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FOUR IRIDOID GLUCOSIDES AND A PHENYLPROPANOID GLYCOSIDE FROM *SESAMUM ANGOLENSE*

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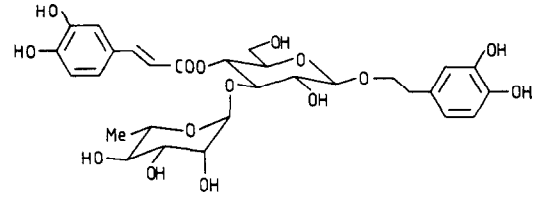
IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—*Sesamum angolense*, Pedaliaceae, iridoid glucosides, phenylpropanoid glycosides, verbascoside.

Abstract—A new iridoid glucoside, sesamoside, isolated from *Sesamum angolense* (Pedaliaceae), has been assigned the structure methyl antirrinoside-4-carboxylate. It was accompanied by the known iridoid glucosides phlomiol, pulchelloside-I and 6β-hydroxyipolamiide, and by the phenylpropanoid glycoside verbascoside. The identities of these compounds were deduced by spectroscopic methods. The structure of sesamoside was confirmed by conversion into its pentaacetate and diisopropylidene derivatives.

INTRODUCTION

As part of our studies on plants used in African traditional medicine we have undertaken the phytochemical investigation of *Sesamum angolense* Welw. (Pedaliaceae). This plant, which grows in tropical Africa, is endowed in particular with hemostatic properties and is used in Malawi to curtail bleeding after tooth removal. Very little is known about its constituents; in fact only the lignans from the seeds have been previously studied [1, 2]. In a recent paper [3] we reported the separation and structure elucidation of four new naphthoxirene derivatives from *S. angolense*. Further studies on the methanolic extract of the root bark afforded a new iridoid glucoside, sesamoside (**5**), together with the known compounds phlomiol (**2**), pulchelloside-I (**3**), 6β-hydroxyipolamiide (**4**) and verbascoside (**1**). We describe here the isolation of these compounds and the establishment of the structure of sesamoside as methyl antirrinoside-4-carboxylate.



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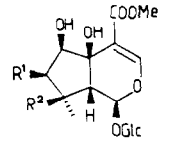
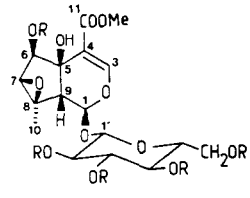


2 R^1 = OH R^2 = OH
3 R^1 = OH R^2 = H
4 R^1 = H R^2 = OH



5 R = H
5a R = Ac

RESULTS AND DISCUSSION

The methanolic extract from the root bark of *S. angolense* was submitted to DCCC [$CHCl_3$–MeOH–*iso*-PrOH–H_2O (5:6:1:4) as solvent system in the ascending mode]. Fifteen fractions (I–XV) were collected. Separation of fraction IV by low-pressure liquid chromatography on RP-8 afforded compounds, **1**, **2** and **3**. Purification of fractions VII and XI by the same technique provided compounds **4** and **5**, respectively. Compound **1** presented the characteristic UV spectrum of a phenylpropanoid glycoside containing caffeate and catechol moieties. It was identified as verbascoside [4, 5] (≡acteoside [6]≡kusaginin [7]) from its ^1H and ^{13}C NMR data [5] and by TLC and HPLC co-chromatography with an authentic sample. Compounds **2**–**5** proved to be four iridoid glucosides differing only in their substitution at C-7 and C-8. Compounds **2**–**4** were shown to have spectroscopic (IR, UV, ^1H and ^{13}C NMR) properties identical to those of phlomiol [8, 9], pulchelloside-I [9, 10] and 6β-hydroxyipolamiide[11] respectively; compound **5** was a new iridoid glucoside.

The MS (D/CI) of **5** showed quasimolecular ions at *m/z* 438 ($[M+NH_4]^+$) and 421 ($[M+H]^+$). The molecular formula was found to be $C_{17}H_{24}O_{12}$. Acid hydrolysis demonstrated the presence of a glucose unit. The UV spectrum of **5** exhibited an intense absorption at 234 nm (log ε, 3.96); the IR spectrum showed bands at 1695 and 1635 cm^{-1}. These data indicated the presence of a conjugated carbonyl function. The ^1H and ^{13}C NMR spectra revealed an iridoid skeleton with a carbomethoxy substituent at C-4 and a methyl group at C-8. The coupling constant of 8.7 Hz observed between H-1 and H-9 (no

