Heptacyclosordarianone, a New Polyketide From *Sordaria* sp., an Endophytic Fungus From *Garcinia polyantha*

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Abstract

One new polyketide derivative, heptacyclosordarianone (I), together with 2 known compounds, heptacyclosordariolone (2) and sordariol (3), were isolated from an extract of *Sordaria* sp. AM-71, an endophytic fungus inhabiting *Garcinia polyantha*. The structures of these compounds were elucidated based on intensive nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry and by comparison with literature data. All compounds were assayed for their antimicrobial and cytotoxic properties. Only compound 2 showed antimicrobial activities against *Bacillus subtilis* DSMZ 704, *Pseudomonas agarici* DSMZ 11810, and *Micrococcus luteus* DSMZ 1605 with minimum inhibitory concentration values of 9.3, 15.5, and 16.9 µg/mL respectively. None of the isolated compounds showed a significant cytotoxic property.

Keywords

*Garcinia*, *Sordaria* sp., polyketide, antimicrobial activity

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Endophytes are rich sources of structurally diverse bioactive metabolites that have a wide range of activities, such as antibacterial, antifungal, antiviral, anti-inflammatory, neuroprotective, and cytotoxic.¹ Over the past 50 years, several medicinally approved drugs have been obtained from fungi, for example, penicillin, the first β-lactam antibiotic.² It has been estimated that less than 1% of bacterial species and less than 5% fungal species are currently known, suggesting that a lot of microorganism species are yet to be discovered. The constituents of fungi from the genus *Sordaria* have attracted attention since the compounds isolated so far from the genus have been shown to possess new carbon skeletons.³,⁴ However, little information on the chemistry and biochemistry of the *Sordaria* genus is available. It has been reported to contain only salicyl aldehyde-type polyketides and their dimers.⁴ Although there are numerous reports on the endophytic fungi of different plants, to the best of our knowledge, there is no study on *Garcinia polyantha* so far. This has been found to be a rich source of bioactive xanthones, benzophenones, and bioflavonoids.⁵,⁶

In our continuing search for new active compounds from endophytic fungi in *Garcinia* plants,¹³,¹⁴ an endophytic fungus *Sordaria* sp. was isolated from *G. polyantha* collected in Kalla mountain, Center Region of Cameroon. The investigation of this endophytic fungus led to the isolation of one new polyketide derivative, heptacyclosordarianone (I), together with 2 known compounds (2 and 3). We describe herein the isolation, structure elucidation, and evaluation of the antimicrobial and cytotoxic activities of these compounds (Figure 1).
Results and Discussion

Compound 1 was isolated as a white amorphous powder and was assigned the molecular formula C_{12}H_{12}O_{3} based on the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) ion [M + Na]^+ at m/z 227.0680 (calc. 227.0684), indicating 7 degrees of unsaturation. Its ultraviolet (UV) spectrum in methanol exhibited maxima at 255 and 282 nm indicative of a conjugated chromophore, and its infrared (IR) (Supplemental Figure S12) spectrum with absorptions at 3357 and 1669 cm\(^{-1}\) suggested the presence of a hydroxyl and carbonyl groups, respectively. The proton resonances assigned to the relevant carbon atoms by the heteronuclear single quantum correlation (HSQC) spectrum (Supplemental Figure S1, S2 and S4) showed the presence of 12 carbons, which can be sorted into 1 methyl (\(\delta\) 25.7; 2.27, 3H, s), 2 methylenes (\(\delta\) 31.0; 3.69, 2H, d, \(J\) = 6.0 Hz), (63.2; 5.39, 2H, s), 4 sp\(^2\) methines (\(\delta\) 107.1; 5.95, 1H, t, \(J\) = 6.0 Hz), (114.5; 6.74, 1H, m), (120.8; 6.74, 1H, m), and (129.7; 7.14, 1H, t, \(J\) = 7.8\(\delta\)), 2 olefinic quaternary carbons (\(\delta\) 122.2 and 142.6), and 3 oxygenated sp\(^2\) carbons (\(\delta\) 153.1, 153.4, and 196.3). The above NMR data (Table 1) suggested that compound 1 has a 1,2,3-trisubstituted benzene ring (\(\delta\)H 7.14 (1H, t, \(J\) = 7.8), 6.74 (1H, m) and 6.74 (1H, m)) and 1 carbonyl group. The benzene ring and the carbonyl carbon occupying 5 degrees of unsaturation, suggested that compound 1 possessed an additional ring, including 1 double bond. These NMR data suggested 1 to be a 2-benzoepin derivative, with close similarity to heptacyclosordariolone (2). The main differences observed were the presence of an additional methylene group (\(\delta\) 31.0; 3.69, 2H, d, \(J\) = 6.0), 1 methine group (\(\delta\) 107.1; 5.95, 1H, t, \(J\) = 6.0), 1 oxygenated sp\(^2\) carbon (\(\delta\) 153.4), 1 carbonyl, and the appearance of 1 downfield methyl group (\(\delta\) 25.7; 2.27, 3H, s). The oxepine ring system was confirmed by the heteronuclear multiple bond correlation (HMBC) spectrum (Supplemental Figure S6) where correlations were observed between H-1 (\(\delta_{\text{H}}\) 5.39) and C-3 (\(\delta_{\text{C}}\) 153.4), C-5a (\(\delta_{\text{C}}\) 142.6) and C-9a (\(\delta_{\text{C}}\) 122.2), H-5 (\(\delta_{\text{H}}\) 3.69) and C-9a, C-5a, C-3, and C-4 (\(\delta_{\text{C}}\) 107.1), and between H-4 (\(\delta_{\text{H}}\) 5.95) and C-3, C-5a. The location of the ketone side chain was confirmed to be at C-3 by the HMBC peaks from H-4 to C-3 and C-1’, and from H-2’ (\(\delta_{\text{H}}\) 2.27) to C-3 and C-1’ (\(\delta_{\text{C}}\) 196.3). Together with correlation spectroscopy (COSY) (Supplemental Figure S5), HSQC (Supplemental Figure S4), and HMBC, the structure of compound 1 was determined as 1-(9-hydroxy-1,5-dihydrobenzoc[\(\text{e}\]xepin-3-yl)ethanone, trivially named heptacyclosordarianone, and described here for the first time (Figure 2).

