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TWO NOVEL PHENALENONES FROM *DILATRIS VISCOSA*

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ABSTRACT.—Phytochemical analysis of the EtOH extract of *Dilatris viscosa* (Haemodoraceae) has resulted in the isolation of two novel glucosides, dilatrin [**1**] and lachnanthoside aglycone 1-glucoside [**3**]. The known haemocorin aglycone [**4**] was also identified.

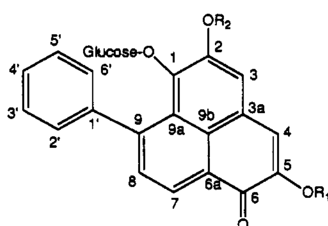
The family Haemodoraceae is generally restricted to the Southern hemisphere, and plants of the family are characterized by having red-pigmented roots; the color is due to phenylphenalenone [as in **1**] and naphthoxanthenone pigments (1). To date, eight of the fourteen genera recognized by the most recent taxonomic study as comprising the family (2) have been found to contain these types of compounds or substances reasonably derived from them, and they are now considered as chemotaxonomic markers for the family (1). Indeed, recent work (3,4) has enabled us to identify, on the basis of chemical studies, two genera incorrectly assigned to the family Haemodoraceae by earlier authorities.

The genus *Dilatris* consists of two species both endemic to the Cape Colony of South Africa. *Dilatris viscosa* Bergius, found on the slopes of Table Mountain, has orange flowers and the red rootstock typical of the family.

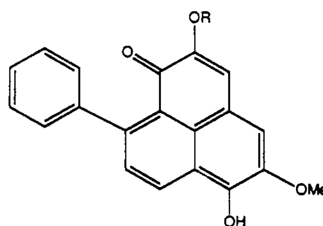
This study has identified two new phenalenone glycosides (**1** and **3**) in extracts of the root system of the plant. The known aglycone **4** of haemocorin has also been isolated. This brings to nine of fourteen the genera of the family that have been found to contain pigments based upon the phenalenone ring system.

RESULTS AND DISCUSSION

A red crystalline compound **1** was isolated from the EtOAc-soluble fraction of the aqueous EtOH extract of *D. viscosa* by preparative tlc. Compound **1** had mp 150–153° from Me_2CO; hreims showed $[M]^+$ at *m/z* 318.0890 for the aglycone corresponding to $C_{20}H_{14}O_4$ (calcd 318.0892) and contained a strong $[M-1]^+$ ion indicative, in this type of compound, of a phenalenone ring system in which a phenyl ring is situated peri to a carbonyl group (1). The fabms showed peaks at *m/z* 481 and 319, suggesting a hexose glycoside. The ^{1}H nmr contained resonances readily assigned to the aromatic aglycone: at δ 3.88 for a single OMe; at δ 8.40 and 7.59, an AM quartet for the protons at H-7 and



1 R_1=Me, R_2=H
3 R_1=R_2=H
5 R_1=R_2=Me



4 R=H
6 R=Me

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H-8; at δ 7.57, a singlet for H-4; a broad singlet at δ 7.34 for the phenyl substituent; and a singlet at δ 7.14 for H-3. The doublet at δ 4.50 with $J=7.8$ Hz implied a glycoside with a β link; this was confirmed by the ^{13}C-nmr shift of the anomeric carbon of δ 103.94 (5). The ir showed ν max at 1630 cm^{-1}, suggesting a non-hydrogen-bonded conjugated carbonyl function. Acid hydrolysis of **1** gave the known aglycone **4**.

The uv spectrum of the glycoside, which was characteristic of the 9-phenylphenalenones (1), showed no bathochromic shift on addition of $AlCl_3$, thus excluding structures having an α-hydroxyketone grouping (as in **4**). The sugar unit could not, therefore, be located at C-6 but could be at C-1 or C-2 of the aglycone. Methylation of the glycoside gave a single monomethyl ether, suggesting that the hexose was at C-1; two isomeric ethers would have been found if the sugar were at C-2 due to the tautomerism of the molecule. The ^{1}H-nmr spectrum of the methylated glycoside **5** showed the resonance for the anomeric proton downfield by 0.5 ppm from its position in the spectrum of the glycoside **1**. We interpreted this as indicating hydrogen bonding between the pyran oxygen of the hexose unit and the OH group at C-2 of the glycoside, so the anomeric proton, and much of the sugar unit, is maintained in the shielding cone of the C-9 phenyl ring. Hydrolysis of **5** gave xiphidone [**6**], a compound previously isolated from *Xiphidium caeruleum* (6) and obtained by hydrolysis of the dimethyl ethers of haemocorin aglycone [**4**] (1).

