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SU3, an oxocycloartane diglucoside from *Sutherlandia humilis*

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ABSTRACT

The structure of a new cycloartane-type triterpene glycoside was determined as 24,25-*O*-β-D-diglucopyranosyl-6α-hydroxycycloart-3-one (SU3) by spectroscopic methods. This is the first cycloartane diglycoside reported from the genus *Sutherlandia* (an important South African traditional medicine and general tonic known as cancer bush). It was isolated from a dwarf form of *S. frutescens*, currently known as *Sutherlandia humilis*.

1. Introduction

Sutherlandia humilis E.Phillips & R.A.Dyer is one of six closely related species of the genus *Sutherlandia* R.Br. ex W.T.Aiton (family Fabaceae) that is endemic to southern Africa and is commonly known as cancer bush (Phillips and Dyer, 1934; Germishuizen and Meyer, 2003). Cancer bush is traditionally used for a myriad of indications, ranging from poor appetite to the prevention and treatment of cancer. The taxonomy, ethnobotany, chemistry and pharmacological properties of cancer bush was recently thoroughly revised and summarized by Van Wyk and Albrecht (2008). There are six species of cancer bush which will soon be reduced to two (Moshe, 1998) and *S. humilis* is often considered to merely represent a dwarf form of the variable *S. frutescens* (L.) R.Br. Users of cancer bush often do not differentiate between species.

Free amino acids, the cyclitol pinitol and flavonoid glycosides have been detected in commercially used *S. microphylla* Burch. ex DC (Van Wyk and Albrecht, 2008). We have previously isolated (Gabrielse, 1996) and later identified two major cycloartane glycosides from this taxon which we called SU1 (Fig. 1) and SU2 (Olivier, Van Heerden, Van Wyk and Albrecht, unpublished work). Whilst Van Wyk and Albrecht (2008) did not give the exact stereochemistry of the hydroxyl groups on C-7 and C-24 of SU1, Olivier clarified this along with the structure of SU2, and found it to be the same as the structures recently published as Sutherlandiosides B and A, respectively (Fu et al., 2008). Herewith, we report on the isolation and characterization of a novel triterpenoid diglucoside, SU3, from *S. humilis* (Fig. 1).

2. Results and discussion

TLC analysis of the methanolic leaf extract of *S. humilis* revealed the presence of triterpenoids SU1 (**1**) and SU2, also known as Sutherlandiosides B and A, respectively (Fu et al., 2008). These two compounds seem to be present in variable concentrations in many, but not all, *Sutherlandia* samples, as was evident from a TLC and HPLC variation study on methanol extracts of a large number of geographically representative provenances of all the species. However, the major compound isolated from *S. humilis* was a triterpenoid of higher polarity, called SU3 (**2**).

The ESI-TOF-MS spectrum of SU3 (**2**) exhibited a $[M + Na]^+$ peak at *m*/*z* 821.4665, corresponding to a molecular formulae of $C_{42}H_{70}O_{14}$. The NMR data gave evidence for the presence of two glucopyranosyloxy moieties and a triterpene aglycone containing six tertiary methyls, one secondary methyls, a ketone, two secondary and one tertiary oxygen-bearing carbons and two methylene protons (δ_H 0.68, 0.49 J = 3.9 Hz) characteristic of the 19-CH_2 of a cycloartane. Correlations observed between the latter two protons and neighbouring carbons in a CIGAR2J3J experiment enabled us to assign C-1, 5, 8, 9, 10 and 11 in the ^{13}C NMR spectrum. The methyl protons assigned to C-28 and C-29 showed long range ^{1}H, ^{13}C coupling to each other, C-5 and the ketone carbon, thereby establishing a 3-oxo functionality. Correlations observed for H-5 (δ_H 2.08, d, J = 9.9 Hz) confirmed the assignments of C-1, 4, 10, 19,

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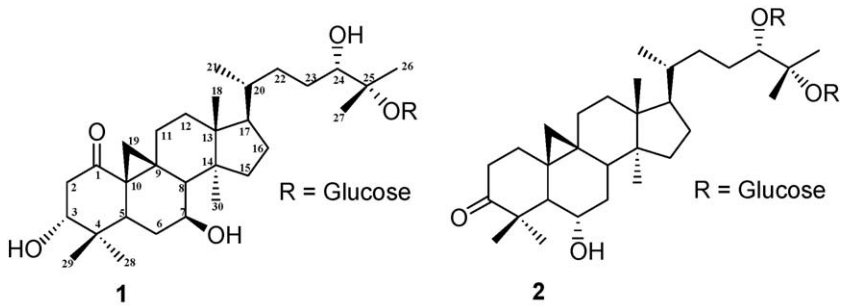

Fig. 1. Chemical structures of compounds **1** and **2**.

