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Homoisoflavanones and spirocyclic nortriterpenoids from three *Eucomis* species: *E. comosa*, *E. schijffii* and *E. pallidiflora* subsp. *pole-evansii* (Hyacinthaceae)[☆]

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Abstract

Three species of the small but ethnomedicinally important genus *Eucomis* (Hyacinthaceae) have been investigated phytochemically. These have yielded a range of homoisoflavanones of the 3-benzyl-4-chromanone, benzylidene and scillascillin types as well as spirocyclic nortriterpenoids. *Eucomis pallidiflora* subsp. *pole-evansii* yielded the novel benzylidene homoisoflavonoid, 8-methoxy-5,6,7-trihydroxy-3-(4'-hydroxybenzylidene)-4-chromanone.

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Keywords: Hyacinthaceae; Homoisoflavonoids; *Eucomis comosa*; *E. schijffii*; *E. pallidiflora* subsp. *pole-evansii*; Benzylidene, scillascillin; Spirocyclic nortriterpenoid; 7-hydroxy-5-methoxy-3-(4'-hydroxybenzyl)-4-chromanone; 5,7-dihydroxy-8-methoxy-3-(4'-hydroxybenzyl)-4-chromanone; Punctatin; (Z)-eucomin; (E)-eucomin; (23S)-17 α ,23-epoxy-3 β ,29-dihydroxy-27-nor-5 α -lanost-8-ene-15,24-dione; 8-methoxy-5,6,7-trihydroxy-3-(4'-hydroxybenzylidene)-4-chromanone; (23S)-17 α ,23-epoxy-3 β ,28,29-trihydroxy-27-norlanost-8-en-24-one

1. Introduction

Eucomis L'Hér. (Hyacinthaceae) is a small African genus of bulbous plants producing inflorescences that are characteristically topped by a conspicuous flat-topped coma of leafy sterile bracts. The loriate to obovate leaves are synanthous. Eight of the ten *Eucomis* species (with fourteen described taxa) are found only in southern Africa (FSA region), with two species extending their range north of the Limpopo River into south tropical Africa (Manning et al., 2004). Species treatments have been provided by Compton (1990) who arranged each overview by environmental parameters, growth and distinguishing features. Whereas all three regional subfamilies of the Hyacinthaceae are in remarkably contentious taxonomic flux (Pfosser and Speta, 1999; Stedje, 2001; Manning et al., 2004),

both morphological and molecular characters concur in circumscribing *Eucomis*. Accordingly, the genus size and nomenclature has not altered substantially in recent years, save for the recognition of *Eucomis pole-evansii* N.E.Br. as a larger, more northerly subspecies of *Eucomis pallidiflora* Bak. (Manning et al., 2004). The genus is found primarily in the summer-rainfall zone where it is a characteristic component of moist grasslands in montane and subalpine areas.

The Hyacinthaceae ranks as one of the most important families in ethnomedicine along the eastern seaboard of southern Africa (Mander, 1998; Von Ahlefeldt et al., 2003). Amongst the seven hyacinthacs occurring within the top seventy most important species marketed by Durban street traders are two *Eucomis* species, *E. autumnalis* (Mill.) Chitt. and *E. bicolor* Bak. (Cunningham, 1988; Mander, 1998). *Eucomis comosa* (Houtt.) Wehrh. (syn. *Eucomis punctata* L'Hér.) has been noted in the ethnomedicinal plant trade both in the Eastern Cape Province (where it is known as *umphompho*) (Dold and Cocks, 2002) and to the north in Durban, KwaZulu-Natal (Von Ahlefeldt et al., 2003). *E. pallidiflora* Bak. subsp. *pole-evansii*

