A Meroisoprenoid, Heptenolides, and C-Benzylated Flavonoids from Sphaerocoryne gracilis ssp. gracilis

Gasper Maeda, Joan J. E. Munissi, Sofia Lindblad, Sandra Duffy, Jerry Pelletier, Vicky M. Avery, Stephen S. Nyandoro,* and Máté Erdélyi*

ABSTRACT: A new meroisoprenoid (1), two heptenolides (2 and 3), two C-benzylated flavonoids (4 and 5), and 11 known compounds (6−16) were isolated from leaf, stem bark, and root bark extracts of Sphaerocoryne gracilis ssp. gracilis by chromatographic separation. The structures of the new metabolites 1−5 were established by NMR, IR, and UV spectroscopic and mass spectrometric data analysis. (Z)-Sphaerodiol (7), (Z)-acethylmelodorinol (8), 7-hydroxy-6-hydromelodienone (10), and dichamanetin (15) inhibited the proliferation of Plasmodium falciparum (3D7, Dd2) with IC50 values of 1.4–10.5 μM, although these compounds also showed cytotoxicity against human embryonic kidney HEK-293 cells. None of the compounds exhibited significant disruption in protein translation when assayed in vitro.

RESULTS AND DISCUSSION

Repeated silica gel column chromatography of the methanolic extracts of the stem and root barks and leaves of Sphaerocoryne gracilis ssp. gracilis, followed by gel filtration on Sephadex LH-20 and further purification on HPLC, gave five new metabolites (1−5) and 11 known compounds (6−16, Figure S1, Supporting Information). The structures of the new meroisoprenoid (1), two heptenolides (2 and 3), and two C-benzylated flavonoids (4 and 5) were established using NMR spectroscopic and mass spectrometric techniques. The identities of the known compounds 3′-hydroxygracinol (6),7 (Z)-sphaerodiol (7),11,12 (Z)-acethylmelodorinol (8),10,11,13 (Z)-melodorinol (9),10−12 7-hydroxy-6-hydromelodienone (10),7 pinocembrin (11),11,14 5,7-dihydroxyflavone (12),15 chamanetin (13),16 isochamanetin (14),16 dichamanetin (15),16 and polycarpol (16)16 were confirmed by comparing their spectroscopic data with those previously reported.

Compound 1 was obtained as a colorless oil and was assigned the molecular formula C21H25O8 based on HRESIMS (Figure S9, Supporting Information) and NMR data (Table 1, Figures S2−S8, Supporting Information). Its UV spectrum showed absorptions at λmax 215, 230, and 275 nm, consistent with a conjugated π-system encompassing a benzene ring.
addition, the 1H NMR spectrum indicated the presence of Hz) with chemical shifts compatible with an carbonyl system. Based on HMBC correlations (Table 1, Figures S2 and S3, Supporting Information) these were established as carbonyl functionalities, 7.75) showed 3 overlapping signals. Overlapping signals.

Table 1. NMR Spectroscopic Data (1H at 800 MHz, 13C at 200 MHz, CDCl3) of Gracidiol (1)

| position | δC type | δH | J (Hz) | HMBC, H→C |
|----------|---------|-----|--------|----------|
| 1        | 66.3, H-C-O | 4.69 | dd (11.8, 2.8) | C-7" |
| 2        | 73.6, H-C-O | 3.96 | dd (7.8, 2.8) | C-1, C-3, C-4, CH3 |
| OH-2/3   |         | 3.49 | s       |          |
| 3        | 73.4, C-O | 2.00 | s       | C-2, C-4 |
| 4        | 68.9, H-C-O | 4.48 | d (11.5) | C-2, C-3, CH3, C-9' |
|          |         | 4.20 | d (11.5) | C-2, C-3, CH3, C-9' |
| 1'       | 134.2, C  | 128.4, CH | 7.54 | m       | C-3/5', C-4', C-7' |
| 3'/5'    | 129.1/130.8 | CH | 7.39- | m       | C-1', C-2/6' |
| 4'       | 130.8/130.9 | CH | 7.39- | m       | C-2/6' |
| 7'       | 146.4, CH | 7.75 | d (16.0) | C-1', C-2/6', C-8', C-9' |
| 8'       | 117.2, CH | 6.47 | d (16.0) | C-1', C-7', C-9' |
| 9'       | 167.6, C=O | 134.2, C | 8.05 | m       | C-4', C-7' |
| 10'      | 129.9, CH | 8.40 | m       | C-2/6', C-4' |
| 11'      | 132.4, CH | 7.56 | m       | C-2/6' |
| 12'      | 167.3, C=O | 7.75 | s       |          |

*Overlapping signals.

