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Article

Prenylated Flavonoids from the Roots of *Tephrosia rhodesica*

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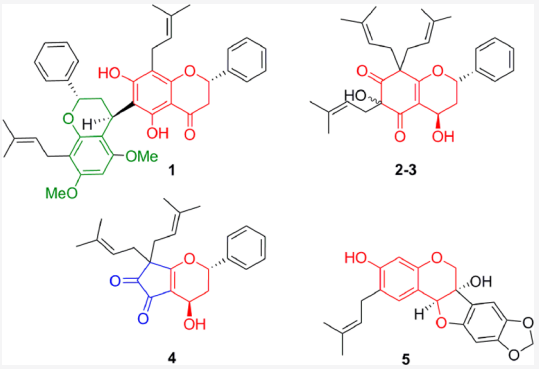
ABSTRACT: Five new compounds—rhodimer (**1**), rhodiflavan A (**2**), rhodiflavan B (**3**), rhodiflavan C (**4**), and rhodacarpin (**5**)—along with 16 known secondary metabolites, were isolated from the CH_2Cl_2−CH_3OH (1:1) extract of the roots of *Tephrosia rhodesica*. They were identified by NMR spectroscopic, mass spectrometric, X-ray crystallographic, and ECD spectroscopic analyses. The crude extract and the isolated compounds **2**−**5**, **9**, **15**, and **21** showed activity (100% at 10 μg and IC_{50} = 5−15 μM) against the chloroquine-sensitive (3D7) strain of *Plasmodium falciparum*.



Tephrosia (Leguminosae) is a tropical and subtropical genus consisting of more than 400 species, 30 of which are native to Kenya.[1] These have provided a variety of new flavonoids, including flavanones, flavones, chalcones, pterocarpans, and rotenoids, most of them prenylated and showing promising antiplasmodial, antifeedant, antileishmanial, estrogenic, antitumor, and antimicrobial activities.[2−6] As part of our ongoing work on the genus *Tephrosia*,[6,7] we now report the first phytochemical investigation of the roots of *Tephrosia rhodesica*, which showed antiplasmodial activity. The investigation of the root extract led to the isolation of five new compounds (**1**−**5**) and 16 known compounds (**6**−**21**). Herein, we describe their isolation, characterization, and activity against the chloroquine-sensitive (3D7) strain of *Plasmodium falciparum*.

RESULTS AND DISCUSSION

Applying silica gel gravity column chromatography, followed by Sephadex LH-20 gel filtration and preparative reverse-phase HPLC, 21 secondary metabolites were isolated from the roots of *T. rhodesica*. The structures of the isolated secondary metabolites were determined by NMR spectroscopic, mass spectrometric, and single-crystal X-ray diffractometric analyses. In addition to the five new secondary metabolites (**1**−**5**), the known tephrowatsin B (**6**),[8] tephrinone (**7**),[9] glabranin (**8**),[10] quercetol B (**9**),[11] maackiain (**10**),[12] 6a-hydroxymaackiain (**11**),[13] pisatin (**12**),[14] tephrosin (**13**),[15] rotenone (**14**),[15] 6-hydroxyrotenone (**15**),[16] 12a-hydroxyrotenone (**16**),[17] hildecarpin (**17**),[18] 3-hydroxy-2-methoxy-8,9-methylenedioxypterocarpene (**18**),[19] isoliquirtigenin (**19**),[20] D-pinitol (**20**),[21] and tephrowatsin A (**21**)[8] were identified by comparison of their observed and reported spectroscopic data (see pages S22−S34 in the Supporting Information).

Compound **1** was isolated as white crystals from CH_2Cl_2−CH_3OH (1:1) solution. Its molecular formula was deduced as $C_{42}H_{44}O_7$ based on HREIMS ($[M]^+$ *m*/*z* 660.3095, calcd 660.3087) and NMR data analyses (see Table 1, as well as Figures S1−S6 in the Supporting Information). The ^1H NMR signals at δ_H5.43 (H-2), 5.06 (H-2′′′), 2.84 and 3.07 (H-3a-b), 2.18 and 2.31 (H-3′′′), and 4.66 (H-4′′′), along with the ^{13}C NMR signals at δ_C78.6 (C-2), 75.4 (C-2′′′), 43.7 (C-3), 36.9 (C-3′′′), 196.3 (C-4), and 26.8 (C-4′′′) were consistent with a flavanone-flavan dimer core structure[22,23] that was corroborated by the UV absorptions of λ_{max} = 230, 290, and 350 nm. The NMR data (Table 1) indicated the presence of two sets of unsubstituted aromatic rings, two sets of prenyl groups, two hydroxy, and two methoxy groups. This is consistent with a 5-hydroxyflavanone moiety possessing an unsubstituted ring B, along with a ring A substituted with prenyl at C-8 (δ_C 108.1) and two hydroxy groups, δ_H 12.61 (HO-5) and 6.80 (HO-7) at C-5 (δ_C 159.3) and C-7 (δ_C 162.8). Accordingly, C-6 (δ_C 109.8) connects the flavanone to the flavan moiety. Whereas the placement of a hydroxy group at C-7 was based on biogenetic considerations,[24] and on the chemical shift of the HO-5 hydrogen (δ_H 12.61), suggesting its involvement in an intramolecular hydrogen bond; the placement of the prenyl group at C-8 (δ_C 108.1) rather than at C-6 (δ_C 109.8) was

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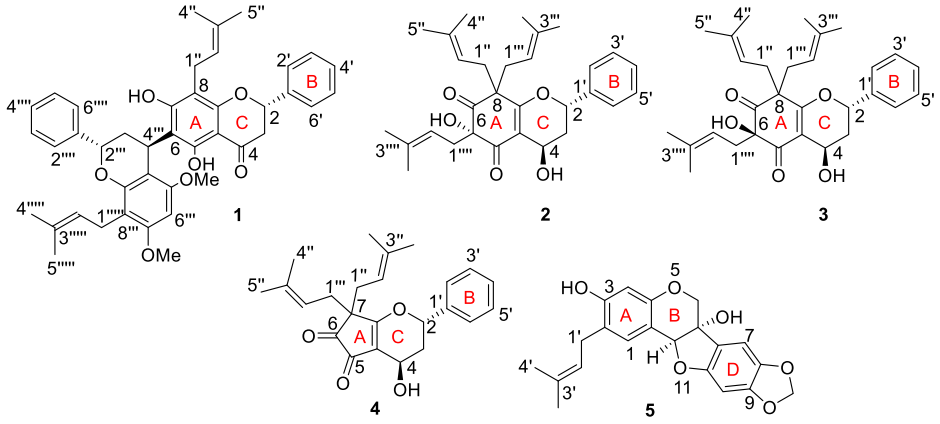