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(N.E.Br.) Reyneke ex J.C.Manning has not yet been identified in trade although it has been documented as an ethnomedicinal subject: unspecified South African user-groups were reported by Watt (1967) to treat mental diseases using this plant. *Eucomis schiffii* Reyneke has not yet been recorded as an ethnomedicinal subject. Gerstner (1939) recorded *E. comosa* as a Zulu ethnomedicine under the name *ubuhlungu-becanti*, noting that bulb preparations were used to treat rheumatism. Watt and Breyer-Brandwijk (1962) documented a similar anti-rheumatic application of bulb decoctions by the Xhosa, a practice more recently confirmed by Simon and Lamla (1991). The trans-cultural use of bulbs of the same species to treat rheumatism, a disorder marked by inflammation and attended by pain, suggests a rational basis for the selection of *E. comosa* in ethnomedicine. Bulb decoctions of *E. comosa* are further administered as enemas to infants during teething; a frequently noted side effect of such treatment is the development of a rash (Watt and Breyer-Brandwijk, 1962). *E. comosa* is considered potentially toxic by some researchers (Hutchings and Terblanche, 1989). The Xhosa name for the plant is reportedly *ubuhlungu bechanti* (Simon and Lamla, 1991).

The phytochemistry of five species of *Eucomis* has been investigated previously: *E. comosa* and *E. bicolor* yielded a range of homoisoflavonoids (Adinolfi et al., 1985a; Heller et al., 1976), *E. comosa* the chromanone, 5,7,8-trihydroxy-4-chromanone (Heller and Tamm, 1978), *E. montana* Compton a range of

homoisoflavonoids and a spirocyclic nortriterpenoid (Koorbanally et al., 2006), *E. pallidiflora* subsp. *pole-evansii* the spirocyclic nortriterpene eucosterol, and *E. autumnalis* a range of homoisoflavonoids and spirocyclic nortriterpenoids (Ziegler and Tamm, 1976; Silayo et al., 1999) as well as the two benzopyranones, autumnariol and autumnariniol (Sidwell et al., 1971). The correct identification of these latter two compounds has been questioned in the light of the recent isolation of the isomeric methylxanthenes from another subfamily representative, *Drimiopsis maculata* Lindl. (Mulholland et al., 2004). The present investigation sought to profile phytochemically the unworked species *E. schiffii*, to re-investigate *E. comosa* and *E. pallidiflora* subsp. *pole-evansii*, and to determine whether constituents could serve as chemotaxonomic markers at genus or species levels.

2. Materials and methods

2.1. Plant materials

Fresh bulbs of *E. comosa* (Houtt.) Wehrh. var. *comosa* (3.9 kg) were purchased from the Warwick Triangle ethnomedicinal market in Durban. Specimens were grown to flowering prior to harvesting, and a voucher (*N. Crouch 940*, NH) was accessioned for verification purposes. Bulbs of flowering plants of *E. pallidiflora* Bak. subsp. *pole-evansii* (N.E.Br.) Reyneke ex

