: Important H/C interactions observed in the HMBC spectrum of cleistenolide (**1**).

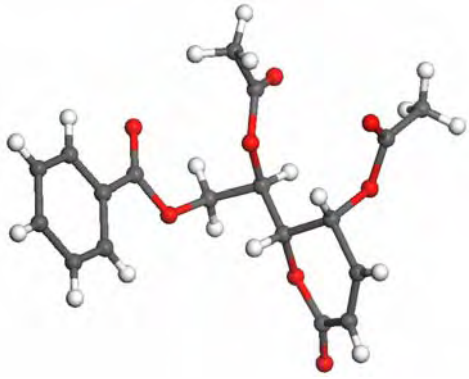


**Figure 2**: Crystal structure of cleistenolide (**1**).

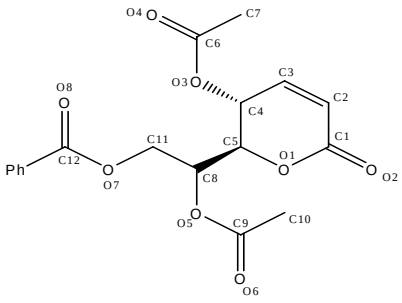


**Figure 3**: Structure of cleistenolide (**1**) as deduced from X-ray diffraction analysis.

Structure **1** for cleistenolide {HRMS $[M]^+$ at *m/z* 362.1034, $C_{18}H_{18}O_8$} was deduced from IR, $^1H$ and $^{13}C$ NMR spectroscopic, and mass spectral data. The structure was further elaborated from H/H COSY and HMBC H/C interactions (Figure 1). The small $^2J_{5,6}$ value of 2.7 Hz revealed in the $^1H$ NMR spectrum entailed a *cis* (*axial/equatorial*) substitution at C-5 and C-6, and hence an H-5/H-6 dihedral angle of close to 90º, as further derived from single crystal X-ray analysis, which also confirmed structure **1**. The X-ray crystal structure is shown in Figures 2 and 3. Cleistenolide undergoes weak (C-H as a donor) intermolecular hydrogen bonding [O2···C5, 2.405 Å; O6···C10, 2.417 Å; O8···C10, 2.493 Å].

Structure **2** for the other novel compound, cleistodienol, was established based on $^1H$ and $^{13}C$ NMR spectroscopic, and mass spectral data {$[M]^+$ at *m/z* 346.1072, $C_{18}H_{18}O_7$}. Thus, apart from the presence of an additional enolic methine proton signal and absence of epoxy proton resonances, the $^1H$ NMR spectral data for **2** resembled very closely those previously reported for the cyclohexane epoxides (+)-β-senepoxide (**4**) and (+)-pandoxide (**5**) [2,17]. In addition, as for **4** and **5** ($J_{2,3}$ = 8.5 Hz) [2,17], the $J_{5,6}$ value of 8.2 Hz observed in the $^1H$ NMR spectrum of **2** indicated an *axial/axial* configuration for H-5/H-6, and hence an *equatorial/equatorial* stereochemistry for the C-5 and C-6 oxygenated substituents, as further indicated by H/H COSY and HMBC H/C interactions (Figure 4). The $^1H$ NMR spectrum of **2** also exhibited a $J_{2,3}$ value of 4.2 Hz, which indicated an H-2/H-3 dihedral angle close to 90º [20], hence establishing a $\beta_{axial}$ configuration for H-2 (molecular models). The long range $J_{6,7}$ value (2.1 Hz) observed in the $^1H$ NMR spectrum indicated a *W*-type configuration for H-6/H-7, which would be achievable only if the C-1 double bond had a *Z* configuration (molecular models), as further deduced from HMBC interactions (Figure 4).

