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6′-*O*-Coumaroylaloesin from *Aloe castanea* — a taxonomic marker for *Aloe* section *Anguialoe*

Fanie R. van Heerden [a,*], Alvaro M. Viljoen [b], Ben-Erik van Wyk [b]

[a] *Department of Chemistry and Biochemistry, Rand Afrikaans University, PO Box 524, Auckland Park, 2006, South Africa*
[b] *Department of Botany, Rand Afrikaans University, PO Box 524, Auckland Park, 2006, South Africa*

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Abstract

The structure of 6′-*O*-coumaroylaloesin [2-acetonyl-8-(6-*O*-coumaroyl-β-D-glucopyranosyl)-7-hydroxy-5-methylchromone], a mono-ester chromone derivative in which only the 6-position of the glucosyl moiety is esterified, was determined by spectroscopic methods. The compound is a unique chemotaxonomic character restricted to the six species in *Aloe* section *Anguialoe*. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Aloe castanea*; *Anguialoe*; Asphodelaceae; Chromone; 6′-*O*-Coumaroylaloesin; Chemotaxonomy

1. Introduction

A survey of the leaf compounds of 380 species of *Aloe* (Viljoen, 1999) indicates that secondary metabolites are a valuable source of taxonomic information for the genus *Aloe* and are useful at the infrageneric level (Viljoen et al., 1996, 1998, 1999; Viljoen and Van Wyk, 1999). All six species of *Aloe* section *Anguialoe* share a single, unique apomorphy, the sessile flowers. Indeed, Reynolds (1950) believed that this group of aloes is so well defined by the sessile campanulate flowers and the distinct inflorescences that he afforded them sectional status in the taxonomic hierarchy of *Aloe* classification. The species are *A. alooides* (Bolus) van Druten (syn. *A. recurvifolia*), *A. castanea* Schönland, *A. dolomitica* Groenewald, *A. spicata* Linné (*fil.*) (syn. *A. sessiliflora*), *A. vryheidensis* Groenewald and *A. tauri* L.C. Leach. *A. dolomitica* is sometimes considered to fall within the variation described for *A. vryheidensis* (Van Wyk and Smith, 1996). The more recently described *A. tauri,* which has a close affinity with *A. spicata*, was added by Leach (1968) to the original sectional circumscription of Reynolds (1950). In this paper, we report the structure of a novel chromone that is a marker metabolite restricted to the section *Anguialoe*, thus providing chemotaxonomic corroboration for the presumed monophyly of the section, hitherto based on morphological evidence alone. This chromone could not be detected in any of the other 240 *Aloe* species analysed by Viljoen (1999).

2. Results and discussion

Fig. 1 illustrates the HPLC profiles for the six species in *Aloe* section *Anguialoe*. The identities of aloin A (**1**), aloin B (**2**) and aloesin (**3**) were confirmed by HPLC comparison with authentic standards available to us from previous studies. Apart from these compounds, the presence of four unidentified chromones was also observed. Three of these compounds (corresponding to peaks 2, 6 and 7 in Fig. 1) are not always present in all representatives of the section *Anguialoe*, hence only the reliable chemotaxonomic marker that corresponds to peak number 4, was isolated. This compound, which could not be matched on HPLC with any of the known *Aloe* metabolites, was identified as 6′-*O*-coumaroylaloesin (**4**).

The structural elucidation of **4** and **5** were based on spectroscopic evidence, with NMR spectroscopy making the most important contribution. NMR assignments were based on ^{1}H, ^{13}C, COSY, HETCOR and selective ^{1}H,^{1}H-decoupling experiments. In order to assign the ^{1}H NMR spectra, it was necessary to obtain the spectra with both DMSO-d_6 and DMSO-d_6 + D_2O as solvents.

* Corresponding author. Fax.: +27-11-489-2605.
E-mail address: frvh@na.rau.ac.za (F.R. van Heerden).