28 and 29 and the presence of a hydroxyl function on C-6. The assignment of the 6_{eq}-OH is corroborated by correlations observed in the COSY spectrum and the coupling pattern observed for H-6. The NMR assignments of the A and B rings of SU3 (**2**) are in good agreement with literature data for 3-oxocycloartanes (Inanda et al., 1995) and 6α-hydroxycycloartanes (Radwan et al., 2004), respectively.

The C-17 side chain of SU3 (**2**) is closely related to that of SU1 (**1**) (Fu et al., 2008), the difference being that in **2** both the hydroxyls at C-24 and C-25 are glucosylated. HMBC correlations between the

Table 1
NMR spectroscopic data (300 MHz, methanol-d4) for SU1 (**1**) and SU3 (**2**).

Position	SU1 (**1**)		SU3 (**2**)			
	δ_C, mult.	δ_H (J = Hz)	δ_C, mult.	δ_H (J = Hz)	COSY[a]	CIGAR[a]
1	213.0, qC		32.7	1.42, m	1b	5, 19a, 19b
				2.14, m	1a	
2	48.7, CH_2	a: 3.01 dd (14.3, 4.5)	36.4	2.44 td (14.4, 12.0)	2b	
		b: 2.41 dd (14.3, 4.5)		2.72 td (14.1, 11.1)	2a	
3	78.8, CH	3.75 t (4.8)	220.0			2a, 2b, 28, 29
4	40.1, qC		51.5			5, 28, 29
5	38.6, CH	2.48 dd (14.0, 3.6)	54.3	2.08 d (9.9)	6	19a, 19b, 28, 29
6	30.8, CH_2	1.90 m, 1.28 m	70.7	3.48 td (9.9, 3.9)	5, 7a, 7b	5, 7
7	69.3, CH	3.75 m	38.3	1.49 m		6
8	51.2, CH	2.12 d (3.6)	49.6	3.36 m		7, 18, 19a, 19b
9	30.6, qC		22.3			7, 19a, 19b
10	40.4, qC		29.3			2a, 2b, 5, 7, 19a, 19b
11	29.0, CH_2	1.88 m	27.1	1.13 m	12	19a, 19b
12	33.8, CH_2	1.67 m, 1.53 d (3.0)	34.0	1.72 br d (3.6)	11	18
13	46.4, qC		46.4			18, 30
14	50.5, qC		49.6			18, 30
15	34.4, CH_2	1.39 td (10.5, 2.1)	36.9	1.42 m	16 a, 16b	30
16	28.7, CH_2	1.90 m	29.1	a: 1.43 m	15	
				b: 2.08 br d (9.9)	15	
17	53.0, CH	1.68 td (9.3, 2.2)	53.9	1.66 d (3.9)	16a, 16b	18, 21
18	15.6, CH_3	1.01 s	20.0	1.05 s		
19	24.6, CH_2	a: 0.78 d (4.5)	31.5	a: 0.49 d (3.9)		5
		b: 1.47 d (4.5)		b: 0.68 d (3.9)		
20	38.0, CH	1.48 m	37.6	1.45 t (5.7)	17, 21, 22a, 22b	21
21	19.3, CH_3	0.96 d (6.3)	18.9	0.94 d (6.3)	20	
22	35.1, CH_2	1.90 m	34.3	1.74 m	20	
23	29.3, CH_2	1.68 m	29.1	1.37 m	24	
24	79.5, CH	3.36 br d (9.3)	87.5	3.63 dd (9.0, 1.5)	23	26, 27, 1″
25	81.9, qC		83.4			26, 27, 1″
26	23.8, CH_3	1.29 s	24.8	1.30 s		27
27	21.3, CH_3	1.26 s	20.0	1.35 s		26
28	24.8, CH_3	1.04 s	28.6	1.37 s		29
29	21.7, CH_3	0.97 s	20.4	1.24 s		5, 28
30	18.9, CH_3	1.01 s	18.9	1.06 s		
1′	98.6, CH	4.56 d (7.8)	98.3	4.57 d (7.8)	2′	2′
2′	75.1, CH	3.21 br t (7.8)	75.7	3.23 t (9.3)	1′, 3′	3′
3′	78.1, CH	3.35 t (8.6)	77.8	3.35 m	4′	2′
4′	71.6, CH	3.38 m	71.8	3.32 m	3′, 5′	3′, 5′
5′	77.7, CH	3.32 m	77.7	3.31 m	4′, 6′a, 6′b	1′, 4′, 6′a, 6′b
6′	62.6, CH_2	a: 3.69 dd (11.9, 5.1)	62.8	a: 3.68 dd (4.8, 3.3)	5′	
		b: 3.87 dd (11.7, 2.1)		b: 3.88 br d (10.5)	5′	
1″			104.7, CH	4.81 d (7.8)	2″	2″
2″			75.2, CH	3.23 t (9.3)	1″, 3″	3″
3″			78.5, CH	3.51 dd (8.1, 3.9)	4″	2″
4″			72.0, CH	3.32 m	3″, 5″	3″, 5″
5″			77.7, CH	3.31 m	4″, 6″a, 6″b	1″, 4″, 6″a, 6″b
6″			63.3, CH_2	a: 3.72 dd (4.8, 3.3)	5″	
				b: 3.91 br d (9.6)	5″	