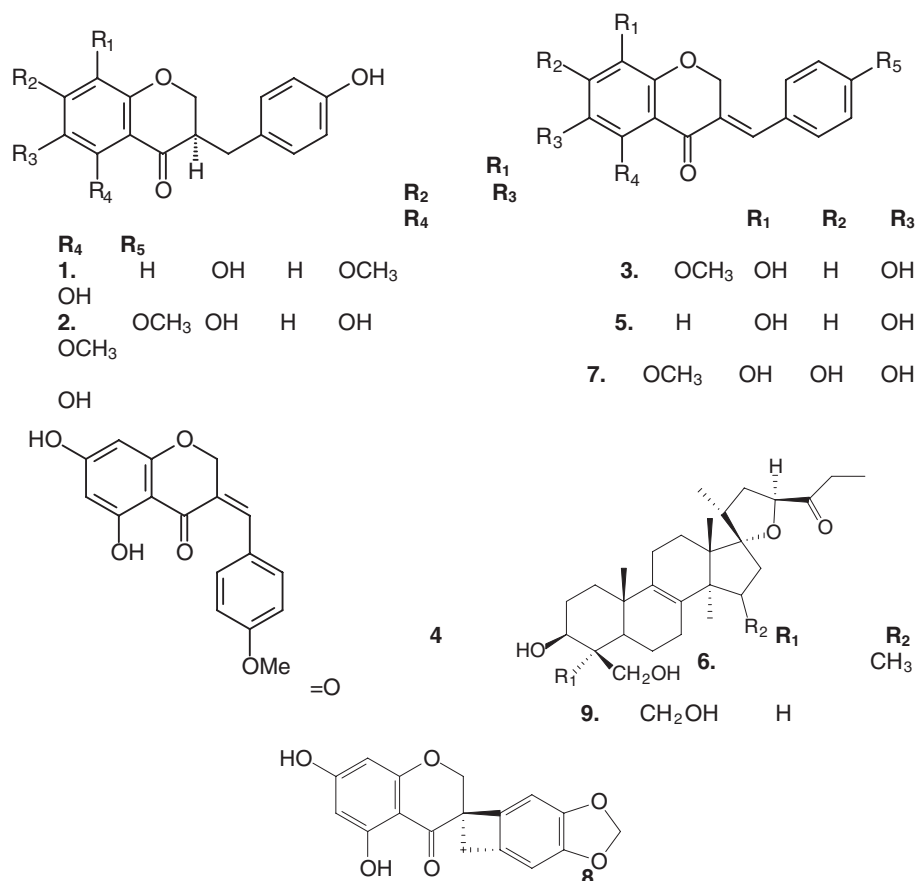


Fig. 1. Isolates of *Eucomis comosa*, *E. schiffii* and *E. pallidiflora* subsp. *pole-evansii*.

J.C.Manning (2.95 kg) were collected from a marsh at Mac Mac Pools in Mpumalanga (*N. Crouch 856*, NH). *E. schijffii* Reyneke bulbs (728 g) were harvested from a flowering population in the southern KwaZulu-Natal Drakensberg (*N. Crouch 836*, NH).

2.2. Extraction and fractionation

The air-dried bulbs of *E. comosa* were chopped and extracted with methanol (2.5 l) on a Labcon shaker for 48 h. The extract (354 g) was concentrated under reduced pressure and extracted with ethyl acetate (4 × 250 ml) to yield a residue (210 g). Chromatographic separation of the residue was achieved using gravity column chromatography over silica gel (130 g, Merck 9385), with a hexane/ethyl acetate step gradient (100% hexane → 100% EtOAc). This process yielded compound **1** (9 mg) (EtOAc:hexane / 1:4), compound **2** (27 mg) (EtOAc:hexane / 1:2), compound **3** (9 mg) (EtOAc:hexane / 1:1), compound **4** (12 mg) (EtOAc:hexane / 1:1) and compound **5** (3 mg) (EtOAc:hexane / 1:1).

The bulbs of *E. pallidiflora* subsp. *pole-evansii* and *E. schijffii* were separately air-dried, chopped and extracted successively with dichloromethane (2.5 l) and methanol (2.5 l) under conditions of continuous agitation over a period of 48 h. The dichloromethane extract of *E. pallidiflora* subsp. *pole-evansii* (34 g) was subjected to column chromatography (2% MeOH in CH₂Cl₂) over silica gel (130 g, Merck 9385) to yield compound **6** (293.6 mg) and compound **7** (11.2 mg). The dichloromethane extract of *E. schijffii* (8.2 g) was subjected to column chromatography to yield compounds **8** (32.6 mg) (100% dichloromethane) and **9** (14.6 mg) (20% EtOAc in CH₂Cl₂). The ¹H NMR spectra of the crude methanol extracts revealed only sugars, and so were not examined further.

2.3. Structure determinations

IR spectra were recorded with a Nicolet Impact 400 D spectrometer on sodium chloride plates and calibrated against an air background. HRMS were obtained using a Kratos High Resolution MS 9/50 spectrometer at the Cape Technikon. ¹H and ¹³C NMR spectra were recorded in CDCl₃ or CD₃OD on a Varian Unity Inova 400 MHz NMR spectrometer. UV spectra were recorded in methylene chloride on a Varian DMS 300 UV–visible spectrometer. Structures were determined following subsequent comparison against literature values (cited below); accordingly, a number of known homoisoflavonoids and spirocyclic nortriterpenoids were identified, as was a novel homoisoflavonoid (Fig. 1).

3. Results and discussion

A total of seven homoisoflavonoids of varying sub-classes were isolated from three species of *Eucomis*, including a novel benzylidene type. Two spirocyclic nortriterpenoids were also identified; overall these findings are consistent with earlier findings for *Eucomis* and the subfamily Hyacinthoideae in the FSA region (Pohl et al., 2000). The current investigation brings to thirty-nine the number of constituents identified from the six *Eucomis* species phytochemically characterised to date. These

include four classes of homoisoflavonoids, as well as benzopyranones, spirocyclic nortriterpenoids, acids, a saponin glycoside and a chromanone.