whereas its IR spectrum indicated O–H (3424 cm⁻¹), C=O/C=O (1641 cm⁻¹), and C–O (1265 cm⁻¹) stretches.11,16 The NMR data (Table 1, Figures S2 and S3, Supporting Information) suggested to have two carbonyl functionalities, two aromatic systems, and four oxygen-substituted aliphatic groups. The NMR spectra showed characteristic signals for a benzyloxy moiety, that is H-2/6" (δH 8.05, δC 129.6), H-3/5" (δH 7.45, δC 128.6), and H-4" (δH 7.56, δC 133.4). In addition, the 1H NMR spectrum indicated the presence of trans-disposed olefinic protons at δH 7.75 and δH 6.47 (J = 16.0 Hz) with chemical shifts compatible with an α,β-unsaturated carbonyl system. Based on HMBC correlations (Table 1, Figure S7, Supporting Information) these were established as being part of a cinnamoyl moiety. Thus, the β-proton (δH 7.75) showed 3JHC HMBC correlations with the aromatic carbons C-2/6’ (δC 129.9) and the carbonyl carbon C-9’ (δC 167.6) of the cinnamoyl moiety. The aromatic proton signals at δH 7.39–7.40 were assigned to H-3/5’ and H-4’, while the signal at δH 7.54 to H-2/6’. Moreover, the HSQC spectrum (Figure S6, Supporting Information) indicated two sets of oxymethylene units with diastereotopic protons. One of these was part of an ABX spin system (COSY) encompassing protons H-1a (δH 4.69), H-1b (δH 4.47), and H-2 (δH 3.96). These diastereotopic protons gave HMBC cross-peaks to C-7” (δC 167.3) and were hence linked to the benzyloxy unit. The second set of diastereotopic protons H-4a (δH 4.48) and H-4b (δH 4.20) showed only a geminal coupling, 2J = 11.5 Hz, and were shown to be linked to the oxygenated quaternary carbon C-3 (δC 73.4) on one side and to the cinnamoyl unit on the other, as indicated by their HMBC cross-peaks to C-2 (δC 73.6), C-3 (δC 73.4), and C-9’ (δC 167.6). The position of CH3-C-3 (δH 1.34; δC 20.0) was deduced based on its HMBC cross-peak to the oxy-quaternary chiral carbon C-3 (δH 73.4). Hence, compound 1 was concluded to be constituted by an isoprenyl unit attached to a cinnamoyl unit on one end and a benzyloxy moiety on the other end.

The relative configurations of C-2 (δC 73.6) and C-3 (δC 73.4) might be indicated by the NOESY correlation (Figure S5, Supporting Information) of CH3-C-3 (δH 1.34) with oxymethylene proton H-2 (δH 3.96), suggesting the erythro relationship of the hydroxy groups at C-2 and C-3. As the C-2–C-3 bond freely rotates, the determination of the relative configuration of these carbons cannot be trusted and should be corroborated by other techniques. Based on the above spectroscopic evidence, however, this new natural product, gracidiol (1), isolated from the leaves of S. gracilis sp. gracilis, was characterized as 4-cinnamoyloxy)-2β,3,β-dihydroxy-3α-methylbutyl benzoate. From the biogenesis point of view, the compound is envisaged to be a meroisoprenoid formed through both mevalonic and shikimate pathways. It is unprecedented in the genus Sphaerocoryne.

Compound 2 was obtained as a colorless oil. Its molecular formula was determined as C9H10O5 based on HRESIMS (Figure S17, Supporting Information) and NMR (Table 2) analyses. Its UV spectrum showed an absorption at λmax 270 nm, consistent with a conjugated system.16 The broad IR absorption band at 3446 cm⁻¹ is typical of a hydroxy functionality, whereas those at 1743 and 1781 cm⁻¹ are compatible with the α,β-unsaturated carbonyls of butenolides.11,16 The chemical shifts of the olefinic protons resonating at δH 6.27 (H-2) and δH 7.38 (H-3) were also consistent with an α,β-unsaturated carbonyl moiety. The NMR spectroscopic
Table 2. NMR Spectroscopic Data (1H at 800 MHz, 13C at 200 MHz, CDCl3) of 7-Acetylspathaerodol (2)

| position | δC, type | δH | J (Hz) | HMBC, H→C |
|----------|----------|-----|--------|-----------|
| 1        | 168.9, C=O | | | |
| 2        | 121.1, CH | 6.27 | d (5.5) | C-1, C-3, C-4 |
| 3        | 143.7, CH | 7.38 | d (5.5) | C-1, C-2, C-4, C-5 |
| 4        | 150.1, C  | | | |
| 5        | 112.9, CH | 3.33 | d (8.0) | C-3, C-4, C-6, C-7 |
| 6        | 65.9, C=O | 5.03 | dd (8.0, 6.8, 3.8) | C-4, C-5, C-7 |
| 7        | 67.2, H2C=O | 4.24 | dd (11.5, 3.8) | C-5, C-6, O-Ac-7 (C=O) |
|          |          | 4.18 | dd (11.5, 6.8) | C-5, C-6, O-Ac-7 (C=O) |
| OAc-7    | 171.2, C=O | | | |
|          | 20.9, CH3 | 2.11 | s | OAc-7 (C=O) |

Table 3. NMR Spectroscopic Data (1H at 800 MHz, 13C at 200 MHz, CDCl3) of (Z)-2′-Hydroxyacetylmelodorinol (3)

| position | δC, type | δH | m, J (Hz) | HMBC, H→C |
|----------|----------|-----|-----------|-----------|
| 1        | 168.5, C=O | | | |
| 2        | 121.9, CH | 6.30 | d (5.5) | C-1, C-3, C-4 |
| 3        | 143.4, CH | 7.38 | d (5.5) | C-1, C-2, C-4 |
| 4        | 150.9, C  | | | |
| 5        | 108.6, CH | 5.31 | d (7.9) | C-3, C-4, C-6, C-7 |
| 6        | 67.2, H2C=O | 6.15 | dd (7.9, 6.1, 3.9) | C-4, C-5, C-7, OAc-6 (C=O) |
| OAc-6    | 169.9, C=O | | | |
|          | 21.0, CH3 | 2.11 | s | OAc-6 (C=O) |
| 7        | 65.0, H2C=O | 4.61 | dd (11.7, 3.9) | C-5, C-6, C-7' |
|          |          | 4.55 | dd (11.7, 6.1) | C-6, C-7, O-Ac-7 (C=O) |

