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FLAVONOIDS OF DRAGON'S BLOOD FROM *DRACAENA CINNABARI*

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Key Word Index—*Dracaena cinnabari*; Agavaceae; homoisoflavans; flavans; dihydrochalcones; chalcone; flavone; flavan-4-one.

Abstract—In addition to 7-hydroxy-3-(4-hydroxybenzyl)-8-methoxychroman, 3-(4-hydroxybenzyl)-7,8-methylenedioxychroman, 7-hydroxy-3-(4-hydroxybenzyl)chroman, (±)-7,4′-dihydroxy-3′-methoxyflavan, (2*S*)-7-hydroxyflavan, 4,4′-dihydroxy-2-methoxydihydrochalcone, 4,4′-dihydroxy-2′-methoxychalcone, 7,4′-dihydroxyflavone and (2*S*)-7-hydroxyflavan-4-one, three new flavonoids have been isolated from the resin, called 'dragon's blood', of *Dracaena cinnabari*, the structures of which have been elucidated as 7-hydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman, (2*S*)-7,3′-dihydroxy-4′-methoxyflavan and 4-hydroxy-2-methoxydihydrochalcone.

INTRODUCTION

Dracaena cinnabari Balf. fil. is known as the dragon's blood tree, and is endemic to the Socotra Island of Yemen [1]. Phytochemical studies of the genus *Dracaena* have previously led to the isolation of several flavonoids from *D. draco* [2] and *D. loureiri* [3–5]. The resin of *D. cinnabari*, which is called dragon's blood, has been known for a long time in folk medicine as an astringent in diarrhoea and dysentery, as well as an antiseptic, haemostatic and antiulcer remedy [6]. Eight flavonoids have been recently reported [7] from dragon's blood, three of which, homoisoflavans, have shown antioxidative activity [8].

The present study showed that dragons's blood contained 7-hydroxy-3-(4-hydroxybenzyl)-8-methoxychroman (**2**), 3-(4-hydroxybenzyl)-7,8-methylenedioxychroman (**3**), 7-hydroxy-3-(4-hydroxybenzyl)chroman (**4**), (2*S*)-7-hydroxyflavan (**7**), 4,4′-dihydroxy-2-methoxydihydrochalcone (**9**), and (2*S*)-7-hydroxyflavan-4-one (**12**), already found in the same resin by Suchý *et al.* [7]. Furthermore, (±)-7,4′-dihydroxy-3′-methoxyflavan (**5**) [9, 10], 4,4′-dihydroxy-2′-methoxychalcone (**10**) [5], and 7,4′-dihydroxyflavone (**11**) [5, 11] and three new flavonoids, 7-hydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman (**1**), (2*S*)-7,3′-dihydroxy-4′-methoxyflavan (**6**) and 4-hydroxy-2-methoxydihydrochalcone (**8**) were obtained, the structures of which have been elucidated as outlined below.

RESULTS AND DISCUSSION

The elemental composition of **1** was shown to be $C_{17}H_{18}O_4$ by high resolution-mass spectrometry. The EI-mass spectrum displayed fragment ions at *m/z* 138 and 149, characteristic of a 3-benzylchroman derivative [12]. The fragment ion at *m/z* 138 revealed the presence of a hydroxymethoxybenzyl group and the ion at *m/z* 149, the presence of a hydroxy group on ring A.

The ^{1}H NMR signals (Table 1, numbered according to ref. [3]) of the hydroxymethoxybenzyl residue at δ6.65 and 6.78 showed an *ortho*-relationship, and the signals at δ6.65 and 6.77 the *meta*-position of two protons. In the NOE difference spectrum, irradiation at δ3.88 (methoxy) gave positive enhancement for the doublet at δ6.78 indicating proximity to the *ortho*-coupled proton. Furthermore, the chemical shifts of two oxygenated aromatic carbon atoms at δ145.0 and 145.5 (Table 2) are in agreement with the assumption that these atoms are bound to each other [13]. These observations suggested the substitution pattern of the benzyl group. Coupling constants and ^{1}H–^{1}H COSY 2D measurements indicated that the two aromatic protons at δ6.34 and 6.84 are *ortho*-related, one of which (δ6.34) is also *meta*-oriented to a proton, at δ6.30. The ^{13}C signal at δ130.5 (C-5) indicated the C-7 position for the hydroxy group of ring A. In addition, the high-field chemical shift of C-8 (δ103.0) is caused by two *ortho*-related oxygenated carbons. ^{1}H–^{1}H COSY 2D spectra were in accordance with an aliphatic O-CH_2-$CH(CH_2)_2$ spin system, and the two H-9 signals were assigned by an NOE between these protons and H-6′. The homoisoflavan **1** was previously synthesized as a racemate [14].