Table 1. NMR Spectroscopic Data (800 MHz, $CDCl_3$) for Rhodimer (1)

position	$\delta_{C,\,type}$	δ_H (J, in Hz)	HMBC
2	78.6, CH	5.43, dd (2.9, 13.1)	C-4, C-1′, C-2′/6′, C-8a
3	43.7, CH_2	2.84, dd (2.9, 17.1) 3.07, dd (13.1, 17.1)	C-2, C-4, C-1′, C-4, C-4a, C-1′
4	196.3, C═O		
4a	100.4, C		
5	159.3, C−O		
6	109.8, C		
7	162.8, C−O		
8	108.1, C		
8a	157.6, C−O		
1′	139.1, C		
2′/6′	125.9, CH	7.47, m	C-2, C-2′/6′, C-3′/5′
3′/5′	128.7, CH	7.43, m	C-1′, C-3′/5′
4′	128.5, CH	7.39, m	C-2′/6′
1″	21.8, CH_2	3.18, br t	C-7, C-8, C-8a, C-3″, C-2″
2″	122.5, CH	5.15, br t	C-1″, C-4″, C-5″
3″	131.6, C		
4″	17.8, CH_3	1.57, s	C-3″, C-2″, C-5′
5″	25.8, CH_3	1.65, s	C-3″, C-2″, C-4′
OH-5		12.61, s	C-5, C-6, C-4a, C-4, C-7
OH-7		6.80, s	C-6, C-8, C-8a, C-7, C-4a
2‴	75.4, CH	5.06, dd (2.0, 11.5)	C-4‴, C-1⁗, C-2⁗/6⁗, C-8a
3‴	36.9, CH_2	2.18, dt (2.0, 6.0, 14.0) 2.31, dt (2.0, 11.5, 14.0)	C-1⁗, C-4‴, C-6, C-4‴, C-4‴a, C-6
4‴	26.8, CH	4.66, dd (2.0, 6.0)	C-2‴, C-3‴, C-4‴a, C-5‴, C-8‴a, C-5, C-6, C-7
4‴a	102.8, C		
5‴	157.5, C−O		
6‴	88.4, CH	6.17, s	C-4‴, C-4‴a, C-5‴, C-7‴, C-8‴, C-1⁗⁗ (w)
7‴	158.6, C−O		
8‴	110.5, C		
8‴a	154.3, C−O		
1⁗	141.4, C		
2⁗/6⁗	126.0, CH	7.38, m	C-2‴, C-2⁗/6⁗, C-3⁗/5⁗
3⁗/5⁗	128.3, CH	7.34, m	C-3⁗/5⁗, C-1⁗
4⁗	127.6, CH	7.29, m	C-2⁗/6⁗
1⁗⁗	21.9, CH_2	3.35, br t	C-8‴, C-8a‴, C-7‴, C-3⁗⁗, C-2⁗⁗
2⁗⁗	123.2, CH	5.24, br t	C-1⁗⁗, C-4⁗⁗, C-5⁗⁗
3⁗⁗	130.8, C		
4⁗⁗	17.8, CH_3	1.66, s	C-2⁗⁗, C-3⁗⁗, C-5⁗⁗
5⁗⁗	25.9, CH_3	1.68, s	C-2⁗⁗, C-3⁗⁗, C-4⁗⁗
OMe-5‴	55.9, C−O	3.73, s	C-5‴
OMe-7‴	55.8, C−O	3.87, s	C-7‴

based on the HMBC correlation of CH_2−1″ (δ_H 3.18) with C-7 (δ_C 162.8), C-8 (δ_C 108.1), C-8a (δ_C 157.6), C-3″ (δ_C 131.6), C-2″ (δ_C 122.5), and of H-2 (δ_H 5.43) with C-4 (δ_C 196.3), C-1′ (δ_C 139.1), C-2′/C-6′ (δ_C 125.9), C-8a (δ_C 157.6). The identity of the other half of the molecule was established as a 5,7-dimethoxy-8-prenylflavan moiety based on the NMR data (Table 1). Hence, its ^1H NMR spectrum revealed the presence of an unsubstituted aromatic ring (ring

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E) [δ_H 7.38 (H-2′′′′/6′′′′), 7.34 (H-3′′′′/5′′′′), 7.29 (H-4′′′′)], two methoxy groups at δ_H 3.73 (OMe-5′′′) and 3.87 (OMe-7′′′) and a prenyl side chain [δ_H 3.35 (H-1′′′′′), 5.24 (H-2′′′′′), 1.66 (H-4′′′′′), and 1.68 (H-5′′′′′)], and a singlet at δ_H 6.17 (H-6′′′). The placement of the two methoxy groups was established based on the HMBC correlation of H-6′′′ (δ_H 6.17) with C-4′′′ (δ_C 102.8), C-5′′′ (δ_C 157.5), and C-7′′′ (δ_C 158.6). The prenyl group was positioned at C-8′′′ (δ_C 110.5) based on the HMBC cross-peaks of H_2-1′′′′′ (δ_H 3.35) to C-8′′′ (δ_C 110.5), C-8a′′′ (δ_C 154.3), and C-7′′′ (δ_C 158.6). Methoxy groups were placed at C-5′′′ (δ_C 157.5) and C-7′′′ (δ_C 158.6) upon biogenetic considerations,[24] as well as based on the HMBC correlations of MeO-5′′′ (δ_H 3.73) to C-5′′′ (δ_C 157.5), and of MeO-7′′′ (δ_H3.87) to C-7′′′ (δ_C 158.6). This assignment was corroborated by the NOEs of MeO-5′′′ (δ_H 3.73) and MeO-7′′′ (δ_H 3.87) with H-6′′′ (δ_H 6.17). The C-6 (δ_C 109.8) to C-4′′′ (δ_C 26.8) linkage of the flavanone and the flavan moieties was confirmed by the HMBC correlations of H-4′′′ (δ_H4.66) with C-5 (δ_C 159.3), C-6 (δ_C 109.8), and C-7 (δ_C 162.8), and of H_2-3′′′ (δ_H 2.18 and 2.31) with C-6 (δ_C 109.8). Because of its three stereocenters at C-2, C-2′′′, and C-4′′′, **1** has eight possible stereoisomers. The large $^3J_{2,3}$ = 13.1 Hz indicated H-2 to be axial, and thereby ring B to be equatorial. Based on the available literature on the flavanones of this genus, C-2 is expected to be *S*-configured.[1,25] Similarly, the large $^3J_{2''',3'''}$ = 11.5 Hz suggests H-2′′′ to be axial, and accordingly ring B of the flavan moiety to be equatorially oriented. Furthermore, based on the literature,[25] C-2′′′ is also expected to be *S*-configured as in the flavanones and flavans of this genus. The $^3J_{3a''',4'''}$ = 2.0 suggests the axial−equatorial or equatorial−equatorial orientation of the involved protons, whereas $^3J_{3b''',4'''}$ = 6.0 Hz gauche orientation, suggesting H-4′′′ to be axially oriented and, consequently, the flavan moiety to occupy the more favorable equatorial orientation with C-4′′′ being *R*-configured.[25] The NOE correlation of H-2′′′ (δ_H5.06) and HO-7 (δ_H 6.80) reveals the flavanone and H-2′′′ to be oriented in the same direction. This absolute configuration (2*S*,2′′′*S*,4′′′*R*), proposed on the basis of biogenetic considerations and NMR analyses was subsequently confirmed by single-crystal X-ray crystallography (Figure 1). Thus, based on the above spectroscopic data, this new compound, rhodimer (**1**), was characterized as (2*S*,2′′′*S*,4*R*)-5,7-dihydroxy-5′′′,7′′′-dimethoxy-8,8′′′-bis(3-methylbut-2-en-1-yl)-2′,2′′′-diphenyl-[4′′′,6-bichroman]-4-one.