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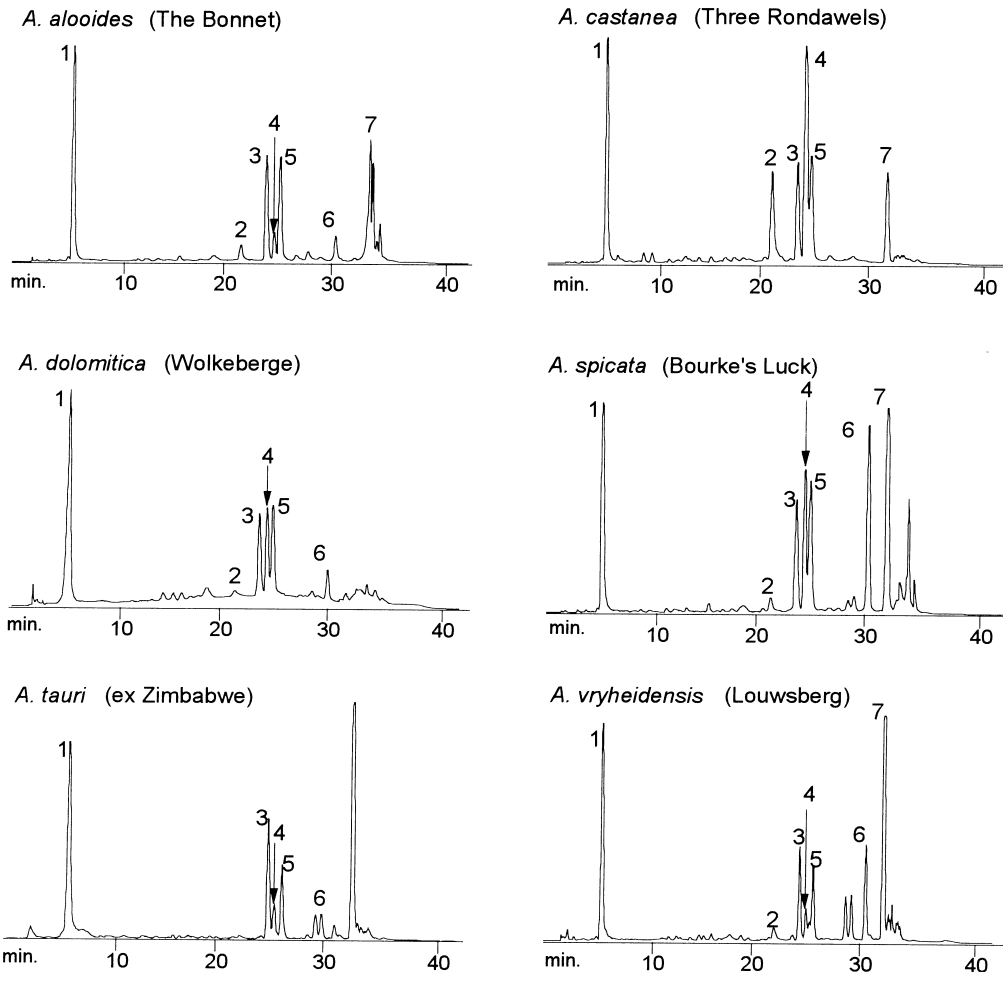
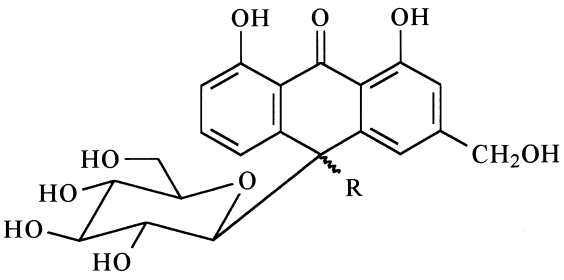


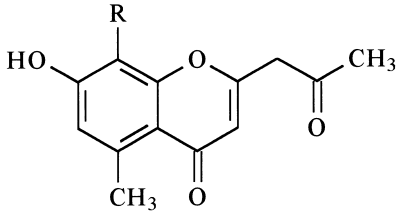
Fig. 1. HPLC chromatogram of the six species on *Aloe* section *Anguialoe*. 1, aloesin; 2, unidentified caffeoyl chromone; 3, aloin B; 4, 6′-*O*-coumaroylaloesin; 5, aloin A; 6 and 7, unidentified cinnamoyl chromones.