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coupling in compounds **2**–**4**), the ^{13}C NMR chemical shift of the C-10 methyl group (17.8 ppm) and the upfield shift (compared with **2**) of C-7 and C-8 were in good agreement with the values published for other iridoid glucosides bearing a β epoxide function at C-7 and C-8 [12–14]. Acetylation of **5** with acetic anhydride/pyridine at room temperature afforded a pentaacetate **5a** which still showed hydroxy absorption in the IR spectrum. In the ^1H NMR spectrum of **5a**, a paramagnetic shift was observed for H-6 (4.32 to 5.35 ppm) whilst H-7 remained practically unchanged. These results are consistent with the presence on the cyclopentane ring of a secondary OH at C-6 and a tertiary OH at C-5.

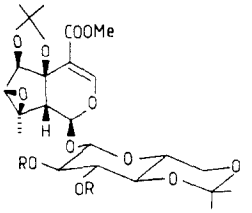
The β configuration of the hydroxy group at C-6, inferred at first from ^{13}C NMR data [13, 14] was confirmed by treatment of **5** with $SnCl_2$ and 2,2-dimethoxy-propane in acetone which afforded the di-*O*-isopropylidene derivative **6**. Acetylation of **6** gave the diacetate **6a**. The absence in **6a** of a free OH group as well as the lack of a downfield shift for the resonance of H-6 (4.86 and 4.84 in **6** and **6a** resp.) proved that an *O*-isopropylidene group is linked to O-5 and O-6 *cis* to each other. The structure of sesamoside is therefore established as methyl antirrinoside-4-carboxylate.

Sesamoside is, to our knowledge, the first example of an iridoid glucoside containing an epoxide function at C-7 and C-8 together with a carbomethoxy group at C-4. In this context, it is interesting to point out the significant decrease of the coupling constant $J_{1,9}$ in going from **5** to its diisopropylidene derivative **6** (8.7 and 1.4 Hz resp.). This effect is not actually mentioned in the derivatisation of other epoxy-iridoids bearing OH groups at C-5 and C-6, for instance antirrinoside [15]. Except verbascoside, which has been recently isolated from *Harpagophytum procumbens* [16], all the compounds described here are found for the first time in the Pedaliaceae. In this plant family, iridoid glucosides were previously known to occur only in the genus *Harpagophytum* [17, 18].

Tests are now in progress to determine if these compounds could be responsible for the hemostatic properties of the plant.

EXPERIMENTAL

General. DCCC: Büchi 670 DCC chromatograph (294 tubes; i.d. 2.7 mm). Prep. LPLC: Lobar RP-8 column (40–63 μm; i.d. 2.5 × 27 cm), equipped with a Duramat-80 pump. TLC: silica gel precoated A1 sheets (Merck) with $CHCl_3$–MeOH–H_2O 13:7:1 (system 1) or *n*-BuOH–AcOH–H_2O 4:1:1 (system 2). Mps: uncorr. ^1H and ^{13}C NMR: at 200 and 50.5 MHz resp.; chemical shifts in δ relative to TMS. D/CIMS: quadrupole instrument with NH_3 as reactant gas; positive ion mode.



6 R = H
6a R = Ac

Plant material. S. angolense was collected near Kasungu (Malawi). A voucher specimen of the plant material is retained at the herbarium, Chancellor College, University of Malawi, Zomba.

Extraction and isolation. The powdered root bark of *S. angolense* (121 g) was extracted at room temp. with CH_2Cl_2 followed by MeOH. A portion (3 × 3.0 g) of the MeOH extract (14.4 g) was separated into 15 fractions by droplet counter-current chromatography (DCCC) with $CHCl_3$–MeOH-*iso*-PrOH–H_2O 5:6:1:4 in the ascending mode. A part (2 × 130 mg) of fraction IV (552 mg) was submitted to LPLC on RP-8; elution with MeOH–H_2O 1:9 afforded **2** (131 mg) and **3** (19 mg); subsequent elution with MeOH–H_2O 8:17 provided **1** (23 mg). Compounds **4** (31 mg) and **5** (50 mg) were obtained by the same technique from fraction VII (67 mg) and from 100 mg of fraction XI (181 mg) resp., using MeOH–H_2O 3:17 as solvent system.

Acid hydrolysis. Compound **5** (2 mg) was refluxed in 2 N HCl for 3.5 hr. The mixture was extracted with Et_2O (2 × 10 ml) and *n*-BuOH (2 × 10 ml). TLC examination of the org. layers revealed complete decomposition of the aglycone. The aq. layer was adjusted to pH 5 with $NaHCO_3$. After evapn, the sugar was extracted from the residue with pyridine and analysed by TLC on silica gel with AcOEt–MeOH–H_2O–AcOH 13:3:3:4; detection with *p*-anisidine phthalate.

