# Dihydro-β-agarofuran Sesquiterpenes and Pentacyclic Triterpenoids from the Root Bark of *Osyris lanceolata*

Elizabeth M. O. Yeboah,[†] Runner R. T. Majinda,*[,†] Anders Kadziola,[‡] and Alfred Muller[§]

*Department of Chemistry, University of Botswana, P/Bag 0022, Gaborone, Botswana, Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark, and Chemistry Department, University of Johannesburg, APK Campus, PO Box 524, Auckland Park, 2006, South Africa*

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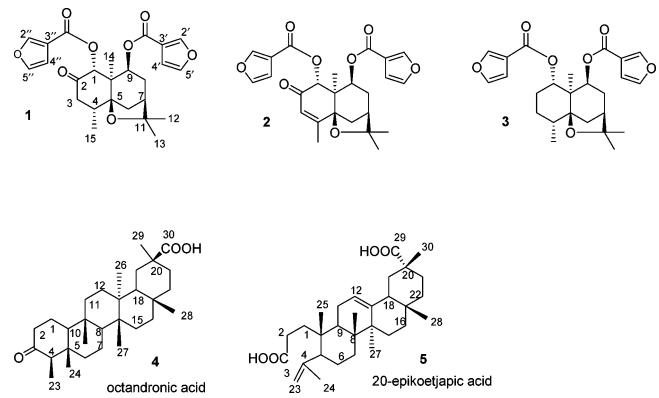
Three new dihydro-β-agarofuran polyesters, 1α,9β-difuranoyloxy-2-oxodihydro-β-agarofuran (**1**), 1α,9β-difuranoyloxy-2-oxo-3-enedihydro-β-agarofuran (**2**), and 1α,9β-difuranoyloxydihydro-β-agarofuran (**3**), have been isolated from the $CHCl_3$ extract of the root bark of *Osyris lanceolata*, together with two known pentacylic triterpenoids, **4** and **5**. Compounds **1**−**5** did not scavenge the DPPH radical within 30 min of reaction time. All five compounds displayed antifungal activity against *Candida albicans*. Compounds **1**, **3**, **4**, and **5** showed antibacterial activity against the Gram-positive *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*, with **4** and **5** being the most active. Compound **2** displayed weak antibacterial activity only against *Escherichia coli.*

*Osyris lanceolata* Hochst. & Steud. (Santalaceae), known also as African sandalwood, is a small hemiparasitic tree that grows on rocky slopes in the northern and southeastern parts of Botswana. It is also distributed in northern, eastern, central, and southern Africa, southwestern Europe, and parts of Asia. *O. lanceolata* has several synonyms including *O. abyssinica*, *O. quadripartita,* and *O. tenuifolia*.[1] Extracts of different parts of the plant are used in traditional medicine to treat a variety of conditions including gynecological disorders, venereal diseases, cancer, and malaria, and as a lactation-inducing agent.[2] The DPPH radical scavenging activity and total phenolic content of different extracts of the root bark of *O. lanceolata* have recently been reported.[3] Previous studies on the chemical composition are limited, and there is only one report on a number of sesquiterpenes identified from the essential oil of the dry wood of *O. tenuifolia*.[4] Phytochemical work on the $CHCl_3$ extract of the root bark of *O. lanceolata* has resulted in the isolation of three new dihydro-β-agarofuran-type sesquiterpene polyesters and two known pentacyclic triterpenoids, octandronic acid (**4**)[5] and 20-epikoetjapic acid (**5**).[6] The structures of the isolated compounds were investigated using IR, MS, and NMR ($^1H$, $^{13}C$, DEPT, HMQC, HMBC, $^1H-^1H$ COSY, and NOESY), with the structure of **1** being confirmed by X-ray crystallographic analysis. Compound **4** was isolated from the stems and leaves of *Catha cassinoides* (Celastraceae) by Betancor et al. in 1980,[5] and although some $^1H$ NMR and MS data were given, the $^{13}C$ NMR data of this compound have not been reported. Although a large number of natural dihydro-β-agarofuran derivatives have been documented, the majority have been isolated from plants belonging to the family Celastraceae.[7] Dihydro-β-agarofuran has been reported as a component of sandalwood oil (*Santalum album* L. of the family Santalaceae).[8] Thus, this work has chemotaxonomic significance in being the first report of naturally occurring dihydro-β-agarofuran derivatives from the family Santalaceae and from the genus *Osyris*. Dihydro-β-agarofuran derivatives have received much attention from medicinal chemists due to their wide range of biological activities, e.g., insecticidal, cytotoxic, anti-inflammatory, multidrug resistance (MDR) reversal, immunosuppressive, antiviral, anti-HIV, and anti-HSV.[7] This paper describes the isolation and structure elucidation of the new dihydro-β-agarofuran derivatives **1**−**3** and the known triterpenoids **4** and **5** together with an evaluation of their antioxidant and antimicrobial potentials. The antioxidant potential of compounds **1**−**5** and the $CHCl_3$ extract (CE) was evaluated over an 8 h period using a semiquantitative TLC-DPPH radical scavenging method,[3] while the antimicrobial potential was evaluated by the agar overlay technique.[9]




The $CHCl_3$ extract of the root bark of *O. lanceolata* yielded three new dihydro-β-agarofuran sesquiterpene polyesters (**1**−**3**) and two known pentacyclic triterpenoids, **4** and **5**.