The ethyl acetate extract of the bulbs of *E. comosa* yielded compound **1**, 7-hydroxy-5-methoxy-3-(4'-hydroxybenzyl)-4-chromanone (Finckh and Tamm, 1970), compound **2**, 5,7-dihydroxy-8-methoxy-3-(4'-hydroxybenzyl)-4-chromanone (3,9-dihydropunctatin) (Adinolfi et al., 1985b), compound **3**, punctatin (Corsaro et al., 1992), compound **4**, (*Z*)-eucomin (Heller et al., 1976) and compound **5**, (*E*)-eucomin (Heller et al., 1976). *E. pallidiflora* subsp. *pole-evansii* yielded compound **2**, 3,9-dihydropunctatin, compound **6**, the spirocyclic nortriterpenoid eucosterol, (23*S*)-17 α ,23-epoxy-3 β ,29-dihydroxy-27-nor-5 α -lanost-8-ene-15,24-dione (Ziegler and Tamm, 1976) and compound **7**, the novel 3-benzylidene-4-chromanone, 8-methoxy-5,6,7-trihydroxy-3-(4'-hydroxybenzylidene)-4-chromanone. The elucidation of the structure of compound **7**, here accorded the trivial name pole-evansin, is discussed below. *E. schijffii* yielded compound **8**, the 3-spirocyclobutene-ring homoisoflavonoid, scillascillin (Kawasaki et al., 1973), as well as compound **6**, eucosterol, and compound **9**, (23*S*)-17 α ,23-epoxy-3 β ,28,29-trihydroxy-27-norlanost-8-en-24-one (Adinolfi et al., 1984). The structure of pole-evansin (compound **7**) was determined, using NMR and CD data, to be a novel homoisoflavanone of the benzylidene type. The mass spectrum indicated a molar mass of 330.07387 g mol⁻¹ which concurred with a molecular formula of C₁₇H₁₄O₇. A fragment peak was seen at *m/z* 107 indicating the presence of a hydroxytropylium ion. The appearance of the resonances at δ 5.32 (2H, *s*, 2H-2) and δ 7.72 (1H, *s*, H-9) in the ¹H NMR spectrum, as well as the appearance of an α,β -unsaturated carbonyl carbon resonance at δ 185.3 (C-4, C) in the ¹³C NMR spectrum, indicated the presence of a 3-benzylidene (i.e. a 3(9)-unsaturated) system (Kirkiacharian et al., 1984). In the COSY spectrum, the 2H-2 resonance was seen to be weakly coupled to H-9. The ¹H NMR spectrum showed a pair of doublet proton resonances, each integrating to two protons, at δ 7.14 (*J*=8.4 Hz) and δ 6.83 (*J*=8.4 Hz), which were assigned to H-2'/6' and H-3'/5' respectively. The corresponding methine carbon resonances were observed at δ 132.3 and δ 115.8. The C-2'/6' carbon resonance showed a correlation in the HMBC spectrum to H-9, and C-9 (δ 137.7), in turn, showed a correlation in the HMBC spectrum to 2H-2. The C-4 carbon resonance was seen to be correlated in the HMBC spectrum with 2H-2 and H-9. In the NOESY spectrum the proton resonance assigned to H-2'/6' showed a correlation to H-3'/5', H-9 and 2H-2. The fact that a correlation was not observed in the NOESY spectrum between H-9 and 2H-2, revealed that this compound must be the (*E*)-isomer. Further, the chemical shifts for C-2 (δ 67.7) of this 3-benzylidene-4-chromanone agreed with the value for eucomin (Kirkiacharian et al., 1984), thus confirmed the same *E* configuration to be assigned to this derivative. Furthermore, an *E*-orientation of the double bond was indicated by the position of the H-2 and H-9 proton signals at δ 5.32 and δ 7.72 respectively (Bohler and Tamm, 1967). The A ring substitution pattern was deduced by UV absorption, whereby positive bathochromic