Verbascoside (**1**). Brownish, amorphous powder. TLC (silica gel, system 2): R_f 0.64; D/CIMS (NH_3): *m/z* 642 ($[M+NH_4]^+$), 626, 625 ($[M+H]^+$), 496 ($[(M+NH_4)-146]^+$), 480 ($[(M+NH_4)-162]^+$), 463 ($[(M+H)-162]^+$), 334 ($[(M+NH_4)-308]^+$), 326 ($[(M+NH_4)-316]^+$).

Phlomiol (**2**). Amorphous, white powder. TLC (silica gel, system 1): R_f 0.22; D/CIMS (NH_3): *m/z* 456 ($[M+NH_4]^+$), 438 ($[M]^+$), ($[(M+NH_4)-18]^+$), 421 ($[(M+H)-18]^+$), 403 ($[(M+H)-36]^+$), 294 ($[(M+NH_4)-162]^+$), 276 ($[M-162]^+$).

Pulchelloside-I (**3**). Amorphous, white powder. TLC (silica gel, system 1): R_f 0.31; D/CIMS (NH_3): *m/z* 440 ($[M+NH_4]^+$), 423 ($[M+H]^+$), 422 ($[M]^+$), ($[(M+NH_4)-18]^+$), 405 ($[(M+H)-18]^+$), 280, 260 ($[M-162]^+$).

6β-hydroxyipolamiide (**4**). Colourless plates from MeOH–H_2O–Me_2CO, mp 192–194° (lit [11] mp 192–193°). TLC (silica gel, system 1) R_f 0.37; D/CIMS (NH_3): *m/z* 440 ($[M+NH_4]^+$), 423 ($[M+H]^+$), 405 ($[(M+H)-18]^+$), 278 ($[(M+NH_4)-162]^+$), 260 ($[M-162]^+$).

Sesamoside (*methyl antirrinoside-4-carboxylate*, **5**). Amorphous, white powder. TLC (silica gel, system 1) R_f 0.45; D/CIMS (NH_3): *m/z* 438 ($[M+NH_4]^+$), 421 ($[M+H]^+$), 403 ($[(M+H)-18]^+$), 276 ($[(M+NH_4)-162]^+$), 258 ($[M-162]^+$); UV λ_{max}^{MeOH} nm (log ε): 234 (3.96); IR ν_{max}^{KBr} cm^{-1}: 3400, 1695 (C=O conjugated), 1635 (C=C conjugated), 1440, 1300 (C–O ester), 1075, 1045; ^1H NMR (CD_3OD): δ 7.58 (1H, *s*, H-3), 5.50 (1H, *d* $J_{1,9}$ = 8.7 Hz, H-1), 4.72 (1H, *d*, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.32 (1H, *d*, $J_{6,7}$ = 1.4 Hz, H-6), 3.75 (3H, *s*, COOMe-11), 3.46 (1H, *d*, H-7), 2.52 (1H, *d*, $J_{1,9}$ = 8.7 Hz, H-9), 1.51 (3H, *s*, 3H-10); ^{13}C NMR: see Table 1.

Sesamoside pentaacetate (**5a**). Treatment of **5** (20 mg) with Ac_2O (0.5 ml) in dry pyridine (1.5 ml) overnight at room temp. afforded after the usual work-up 20.4 mg of **5a**. Colourless prisms from CH_2Cl_2–Et_2O *n*-hexane, mp 151–153°; D/CIMS (NH_3): *m/z* 648 ($[M+NH_4]^+$), 631 ($[M+H]^+$), 613 ($[(M+H)-18]^+$), 606 ($[(M+NH_4)-42]^+$), 571 ($[(M+H)-60]^+$), 331; IR ν_{max}^{KBr} cm^{-1}: 3500, 1750, 1635; ^1H NMR ($CDCl_3$): δ 7.41 (1H, *s*, H-3), 5.35 (1H, *d*, $J_{6,7}$ = 1.8 Hz, H-6), 5.3–4.9 (5H, *m*, H-1, H-1', H-2', H-3', H-4'), 4.22 (2H, *d*, $J_{5',6'}$ = 3.2 Hz, 2H-6'), 3.72 (1H, *dt*, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'}$ = 3.2 Hz, H-5'), 3.68 (3H, *s* COOMe-11), 3.44 (1H, *d*, H-7), 2.58 (1H, *d*, $J_{1,9}$ = 8.2 Hz, H-9), 2.19, 2.08, 2.03, 2.01, 2.00 (together 15 H, 5*s*, COMe), 1.48 (3H, *s*, 3H-10); ^{13}C NMR: see Table 1.