The homoisoflavans **1**–**4** displayed positive circular dichroism at about 280 nm (**1** $\Delta\varepsilon_{282}$ + 0.41, **2** $\Delta\varepsilon_{276}$ + 0.20, **3** $\Delta\varepsilon_{286}$ + 0.50, **4** $\Delta\varepsilon_{279}$ + 0.74, all measurements in MeOH) indicating the same absolute configurations. It was attempted to determine the absolute configurations by X-ray analysis, but this was not successful.

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Table 1. ^{1}H NMR spectral data of compounds **1–3** ($CDCl_3$, 500 MHz)

H	1		2		3	
2eq	4.15	(15.5; 3.1; 1.5)	4.24	(10.5; 3.1; 1.5)	4.24	(10.7; 3.0; 1.5)
2ax	3.78	(10.6; 8.4)	3.83	(10.5; 8.4)	3.87	(10.6; 8.2)
3	2.25	*m*	2.25	*m*	2.26	*m*
4eq	2.71	(15.9; 5.2)	2.73	(15.3; 5.2)	2.76	(15.9; 5.2)
4ax	2.41	(15.9; 8.9)	2.45	(15.9; 8.5)	2.46	(15.9; 8.6)
5	6.84	(8.0)	6.62	(8.4)	6.48	(8.1)
6	6.34	(8.0; 2.5)	6.47	(8.4)	6.40	(8.1)
8	6.30	(2.5)	—	—	—	—
9a	2.60	(13.7; 7.3)	2.62	(13.9; 7.5)	2.65	(13.7; 7.3)
9b	2.51	(13.7; 7.7)	2.56	(13.9; 7.5)	2.55	(15.9; 7.9)
2′	6.77	(2.0)	7.05	(6.4; 2.1)	7.06	(6.6; 1.8)
3′	—	—	6.77	(6.4; 2.1)	6.78	(6.6; 1.8)
5′	6.78	(8.2)	6.77	(6.4; 2.1)	6.78	(6.6; 1.8)
6′	6.65	(8.2; 2.0)	7.05	(6.4; 2.1)	7.06	(6.6; 1.8)
OMe	3.88	*s*	3.88	*s*	—	—
O-CH_2-O	—	—	—	—	5.94	(1.2)

Coupling constants (*J* in Hz) in parentheses.

Table 2. ^{13}C NMR spectral data of compounds **1–3***

C	1	2	3
2	69.9	70.9	70.2
3	34.1	35.7	34.2
4	30.3	31.4	30.5
4a	113.8	115.1	116.8
5	130.5	125.4	121.8
6	107.8	109.2	101.3
7	155.3	149.6[a]	146.8[a]
8	103.0	136.7	134.1
8a	154.7	149.1[a]	139.0[a]
9	37.3	37.9	36.9
1′	132.6	131.6	131.3
2′	115.1	130.9	130.1
3′	145.0	116.1	115.3
4′	145.5	156.8	153.9
5′	116.6	116.1	115.3
6′	120.4	130.9	130.1
OMe	56.0	60.9	
O-CH_2-O			101.3

*1 and 3 in $CDCl_3$, 2 in CD_3OD; 125 MHz.
[a]May be exchanged.

Table 3. ^{1}H NMR spectral data of compounds **5**, **5a**, **6** and **6a** ($CDCl_3$, 500 MHz)

H	5	5a*	6	6a*
2	4.94	5.02	4.94	4.99
3	2.0–2.2	2.0–2.2	2.0–2.2	2.0–2.2
4	2.7–3.0	2.7–3.0	2.6–3.0	2.7–3.0
5	6.92	7.08	6.92	7.06
6	6.39	6.62	6.39	6.60
8	6.38	6.65	6.38	6.62
2′	6.94	7.05	6.99	7.11
5′	6.90	7.04	6.86	6.97
6′	6.90	6.96	6.90	7.24
OMe	3.90	3.85	3.89	3.84
OAc		2.28; 2.32		2.28; 2.32

*$J_{2,3}$ = 10.5; 2.4 Hz, $J_{5,6}$ = 8.2 Hz, $J_{6,8}$ = 2.4 Hz, $J_{2',6'}$ = 2.1 Hz, $J_{5',6'}$ = 8.5 Hz.

