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## Secoiridoids and Iridoids from *Morinda asteroscepa*

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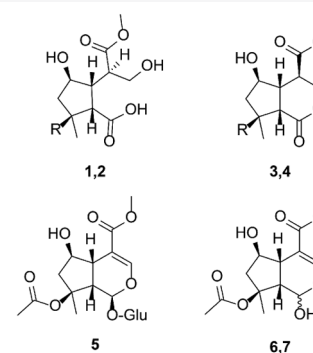
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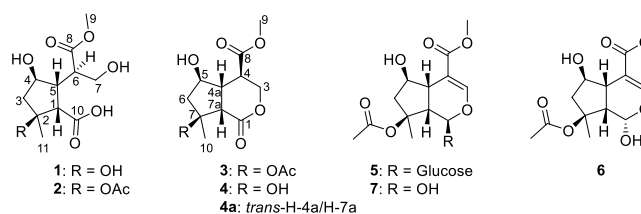
Supporting Information

**ABSTRACT:** The new 2,3-secoiridoids morisecoiridoic acids **1** and **2**, the new iridoid 8-acetoxyepishanzilactone (**3**), and four additional known iridoids (**4–7**) were isolated from the leaf and stem bark methanol extracts of *Morinda asteroscepa* using chromatographic methods. The structure of shanzilactone (**4**) was revised. The purified metabolites were identified using NMR spectroscopic and mass spectrometric techniques, with the absolute configuration of **1** having been established by single-crystal X-ray diffraction analysis. The crude leaf extract (10  $\mu\text{g/mL}$ ) and compounds **1–3** and **5** (10  $\mu\text{M}$ ) showed mild antiplasmodial activities against the chloroquine-sensitive malaria parasite *Plasmodium falciparum* (3D7).



*Morinda asteroscepa* K. Schum is a small tree belonging to the family Rubiaceae and is endemic to Tanzania and Malawi. It has a limited distribution in Eastern Tanzania and in the Nchisi Forest in Malawi.<sup>1,2</sup> The plant is classified as a vulnerable species in the International Union of Conservation of Nature (IUCN) Red List of Threatened Plant Species.<sup>1,2</sup> *M. asteroscepa*, locally known as “muromberombe” in Tanzania, is used in folk medicine as an antimalarial and anticonvulsant, especially for children.<sup>2</sup> Previous reports of phytochemical investigations of *Morinda* species have revealed the genus to accumulate iridoids, glycosides, flavonoids, and anthraquinones, some of which are known for antidiabetic,<sup>3</sup> antioxidant,<sup>4,5</sup> antimicrobial,<sup>6–8</sup> antiviral,<sup>7</sup> antitumor,<sup>6,9–11</sup> anti-inflammatory,<sup>6,12</sup> immune-enhancing,<sup>6</sup> antiosteoporotic,<sup>13</sup> anti-malarial,<sup>14</sup> antihistamine, antihelminthic, and analgesic<sup>6</sup> activities. Some other species of this genus, including *M. citrifolia* (Noni), are used in folk medicine for the treatment of abdominal colic, cancer, hypertension, malaria, and various types of fever.<sup>15–17</sup> *M. asteroscepa* has not been phytochemically investigated before. Owing to the previously reported bioactive secondary metabolites of the genus and the medicinal uses of its species, it was included in our ongoing phytochemical investigation of rare and endemic Tanzanian medicinal plants.<sup>18–23</sup> Herein, we report the isolation, identification, and determination of the antiplasmodial activities of the new 2,3-secoiridoids **1** and **2**, the new iridoid **3**, and four additional known iridoids, **4–7**.

HPLC afforded the new secoiridoid **1** and the four known iridoids **4–7**. The new natural product **1**, along with two additional new compounds, **2** and **3**, were isolated from the CH<sub>3</sub>OH extract of the leaves. The compounds were identified using NMR spectroscopic and mass spectrometric techniques and were further verified by CSEARCH,<sup>24</sup> and the structure of **1** was also confirmed by single-crystal X-ray crystallographic analysis.



Compound **1**  $\{[\alpha]_D^{20} -37.5$  (*c* 2.0, CH<sub>3</sub>OH) $\}$ , a major metabolite of the stem bark and the leaf extracts, was obtained as a white solid and was assigned the molecular formula C<sub>11</sub>H<sub>18</sub>O<sub>7</sub> based on HRESIMS analysis (*m/z* [M + H]<sup>+</sup> 263.1137, calcd 263.1131). The <sup>1</sup>H NMR data (Table 1) showed signals for two sets of diastereotopic protons at  $\delta_H$  3.99 (H-7a), 3.85 (H-7b) and  $\delta_H$  2.42 (H-3a), 1.72 (H-3b), for four methine protons at  $\delta_H$  4.40 (H-4), 2.87 (H-6), 2.78 (H-1), and

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### RESULTS AND DISCUSSION

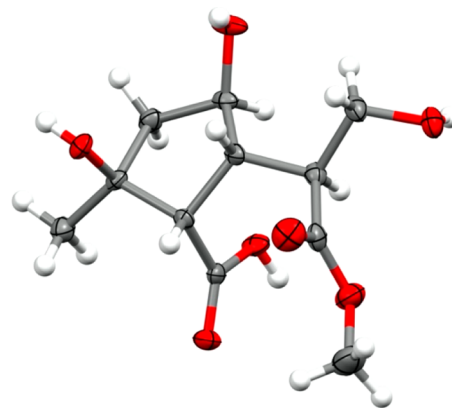
Repeated gravitational column chromatographic separation of the CH<sub>3</sub>OH extract of the stem bark of *M. asteroscepa* over silica gel followed by purification using Sephadex LH-20 and