Compound **1** was obtained as pale yellow crystals that displayed a molecular ion peak at *m/z* 456.1754 in the HR-TOFEIMS, suggesting the molecular formula $C_{25}H_{28}O_8$ and 12 degrees of unsaturation. Its IR spectrum showed carbonyl absorptions at 1727, 1713 $cm^{-1}$ (esters) and 1697 $cm^{-1}$ (carbonyl) together with a C−O absorption at 1314 $cm^{-1}$, confirming the presence of ester functional groups. The $^{13}C$ NMR spectrum showed two ester carbonyls at δ 161.9 and 161.6, which, on the basis of the $^1H$ NMR and $^{13}C$ NMR data, were identified as a pair of furanoyl esters with similar chemical shifts (Table 1). The presence of the furanoyl esters was supported by observed MS fragment peaks at *m/z* 344 [M − furanoic acid]$^+$, 232 [M − 2 × furanoic acid]$^+$, and 112 [furanoic acid]$^+$. The remaining 15 signals in the $^{13}C$ NMR spectrum were sorted, using the DEPT spectrum, into four methyls at δ 18.7, 19.5, 24.2, and 30.2, three methylenes at δ 31.7, 36.3, and 44.0, two methines at δ 43.7 and 44.2, two oxymethines at δ 73.5 and 77.6, one quaternary carbon at δ 52.9, two oxygenated quaternary carbons at δ 83.0 and 86.6, and one carbonyl carbon at δ 205.1. In addition, the $^1H$ NMR spectrum showed that three of the methyl groups were tertiary (δ 1.27 s, 1.30 and 1.46 s) and one was secondary at δ 1.08 d (7.8), while the signals observed at δ 4.98 (d, 1H, $J$ = 6.0

* To whom correspondence should be addressed. Tel: 267 355-2503. Fax: 267 355-2836. E-mail: mail to: majindar@mopipi.ub.bw.
[†] University of Botswana.
[‡] University of Copenhagen.
[§] University of Johannesburg.

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**Table 1.** $^{13}C$ (75.5 MHz) and $^{1}H$ (300 MHz) NMR Data ($CDCl_3$) for Compounds **1**−**3**

| | $\delta_C$, mult.[a] | | | $\delta_H$ ($J$ in Hz) [b] | | |
|---|---|---|---|---|---|---|
| carbon | **1** | **2** | **3** | **1** | **2** | **3** |
| 1 | 77.6, CH | 75.3, CH | 74.2, CH | 6.01, s | 6.18, s | 5.55, dd (12.0, 4.2) |
| 2 | 205.1, qC | 193.0, qC | 22.0, $CH_2$ | | | 1.98, m |
| | | | | | | 1.68, m |
| 3 | 44.0, $CH_2$ | 127.5, CH | 27.0, $CH_2$ | 2.25, dd (12.9, 1.2) | 6.04, q (1.2) | 2.22, m |
| | | | | 3.36, dd (12.6, 8.1) | | 1.46 br, d (13.5) |
| 4 | 44.1, CH | 155.4, qC | 40.0, CH | 2.44, qui. d (7.8, 1.2) | | 1.87, m |
| 5 | 86.5, qC | 85.2, qC | 87.6, qC | | | |
| 6 | 31.6, $CH_2$ | 33.2, $CH_2$ | 36.6, $CH_2$ | 2.16, 2.11, m | 2.56, ddd (12.6, 4.5, 2.3) | 1.90, m |
| | | | | | 2.00, d (12.6) | 2.05, m |
| 7 | 43.6, CH | 43.6, CH | 43.8, CH | 2.13, m | 2.25, m | 2.01, m |
| 8 | 36.3, $CH_2$ | 30.6, $CH_2$ | 31.7, $CH_2$ | 2.25, d (12.6) | 2.32, dd (6.3, 3.0) | 2.08, m (2H) |
| | | | | 2.03, d (12.3) | 2.17, m | |
| 9 | 73.5, CH | 70.8, CH | 73.9, CH | 4.98, d (6.0) | 5.11 br, d (6.3) | 4.92, d (6.3) |
| 10 | 52.8, qC | 48.5, qC | 48.0, qC | | | |
| 11 | 83.0, qC | 83.7, qC | 82.0, qC | | | |
| 12 | 24.1, $CH_3$ | 23.9, $CH_3$ | 24.2, $CH_3$ | 1.48, s | 1.51, s | 1.38, s |
| 13 | 30.1, $CH_3$ | 30.4, $CH_3$ | 30.2, $CH_3$ | 1.32, s | 1.39, s | 1.22, s |
| 14 | 19.4, $CH_3$ | 19.4, $CH_3$ | 18.6, $CH_3$ | 1.25, s | 1.21, s | 1.30, s |
| 15 | 18.6, $CH_3$ | 19.4, $CH_3$ | 17.8, $CH_3$ | 1.08, d (7.8) | 2.07, d (1.5) | 1.10, d (7.8) |
| a | 161.9, qC | 162.0, qC | 162.3, qC | | | |
| −O−C═O | | | | | | |
| 2′ | 148.0, CH | 148.4, CH | 148.2, CH | 7.85, dd (1.5, 0.6) | 7.88, dd (1.5, 0.6) | 7.93, d (0.9) |
| 3′ | 118.4, qC | 118.7, qC | 119.2, qC | | | |
| 4′ | 109.6, CH | 109.7, CH | 109.8, CH | 6.53, dd (1.8, 0.6) | 6.52, dd (2.0, 0.8) | 6.61, d (1.5) |
| 5′ | 143.3, CH | 143.5, CH | 143.2, CH | 7.33, m | 7.32, m | 7.33, m |
| b | 161.6, qC | 161.5, qC | 162.3, qC | | | |
| −O−C═O | | | | | | |
| 2″ | 147.7, CH | 147.7, CH | 147.1, CH | 7.89, dd (1.5, 0.6) | 7.83, dd (1.5, 0.6) | 7.75, d (0.9) |
| 3″ | 118.9, qC | 118.5, qC | 119.7, qC | | | |
| 4″ | 109.5, CH | 109.6, CH | 109.5, CH | 6.57, dd (1.8, 0.6) | 6.57, dd (2.0, 0.8) | 6.48, d (1.5) |
| 5″ | 143.5, CH | 143.4, CH | 143.2, CH | 7.33, m | 7.32, m | 7.30, m |