Table 1. \(^1\text{H}\) (500 MHz) and \(^{13}\text{C}\) (125 MHz) Nuclear Magnetic Resonance Spectroscopic Data of Compounds 1 in Deuterated Chloroform and 2 in Deuterated Methanol (\(\delta\) in ppm, \(J\) in Hz).

| Position | \(\delta_{\text{H}}\) | \(\delta_{\text{C}}\) | \(\delta_{\text{H}}\) | \(\delta_{\text{C}}\) |
|----------|----------------|----------------|----------------|----------------|
| 1        | 5.39 (s)       | 63.2           | 4.98 (d, \(J\) = 13.7) | 56.4          |
|          |                |                | 4.44 (d, \(J\) = 13.7) |                |
| 3        |                | 153.4          |                | 103.9         |
| 4        | 5.95 (t, \(J\) = 6.0) | 107.1          | 5.77 (d, \(J\) = 12.6) | 130.1         |
| 5        | 3.69 (d, \(J\) = 6.0) | 31.0           | 6.59 (d, \(J\) = 12.6) | 131.0         |
| 5a       |                | 142.6          |                | 136.7         |
| 6        | 6.74 (m\(^{a}\)) | 120.8          | 6.65 (brd, \(J\) = 8.0) | 114.0         |
| 7        | 7.14 (t, \(J\) = 7.8) | 129.7          | 7.00 (t, \(J\) = 7.9) | 127.6         |
| 8        | 6.74 (m\(^{a}\)) | 114.5          | 6.74 (brd, \(J\) = 7.5) | 121.6         |
| 9        |                | 153.1          |                | 153.5         |
| 9a       |                | 122.2          |                | 126.3         |
| 1’       |                | 196.3          | 3.98 (q, \(J\) = 6.5) | 66.7          |
| 2’       | 2.27 (s)       | 25.7           | 0.90 (d, \(J\) = 6.5) | 14.9          |
| 9-OH     |                | 5.11 (s)       |                |                |

*Overlapped signals determined by heteronuclear single quantum correlation and heteronuclear multiple bond correlation spectra.

The known compounds (Figure 1) were identified as heptacyclosordariolone (2) and sordanol (3). None of the isolated compounds has been previously reported from the host plant.

Based on an agar diffusion method, the antibacterial activity of all compounds was tested against *Escherichia coli* DSMZ 1058, *Bacillus subtilis* DSMZ 704, *Micrococcus luteus* DSMZ 1605, 

Figure 1. Chemical structures of compounds 1-3

Table 1. \(^1\text{H}\) (500 MHz) and \(^{13}\text{C}\) (125 MHz) Nuclear Magnetic Resonance Spectroscopic Data of Compounds 1 in Deuterated Chloroform and 2 in Deuterated Methanol (\(\delta\) in ppm, \(J\) in Hz).

Figure 2. Key heteronuclear multiple bond correlation and correlation spectroscopy correlations of compound 1.
**Pseudomonas agarici** DSMZ 11810, and *Staphylococcus warneri* DSMZ 20036. Compound 2 showed interesting activities with minimum inhibitory concentration (MIC) values of 9.3 µg/mL for *B. subtilis*, 15.5 µg/mL for *P. agarici*, and 16.9 µg/mL for *M. luteus*, highlighting its possible use as a lead in the control of bacterial infections. Gentamycin was used as a reference drug.