**Table 1.**  $^1\text{H}$ ,  $^{13}\text{C}$ , and HMBC NMR Spectroscopic Data for **1** (800 MHz, Methanol- $d_4$ )

position	$\delta_{\text{C}}$ , type	$\delta_{\text{H}}$ , m (J in Hz)	HMBC, H $\rightarrow$ C
1	61.9, CH	2.78, dd (6.8, 1.9)	C-1, C-2, C-3, C-4, C-5, C-10
2	79.9, C–O		
3	49.6, CH <sub>2</sub>	2.42, dd (14.6, 9.6) 1.72, ddd (14.6, 2.5, 1.9)	C-2, C-4, C-5, C-11 C-1, C-2, C-4, C-5
4	76.7, HC–OH	4.40, ddd (9.6, 7.3, 2.5)	C-2, C-3, C-5, C-6
5	50.2, CH	2.70, ddd (11.4, 7.3, 6.8)	C-1, C-3, C-4, C-6, C-7, C-8
6	51.0, CH	2.87, ddd (11.4, 8.2, 4.9)	C-1, C-4, C-5, C-7, C-8
7	63.9, H <sub>2</sub> C–OH	3.99, dd (11.2, 4.9) 3.85, dd (11.2, 8.2)	C-5, C-6, C-8 C-5, C-6, C-8
8	175.9, OC=O		
9	51.9, OCH <sub>3</sub>	3.69, s	C-8
10	175.8, COOH		
11	25.5, CH <sub>3</sub>	1.29, s	C-1, C-2, C-3

2.70 (H-5), methyl group protons at  $\delta_{\text{H}}$  1.29 (H-11), and methoxy group protons at  $\delta_{\text{H}}$  3.69 (H-9), as confirmed by multiplicity-edited HSQC data. The COSY and TOCSY coupling patterns of these signals suggested the presence of a cyclic structure; thus, H-3a/b ( $\delta_{\text{H}}$  2.42 and 1.72) couples to H-4 ( $\delta_{\text{H}}$  4.40) with  $^3J_{\text{HH}}$  2.5 and 9.6 Hz, H-4 to H-5 ( $\delta_{\text{H}}$  2.70) with  $^3J_{\text{HH}}$  7.3 Hz, and H-5 to H-1 ( $\delta_{\text{H}}$  2.78) with  $^3J_{\text{HH}}$  1.9 Hz. In addition H-1 ( $\delta_{\text{H}}$  2.78) gave  $^3J$  HMBC correlation to C-3 ( $\delta_{\text{C}}$  49.6), and H-3 ( $\delta_{\text{H}}$  2.42) to C-1 ( $\delta_{\text{C}}$  61.9). The  $^{13}\text{C}$  NMR spectrum indicated the presence of 11 magnetically non-equivalent carbons, of which two were carbonyls at  $\delta_{\text{C}}$  175.8 (C-10) and 175.9 (C-8) with their chemical shifts being compatible with carboxylic acid or ester functionalities. An oxygenated tertiary carbon at  $\delta_{\text{C}}$  79.9 (C-2), two additional oxygenated carbons at  $\delta_{\text{C}}$  76.7 (C-4) and 63.9 (C-7), a methoxy carbon at  $\delta_{\text{C}}$  50.2 (C-9), and five aliphatic carbons at  $\delta_{\text{C}}$  25.5 (C-1), 61.9 (C-1), 49.6 (C-3), 50.2 (C-5), and 51.0 (C-6) were also detected. The cyclic structure of **1** was confirmed by the HMBC cross-peaks of H-11 ( $\delta_{\text{H}}$  1.29), H-4 ( $\delta_{\text{H}}$  4.40), H-3a,b ( $\delta_{\text{H}}$  2.42, 1.72), and H-1 ( $\delta_{\text{H}}$  2.78) with the oxygenated tertiary carbon C-2 ( $\delta_{\text{C}}$  79.9), after the C-3–C-4–C-5–C-1 connectivity was established based on COSY, TOCSY, and HMBC data. Hence, HMBC cross-peaks were observed between H-3a,b ( $\delta_{\text{H}}$  2.42, 1.72) and C-5 ( $\delta_{\text{C}}$  50.2), while H-5, ( $\delta_{\text{H}}$  2.70) showed HMBC correlation to C-1 ( $\delta_{\text{C}}$  61.9). Proton H-1 ( $\delta_{\text{H}}$  2.78) exhibited a  $^4J_{1,3b} = 1.9$  Hz *W*-coupling, observed by  $^1\text{H}$  NMR, COSY, and TOCSY spectra, compatible with the proposed cyclic structure. The position of CH<sub>3</sub>O-9 ( $\delta_{\text{H}}$  3.67) was revealed by its HMBC correlation to C-8 ( $\delta_{\text{C}}$  175.9), confirming the presence of an ester moiety. The position of the ester carbonyl was established by its HMBC correlation to the diastereotopic protons H-7a,b ( $\delta_{\text{H}}$  3.99, 3.85) and H-5 ( $\delta_{\text{H}}$  2.70). Proton H-6 ( $\delta_{\text{H}}$  2.87) showed scalar couplings to H-7a,b ( $\delta_{\text{H}}$  3.99, 3.85) and H-5 ( $\delta_{\text{H}}$  2.70), indicating the fragment C-7–C-6–C-5. The carbonyl carbon C-10 ( $\delta_{\text{C}}$  175.8) was established to be within a three-bond distance to H-5 ( $\delta_{\text{H}}$  2.70) by HMBC spectroscopy. NOE correlations between H<sub>3</sub>-11 ( $\delta_{\text{H}}$  1.29) and H-3a ( $\delta_{\text{H}}$  2.42) and between H-3a ( $\delta_{\text{H}}$  2.42) and H-4 ( $\delta_{\text{H}}$  4.40) indicated these protons to be cofacial. Proton H-4 ( $\delta_{\text{H}}$  4.40) showed NOE correlations with the side chain protons H-6 ( $\delta_{\text{H}}$  2.87) and H-

