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A GERANIOL GLYCOSIDE FROM *HYPOXIS ACUMINATA**

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Key Word Index—*Hypoxis acuminata*; Hypoxidaceae; terpenoid glycoside; acuminoside; geraniol; apiofuranose; high-field NMR.

Abstract—A new terpenoid glycoside, acuminoside, was isolated from *Hypoxis acuminata*. The aglycone, geraniol is linked to glucose, which in turn is connected to an apiofuranose residue.

INTRODUCTION

Pharmaceutical interest in the genus *Hypoxis* stems from its use as a folk medicine by the indigenous people of eastern and southern Africa [2]. Extracts from these plants are sold commercially as a remedy for prostrate hypertrophy. More recently claims have been made that methanolic extracts of *H. rooperi* possess anti-cancer activity [3, 4].

Some of the known chemical constituents of *Hypoxis* plants are zeatin and zeatin glycoside [5], hypoxoside, which is an unusual bis-(3,4-dihydroxyphenyl)pent-1-en-4-yne glycoside [6, 7], and the related compounds nyasoside [8] and nyasicoside [9]. Herein we report the isolation of a new geraniol glycoside, named acuminoside (**1**), from the rhizomes of *H. acuminata* Bak.

RESULTS AND DISCUSSION

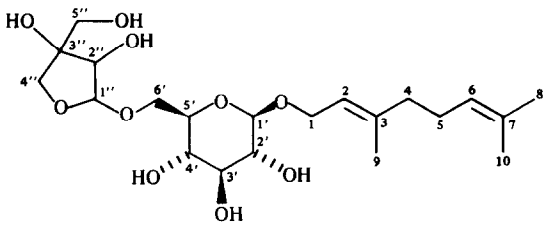
Preparative HPLC (PrepPAK 500/C_{18}) separation of the methanolic plant extract yielded hypoxoside and acuminoside (**1**). Hydrolysis of acuminoside with cellulase for 48 hr yielded glucose and two other compounds which were not identified.

Extensive highfield (500 MHz) NMR experiments on acuminoside in Me_2CO-d_6, MeOH-d_4 and D_2O were the essence of its structure elucidation. Besides standard ^1H NMR and coupled and PND ^{13}C NMR experiments, use was made of proton decoupling experiments, HETCORR [10], DEPT [11] and SPI [12] to indicate through-bond ^1H–^1H and ^1H–^{13}C connectivities. The results are summarized in Table 1.

The wealth of NMR data unambiguously identifies the aglycone as geraniol. Proton–proton decoupling experiments indicated two discrete spin-systems characteristic of geraniol. Both systems contain an olefinic proton, the one (δ_H 5.362) coupled to the non-equivalent geminal protons of an oxygen-bearing methylene group, and the other (δ_H 5.103) to a methylene group which in turn couples to another vinylic methylene group. The ^{13}C NMR chemical shifts are in good agreement with shifts reported for geraniol glycosides [13] and geraniol [14], the latter, with the expected differences at C-1, C-2 and C-3. The possibility of the aglycone being nerol is excluded by the chemical shifts of C-4 and C-9 [13].

The glucose moiety is shown to be β-glucopyranose by ^1H NMR. The coupling constants between the five ring protons are 7.8 Hz and greater, indicating that they are all axial.

The identity of the remaining sugar moiety as well as the positions of the two ether linkages between the two sugars and geraniol were established by four selective population inversion (SPI) experiments [12]. SPI is useful for indicating connectivity through two or three bonds between a selected proton and carbon nuclei. The multiplicities, chemical shifts and $^1J_{CH}$ values of the five remaining carbon signals imply an apiofuranoside: Two methine signals, one belonging to the anomeric carbon and the other to a hydroxylated carbon atom; one quarternary signal; and two methylene signals, one with magnetically equivalent protons and the other with non-equivalent protons. The two methine protons are vicinal with a small coupling constant, indicating *anti* orientation on a furanose ring. Since H–2″ does not couple with either of the methylene groups, the quaternary carbon must be C–3″. In SPI-experiment C (see Table 1), irradiation of H-1″ affected C-3″ through three bonds and methylene carbon C-4″ through three bonds across the furanose oxygen atom. Attachment of the furanose anomeric position to C-6′ of the glucose moeity is indicated by



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*Part 3 in the series 'Medicinal Plants of Southern Africa'. For Part 2, see ref. [1].

