PERGAMON

Phytochemistry 51 (1999) 943–946

PHYTOCHEMISTRY

Homoisoflavanones from three South African *Scilla* species

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Received 10 December 1998; received in revised form 3 March 1999; accepted 3 March 1999

Abstract

The bulbs of three South African *Scilla* species have been investigated. *Scilla kraussii* yielded 5,7-dihydroxy-3-(4-hydroxy-4-methoxybenzyl)chroman-4-one, *Scilla dracomontana* yielded the novel 5,7-dihydroxy-6-methoxy-3-(3-methoxybenzyl)chroman-4-one and also eucomol and 5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl)chroman-4-one. *Scilla natalensis* again yielded 5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl)chroman-4-one and 5,7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one.

Keywords: *Scilla natalensis*; *S. dracomontana*; *S. kraussii*; Hyacinthaceae; Homoisoflavanones; 5,7-dihydroxy-3-(4-hydroxy-4-methoxybenzyl)chroman-4-one; 5,7-dihydroxy-6-methoxy-3-(3-methoxybenzyl)chroman-4-one; Eucomol; 5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl)chroman-4-one; 5,7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl) chroman-4-one

1. Introduction

In South Africa the essentially Eurasian genus *Scilla* L. (Hyacinthaceae) is represented by at least six species, including *Scilla natalensis* Planch., *Scilla kraussii* Bak. and *Scilla dracomontana* Hilliard and Burtt. The polymorphic character of *S. natalensis* has resulted in some authors refusing to uphold the taxonomic status of *S. kraussii* and *S. dracomontana* (Jessop, 1970; Reid, 1993) although recent reports afford them full recognition (Pooley, 1998). Previous studies on representatives of *Scilla* have shown them to contain triterpenoid (Mimaki et al., 1993) and cardiac (Kamano & Petit, 1974) glycosides as well as homoisoflavanones (Kuono, Komori & Kawasaki, 1973; Heller & Tamm, 1981; Bangani, Crouch & Mulholland, 1999) and stilbenoids (Bangani, Crouch & Mulholland, 1999). The non-polar fractions of three South African species have been investigated in this work. *Scilla* species are widely used as medicinal plants in South Africa. Traditional use of *S. natalensis* has been widely documented: it is used by the Zulu as a purgative (Gerstner, 1939) and to facilitate labour at term (Gerstner, 1941) although the plant has been reported as toxic to sheep (Kellermann, Coetzer & Naude, 1988). The Sotho eat cooked bulbs as an aperient, use bulb decoctions in enemas for the treatment of internal tumours, and treat lung sickness in cattle (Jacot Guillarmod, 1971). The powdered bulbs are rubbed over sprains and fractures by the Southern Sotho and the Tswana rub powdered bulbs into the back, joints and other body parts with the belief that it makes them strong and resilient to witchcraft (Watt & Breyer-Brandwijk, 1962). Plant material was collected and identified by N.C. and voucher specimens lodged at the Natal Herbarium. Two samples of *S. natalensis* were obtained, the first specimen (KZN) purchased from the Warwick Triangle herbal market in Durban, KwaZulu-Natal. A second collection (MPL) was made in Lydenburg, Mpumalanga. *S. dracomontana* was col-

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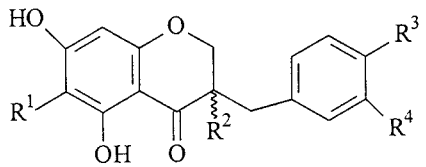
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00154-5

lected near Underberg in the Drakensberg mountains and *S. kraussii* near Port Edward on the KwaZulu-Natal south coast. Five homoisoflavanones, one of which has not been described previously, were isolated. Structural elucidation was performed using NMR, UV (including use of shift reagents) and MS techniques. Homoisoflavanones have been shown to have anti-inflammatory (Della Loggia, Del Negro, Tubaro, Barone & Parrilli, 1989), anti-allergenic, anti-histaminic, anti-mutagenic and angioprotective properties as well as being potent phosphodiesterase inhibitors (Amschler et al., 1996). The isolation of homoisoflavanones validates the rational ethnomedical use of *Scilla natalensis,* particularly with respect to the treatment of strains (Felhaber, 1997), sprains, fractures (Watt & Breyer-Bradwijk, 1962) and cancers (Jacot Guillarmod, 1971).