7a ( $\delta_{\text{H}}$  3.99). Proton H-11 ( $\delta_{\text{H}}$  1.29) also showed a weak NOE correlation with H-1 ( $\delta_{\text{H}}$  2.78), indicating their *trans*-relationship (strong NOE would be expected for the *cis*-isomer). Compound **1** crystallized from a 1:1 *iso*-hexane–EtOAc mixture at room temperature. The compound was analyzed by single-crystal X-ray diffraction, confirming its structure, including the absolute configurations of the five stereogenic carbons (Figure 1). Therefore, the new mor-

**Figure 1.** X-ray crystal ORTEP drawing of morisecoic acid A (**1**, CCDC 1963302). The thermal displacement parameters are shown at the 60% probability level.

isecoic acid A (**1**) was characterized as (1*S*,2*S*,4*R*,5*R*)-2,4-dihydroxy-5-[(*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl]-2-methylcyclopentane-1-carboxylic acid.

Compound **2**  $\{[\alpha]_{\text{D}}^{20} -25.0$  (*c* 2.0, CH<sub>3</sub>OH) $\}$ , isolated from the CH<sub>3</sub>OH extract of the leaves, was obtained as white solid and was assigned the molecular formula C<sub>13</sub>H<sub>20</sub>O<sub>8</sub> based on HRESIMS data analysis (*m/z* [M + H]<sup>+</sup> 305.1204, calcd 305.1236).  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Table 2) together with 2D spectra showed similar spectroscopic features to those

**Table 2.**  $^1\text{H}$ ,  $^{13}\text{C}$ , and HMBC NMR Spectroscopic Data for **2** (600 MHz, Methanol- $d_4$ )

position	$\delta_{\text{C}}$ , type	$\delta_{\text{H}}$ , m (J in Hz)	HMBC, H $\rightarrow$ C
1	59.6, CH	3.24, dd (6.8, 1.9)	C-2, C-3, C-4, C-5, C-6, C-10, C-11
2	90.2, C–O		
3	47.2, CH <sub>2</sub>	2.43, dd (14.6, 9.2) 2.35, ddd, (14.6, 2.8, 1.9)	C-1, C-2, C-4, C-5, C-11
4	75.9, HC–OH	4.40, ddd (9.2, 7.3, 2.8)	C-1, C-2, C-3, C-5, C-6
5	49.9, CH	2.50, ddd (11.6, 7.3, 6.8)	C-1, C-3, C-4, C-6, C-7, C-8
6	50.9, CH	2.83, ddd (11.6, 8.0, 4.9)	C-1, C-4, C-5, C-7, C-8
7	63.9, H <sub>2</sub> C–OH	3.96, dd (11.2, 4.9) 3.82, dd (11.2, 8.0)	C-5, C-6, C-8
8	175.9, OC=O		
9	52.1, OCH <sub>3</sub>	3.68, s	C-8
10	174.6, COOH		
11	21.2, CH <sub>3</sub>	1.54, s	C-1, C-2, C-3
13	172.3, OC=O		
14	22.5, CH <sub>3</sub>	2.03, s	C-13

observed for compound **1**, except for additional signals due to its acetoxy functionality ( $\delta_{\text{H}}$  2.02, 3H;  $\delta_{\text{C}}$  22.5 and 172.3) and associated minor chemical shift differences. The position of the acetoxy group was assigned to C-2 due to its deshielded chemical shift ( $\delta_{\text{C}}$  79.9 in **1**,  $\delta_{\text{C}}$  90.2 in **2**). Other noticeable chemical shift differences as compared to **1** were detected for H-3b ( $\delta_{\text{H}}$  1.72 to  $\delta_{\text{H}}$  2.35) and H-1 ( $\delta_{\text{H}}$  2.78 to  $\delta_{\text{H}}$  3.24) and for the corresponding carbons being shielded from  $\delta_{\text{C}}$  49.6 to  $\delta_{\text{C}}$  47.2 (C-3) and  $\delta_{\text{C}}$  61.9 to  $\delta_{\text{C}}$  59.6 (C-1). As for compound **1**, H-1 ( $\delta_{\text{H}}$  3.24) of **2** also exhibited a  $^4J_{1,3b} = 1.9$  Hz *W*-coupling. The similar NOESY correlations of compound **2** (Figure S14, Supporting Information) to those observed for **1**, along with both being levorotatory, indicated **1** and **2** to have the same absolute configurations. Based on the spectroscopic evidence, the new morisecoirdoic acid **B** (**2**) was characterized as (1*S*,2*S*,4*R*,5*R*)-2-acetoxy-4-hydroxy-5-[(*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl]-2-methylcyclopentane-1-carboxylic acid.