The glycoside **1** was resistant to hydrolysis by α- or β-glucosidase and galactosidase; however, acid hydrolysis gave a sugar which by tlc could have been either glucose or galactose. The ^{13}C-nmr signals for the sugar unit of **1** provided presumptive evidence that the sugar was a β-linked glucose unit; this was confirmed by a refocused ^{13}C INEPT experiment, which indicated only a single methylene carbon. The identity of the aldohexose was established by 500 MHz ^{1}H nmr, which resolved the sugar protons and showed that all the vicinal coupling constants but one were greater than 6 Hz, implying a diaxial coupling; only glucose could give rise to this spectrum. All the proton resonances for the sugar residue were seen to be shifted upfield (by up to 1.5 ppm) relative to those in other β-phenylglucosides (e.g., salicin); this is taken to be further evidence that the sugar is at C-1 of the phenalenone ring and lies within the shielding cone of the phenyl substituent. The ^{13}C chemical shift assignments for **1** are based upon a detailed investigation of the ^{13}C spectrum of **2**, one of the two isomeric dimethylethers of haemocorin aglycone. The analysis for **2** was made on the basis of a first order analysis of the 1D nmr spectrum and an interpretation of the 2D homo- or hetero-COSY and NOESY spectra. Computer molecular modeling was used to search for minimum energy conformations using the Biosym Insight II/DISCOVER program package incorporating an empirical forcefield and the consistent valence forcefield on a Silicon Graphic 4D/70GT workstation. The preferred dihedral angle between the benzene ring and the phenalenone ring was examined using the bond-rotary search technique at intervals of 5°; the most energetically preferred one is about 56°.

In the 1D ^{1}H-nmr spectrum, the doublet peak located most downfield and integrating for one proton could be easily assigned to H-7 (δ 8.62). The corresponding strong ^3J crosspeaks in the 2D COSY spectrum were observed for H-7 and H-8 (δ, 7.56). Similarly, we could assign the chemical shifts of the protons H-3 (δ 6.87) and H-4 (δ 7.48) based on their chemical environments and integration; these assignments were later confirmed by the long-range benzylic ^4J coupling between H-3 and H-4 seen in the 2D COSYLR spectrum. The most upfield peak, integrating for three protons, was assigned to the 12-MeO proton resonances (δ 3.23), which is upfield shifted by ca. 1.0 ppm compared with the other two MeO groups (about δ 4.0). This is attributed to the shielding effect of the aromatic ring. Differentiation between the 10-MeO and 11-MeO

was made on the basis of the nOe seen between H-3 or H-4 and the adjacent MeO groups in the 2D NOESY spectrum (Figure 1): two weak nOe crosspeaks were observed for the 10-MeO (δ 4.00) with H-4 and for the 11-MeO (δ 3.96) with H-3. The singlet peak with high intensity and integrating for five protons is typical for an aromatic ring with a single substituent. The proton assignments are summarized in Table 1.

The hetero-COSY spectrum gave the C-H connections and located the protonated carbons as shown in Table 1. The assignment of the nonprotonated carbons was made using broad-band decoupled ^{13}C and DEPT spectra. The chemical shifts of some carbons (C-6a, C-9a, and C-9b) were assigned based on their chemical environments and the

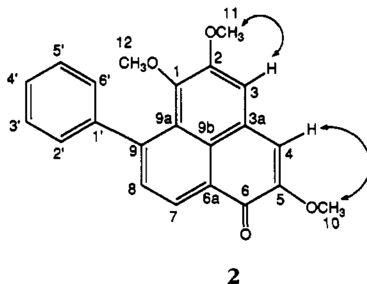

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FIGURE 1. Arrows indicate nOe effects.

partial atomic charge calculated by the semi-empirical calculation method, MOPAC (AM1) (7). The assignments of the ^{13}C resonances are summarized in Table 1. These observations confirmed structure **1** for the major glycoside of *D. viscosa*, and we have named **1** as dilatrin.

A minor glycoside was also isolated from the EtOAc fraction of the EtOH extract of *D. viscosa*. The compound **3** crystallized from MeOH as red needles, mp 189–191°; the eims showed $[M]^+$ at *m/z* 304.0734 for the aglycone corresponding to $C_{19}H_{12}O_4$ (calcd 304.0735), with a strong $[M-1]^+$ base peak. The fabms indicated the presence of a hexose sugar in the glycoside. The ^1H nmr of **3** was very similar to that of **1**, lacking only the MeO resonance as implied by the formula; the anomeric linkage was β. Methylation of **3** gave **5**, demonstrating that the new compound was the C-1 glucoside of lacnanthoside aglycone (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were determined in Me_2CO-d_6, unless otherwise stated, with a Bruker 200 MHz or 500 MHz spectrometer. The chemical shifts are given in ppm relative to TMS. Hreims was recorded on a KRATOS MS 902 double focusing magnetic sector mass spectrometer. Fabms was recorded on a MAT 731 mass spectrometer; samples were applied in a matrix of dithiothreitol and dithioerythritol. Ir spectra were determined on a Beckman Acculab 3 in KBr. Uv spectra were obtained on a Varian CARY 2290 spectrophotometer in MeOH. Mp's were determined on a Koefler hot-stage apparatus and are uncorrected.