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Table 1. ^1H and ^{13}C NMR data of acuminoside (1)

^{13}C					^1H*			
$^1J_{CH}$†	δ_C‡§	Nucleus	SPI¶ correlation		H	δ_H§	$^nJ_{H,H}$ (Hz)	Decoupling¶ correlation
142.4	64.47 *t*	1		A	1a	4.297 *dd*	$^2J_{1,1}=11.9$, $^3J_{1a,2}=6.3$	a
				B	1b	4.230 *dd*	$^2J_{1,1}=11.9$, $^3J_{1b,2}=7.8$	a
154.8	119.85 *d*	2	b		2	5.362 t	$^3J_{2,1a}\simeq{}^3J_{2,1b}\simeq6.8$	A
—	142.99 *s*	3	b					
126.4	39.84 *t*	4			4	2.047 *t*	$^3J_{4,5}=7.5$	B
126.2	26.69 *t*	5			5	2.117 *q*	$^3J_{5,4}\simeq{}^3J_{5,6}\simeq7.1$	Bc
149.7	124.72 *d*	6			6	5.103 *t*	$^3J_{6,5}=6.9$	Cb
—	132.18 *s*	7						
123.7	25.88 *q*	8			8	1.670 *s*		
126.2	16.41 *q*	9			9	1.688 *s*		
124.4	17.89 *q*	10			10	1.603 *s*		
158.1	101.35 *d*	1′	a		1′	4.261 *d*	$^3J_{1',2'}=7.8$	d
143.5	73.62 *d*	2′			2′	3.169 *t*	$^3J_{2',1'}\simeq{}^3J_{2',3'}\simeq8.4$	De
141.0	76.48 *d*	3′			3′	3.324 *t*	$^3J_{3',2'}\simeq{}^3J_{3',4'}\simeq9.1$	Ed
144.9	69.90 *d*	4′			4′	3.264 *t*	$^3J_{4',3'}\simeq{}^3J_{4',5'}\simeq9.2$	E
141.4	77.26 *d*	5′			5′	3.353 *ddd*	$^3J_{5',4'}=9.8$, $^3J_{5',6'b}=6$, $^3J_{5',6'a}=2.0$	fe
143.0	67.72 *t*	6′	c		6′a	3.979 *dd*	$^2J_{6',6'}=11.1$, $^3J_{6'a,5'}=2$	F
					6′b	3.591 *dd*	$^2J_{6',6'}=11.1$, $^3J_{6'b,5'}=6.1$	f
171.7	109.59 *d*	1″		C	1″	4.999 *d*	$^3J_{1'',2''}=2.3$	g
148.7	75.26 *d*	2″	d		2″	3.888 *d*	$^3J_{2'',1''}=2.3$	G
—	79.91 *s*	3″	cd					
146.8	74.21 *t*	4″	c		4″a	3.963 *d*	$^2J_{4'',4''}=9.6$	Fh
					4″b	3.759 *d*	$^2J_{4'',4''}=9.6$	Hf
142.4	65.95 *t*	5″		D	5″	3.570 *s*		

*MeOH-d_4.
†Me_2CO-d_6.
‡D_2O.
§Multiplicity of undecoupled signals.
¶Upper case letter indicates irradiated nucleus for which the same letter in the lower case indicates the affected nuclei in each experiment.

C-6′ also being affected. The apiofuranose constitution is confirmed by SPI-experiment D in which the equivalent methylene protons (H-5″) were irradiated, affecting C-2″ and C-3″. The carbon nuclei chemical shifts of this moiety correlate well with those of published apiofuranosides [15].

The above accounts for all ^{13}C signals and it only remained to prove the position of attachment of the geraniol moiety to the disaccharide. This was achieved by another SPI-experiment (SPI experiment A) where irradiation at one of the geminal H-1 protons affected C-1′, indicating attachment to the anomeric position of glucose.

Mass spectrometry confirms the presence of the apiose (*m/z* 133), geraniol (*m/z* 153) and the apiose-glucose disaccharide (*m/z* 295) fragments.

EXPERIMENTAL

Mps are uncorrected. NMR spectra were recorded at 500 and 125 MHz for protons and ^{13}C nuclei, respectively. The chemical shifts are δ with respect to TMS, referenced to the solvent signal (MeOH-d_4 δ_H 3.30; Me_2CO-d_6 δ_H 2.03, δ_C 29, 80; and for D_2O a drop of Me_2CO-d_6 was used).

