Pergamon

0031–9422(95)00744–X

Phytochemistry, Vol. 41, No. 6, pp. 1547–1551, 1996

0031–9422/96 $15.00 + 0.00

PLICATALOSIDE, AN *O,O*-DIGLYCOSYLATED NAPHTHALENE DERIVATIVE FROM *ALOE PLICATILIS*

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(*Received in revised form* 12 *September* 1995)

Key Word Index—*Aloe plicatilis*; Liliaceae; tree aloe; plicataloside; 2,8-*O,O*-di-(*β*-D-glucopyranosyl)-1,2,8-trihydroxy-3-methylnaphthalene.

Abstract—Analysis of the leave exudate of *Aloe plicatilis* resulted in the isolation of a new compound designated plicataloside. Its structure, 2,8-*O,O*-di-(*β*-D-glucopyranosyl)-1,2,8-trihydroxy-3-methylnaphthalene, was established by spectral and chemical methods.

INTRODUCTION

As part of a chemotaxonomic survey of the leaf and root chemistry of the genus *Aloe*, we found an unknown major compound in the leaf exudate of *A. plicatilis*, with an interesting UV/VIS spectrum quite unlike those of the commonly encountered chromone-, phenylpyrone-, flavonoid- or anthraquinone derivatives [1]. A literature search failed to reveal any information on the chemistry of *A. plicatilis*. In a TLC survey of several *Aloe* species, Reynolds [2] detected two, as yet, unidentified 'purple-staining zones' in the leaf exudate of this species. The unusual leaf exudate reflected the taxonomic isolation of *A. plicatilis*, which suggested that the chemical structure may provide clues about the natural affinities of the species. This paper deals with the structural elucidation of the *O,O*-diglucosylated naphthalene derivative, designated plicataloside (**1**).

RESULTS AND DISCUSSION

Analytical HPLC and TLC of the exudate of *A. plicatilis* revealed the presence of only one major compound (**1**), the purification of which was effected by low-temperature flash chromatography over silica gel in a water–methanol–ethyl acetate mixture. The presence of a naphthalene-type chromophore in plicataloside (**1**) was suggested by the absorption maxima and fine structure of its UV-VIS spectrum [3]. A strong peak at *m/z* 515, equivalent to protonated **1** and little fragmentation, was evident in the FAB-mass spectrum of plicataloside (**1**). The EI-mass spectrum of the compound showed no molecular ion but a prominent fragment ion at *m/z* 190, corresponding to the aglycone moiety resulting from the loss of two glycosyl units.

The structure of plicataloside (**1**), $C_{23}H_{30}O_{13}$, in $(CD_3)_2SO$ was determined from the one-dimensional ^1H and ^{13}C NMR spectra and by using COSY [4], TOCSY [5], ROESY [6], HMQC [7] and HMBC [8]. The 500 MHz ^1H and 125 MHz ^{13}C NMR data of plicataloside (**1**) (Table 1) could be readily interpreted in terms of a diglycosylated methylnaphthalene-triol. A COSY experiment revealed the correlation of the hydroxy protons with the corresponding glycoside protons. The resonances belonging to a specific glycoside unit were determined from a TOCSY experiment. Spectral analysis of the ^1H NMR spectrum of the individual glycoside moieties using the PERCH integrated software package [9] gave the chemical shifts and coupling constants. In the COSY and TOCSY experiments, cross-peaks were observed between the methyl proton signal and the aromatic proton singlet, indicating that the methyl group was next to and on the same aromatic ring as the proton resonating at $\delta_H 7.158$. The proton-bearing ^{13}C resonances were assigned with a HMQC experiment. A HMBC experiment was used to assign the quaternary carbon atom signals and to determine the structure of plicataloside (**1**) unambiguously. The methyl protons