PLANT MATERIAL.—*D. viscosa* was collected in 1986 from Table Mountain, Cape Town, South Africa. The plants were dried and powdered. Voucher specimens are lodged in the herbarium at Kirstenboch Botanical Garden in Cape Town.

EXTRACTION AND FRACTIONATION OF *D. VISCOSA*.—The dried powdered plant (460 gm) was exhaustively extracted by cold maceration using EtOH (2×2 liters). The extract was concentrated under vacuum, and the concentrated EtOH extract (100 ml) was suspended in H_2O (220 ml), transferred completely into a separatory funnel, and partitioned sequentially into petroleum ether, $CHCl_3$, and EtOAc.

Haemocorin aglycone [**4**].—The $CHCl_3$ fraction (5 g) was subjected to chromatographic fractionation using a chromatotron (3 runs, each using a 0.5 mm Si gel coated rotor and 1.67 g of the extract). The rotor

TABLE 1. ^{1}H nmr (200 MHz) and ^{13}C nmr (75 MHz) Chemical Shift Assignments for Compound **1** [in $(CD_3)_2CO$] and ^{13}C nmr (75 MHz) Chemical Shift Assignments for Compound **2** (in $CDCl_3$).

Compound				
2				**1**
Proton	Chemical shift δ	Carbon	Chemical shift δ	Chemical shift δ
—	—	C-1	150.37	149.15
—	—	C-2	146.29	148.63
H-3	6.87	C-3	112.90	115.27
—	—	C-3a	121.09	125.08
H-4	7.48	C-4	118.14	121.68
—	—	C-5	152.47	153.30
—	—	C-6	179.88	181.84
—	—	C-6a	142.82	141.88
H-7	8.62	C-7	128.71	130.07
H-8	7.54	C-8	130.82	131.70
—	—	C-9	146.18	145.25
—	—	C-9a	126.80	126.64
—	—	C-9b	128.58	129.7
H-10	4.00	C-10	56.75	56.22
H-11	3.96	C-11	55.59	—
H-12	3.23	C-12	60.69	—
—	—	C-1′	143.13	144.80
H-2′, -6′	7.39	C-2′, -6′	127.00	127.80
H-3′, -5′	7.39	C-3′, -5′	126.77	128.64
H-4′	7.39	C-4′	128.58	129.12
		Glucose		
—	—	C_1	—	103.94
—	—	C_2	—	74.71
—	—	C_3	—	77.64
—	—	C_4	—	71.43
—	—	C_5	—	77.95
—	—	C_6	—	62.77

was eluted by toluene, and fractions of 100 ml were collected. Based on preliminary tlc analysis, similar fractions were combined.

One fraction, which on evaporation gave 200 mg of product, showed the presence of one major spot on tlc. On purification by rechromatography over a Si gel column (1×10 cm, 10 g) using $CHCl_3$ as eluent, and crystallization from Me_2CO, 30 mg of compound **4** as black crystals, mp 227° were obtained: hreims *m/z* $[M]^+$ 318.0893 ($C_{20}H_{14}O_4$ requires 318.0888); ir ν max 3500–3300, 1610 cm^{-1}. Compound **4** was shown to be identical to the aglycone of haemocorin [mp, mixed mp, ir, ^{1}H-nmr formation of methyl ethers (8)].