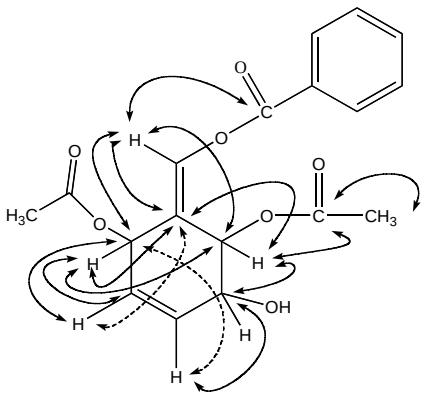


**Figure 4**: Important H/C interactions observed in the HMBC plot for cleistodienol (**2**).

Spectral data established structure **3** for (*Z*)-(+)-5-(2,3-dihydroxy-propylidene)-5*H*-furan-2-one [12,13,15], the *Z* geometry of the exocyclic double bond being deduced from NOE interactions. The $^1$H NMR spectrum of *iso*-acetyl melodorinol indicated a $^5J_{2,5}$ value of 1.8 Hz and hence establishing the H-2/H-5 *transoid* stereochemistry [12,13].

Cleistenolide exhibited *in vitro* antibacterial activity against *Staphylococcus aureus* and *Bacillus anthracis* and antifungal activity against *Candida albicans*. Cleistodienol demonstrated *in vitro* cytotoxicity in the brine shrimp test [21], exhibiting an $LD_{50}$ value of 0.09 μg/mL. It also showed *in vitro* antibacterial activity against *Staphylococcus aureus* and *Bacillus anthracis* and antifungal activity against *Candida albicans*, thus corroborating the traditional use of the plant extract for ailments related to bacterial infections [11].

Compound **3** and its derivatives, including melodorinol that showed cytotoxicity (brine shrimp test), antibacterial activity against *Staphylococcus aureus* and *Bacillus anthracis*, and antifungal efficacy against *Candida albicans*, belong to a series of antitumour heptanoids previously isolated from *Melodorum* (*Sphaerocoryne*) species [12].

## Experimental

***General experimental procedures:*** FTIR: $CHCl_3$; UV: MeOH; $^1$H NMR at 300 MHz and $^{13}$C NMR at 75 MHz in $CDCl_3$, internal standard TMS ($^1$H NMR) and solvent signals ($^{13}$C NMR); EIMS at 70 eV with direct injection and CIMS: $NH_3$, only key ions are given; TLC on plastic plates (Kieselgel $60F_{254}$), detection: UV and anisaldehyde/heat [22]; CC: Silica gel 60; gel filtration: Sephadex® LH-20 ($CHCl_3$/MeOH, 1:1 v/v); reversed phase (RP-18); optical rotation in $CHCl_3$.

***Plant materials:*** Fruits, leaves, stem and root barks of *Cleistochlamys kirkii* Oliver were collected in November 2001 from Weme forest reserve in Rufiji flood plains, Rufiji district in Tanzania. The plant species was authenticated at the Herbarium of the Department of Botany, University of Dar es Salaam where a voucher specimen is preserved (H.O. Sulemani/Mwasumbi/Lyaruu Coll. No. 1026).

***Extraction and isolation:*** Air-dried pulverized plant materials were soaked consecutively in light petroleum, $CH_2Cl_2$ and EtOH (2 x 48 h) at room temperature and the combined extracts from leaves, on partitioning by vacuum liquid chromatography (VLC) (light petroleum/EtOAc gradient) and then silica gel CC (light petroleum/EtOAc, 7:3 v/v), gave fractions containing cleistenolide (**1**) and cleistodienol (**2**), which were purified by recrystallization (MeOH) and gel filtration (Sephadex LH-20), respectively. Cleistenolide was also isolated from the medium polar VLC fractions of the light petroleum and $CH_2Cl_2$ extracts of the stem and root barks upon repeated chromatography, as above, in addition to benzoyl melodorinol and (-)-1,6-desoxy-β-senepoxide (fruits only), (*Z*)-(+)-5-(2,3-dihydroxy-propylidene)-5*H*-furan-2-one and melodorinol (fruits and stem bark), acetyl melodorinol, pinocembrin and polycarpol (stem bark only), and *iso*-acetyl melodorinol (fruits and root bark).