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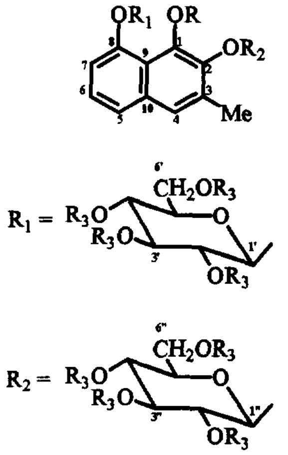

1 : R = R_3 = H

2 : R = H; R_3 = Ac

3 : R = R_3 = Ac

correlate with C-4 and the ^{13}C resonances at δ_C133.44 and δ_C 139.63. In the proton-coupled ^{13}C NMR spectrum the signal at δ_C 133.44 appeared as a quartet with $^{>1}J$(C, H) = 6.0 Hz, assigning this resonance to a carbon atom bearing a methyl group. The resonance at δ_C 139.63 must, therefore, arise from another carbon atom (C-2) three bonds away from the methyl protons. With a mixing delay of 50 ms in the HMBC experiment, the intensities of cross-peaks resulting from vicinal coupled ^{13}C and ^1H nuclei are more intense than those between nuclei with smaller couplings [10]. We observed that H-4 correlates with C-2, C-5 and the signal at δ_C114.85. Cross-peaks were observed from H-6 to two quaternary carbon signals at δ_C 154.02 and δ_C132.76, which must arise from carbon atoms (C-8 and C-10) three bonds from H-6. Intense cross-peaks were noted between H-7 and C-9 and C-5 and a correlation of a lower intensity with the resonance at δ_C 154.02, thereby assigning this signal to C-8 and the resonance at δ_C132.76 to C-10. The remaining quaternary carbon signal at δ_C 114.14 is assigned to C-1. With all the quaternary carbon resonances assigned the substitution positions of the two glycoside units follow directly from the HMBC results. Correlations were observed between H-1′ and C-2 and between H-1″ and C-8. The remaining hydroxyl group must be connected to C-1. Further evidence for the structure and an indication of the stereochemistry were obtained from a ROESY experiment, e.g. correlation between H-4 and H-6 substantiated the existence of the naphthalene moiety.

Acetylation of plicataloside (**1**) under a variety of conditions furnished two acetylated products, **2** and **3**, which were isolated by reverse-phase HPLC. The ^1H and ^{13}C NMR data of these two products are shown in Table 2. The data showed that **2** and **3** resulted from the acetylation of eight and nine hydroxy groups, respectively. Interestingly, **2** exhibited a sharp one-proton singlet at δ_H 8.223, which disappeared on addition of D_2O, indicating the presence of a phenolic hydroxy group. The ^1H NMR spectrum of compound **3** exhibited broad signals, possibly attributable to restricted motion caused by steric congestion of the bulky substituents.

Several attempts to effect acid-catalysed hydrolysis of plicataloside (**1**) in an inert atmosphere resulted in rapid blackening of the solution. Chromatography of the reaction mixture failed to furnish any of the expected aglycone. This may be due to the known oxidation sensitivity of naphthalene polyols [11, 12].

EXPERIMENTAL

General. Mp: uncorr.; NMR: 500.13 MHz and 125.76 MHz for ^1H and ^{13}C, respectively. Sample concentrations were typically 12 mg ml^{-1} in $(CD_3)_2SO$ or $CDCl_3$ and spectra were recorded in 5-mm tubes at 30°; 1D NMR: 32 K data points with sweep widths of 10.5 ppm for ^1H and 200 ppm for ^{13}C. Standard Bruker microprograms were used for performing the 2D experiments. The following mixing times were used: TOCSY 150 ms, ROESY 250 ms, HMBC 50 ms. EI-MS and FAB-MS (glycerol matrix, Ar bombarding gas) were recorded on a VG-7070 E spectrometer. CC: silica gel (Merck, 230–400 mesh); TLC: (Merck, $60F_{254}$).