Dilatrin [**1**].—The EtOAc fraction (4 g) was chromatographed on a Si gel column (20×2 cm, 100 g) starting with $CHCl_3$ as eluent. Fractions of 200 ml were collected. Increasing the polarity with MeOH afforded fractions containing two compounds: one major, one minor. These fractions were subjected to preparative Si gel tlc [20×20 cm, EtOAc-HCOOH-H_2O (12:1:1)]. The band for each compound was removed from the wet plates, and the Si gel was exhaustively extracted with MeOH. Evaporation of the eluate containing the band of lower R_f afforded the major compound **1**. Similar treatment of the higher band afforded a very minor compound **3**. Crystallization of **1** from Me_2CO gave prismatic orange-red crystals (15 mg) with mp 150–153°; hreims *m/z* (%) $[M]^+$ 318.0890 (30%) ($C_{20}H_{14}O_4$, calcd 318.0892), $[M-1]^+$ 317 (50%). Fabms showed $[MH]^+$ at *m/z* 481 and 319: the former for the protonated glycoside and the latter for the protonated aglycone. Ir ν max 3600–3200, 1630 cm^{-1}; uv λ max 267, 356 (sh), 372, and 466 nm; with $AlCl_3$ no change; in NaOMe 292, 382, 540 nm; ^{1}H nmr δ 8.40 (1H, d, J=7.7 Hz, H-7), 7.57 (1H, s, H-4), 7.59 (1H, d, J=7.7 Hz, H-8), 7.34 (phenyl 5H, broad s), 7.14 (1H, s, H-2), 4.50 (1H, d, J=7.8 Hz, H-1′), 3.85 (3H, s, MeO), 3.6–3.03 (overlapped signals). At 500 MHz (DMSO-d_6), δ 4.63 (1H, d, J=7.7, H-1′), 3.85 (3H, s, MeO), 3.45 (1H, dd, J=4.7 and 11 Hz, H-6′), 3.24 (1H, dd, J=6.1 and 11 Hz,

H-6′), 2.99 (1H, t, J=8.9 Hz, H-3′), 2.78–2.82 (1H, m, H-5′), 2.70 (1H, dd, J=9.1 and 7.5 Hz, H-2′), 2.21 (1H, dd, J=8.5 and 8.5 Hz, H-4′); ^{13}C nmr see Table 1; ^{13}C-nmr refocused INEPT showed all the carbon signals from the sugar inverted except for one at 62.77 ppm.

Monomethyl dilatrin [**5**].—Compound **1** (5 mg) was methylated by adding an excess of ethereal CH_2N_2. After 12 h, the Et_2O was evaporated, and the residue was crystallized from Et_2O to afford 5 mg of fine red crystals: mp 166–168°; ^{1}H nmr δ 8.46 (1H, d, J=7.8 Hz, H-7), 7.83 (1H, s, H-4), 7.52 (1H, d, J=7.8 Hz, H-8), 7.37 (5H, m, phenyl ring), 7.21 (1H, s, H-2), 5.08 (1H, d, J=7.7 Hz, H-1′), 4.03 (3H, s, OMe), 3.91 (3H, s, OMe), 3.56–2.88 (sugar Hs, complex m).

Xiphidone [**6**].—Compound **5** (2 mg) was heated with 2 ml of 1% aqueous HCl for 5 min in an H_2O bath and cooled, and the mixture was extracted exhaustively with $CHCl_3$. The extract was dried over anhydrous Na_2SO_4, then evaporated to leave a dark residue. On crystallization from EtOH it afforded 1.3 mg of purple crystals with mp 170–171°; [lit. (6) mp 170–172°]; ^{1}H nmr δ 8.70 (1H, d, J=7.7 Hz, H-7), 7.55 (1H, d, J=7.7 Hz, H-8), 7.41 (5H, s, phenyl ring), 7.37 (1H, s, H-3), 6.89 (1H, s, H-4), 6.28 (1H, s, exchangeable with D_2O), 4.02 (3H, s, OMe), and 3.96 (3H, s, OMe). On addition of D_2O additional smaller peaks due to the tautomer appeared at δ 8.55 (1H, d, J=7.7 Hz, H-7), 6.79 (1H, s, H-4), 4.08 (3H, s, OMe), 3.85 (3H, s, OMe). Hreims *m/z* 332.1048 ($C_{21}H_{16}O_4$ requires 332.1044); methylation of the resulting aglycone gave two permethyl ethers identical to those obtained by methylation of haemocorin aglycone [**4**].

Lachnanthoside aglycone 1-glycoside [**3**].—Compound **3** was isolated from the EtOAc fraction after cc on Si gel followed by preparative tlc of major fractions. Crystallization from MeOH gave 2 mg of compound **3** as red needles: mp 189–191°; hreims *m/z* 304.0734 ($C_{19}H_{12}O_4$ calcd 304.0735), a strong peak at 303; fabms *m/z* $[M+H]^+$ 467, $[M-H]^+$ 465; ^{1}H nmr δ 8.50 (1H, d, J=7.8 Hz, H-7), 7.66 (1H, d, J=7.8 Hz, H-8), 7.60 (1H, s, H-3), 7.35 (5H, broad s, phenyl ring), 7.17 (1H, s, H-4), 4.53 (1H, d, J=7.4 Hz, H-1′), 3.20–2.00 (sugar Hs, complex m). Methylation of 1 mg of **3** with CH_2N_2 afforded compound **5**, identical (mp, tlc, co-tlc, ^{1}H nmr) to the one obtained from methylation of compound **1**. Acid hydrolysis of **5** gave xiphidone [**6**], identical to the product obtained from acid hydrolysis of the methyl ether of compound **1**.

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