Xanthones from *Drimiopsis maculata*

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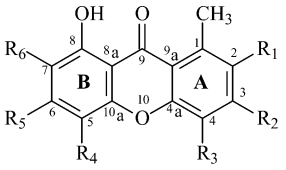
Six new xanthones, drimiopsins A−F (**1**−**6**), have been isolated from the South African *Drimiopsis maculata*. The structures of these compounds were difficult to elucidate due to the lack of correlating protons seen in the NMR spectra, and INADEQUATE spectra were used to confirm the structures. Xanthones have not previously been reported from the Hyacinthaceae.

The genus *Drimiopsis* Lindl. (Hyacinthoideae: Hyacinthaceae) is endemic to sub-Saharan Africa, where it is represented by approximately 20 species, at least five of which occur in southern Africa. *Drimiopsis maculata* Lindl., the only South African species from this genus reportedly employed in ethnomedicine, is found along the eastern coastal region. The plant is traded by the Zulu as "inJobo"[1] but is also known as "ucibicibane" (Zulu)[2] or "intshwilisa" (Xhosa).[3] A warmed infusion of the bulbs is administered by the Zulu as an enema to children suffering from stomach complaints.[2] For the relief of constipation in children, the bulbs may also be chopped and ingested with food.[3] A recipe for the mucilage-rich purging medicine prepared from the bulbs of *D. maculata* has been documented;[4] this infusion was reportedly being used to treat an alleged Xhosa disease of newborn infants known as "ipleyiti". Sheep toxicity tests using fresh plants in the flowering and seeding stages proved negative.[5]

Of the 14 genera belonging to the southern African Hyacinthoideae, only *Eucomis* L'Hérit and *Merwilla* Speta (syn. *Scilla* L.) have been extensively studied and have been found to typically contain 3-benzylchroman-4-ones (homoisoflavanones)[6] and eucosterol derivatives.[7] In a preliminary study of this *Drimiopsis maculata* species the known scillascillins, 5,3′-dihydroxy-4′,7-dimethoxyspiro[2*H*-1-benzopyran-3(4*H*,7′-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one and 5,7,3′-trihydroxy-4′-methoxyspiro[2*H*-1-benzopyran-3(4*H*),7′-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one were isolated.[8] As there appeared to be different types of interesting compounds present in small quantities, a second plant collection was made, and six new xanthones, drimiopsins A−E (**1**−**6**), were also isolated. Structures were determined using NMR and MS techniques.

Previously, the dibenzo-α-pyranones, autumnariol and autumnariniol, were reported from the bulbs of *Eucomis autumnalis* (Mill.) Chitt. (Hyacinthoideae: Hyacinthaceae).[9] However, NMR data in this publication were incomplete, and in light of our findings, it is likely that the structures of these compounds were incorrectly assigned and are actually the corresponding xanthones.

The FABMS of drimiopsin A (**1**) gave a molecular ion peak at *m*/*z* 318.07411, indicating a molecular formula of $C_{16}H_{14}O_7$. The IR spectrum showed a strong carbonyl stretch at 1633 cm^{-1}. The ^{1}H NMR spectrum (Table 1) indicated the presence of two methoxy group proton resonances (δ 3.78, 3.73, 3H, each), an aromatic methyl group proton resonance (δ 2.70, 3H), and a pair of *meta*-coupled proton resonances at δ 6.62 (J = 2.38 Hz) and 6.67 (J = 2.38 Hz). The ^{13}C NMR spectrum showed 12 resonances ascribable to aromatic carbons of two rings, two methoxy group carbon resonances (δ 61.7, 60.7), a methyl group carbon resonance (δ 23.7), and a carbonyl carbon resonance at δ 182.5. The chemical shift of this carbonyl resonance indicated a xanthone structure rather than an isomeric dibenzo-α-pyranone structure.[10] The structure of ring A was determined using the COSY and NOESY spectra. The methyl group proton singlet (δ 2.70, 3H) showed correlations in the COSY and NOESY spectra with the proton resonance at δ 6.62 and so were placed at C-1 and C-2, respectively. The *meta*-coupled proton resonance at δ 6.67 was thus assigned as H-4. As no NOESY correlations were seen between either of the methoxy group proton resonances and H-2 or H-4, a hydroxy group was placed at C-3. The molecular formula indicated a fully substituted ring B, and an INADEQUATE NMR experiment was used to place the remaining two methoxy and two hydroxy groups on the ring and to confirm the xanthone structure. This experiment indicated that methoxy groups were present at C-5 and C-7 and hydroxy groups occurred at C-6 and C-8. The presence of a hydroxy group at C-8 was confirmed by the phenolic proton resonance at δ 13.23, indicative of a phenolic proton that is hydrogen bonded to the neighboring carbonyl group.[11] Thus, structure **1** was assigned to drimiopsin A. All ^{13}C NMR resonances could be assigned using the INADEQUATE spectrum and are shown in Table 1.