Plant material. Leaf exudate samples were collected near Worcester in the south-western Cape. Copious amounts of a watery exudate, which soon solidified to a pale yellow wax-like solid, was taken from three different plants, and also from leaves of various ages (old, medium and young). Analytical studies showed that the three different individuals and the various leaf samples were all practically identical. The same applied to several extracts obtained from cultivated specimens. In view of the striking morphological features of *A. plicatilis*, which makes it impossible to be mistaken for any other species, we deemed it unnecessarily destructive to collect a voucher specimen.

Extraction and isolation. The yellow wax-like solid from *A. plicatilis* was treated with MeOH–Et_2O (2:1) and stirred for 30 min at 0°. After filtration, the solvent was removed *in vacuo* to provide a brown residue which contained one major compound with R_f 0.53 (EtOAC–MeOH–H_2O, 7:2:1). This (1 g) was adsorbed on silica gel and flash chromatographed on silica gel (100 g) at low temp (− 15°) in EtOAc–MeOH–H_2O (8:1:1). The separation was monitored by TLC and analytical HPLC.

2,8-O,O-*Di-(β-D-glucopyranosyl)*-1,2,8-*trihydroxy*-3-*methylnaphthalene* (**1**). Crystals, mp 197–199° (31%); $[\alpha]_D^{20}$ − 199.1° (DMSO, *c* 1,0); UV λ_{max}^{DMSO} (log ε) nm: 284sh (3.98), 305 (4.05), 318 (3.97), 334 (3.89); H^1 (DMSO-d_6) and ^{13}C NMR ($CDCl_3$): Table 1; EIMS (rel. int.) *m/z*: 190, 0634 (calcd for $C_{11}H_{10}O_3$, 190, 0630); FABMS *m/z*: 515 $[M + 1]^+$.

Acetylation of plicataloside (**1**). Plicataloside (**1**) (100 mg), Ac_2O (0.5 ml) and pyridine (0.5 ml) in CH_2Cl_2

Table 1. ^1H (500.13 MHz) and ^{13}C (125.76 MHz) NMR data for **1**

	^1H		^{13}C		
C/H	$\delta_H^{\dagger}$	J (Hz)	$\delta_C^{\ddagger}$	^1J§ (Hz)	$^{>1}J$ (Hz)
1	9.3‡(OH)	—	144.14 *S*	—	—
2	—	—	139.63 *Sm*	—	—
3	—	—	133.44 *Sq*	—	6.0
4	7.158 *s*	—	118.79 *Dm*	160.5	—
5	7.385 *dd*	8.29 (5,6)	121.89 *Dm*	161.9	—
6	7.254 *dd*	0.89 (5,7)	125.21 *D*	162.6	—
7	7.199 *dd*	7.71 (6,7)	109.74 *Dd*	161.7	9.5
8	—	—	154.02 *Sd*	—	7.8
9	—	—	114.85 *Sm*	—	—
10	—	—	132.76 *Sdm*	—	8.4
11	2.391 *s*	0.55 (4,11)	17.73 *Qd*	127.6	5.3
1′	4.818 *d*	7.58 (1′,2′)	104.19 *Dm*	166.3	—
2′	3.309 *dd*	8.75 (2′,3′)	74.23 *Dm*	144.3	—
2′-OH	5.5	4.84 (3′,OH)	—	—	—
3′	3.258 *td*	8.75 (3′,4′)	76.35 *Dm*	143.9	—
3′-OH	5.019 *d*	5.28 (4′,OH)	—	—	—
4′	3.159 *td*	9.62 (4′,5′)	69.91 *Dm*	144.9	—
4′-OH	4.910 *d*	2.07 (5′,6′a)	—	—	—
5′	3.087 *dddd*	5.70 (5′,6′b)	77.08 *Dm*	141.2	—
6′(a)	3.607 *ddd*	− 11.68 (6′a,6′b)	60.93 *Tm*	139.9	—
(b)	3.426 *dt*	5.91 (6′a,OH)	—	—	—
6′-OH	4.305 *t*	5.91 (6′b,OH)	—	—	—
1″	4.985 *d*	7.69 (1″,2″)	102.69 *Dm*	165.0	—
2″	3.388 *dd*	8.72 (2″,3″)	73.61 *Dm*	142.8	—
2″-OH	5.5	5.09 (3″,OH)	—	—	—
3″	3.330 *td*	8.72 (3″,4″)	76.20 *Dm*	145.6	—
3″-OH	5.107 *d*	5.47 (4″,OH)	—	—	—
4″	3.206 *td*	9.59 (4″,5″)	69.85 *Dm*	144.9	—
4″-OH	5.061 *d*	1.93 (5″,6″a)	—	—	—
5″	3.407 *dddd*	6.03 (5″,6″b)	77.60 *Dm*	140.9	—
6″(a)	3.756 *ddd*	− 11.92 (6″a,6″b)	60.74 *Tm*	139.6	—
(b)	3.514 *dt*	− 5.82 (6″a,OH)	—	—	—
6″-OH	4.646 *t*	5.82 (6″b,OH)	—	—	—