[a] Multiplicities were determined by the DEPT sequence. [b] $^{1}H$ chemical shifts were assigned on the basis of the HMQC experiment.

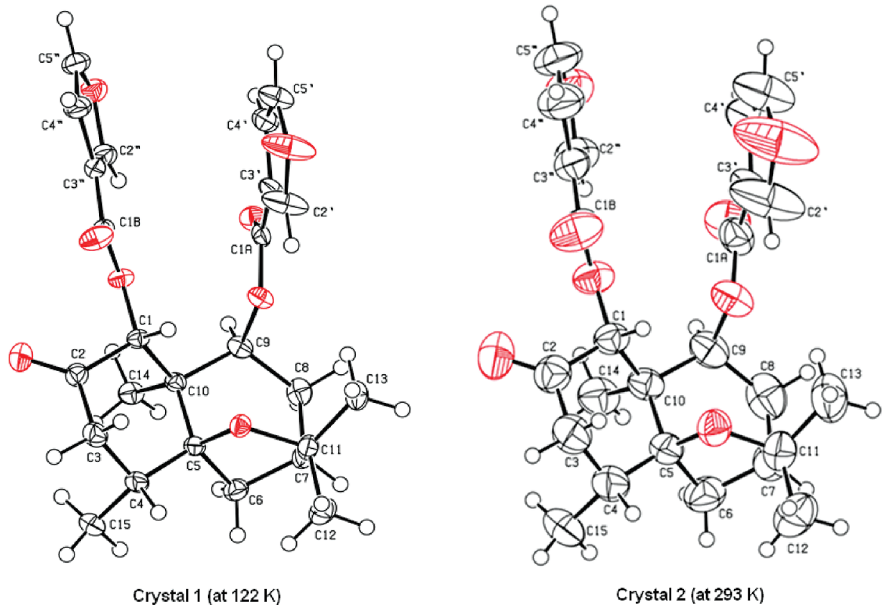


**Figure 1.** ORTEP drawings of compound **1**.

Hz) and 6.01 (s, 1H) were assigned to the two protons attached to the carbons bearing ester groups at $\delta$ 73.5 and 77.6, respectively. Analysis of all the $^{13}C$ and $^{1}H$ NMR chemical shifts (Table 1) suggested a sesquiterpene with a dihydro-$\beta$-agarofuran parent skeleton with two furanoyl esters attached. The chemical shifts of the 15 carbons were indeed similar to those of reported dihydro-$\beta$-agarofuran polyolesters.[10] The positions of the ester groups were located at C-1 and C-9 from the fact that the protons at $\delta$ 4.98 and 6.01 each had an HMBC correlation with C-10 ($\delta$ 52.9), and in addition, the proton at $\delta$ 4.98 had a $^{1}H-^{1}H$ COSY correlation with H-8, while the proton at $\delta$ 6.01 had long-range COSY correlations with H-3 and $H_3$-14, suggesting the assignment of the $\delta$ 4.98 resonance to H-9 and the $\delta$ 6.01 resonance to H-1. The carbonyl carbon at $\delta$ 205.1 was assigned to C-2 due to its HMBC correlations with H-1, $H_2$-3, and H-4. The structure of **1** was further studied by X-ray crystallography (Figure 1). Two independent crystallographic analyses were carried out, and the findings are summarized in Table 2. Both analyses confirmed the location of the carbonyl group at C-2 and the furanoyl ester groups at C-1 and C-9. The orientation of the ester groups was observed from the X-ray crystal structure to be 1$\alpha$ and 9$\beta$, and this was confirmed by the correlations in the NOESY experiment (Figure 2). Compound **1** was identified as a