**(-)-5-Acetoxy-6-(1-benzoyloxy-2-acetoxyethyl)-pyr-3-en-2-one** (**Cleistenolide**, **1**)

Yield: 140 mg (0.02%).
anisaldehyde – yellow → orange → green.
White needles.
MP: 130-134ºC.
$[\alpha]_D$: – 63.54º (*c* 0.7, $CHCl_3$).
IR $\nu_{max}$: 3031, 1742, 1721, 1615, 1270, 1113, 875 $cm^{-1}$.
UV $\lambda_{max}$: 270, 242 nm.
$^1$H NMR: δ 2.08 (3H, s, 5-OCOC$H_3$), 2.13 (3H, s, 7-OCOC$H_3$), 4.54 (1H, dd, $J_{8,8}$ = 12.5, $J_{7,8A}$ = 2.4 Hz, H-8A), 4.82 (1H, dd, $J_{6,7}$ = 9.7, $J_{5,6}$ = 2.7 Hz, H-6), 4.93 (1H, dd, $J_{8,8}$ = 12.5, $J_{7,8B}$ = 4.4 Hz, H-8B), 5.42 (1H, dd, $J_{4,5}$ = 6.1, $J_{5,6}$ = 2.7 Hz, H-5), 5.53 (1H, ddd, $J_{6,7}$ = 9.6, $J_{7,8B}$ = 4.4, $J_{7,8A}$ = 2.4 Hz, H-7), 6.30 (1H, d, $J_{3,4}$ = 9.7 Hz, H-3), 7.02 (1H, dd, $J_{3,4}$ = 9.7, $J_{4,5}$ = 6.1 Hz, H-4), 7.49 (2H, ddd, *J* = 7.7, 7.5, 1.8 Hz, H-3' and H-5'), 7.60 (1H, m, H-4'), 8.05 (2H, dd, *J* = 7.6, 1.9 Hz, H-2' and H-6');
$^{13}$C NMR: δ 170.35 (5-O<u>C</u>OCH$_3$), 170.03 (7-O<u>C</u>OCH$_3$), 166.42 (8-O<u>C</u>OPh), 161.54 (C-2), 140.10 (C-4), 133.68 C-4'), 130.09 (C-2' and C-6'), 130.02 (C-1'), 128.89 (C-3' and C-5'), 125.77 (C-3), 75.92 (C-5), 68.03 (C-7), 62.40 (C-8), 60.13 (C-6), 21.05 (7-<u>C</u>H$_3$COO) and 20.84 (5-<u>C</u>H$_3$COO).
MS, *m/z* (% rel. int.): 362 ([M]$^+$, 14), 303 (38), 302 (66), 243 (7), 106 (10) and 105 (100); HRMS, *m/z* 362.1034 ([M]$^+$, $C_{18}H_{18}O_8$).

**(-)-2,6-Diacetoxy-5-hydroxy-cyclohex-3-enylidenemethyl benzoate** (**Cleistodienol**, **2**)

Yield: 45 mg (0.006%).
anisaldehyde – yellow → orange → green.
Brown oil.
$[\alpha]_D$: – 96º (*c* 0.02, $CHCl_3$).
IR $\nu_{max}$: 3446, 3041, 1750, 1715, 1275, 705 $cm^{-1}$.
UV $\lambda_{max}$: 280, 272, 242 nm.
$^1H$ NMR: δ 2.03 (3H, s, 6-$OCOCH_3$), 2.06 (3H, s, 2-$OCOCH_3$), 4.28 (1H, dd, $J_{5,6}$ = 8.2, $J_{3,5}$ = 1.8 Hz, H-5), 5.70 (1H, dd, $J_{5,6}$ = 8.2, $J_{6,7}$ = 2.1 Hz, H-6), 5.88 (1H, ddd, $J_{3,4}$ = 10.0, $J_{2,3}$ = 4.2, $J_{3,5}$ = 1.8 Hz, H-3), 5.96 (1H, dd, $J_{3,4}$ = 10.0, $J_{4,5}$ = 1.8 Hz, H-4), 6.53 (1H, dd, $J_{2,3}$ = 4.2, $J_{2,7}$ = 0.65, H-2), 7.52 (2H, ddd, *J* = 7,8, 7.5, 1.8 Hz, H-3' and H-5'), 7.59 (1H, dd, $J_{6,7}$ = 2.1, $J_{2,7}$ = 0.65 Hz, H-7), 7.64 (2H, m, H-4') and 8.18 (2H, dd, *J* = 7.6, 1.8 Hz, H-2' and H-6');
$^{13}C$ NMR: δ 170.55 (6-O<u>C</u>$OCH_3$), 170.35 (2-O<u>C</u>$OCH_3$), 163.29 (7-O<u>C</u>OPh), 134.87 (C-4), 134.41 (C-1'), 133.14 (C-7), 131.29 (C-4'), 130.62 (C-2' and C-6'), 128.92 and 129.15 (C-3' and C-5'), 125.21 (C-3), 116.78 (C-1), 73.07 (C-6), 72.96 (C-5), 64.11 (C-2), 21.54 (2-<u>C</u>$H_3COO$) and 20.84 (6-<u>C</u>$H_3COO$);
MS *m/z* (% rel. int.): 346 ($[M]^+$, 7) 105 (78), 123 (34) 241 (45) and 227 (29); HRMS, *m/z* 346.1072 ($[M]^+$, $C_{18}H_{18}O_7$).