Compound **5** is also known as a racemate [9] and as the (2*S*)-compound [10]. As the previous arguments for structural determination are not convincing, we describe the structure elucidation of the racemate. The EI-mass spectrum of **5** displayed, in addition to the molecular ion peak at *m/z* 272, fragment ions at *m/z* 123, 149 and 150, characteristic of a flavan with a hydroxy group in ring A and hydroxy and methoxy groups in ring B [15] (see Experimental).

In the ^{1}H NMR spectrum of the acetyl derivative **5a** (Table 3), the signals of the acetoxymethoxybenzyl residue at δ6.96 and 7.04 showed *ortho*-coupling, the signals at δ6.96 and 7.05 *meta*-coupling. The chemical shifts of two oxygenated aromatic carbon atoms in **5** at δ147.2 and 148.9 (Table 4, numbered according to ref. [16]) indicated that these atoms are connected [13]. In the NOE difference spectrum of **5a** irradiation at δ3.85 (methoxy) gave positive enhancement for the signal at δ7.05 indicating proximity to the proton, which only showed *meta*-coupling. These data suggested the 4′-hydroxy-3′-methoxy substitution. The C-7 position of another hydroxy group was determined analogously as with **1**, except that the ^{1}H NMR data of the acetyl derivative **5a** were again analysed (Tables 3 and 4).

The elemental composition of **6** was shown to be $C_{16}H_{16}O_4$ by high resolution-mass spectrometry. The mass spectrometric fragmentations of **5** and **6** were very similar, suggesting isomeric structures (see Experimental). The ^{1}H- and ^{13}C-signals for rings A and C of **5** and **6**

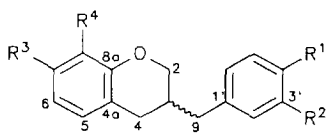
Table 4. ^{13}C NMR spectral data of compounds **5** and **6** (CD_3OD, 125 MHz)

C	5	6
2	79.2	78.7
3	25.5	25.3
4	31.4	31.3
5	130.4	130.9
6	109.1	109.0
7	157.1	157.0
8	104.0	104.0
9	157.5	157.5
10	114.3	114.3
1′	134.9	136.3
2′	110.9	112.5
3′	148.9	147.4
4′	147.2	148.5
5′	115.9	114.1
6′	119.9	118.5
OMe	56.4	56.4

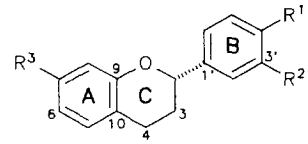
(Tables 3 and 4) were identical, in accordance with identical partial structures. The spectral assignments were supported by HETCOR spectra. As in the case of **5**, the ^1H NMR spectrum of the acetyl derivative **6a** was better resolved than that of **6** (Table 3). The signal of the acetoxymethoxybenzyl residue at δ7.24 indicated *ortho*-coupling to the signal at δ6.97, and *meta*-coupling to the signal at δ7.11. The chemical shifts of two oxygenated aromatic carbon atoms in **6** at δ147.4 and 148.5 (Table 4) indicated their *ortho*-relationship [13]. Thus, compound **6** possesses the same substitution pattern as **5** and must differ in the position of the *O*-methyl group. Flavan **6** was previously synthesized as the racemate [17].

The elemental composition of **8** was shown to be $C_{16}H_{16}O_3$ by high resolution-mass spectrometry. The fragments at *m/z* 105 and 137 suggested the presence of benzoyl and hydroxymethoxybenzyl ions formed by α and β cleavage in agreement with high resolution data.

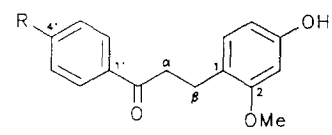
The ^1H NMR spectrum showed in addition to the five protons of the unsubstituted phenyl group, three aromatic protons, which appeared as an ABC system at



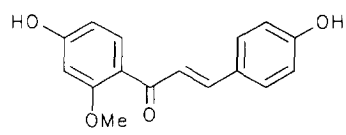
	R^1	R^2	R^3	R^4
1	OMe	OH	OH	H
2	OH	H	OH	OMe
3	OH	H	O-CH_2-O	
4	OH	H	OH	H



	R^1	R^2	R^3
5*	OH	OMe	OH
5a*	OAc	OMe	OAc
6	OMe	OH	OH
6a	OMe	OAc	OAc
7	H	H	OH



8 R = H
9 R = OH



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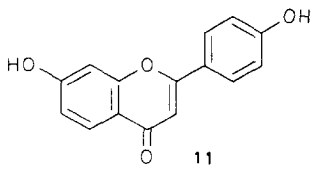