**Table 2.** X-ray Diffraction Parameters and Statistics of Compound **1**

| | CCDC[a] 746822/1 | CCDC 746823/2 |
|---|---|---|
| empirical formula | $C_{25}H_{28}O_8$ | $C_{25}H_{28}O_8$ |
| $M_r$ | 456.47 | 456.47 |
| $T$ [K] | 122(2) | 293(2) |
| $\lambda$ [Å] | 0.71073 | 0.71073 |
| cryst syst | orthorhombic | orthorhombic |
| space group | $P2_12_12_1$ | $P2_12_12_1$ |
| $a$ [Å] | 10.8670(11) | 11.0809(7) |
| $b$ [Å] | 12.7970(15) | 12.7585(8) |
| $c$ [Å] | 16.6470(19) | 16.7696(11) |
| α [deg] | 90 | 90 |
| $\beta$ [deg] | 90 | 90 |
| $\gamma$ [deg] | 90 | 90 |
| $V$ [Å$^3$] | 2315.0(4) | 2370.8(3) |
| $Z$ (no. of molecules/unit cell) | 4 | 4 |
| $\rho_{calc}$ [g × cm$^{-3}$] | 1.310 | 1.279 |
| $\mu_{MoK\alpha}$ [mm$^{-1}$] | 0.098 | 0.095 |
| $F$(000) | 968 | 968 |
| cryst size [mm$^3$] | 0.36 × 0.25 × 0.11 | 0.48 × 0.34 × 0.24 |
| $\theta$ range [deg] | 2.01−27.51 | 2.20−28.44 |
| reflns collected | 60 561 | 11 308 |
| unique data | 5330 [$R_{int}$ = 0.0443] | 5754 [$R_{int}$ = 0.0416] |
| obsd data [$I > 2\sigma(I)$] | 4888 | 2799 |
| GOF on $F^2$ | 1.023 | 0.990 |
| $R$ indices (all data) | R1 = 0.0422 | R1 = 0.1352 |
| | wR2 = 0.0953 | wR2 = 0.1397 |
| larg diff peak/hole [e Å$^{-3}$] | 0.235/−0.156 | 0.129/−0.166 |

[a] Cambridge Crystallographic Data Centre, http://www.ccdc.cam.ac.uk.

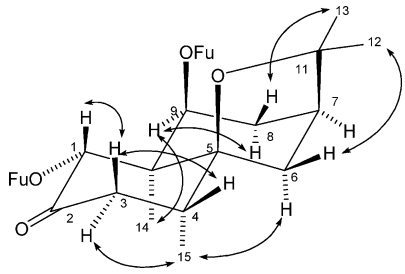


**Figure 2.** Important NOESY correlations for compound **1**.

6-deoxy-2-oxocelorbicol type of dihydro-$\beta$-agarofuran from group 1b of the classification given by Gao et al.[7] Only one member of this group, triptogelin E-4, with one acetate and one benzoate ester moiety, has been reported,[10] and its $^{13}$C and $^1$H NMR chemical shifts for the parent skeleton, including a quintet of doublets for H-4, are in close agreement with compound **1**, with the differences being in the chemical shifts of the different ester groups. Compound **1** was thus characterized as 1α,9$\beta$-difuranoyloxy-2-oxodihydro-$\beta$-agarofuran.

Compound **2**, obtained as fine colorless needles, analyzed for a molecular formula of $C_{25}H_{26}O_8$ by HR-TOFEIMS. The $^1$H and $^{13}$C NMR spectroscopic data (Table 1) revealed that **2** possessed an identical dihydroagarofuran skeleton to that of **1**. The difference in the $^1$H NMR spectrum of **2** was due to the presence of a double bond located between C-3 and C-4, giving rise to a vinylic proton signal at $\delta$ 6.04, which resonated as a quartet with allylic coupling to $H_3$-15. The presence and location of the double bond were confirmed by signals in the $^{13}$C NMR spectrum at $\delta$ 155.4 and 127.5, suggesting a dialkylated and monoalkylated olefinic carbon, respectively. The regiosubstitution of the ester groups was deduced from HMBC and COSY correlations to be the same as in compound **1**. Their orientation was deduced to be the same as in **1** (1α, 9$\beta$) on the basis of NOESY correlations between H-1 and H-3$_{ax}$, H-9 and H-8$_{ax}$, and H-9 and $H_3$-14. Compound **2** was identified as the dehydro analogue of **1** and was characterized as 1α,9$\beta$-difuranoyloxy-2-oxo-3-enedihydro-$\beta$-agarofuran.