**(4*Z*)-5-(2,3-Dihydroxy-propylidene)-5H-furan-2-one** (**3**)

Yield: 25 mg (0.013%).
Colourless oil.
$[\alpha]_D$: +13º (*c* 0.17, $CHCl_3$).
anisaldehyde – yellow → green.
IR, $\nu_{max}$: 3406, 2919, 1775, 1722, 1456, 1373, 1105, 1013, 922 $cm^{-1}$.
$^1H$ NMR: δ 3.75 (1H, dd, $J_{7,7}$ = 11.2, $J_{6,7A}$ = 6.7 Hz, H-7A), 3.94 (1H, dd, $J_{7,7}$ = 11.2, $J_{6,7B}$ = 3.4 Hz, H-7B), 5.0 (1H, ddd, $J_{5,6}$ = 7.9, $J_{,7A6}$ = 6.7, $J_{6,7B}$ = 3.4 Hz, H-6), 5.51 (1H, d, $J_{5,6}$ = 7.9 Hz, H-5), 7.50 (1H, d, $J_{2,3}$ = 5.5 Hz, H-3) and 6.37 (1H, d, $J_{2,3}$ = 5.5 Hz, H-2).
$^{13}C$ NMR: δ 169.39 (C-1), 150.21 (H-4), 144.06 (C-3), 121.18 (C-2), 114.21 (H-5), 68.28 (C-6) and 66.03 (C-7).
CIMS, *m/z* (% rel. int.): 156 ($[M]^+$, ~ 2), 126 (70), 125 (100), 98 (27), 97 (93) and 69 (21).

***X-ray diffraction analysis:*** A white rod shaped crystal of cleistenolide (**1**), $C_9H_9O_4$, 0.40 x 0.20 x 0.20 mm was mounted on a glass fiber and intensity data were collected at 90 (2) K. Z = 4, MW = 181.16, monoclinic, $P2_1$, a = 10.557(10) Å, b = 5.364(5) Å, c = 15.544(15) Å, β = 106.659(17)°, V = 843.4(14) $Å^3$, $D_x$ = 1.427 $g/cm^3$, Total reflections = 5164, Independent reflections = 1348, Final R factor [$I > 2\sigma(I)$] = 0.0623. Data were collected on a Bruker SMART CCD diffractometer with Mo Kα radiation (0.71073 Å). The structures were solved by direct methods and refined by full matrix least-squares methods with the SHELXTL-97 package. All hydrogen atoms were generated geometrically while all non-hydrogen atoms were refined anisotropically.

Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 634224. Copies of the data can be obtained, free of charge, on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Tel: (44) 01223 762910, Facsimile: (44) 01223 336033, email: deposit@ccdc.cam.ac.uk).

***Biological assay:*** Brine shrimp tests, and antibacterial and antifungal assays were carried out as reported in the literature [21,23]. In the antibacterial assays *Staphylococcus aureus* (strain NCTC 6571) and *Bacillus anthracis* (strain NCTC 10073) were used at a minimum inhibitory concentration (MIC) of 10 μg/mL and Chloramphenicol (10 μg/mL) as the standard drug. *Candida albicans* (strain HG 392) was used in the antifungal tests, at a minimum inhibitory concentration (MIC) of 10 μg/mL and Ketaconazole as the standard antifungal agent.

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