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ALOENIN B, A NEW DIGLUCOSYLATED 6-PHENYL-2-PYRONE FROM KENYA ALOE[1]

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ABSTRACT.—A new 6-phenyl-2-pyrone *O,O*-diglucoside, aloenin B, was isolated from commercial Kenya aloe. Its structure, 6- { 2-*O*-β-D-[2-*O*-(*E*)-*p*-coumaroyl] glucopyranosyl-4-*O*-β-D-glucopyranosyl-6-methyl } phenyl-2-pyrone (**1**), was established by spectral methods and chemical transformations.

As a part of systematic chemical studies on aloe,[2] the water soluble constituents of a commercial sample from Kenya were investigated. In addition to aloins A and B (30%) (2,3) and aloeresin D (12.5 %) (4), a new *O,O*-diglucoside for which we propose the name aloenin B[3] (**1**) was isolated in 13.5 % yield.

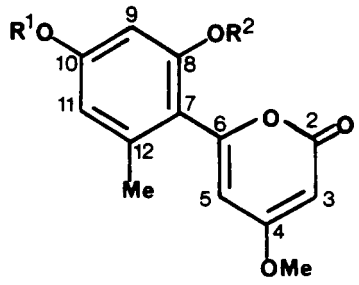
Aloenin B (**1**) was obtained as an amorphous powder. The molecular formula $C_{34}H_{38}O_{17}$ and the presence of two hexose residues, one of them bearing a *p*-coumaroyl group, were derived by fdms: fragments at *m/z* 718 $[M]^+$, 572 $[M-C_9H_6O_2]^+$, 556 $M-C_6H_{10}O_5]^+$, 410 $[M-C_9H_6O_2-C_6H_{10}O_5]^+$, and 248 $[M-C_9H_6O_2-2C_6H_{10}O_5]^+$, likely corresponding to the aglycone.

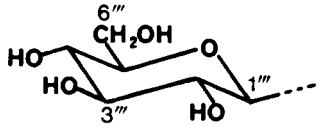
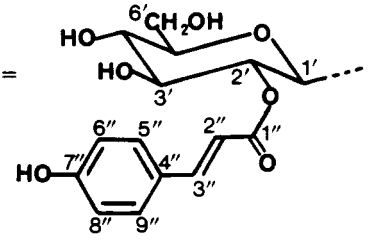
Uv and ir spectra of aloenin B revealed strong similarities with those of 4-methoxy-2-pyrones (6) and of aloenin A (**2**) (formerly aloenin), previously isolated from *Aloe arborescens* var. *natalensis* Berger (5). This fact, together with the molecular weight of the aglycone, allowed structure **1** (or the isomeric one having the acylated and non-acylated glucosyl residues in inverted position) to be assigned to aloenin B. This was confirmed by spectral evidence, as shown in Tables 1 and 2, in which ^{1}H- and ^{13}C-chemical shifts of aloenin B are listed together with those of other structurally related 6-phenyl-2-pyrones. Assignments in Tables 1 and 2 are mainly based on analogies of chemical shifts and coupling constants with those found for the corresponding signals in 4-methoxy-2-pyrones (7-9) and in aloenin A (5,10). Proton-proton coupling constants were confirmed by homonuclear decoupling, and ^{13}C assignments were supported by off-resonance and proton-coupled spectra. In addition, selective proton decoupling experiments were carried out to solve the ambiguities in the assignments of some C-13 signals (e.g., for H-9 vs. H-11, C-9 vs. C-1″ in **1**, etc.). Convincing evidence in favor of structure **1**, in which the non-acylated glucosyl group is linked to the 10-*O*-position, came from differential ^{1}H-nOe experiments performed on compound **4** prepared from

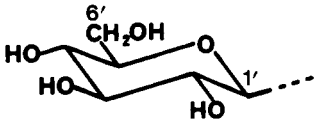
[1]Part 4 in the series "Studies on Aloe." For Part 3, see G. Speranza *et al.* (4).

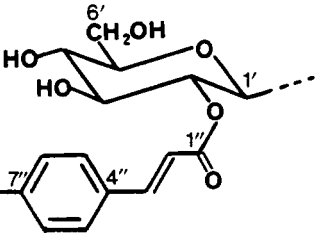
[2]Aloe is the solid residue obtained by evaporating the liquid which drains from cut leaves of *Aloe* spp. (Liliaceae). The drug is mainly prepared from *Aloe ferox* Mill., its hybrids (Cape and Kenya aloe), and *Aloe vera* L. (Curaçao aloe) (1).