Cell-based cytotoxicity assays of all isolated compounds against the human cervix carcinoma cell line KB-3-1 in a resazurin assay was undertaken according to our previous study. However, all the tested compounds were found to be inactive against the KB-3-1 cell line.

**Experimental**

**General Experimental Procedures**

IR and UV spectra were respectively recorded with JASCO A-302 IR, and Jasco J-20A, Shimadzu UV mini-1240 spectrophotometers. 1H, 13C, and 2D-NMR spectra were recorded on Bruker AMX-500 and Jeol ECZ-600 spectrometers. Proton and carbon chemical shifts are reported in δ (ppm) with reference to TMS. Coupling constants (J) are given in Hz. Homonuclear 1H-1H connectivity was determined by COSY 45° experiment. One-bond 1H-13C connectivities were determined by HMOC. 1H-13C connectivity over 2 or 3 bonds was detected by HMBC. The ESI-MS was recorded on a Double-Focusing Mass Spectrometer (Varian MAT 311A). HR-ESI-MS were recorded on JEOL HX 110 and Synapt G2 Mass Spectrometers. Column chromatography was carried out on silica gel 60 (70, 230, and 240-300 mesh sizes, E. Merck) and ODS (Fuji Silysia, Japan). Precasted silica gel thin-layer chromatography plates supported on aluminum sheets (E. Merck, F254) were used to check the purity of compounds, and compounds were detected either by spraying with 10% vanillin in sulfuric acid followed by heating, or by UV irradiation. The vanillin/sulfuric acid spray reagent was prepared by dissolving 1.0 g of vanillin in 100 mL of concentrated sulfuric acid.

**Fungal Material**

The fungal strain *Sordaria* sp. AM-71 was isolated from the stem bark of *G. polyantha*, a plant collected in Kala mountain in Yaoundé, Center Region of Cameroon. The plant material was authenticated by Mr NANA Victor of the National Herbarium of Cameroon in Yaoundé, where a voucher specimen (21337/SRF/Cam/Mt Kala) is deposited. The stem bark samples were aseptically cleaned successively with 70% ethanol (EtOH) for 1 minute, 5% sodium hypochlorite for 5 minutes, and 70% EtOH for 1 minute, then rinsed in sterile water 2 times. The aseptically clean samples were dried on sterilized paper and cut into 1-cm pieces. The pieces were placed on plates of Potato-Dextrose-Agar (PDA) containing chloramphenicol (100 mg/L). After incubation at 25 °C for 7 days, the hyphal tips of the fungi on the plates were removed from the agar plates and transferred to new PDA plates (slant). The strain AM-71 was isolated and grown on slants of PDA as silver to black colored cultures. This strain was identified as *Sordaria* sp. by BEX Co. LTD., Japan, using DNA analysis of the 18S rDNA regions and deposited at the laboratory in the Faculty of Agriculture of Yamagata University, Japan.

**Extraction and Isolation**

*Sordaria* sp. was cultivated in 10 Erlenmeyer flasks (1000 mL each) each containing 50 g of sterile steamed unpolished rice. Each flask received about 5 small pieces of mycelium from a PDA plate under sterile conditions. After 4 weeks growing at 25 °C, the moldy unpolished rice was extracted with ethyl acetate (EtOAc, 2.0 L), and the extract taken to dryness. The resulting concentrate was further partitioned into n-hexane (0.5 L), EtOAc (0.5 L), and aqueous layers (0.5 L). The EtOAc layer (4.32 g) was chromatographed on a silica gel column with stepwise elution with n-hexane-EtOAc (100:0-100, each 300 mL) and EtOAc/MeOH (50:50, 0:100, each 300 mL), respectively, to afford fractions A (0%-20% n-hexane/EtOAc, 0.7 g), B (30%-50% n-hexane/EtOAc, 60.0 mg), C (90%-100% n-hexane/EtOAc, 40.0 mg), D (0%-10% EtOAc/MeOH 1.3 g), E (20%-30% EtOAc/MeOH, 1.2 g), and F (40%-100% EtOAc/MeOH, 0.9 g). Fraction A (0.7 g) was further chromatographed on a silica gel column using stepwise elution with chlorofom (CHCl₃)-EtOAc to afford fractions A1-A6. Fractions A3 and A5 (CHCl₃-EtOAc, 50:50 and 40:60, 60 mg) were further separated by octadecyl silica gel column chromatography eluting with acetonitrile-water (60:40) to give sordariol (2) (15.2 mg). Fractions B and C were combined and further rechromatographed on silica gel using a gradient system of CHCl₃/EtOAc (100:0-0:100) and EtOAc/MeOH (50:50-0:100) to afford fractions B1-B-6. Fraction B-3 (CHCl₃/EtOAc, 50:50-43.0 mg) was purified by ODS column chromatography using a gradient of water-methanol (20:80) to afford compounds 1 (7.0 mg) and 3 (15.0 mg).