2. Results and discussion

All three species investigated contained homoisoflavanones. Three compounds were isolated from *S. dracomontana*.



Cpd	R^1	R^2	R^3	R^4
1	OCH_3	H	OCH_3	H
2	OCH_3	H	OH	H
3	H	OH	OCH_3	H
4	H	H	OCH_3	OH
5	OCH_3	H	OCH_3	OH

The HRMS spectrum of compound **1** indicated a molecular formula of $C_{18}H_{18}O_6$. The COSY spectrum indicated the compound was a homoisoflavanone with resonances ascribable to the two protons at C-2 occurring at δ4.10 and δ4.27, the proton at C-3 occurring as a multiplet at δ2.85 and the two protons at C-9 each occurring as double doublets at δ2.73 and δ3.26. Similar resonances were present in compounds **2** and **3**, indicating these compounds were also homoisoflavanones. A keto group at C-4 was indicated by a resonance at δ200.1 in the ^{13}C NMR spectrum. A singlet, integrating to six protons occurred at δ3.81, indicating two methoxy groups. A pair of doublets, each integrating to two protons at δ7.19 and δ6.90 (J 8.7 Hz) indicated a *para*-disubstituted ring B. A peak at m/z 121 corresponding to a methoxybenzyl/tropylium ion showed that the substituent present at C-4′ was one of the methoxy groups (Heller & Tamm, 1981). This was confirmed by a NOE experiment. Irradiation of the methoxy proton resonance at δ3.81 gave an enhancement of the H-3′, H-5′ doublet at δ6.90. A singlet at δ5.95 indicated that ring A was penta-substituted. The molecular formula indicated two hydroxy groups. Bathochromic shifts with NaOAc (+36 nm) and $AlCl_3$ (+23 nm) indicated hydroxy groups at C-7 and C-5 respectively (Heller & Tamm, 1981). The second methoxy group could be placed at either C-6 or C-8. In 3,9-dihydroeucomnalin, which is a 5,7-dihydroxy-6-methoxy compound, C-6 occurs at δ130.4 and C-8 at δ95.9. In 3,9-dihydropunctatin, which is a 5,7-dihydroxy-8-methoxy compound, C-6 occurs at δ97.2 and C-8 at δ130.1 (Adinolfi, Lanzetta, Laonigro, Parrilli & Breitmaier, 1986). In the ^{13}C NMR spectrum of **1**, C-6 and C-8 occurred at δ131.4 and δ95.8 respectively indicating the same substitution as in 3,9-dihydroeucomnalin. Thus **1** is 5,7-dihydroxy-6-methoxy-3-(4-methoxybenzyl)chroman-4-one. This compound has not been reported previously.

Compound **2** was found to be the related 4′-hydroxy analogue of **1**. This compound has been isolated previously from *Eucomis autumnalis* (Hyacinthaceae) (Sidwell & Tamm, 1970). The ^1H NMR spectra of **3** did not show the 2H-2, H-3, 2H-9 coupled system. Instead it showed a pair of doublets ascribable to the two protons at C-2, and a doublet integrating to two ascribed to the two protons at C-9. The C-3 methine resonance which occurred at about δ49 in the other homoisoflavonoids isolated, occurred as a fully substituted resonance at δ73.5 in **3** indicating a tertiary hydroxy group at C-3. This compound was identified as eucomol which has previously been isolated from *Eucomis bicolor* (Hyacinthaceae) (Böhler & Tamm, 1967).

One homoisoflavanone, 5,7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one), **4**, was isolated from *S. kraussii*. This compound has been reported previously to occur in *Muscari comosum* (Hyacinthaceae) (Adinolfi et al., 1985). Two homoisoflavanones were isolated from *S. natalensis*. The first (from the KZN extract) was found to be **2**, which was also found in *Scilla dracomontana*, and the second (from the MPL extract), **5**, which is the 3′-hydroxy derivative of **1**. Compound **5** has been isolated previously from *Muscari armeniacum* (Hyacinthaceae) (Adinolfi et al., 1987).