	R_1	R_2	R_3	R_4	R_5	R_6
1	H	OH	H	OCH_3	OH	OCH_3
2	H	OH	H	OCH_3	OCH_3	OCH_3
3	H	OH	H	H	OH	OCH_3
4	H	OH	OCH_3	OCH_3	OH	H
5	H	OH	H	OCH_3	OH	H
6	H	OCH_3	H	H	OH	OCH_3

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Table 1. ^{1}H and ^{13}C NMR Data for Drimiopsins A−F (**1**−**6**) (400 MHz) (*J* values in Hz)

	1			**2**			**3**			**4**			**5**			**6**		
position	^{1}H[a]	^{13}C	HMBC H→C	^{1}H[a]	^{13}C	HMBC H→C	^{1}H[a]	^{13}C	HMBC H→C	^{1}H[a]	^{13}C	HMBC H→C	^{1}H[b]	^{13}C	HMBC H→C	^{1}H[b]	^{13}C	HMBC H→C
1		143.4			143.6			142.7			138.4			143.6			144.2	
2	6.62d (2.38)	117.0	3, 9a, 1-CH_3	6.63d (2.12)	117.5	1, 4, 9a	6.58d[c]	116.4	9a, 1-CH_3	6.67	116.8	4, 9a	6.60d (2.19)	116.0	3, 4, 1-CH_3	6.73d (2.56)	116.7	
3		163.6			164.7			166.0			156.5			163.1			165.6	
4	6.67d (2.38)	101.2	2, 3, 4a, 9a	6.67d (2.12)	101.3	2, 3, 4a, 9a	6.58d[c]	100.6	4a, 9a		134.2		6.68d (2.19)	100.4	3, 4a	6.88d (2.56)	99.6	
4a		159.4			159.7			163.3			155.9			159.4			160.9	
5		127.7			132.1		6.28s	93.5	7, 10a		131.9			127.1		6.18s	99.1	4a
6		152.4			153.5			159.0			159.0			157.5			161.6	
7		131.3			135.4			130.8		6.39s	94.4	5, 6, 8, 8a	6.16s	97.5	5, 6, 8, 8a		124.5	
8		150.8			150.9			154.6			153.7			158.7			150.1	
8a		101.8			105.2			102.0			104.0			102.7			98.9	
9		182.5			182.8			182.5			183.9			182.3			182.6	
9a		111.2			110.9			110.5			113.0			111.5			114.0	
10a		145.2			145.1			151.9			153.4			149.2			143.9	
1-CH_3	2.70s	23.7	1,2,4a, 9, 9a	2.70s	23.7	1, 2, 4, 9a	2.69s	23.2	1, 2, 9a	2.65s	23.2	1, 2, 9a	2.77s	22.4	1, 2, 9a	2.80s	23.6	1, 2, 9a
3-OCH_3																3.91s	56.3	3
4-OCH_3										3.79s	61.7	4						
5-OCH_3	3.78s	61.7	5	3.82s	62.3	5				3.70s	60.9	5	3.86s	60.6	5, 6			
6-OCH_3				3.99s	62.0	6												
7-OCH_3	3.73s	60.7	7	3.77s	61.2	7	3.68s	60.0	7							3.88s	61.8	7
8-OH	13.23s			13.20s		7, 8, 8a	13.51s		7, 8, 8a	13.45s			13.10s			*d*		

[a] Spectrum measured in DMSO. [b] Spectrum measured in CD_3OD. [c] Peaks superimposed; *J* could not be determined. [d] Sample acquired in CD_3OD, resonance not observed.

The structures of ring A of drimiopsins B, C, and E (**2**, **3**, **5**) were identical to that of drimiopsin A (**1**). The ^{1}H NMR spectrum of **2**, $C_{17}H_{16}O_7$, showed the presence of three methoxy group proton resonances at δ 3.82, 3.99, and 3.77 and a phenolic proton resonance at δ 13.20, ascribed to the hydrogen-bonded proton of the hydroxy group at C-8. The INADEQUATE NMR spectrum confirmed that the methoxy groups occurred at C-5, C-6, and C-7. This was confirmed by a NOESY NMR experiment, which showed correlations between H-4 and the protons of the methoxy group at C-5 and between the protons of the methoxy groups at C-5 and C-6, and C-6 and C-7. Thus structure **2** was assigned to drimiopsin B.