Table 1
NMR data (400 MHz, CDCl₃) for compound 7, pole-evansin

	¹ H	¹³ C	COSY	HMBC (C → H)	NOESY
2	5.32 (2H, s)	67.7	H-9	H-9	H-2''/6', 8-OCH ₃
3	–	126.4	–	2H-2, H-9	–
4	–	185.3	–	2H-2, H-9	–
4a	–	*	–	*	–
5	–	*	–	*	–
6	–	102.6	–	7-OH	–
7	–	*	–	*	–
8	–	17.8	–	7-OH, 8-OCH ₃	–
8a	–	153.0	–	2H-2	–
9	7.72 (1H, s)	137.7	2H-2	2H-2, H-2''/6'	H-2''/6'
1'	–	125.6	–	H-3'/5'	–
2'	7.14 (1H, d, J=8.4 Hz)	132.3	H-3'/5'	H-9, H-2''/6'	2H-2, H-9, H-3'/5'
3'	6.83 (1H, d, J=8.4 Hz)	115.8	H-2''/6'	H-3'/5'	H-2''/6'
4'	–	158.8	–	H-2''/6', H-3'/5'	–
5'	6.83 (1H, d, J=8.4 Hz)	115.8	H-2''/6'	H-3'/5'	H-2''/6'
6'	7.14 (1H, d, J=8.4 Hz)	132.3	H-3'/5'	H-9, H-2''/6'	2H-2, H-9, H-3'/5'
8-OCH ₃	3.72 (3H, s)	61.2	–	–	2H-2
7-OH	6.01 (1H, s)	–	–	–	–

*Carbon resonances and HMBC correlations indeterminable from spectra.

shifts were observed with the addition of both NaOAc (+30 nm) and AlCl₃ (+50 nm). This indicated that hydroxy groups were present at C-5 and C-7. The ¹H NMR spectrum also showed the presence of a singlet resonance integrating to three protons at δ 3.72, which indicated a methoxy group. This was placed at C-8 (δ 127.8) as it showed a NOESY correlation to 2H-2. A model was built in order to confirm that this NOESY correlation was possible. From the NMR data compound 7 was identified as the novel 3-benzylidene-4-chromanone, (*E*)-8-methoxy-5,6,7-trihydroxy-3-(4-hydroxybenzylidene)-4-chromanone.

Pole-evansin: (11.2 mg), yellow amorphous, (C₁₇H₁₄O₇) EIMS: *m/z*: 330.07387 (calc. 330.073955): 330, 316, 298, 167, 107, 71, 57, 55, 44, 28. IR: *ν*_{max} (NaCl) cm⁻¹: 3381, 2917,

2846, 1634, 1514, 1378, 1307, 1170, 1034. UV *λ*_{max} (MeOH) nm (log ε): 354 (3.73). With NaOAc: 384. With AlCl₃: 404. NMR data are given in Table 1.

In view of the use of *Eucomis* to treat disorders marked by inflammation and pain (e.g. rheumatism) three homoisoflavonoids (compounds 2, 7 and 8) isolated from these *Eucomis* species (Table 2) were, in previous work (Du Toit et al., 2005), screened for anti-inflammatory activity using indomethacin as a standard. The inhibition of prostaglandin synthesis in microsomal cells was evaluated followed by screening for specific inhibition of cyclooxygenase enzymes (COX-1 and COX-2). None of these compounds showed even moderate anti-inflammatory activity based on, particularly, COX-1 inhibition, nor was significant anti-inflammatory