The UV spectrum of **4** is characteristic of a coumaroyl ester of the chromone aloesin (**3**), and is almost identical to that of aloeresin A (**5**). The M_r of **4** was determined by FAB-mass spectrometry ($[M+H]^+$ m/z 541). The ^1H and ^{13}C NMR data of the key structural features of **4**, viz., the acetonyl, γ-pyrone, 5-Me, 7-OH and 8-*C*-glucoside were in close agreement with those reported for aloesin (**3**) (Haynes and Holdsworth, 1970) and aloeresin A (**5**) (Makino et al., 1974; Speranza et al., 1985). The absolute configuration of the glucose moiety was not determined, but was assumed as D, as is the case for the other known chromone-*C*-glycosides. The presence of a *p*-coumaroyl ester (δ_H 7.44 and 6.25, $J=15.9$ Hz; δ_C 144.9 and 125.1: *trans*-α,β-unsaturated carbonyl; δ_H 7.39 and 6.73; δ_C 130.3 and 115.7: *p*-substituted phenol) suggested that **4** must be an isomer of aloeresin A (**5**). A careful inspection of the ^1H,^1H-decoupled spectra of **4** with DMSO-d_6/D_2O as solvent, revealed that the doublet and multiplet resonating at δ 4.49 and 4.05, respectively, can be assigned to the two C-6 protons, and from their chemical shifts (compared to those of aloesin), it can be deduced that the coumaroyl moiety is attached to C-6. In most glycosides, the two 6-protons are part of an ABX system, but in the spectrum of **4**, one of the C-6 protons (δ_H 4.41) is observed as a doublet only. This phenomenon can probably attributed to restricted rotation around C5–C6 bond caused by the 6-ester group, and that the carbohydrate has a conformation in which the dihedral angle between one of the C-6 protons and H-5 is close to 90°. Based on this evidence, the structure of **4** was assigned as 6′-*O*-coumaroylaloesin [2-acetonyl-8-(6-*O*-coumaroyl-β-D-glucopyranosyl)-7-hydroxy-5-methylchromone]. This is the structure that was originally assigned to aloeresin A (Wagner, 1970), but has, after the revision of the structure of aloeresin A (**5**) (Makino et al., 1974; Gramatica et al., 1982), not been assigned to a metabolite of any *Aloe* species.

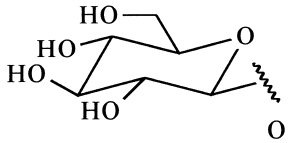
It is of interest to note that in our ^{13}C NMR spectra of both aloesin and 6′-*O*-coumaroylaloesin, the signals of the carbons (C-8, 8a, 1′ and 6′) in close proximity of the C-8–C-1′ bond are observed as broad peaks with low intensities. This observation does imply restriction



1: R= α-H
2: R= β-H



3: R =



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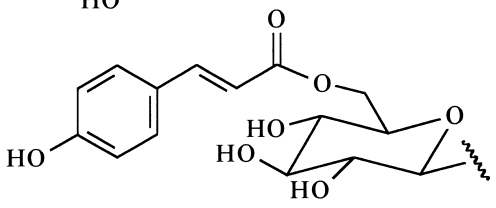
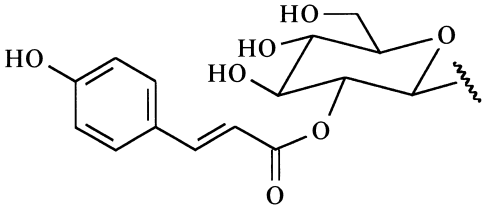


5: R =



of rotation around the C-8–C-1′ bond in aloesin (**3**) and its ester derivatives, an observation that has to our knowledge, not been recorded before.