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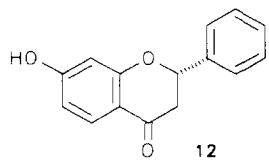

12

*racemate

δ6.33 (*dd*, J = 7.9, 2.4 Hz), 6.41 (*d*, J = 2.4 Hz) and 7.01 (*d*, J = 8.2 Hz) (see Experimental) assigned to H-5, H-3 and H-6, respectively. The chemical shifts of the oxygenated aromatic carbon atoms of ring B at δ155.3 and 158.5 excluded their connection [13]. Location of a methoxy group either at position C-2 or C-4 was determined by an NOE difference experiment. Irradiation at δ3.78 (methoxy) gave positive enhancement only for the signal at δ6.41, in agreement with a 2′-methoxy group. H_2-α and H_2-β were assigned by comparison with literature data of related compounds [4]. The structure of **8** was verified by a long-range ^1H–^1H COSY spectrum which showed a strong correlation signal between H_2-β and H-6.

^{13}C NMR data for **2** and **3** are not previously reported, furthermore, the literature for **3** [7] is hardly accessible. Therefore, we report our results for these compounds (see Tables 1 and 2 as well as Experimental).

EXPERIMENTAL

Plant material. Dragon's blood from *Dracaena cinnabari* was collected in Socotra Island of Yemen in summer, 1992. A voucher specimen of the resin is deposited at the Institute of Plant Biochemistry, Halle.

TLC. On silica gel 60 F_{254} using *n*-hexane–EtOAc (3:2) [R_f(1)] or $CHCl_3$–MeOH (19:1) [R_f(2)] for development, and vanillin–H_3PO_4 (120°) for detection.

Extraction and isolation. The powdered resin (500 g) was successively extracted with *n*-hexane, $CHCl_3$ and MeOH. Evapn of solvents *in vacuo* gave residues of 2.8, 55 and 200 g, respectively. The $CHCl_3$ extract was repeatedly subjected to silica gel chromatography (Merck 60, 0.063–0.20 mm), eluting with *n*-hexane with increasing amounts of EtOAc. The following compounds were isolated in the indicated yields: **7** (0.48%), **3** (0.17%), **8** (0.02%), **12** (0.03%), **2** (1.0%), **1** + **5** (0.3%), **6** (0.66%), **4** (0.12%), **9** (0.15%). Compounds **1** (0.12%) and **5** (0.17%) were sepd by HPLC (Lichrospher 100 RP-18, column 125 × 4 mm, flow rate 1 ml min^{-1}, mobile phase MeOH–0.2% HOAc, 9:11, detection 280 nm). **10** (0.3%) and **11** (0.2%) were isolated from the MeOH extract by silica gel chromatography (Merck 60, 0.063–0.20 mm) with *n*-hexane–EtOAc (3:2) and toluene–EtOAc–HOAc (60:30:1), respectively.

7-*Hydroxy*-3-(3-*hydroxy*-4-*methoxybenzyl*)*chroman* (**1**). Amorphous, $[\alpha]_D^{22}$ + 27.9° (MeOH; *c* 0.20), R_f(2) 0.45. UV λ_{max}^{MeOH} nm (log ε): 211 (4.17), 223 (3.97), 282 (3.66). EI-MS (70 eV) *m/z* (rel. int.): 286.1214 $[M]^+$ ($C_{17}H_{18}O_4$, calcd 286.1223) (13), 176 (7), 149 (51), 138 (40), 125 (13).

7-*Hydroxy*-3-(4-*hydroxybenzyl*)-8-*methoxychroman* (**2**). Amorphous, $[\alpha]_D^{28}$ + 49.5° ($CHCl_3$; *c* 0.30) (lit. + 36.93° ($CHCl_3$) [2]), R_f(2) 0.45. UV λ_{max}^{MeOH} nm (log ε): 231 (3.40), 278 (3.50).