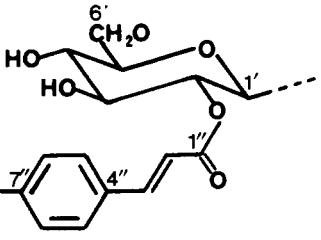
[3]This name is suggested by structural similarities between **1** and aloenin (**2**) (5), which is called aloenin A in the present paper.

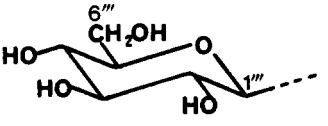
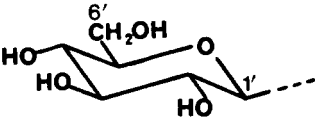


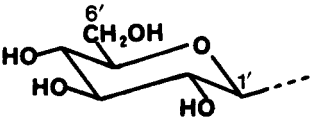
1 R^1=  R^2= 

2 R^1=H, R^2= 

3 R^1=H, R^2= 

4 R^1=Me, R^2= 

5 R^1=  R^2= 

6 R^1=Me, R^2= 

7 R^1=H, R^2=H

aloenin B (**1**) via enzymatic or acid hydrolysis to **3** followed by methylation with CH_2N_2. Irradiation of the singlet at δ 3.74 caused intensity enhancement of both the singlets at δ 6.47 (5.8 %) and at δ 6.61 (3.5 %) due to aromatic protons in the 11- and 9- positions, respectively. This allowed the irradiated methoxy group of **4** and the non-acylated glucosyl residue of **1** to be located in the 10-position. By contrast, when the pyrone methoxy group was irradiated, only the signal of H-3 was enhanced (3.5 %). This fact, however, could be interpreted in terms of a preferred orientation of the methyl group (11) in agreement with a previous observation concerning aloenin A derivatives (12). Finally, on irradiation of the methoxy group present in the cinnamoyl residue of **4**, an nOe value of ca. 3% was observed for the two equivalent *ortho*-protons.

The β-configuration of the two D-glucosyl moieties of **1**, one of which esterified in the 2-*O*-position, was inferred by the coupling constant values for the anomeric protons (see Table 1) (8 *Hz*) as well as for the anomeric carbon atoms $^1J[^{13}CH(1')]=$ $^1J[^{13}CH(1''')]=160$ Hz) (13).

TABLE 1. ^{1}H-nmr Spectral Data of Aloenin B (**1**) and its Derivatives[a]

Proton(s) No.	Compound					
	1[b]	**2**[c]	**3**[b]	**4**[b]	**5**[b]	**6**[b]
3	5.52 (d, 2.2)	5.47 (d, 2.5)	5.47 (d, 2.2)	5.49 (d, 2.2)	5.63 (d, 2.2)	5.61 (d, 2.2)
5	5.79 (d, 2.2)	6.15 (d, 2.5)	5.77 (d, 2.2)	5.82 (d, 2.2)	6.25 (d, 2.2)	6.24 (d, 2.2)
9	6.63 (d, 2.0)	6.62 (d, 2.2)[d]	6.43 (d, 2.0)	6.61 (d, 2.0)	6.71 (d, 1.8)	6.65 (d, 2.0)
11	6.48 (d, 2.0)	6.45 (d, 2.2)[d]	6.30 (d, 2.0)	6.47 (d, 2.0)	6.60 (d, 1.8)	6.56 (d, 2.0)
4-OCH_3	3.69	3.86	3.68	3.67	3.85	3.82[e]
10-OCH_3				3.74		3.75[e]
12-CH_3	2.09	2.19	2.04	2.10	2.18	2.17
1′	5.06 (d, 8.0)		4.99 (d, 8.0)	5.09 (d, 8.0)	4.91 (d, 7.0)[e]	4.88 (d, 7.5)
2′	4.78 (dd, 8.0)		4.74 (dd, 8.0)	4.79 (dd, 8.0)		
2″	6.13 (d, 16.0)		6.13 (d, 16.0)	6.23 (d, 16.0)		
3″	7.38 (d, 16.0)		7.36 (d, 16.0)	7.40 (d, 16.0)		
5″,9″	7.44 (d, 8.5)		7.43 (d, 8.5)	7.55 (d, 8.5)		
6″, 8″	6.74 (d, 8.5)		6.73 (d, 8.5)	6.90 (d, 8.5)		
7″-OCH_3				3.77		
1′	4.87 (d, 8.0)				4.90 (d, 7.5)[e]	

[a]Multiplicities (d, doublet; dd, doublet of doublets) and coupling constants (Hz) are given in parentheses. Other signals are singlets.