data of 2 (Table 2, Figures S10–S16, Supporting Information) were diagnostic for a heptenolid moiety consisting of ABMX \([\delta_H 4.24 (H-7a), \delta_H 4.18 (H-7b), \delta_H 5.03 (H-6), and \delta_H 5.33 (H-5)]\) and \(\alpha\beta\)-unsaturated \([\delta_H 7.38 (H-3) and \delta_H 6.27 (H-2)]\) spin systems, resembling that of compound 7.\(^{11,17}\) Comparison of its NMR data suggested 2 to be a close derivative of \((Z)\)-spathaerodol \((7)\), with additional NMR signals observed at \(\delta_H 2.11 and \delta_C 20.9\) and \(\delta_C 171.2\) \((OAc-7)\), suggesting the presence of an additional acetyl group. The position of the acetyl moiety at C-7 was established from the HMBC (Table 2; Figure S16, Supporting Information) cross-peaks of the C-7 methylene protons \((\delta_H 4.24 and \delta_H 4.18)\) to \(\delta_C 171.2\) \((OAc-7)\). NOESY correlations (Figure S14, Supporting Information) of H-3 \((\delta_H 7.38)\) to H-5 \((\delta_H 5.33)\) indicated the exocyclic double-bond geometry is Z. Its configuration at C-6 was the same as in compound 7, based on the comparable sign and magnitude of optical rotation.\(^{10,11}\) Based on the above evidence, the new compound \((Z)\)-7-acylspathaerodol \((2)\) was characterized as \((S,Z)\)-2-hydroxy-3-(3-oxofuran-2(3H)-ylidene)propyl acetate, the acetylated derivative of \((Z)\)-spathaerodol \((7)\).\(^{11}\) Both compounds were obtained from the stem and the root bark methanol extracts of \(S. gracilis\) sp. gracilis.

Compound 3 was obtained as a colorless oil and was assigned the molecular formula \(C_{22}H_{20}O_7\), based on analysis of its HRESIMS (Figure S25, Supporting Information) and NMR (Table 3, Figures S18–S24, Supporting Information) data. Its IR spectrum showed absorption bands at 3417 and 1639 cm\(^{-1}\), indicating the presence of \(O\rightarrow H\) and \(C\equiv C\) functionalities, respectively.\(^{16}\) The UV absorptions at \(\lambda_{max} 205 and 240\) nm along with the NMR data were compatible with the presence of a benzene moiety and an \(\alpha\beta\)-unsaturated carbonyl system, which was corroborated by NMR (vide infra).\(^{16}\) The \(^1\)C NMR spectrum (Table 3, Figure S19, Supporting Information) showed 16 signals, which were sorted with the aid of the HSQC spectrum (Figure S23, Supporting Information) into an oxymethine, an oxymethylene, an oxomethyl, three carbonyls, and 10 sp\(^2\) carbons. Six sp\(^2\) carbons were assigned to an aromatic ring and four to two olefinic bonds (Table 3). The \(^1\)H NMR spectrum of 3 (Table 3, Figure S18, Supporting Information) displayed signals at \(\delta_H 4.61 (H-7a), \delta_H 4.55 (H-7b), \delta_H 5.31 (H-5), and \delta_H 6.15 (H-6)\) corresponding to an ABMX spin system, as revealed by COSY and TOCSY spectra (Figures S20 and S22, Supporting Information), resembling that observed for compound 2. The chemical shifts of the olefinic protons \(\delta_H 6.30 (H-2) and \delta_H 7.38 (H-3)\) were also similar to those of 2 and hence compatible with an \(\alpha\beta\)-unsaturated carbonyl moiety. The above spectroscopic features are reminiscent of a butenolide system with an extension of an alicyclic three-carbon skeleton forming a heptenolid moiety, which has previously been reported for similar metabolites.\(^{4–7,10,11}\) Overall, the NMR data of 3 resembled that of \((Z)\)-melodorinol \((8)\) and of related compounds\(^{4–7,10,11}\) with the only difference being the ortho-hydroxy group substitution of its benzoyloxy group. The placement of this hydroxy group functionality was established by the ABCD coupling pattern of the aromatic signals \((1H NMR, COSY, and TOCSY)\) and by HMBC correlations (Table 2, Figure S24, Supporting Information). NOESY correlations (Figure S22, Supporting Information) of H-3 \((\delta_H 7.38)\) to H-5 \((\delta_H 5.31)\) revealed the Z-geometry of the exocyclic C-4, C-5 double bond, while the NOE of H-5 \((\delta_H 5.31)\) and H-6 \((\delta_H 6.15)\) might indicate the \(\beta\)-orientation of OAc-6. Whereas the latter assignment may be corroborated by the configuration of closely related compounds previously isolated from this genus,\(^{4–7,10,11}\) the C-5 to C-6 bond can freely rotate, making a purely NOE-based assignment unreliable. Nonetheless, the configuration of compound 3 was determined as being similar to those of \((Z)\)-acylmelodorinol \((8)\) and \((Z)\)-melodorinol \((9)\), which were also isolated in the present study. The absolute configuration of the latter was recently determined by single-crystal X-ray diffraction analysis.\(^{17}\) Based on the above evidence, this new natural product, \((Z)\)-2′-hydroxyacetylmelodorinol \((3)\), was characterized as \((S,Z)\)-2-acetoxy-3-(3-oxofuran-2(3H)-ylidene)propyl 2-hydroxybenzoate.