Isolation of geraniol apiofuranosyl-β-glucopyranoside (**1**). *Hypoxis acuminata* plants were harvested at the Grand Central Airport, midway between Johannesburg and Pretoria, South Africa, at the beginning of Dec. 1984. They were dried in an air-circulating oven at under 100° to constant weight (*ca* 6 hr), then extracted with cold MeOH for 30 min. The solvent was removed *in vacuo* and the glassy residue milled and chromatographed by prep. HPLC using MeOH–H_2O (1:1) on a reversed phase column (PrepPAK-500/C_{18}), to yield hypoxoside, and on elution with MeOH, acuminoside, mp 39–41° (amorphous); $[\alpha]_D^{19}$ −94.6° (MeOH; *c* 0.93); IR ν_{max}^{nujol} cm^{-1}: 1667 and 1658 (C=C); EIMS (70 eV) *m/z* (rel. int): 295 $[M-C_{10}H_{17}O]^+$ (0.45) (Found *m/z* 295.102. $C_{11}H_{19}O_6$ requires 295.103), 153 $[C_{10}H_{17}O]^+$ (5.3), 133 $[C_5H_9O_4]^+$ (33), 115 $[133-H_2O]^+$ (24), 81 $[C_5H_5O]^+$ (39) and 69 $[C_5H_9]^+$ (100%); ^1H and ^{13}C NMR: see Table 1.

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ISOLATION AND PREPARATION OF TWO LONGIPINENE DERIVATIVES FROM *STEVIA SUBPUBESCENS*

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Key Word Index—*Stevia subpubescens*; Compositae; sesquiterpenes; longipinene derivatives; isolation; preparation.

Abstract—The roots of *Stevia subpubescens* Lag. afforded longicyclene, longipin-2-ene-7β,9α-diol-1-one diangelate and longipin-2-ene-7β,9α-diol-1-one 7-angelate-9-seneciate. A synthetic pathway involving the protection of the C-7 hydroxyl group of longipin-2-ene-7β,9α-diol-1-one with *p*-nitrobenzoyl chloride allowed the preparation of the 7-angelate-9-seneciate, while the diangelate was prepared by direct esterification.

INTRODUCTION

Many diesters of longipin-2-ene-7β, 9α-diol-1-one (**1**) have been found as constituents of *Stevia* species [1, 2], although in most of the cases they appear as complex mixtures. This fact hinders their isolation and complete characterization [3, 4]. In contrast, good yields of diol **1** [5] are easily achieved by hydrolysis of natural ester mixtures. Thus, it seems attractive to develop methodology for the selective preparation of such diesters starting from **1**. The present paper reports the isolation of two of these longipinene derivatives (**2** and **7**) [4, 6] from the Mexican plant *S. subpubescens* Lag., as well as their preparation in substantial amounts which allowed their detailed characterization.

RESULTS AND DISCUSSION

Chromatography of the hexane extracts of the roots of *S. subpubescens* afforded longicyclene [7], longipin-2-ene-7β,9α-diol-1-one diangelate (**2**), and longipin-2-ene-7β,9α-diol-1-one 7-angelate-9-seneciate (**7**) [4, 6]. The preparation of the diangelate **2** was achieved by treatment of **1** with angeloyl chloride [8] in methyl cyanide. Under these conditions, tiglic esters, which are usual by-products in several preparations of angelic esters [9, 10], were not obtained. To prepare the 7-angelate-9-seneciate (**7**), a synthetic pathway for placing each ester residue at the required position was developed. Esterification of **1** with *p*-nitrobenzoyl chloride afforded the monoester **3** as the main product and the diester **4** in small amounts. The positional assignment of the *p*-nitrobenzoate group in **3** was deduced from the ^1H NMR chemical shifts and multiplicities of H-7 and H-9 by comparison with those of related monoesters [2, 5]. The difference in chemical reactivity between the hydroxyl groups at C-7 and C-9 is explained by the seven-membered ring conformation of **1** [11] in which the hydroxyl group at C-7 has a *pseudo*-equatorial orientation and that at C-9 has a *pseudo*-axial one. Treatment of **3** with senecioyl chloride yielded the