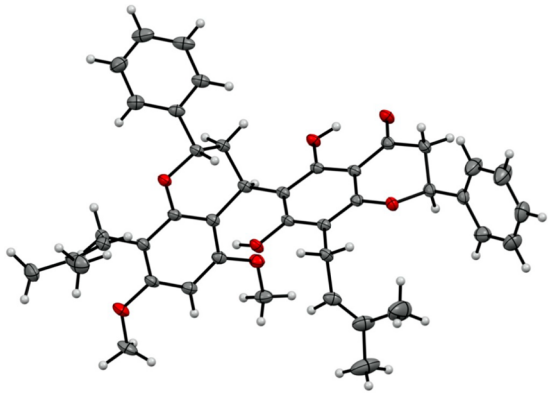

Figure 1. Single crystal X-ray structure of compound **1** with the thermal displacement parameters at 40% probability level.

Compound **2** was isolated as an oily paste and was assigned the molecular formula $C_{30}H_{38}O_5$, based on HRESIMS ([M + H]$^+$ *m*/*z* 479.2493, calcd 479.2753) and NMR data analyses (see Table 2, as well as Figures S9−S14 in the Supporting Information). The ^{1}H NMR signals at δ_H 5.22 (H-2), 1.80 (H-3$_{ax}$), 2.31 (H-3$_{eq}$), and 4.99 (H-4), along with the ^{13}C NMR signals at δ_C76.1 (C-2), 37.0 (C-3), and 57.4 (C-4), suggested it to be a flavan-4-ol derivative, which was corroborated by the UV absorptions λ_{max} = 240 and 290 nm. The characteristic flavan-4-ol ring C of **2** was confirmed by the HMBC correlations of H-4 (δ_H 4.99) with C-2 (δ_C 76.1), C-3 (δ_C 37.0), C-5 (δ_C 194.7), C-4a (δ_C 114.9), and C-8a (δ_C 171.0), and by the COSY correlations of H-2 (δ_H 5.22) with CH_2−3 (δ_H 2.31 and 1.80), and of the latter with H-4 (δ_H 4.99). The six methyl (H_3-4′′, H_3-4′′′, H_3-4′′′′, H_3-5′′, H_3-5′′′, and H_3-5′′′), along with the three methine signals (H-2′′, H-2′′′, and H-2′′′′) were diagnostic for three prenyl groups. The NMR signals of H-2′/6′, H-3′/5′, and H-4′ (Table 2) further suggested ring B to be unsubstituted and, hence, all three prenyl groups to be connected to ring A. This ring is oxygenated at C-5 (δ_C 194.7) and C-7 (δ_C 206.4), with their placement having been derived from biogenetic considerations.[24] An additional oxygenation is presented at C-6 (δ_C 83.3), which uncharacteristically is an sp^3 hybridized carbon. Prenyl substitution at this carbon was confirmed by the HMBC correlation of H-2′′′′ (δ_H 4.99) with C-6 (δ_C 83.3). The location of the other two prenyl groups at C-8 (δ_C 58.3), also sp^3 hybridized, was established from the ^3J HMBC correlations of H-2′′ (δ_H 5.03, *m*) and H-2′′′ (δ_H 4.66, *m*) with C-8 (δ_C 58.3). Moreover, CH_2−1′′ (δ_H 2.60 and 2.81) and CH_2−1′′′ (δ_H 2.46 and 2.77) showed HMBC cross-peaks with C-7 (δ_C 206.4) and C-8a (δ_C 171.0), whereas CH_2−1′′′′ (δ_H 2.71 and 2.25) with C-5 (δ_C 194.7), confirming that ring A is fully substituted. Based on the above data, compound **2** was characterized as 4,6-dihydroxy-6,8,8-tris(3-methylbut-2-en-1-yl)-2-phenyl-2,3,4,8-tetrahydro-5*H*-chromene-5,7(6*H*)-dione. Its configurational assignment is discussed below, together with that of the structurally closely related compound **3**.

Compound **3** was isolated as an oily paste and was assigned the molecular formula $C_{30}H_{38}O_5$ based on HRESIMS ([M + H]$^+$ *m*/*z* 479.2493, calcd 479.2753) and NMR data (see Table 2, as well as Figures S16−S21 in the Supporting Information). The ^{1}H NMR signals at δ_H 5.24 (H-2), 2.00 and 2.30 (CH_2−3), and δ_H4.55 (H-4) and ^{13}C NMR signals at δ_C76.2 (C-2), 36.5 (C-3), and 59.5 (C-4) suggested **3** to be a flavan derivative with identical core structure, 4,6-dihydroxy-6,8,8-tris(3-methylbut-2-en-1-yl)-2-phenyl-2,3,4,8-tetrahydro-5*H*-chromene-5,7(6*H*)-dione to compound **2**. The highly similar NMR data of **2** and **3** (Table 2) indicated these compounds to be stereoisomers.