Compound 4 was obtained as a white powder and was assigned the molecular formula \(C_{22}H_{19}O_6\), based on the HRESIMS (Figure S33, Supporting Information) and NMR (Table 4) data. Its UV absorptions found at \(\lambda_{max} 210, 290, and 320\) nm are diagnostic of dihydroflavones.\(^{18}\) The IR absorptions at 3430, 1641, and 1255 cm\(^{-1}\) corresponded to
The signals at δH 5.8a (S31, Supporting Information) along with the COSY spectrum (Figure S28, Supporting Information) indicated an ABX coupling pattern with the help of the HSQC experiment (Figure S39, Supporting Information) of H-7 to the aromatic protons of a dihydrobenzyl group. The coupling pattern of the protons of the C-benzyl moiety supported the 2′- and 3′-hydroxy groups as being ortho to each other. The linkage of the dihydrobenzyl group at C-6 instead of C-8 of the dihydroflavone moiety was deduced from the 1H,13C HMBC of H-7′ (δH 3.87) to C-5′ (δC 160.7) (Table 4, Figure S31, Supporting Information). In the singlet at δH 6.03 was assigned to C-8 (instead of C-6 as in 5). Moreover, HMBC cross-peaks of H-8 (δH 6.03) and H-2 (δH 5.38) to C-8a (δC 161.4) supported the placement of the dihydrobenzyl moiety at C-6. The overlapping signals at δH 7.42–7.43 (4H) were assigned to the ortho (H-2′/6′) and meta (H-3′/5′) protons, and that at δH 7.38 (1H) to the para proton (H-4′) of ring B. The signal at δH 12.89 signal was ascribed to OH-5, with its chemical shift being deshielded due to intramolecular hydrogen bonding with the nearby carbonyl group. The levorotatory specific rotation and the ECD spectrum (Experimental Section) of this flavonoid suggested the S-configuration at C-2. Based on the features mentioned above, the new compound 3′-hydroxyisochamanetin (4) was characterized as (S)-6-(2,3-dihydrobenzyl)-5,7-dihydroxy-2-phenylchroman-4-one, an isochamanetin derivative.

Compound 5 was obtained as a yellow powder. Its molecular formula, C22H18O8, was determined based on HRESIMS (Figure S40, Supporting Information) and NMR (Table 5).

### Table 5. NMR Spectroscopic Data (1H at 800 MHz, 13C at 200 MHz, CD3OD) of 2,3-Dehydro-3′-hydroxychamanetin (5)

| position | δH | J (Hz) | δC | HMBC, H→C |
|----------|-----|--------|-----|-----------|
| 2        | 165.8 | s       | C-2, C-4, C-4a, C-1′ |
| 3        | 105.6 | CH      | C-2′, C-4′, C-6, C-7, C-8a |
| 4        | 145.0 | s       | C-4, C-4a, C-6, C-7, C-8a |
| 5        | 120.4 | dd      | C-7, C-8, C-8a, C-1′, C-2′ |
| 6        | 142.2 | CH      | C-2′, C-3′, C-4′, C-6′ |
| 7        | 128.3 | s       | C-2′, C-3′, C-4′, C-5′ |
| 8        | 146.0 | CH      | C-2′, C-3′, C-4′, C-5′ |
| 9        | 113.5 | dd      | C-7, C-8, C-8a, C-1′, C-2′ |

**O–H, C=C aromatic, and C–O stretches, respectively.** The 1H NMR spectrum (Table 4, Figure S26, Supporting Information) along with the COSY spectrum (Figure S28, Supporting Information) indicated an ABX coupling pattern for H-3′α (δH 3.08), H-3′β (δH 2.84), and H-2 (δH 5.38), typical of the ring C of flavanones. An AB spin system integrating to two protons at δH 3.87 was assigned to the methyl benzylic protons H-7″ with the help of the HSQC experiment (Figure S30, Supporting Information). The signals at δH 6.74 (C-4′/C-6′) and δH 7.05 (C-5′) were deduced as belonging to aromatic protons of a dihydrobenzyl group. The coupling pattern of the protons of the C-benzyl moiety supported the 2′- and 3′-hydroxy groups as being ortho to each other. The linkage of the dihydrobenzyl group at C-6 instead of C-8 of the dihydroflavone moiety was deduced from the 1H,13C HMBC of H-7′ (δH 3.87) to C-5′ (δC 160.7) (Table 4, Figure S31, Supporting Information). In the singlet at δH 6.03 was assigned to H-8 (instead of C-6 as in 5). Moreover, HMBC cross-peaks of H-8 (δH 6.03) and H-2 (δH 5.38) to C-8a (δC 161.4) supported the placement of the dihydrobenzyl moiety at C-6. The overlapping signals at δH 7.42–7.43 (4H) were assigned to the ortho (H-2′/6′) and meta (H-3′/5′) protons, and that at δH 7.38 (1H) to the para proton (H-4′) of ring B. The signal at δH 12.89 signal was ascribed to OH-5, with its chemical shift being deshielded due to intramolecular hydrogen bonding with the nearby carbonyl group. The levorotatory specific rotation and the ECD spectrum (Experimental Section) of this flavonoid suggested the S-configuration at C-2. Based on the features mentioned above, the new compound 3′-hydroxyisochamanetin (4) was characterized as (S)-6-(2,3-dihydrobenzyl)-5,7-dihydroxy-2-phenylchroman-4-one, an isochamanetin derivative.