It is known that esters of carbohydrates are prone to migration under basic conditions, and on isolating different esters of a glycoside, it is important to consider whether the product is not an artefact originating from migration of the ester moiety. In our hands, we could isolate aloeresin A (**5**) under the same experimental conditions decribed here for anthrone **1**, which suggests that **4** is not a rearrangement product of **5**, a metabolite that has been isolated from several *Aloe* species. Furthermore, in a recent communication Park et al. (1997) reported the intramolecular acyl transfer from the 7-hydroxyl group of an aloesin derivative to the 2′-hydroxyl group of the glucose moiety. In their experiments, using either Et_3N or DMAP as catalyst, they isolated only the 2′-acyl derivatives and do not report the formation of any other glucosyl esters. These results suggest that the migration of an acyl group from *O*-2 to any of the other hydroxyl groups of the carbohydrate moiety of an aloesin derivative, is not a facile process. Therefore, we conclude that **4**, to which the structure 6′-*O*-coumaroylaloesin is assigned, does occur naturally in *A. castanea* and is not an artefact formed by rearrangement of aloeresin A (**5**).

3. Experimental

NMR spectra were recorded in DMSO-d_6 using TMS as internal standard at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts are reported in δ units and coupling constants (J) in Hz. Analytical HPLC analysis was performed on a C_{18} column (5 μ, 4.5×250 mm, flow 1 mL min^{-1}) with the following gradient system: 30% MeOH in H_2O (1 min), 30–60% MeOH in H_2O (25 min) and 60–100% MeOH (2 min). Peaks were detected with a photodiode array detector. Analytical TLC was carried out on Merck Silica 60 glass plates using the solvent system EtOAc–MeOH–H_2O (100:16.6:13.5). Flash chromatography was performed on Merck Silica gel 60 (40–63 μM).

3.1. Isolation of metabolites

Aloe castanea Schönland was collected at the 'Three Rondawel viewsite' in Mpumalanga, South Africa. The exudate of the leaves (1.1 kg) was collected over a period of 24 h at room temp and suspended in methanol. After filtration and evapn. of the solvent, a yellow residue (15 g) was obtained. Analytical HPLC analysis confirmed the presence of aloesin (**3**, R_t 5.62 min, rel. yield 22%), an unidentified chromone (R_t 21.31 min, 10%) aloin B (**2**, R_t 23.71 min, 11%), **4** (R_t 24.42 min, 27%) aloin A (**1**, R_t 24.99 min, 13%) and an unidentified chromone (R_t 32.12 min, 10%). Aloesin (**3**), aloin A (**1**) and B (**2**) were identified by HPLC comparison with authentic standards available to us from previous studies. A portion of the exudate (5.8 g) was subjected to flash column chromatography using solvent system $CHCl_3$–MeOH (8:2) to yield 6′-*O*-coumaroylaloesin (**4**) (236 mg) and aloesin (280 mg). **4** was isolated as a light-yellow amorphous solid (236 mg). $[\alpha]_D^{25}$ −50.6° (*c* 0.8 in EtOH). FABMS: *m/z* 541 $[M+H]^+$; EIMS *m/z* (rel. int):358 (7), 340 (7), 304 (17), 245 (65), 203 (16), 164 (100), 147 (48), 120 (21), 91 (28), 89 (11), 77 (13), 65 (19), 63 (10), 43 (76). λ_{max}^{MeOH} nm: 211, 225, 248, 260. ^{1}H NMR (DMSO-d_6): δ 10.60 (1H, *s*, OH), 10.00 (1H, *s*, OH), 7.53 (1H, *m*, H-3″, 5″, 9″), 6.76 (2H, *d*, J=8.4 Hz, H-6″, 8″), 6.68 (1H, *s*, H-6), 6.41 (*d*, J=15.9 Hz, H-2″), 6.10 (*s*, H-3), 5.21 (1H, *br.s*, OH), 5.00 (1H, *br.s*, OH), 4.74 (2H, *br.s*, OH, H-1′),