*Solvent $(CD_3)_2SO$.

†Relative to $(CH_3)_4Si$ measured from internal $(CHD_2)_2SO$ and corrected using δ_H $[(CH_3)_4Si] = \delta_H(CHD_2)_2SO + 2.49$.

‡Relative to $(CH_3)_4Si$ measured from internal $(CD_3)_2SO$ and corrected using δ_C $[(CH_3)_4Si] = \delta_C(CD_3)_2SO + 39.5$.

§Coupling constants in Hz. Capital letters refer to the pattern resulting from directly bonded (C,H) coupling $[^1J(CH)]$ and lower-case letters to that from (C,H) couplings over more than one bond $[^{>1}J(CH)]$.

Table 2. ^{1}H (500.13MHz) and ^{13}C (125.76 MHz) NMR data for **2** and **3**

	2					**3**	
	^{1}H		^{13}C			^{13}C	
H/C	$\delta_{\mathrm{H}}^{\dagger}$	J (Hz)	$\delta_{\mathrm{C}}^{\ddagger}$	^{1}J (Hz)	$^{>1}J$ (Hz)	$\delta_{\mathrm{H}}^{\dagger}$	J (Hz)
1(OH)	8.233 *s* (OH)	—	144.12 *S*	—	—	—	—
2	—	—	139.29 *Sm*	—	—	—	—
3	—	—	135.04 *Sq*	—	6.1	–	—
4	7.080 *s*	—	119.29 *Dm*	160.5	—	7.460	—
5	7.371 *dd*	7.91 (5,6)	123.43 *Dm*	159.6	—	7.421	8.00 (5,6)
6	7.184 *t*	0.55 (5,7)	124.49 *D*	160.5	—	7.268	7.70 (6,7)
7	6.828 *dd*	7.66 (6,7)	107.76 *Dd*	160.2	8.3	6.960	—
8	—	—	152.72 *Sd*	—	11.0	—	—
9	—	—	114.78 *Sm*	—	—	—	—
10	—	—	133.51 *Sd*	—	8.6	—	—
11	2.362 *d*	0.56 (4,11)	17.58 *Qd*	128.4	5.0	2.404	—
1′	5.264 *d*	7.60 (1,2)	101.12 *Dm*	168.2	—	5.32	—
2′	5.330 *m*	8.01 (2,3)	71.81 *Dd*	153.8	6.1	—	—
3′	5.304 *m*	9.14 (3,4)	72.98 *Dm*	150.0	—	5.12	—
4′	5.146 *tm*	10.04 (4,5)	68.75 *Dm*	150.5	—	—	—
5′	3.606 *ddd*	4.63 (5,6a)	71.53 *Dm*	144.6	—	3.634	—
6′a	4.188 *dd*	2.60 (5,6b)	61.77 *Tm*	147.9	—	4.200	—
b	4.007 *dd*	– 12.20 (6a,6b)	—	—	—	4.007	—
1″	5.394 *d*	7.60 (1,2)	98.93 *Dm*	164.6	—	5.32	—
2″	5.402 *m*	7.46 (2,3)	70.91 *Dd*	154.3	5.6	—	—
3″	5.357 *m*	8.77 (3,4)	72.38 *Dm*	154.0	—	5.12	—
4″	5.228 *dd*	9.96 (4,5)	68.20 *Dm*	153.6	—	—	—
5″	3.923 *ddd*	4.66 (5,6a)	72.35 *Dm*	144.6	—	3.823	—
6″a	4.246 *dd*	3.04 (5,6b)	61.48 *Tm*	148.5	—	4.255	—
b	4.226 *dd*	– 12.43 (6a,6b)	—	—	—	4.181	—
$\mathrm{O\overset{\overset{O}{\Vert}}{C}\underline{CH}_3}$	2.165 *s*	—	20.80 *Q*	—	—	2.099 *s*	—