Besides the new compounds 1–5, C-benzylated flavonoids 6 and 13–15, heptenolides 7, 12, 18, 19, 12, and 10, flavonoids 11 and 12, and triterpenoid 16 were also isolated and identified by comparison of their spectroscopic features to those previously published. The natural occurrence of heptenolides, such as 2, 3, and 7–9, is limited so far only to the members of the Uvaria tribe within the family Annonaceae. Compounds 11 and 12 appear to be biogenetic data. The UV absorption at λmax 280 nm was in agreement with a flavone, while the IR absorptions were consistent with hydroxy (3430 cm−1), aromatic (1642 cm−1), and C–O (1260 cm−1) stretches. The NMR spectroscopic data (Table S, Figures S33–S39, Supporting Information) were comparable to those of 4, except the absence of an ABX spin system in the 1H NMR spectrum for ring C. Instead, 5 showed a singlet at δH 6.69 corresponding to the olefinic H-3 of a flavone moiety. The 1H,13C HMBC signals (Table 5, Figure S39, Supporting Information) of H-7″ (δH 4.16) to C-7 and C-8a indicated a dihydrobenzyl group to be substituted at position C-8, instead of C-6 as in compound 4. The 2D NMR data (Figures S35–S39, Supporting Information) of 5 showed resemblances to those of 4 and were in agreement with the established structure. Based on the above spectroscopic features, the new compound, 2,3-dehydro-3′-hydroxychamanetin (5), was characterized as 8-(2,3-dihydrobenzyl)-5,7-dihydroxy-2-phenyl-4H-chromen-4-one.
precurors of 4−6 and 13−15. Flavonoids, such as 4−6 and 13−15, containing one or two hydroxylated C-benzyl groups on ring A as well as hydroxylated C-benzyl units that extend in a linear fashion, have been isolated from numerous species in the Annonaceae.8,18,21 Heptenolides10−13 and C-benzylated flavonoids3,18,21,22 are known to possess diverse bioactivities.

As part of an ongoing search for bioactive phytochemicals, the compounds isolated from S. gracilis ssp. gracilis were screened for antimalarial activity against the chloroquine-sensitive (3D7) and the chloroquine-resistant (Dd2) strains of P. falciparum. Compounds showing promising antimalarial activities, <10 μM, were further screened for cytotoxicity using HEK-293 human embryonic kidney cells. Antimalarial activities of IC50 1.4 to >40 μM (Table 6), with 7-hydroxy-

Table 6. Antiplasmodial and Cytotoxic Activities of Compounds Isolated from Sphaerocoryne gracilis ssp. gracilis

| Compound | 3D7 | Dd2 | HEK-293 | SIHEK293/3D7 |
|----------|-----|-----|---------|-------------|
| (Z)-sphaerodial (7) | 10.5e | NT | NT | 3 |
| (2)-acetylhemodiolinol (8) | 4.8e | 6.7a | 11.8e | 3 |
| (7)-melowlerinol (9) | 3.7a | NT | NT | 3 |
| 7-hydroxy-6-hydroxylidione (10) | 1.4a | 4.1a | 100bc | 3 |
| dichametin (15) | 9.3a | 6.7a | 75b | 3 |
| pyrimethamine | 0.0025 | 4.2 | 1688 | 3 |
| chloroquine | 0.0045 | 0.046 | 60b | >4000b |
| pyronaridine | 0.0036 | 0.0075 | 1.8 | 494.5 |
| puromycin | 0.023 | 0.045 | 13.6 | 55 |
| artemesine | 0.00004 | 0.0094 | 75c | >22.000b |
| DHA | 0.0000 11 | 0.005 15 | 50c | >22.000b |

“IC50: Percentage growth inhibition at 40 μM. IA = inactive at 40 μM. NT = not tested. Percentage growth inhibition at 20 μM. The inhibitory activities are given as the mean value of at least two independent measurements. IC50 values were determined for one biological replicate in duplicate.

6-hydroxylidione (10) being the most active isolated constituent, were observed [IC50 1.4 (3D7) and 4.1 μM (Dd2)]. However, 10 also showed cytotoxicity against HEK-293 mammalian cells (100% at 40 μM). (2)-Acetylhemodiolinol (8), melowlerinol (9), and dichametin (15) showed low selectivities. Compounds 4, 5, and 12−14 inhibited plasmoidal growth moderately (Table 6) at a 20 μM concentration. All other compounds showed very weak or no antimalosomal activities at 40 μM. The crude methanol extracts of the leaves, stem bark, and root bark of S. gracilis ssp. gracilis and the isolated 4, 5, 6, 9, 10, and 12 were tested further for the inhibition of cap-dependent and independent translation initiation using a luciferase model17,25 but none showed significant translational inhibitory activity.

In conclusion, a new meroisoprenoid (1), two new heptenolides (2 and 3), two new C-benzylated flavonoids (4 and 5), and 11 known flavonoids, heptenolides and triterpenoids (6−16), were isolated from S. gracilis ssp. gracilis. The isolation of heptenolides from this plant is of significance, as these compounds have so far been reported to be restricted to the Uvariae tribe of the family Annonaceae. In line with previous reports, some of the isolated heptenolides and flavonoids (7−10 and 15, Table 6) showed antimalosomal activities that may be interesting for drug development.