Table 2
Occurrence of compounds isolated from current *Eucomis* subjects

Cpd / *Class	Current <i>Eucomis</i> investigation	Previous <i>Eucomis</i> investigations	Non- <i>Eucomis</i> occurrence
1 / HIFa	<i>E. comosa</i>	<i>E. comosa</i> (Finckh and Tamm, 1970)	–
2 / HIFa	<i>E. comosa</i> , <i>E. pallidiflora</i> subsp. <i>pole-evansii</i>	<i>E. comosa</i> (Finckh and Tamm, 1970)	–
3 / HIFb	<i>E. comosa</i>	<i>E. comosa</i> (Finckh and Tamm, 1970)	–
4 / HIFb	<i>E. comosa</i>	<i>E. comosa</i> , <i>E. bicolor</i> (Bohler and Tamm, 1967)	–
5 / HIFb	<i>E. comosa</i>	<i>E. montana</i> (Koorbanally et al., 2006) <i>E. bicolor</i> (Bohler and Tamm, 1967; Heller et al., 1976)	–
6 / SN	<i>E. pallidiflora</i> subsp. <i>pole-evansii</i> , <i>E. schiffii</i>	<i>E. comosa</i> , <i>E. pallidiflora</i> subsp. <i>pole-evansii</i> , <i>E. bicolor</i> , <i>E. autumnalis</i> (Ziegler and Tamm, 1976)	<i>L. ovatifolia</i> (Langlois, 2003)
7 / HIFb	<i>E. pallidiflora</i> subsp. <i>pole-evansii</i>	–	–
8 / HIFc	<i>E. schiffii</i>	–	<i>Muscari neglectum</i> (Barone et al., 1988) <i>Scilla scilloides</i> (Kawasaki et al., 1973)
9 / SN	<i>E. schiffii</i>	<i>E. montana</i> (Koorbanally et al., 2006)	<i>Ledebouria ovatifolia</i> (Langlois, 2003) <i>Mervilla plumbea</i> (Moodley et al., 2004) <i>Muscari comosum</i> (Parrilli et al., 1980; Adinolfi et al., 1984) <i>Scilla zebrina</i> (Mulholland et al., 2006)

*Class: HIF = homoisoflavanone; HIFa = 3-benzyl-4-chromanone type; HIFb = benzylidene type; HIFc = scillascillin type; SN = spirocyclic nortriterpenoid.

activity observed when inhibition of prostaglandin synthesis in microsomal cells was considered. By contrast, earlier work (Taylor and Van Staden, 2002) demonstrated significant inhibition of COX-1 and COX-2 enzymes by extracts of the bulbs of *E. pallidiflora* subsp. *pole-evansii* and *E. comosa*. Accordingly, data obtained from the compound-specific investigations of Du Toit et al. (2005) failed to confirm homoisoflavonoid presence as the basis for the ethno-selection of *Eucomis* to treat a variety of pain and inflammation-related ailments. Based on the earlier findings of Della Loggia et al. (1989), positive anti-inflammatory activity had been anticipated. However, not all isolates from the three *Eucomis* species (Table 2) were screened by Du Toit and co-workers (based on structure-related criteria), in which case other homoisoflavonones with significant anti-inflammatory activity could still be responsible. In particular, compounds 1, 3, 4 and 5, all homoisoflavonones from the anti-rheumatic *E. comosa* should be considered for further evaluation.

Many of the thirty-nine *Eucomis* constituents so far isolated have been found in other genera of the Hyacinthoideae (Pohl et al., 2000) and several compounds are common to more than one *Eucomis* taxon (Table 2). Of the nine compounds isolated in the current investigation, seven (compounds 1–7) have only been found within the genus *Eucomis*. The remaining two (compounds 8 and 9) are not known to occur beyond the Hyacinthoideae of the Hyacinthaceae (Table 2) and so are not of taxonomic value beyond the chemo-circumscription of the subfamily. The present study served to confirm the findings of Bohler and Tamm (1967) and Finckh and Tamm (1970) for isolates from *E. comosa*, a number of which (compounds 1 and 3) appear restricted to this species. The earlier isolation of compound 6 (eucosterol) from *E. pallidiflora* subsp. *pole-evansii* by Ziegler and Tamm (1976) was also confirmed. Eucosterol is restricted to the genus *Eucomis*: of the six species characterised phytochemically to date only one (*E. montana*) has not yielded this isolate. From *E. pallidiflora* subsp. *pole-evansii*, the novel compound 7 may presently be considered a marker at the subspecies level; species level indicators were not found for the previously unworked taxon *E. schijffii* which yielded three known isolates. Earlier work undertaken by Tamm's group made use of plant materials sourced through a commercial nursery in Holland. Accordingly it is not possible to interpret differences between historical and current findings within the context of geographic or seasonal variation.

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