Compounds **2** ([α]$_D$ +66.1) and **3** ([α]$_D$ +17.5) are both dextrorotatory (for details, see the Experimental Section) and, hence, are diastereomers (as enantiomers would be expected to rotate plan-polarized light in opposite directions). The large coupling constant $^3J_{2,3ax}$ (12.6 Hz in both **2** and **3**) is consistent with H-2 being axial and ring B of both compounds to occupy the more favorable equatorial orientation.[25] The $^3J_{3ax,4}$ (4.2 Hz for **2**, and 3.7 Hz for **3**) and $^3J_{3eq,4}$ (1.9 Hz in **2**, 2.3 Hz in **3**) are small, suggesting H-4 to be equatorial and hence HO-4 to be axial in both compounds, according to Pouget et al.[26] This is further corroborated by the 3.4 Å H-2−H-4 interatomic distance of **3**, estimated based on the NOE cross-peak intensities using H-3a/b as an internal reference (1.8 Å; see

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Table 2. NMR Spectroscopic Data (800 MHz, $CDCl_3$) for Rhodiflavan A (2) and Rhodiflavan B (3)

	2			3		
position	δ_C, type	δ_H, m (J in Hz)	HMBC	δ_C, type	δ_H, m (J in Hz)	HMBC
2	76.1, CH	5.22, dd (12.6, 2.3)	C-3, C-4, C-1′, C-2′, C-6′	76.2, CH	5.24, dd (12.6, 2.3)	C-3, C-4, C-1′, C-2′, C-6′
3	37.0, CH_2	2.31, ddd (14.6, 2.3, 1.9)	C-4, C-4a	36.5, CH_2	2.30, dt (14.6, 2.3, 2.0)	C-4, C-4a
		1.80, ddd (14.6, 12.6, 4.2)	C-1′, C-2		2.00, ddd (14.6, 12.6, 3.6)	C-2, C-1′
4	57.4, CH	4.99, m	C-2, C-3, C-5, C-4a, C-8a	59.5, CH	4.55, dd (2.0, 3.6)	C-2, C-4a, C-8a, C-5
4a	114.9, C			114.2, C		
5	194.7, C−O			197.0, C−O		
6	83.3, C−OH			83.4, C−OH		
7	206.4, C═O			207.2, C═O		
8	58.3, C			58.3, C		
8a	171.0, C−O			171.0, C−O		
1′	138.9, C			139.2, C		
2′, 6′	125.9, CH	7.40, m	C-2, C- 2′, C-4′, C-6′	125.9, CH	7.40, m	C-2, C-4′, C-2′, C-6′
3′, 5′	128.7, CH	7.43, m	C-1′, C-3′, C-5′	128.7, CH	7.43, m	C-1′, C-3′, C-5′
4′	128.5, CH	7.39, m	C-1′, C-2′, C-6′	128.5, CH	7.39, m	C-1′, C-2′, C-6′
1″	35.0, CH_2	2.81, dd (14.2, 9.2)	C-7, C-8, C-8a, C-2″, C-3″, C-1‴	34.5, CH_2	2.89, dd (14.2, 9.8)	C-7, C-8, C-8a, C-2″, C-3″, C-1‴
		2.60, dd (6.3, 14.2)	C-7, C-8, C-8a, C-2″, C-3″, C-1‴		2.59, m	C-7, C-8, C-8a, C-2″, C-3″, C-1‴
1‴	38.5, CH_2	2.77, m	C-7, C-8, C-8a, C-2‴, C-3‴, C-1″	38.5, CH_2	2.69, dd (13.5, 7.3)	C-7, C-8, C-8a, C-2‴, C-3‴, C-1″
		2.46, dd (13.7, 8.2)	C-7, C-8, C-8a, C-2‴, C-3‴, C-1″		2.43, dd (13.5, 8.7)	C-7, C-8, C-8a, C-2‴, C-3‴, C-1″
1⁗	38.5, CH_2	2.71, dd (14.9, 6.3)	C-6, C-5, C-2⁗	38.2, CH_2	2.63, dd (14.7, 8.7)	C-6, C-5, C-2⁗
		2.25, dd (15.0, 5.7)	C-6, C-5, C-2⁗		2.18, m	C-6, C-5, C-2⁗
2″	118.8, CH	5.03, m	C-1″, C-4″, C-5″	118.8, CH	5.13, m	C-8, C-1″, C-4″, C-5″
2‴	117.4, CH	4.66, m	C-8, C-1‴, C-4‴, C-5‴	117.4, CH	4.71, m	C-8, C-1‴, C-4‴, C-5‴
2⁗	115.6, CH	4.99, m	C-6, C-4⁗, C-5⁗	115.5, CH	4.97, m	C-6, C-4⁗, C-5⁗
3″	136.0, C			136.0, C		
3‴	136.9, C			136.9, C		
3⁗	136.9, C			136.9, C		
4″	26.0, CH_3	1.64, s	C-1″, C-2″, C-5″	18.0, CH_3	1.61, s	C-1″, C-2″, C-5″
4‴	18.1, CH_3	1.52, s	C-1‴, C-2‴, C-5‴	17.8, CH_3	1.52, s	C-1‴, C-2‴, C-5‴
4⁗	26.0, CH_3	1.71, s	C-2⁗, C-3⁗, C-5⁗	25.9, CH_3	1.71, s	C-2⁗, C-3⁗, C-5⁗
5″	18.1, CH_3	1.59, s	C-1″, C-2″, C-4‴	26.0, CH_3	1.68, s	C-1″, C-2″, C-4‴
5‴	25.8, CH_3	1.55, s	C-1″, C-2″, C-4‴	25.7, CH_3	1.56, s	C-1″, C-2″, C-4‴
5⁗	17.9, CH_3	1.55, s	C-2⁗, C-3⁗, C-4⁗	17.9, CH_3	1.54, s	C-2⁗, C-3⁗,C-4⁗