### Experimental Section

#### General Experimental Procedures

Melting points were determined with a Büchi B-545 melting point instrument. The optical rotation and circular dichroism for compounds possessing chiral centers were determined using a 341LC OROT polarimeter (589 nm temp 20.0 °C) and a JASCO J-715 spectrometer, respectively. The UV measurements were done using a 264 UV−vis spectrophotometer. A MIR 450FT-IR spectrometer was used to record the IR spectra. NMR spectra were acquired on a Bruker Avance III HD 800 NMR MHz spectrometer and analyzed with the software MestReNova (v10.0.0). Structural assignments were based on 1H NMR, 13C NMR, COSY, TOCSY, NOESY, HSQC, and HMBC spectra. LC-MS (ESI) spectra were acquired with a PerkinElmer PE SCIEX API 150 EX instrument equipped with a Turbolon spray ion source and a Gemini 5 mm RP-C18 110 Å column, using a gradient of H2O−CH3CN (80:20 to 20:80) in the presence of 0.2% HCO2H and a separation time of 8 min. HRESIMS were obtained with a Q-ToF-LC/MS spectrometer with a lock mass ESI source (Stenhagen Analysis Lab AB, Gothenburg, Sweden), using a 2.1 × 30 mm 1.7 μm RP-C18 column and an elution gradient of H2O−CH3CN (5:95 to 95:5, with 0.2% HCO2H). Analytical TLC was performed on aluminum plates precoated with silica gel 60 F254 (Merck). After development with an appropriate solvent system, the plates were evaluated under UV light (254 and 366 nm) and then sprayed with anisaldehyde reagent, prepared by mixing 3.5 mL of 4-anisaldehyde with 2.5 mL of concentrated H2SO4, 4 mL of glacial HOAc, and 90 mL of MeOH. The plates were then heated for the identification of UV-negative compounds and assessment of the color change of the UV-positive spots. Column chromatography was carried out using silica gel 60 (230−400 mesh), and gel filtration was performed over Sephadex LH-20 (Pharmacia) suspended in CH3Cl−MeOH (1:1). Preparative HPLC was performed on a Waters 600E system using Chromatix software (Pikron Ltd.) and an RP-C18 Kromasol column (250 mm × 25 mm) with a H2O−MeOH gradient (70:30 to 100:0) for 20−40 min at a flow rate of 7 mL/min.

#### Plant Material

The stem bark, root bark, and leaves of Sphaerocoryne gracilis ssp. gracilis were collected in June 2014 from Pugu Forest Reserve, Kisarawe District, Pwani Region, Tanzania, at GPS location S 06° 53′ 28.4″, E 03° 05′ 56.3″ at an elevation of 269 m. The plant was identified in the field and authenticated by Mr. F. M. Mbago, a senior taxonomist of the Herbarium, Botany Department of University of Dar es Salaam. The voucher specimen is deposited there with reference number FMM-3670.

#### Extraction and Isolation

Samples of the stem bark, root bark, and leaves were air-dried for 2 weeks and then pulverized to obtain about 2 kg of each. The materials were then separately soaked twice, consecutively, in methanol for 48 h. The extracts were then filtered and concentrated in vacuo on a rotary evaporator, affording 70.0, 43.0, and 73.0 g of leaf, stem bark, and root bark extracts, respectively.

The leaf extract (65.0 g) was adsorbed on silica gel and subjected to gravity column chromatography, using a elution gradient from 30% EtOAc−water (100% to 30% EtOAc−methanol), to obtain 13 fractions of approximately 200 mL each. Fractions 4−6, obtained with 50% EtOAc−hexane, were found to consist of the same compounds identified by TLC analysis. They were therefore combined and subjected to further gravitational column chromatography, using dry packing and elution with 30% EtOAc−hexane, resulting in 25 fractions of ~80 mL each. TLC analysis enabled the combination of fractions 16−21, which were then subjected to separation on Sephadex, eluted with 1:1 MeOH−CH2Cl2, to obtain 23 subfractions of ~2 mL each. After TLC analysis, the combination of subfractions 7−10 gave 1.3 mg of 5,7-dihydroxyflavone (12), and fractions 13−16 gave 5.8 mg of 3′-hydroxyscolamarin (4). Subfractions 17−20 were combined for further separation on a Sephadex column, also eluted with 1:1 MeOH−CH2Cl2, resulting in 32 fractions of ~1 mL each. TLC analysis also enabled the combination of fractions 15−23, which were then subjected to preparative HPLC, using 70:30 Milli Q water−methanol, to obtain gracidiol (1, 4.5 mg). Preparative HPLC of fractions 24−29, using the same conditions, gave three peaks.
representing 7-hydroxy-6-hydromelodione (10), (Z)-acetylmelodorinol (8), and (Z)-2′-hydroyacetamylodinol (3). The combined fractions 8–10 from the initial column were subjected to gravity column chromatography, eluted with 50% EtOAc–i-hexane. This yielded 12 fractions, of which fraction 6 was purified further on a Sephadex column, MeOH–CH2Cl2 (1:1), giving 32 fractions, of which fractions 10–18 gave 75.7 mg of (Z)-melodorinol (9), while fractions 29–32 yielded 1 mg of 5,7-dihydroxy-8-(2,3-dihydroxybenzyl)flavone (5). Fraction 11, obtained from the initial column, was separated over Sephadex by elution with 1:1 MeOH–CH2Cl2, yielding 15 fractions. Fractions 9–11 contained complex, inseparable mixtures of compounds, which were not further analyzed, while fractions 12–14 were separated once again, as described above, yielding 186.5 mg of 3′-hydroxygracinol (6).