Compound **3**  $\{[\alpha]_{\text{D}}^{20} +35.0$  (*c* 2.0, CH<sub>3</sub>OH) $\}$ , isolated from the leaf CH<sub>3</sub>OH extract of *M. asteroscepa*, was obtained as a white solid and was assigned the molecular formula C<sub>13</sub>H<sub>18</sub>O<sub>7</sub> based on HRESIMS data analysis (*m/z* [M + H]<sup>+</sup> 287.1115, calcd 287.1131). The <sup>1</sup>H, <sup>13</sup>C (Table 3), COSY, TOCSY,

**Table 3.** <sup>1</sup>H, <sup>13</sup>C, and HMBC NMR Spectroscopic Data for **3** (800 MHz, Methanol-*d*<sub>4</sub>)

position	$\delta_{\text{C}}$ , type	$\delta_{\text{H}}$ , <i>m</i> ( <i>J</i> in Hz)	HMBC, H→C
1	171.8, OC=O		
3	68.5, H <sub>2</sub> C–O	4.53, dd (12.2, 4.0) 4.51, ddd, (12.2, 5.3, 1.2)	C-1, C-4, C-4a, C-8
4	42.5, CH	2.90, ddd (5.3, 5.3, 4.0)	C-3, C-4a, C-5, C-7a, C-8
4a	45.6, CH	2.80, dddd (12.0, 7.9, 5.3, 1.2)	C-3, C-4, C-5, C-6, C-7a, C-8
5	74.2, HC–OH	4.08, ddd (9.4, 7.9, 6.4)	C-4, C-4a, C-6, C-7, C-7a
6	46.4, CH <sub>2</sub>	2.59, dd (12.8, 6.4) 2.25, dd (12.8, 9.4)	C-4a, C-5, C-7, C-7a, C-10
7	86.9, C–O		
7a	51.4, CH	3.73, d (12.0)	C-1, C-4, C-4a, C-5, C-6, C-7, C-10
10	23.1, CH <sub>3</sub>	1.50, s	C-7, C-8, C-9
8	173.5, OC=O		
9	53.0, OCH <sub>3</sub>	3.77, s	C-8
11	172.0, OC=O		
12	22.1, CH <sub>3</sub>	2.04, s	C-1a

NOESY, HSQC, and HMBC NMR spectra of **3** indicated high structural similarity of its substituted cyclopentane moiety to those of **1** and **2**. However, significant spectroscopic differences associated with its C-3 ( $\delta_{\text{C}}$  68.5), H-3a ( $\delta_{\text{H}}$  4.53), and H-3b (4.51) were observed. Unlike compounds **1** and **2**, whose diastereotopic protons, H-7a,b did not show HMBC cross-peaks with the carboxylic carbon C-10, the corresponding diastereotopic C-3 protons of **3** ( $\delta_{\text{H}}$  4.53 and 4.51) gave  $^3J$  HMBC correlations with the carbonyl C-1 ( $\delta_{\text{C}}$  171.8), in addition to a second carbonyl at  $\delta_{\text{C}}$  173.5 (C-8). This suggested the presence of a second cyclic moiety, identified as a  $\delta$ -lactone ring. Compound **2** may be converted to **3** by the intramolecular esterification of HO-7 and the C-10 hydroxycarbonyl group, resulting in a  $\delta$ -lactone. The lactonization to form the iridolactone moiety led to changes in chemical shifts

and multiplicities (Table 3). Hence, concomitant with the disappearance of the  $^4J_{6b,7a}$  coupling, present in **1** and **2** ( $^4J_{1,3b}$ ), the  $^4J_{3b,4a} = 1.2$  Hz *W*-coupling was evident in **3**. Its  $J_{4a,7a}$  value increased to the upper limits of the *cis*-form, 12.0 Hz, due to an eclipsed relationship ( $\theta = 0^\circ$ ), unlike in compounds **1** and **2**, in which these protons exhibited an intermediate *cis*-coupling (*gauche* relationship,  $J_{1,5} = 6.8$  Hz). Such a large coupling (12.0 Hz) can also be observed for *trans*-disposed ring protons having an *anti*-relationship. However, the *cis*-orientation of H-4a ( $\delta_{\text{H}}$  2.80) and H-7a ( $\delta_{\text{H}}$  3.73) was supported by their strong NOE correlation that confirmed the relative configurations of the corresponding stereogenic carbons. The NOE correlation of H-4a ( $\delta_{\text{H}}$  2.80) with H-6b ( $\delta_{\text{H}}$  2.25) and H-3b ( $\delta_{\text{H}}$  4.51) indicated these protons to be on the same convex side of the molecule. A second set of strong NOE correlations between H-10 ( $\delta_{\text{H}}$  1.50) and H-6a ( $\delta_{\text{H}}$  2.59), H-5 ( $\delta_{\text{H}}$  4.08), H-4 ( $\delta_{\text{H}}$  2.90), and H-3a ( $\delta_{\text{H}}$  4.53) revealed another set of protons in *cis*-relationship, covering the concave side of the molecule. The CH<sub>3</sub>-10 hydrogens ( $\delta_{\text{H}}$  1.50) showed a weak NOE correlation to H-7a ( $\delta_{\text{H}}$  3.73), but did not show NOE correlation to H-4a ( $\delta_{\text{H}}$  2.80), confirming the *anti*-orientation of Me-10 and H-7a. On the basis of the above spectroscopic data, the new iridoid 8 $\beta$ -acetoxy-epishanzilactone (**3**) was characterized as methyl (4 $\alpha$ ,4 $\alpha\beta$ ,5 $\alpha$ ,7 $\alpha$ ,7 $\alpha\beta$ )-7-acetoxy-5-hydroxy-7-methyl-1-oxooctahydrocyclopenta[*c*]pyran-4-carboxylate.