Figure S23 in the Supporting Information). This distance was computed to 2.3 Å for the (2*S**,4*S**) diastereomer, and 3.7 Å for the (2*S**,4*R**) configured C-ring, using Boltzmann averaging of the H-2−H-3 distances of the output conformers of Monte Carlo conformational searches (see Experimental Section for details; this distance was not measured for **2**, because of signal overlap). Based on biogenetic considerations discussed for compound **1** and the NMR data, compounds **2** and **3** are C-6 epimers and possess a (2*S*,4*R*) absolute configuration. In order to determine their absolute configuration, their electronic circular dichroism (ECD) spectra (Figure 2) were recorded. The observed Cotton effects supported the ORD-based suggestion of the diastereomeric relationship of **2** and **3** as enantiomers are expected to show mirror-image ECD spectra. The comparison of the experimental spectra with those reported for flavan-4-ols[11] did not allow configurational assignment, because of the dissimilarity of rings A of **2** and **3**, compared to those of the reported compounds. Consequently, the Boltzmann-weighted ECD spectra of the possible diastereomers were calculated using

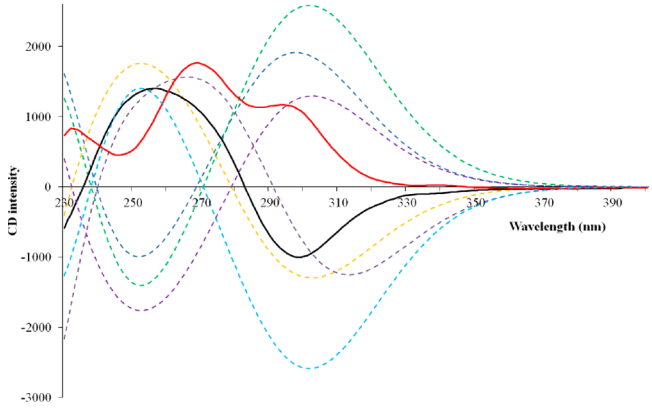

Figure 2. ECD spectra of compounds **2** (black) and **3** (red) along with the calculated spectra of the stereoisomers [(2*S*,4*R*,6*S*) yellow, (2*S*,4*R*,6*R*) blue, (2*S*,4*S*,6*R*) green, (2*S*,4*S*,6*S*) lilac, (2*R*,4*S*,6*R*) pink, (2*R*,4*S*,6*S*) light blue]. See details of the ECD calculations in the Experimental Section.

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Table 3. ^{1}H and ^{13}C NMR Spectroscopic Data (800 MHz, $CDCl_3$) for Rhodiflavan C (4)

position	δ_C, type	δ_H, m (J in Hz)	HMBC
2	78.8, CH	5.45, dd (12.3, 2.2)	C-3, C-4, C-1′, C-2′/C-6′
3	37.0, CH_2	2.31, dt (14.8, 2.3)	C-4, C-4a
		2.02, ddd (14.9, 12.3, 3.5)	C-2, C-1′
4	55.6, CH	4.86, m	C-2, C-5, C-4a, C-7a
4a	125.9, C		
5	184.5, C═O		
6	203.4 C═O		
7	52.9, C,		
7a	188.6, C−O		
1′	137.9, C		
2′, 6′	126.3, CH	7.40, m	C-2, C-2′, C-4′, C-6′
3′, 5′	128.9, CH	7.46, m	C-1′, C-2′, C-3′, C-5′, C-6′
4′	129.1, CH	7.43, m	C-2′, C-6′, C-3′, C-5′
1″	32.8, CH_2	2.51, dd (14.3, 7.2)	C-5, C-6, C-7, C-7a, C-2″, C-3″, C-1‴
		2.42, dd (14.3, 7.9)	C-5, C-6, C-7, C-7a, C-2″, C-3″, C-1‴
1‴	32.6, CH_2	2.56, dd (14.2, 7.2)	C-5, C-6, C-7, C-7a, C-2‴, C-3‴, C-1″
		2.44, dd (14.2, 7.9)	C-5, C-6, C-7, C-7a, C-1″, C-2‴, C-3‴
2″	116.9, CH	4.86, m	C-8, C-1″, C-4″, C-5″
2‴	116.9, CH	4.86, m	C-8, C-1‴, C-4‴, C-5‴
3″	136.6, C		
3‴	136.8, C		
4″	25.9, CH_3	1.61, s	C-1″, C-2″, C-5″
4‴	25.9, CH_3	1.61, s	C-1″, C-2″, C-4‴
5″	17.9, CH_3	1.57, s	C-1‴, C-2‴, C-5‴
5‴	17.8, CH_3	1.56, s	C-1″, C-2″, C-4‴

Table 4. ^{1}H and ^{13}C NMR Spectroscopic Data (800 MHz, $CDCl_3$) of Compound 5

position	δ_C, type	δ_H, m (J in Hz)	HMBC
1	131.8, CH	7.20, s	C-3, C-11a, C-4a, C-1′
2	121.8, C		
3	155.8, C−O		
4	104.0, CH	6.40	C-2, C-3, C-11b, C-4a
4a	154.1, C−O		
6	69.6, CH_2	3.95, d (11.5)	C-4a, C-6b, C-11a
		4.15, d (11.5)	C-4a, C-6a, C-6b, C-11a
6a	77.2, C−H		
6b	119.0, C		
7	103.0, CH	6.80, s	C-6a, C-8, C-9, C-10a
8	142.4, C−O		
9	149.7, C−O		
10	94.2, CH	6.81, s	C-8, C-9, C-6b, C-10a
10a	149.7, C		
11a	84.8, C−H	5.25, s	C-1, C-4a, C-6, C-6a, C-11b, C-10a
11b	112.1, C		
OCH_2O-8, 9	101.5, CH_2	5.91, d (1.4) 5.94, d (1.4)	C-8, C-9
1′	29.2, CH_2	3.32, d (7.2)	C-1, C-3, C-2′, C-3′
2′	121.8, CH	5.31, m	C-4′, C-5′, C-1′
3′	135.8, C		
4′	17.9, CH_3	1.78, s	C-2′, C-3′, C-5′
5′	25.8, CH_3	1.78, s	C-2′, C-3′, C-4′