The root bark extract (680.0 g) was subjected to vacuum liquid chromatography under gradient elution (20% EtOAc–petroleum ether to 10% MeOH–EtOAc) to obtain 10 fractions of ~500 mL each, which were analyzed with TLC and developed using EtOAc–petroleum ether at ratios of 2.8, 5.5, 7.5, 2.5, and 9.1. Fraction 3, obtained at 50% EtOAc–petroleum ether, was further separated on a Sephadex column, as above, giving 22 fractions. Following TLC analysis, fractions 14 and 15 were combined and subjected to gravity column chromatography using an elution gradient from 30% to 50% EtOAc–i-hexane, giving 45 fractions of 10 mL each. Fractions 44 and 45 contained complex, inseparable mixtures of compounds and were not further analyzed. Fraction 4 from the initial column, obtained using 50–75% EtOAc–i-hexane, was subjected to gravity column chromatography, using gradient elution from 40% to 75% EtOAc–i-hexane, yielding pinocembrin (11, 16.7 mg). Fraction 6, obtained from elution with 3% MeOH–EtOAc, underwent subsequent separation on the Sephadex column (1:1 MeOH–CH2Cl2) and silica gel column chromatography (75% EtOAc–i-hexane), yielding (Z)-sphaerodiol (7, 2.1 mg). The polar fraction, 8 (obtained at 10% MeOH–EtOAc), gave (Z)-7-acetylsphaerodiol (2, 19.8 mg).

The crude methanol extract of the stem bark (43.0 g) was fractionated chromatographically and purified as described above, leading to the isolation of (Z)-7-acetylsphaerodiol (2, 32.0 mg), 3′-hydroxygracinol (6, 89.0 mg), (Z)-sphaerodiol (7, 24.0 mg), (Z)-acetylmelodorinol (8, 63.0 mg), (Z)-melodorinol (9, 15.0 mg), 5,7-dihydroxyavone (12, 18.7 mg), chamanetin (13, 64.8 mg), isochamanetin (14, 23.6 mg), dichamanetin (15, 126.8 mg), and polycarpol (16, 18.0 mg).

Gracidiol (1): colorless oil; [α]20 D +52.0 (c 0.15, MeOH); ECD (MeOH, $\lambda_{\text{max}}$ (Δε) = 255 (3.07), 250 (3.02), 248 (3.04), 240 (2.77), 238 (2.77), 225 (2.77)) and CD (MeOH) $\lambda_{\text{max}}$ (Δε) = 270 (4.41) nm; IR (KBr) $\nu_{\text{max}}$ 3500, 3400, 2195, 1740, 1642, 1265, 740 cm$^{-1}$; H and 13C NMR data, see Table 1; ESIMS m/z 377.5 [M + H]+, 376.5, 267.0, 149.4, 111.5, 99.1, 74.1, 69.3; HRESIMS [M + H]+ m/z 377.1012 (calcd for C20H17O7 377.1025). Antiplasmodial and Cytotoxicity Assays. Antiplasmodial activity was determined using a high-content imaging assay, as described previously. The cytotoxicity of the antiplasmodial compounds was evaluated against human embryonic kidney cells (HEK-293) following an established protocol. Human red blood cells for culture of P. falciparum were provided by the Australian Red Cross Blood Bank in accordance with their routine Material Transfer Agreement (MTA) for nonclinical blood product supply. These bioassay studies were approved by the Griffith University Biosafety and Human Ethics Committee (GU ref no. ESEK/03/12/HREC/03/08/11019).

Translation Inhibition Assay. Luciferase inhibition activity assays were performed following standard procedures described previously.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b00721.

Structures and tables containing 1H and 13C NMR data for the known compounds 6–16; 1D and 2D NMR spectra and HRESIMS for the new compounds 1–5 (PDF)

### AUTHOR INFORMATION

**Corresponding Authors**

Stephen S. Nyandoro — Chemistry Department, College of Natural and Applied Sciences, University of Dar es Salaam, Dar es Salaam, Tanzania; Department of Chemistry and Molecular Biology, University of Gothenburg, SE-412 96 Gothenburg, Sweden; Phone: +255-754-206560; Email: nyandoro@udsm.ac.tz

Máte Erdélyi — Department of Chemistry and Molecular Biology, University of Gothenburg, SE-412 96 Gothenburg, Sweden; Department of Chemistry — BMC, Uppsala University, SE-751 23 Uppsala, Sweden; orcid.org/0000-0003-0359-5970; Phone: +46-18-4713810; Email: mate.erdelyi@kemi.uu.se

**Authors**

Gasper Maeda — Chemistry Department, College of Natural and Applied Sciences, University of Dar es Salaam, Dar es Salaam, Tanzania; Department of Chemistry and Molecular Biology, University of Gothenburg, SE-412 96 Gothenburg, Sweden

Joan J. E. Munissi — Chemistry Department, College of Natural and Applied Sciences, University of Dar es Salaam, Dar es Salaam, Tanzania

Sofia Lindblad — Department of Chemistry and Molecular Biology, University of Gothenburg, SE-412 96 Gothenburg, Sweden; Department of Chemistry — BMC, Uppsala University, SE-751 23 Uppsala, Sweden

Sandra Duffy — Discovery Biology, Griffith Institute for Drug Discovery, Griffith University, Nathan Qld 4111, Australia

Jerry Pelletier — Department of Biochemistry, McGill Institute for Drug Discovery, Montreal, QC H3G 1Y6, Canada

Vicky M. Avery — Discovery Biology, Griffith Institute for Drug Discovery, Griffith University, Nathan Qld 4111, Australia
Complete contact information is available at:
https://pubs.acs.org/10.1021/acs.jnatprod.9b00721

Notes
The authors declare no competing financial interest.
Original FIDs are available, open access, at Zenodo with DOI: 10.5281/zenodo.3355165.