3. Experimental

Bulbs of *S. dracomontana* Hilliard and Burtt. (1.2 kg) were collected from Bamboo Mountain, Underberg in the KwaZulu-Natal Drakensburg and a

voucher deposited (*Crouch 754*, NH). *S. kraussii* Bak. (1.3 kg) (*N. Crouch and D. Mulholland 770*, NH) was collected in the Umtamvuna Nature Reserve on the KwaZulu-Natal south coast. Two samples of *S. natalensis* Planch. were obtained. The first batch (1.3 kg)(KZN) (*Bangani and Crouch 1*, NH) were purchased at the Warwick Triangle herbal market in Durban and the second (1.3 kg) (MPL) was collected at Lydenburg, Mpumalanga (*Crouch 753*, NH).

In each case, bulbs were cut into pieces, dried overnight, and shaken in methanol (2.5 L) for 24 h. After removal of methanol, water was added and the crude extract extracted with hexane, ether and ethyl acetate. After CC the hexane extract of *S. dracomontana* yielded **3** (12 mg) and the ether extract yielded **1** (28 mg) and **2** (15 mg). The ether extract of *S. kraussii* yielded **4** (24 mg) and the hexane extracts of the KZN and MPL samples of *S. natalensis* yieded **2** (19 mg) and **5** (23 mg) respectively. Compound **1** has not been reported previously, but compounds **2**–**5** are known and structures were confirmed by comparison with literature data (Adinolfi, Lanzetta, Laonigro, Parrilli & Breitmaier, 1986; Sidwell & Tamm, 1970; Böhler & Tamm, 1967; Adinolfi et al., 1985; Adinolfi et al., 1987). NMR spectra were recorded in CD_3OD.

*3.2. 5,7-dihydroxy-6-methoxy-3-(4-methoxybenzyl) chroman-4-one (**1**)*

Yellow vitreous solid (28 mg). HRMS: M^+ at m/z 330.1100 ($C_{17}H_{18}O_6$ requires 330.1103). EIMS: 330(38), 167(6), 149(6), 121(100). ^1H NMR (300 MHz, CD_3OD) δ 7.19 (2H, *d*, $J=8.7$ Hz, H-2′,6′), 6.90 (2H, *d*, $J=8.7$ Hz, H-3′,5′), 5.95 (*s*, H-8), 4.27, 4.10 (AB of ABX, $J=4.5$, 6.9, 11.4, 2H-2), 3.81(6H, *s*, $2\times OCH_3$), 3.26 (*dd*, $J=9.9$, 13.5 Hz, H-9A), 2.85 (*m*, H-3), 2.73 (*dd*, $J=4.5$, 13.5 Hz, H-9B). ^{13}C NMR (75 MHz, CD_3OD) δ 200.1 s (C-4), 160.7 s (C-2), 160.1 s (C-8a), 160.0 s (C-4′), 156.5 s (C-5), 131.4 s (C-6), 131.4 s (C-1′), 131.2 d (C-2′,6′), 115.1 d (C-3′,5′), 103.0 s (C-4a), 95.8 d (C-8), 70.3 t (C-2), 61.0 q (OCH_3 at C-6), 55.7 (OCH_3 at C-4′), 32.9 t (C-9), (C-3 obscured by solvent peak). IR ν_{max} (NaCl) cm^{-1}: 3375, 2940, 2850, 1655, 1591, 1527, 1464, 1306, 1171. UV λ_{max} nm (log ϵ): 293 (4.58), 312 ($+AlCl_3$), 327 ($+NaOAc$). $[\alpha]_D=-100^\circ$ ($c=0.025$ g/100 ml, CH_3OH).

Acknowledgements

This research was funded by the University of Natal Research Fund and the Foundation for Research Development. N. Crouch gratefully acknowledges the support of the Mazda Wildlife Fund, and the DEAT in their sponsorship of MEDBASE. We thank the Natal and Mpumalanga Parks Boards for permits to collect plant materials. V. Bangani is grateful for bursaries from the Foundation for Research Development and the Mellon Foundation. We thank Mrs A. Hutchings for initial discussions and Dr P. Boshoff for providing mass spectra.

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