**Table 3.** $^{13}$C (150.9 MHz) and $^1$H (600 MHz) NMR Data ($CDCl_3$) for Compound **4**

| carbon | $\delta_C$, mult. | $\delta_H$ ($J$ in Hz) | HMBC[a] |
|---|---|---|---|
| 1 | 22.3, $CH_2$ | 2.01, 1.95, m | H-2 |
| 2 | 41.5, $CH_2$ | 2.28−2.35, m | H-4, H-23 |
| 3 | 213.2, qC | | |
| 4 | 58.2, CH | 2.26, q (6.6) | H-6, H-23, H-24 |
| 5 | 42.1, qC | | H-23, H-25, H-2 |
| 6 | 41.2, $CH_2$ | 2.42, 1.75, m | |
| 7 | 18.2, $CH_2$ | 1.40−1.50, m | |
| 8 | 53.1, CH | 1.39, m | H-15, H-25, H-26 |
| 9 | 37.5, qC | | H-25, H-27 |
| 10 | 59.4, CH | 1.53, m | H-4, H-6, H-7, H-24, H-25 |
| 11 | 35.6, $CH_2$ | 1.38, 1.27, m | H-25, H-27 |
| 12 | 30.3, $CH_2$ | 1.30, 1.50, m | H-27 |
| 13 | 39.7, qC | | H-18, H-26, H-27 |
| 14 | 38.0, qC | | H-26, H-27 |
| 15 | 32.8, $CH_2$ | 1.58, 1.28, m | H-26 |
| 16 | 35.4, $CH_2$ | 1.54, 1.38, m | H-28 |
| 17 | 29.5, qC | | H-28 |
| 18 | 42.5, CH | 1.63, m | H-27, H-28 |
| 19 | 31.2, $CH_2$ | 2.01, 1.98 m | H-29 |
| 20 | 40.3, qC | | H-18, H-29 |
| 21 | 28.2, $CH_2$ | 2.48, td (14.4, 5.4); 1.40, m | H-29 |
| 22 | 38.2, $CH_2$ | 1.50, 1.05, m | H-26, H-28 |
| 23 | 6.8, $CH_3$ | 0.89, d (6.6) | H-4 |
| 24 | 14.6, $CH_3$ | 0.74, s | H-4 |
| 25 | 17.7, $CH_3$ | 0.88, s | |
| 26 | 20.9, $CH_3$ | 1.00, s | |
| 27 | 17.7, $CH_3$ | 1.06, s | H-18 |
| 28 | 32.0, $CH_3$ | 1.08, s | |
| 29 | 31.9, $CH_3$ | 1.33, s | H-18 |
| 30 | 184.7, qC | | H-29, H-19, H-21 |

[a] HMBC correlations are from carbon stated to indicated protons.

Compound **3** was obtained as a yellow paste that analyzed for a molecular formula of $C_{25}H_{30}O_7$ by HR-TOFEIMS. Analysis of the close similarities and the differences found in the $^1$H, $^{13}$C, and NOESY NMR spectra of **3** in comparison with **1** and **2** revealed that **3** was the deoxodihydro analogue of **1** with the same orientation of the ester groups. Thus, **3** was characterized as 1α,9$\beta$-difuranoyloxydihydro-$\beta$-agarofuran.

The two known metabolites were characterized by comparison of their spectroscopic data with literature values as 3-oxo-D:A-friedooleanan-30-oic acid or octandronic acid (**4**)[5,11] and 20-epikoetjapic (**5**).[6]

In the semiquantitative TLC-DPPH radical scavenging assay, using gallic acid (GA) and ascorbic acid (AS) as standards, compounds **1**−**5** did not scavenge DPPH radical within 30 min (Table 4). However, **1**−**3** showed weak activity after 2 h and further activity after 8 h, indicating slow antioxidant kinetics. Compounds **4** and **5** showed activity only after 8 h, while crude CE showed moderate activity within 30 min (at MIQ 10 $\mu$g) and also displayed slow radical scavenging kinetics as previously observed.[3] Plant derivatives with slow antioxidant kinetics such as **1**−**3** and CE could find application for use in formulations where protection against free radicals is required over several hours.[12]

Antimicrobial evaluation (Table 5) showed that, of the five compounds, **5** displayed the strongest activity, comparable to standards, against *Staph. aureus* and *C. albicans* and moderate activity against *B. subtilis*, *P. aeruginosa*, and *E. coli*. Compound **4** showed moderate activity against *B. subtilis*, *P. aeruginosa*, and *C. albicans* and weak activity against *Staph. aureus*. The $CHCl_3$ extract showed moderate activity against the fungus (*C. albicans*) and weak to moderate activity against both Gram-positive and Gram-negative bacteria. Compounds **3** and **1** were active against all the bacteria and the fungus tested, while **2** showed weak activity against *C. albicans* and against Gram-negative bacteria (*P. aeruginosa* and *E. coli*) but no activity against Gram-positive bacteria (*B. subtilis* and *Staph. aureus*). The antimicrobial activity of the

**Table 4.** TLC-DPPH Radical Scavenging Activity of Compounds **1**−**5**[a]

| sample | minimum radical scavenging quantities in μg (intensity of spot) 30 min | 2 h | 8 h |
|---|---|---|---|
| **1** | NA | 10 (W) | 10 (M) |
| **2** | NA | 50 (W) | 10 (W) |
| **3** | NA | 10 (W) | 10 (M) |
| **4** | NA | NA | 10 (W) |
| **5** | NA | NA | 10 (W) |
| CE | 10 (M) | 10 (M) | 0.5 (W) |
| GA | 0.01(M) | 0.01(M) | 0.01 (M) |
| AS | 10 (S) | 0.5 (W) | 0.01 (W) |

[a] NA = not active at 100 μg, W = weak, M = moderate, S = strong CE = chloroform extract, GA = gallic acid, AS = ascorbic acid.