Table 1. ^{13}C NMR data of sesamoside (**5**) (CD_3OD) and sesamoside pentaacetate (**5a**) ($CDCl_3$)

C	5*	5a
1	96.75	96.03[a]
3	155.37	152.25
4	112.95	111.41
5	74.92	73.41
6	77.50	75.89
7	65.93	62.51
8	63.75	61.73
9	54.34	51.49
10	17.85	17.15
11	168.95	165.91
OMe	52.28	51.70
1′	99.86	94.75[a]
2′	74.64	70.56
3′	77.66[a]	72.31[b]
4′	71.72	67.96
5′	78.67[a]	72.18[b]
6′	63.01	61.31
COOMe		170.69, 170.40, 170.03, 169.24 (2x)
COOMe		21.11, 20.78, 20.63 (2x), 20.58

Values with the same superscript in each column are interchangeable.

DEPT sequences allowed distinction of carbon multiplicities.

*C–H connectivities by the use of HETCOR experiments.

5,6-4′,6′-*Bis*-O-*isopropylidene-sesamoside* (**6**). Compound **5** (5 mg) and $SnCl_2$ (10 mg) were dissolved in Me_2CO. A small amount of 2,2-dimethoxy-propane (2 × 5 drops) was added after 30 min and 3 hr. After stirring for 4 hr at room temp., the solution was treated with aq. satd $NaHCO_3$. The mixture was filtered, evapd to dryness and the residue passed through a small silica gel column (i.d. 5 × 40 mm) with $CHCl_3$–MeOH 17:3. Purification of the crude product (5.6 mg) by HPLC on a Novapak C-18 column (4 μm, i.d. 3.9 × 150 mm) with MeOH–H_2O 4:6 (1 ml/min) provided pure **6** (2.7 mg) as a white, amorphous powder. D/CIMS (NH_3): *m/z* 518 ($[M+NH_4]^+$), 501 ($[M+H]^+$), 485, 460, 443, 316 ($[(M+NH_4)-202]^+$), 299 ($[(M+H)-202]^+$), 220 ($[Glc+40]^+$); ^1H NMR ($CDCl_3$): δ7.63 (1H, *s*, H-3), 5.65 (1H, *d*, $J_{1,9}$ = 1.4 Hz, H-1), 4.86 (1H, *d*, $J_{6,7}$ = 2.8 Hz, H-6), 4.61 (1H, *d*, $J_{1',2'}$ = 7.8 Hz, H-1′); 3.74 (3H, *s*, COOMe-11), 3.30 (1H, *d*, $J_{6,7}$ = 2.8 Hz, H-7), 2.80 (1H, *d*, H-9), 1.56, 1.53, 1.52, 1.45, 1.43 (together 15H, 5 *s*, 5 Me).

Diacetate of **6** (**6a**). Compound **6** (2 mg) was treated with Ac_2O (0.1 ml) in dry pyridine (0.1 ml) for 4 hr at room temp. Purification of the crude product (1.6 mg), as described for **6**, with MeOH–H_2O 1:1, afforded 0.8 mg of pure **6a** as a white amorphous powder. D/CIMS (NH_3): *m/z* 602 ($[M+NH_4]^+$), 287, 202; ^1H NMR ($CDCl_3$): δ7.56 (1H, *s*, H-3), 5.59 (1H, *d*, $J_{1,9}$ = 1.2 Hz, H-1), 4.84 (1H, *s*, $J_{6,7}$ = 2.8 Hz, H-6), 5.2–4.7 (3H, *m*, H-1′, H-2′, H-3′), 3.99 (1H, *dd*, $J_{5',6'eq}$ = 5.3, $J_{6'ax,6'eq}$ = 10.0 Hz, H-6′eq), 3.9–3.6 (2H, *m*, H-6′ax, H-4′), 3.72 (3H, *s*, COOMe-11), 3.4 (1H, *m*, H-5′), 3.28 (1H, *d*, $J_{6,7}$ = 2.8 Hz, H-7), 2.73 (1H, *d*, H-9), 2.03, 1.91 (together 6H, 2*s*, COMe), 1.51, 1.46 (2 ×), 1.40, 1.39 (together 15H, 4*s*, 5Me).

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