Apart from the ring A resonances, which are very similar to those of drimiopsins A (**1**) and B (**2**), the ^{1}H NMR spectrum of **3**, $C_{15}H_{12}O_6$, showed the presence of a methoxy group proton resonance at δ 3.68, a single proton resonance at δ 6.28, and a downfield phenolic group proton resonance at δ 13.51, which indicated the presence of a hydroxy group at C-8. On biogenetic grounds, where protons are present in methylxanthones, they are expected to occur at C-2, -4, -5, and -7;[12] thus it was expected that the single proton on the B ring should be present at either C-5 or C-7. The proton was assigned as H-5 due to HMBC correlations seen between C-6, C-7, C-8a, and C-10a to this proton. The methoxy group was placed at C-7 and a hydroxyl group was placed at the remaining C-6 position, as there were no correlations seen in the NOESY spectrum between H-5 and the methoxy group proton resonance. Further evidence for this was the ^{13}C NMR shift of the methoxy group carbon resonance. The chemical shift of methoxy group carbon with protons at one of the *ortho* positions occurs at ~55 ppm.[13] The shift of the methoxy group was δ_C 60.0, typical of a methoxy group with *ortho* hydroxy groups. Thus structure **3** was assigned to drimiopsin C.

The ^{1}H NMR spectrum of **4**, $C_{16}H_{14}O_7$, showed two aromatic proton singlets at δ 6.67 and δ 6.39 instead of the *meta*-coupled doublets seen in compounds **1**−**3**, two methoxy group proton resonances at δ 3.79 and 3.70, the usual C-1 methyl group proton resonance at δ 2.65, and the C-8 phenolic group proton resonance at δ 13.45. The COSY and NOESY spectra showed a correlation between the proton resonance at δ 6.67 and the methyl group proton resonance, and hence this proton was assigned as H-2. As no correlations were seen with the second proton resonance in the COSY spectrum, the second proton was assigned to ring B. No further correlations were seen with H-2 in the NOESY spectrum, so a hydroxy group was placed at C-3. This was confirmed by HMBC correlations between C-4 and the methoxy group proton resonances at δ 3.79 and the H-2 resonance. A hydroxy group was placed at C-8 in accordance with the downfield resonance at δ 13.45 ascribed to the proton of the hydroxy group at C-8 observed in the ^{1}H NMR spectrum of **4**. A weak correlation between the C-4 methoxy group proton resonance and the second methoxy group at δ 3.70 indicated that the second methoxy group was present at C-5. On biogenetic grounds, the remaining proton was placed at C-7, and this placement was confirmed by a ^3J correlation between C-7 (δ 94.4) and the C-8 hydroxy group proton in the HMBC spectrum. The remaining hydroxy group was placed at C-6. Thus structure **4** was assigned to drimiopsin D.

Drimiopsin E (**5**), $C_{15}H_{12}O_6$, had the same ring A structure as drimiopsins A−C (**1**−**3**). The ^{1}H NMR spectrum showed an additional single proton resonance at δ 6.16, a methoxy group proton resonance at δ 3.86, and the usual C-8 phenolic group proton resonance at δ 13.10. The

molecular formula indicated the presence of an additional hydroxyl group. The proton resonance was assigned as H-7, a hydroxyl group was placed at C-6, and the methoxy group was placed at C-5. The assignment of the proton resonance as H-7 was supported by the HMBC correlations seen from C-8 and C-8a to H-7. Methoxy groups with protons at one of the *ortho* positions occur at approximately 55 ppm. However, the methoxy group carbon resonance of drimiopsin E (**5**) occurred at δ 60.6, which confirmed a neighboring hydroxy group substituent, and the methoxy group was therefore placed at C-5.[13] Thus, structure **5** was assigned to drimiopsin E.

Ring A of drimiopsin F (**6**), $C_{16}H_{14}O_6$, had the same substitution pattern as drimiopsins A−C (**1**−**3**) except that instead of a hydroxyl group, **6** has a methoxy group at C-3. This was indicated by the NOESY correlations seen between the protons of the methoxy group at δ 3.91 and the *meta*-coupled protons at δ 6.73 (J = 2.56 Hz) and 6.88 (J = 2.56 Hz). A hydroxy group was placed at C-8 due to the appearance of the carbonyl carbon of C-9 at δ 182.6,[11] as found for drimiopsins A−E (**1**−**5**). The single proton resonance was assigned to C-5 due to a HMBC correlation with C-4a. The remaining methoxy and hydroxy groups were placed at C-7 and C-6, respectively, since no correlations were seen in the NOESY spectrum between H-5 and the methoxy group proton resonance. Thus structure **6** was assigned to drimiopsin F.