the TD-DFT method (see Experimental Section for details), and compared to those obtained experimentally for **2** and **3** (Figure 2). The experimental ECD spectrum of **2** showed a negative Cotton effect at ca. 300 nm, positive at 260, and negative at 230 nm, best matched with the calculated spectrum of the (2*S*,4*R*,6*S*) isomer. Nonetheless, the mirror imaged spectrum of the (2*S*,4*R*,6*R*) isomer, corresponding to a (2*R*,4*S*,6*S*) configuration, shows similar Cotton effects. Furthermore, the calculated spectra of the (2*S*,4*R*,6*S*) and (2*S*,4*S*,6*S*) isomers are remarkably similar, and the same goes for the other two calculated isomers. None of these show agreement with the experimentally obtained ECD spectrum of **3**. Based on the above spectroscopic evidence, the new compounds rhodiflavan A (**2**) and B (**3**) are characterized as diastereomers of 4,6-dihydroxy-6,8,8-tris(3-methylbut-2-en-1-yl)-2-phenyl-2,3,4,8-tetrahydro-5*H*-chromene-5,7(6*H*)-dione, with (2*S**,4*R**,6*S**) and (2*S**,4*R**,6*R**) relative configurations, respectively.

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Compound **4** was isolated as an oily paste. Its molecular formula was deduced as $C_{24}H_{28}O_4$ based on HREIMS (m/z $[M]^+$ 381.2062, calcd 381.2066) and NMR data (see Table 3, as well as Figures S23−S28 in the Supporting Information) analyses. The ^{1}H NMR signals at δ_H5.45 (H-2), 2.02 (H-3$_{ax}$), 2.31 (H-3$_{eq}$), and 4.86 (H-4), the ^{13}C NMR signals at δ_C78.8 (C-2), 37.0 (C-3), and 55.6 (C-4), along with the UV λ_{max} at 230 and 270 nm suggested compound **4** to have an analogous ring C to those of **2** and **3**. Its ^{1}H NMR signals at δ_H 7.4 (H-2′/6′), 7.46 (H-3′/5′), and 7.43 (H-4′) indicated ring B to be unsubstituted. The lack of aromatic protons for ring A suggested it to be fully substituted. As rings B and C were fully assigned, the carbonyl groups at δ_C 184.5 (C-5) and 203.4 (C-6), and the two prenyl groups (see Table 3 for signals 1′′−5′′, and 1′′′−5′′) could only be related to ring A. The HMBC correlations of H-2′′ (δ_H 4.86) and H-2′′′ (δ_H 4.86) with C-7 (δ_C 52.9) revealed the two prenyl groups to be both attached to C-7. Both methylene groups CH_2−2′′ (δ_H 4.86) and CH_2−2′′′ (δ_H 4.86) showed HMBC correlation to δ_C 188.6 (C-7a), δ_C 203.4 (C-6), and δ_C 52.9 (C-7), indicating the placement of the two carbonyl groups at C-5 and C-6, which, being a five-membered ring, is unique for the genus *Tephrosia*. The difference in the chemical shifts of C-5 (δ_C 184.5) and C-6 (δ_C 203.4) is rationalized by the strong intramolecular hydrogen bond of HO-4 and the oxygen of the C-5 carbonyl group, and C-5 has strong conjugation with C-7a through the 4a (7a) double bond. The observed mass and NMR data (vide supra), the UV spectrum only showing a benzenoid band, and the HMBC of H-4 (δ_H 4.86) with C-5 (δ_C 184.5) corroborate the proposed structure. It is optically active ($[\alpha]_D$ +90 (*c* 0.001, CH_2Cl_2), and its ECD spectrum (see Figure S30 in the Supporting Information) shows a positive Cotton effect at λ 300−340 nm and a negative one at 275 nm. Based on the similarity of the NMR data (δ, *J*, Table 3) of H-2, H-3, and H-4 (ring C) of **4** to those of compounds **2** and **3** (Table 2), compound **4** was assigned the (2*S**,4*R**) relative configuration. Therefore, based on the above spectroscopic data, this unique compound, rhodiflavan C (**4**), was characterized as (2*S**,4*R**)-4-hydroxy-7,7-bis(3-methylbut-2-en-1-yl)-2-phenyl-2,3,4,7-tetrahydrocyclopenta[b]pyran-5,6-dione.

Compound **5** was isolated as an amorphous solid and was assigned the molecular formula $C_{21}H_{20}O_6$, based on HRESIMS ($[M + H]^+$ m/z obs 369.1367) and NMR data (see Table 4, as well as Figures S31−S36 in the Supporting Information) analyses. It showed characteristic UV (λ_{max} 230, 190, 310, and 350 nm), ^{1}H NMR [δ_H 3.95 and 4.15 (CH_2−6), 5.25 (H-11a)], and ^{13}C NMR data [δ_C 69.6 (C-6), 77.2 (C-6a), 84.8 (C-11a) for a 6a-hydroxypterocarpan skeleton. The NMR data further indicated the presence of a prenyl group [δ_H 3.32 (H_2-1′), 5.31 (H-2′), 1.78 (H_3-4′ and H_3-5′), and δ_C 29.2 (C-1′) 121.8 (C-2′), 135.8 (C-3′), 17.9 (C-4′), 25.8 (C-5′)] connected to C-2, as revealed by HMBC cross-peaks of H-1 (δ_H 7.20) with C-1′ (δ_C 29.2), and of CH_2−1′ (δ_H 3.32) with C-1 (δ_C 131.8) and C-3 (δ_C 155.8). The position of the prenyl group was confirmed by the NOE correlation between H-1 (δ_H 7.20) and CH_2−1′ (δ_H 3.32). Hence, ring A is substituted with a prenyl group at C-2 and a hydroxy group at C-3. Its C-1 and C-4 are unsubstituted as evidenced by the two singlets in its ^{1}H NMR data. In ring D, the NMR data (Table 4) indicated the presence of an 8, 9-methylenedioxy group (δ_H 5.91 and 5.94; δ_C 101.5) and two *p*-substituted aromatic protons at δ_H 6.80 (H-7) and δ_H 6.81 (H-10). The NMR spectra of this compound showed resemblance to those of previously published pterocarpans.[7,27−29] The absolute configuration at the B/C-ring junction was determined as (6a*S*,12a*S*), based on the ECD spectrum (see Figure S34 in the Supporting Information), which exhibited a positive and negative Cotton effect at 309 nm and 241 nm, respectively.[30] Based on the above spectroscopic data, this new compound, rhodacarpin (**5**), was characterized as (6a*S*,12a*S*)-2-(3-methylbut-2-en-1-yl)-6*H*-[1,3]dioxolo[4′,5′:5,6]benzofuro[3,2-*c*]chromene-3,6a-(12a*H*)-diol.