Compound **4**, obtained as a white solid from the CH<sub>3</sub>OH extract of the leaves. Its ESIMS spectrum was compatible with the molecular formula C<sub>11</sub>H<sub>16</sub>O<sub>6</sub> (*m/z* 245.2 [M + H]<sup>+</sup>). The NMR data (Table 4) revealed high structural similarities to **3**,

**Table 4.** <sup>1</sup>H, <sup>13</sup>C, and HMBC NMR Spectroscopic Data for **4** (800 MHz, Methanol-*d*<sub>4</sub>)

position	$\delta_{\text{C}}$ , type	$\delta_{\text{H}}$ , <i>m</i> ( <i>J</i> in Hz)	HMBC, H→C
1	172.5, OC=O		
3	66.9, H <sub>2</sub> C–O	4.42, dd (12.1, 4.0) 4.40, ddd (12.1, 5.8, 1.2)	C-1, C-4, C-4a, C-8
4	40.6, CH	3.19, ddd (10.0, 5.8, 4.0)	C-3, C-4a, C-5, C-7a, C-8
4a	46.1, CH	2.86, dddd (10.0, 8.0, 6.0, 1.2)	C-3, C-4, C-5, C-6, C-7a, C-8
5	71.2, HC–OH	4.11, ddd (10.0, 8.0, 6.0)	C-4, C-4a, C-6, C-7, C-7a
6	50.6, CH <sub>2</sub>	2.06, dd (12.1, 6.0) 1.94, dd (12.1, 10.0)	C-4a, C-5, C-7, C-7a, C-10
7	78.6, C–OH		
7a	55.1, CH	3.34, d (12.2)	C-1, C-4, C-4a, C-5, C-6, C-7, C-10
10	25.9, CH <sub>3</sub>	1.29, s	C-6, C-7, C-7a
8	172.6, OC=O		
9	52.0, OCH <sub>3</sub>	3.77, s	C-8

indicating a substituted cyclopenta[*c*]pentanolactone iridoid core, arising from cyclization of hydroxy groups on C-1 and C-3.<sup>25–29</sup> Unlike compound **3**, this iridoid had a hydroxy instead of an acetoxy functionality at C-7, with minor chemical shift displacements being indicative of the position of substitution (Table 4). The C-3 methylene protons ( $\delta_{\text{H}}$  4.40 and 4.42) exhibited  $^3J$  HMBC correlation with the carboxylic carbon C-1 ( $\delta_{\text{C}}$  172.5), proving the esterification into an iridolactone moiety, which was further corroborated by the *W*-coupling  $^4J_{3b,4a} = 1.2$  Hz. In addition, H-4a ( $\delta_{\text{H}}$  2.86) showed a large  $^3J_{4a,7a}$  value of 12.2 Hz, supporting the eclipsed *cis*-orientation

of the involved protons, and a strong NOE correlation to H-7a ( $\delta_{\text{H}}$  3.34), further corroborating the *cis*-orientation of these protons. Furthermore, this was also supported by the NOE correlation observed of the *cis*-oriented H-4a ( $\delta_{\text{H}}$  2.86) with H-6b ( $\delta_{\text{H}}$  1.94) and H-3b ( $\delta_{\text{H}}$  4.40) on the convex side. For the concave side, the strong NOEs of H<sub>3</sub>-10 ( $\delta_{\text{H}}$  1.29) to H-6a ( $\delta_{\text{H}}$  2.06), H-5 ( $\delta_{\text{H}}$  4.11), and H-3a ( $\delta_{\text{H}}$  4.42) revealed these protons to be *cis*-oriented as well. Similar to **3**, H<sub>3</sub>-10 ( $\delta_{\text{H}}$  1.29) of **4** showed weak NOE correlation to H-7a ( $\delta_{\text{H}}$  3.34), but did not show NOE to H-4a ( $\delta_{\text{H}}$  2.86). Based on the spectroscopic evidence above, this natural product, shanzilactone (**4**), was characterized as methyl (4 $\alpha$ ,4 $\alpha$ ,5 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ )-5,7-dihydroxy-7-methyl-1-oxooctahydrocyclopenta[*c*]pyran-4-carboxylate. Shanzilactone was originally reported from *Mussaenda incana*<sup>25</sup> with **4a** as its structure. Its H-4a and H-7a were suggested to be in a *trans*-disposed orientation, based on the  $J_{5,9} = 12.0$  Hz, however without NOE data analysis. As indicated by the above analysis, particularly by the strong NOE observed between H-4a ( $\delta_{\text{H}}$  2.86) and H-7a ( $\delta_{\text{H}}$  3.34), the NOEs of H-6b ( $\delta_{\text{H}}$  1.94) with H-4a ( $\delta_{\text{H}}$  2.86) and H-7a ( $\delta_{\text{H}}$  3.34), and the absence of an NOE from H-6a ( $\delta_{\text{H}}$  2.06) to H-4a or H-7a, the earlier reported structure is incorrect. Neither is the similar chemical shift of H-4a ( $\delta_{\text{H}}$  2.86) and H-7a ( $\delta_{\text{H}}$  3.34) compatible with a *trans*-orientation of these protons. The  $J_{4a,7a}$  value of 12.0 Hz is compatible with the proposed eclipsed *cis*-orientation of these protons. The structure of **4a** is therefore revised to **4**.