■ ACKNOWLEDGMENTS
The Swedish Research Council (2016-05857) and the Australian Research Council (VMA, grant no. LP120200557) are gratefully acknowledged for financial support. S.S.N. is grateful to the Swedish Institute for a postdoctoral research award (00045/2014). We thank Mr. Frank M. Mbago, curator at the Herbarium of the Department of Botany, University of Dar es Salaam, for locating and identifying the investigated plant species. We thank the Australian Red Cross Blood Bank for the supply of human blood for parasite culture. The Swedish NMR Centre is acknowledged for access to an 800 MHz spectrometer.

■ REFERENCES
(1) IUCN. The IUCN Red List of Threatened Species, 2009.
(2) Verdcourt, B. Flora of Tropical East Africa; Annonaceae, Vol. 5; Royal Botanic Gardens: Kew, Richmond, UK, 1971; p 131.
(3) Bick, I.; Preston, N. Aust. J. Chem. 1971, 24, 2187–2188.
(4) Jung, J.; Chang, C.-J.; Smith, D.; McLaughlin, J.; Pummangura, S.; Chaichantipyuth, C.; Patarapanich, C. J. Nat. Prod. 1991, 54, 500–505.
(5) Jung, J.; Pummangura, S.; Chaichantipyuth, C.; Patarapanich, C.; Fanwick, P.; Chang, C.-J.; McLaughlin, J. Tetrahedron 1990, 46, 5043–5054.
(6) Tuchinda, P.; Udchachon, J.; Reutrakul, V.; Santisuk, T.; Taylor, W. C.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. Phytochemistry 1991, 30, 2685–2689.
(7) Momburi, S. W. New Natural Products and Other Constituents of Sphaerocornye gracilis spp. gracilis, M.Sc. Thesis, University of Dar es Salaam, Tanzania, 1998.
(8) Nkunya, M. H. Pure Appl. Chem. 2005, 77, 1943–1955.
(9) Novac, O.; Guenier, A. S.; Pelletier, J. Nucle. Acids Res. 2004, 32, 902–915.
(10) Nyandoro, S. S.; Munissi, J. J.; Gruhonjic, A.; Duffy, S.; Pan, F.; Puttreddy, R.; Hollera, J. P.; Fitzpatrick, P. A.; Pelletier, J.; Avery, V. M.; Rissanen, K.; Erdelyi, M. J. Nat. Prod. 2017, 80, 114–125.
(11) Samuel, S. M.; S. J. M.; Nkunya, M. H. H.; Irungu, B. N.; Moshi, M. J.; Moulton, B.; Luisi, B. S. Nat. Prod. Commun. 2007, 2, 737–741.
(12) Shen, C.; Chou, S.; Chou, C.; Hop, L. K. Tetrahedron: Asymmetry 1996, 7, 3141–3146.
(13) Hasan, C. M.; Healey, T. M.; Waterman, P. G.; Schwalbe, C. H. J. Chem. Soc., Perkin Trans. 1 1982, 2807–2812.
(14) Ching, A. Y. L.; W. T.; S.; Sukari, M. A.; Lian, G. E. L.; Rahman, M. M.; Khalid, K. Malays. J. Anal. Sci. 2007, 1, 154–159.
(15) Chavi, Y.; Siripit, P.; Bungon, S. Arch. Pharmacal Res. 2009, 32, 1179–1184.
(16) Joshi, V.; Chamoli, R. Bull. Chem. Soc. Ethiop. 2010, 24, 467–471.
(17) Nyandoro, S. S.; Munissi, J. J.; Gruhonjic, A.; Duffy, S.; Pan, F.; Puttreddy, R.; Hollera, J. P.; Fitzpatrick, P. A.; Pelletier, J.; Avery, V. M.; Rissanen, K.; Erdelyi, M. J. Nat. Prod. 2017, 80, 114–125.
(18) Pan, L.; Mathew, S.; Lantvit, D. D.; Zhang, X.; Ninh, T. N.; Chai, H.; Carcache de Blanco, E. J.; Soeiarto, D. D.; Swanson, S. M.; Kinghorn, A. D. J. Nat. Prod. 2011, 74, 2193–2199.
(19) Slade, D.; Ferreira, D.; Marais, J. P. Phytochemistry 2005, 66, 2177–2215.
(20) Antus, S.; Baitzgacs, E.; Kajtar, J.; Snatzke, G.; Tokes, A. L. Liebigs Ann. Chem. 1994, 1994, 497–502.
(21) Achenbach, H.; Höhn, M.; Waibel, R.; Nkunya, M. H.; Jonker, S. A.; Muhie, S. Phytochemistry 1997, 44, 359–364.
(22) Holzhauser, S.; Freiwald, A.; Weise, C.; Multhaup, G.; Han, C. T.; Sauer, S. Angew. Chem., Int. Ed. 2013, 52, S171–S174.
(23) Novac, O.; Guenier, A. S.; Pelletier, J. Nucl. Acid Res. 2004, 32, 902–915.
(24) Duffy, S.; Avery, V. M. Am. J. Trop. Med. Hyg. 2012, 86, 84–92.