[a] COSY and CIGAR correlations are from proton(s) stated to the indicated carbon.

anomeric protons of the β-D-glucopyranose units with C-24 and C-25 confirmed the position of these sugars. The anomeric ^{1}H NMR doublet of the C-24 glucosyl moiety is substantially broadened, most likely because of restricted rotation around the sterically hindered C-24 glycosidic bond. Therefore, the structure of SU3 was established as shown in **2.**

SU3 (**2**) is similar in structure to those of SU1 and SU2 (the Sutherlandiosides B and A previously isolated from *S. frutescens*) (Fu et al., 2008) and also to various triterpenoids isolated from *Astragalus* species (Bedir et al., 1998, 2000; Özipek et al., 2005). These triterpenoids are responsible for the bitter taste and the *amarum* effect (Van Wyk and Wink, 2004) of *Sutherlandia* and may also provide a rationale for the numerous other medicinal uses that have been recorded. It was also reported that cycloartanes with hydroxylation at C-24 and a 3-oxo group (the latter is true for SU3) exhibit a powerful inhibitory ability toward skin carcinogenesis (Kikuchi et al., 2007). Further studies on the triterpenoid variation and biological activity of *Sutherlandia* triterpenoids will undoubtedly yield interesting results.

3. Experimental section

3.1. General experimental procedures

ESI-MS measurements were obtained on a Waters API Q-TOF Ultima instrument in positive ionization mode. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^{1}H NMR and ^{13}C NMR experiments were performed on a Varian Unity Inova 300. C18 reverse phase gel (Chromabond, Machery Nagel) and silica gel (Kieselgel GF_{254} 15 μm, Merck) were used for column chromatography and silica gel 60 F_{254} on aluminum sheets (Merck) for thin-layer chromatography (TLC). The solvent system used for TLC was chloroform:methanol:water:acetic acid (60:30:8:6). Plates were sprayed with 5% ethanolic sulfuric acid followed by 1% ethanolic vanillin and baked for 5 min at 100 °C.

3.2. Plant material

The plant material was collected near Barrydale, South Africa in September 1996 and identified by B.-E. van Wyk. A voucher specimen (Palmer 17a) was deposited in the Herbarium, Department of Botany and Plant Biotechnology at the University of Johannesburg (JRAU).

3.3. Extraction and isolation

SU3 (**2**) was isolated from a methanolic extract obtained by stirring 2.8 g of ground dried leaf material in 30 mL of methanol for 18 h. After filtration, the extract was dried under reduced pressure at 40 °C to yield 36.4 mg of extract. This extract was subjected to flash column chromatography and eluted with a gradient system of chloroform:methanol:ethyl acetate (65:20:15 and 40:50:10). The fraction containing **2** was subjected to further flash column chromatography, this time using chloroform:methanol:water (60:20:2.5) as solvent system, to yield 2.7 mg of the pure amorphous compound (*Rf*: 0.45).

3.4. Compound 2 (SU3)

24,25-*O*-*β*-D-Diglucopyranosyl-6α-hydroxycycloart-3-one (**2**): white amorphous solid; $[\alpha]_{25}^{D} + 50.0°$ (*ca.* 0.28 g/100 mL, MeOH); positive ESI-TOF-MS: $m/z = 821.4665$ $[M + Na]^{+}$ (calc. for $C_{42}H_{70}O_{14}Na$: 821.4663); ^{1}H and ^{13}C NMR data, (^{1}H: 300 MHz; ^{13}C: 75 MHz, CD_3OD) see Table 1.

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