**Heptacyclorsordarione (1).** White amorphous powder; IR (KBr): 3357 (-OH) and 1669 (-C = O) cm⁻¹; HREIMS: m/z 227.0680 [M + Na]+, (calcd. for C₁₂H₁₂O₃Na, m/z 227.0684). 1H-NMR (CDCl₃, 500 MHz), δ (ppm): 2.27 (3H, s, H-2′), 3.69 (2H, d, J = 6.0, H-6), 5.39 (2H, s, H-1′), 5.95 (1H, t, J = 6.0, H-4), 6.74 (2H, m, H-6,8), 7.14 (1H, t, J = 7.8, H-7). 13C-NMR (CDCl₃, 150 MHz), δ (ppm): 25.7 (C-2′), 31.0 (C-5), 63.2 (C-1), 107.1 (C-4′), 114.5 (C-8′), 120.8 (C-6′), 122.2 (C-9′), 129.7 (C-7′), 142.6 (C-5a), 153.1 (C-9), 153.4 (C-3′), 196.3 (C-1′) (Table 1).

**Heptacyclorsdarionone (2).** Amorphous powder. 1H and 13C NMR: see Supplemental Figures S8 and S9.

**Sordanol (3).** Yellow powder. 1H and 13C NMR: see Supplemental Figures S10 and S11.
Antimicrobial Assay

Antimicrobial activity of compounds 1-3 was determined against a set of microorganisms using the agar diffusion technique.\textsuperscript{15,17} Either nutrient broth agar or Trypticase Soy Broth (depending on the strain), together with 0.2 mL of a bacterial suspension from a culture with an optical density between 0.05 and 0.1, was plated in a Petri dish. A sterile paper disk (6 mm diameter) with 25 µL (1 mg/mL) of test solution was placed on each bacteria plate using sterile forceps and incubated at optimal temperatures; 25 µL (500 µg/mL) of gentamycin was used as the reference. The inhibition zones around the paper disks were measured after 24 hours in mm. Each test was conducted 2 or 3 times. Inhibitory zones of 6.5-8.0 mm were considered to reflect mediocre activity and inhibitory zones greater than 10.0 mm as significant activity. MIC values were determined only for active test compounds.

Cell culture was done overnight at different temperatures with the culture media depending on the strain. The cell suspension was prepared to an optical density (OD 600) of 0.05. The culture medium (100 µL) per well in a 96-well plate was dispensed for suspension cells. Dilution series of the test compounds were prepared from stock solution in dimethylsulfoxide (DMSO)/culture media (ca. 1:10) to concentrations of either 250 µg/mL or 500 µg/mL. Each concentration was tested in 2 or 4 replicates. The positive control was the strain without drugs, and gentamycin (250 µg/mL-0.12 µg/mL) was used as the reference drug. The cell suspension (100 µL) was pipetted into each well except for the negative control (a total of 200 µL in each well). The plates were shaken on a plate shaker for 8 hours at optimal temperature and measured using a Tecan reader at 600 nm. MIC values were calculated as a sigmoidal dose-response curve using GRAPHPAD PRISM 4.03.

Cytotoxicity Assay

The cytotoxicity of 1-3 against the human cervix carcinoma cell line KB-3-1 was evaluated as previously reported.\textsuperscript{16} The KB-3-1 cells were cultivated in 1 layer in Dulbecco’s Modified Eagle medium with glucose (4.5 g/L), l-glutamine, sodium pyruvate, and phenol red, supplemented with 10% fetal bovine serum (FBS). The cells were then maintained at 37 °C in 5.3% carbon dioxide (CO\textsubscript{2}) humidified air. The cells were isolated at 70% confluence, treated with trypsin solution (0.5%), and placed in sterile 96-well plates at a density of 10 000 cells in 100 mL medium per well on the day before the test. The compounds were diluted from stock solutions in DMSO at concentrations of 100 mM, 50 mM, or 25 mM. Stock solutions were then diluted with culture medium (10% FBS [KB-3-1]) down to pM range and added to the wells. Each concentration was tested in 6 replicates. Dilution series were prepared by pipetting liquid from well to well. The control was made up of the same concentration of DMSO as the first dilution. After incubation for 72 hours at 37 °C in 5.3% CO\textsubscript{2}/humidified air, 30 mL of an aqueous resazurin solution (175 mM) was added to each well. The cells were incubated under the same conditions for 5 hours and fluorescence measured thereafter. The half-maximal inhibitory concentration values (the drug concentrations at which the cell viability is 50%) were calculated as a sigmoidal dose-response curve using GRAPHPAD PRISM 4.03.
Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material
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