**Table 5.** Antimicrobial Activities of Compounds **1**−**5** by the Agar Overlay Technique[a]

| | microorganisms and minimum inhibiting quantities (MIQ) of the compounds (μg) | | | | |
|---|---|---|---|---|---|
| | Gram-positive bacteria | | Gram-negative bacteria | | fungus |
| | *B. subtilis* | *Staph. Aureus* | *P. aeruginosa* | *E. coli* | *C. albicans* |
| sample | | | | | |
| **1** | 10 | 50 | 10 | 10 | 0.5 |
| **2** | NA | NA | NA | 50 | 10 |
| **3** | 0.5 | 10 | 0.5 | 10 | 0.01 |
| **4** | 0.01 | 0.5 | 0.01 | 0.01 | 0.01 |
| **5** | 0.01 | 0.001 | 0.01 | 0.01 | 0.001 |
| CE | 0.5 | 10 | 0.5 | 0.5 | 0.01 |
| ChP | 0.001 | 0.001 | 0.001 | 0.001 | |
| Mic | | | | | 0.001 |

[a] CE = chloroform extract, ChP = chloramphenicol, Mic = miconazole NA = not active at 100 μg.

agarofurans **1**−**3** appeared to be influenced by the presence or absence of an α,β-unsaturated carbonyl system. Compound **2**, with its α,β-unsaturated carbonyl, was observed to be the least active. Absence of the C-3−C-4 double bond (as in **1**) increased antimicrobial activity, while absence of the double bond as well as the carbonyl group at C-1 (as in **3**) further promoted antimicrobial activity. Good antibacterial activity for **5** (20-epikoetjapic acid) and its epimer, koetjapic acid, has previously been observed.[6]

## Experimental Section

**General Experimental Procedures.** Melting points (uncorrected) were recorded on a Stuart Scientific (SMP1) melting point apparatus. Optical rotation values were recorded at 20 °C in $CHCl_3$ using an Autopol IV automatic polarimeter. UV spectra were obtained in $CHCl_3$, using a Shimadzu UV-2101PC UV−vis scanning spectrophotometer. FTIR spectra of solid samples were taken on a Perkin-Elmer Spectrum 100 spectrophotometer fitted with a universal attenuated total reflectance (UATR) sampling device. $^1$H NMR at 300 or 600 MHz, $^{13}$C NMR at 75.5 or 150.9 MHz, DEPT, COSY, HMQC, and HMBC spectra were recorded in $CDCl_3$ using TMS as internal standard on Bruker Avance DPX 300 or DRX 600 spectrometers. HR-TOFEIMS spectra were measured using a Waters GCT Premier mass spectrometer. Column chromatography was carried out using silica gel 60 (0.040−0.063 mm, Merck). Preparative TLC was performed with silica gel 60 $PF_{254+366}$ (Merck). Analytical TLC was carried out on precoated silica gel 60 $F_{254}$ aluminum sheets (0.25 mm layer, Merck.). Visualization of chromatograms was done with UV light at λ 254 and 366 nm and with vanillin−$H_2SO_4$ spray.

**Plant Material.** The roots of *O. lanceolata* were obtained from Otse, Botswana, in August 2007. The root bark was separated from the root wood and air-dried for two weeks and then ground into powder.

**Extraction and Isolation.** The powdered root bark (960 g) was extracted exhaustively and sequentially with *n*-hexane, $CHCl_3$, MeOH, and 90% MeOH/$H_2O$. The crude $CHCl_3$ extract (80 g) was adsorbed on 100 g of a mixture of fine silica gel 60 (0.040−0.063 mm), coarse silica gel 100 (0.2−0.5 mm), and acid washed sand and applied onto a glass column (70 × 8 cm) packed with 600 g of silica gel 60 (0.040−0.063 mm). The column was gradiently eluted with petroleum ether (60−80 °C) containing increasing proportions of $CHCl_3$ up to 100%. Elution was continued with $CHCl_3$ containing increasing proportions of MeOH up to 30%. Ninety-six fractions (250 mL each) were collected, concentrated under reduced pressure at 35 °C, and on the basis of their TLC profiles, combined to give 11 subfractions, 1A−11A, as follows: 1A (1−4), 2A (5 and 6), 3A (7−16), 4A (17−23), 5A (24−34), 6A (35−41), 7A (42−55), 8A (56−73), 9A (74−78), 10A (79−86), and 11A (87−96). Analytical TLCs of the fractions using $CHCl_3$/*n*-hexane/acetone (6:3:1) showed 7A to have the highest number of bands. Fraction 7A (25.0 g, yellow paste) was adsorbed on ∼30 g of silica gel, then loaded onto a column packed with 300 g of silica gel in petroleum ether/acetone (6:3) and eluted isocratically with the same solvent system. Eighty-four fractions (100 mL each) were collected, out of which fractions 1−8 did not show any spots on TLC and were therefore discarded. The remaining fractions were combined to give six subfractions, 7A1−7A6, as follows: 7A1 (9−12), 7A2 (13−16) 7A3 (17−24), 7A4 (25−37), 7A5 (38−47), and 7A6 (48−84). A white solid formed in subfraction 7A5, which was filtered off and washed with petroleum ether/acetone (6:3) to give compound **5** as an amorphous, white solid (50 mg, $R_f$ = 0.27). The filtrate of subfraction 7A5 was concentrated under reduced pressure to a mass of 6.0 g, which was subjected to silica gel column chromatography and eluted isocratically using petroleum ether/acetone (8:2). Forty-five subfractions of 50 mL each, 7A5(1)−7A5(45), were obtained with different types of crystals forming in the conical flasks. The crystals were filtered off and analyzed by TLC using *n*-hexane/acetone, 6:3. Compound **1** crystallized from subfractions 7A5 (13 and 14) as fairly large pale yellow translucent crystals (65 mg, $R_f$ = 0.37) and compound **2** from fractions 7A5 (24 and 25) as fine colorless needles (50 mg, $R_f$ = 0.29). Fractions 7A5 (10 and 11) yielded **3** (40 mg, $R_f$ = 0.55) as a yellow paste, while fraction 7A5(6) yielded **4** as white crystals (15 mg, $R_f$ = 0.49).