The secondary metabolites barlerin (**5**),<sup>26,27</sup> 8-acetylshanzhigenin methyl ester (**6**),<sup>29,30</sup> and 8-acetyl-1-epishanzhigenin methyl ester (**7**)<sup>29,30</sup> were identified by comparison of their spectroscopic features (Tables S1 and S2, Supporting Information) to those previously published. 8-Acetylshanzhigenin methyl ester (**6**) and 8-acetyl-1-epishanzhigenin methyl ester (**7**) were obtained as an isomeric mixture. Compounds **6** and **7** have previously been reported from the family Lamiaceae only,<sup>29,30</sup> and thus this is the first report of their occurrence in the Rubiaceae.

*M. asteroscepa* and other *Morinda* species are used in indigenous medicine for the treatment of malaria, and accordingly some secondary metabolites of the genus were reported to show antiplasmodial activities.<sup>14</sup> The CH<sub>3</sub>OH extract of the leaves (10  $\mu\text{g}/\text{mL}$ ) and the isolated compounds **1**–**3** and **5** (10  $\mu\text{M}$ ) showed insignificant activity against the chloroquine-resistant parasite *Plasmodium falciparum* (3D7) (Table S3, Supporting Information).

In conclusion, two new 2,3-secoiridoids (**1** and **2**) and a new iridoid (**3**) along with four known iridoids (**4**–**7**) were isolated from *M. asteroscepa*. The structure of shanzilactone (**4**) was revised. Some of the isolated compounds showed weak antiplasmodial activities.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were determined using an Autopol II Rudolph Research Analytical WLG polarimeter at 589 nm, at 24.1 °C. NMR spectra were acquired on a Bruker Avance III HD 800 MHz, a Varian 600 MHz Inova, or a Varian 500 MHz VNMR-S spectrometer and were processed using MestReNova-10.0. The structural assignment was based on <sup>1</sup>H and <sup>13</sup>C NMR, COSY, NOESY, HSQC, and HMBC spectra. The solvent residual peak was used for chemical shift referencing (methanol-*d*<sub>4</sub>,  $\delta_{\text{H}}$  3.31 and  $\delta_{\text{C}}$  49.00; CDCl<sub>3</sub>,  $\delta_{\text{H}}$  7.26 and  $\delta_{\text{C}}$  77.16). LC-ESIMS chromatograms were acquired on a PerkinElmer PE SCIEX API 150 EX instrument equipped with a Turbolon spray ion source and a Gemini 5 mm RPC<sub>18</sub> 110 Å column, applying an H<sub>2</sub>O–MeCN, 80:20–20:80 (0.2% formic acid), gradient solvent system with a

separation time of 8 min. HRMS (ESI) was obtained with a Q-TOF/MS spectrometer (Stenhagen Analys Lab AB, Gothenburg, Sweden) using a 2.1  $\times$  30 mm, 1.7  $\mu\text{m}$ , RPC<sub>18</sub> column and a H<sub>2</sub>O–MeCN gradient system (5:95–95:5 gradient and 0.2% HCOOH). Column chromatography (CC) was carried out using silica gel 60 (230–400 mesh). Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F254 (Merck) precoated aluminum plates, which after development with an appropriate solvent system were visualized under UV light (254 and 366 nm) and then sprayed with 4-anisaldehyde reagent (prepared by mixing 3.5 mL of 4-anisaldehyde with 2.5 mL of concentrated H<sub>2</sub>SO<sub>4</sub>, 4 mL of glacial HOAc, and 90 mL of CH<sub>3</sub>OH) followed by heating for identification of UV-negative compounds and detection of the color change of the UV-positive spots. Gel filtration was carried out over Sephadex LH-20 (Pharmacia) suspended in CH<sub>2</sub>Cl<sub>2</sub>–CH<sub>3</sub>OH (1:1). Preparative HPLC was performed on a Waters 600E system using the Chromalan (Pikron Ltd.) software and an RPC<sub>8</sub> Kromasil column (250 mm  $\times$  25 mm) with the solvent system H<sub>2</sub>O–CH<sub>3</sub>OH (gradient, 90:10 to 0:100, for 20–40 min, flow rate of 8–15 mL/min).

**Plant Material.** The stem bark and leaves of *M. asteroscepa* were collected in November 2014 from the Dindira Sakare forest along the path to Manka (altitude 1245 m; GPS 37 M 0436537/9448240) West Usambara, Korogwe District in Tanga Region, Tanzania. The plant was identified by Frank M. Mbago in the field and subsequently authenticated at the Herbarium, Botany Department of the University of Dar es Salaam, where the voucher specimen (FMM 3668) is deposited.