[b]Recorded at 300 MHz in DMSO-d_6 after D_2O exchange except for **4**. Chemical shifts are given in ppm. DMSO was used as internal standard (δ 2.50 from TMS). Signals of glucosyl hydroxy groups were observed in the range δ 3-5 and broad signals of phenolic groups in the range δ 9.5-10 when spectra were run without addition of D_2O.

[c]Spectrum recorded in $(CD_3)_2CO$ (5).

[d]Previously reported without unequivocal assignments (5).

[e]Assignments may be reversed.

To verify that the *p*-coumaroyl group is responsible for the bathochromic shift (54 nm) of aloenin B (**1**) in alkaline solution, whereas a similar shift in aloenin A (**2**) is due to the presence of a phenolic OH in the 10-position (5), compound **1** was treated with CH_2N_2 in MeOH. However, the resulting product, showing no bathochromic shift, was found to be the deacylated aloenin B, i.e. **5**, instead of the expected 7″-O-methylated derivative of **1**. This fact can be explained on the basis of a nucleophilicity enhancement of MeOH in the presence of CH_2N_2 (14). It is worth noting that marked upfield shift appeared in the ^{1}H-nmr spectrum for H-3 and H-5 resonances going from **1** to **5** (Table 1). This is indicative of a strong anistropic effect produced by the cinnamoyl residue on the pyrone protons, as a consequence of the relative position of the two rings in the preferred conformation of **1** in solution.

Taking advantage of the CH_2N_2-catalyzed *trans*-esterification in MeOH, **4** was converted into **6** which was found to be identical with the 10-*O*-methylaloenin A (5) by a comparison of their physical properties. In addition, enzymatic β-glucosidase hydrolysis of **5** afforded aloenin A (**2**) and, in a longer term experiment, aglycone **7**, whose structure had previously been demonstrated by X-ray analysis (5,15).

Thus, structure **1** for aloenin B was unequivocally proved by the above chemical correlation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Ir and uv spectra were obtained on a Perkin-Elmer spectrometer model 681 and on a Perkin-Elmer 554 instrument, respectively. A Bruker CXP 300 spectrometer was used to record ^{1}H-nmr (300 MHz) and ^{13}C-nmr (75.47 MHz)

TABLE 2. ^{13}C-nmr Spectral Data of Aloenin B (**1**) and its Derivatives

Carbon No.	Compound					
	1[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]
2	163.74	165.04	163.77	163.61	163.81	163.78
3	88.09	88.52	87.88	88.02	88.00	87.91
4	170.50	171.53	170.49	170.38	170.80	170.79
5	104.03	104.99	103.92	103.96	104.23	104.20
6	158.75[c]	159.50	157.43[c]	157.04[c]	158.86[c]	157.44[c]
7	115.34	114.85	113.25	114.75	115.50	114.85
8	156.95[c]	158.18	156.16[c]	156.03[c]	157.36[c]	156.40[c]
9	99.89	102.06	99.86	98.85	100.71	100.80
10	155.75	161.44	159.41[d]	160.97[d]	156.10	160.96
11	111.35	112.45	110.54	109.07	111.39	109.21
12	138.69	140.14	138.68	138.86	138.75	138.95
4-OCH_3	56.06	55.83	55.93	55.95[e]	56.19	56.13[d]
10-OCH_3				55.17[e]		55.15[d]
12-CH_3	19.30	20.31	19.25	19.33	19.66	19.66
1′	97.98	103.06	98.43	98.42	99.80[d]	99.31
2′	73.88	74.75	73.86	73.93	73.08[d]	73.17
3′	72.65[d]	78.76[c]	72.74	72.77	76.60[f]	76.72[e]
4′	70.08	71.16	69.70	70.05	69.75[g]	69.78
5′	77.08[e]	78.57[c]	77.10	77.34	76.94[f]	77.11[e]
6′	60.79[f]	62.46	60.42	60.60	60.79	60.73
1″	165.03		164.98	164.87		
2″	113.96		113.96	115.06		
3″	144.57		144.48	144.05		
4″	125.27		125.22	126.75		
5″, 9″	130.08		130.01	129.87		
6″, 8″	115.68		115.64	114.23		
7″-OCH_3	159.69		159.60[d]	160.93[d]		
7″				55.22[e]		
1‴	99.48				100.4[d]	
2‴	73.08[d]				73.16[e]	
3‴	76.66[e]				76.66[f]	
4‴	70.08				69.81[g]	
5‴	77.08[e]				76.94[f]	
6‴	60.72[f]				60.79	

[a]Registred at 75.47 MHz in DMSO-d_6 at room temperature. Chemical shifts are given in ppm. DMSO was used as internal standard (δ 39.50 from TMS).