The crude extract of the roots of *T. rhodesica* and the isolated compounds were tested for antiplasmodial activity using a previously established protocol.[31] The crude extract gave 100% growth inhibition of the chloroquine-sensitive (3D7) strain of *Plasmodium falciparum* at 10 μg/mL concentration, whereas the isolated compounds **2**, **3**, **4**, **5**, **9**, and **15** showed moderate activities (Table 5). Among the tested compounds, **3** showed the highest activity (IC_{50} = 5.7 ± 1.9 μM).[32]

Table 5. In Vitro Antiplasmodial Activities (IC_{50}) of Isolated Compounds against 3D7 Strains of *P. falciparum*[a]

sample	IC_{50}, μM (*Pf*3D7)	LD_{50} (HEK-293)
T. rhodesica (roots) crude extract	100% active at 10 μg/mL	
Rhodiflavan A (**2**)	7.3 ± 1.8	71% at 40 μM
Rhodiflavan B (**3**)	5.7 ± 1.9	34% at 40 μM
Rhodiflavan C (**4**)	7.0 ± 2.4	101% at 40 μM
Rhodacarpin (**5**)	10.2 ± 0.2	27% at 40 μM
Quercetol B (**9**)	7.4 ± 0.3	87% at 40 μM
6-Hydroxyrotenone (**15**)	8.6 ± 2.6	10.9 μM
Tephrowatsin A (**21**)	14.5 ± 0.7	
Chloroquine	0.0047	
Artesunate	0.00067	

[a]Compounds were tested independently either two or three times against *Pf*3D7. All compounds were tested in a single experiment against HEK 293 mammalian cells (LD_{50}).

In conclusion, five new compounds—rhodimer (**1**), rhodiflavan A (**2**), rhodiflavan B (**3**), rhodiflavan C (**4**), and rhodacarpin (**5**)—were isolated from the roots of *Tephrosia rhodesica*, along with 16 known natural products. Rohodimer is an unusual flavanone-flavan dimer, whereas rhodiflavan C has a five-membered A-ring, which is unprecedented for this genus. The crude root extract and several of its isolated constituents showed activities against the chloroquine-sensitive (3D7) strain of *Plasmodium falciparum*.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 341-LC system, whereas ECD experiments were performed on a Jasco Model J-715 spectropolarimeter. UV spectra were recorded on a Specord S600 (Analytik Jena AG) spectrophotometer. Melting points were obtained on a Büchi Melting Point B-545 Switzerland apparatus; NMR spectra were acquired on a Bruker Avance III HD 800 MHz NMR spectrometer equipped with a TCI cryogenic probe and were processed with the MestReNova 10.0 software, using the solvent residual signal ($CDCl_3$ δ_H 7.26; δ_C 77.16) as chemical shift reference. LC-ESIMS data were obtained on a Micromass GC-TOF micro mass spectrometer (Micromass, Wythenshawe, Waters, Inc., U.K.), using direct inlet, and 70 eV ionization voltage. TLC was performed on Merck precoated silica gel 60 F254 plates. Column chromatography was run on silica gel 60 (70−230 mesh). Gel filtration was done on Sephadex LH-20. Preparative HPLC was performed on a Waters 600E instrument using the

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Chromulan (Pikron, Ltd.) software and an RP C_8 Kromasil (250 mm × 55 mm) column eluting with repeated CH_3OH-H_2O (5 to 95) gradients. Single-crystal X-ray data of **1** were collected at 120 K on an Agilent SuperNova dual wavelength diffractometer with a microfocus X-ray source and multilayer optics monochromatized Cu Kα (λ = 1.54184 Å) radiation.

Plant Material. The roots of *Tephrosia rhodesica* were collected in April 2015 from Kilungu Hills in Makueni County, Kenya. The plant specimen was identified by Mr. Patrick C. Mutiso of the School of Biological Sciences, the University of Nairobi. A voucher specimen (Mutiso-842/April 2015) was deposited at the University Herbarium of the University of Nairobi, Kenya.

Extraction and Isolation. The air-dried roots (2 kg) of *T. rhodesica* were ground and extracted with CH_2Cl_2–CH_3OH (1:1) (3 × 2 L) by percolation at room temperature to yield 70 g of a dark brown paste after evaporation of the solvent. A portion of the extract (37 g) was subjected to column chromatography over silica gel (400 g) eluting with a mixture of *iso*-hexane containing increasing amounts of EtOAc. The fraction eluted with 1% EtOAc in *iso*-hexane was purified by preparative HPLC (CH_3OH-H_2O gradient elution) to give tephrowatsin B (**6**, 20 mg).[8] The fraction eluted with 3% EtOAc in *iso*-hexane was purified by column chromatography on a Sephadex LH-20 column (CH_2Cl_2–CH_3OH; 1:1) to give tephrinone (**7**, 200 mg)[9] and glabranin (**8**, 100 mg).[10] The fractions eluted with 5% EtOAc in *iso*-hexane were purified by column chromatography on a Sephadex LH-20 column (CH_2Cl_2–CH_3OH; 1:1), followed by recrystallization from CH_2Cl_2–CH_3OH (1:1) to afford rhodimer (**1**, 15 mg). The fractions eluted with 6% EtOAc in *iso*-hexane were subjected to column chromatography on a Sephadex LH-20 (CH_2Cl_2–CH_3OH (1:1)) system to give quercetol B (**9**, 300 mg).[11] The fractions eluted with 7% EtOAc in *iso*-hexane were purified by column chromatography on a Sephadex LH-20 column (CH_2Cl_2–CH_3OH (1:1)) to give maackiain (**10**, 20 mg),[12] 6a-hydroxymaackiain (**11**, 10 mg),[13] and pisatin (**12**, 5 mg).[14] The fractions eluted with 8% EtOAc in *iso*-hexane were subjected to column chromatography on a silica gel (200 g) with 3% EtOAc in *iso*-hexane to give tephrowatsin A (**21**, 10 mg),[8] with 5% EtOAc in *iso*-hexane to give rhodiflavan A (**2**, 50 mg), and with 10% EtOAc in *iso*-hexane to provide rhodiflavan B (**3**, 20 mg) and rhodiflavan C (**4**, 15 mg), which were further purified by preparative HPLC (CH_3OH–H_2O gradient elution). The fractions eluted with 9%–10% EtOAc in *iso*-hexane were combined and purified by column chromatography on a Sephadex LH-20 column (CH_2Cl_2–CH_3OH; 1:1), and subsequently by preparative HPLC (CH_3OH–H_2O gradient elution) to give tephrosin (**13**, 10 mg),[15] rotenone (**14**, 15 mg),[15] 6-hydroxyrotenone (**15**, 10 mg),[16] 12a-hydroxyrotenone (**15**, 10 mg),[17] rhodacarpin (**5**, 10 mg), hildecarpin (**17**, 10 mg),[18] and 3-hydroxy-2-methoxy-8–9-methylenedioxypterocarpene (**18**, 15 mg).[19] The fractions eluted with 12% EtOAc in *iso*-hexane gave isoliquirtigenin (**19**, 15 mg),[20] and those eluted with 20% EtOAc in *iso*-hexane gave D-pinitol (**20**, 900 mg).[21]