**Extraction and Isolation.** The air-dried and pulverized leaves and stem bark were extracted with CH<sub>3</sub>OH twice for 48 h at room temperature. The extracts were concentrated using a rotatory evaporator at 40 °C, providing 57.9 g of crude stem bark extracts and 38.0 g of leaf extracts. Silica gel CC of the stem crude extract with an increasing amount of EtOAc in isohexane gave 65 fractions, ~200 mL each. The fractions were combined and concentrated based on TLC analysis. Morisecoirdoic acid A (**1**, 204.0 mg) was obtained from the silica gel column of the crude extract and crystallized overnight from the solvent at room temperature. Fractions 25–38 also afforded compound **1**, which eluted with a gradient of isohexane–EtOAc (90:10 to 10:90), followed by 5% CH<sub>3</sub>OH in EtOAc. Fractions 40–51 contained barlerin (**5**, 20.3 mg), which eluted at 10% CH<sub>3</sub>OH in EtOAc. The fractions containing compounds **4**, **6**, and **7** were subjected to further purification. The combination of the impure fractions 22–24 was purified on Sephadex LH-20 with CH<sub>2</sub>Cl<sub>2</sub>–CH<sub>3</sub>OH (1:1), giving 12 subfractions of 30 mL each, followed by additional purification of the subfractions 6–9 over silica gel eluting with EtOAc–isohexane (9:1), which provided shanzhilactone (**4**, 12.6 mg). The combined impure fractions 10–12 were passed through Sephadex LH-20 using CH<sub>2</sub>Cl<sub>2</sub>–CH<sub>3</sub>OH (1:1), yielding 21 subfractions of approximately 30 mL each, of which 3–8 were combined and further purified on an additional Sephadex LH-20 column using CH<sub>2</sub>Cl<sub>2</sub>–CH<sub>3</sub>OH (1:1), yielding fractions from which fractions 5–8 were combined and run on HPLC using H<sub>2</sub>O–CH<sub>3</sub>OH (gradient 90:10 to 0:100), providing the isomeric mixture of **6** and **7** (6.4 mg).

The crude CH<sub>3</sub>OH extract of the leaves (38 g) was fractionated and purified using a similar procedure to that described above, leading to the isolation of morisecoirdoic acid A (**1**, 80.4 mg), morisecoirdoic acid B (**2**, 13.3 mg), and 8-acetoxy-epi-shanzhilactone (**3**, 15.6 mg).

**Morisecoirdoic acid A (1):** white solid;  $[\alpha]_{\text{D}}^{20} -37.5$  (*c* 2.0, CH<sub>3</sub>OH); <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; HRESIMS  $[M + H]^+$  *m/z* 263.1137 (calcd for C<sub>11</sub>H<sub>18</sub>O<sub>7</sub>, 263.1131).

**Morisecoirdoic acid B (2):** white solid;  $[\alpha]_{\text{D}}^{20} -25.0$  (*c* 2.0, CH<sub>3</sub>OH); <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 2; HRESIMS  $[M + H]^+$  (*m/z*) 305.1236 (calcd for C<sub>13</sub>H<sub>20</sub>O<sub>8</sub>, 305.1204).

**8-Acetoxy-epi-shanzhilactone (3):** white solid;  $[\alpha]_{\text{D}}^{20} +35.0$  (*c* 2.0, CH<sub>3</sub>OH); <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 3; HRESIMS  $[M + H]^+$  (*m/z*) 287.1115 (calcd for C<sub>13</sub>H<sub>18</sub>O<sub>7</sub>, 287.1131).

**Shanzhilacone (4):** white, amorphous solid;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 4; MS (ESI)  $[\text{M} + \text{H}]^+$  ( $m/z$ ) 245.2 (calcd for  $\text{C}_{11}\text{H}_{17}\text{O}_6$ , 245.1).

**Barlerin (5):** white, amorphous solid;  $[\alpha]_D^{20}$   $-81.5$  ( $c$  2.0,  $\text{CH}_3\text{OH}$ );  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table S1; MS (ESI)  $[\text{M} + \text{H}]^+$  ( $m/z$ ) 449.4 (calcd for  $\text{C}_{19}\text{H}_{28}\text{O}_{12}$ , 449.4).

**8-Acetylshanzhigenin methyl ester (6):** white, amorphous solid;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table S2; MS (ESI) ( $m/z$ )  $[\text{M} + \text{H}]^+$  287.2 (calcd for  $\text{C}_{13}\text{H}_{18}\text{O}_7$ , 287.1).

**Antiplasmodial Asexual Assay.** The antiplasmodial activity was determined using an imaging-based assay method as previously described.<sup>31</sup> Human red blood cells for the culture of *Plasmodium falciparum* were provided by the Australian Red Cross Blood Bank following their routine MTA for nonclinical blood product supply. The antiplasmodial assay work undertaken is covered by the approval from the Griffith University Biosafety and Human Ethics Committee, GU ref no. ESK/03/12/HREC; 03/08/11019.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00447>.

1D and 2D NMR spectra and HRESIMS for compounds 1–4; NMR data for compound 5–7 (PDF)

X-ray crystal data (CIF)

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## Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