4.49 (1H, *d*, $J=11.4$ Hz, H-6′a), 4.25–3.00 (7H, *m*, H-2′, 3′, 4′, 5′, 6′b, 9), 2.63 (3H, *s*, H-12), 2.19 (3H, *s*, H-11); δ (DMSO-d_6 + D_2O) 7.44 (1H, *d*, $J=15.9$ Hz, H-3″), 7.39 (2H, *d*, $J=8.7$ Hz, H-5″, 9″), 6.73 (2H, d $J=8.7$ Hz, H-6″,8″), 6.58 (1H, *s*, 6-H), 6.26 (1H, *d*, *J* 15.9 Hz, H-2″), 6.06 (1H, *s*, H-3), 4.71 (1H, *d*, $J=9.9$ Hz, H-1′), 4.41 (1H, *d*, $J=11.7$ Hz, H-6′a), 4.05 (3H, *m*, H-6′b, H-9), 3.86 (1H, *t*, H-2′), 3.48 (1H, *m*, H-5′), 3.30 (1H, *m*, H-3′), 3.21 (1H, *m*, H-4′), 2.55 (3H, *s*, H-12), 2.14 (3H, *s*, H-11). ^{13}C NMR (DMSO-d_6): δ 202.3 (C-10), 178.4 (C-4), 166.7 (C-1″), 160.1 (C-2), 159.7 (C-7″), 159.4 (C-7), 157.9 (C-8a), 144.9 (C-3″), 140.4 (C-5), 130.3 (C-5″,9″), 125.0 (C-4″), 115.7 (C-6, 6″,8″), 114.8 (C-4a), 114.0 (C-2″), 112.5 (C-3), 110.7 (C-8), 78.5 (C-3′ or 5′), 78.4 (C-3′ or 5′), 73.3 (C-1′), 70.8 (C-2′) 70.4 (C-4′), 64.8 (C-6′), 47.8 (C-9), 29.6 (C-11), 22.6 (C-12).

Aloesin (**3**) had EIMS *m*/*z* (rel. int.): 394 [M^+] (5), 376 (6), 304 (29), 274 (14), 245 (100), 219 (10), 203 (27), 163 (27), 91 (20), 77 (15), 69 (10), 67 (10), 60 (14), 57 (17), 55 (18), 43 (97). λ_{max}^{MeOH} nm: 215, 244, 263, 295. ^{1}H NMR (DMSO-d_6): δ 10.50 (1H, *br.s*, OH), 6.66 (1H, *s*, H-6), 6.09 (1H, *s*, H-3), 4.91 (1H, *br.s*, OH), 4.69 (1H, *d*, $J=9.0$, H-1′), 4.39 (1H, *br.s*, OH), 3.76 (2H, *s*, H-9), 3.90–3.00 (m, H-2′, 3′, 4′, 5′, 6′), 2.63 (3H, *s*, H-12), 2.21 (3H, *s*, H-11). ^{13}C NMR (DMSO-d_6): δ 202.4 (C-10), 178.5 (C-4), 160.2 (C-2), 159.5 (C-7), 157.8 (C-8a), 140.2 (C-5), 116.4 (C-6), 114.7 (C-4a), 112.4 (C-3), 111.0 (C-8), 81.5 (C-5′), 78.6 (C-3′), 73.5 (C-1′), 71.1 (C-2′), 70.4 (C-4′), 61.4 (C-6′), 47.6 (C-9), 29.8 (C-11), 22.5 (C-12).

Acknowledgements

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