**1α,9β-Difuranoyloxy-2-oxo-dihydro-β-agarofuran (1):** pale yellow crystals (petroleum ether/acetone, 8:2); mp 162−164 °C; $[\alpha]^{20}_D$ +75 (*c* 0.14, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 255 (3.15) nm; IR (UATR crystal) $\nu_{max}$ 3159, 2975, 1727 (C═O), 1713 (C═O), 1697 (C═O), 1573, 1508, 1383, 1315 (C−O), 1158, 1133, 1074, 1003, 968, 872, 758 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; EIMS [*m*/*z*] (% rel int) 456 $[M]^+$ (14.4), 344 [M − furanoic acid]$^+$ (21.3), 232 [M − 2 × furanoic acid]$^+$ (10.1), 179 (11.5), 137 (28.9), 124 (100), 112 [furanoic acid]$^+$ (5.0), 105 (7.0); HR-TOFEIMS *m*/*z* 456.1754 (calcd for $C_{25}H_{28}O_8$, 456.1784).

**1α,9β-Difuranoyloxy-2-oxo-3-enedihydro-β-agarofuran (2):** colorless needles (petroleum ether/acetone, 8:2); mp 232−234 °C; $[\alpha]^{20}_D$ +158 (*c* 0.11, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 255 (3.31), 328 (1.71) nm; IR (UATR crystal) $\nu_{max}$ 3160, 2975, 1728, 1711, 1697 (C═O), 1575, 1508, 1404, 1384, 1316 (C−O), 1157, 1132, 1004, 872, 757, 731 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; EIMS [*m*/*z*] (% rel int) 454 $[M]^+$ (100.0), 439 [M − Me]$^+$ (3.0), 342 [M − furanoic acid]$^+$ (53.1), 275 (89.7), 248 (51.9), 232 [M − 2 × furanoyl]$^+$ (73.7), 230 [M − 2 × furanoic acid]$^+$ (43.9), 187 (25.2), 159 (61.5), 138 (36.3), 120 (40.9), 112 [furanoic acid]$^+$ (16.0), 105 (22.4); HR-TOFEIMS *m*/*z* 454.1620 (calcd for $C_{25}H_{26}O_8$, 454.1628).

**1α,9β-Difuranoyloxydihydro-β-agarofuran (3):** yellow paste; $[\alpha]^{20}_D$ +65 (*c* 0.26, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 258 (2.93) nm; IR (UATR crystal) $\nu_{max}$ 3149, 2929, 1706 (C═O), 1633, 1576, 1508, 1384, 1311 (C−O), 1241, 1157, 1075, 1006, 967, 872, 756 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; EIMS [*m*/*z*] (% rel int) 442 $[M]^+$ (100.0), 427 [M − Me]$^+$ (27.2), 330 [M − furanoic acid]$^+$ (47.6), 315 (24.9), 235 (19.0), 218 [M − 2 × furanoic acid]$^+$ (41.0), 203 (52.1), 159 (36.5), 137 (39.7), 124 (54.7), 112 [furanoic acid]$^+$ (11.0), 105 (33.6); HR-TOFEIMS *m*/*z* 442.2010 (calcd for $C_{25}H_{30}O_7$, 442.1992).

**3-Oxo-D:A-friedooleanan-30-oic acid or octandronic acid (4):** white crystals (petroleum ether/acetone, 8:2), mp 260−262 °C; $[\alpha]^{20}_D$ +23 (*c* 0.094, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log ε) 241 (2.23), 285 (1.79) nm; IR (UATR crystal) $\nu_{max}$ 3500−3200 br. (OH), 2928, 1699 (C═O), 1578, 1450, 1386, 1313 (C−O), 1203, 1153, 1105, 1077, 1004, 872, 755 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 2; EIMS [*m*/*z*] (% rel int) 456 $[M]^+$ (87.7), 410 [M − HCOOH]$^+$ (22.2), 371 (36.6), 303 (12.0), 273 (33.3), 248 (58.9), 189 (36.4), 163 (37.7), 155 (53.1), 147 (27.1), 135 (43.4), 121 (73.0), 109 (100.0), 107 (73.0), 105 (39.3); HR-TOFEIMS *m*/*z* 456.3615 (calcd for $C_{30}H_{48}O_3$, 456.3603).