3-(4-*Hydroxybenzyl*)-7,8-*methylenedioxychroman* (**3**). Needles, mp 141–142° (CH_2Cl_2) (lit. 140° [7]), $[\alpha]_D^{28}$ + 45.8° (MeOH; *c* 0.28) (lit. + 52.8° (MeOH) [7]), R_f (1) 0.56. UV λ_{max}^{MeOH} nm (log ε): 223 (4.17), 277 (3.40). EI-MS (70 eV) *m/z* (rel. int.): 284 $[M]^+$ (100), 177 (22), 176 (44), 151 (30), 150 (22), 149 (18), 147 (17), 135 (15), 107 (51).

7-*Hydroxy*-3-(4-*hydroxybenzyl*)*chroman* (**4**). Needles, mp 153–154° (CH_2Cl_2) (lit. 123 – 124° [3]), $[\alpha]_D^{28}$ + 59.5° (MeOH; *c* 0.26) (lit. + 62.9° (MeOH) [3]), R_f(2) 0.29.

(±)-7,4′-*Dihydroxy*-3′-*methoxyflavan* (**5**). Amorphous (lit. mp 157–158° [9]), $[\alpha]_D^{22}$ ± 0.0° (MeOH; *c* 0.24), R_f(2) 0.45. UV λ_{max}^{MeOH} nm (log ε): 211 (4.54), 222sh (4.49), 281 (3.94); $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 220 (4.40), 245 (4.25), 291 (4.00). EI-MS (70 eV) *m/z* (rel. int.): 272 $[M]^+$ (50), 163 (11), 150 (89), 149 (100), 137 (30), 135 (30), 123 (15), 107 (12).

(2S)-7,3′-*Dihydroxy*-4′-*methoxyflavan* (**6**). Needles, mp 151–152° ($CHCl_3$), $[\alpha]_D^{24}$ − 45.5° (MeOH; *c* 0.30), R_f(2) 0.44. UV λ_{max}^{MeOH} nm (log ε): 213 (4.23), 224 sh (4.18), 282 (3.83); $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 219 (4.30), 287 (3.83); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 219 (4.27), 282 (3.82). EI-MS (70 eV) *m/z* (rel. int.): 272.0979 $[M]^+$ ($C_{16}H_{16}O_4$, calcd 272.0972) (96), 163 (14), 162 (25), 150 (100), 137 (43), 135 (46), 123 (16), 107 (14).

Diacetate (**6a**). Mp 109° (MeOH). EI-MS (70 eV) *m/z* (rel. int.): 356 $[M]^+$ (63), 314 $[M - CH_2CO]^+$ (60), 272 $[M - 2CH_2CO]^+$ (100), 163 (19), 162 (68), 150 (75), 137 (34), 135 (36), 123 (12), 107 (8).

4-*Hydroxy*-2-*methoxydihydrochalcone* (**8**). Oil, $[\alpha]_D^{24}$ ± 0.0° (MeOH; *c* 0.30), R_f (2) 0.57. EI-MS (70 eV) *m/z* (rel. int.): 256.1098 $[M]^+$ ($C_{16}H_{16}O_3$, calcd 256.1096) (90), 179 (8), 151 (11), 137.0598 ($C_8H_9O_2$, calcd 137.0593) (100), 124 (52), 107 (29), 105.0350 (C_7H_5O, calcd 105.0360) (40), 77 (25). ^1H NMR (500 MHz, $CDCl_3$): δ2.96 (2H, *t*, J = 8.2 Hz, H-β), 3.22 (2H, *t*, J = 8.2 Hz, H-α), 3.78 (3H, *s*, OMe), 6.33 (1H, *dd*, J = 7.9; 2.4 Hz, H-5), 6.41 (1H, *d*, J = 2.4 Hz, H-3), 7.01 (1H, *d*, J = 8.2 Hz, H-6), 7.43 (2H, *t*, J = 7.6 Hz, H-3′ and H-5′), 7.54 (1H, *t*, J = 7.5 Hz, H-4′), 7.97 (2H, *d*, J = 7.9 Hz, H-2′ and H-6′). ^{13}C NMR (125 MHz, $CDCl_3$): δ25.2 (C-β), 39.2 (C-α), 55.2 (OMe), 98.8 (C-3), 106.6 (C-5), 121.6 (C-1), 128.1 (C-2′ and C-6′), 128.5 (C-3′ and C-5′), 130.5 (C-6), 132.9 (C-4′), 137.0 (C-1′), 155.3 (C-4), 158.5 (C-2), 200.3 (C=O).

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