Experimental Section

General Experimental Procedures. The melting points of the crystalline compounds were recorded on a Kofler microhot stage melting point apparatus**.** The ultraviolet absorption spectra were obtained on a Varian DMS 300 UV−visible spectrometer, and infrared spectra were recorded using a Nicolet Impact 400D Fourier transform infrared (FT-IR) spectrometer. NMR spectroscopy was carried out on a 400 MHz Varian UNITY-INOVA spectrophotometer, and INADEQUATE spectra were acquired for drimiopsins A (**1**) and B (**2**) in the Varian NMR Applications Laboratory in Darmstadt, Germany, using a 400 MHz Varian UNITY−INOVA spectrophotometer. FABMS were recorded on a Micromass 70-70E mass spectrometer, using a *m*-nitrobenzyl alcohol matrix and xenon as the bombardment gas, with peak matching for drimiopsins A, D, E, and F, while low-resolution mass spectra were obtained for the remaining samples.

Plant Material. The bulbs of *Drimiopsis maculata* Lindl., cultivated in Pretoria, South Africa, were harvested in January 2000, and a voucher was lodged for verification purposes (*Crouch 1000*, NH).

Extraction and Isolation. The material (2585.4 g) was extracted at room temperature for 24 h on a Labcon Mechanical shaker with methylene chloride, and the extract (1.25 g) was separated using gravity column chromatography over silica gel (Merck 9385, solvent $MeCl_2$/EtOAc) to yield the xanthones drimiopsin A, **1** (7.2 mg), drimiopsin B, **2** (8.4 mg), drimiopsin C, **3** (5.7 mg), drimiopsin D, **4** (8.1 mg), drimiopsin E, **5** (9.1 mg), and drimiopsin F, **6** (5.6 mg). Compounds **1**−**4** were eluted using a ratio of 9:1 dichloromethane to ethyl acetate, and compounds **5** and **6** were eluted using a ratio of 8:2 dichloromethane to ethyl acetate.

Drimiopsin A (3,6,8-Trihydroxy-5,7-dimethoxy-1-methylxanthone) (1): yellow powder; mp 218−222 °C; UV (MeOH) λ_{max} (log ϵ) 315 (3.48) nm; IR (NaCl) ν_{max} 3404, 2931, 2858, 1633, 1596, 1463, 1324, 1185, 1052 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 318 [M$^+$] (18), 279 (14), 217 (12), 154 (95), 136 (100), 107 (47), 77 (52); HRMS *m*/*z* 318.07411 (calcd for $C_{16}H_{14}O_7$, 318.07395).

Drimiopsin B (3,8-Dihydroxy-5,6,7-trimethoxy-1-methylxanthone) (2): fine, white powder; UV (MeOH) λ_{max} (log ϵ) 310 (3.48) nm; IR (NaCl) ν_{max} 3380, 2922, 2847, 1644, 1607, 1461, 1250, 1169, 1049 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 332 [M$^+$] (12), 303 (17), 288 (70), 273 (72), 245 (100), 151 (25), 121 (36), 107 (11); FABMS *m*/*z* 332 (calcd for $C_{17}H_{16}O_7$, 332).

Drimiopsin C (3,6,8-Trihydroxy-7-methoxy-1-methylxanthone) (3): off-white powder; mp 222−225 °C; IR (NaCl) ν_{max} 3387, 2918, 2848, 1630, 1622, 1467, 1273, 1158 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRMS *m*/*z* 288.06310 (calcd for $C_{15}H_{12}O_6$, 288.06339).

Drimiopsin D (3,6,8-Trihydroxy-4,5-dimethoxy-1-methylxanthone) (4): fine, white powder; mp 205−208 °C; UV (MeOH) λ_{max} (log ϵ) 311 (3.94) nm; IR (NaCl) ν_{max} 3396, 2921, 2850, 1651, 1614, 1456, 1320, 1168, 1100 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 318 [M$^+$] (100), 303 (77), 287 (24), 260 (53), 107 (16), 77 (32); HRMS *m*/*z* 318.07411 (calcd for $C_{16}H_{14}O_7$, 318.07395).

Drimiopsin E (3,5,8-Trihydroxy-6-methoxy-1-methylxanthone) (5): brown, amorphous material; IR (NaCl) ν_{max} 3429, 2914, 2846, 1644, 1613, 1276, 102 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 288 [M$^+$] (69), 273 (100), 245 (91), 149 (25), 123 (55), 57 (43); HRMS *m*/*z* 288.06310 (calcd for $C_{15}H_{12}O_6$, 288.06339).

Drimiopsin F (6,8-Dihydroxy-3,7-dimethoxy-1-methylxanthone) (6): fine, white crystalline material; mp 214−216 °C; IR (NaCl) ν_{max} 3328, 2923, 1625, 1597, 1507, 1413, 1157, 1035 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 302 [M$^+$] (54), 287 (100), 259 (71), 216 (10); HRMS *m*/*z* 302.07911 (calcd for $C_{16}H_{14}O_6$, 302.07903).

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