**3,4-Seco-4(23),12-oleandiene-3,29-dioic acid or 20-epikoetjapic acid (5):** white, amorphous solid, $[\alpha]^{20}_{D}$ +60 (*c* 0.12, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 241(2.70) nm [lit.[6] $[\alpha]^{25}_{D}$ +32 (*c* 0.19, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 219 (313) nm]; IR, $^1$H and $^{13}$C NMR data were in agreement with reported data;[6] EIMS [*m*/*z*] (% rel int) 470 $[M]^+$ (23.6), 424 $[M - HCOOH]^+$ (8.0), 397 (3.0), 248 (100.0), 204 (7.0), 187 (56.6), 173 (48.5), 147 (18.2), 133 (19.5), 119 (26.7), 107 (32.5); HR-TOFEIMS *m*/*z* 470.3374 $[M]^+$ (calcd for $C_{30}H_{46}O_4$, 470.3396).

**Crystallographic Analysis of Compound 1.** X-ray crystallography was carried out at two different institutions on two different crystals of **1** at 122 and 293 K, respectively, and both analyses confirmed the same structure as illustrated in Figure 1.

**Crystal 1.** Intensity data were collected on a single crystal of **1** at 122 K on a Bruker-Nonius KappaCCD diffractometer equipped with an Oxford Cryostream and graphite-monochromatized Mo Kα radiation. Data were reduced with EvalCCD[13] and the structures solved by direct methods and refined by least-squares against $F^2$ with SHELX97[14,15] as incorporated in the maXus program.[16] Compound **1** crystallizes in the orthorhombic space group $P2_12_12_1$ (no. 19).[17] This space group does not have symmetry elements involving either inversion or mirror imaging. Because of lack of heavy-atom scatters, the absolute configuration of the compound could not be established.[18] All non-hydrogen atoms were refined with anisotropic temperature factors. An ORTEP[19] drawing of compound **1**, crystal 1 is shown in Figure 1a. Hydrogen atoms were found in subsequent difference Fourier maps, and their temperature factors were refined using a riding model.

**Crystal 2.** The X-ray intensity data for a single crystal of compound **1** (i.e., crystal 2) were measured at room temperature (293 K) on a Siemens SMART 1K CCD area detector diffractometer system equipped with a graphite monochromator and a Mo Kα fine-focus sealed tube ($\lambda$ = 0.71073 Å) operated at 1.6 KW power (40 kV, 40 mA). The detector was placed at a distance of 4.00 cm from the crystal. The initial unit cell and data collection was achieved by the SMART-NT software,[20] utilizing COSMO[21] for optimum collection of more than a hemisphere of reciprocal space. A total of 830 frames were collected with a 0.3° scan width in $\omega$ and an exposure time of 30 s frame$^{-1}$. The structure was solved by the direct methods package SIR97[22] and refined using the WinGX software package[23] incorporating SHELXL.[15] An ORTEP[19] drawing of crystal 2 is shown in Figure 1 and is very similar to that of crystal 1, only with slightly enlarged ellipsoids because of the higher temperature for the experiment.

X-ray diffraction parameters and statistics from crystals 1 and 2 are collated in Table 2. Two sets of supplementary crystallographic data for **1** have been deposited with the Cambridge Crystallographic Data Centre under deposition numbers CCDC 746822 and CCDC 746823, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

**Biological Activity Tests. Reagents.** DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent and thiazolyl blue were purchased from Fluka Chemicals (Steinheim, Germany); ascorbic acid (AR) was purchased from UNILAB Chemicals (South Africa); gallic acid, chloramphenicol, and miconazole were obtained from Sigma Chemicals (Steinheim, Germany); nutrient broth, nutrient agar, mycological peptone, and Sabouraud dextrose agar were purchased from OXOID LTD (Hampshire, England); MeOH (AR) and $CHCl_3$ (AR) were purchased from Rochelle Chemicals (South Africa).

**DPPH Radical Scavenging Assay by a Semiquantitative TLC Procedure.** A semiquantitative TLC-DPPH procedure[3] was followed. The time taken for yellow spots to appear against a purple background on the TLC sheets was taken as a measure of the kinetics of the radical scavenging reaction, while the amount of sample that produced the least visible yellow spot was recorded as the minimum quantity required for scavenging DPPH radical. The experiment was performed in duplicate.

**Antimicrobial Activity by the Agar Overlay Method.** The antimicrobial activity of the compounds and the crude $CHCl_3$ extract was evaluated using an adaptation of the agar overlay technique.[10] The microorganisms, two Gram-positive bacteria (*Bacillus subtilis* -ATCC 6633 and *Staphylococcus aureus*ATCC 9144), two Gram-negative bacteria (*Pseudomonas aeruginosa* NCTC 10332 and *Escherichia coli* ATCC 11229), and one fungus (*Candida albicans* ATCC 10231), were obtained from the Department of Biology, Microbiology section, of the University of Botswana. See Supporting Information for a detailed description of the experimental procedure and sample results.

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**Supporting Information Available:** $^1$H and $^{13}$C NMR, COSY, HMQC, HMBC, IR, and HR-TOFEIMS spectra for compounds **1**−**4** and CIF files of X-ray crystallographic data of compound **1** are supplied in the supplementary data. This material is available free of charge via the Internet at http://pubs.acs.org.

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