	2.056 *s*	—	20.62 *Q*	—	—	2.025 *s*	—
	2.044 *s*	—	20.58 *Q*	—	—	2.010 *s*	—
	2.038 *s*	—	20.54 *Q* (3)	—	—	2.003 *s*	—
	2.015 *s* (2)	—	20.52 *Q* (2)	—	—	1.983 *s* (2)	—
	1.994 *s*	—	—	—	—	1.950 *s*	—
	1.958 *s*	—	—	—	—	1.930 *s*	—
	—	—	—	—	—	2.424 *s*	—
$\mathrm{O\underline{\overset{\overset{O}{\Vert}}{C}}CH_3}$	—	—	170.53 *s*	—	—	—	—
	—	—	170.41 *s*	—	—	—	—
	—	—	170.15 *s*	—	—	—	—
	—	—	170.06 *s*	—	—	—	—
	—	—	170.02 *s*	—	—	–	—
	—	—	169.50 *s* (2)	—	—	—	—
	—	—	169.31 *s*	—	—	—	—

*Solvent $CDCl_3$.

†Relative to $(CH_3)_4Si$ measured from internal $CHCl_3$ and corrected using δ_{H} $[(CH_3)_4Si] = \delta_{\mathrm{H}}(CHCl_3) + 7.24$.

‡Relative to $(CH_3)_4Si$ measured from internal $CDCl_3$ and corrected by using $\delta_{\mathrm{C}}[(CH_3)_4Si] = \delta_{\mathrm{C}}(CDCl_3) + 77.0$.

§Coupling constants in Hz. Capital letters refer to the pattern resulting from directly bonded (C,H) couplings [^{1}J(CH)] and lower-case letters to that from (C,H) couplings over more than one bond [$^{>1}J$(CH)].

(3 ml) were stirred for 70 hr at room temp. The solvent was removed under red. press. and the crude product chromatographed in EtOAC–$CHCl_3$ (1:1). RP-HPLC of the eluate indicated the presence of two products which were not sepd by CC on silica gel. Sepn was achieved by prep. RP-HPLC on a Beckman Ultrasphere ODS column (C_{18} reverse phase, 5 μm particle size, 250 × 10 mm; flow rate 4, 5 ml min^{-1}; 1-ml sample loop). The solvent system comprised MeCN–H_2O (11:9) (isocratic). Detection by diode-array detector, 2 channels (A set at 275 ± 35 nm; B set at 365 ± 20 nm). Pure **2** and **3** were obtained (NMR data, see Table 2). The same mixture of

structures was obtained on acid (H_2SO_4)-catalysed acetylation of **1** in AC_2O at room temp for 70 hr.

Acknowledgement—The financial assistance of the South African Foundation of Research Development is gratefully acknowledged.

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