Rhodimer (1). White crystals (CH_2Cl_2–CH_3OH; 1:1); $[\alpha]_D^{20}$ +13.4 (*c* 0.001, CH_3OH); UV (CH_2Cl_2) λ_{max} (log ε) 230 (4.05), 290 (4.25), and 350 (4.30) nm; ECD (*c* 0.05, CH_3OH) λ_{max} ($\Delta\varepsilon$) 314 (10.16), 292 (−40.36), 249 (−23.33), 235 (50.08), 220 (−56.36), 212 (62.23);^{1}H and ^{13}C NMR, see Table 1; HREIMS *m*/*z* 660.3095 $[M]^+$ (calcd for $C_{42}H_{44}O_7$, 660.3087).

Rhodiflavan A (2). Yellow oily paste; $[\alpha]_D^{20}$ +17.5 (*c* 0.001, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 240 (3.05) and 290 (3.40) nm; ECD (*c* 0.05, CH_3OH) λ_{max} ($\Delta\varepsilon$) 300 (−92.25), 257 (85.45), 243 (−119.45); ^{1}H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 479.2493 $[M + H]^+$ (calcd for $C_{30}H_{38}O_5$, 479.2753).

Rhodiflavan B (3). Yellow oily paste; $[\alpha]_D^{20}$ +66.1 (*c* 0.001, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 230 (3.08) and 280 (3.20) nm; ECD (*c* 0.05, CH_3OH) λ_{max} ($\Delta\varepsilon$) 301 (199.73), 257 (−130.29), 225 (65.0); ^{1}H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 479.2493 $[M + H]^+$ (calcd for $C_{30}H_{38}O_5$, 479.2753).

Rhodiflavan C (4). Yellow oily paste. $[\alpha]_D^{20}$ +90.0 (*c* 0.001, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 230 (3.20) and 270 (3.08) nm; ECD (*c* 0.05, CH_3OH) λ_{max} ($\Delta\varepsilon$) 316 (89.0), 284 (−32.21), 245 (91.80), 226 (−26.26), 218 (70.0), 210 (80.5); ^{1}H and ^{13}C NMR, see Table 3; HREIMS *m*/*z* 381.2062 $[M]^+$ (calcd for $C_{24}H_{28}O_4$, 381.2066).

Rhodacarpin (5). White amorphous solid; UV (CH_2Cl_2) λ_{max} (log ε) 230 (3.04), 290 (3.45), 310 (3.42) and 350 (3.10) nm; ECD (*c* 0.05, CH_3OH) λ_{max} ($\Delta\varepsilon$) 309 (12.0), 241 (−82.1), 220 (7.0), 205 (−80); ^{1}H and ^{13}C NMR, see Table 4; HRESIMS *m*/*z* 369.1367 $[M + H]^+$ (calcd for $C_{21}H_{20}O_6$, 369.1338).

***Plasmodium falciparum* Culture.** In vitro parasite culture of the *P. falciparum* strain 3D7 was maintained in RPMI with 10 mM Hepes (Life Technologies), 50 μg/mL hypoxanthine (Sigma) and 5% human serum from male AB plasma and 2.5 mg/mL AlbuMAX II (Life Technologies). Human 0$^+$ erythrocytes were obtained from the Australian Red Cross Blood Service (Agreement No. 13-04QLD-09; 17-06QLD-16). The parasites were maintained at 2%–8% parasitaemia (% P) at 5% hematocrit (% H), and incubated at 37 °C, 5% CO_2, 5% O_2, 90% N_2 and 95% humidity.

***Plasmodium falciparum* Growth Inhibition Assay.** A well-established asexual *P. falciparum* imaging assay was used to determine parasite growth inhibition according to the procedure described by Duffy and Avery.[31]

Computation. A low-energy conformation library of postulated compounds **2** and **3** were generated using Macromodel as implemented in the Macromodel v12.1 Schrödinger suite by performing careful Monte Carlo conformational analysis using MMFF force fields, each with the GB/SA solvation models $CHCl_3$ and H_2O. Elimination of redundant conformations was performed by comparison of heavy atom coordinates applying an RMSD cutoff set to 2.0 Å. Next, all unique conformers were optimized at the B3LYP/6-311++G(3df,2pd) level of theory. The conformers having a Boltzmann weight, calculated using $\Delta H°$, above 1% were selected for subsequent TD-DFT calculations at the same level of theory using 50 singlet excited states. To obtain the Boltzmann-weighted ECD spectrum, the individual spectra were line-broadened using a Gaussian band shape (σ = 0.3 eV). All (TD-)DFT calculations were performed using Gaussian 16, Revision A.03.[33] The solvent was taken into account by using the integral equation formalism model (IEFPCM) as implemented in Gaussian 16 and the dielectric constant for methanol (ε = 32.613). To compensate for the typical underestimation of the transition energies, a 20 nm blue shift was applied on the computed ECD spectra for comparison with the experimental one.[34]

■ ASSOCIATED CONTENT

Supporting Information

CCDC 1987379 has been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk). The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00245.

Original MS and NMR spectra for all compounds, along with the corresponding NMReDATA[35] for the new compounds **1**–**5**; the X-ray structure of **1** (details are freely available on Zenodo at https://10.5281/zenodo.3679345); NMR and MS data for compounds (**1**–**21**) (PDF)

Crystallographic data for **1** (CIF)

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Notes

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