- (1) Lovett, J.; Clarke, G. P.; Morinda asteroscepa. The IUCN Red List of Threatened Species 2019: e.T34858A2856538.
- (2) Hamilton, A. C.; Bensted-Smith, R. *Forest Conservation in the East Usambara Mountains*; IUCN: Tanzania, 1989; Vol. 15.
- (3) Odotuga, A.; Dairo, J.; Minari, J.; Bamiyaye, F. *Res. J. Pharm.* **2010**, *4*, 78–82.
- (4) Sang, S.; Cheng, X.; Zhu, N.; Stark, R. E.; Badmaev, V.; Ghai, G.; Rosen, R. T.; Ho, C.-T. *J. Agric. Food Chem.* **2001**, *49*, 4478–4481.
- (5) Deng, S.; West, B. J.; Jensen, C. *ISRN Anal. Chem.* **2012**, *2012*, 1.
- (6) Phakhodee, W. *Walailak J. Sci. Technol.* **2012**, *9*, 173–188.
- (7) Ali, A.; Ismail, N.; Mackeen, M.; Yazan, L.; Mohamed, S.; Ho, A.; Lajis, N. *Pharm. Biol.* **2000**, *38*, 298–301.
- (8) Rath, G.; Ndonzao, M.; Hostettmann, K. *Int. J. Pharmacogn.* **1995**, *33*, 107–114.
- (9) Takashima, J.; Ikeda, Y.; Komiyama, K.; Hayashi, M.; Kishida, A.; Ohsaki, A. *Chem. Pharm. Bull.* **2007**, *55*, 343–345.
- (10) Jasril, L. N.; Mooi, L. Y.; Abdullah, M. A.; Sukari, M. A.; Ali, A. M. *Asia Pac. J. Mol. Biol. Biotechnol.* **2003**, *11*, 3–7.
- (11) Umezawa, K. *Japan Kokai Tokyo Koho JP* **1992**, *6*, 736.
- (12) Akihisa, T.; Matsumoto, K.; Tokuda, H.; Yasukawa, K.; Seino, K. -i.; Nakamoto, K.; Kuninaga, H.; Suzuki, T.; Kimura, Y. *J. Nat. Prod.* **2007**, *70*, 754–757.
- (13) Wu, Y.-B.; Zheng, C.-J.; Qin, L.-P.; Sun, L.-N.; Han, T.; Jiao, L.; Zhang, Q.-Y.; Wu, J.-Z. *Molecules* **2009**, *14*, 573–583.
- (14) Tamura, S.; Kubata, B. K.; Itagaki, S.; Horii, T.; Taba, M. K.; Murakami, N. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1520–1523.
- (15) Nivas, D.; Gaikwad, D.; Chavan, P. *Ethnopharm.* **2010**, *2*, 1–11.
- (16) West, B.; Deng, S.; Isami, F.; Uwaya, A.; Jensen, C. *Foods* **2018**, *7*, 58.
- (17) Wang, M.-Y.; West, B. J.; Jensen, C. J.; Nowicki, D.; Su, C.; Palu, A. K.; Anderson, G. *Acta Pharm. Sin.* **2002**, *23*, 1127–1141.
- (18) Maeda, G.; Munissi, J. J. E.; Lindblad, S.; Duffy, S.; Pelletier, J.; Avery, V. M.; Nyandoro, S. S.; Erdelyi, M. *J. Nat. Prod.* **2020**, *83*, 316–322.
- (19) Maeda, G.; Van der Wal, J.; Gupta, A. K.; Munissi, J. J. E.; Orthaber, A.; Sunnerhagen, P.; Nyandoro, S. S.; Erdelyi, M. *J. Nat. Prod.* **2020**, *83*, 210–215.
- (20) Nyandoro, S. S.; Maeda, G.; Munissi, J. J. E.; Gruhonjic, A.; Fitzpatrick, P. A.; Lindblad, S.; Duffy, S.; Pelletier, J.; Pan, F.; Puttreddy, R.; Avery, V. M.; Erdelyi, M. *Molecules* **2019**, *24*, 2746.

- (21) Nyandoro, S. S.; Munissi, J. J.; Kombo, M.; Mgina, C. A.; Pan, F.; Gruhonjic, A.; Fitzpatrick, P.; Lu, Y.; Wang, B.; Rissanen, K.; Erdelyi, M. *J. Nat. Prod.* **2017**, *80*, 377–383.
- (22) Nyandoro, S. S.; Munissi, J. J.; Gruhonjic, A.; Duffy, S.; Pan, F.; Puttreddy, R.; Holleran, J. P.; Fitzpatrick, P. A.; Pelletier, J.; Avery, V. M.; Rissanen, K.; Erdelyi, M. *J. Nat. Prod.* **2017**, *80*, 114–125.
- (23) Nyandoro, S. S.; Ndanu, J.; Munissi, J. J.; Gruhonjic, A.; Fitzpatrick, P. A.; Landberg, G.; Lu, Y.; Wang, B.; Pan, F.; Rissanen, K.; Erdelyi, M. *J. Nat. Prod.* **2015**, *78*, 2045–2050.
- (24) Robien, W. *Monatsh. Chem.* **2019**, *150*, 927–932.
- (25) Dinda, B.; Debnath, S.; Majumder, S.; Arima, S.; Sato, N.; Harigaya, Y. *Indian J. Chem.* **2005**, *44B*, 2362–2366.
- (26) Byrne, L.; Sasse, J.; Skelton, B.; Suksamrarn, A.; White, A. *Aust. J. Chem.* **1987**, *40*, 785–794.
- (27) Kanchanapoom, T.; Kasai, R.; Yamasaki, K. *Phytochemistry* **2001**, *58*, 337–341.
- (28) Guo, S.-J.; Gao, L.-M.; Cheng, D.-L. *Pharm.* **2001**, *56*, 178–180.
- (29) Tan, J.-J.; Tan, J.-M.; Tan, C.-H.; Qu, S.-J.; Zhu, D.-Y. *Nat. Prod. Res.* **2012**, *26*, 1213–1217.
- (30) Nyandoro, S. S.; Munissi, J. J.; Gruhonjic, A.; Duffy, S.; Pan, F.; Puttreddy, R.; Holleran, J. P.; Fitzpatrick, P. A.; Pelletier, J.; Avery, V. M. *J. Nat. Prod.* **2017**, *80*, 114–125.
- (31) Duffy, S.; Avery, V. M. *Am. J. Trop. Med. Hyg.* **2012**, *86*, 84–92.