[b]Recorded in C_5D_5N (5).

[c-g]Signals with the same superscript are interchangeable.

spectra. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter with a 10 cm microcell in MeOH solutions. Fdms were recorded on a Varian MAT 311-A mass spectrometer equipped with a combined FI/FD/EI ion source, and fabms on a VG 7070EQ mass spectrometer. Droplet countercurrent chromatography (dccc) was carried out on a Model 670 equipped with 300 standard glass tubes (40 cm×2.7 cm i.d.). Microanalyses were obtained with a Perkin-Elmer 240 elemental analyser. Tlc was performed with silica gel 60 F_{254} precoated plates (Merck, 0.25 mm layer) using EtOAc-EtOH-H_2O (100:20:13) as eluent, unless otherwise indicated; spots were visualized by exposing to uv light (254 nm). Commercial Merck silica gel 60 (230-400 mesh ASTM) was used for column chromatography. Analytical and semipreparative hplc was performed on a Perkin-Elmer 3B liquid chromatograph connected to a variable wavelenght uv detector (Perkin-Elmer LC 75 Spectrophotometric detector); general analytical conditions: column 250×4 mm, LiChrosorb RP-18, 10 μm; flow rate: 1 ml/min; detector λ 280 nm; eluent MeOH/H_2O, linear gradient from 30 to 60% MeOH in 25 min (unless stated otherwise); semi-preparative conditions: column 250×25 mm, LiChrosorb RP-18, 7 μm; flow rate: 15 ml/min; detector λ 340 nm; eluent: MeOH/H_2O, linear gradient form 30 to 60% MeOH in 18 min (unless stated otherwise).

PLANT MATERIAL.—Commercial Kenya aloe used in this study was purchased from Saamer Int., Ltd. (Mombasa, Kenya).

ISOLATION OF ALOENIN B (**1**).—Powdered Kenya aloe (3.0 g) was dissolved in 15 ml of the dccc solvent (1:1 ratio of two phases formed from $CHCl_3$-MeOH-H_2O, 7:13:8). After filtration (Millipore filter, pore size 0.5 m), both phases were subjected to dccc (ascending mode; the lower phase was used as stationary phase; flow 40 ml/h). Separation was monitored by tlc and fractions containing aloenin B (Rf 0.60) as a major component were combined. A further purification by flash chromatography (eluent: EtOAc-EtOH-H_2O, 100:20:13) afforded a yellowish compound (400 mg) which was found to be pure on tlc and analytical hplc (Rt 15 min). Mp 186-188°; $[\alpha]^{30}D$ −24.9° (c 0.22, MeOH); ir νmax (KBr) 3400, 1700, 1680, 1630, 1560 cm^{-1}; uv λ max (MeOH) 205 (log ε 4.75), 224sh (4.48), 298 (4.44), 308 nm (4.45); ^1H nmr see Table 1; ^{13}C nmr see Table 2; fabms *m/z* 741 $(M+Na)^+$, 719 $(M+H)^+$, 411, 309, 249. Calcd for $C_{34}H_{38}O_{17}\cdot 1.5\ H_2O$: C,54.76; H,5.54%. Found: C,54.88; H,5.67%.

METHANOLYSIS OF ALOENIN B (**1**) TO **3**.—Aloenin B (**1**) (50 mg) was heated with 3% HCl in MeOH (25 ml) for 1 h. After cooling, the mixture was neutralized with solid $NaHCO_3$, filtered and evaporated. The residue was diluted with H_2O and extracted with *n*-BuOH. The organic layer, showing a major spot with Rf 0.80 on tlc and a major peak on hplc with Rt 18 min was evaporated in vacuo, redissolved in H_2O (20 ml) and desalted by passing through a column of Amberlite XAD-7 (wet volume, 100 ml). Elution with MeOH followed by evaporation of the solvent afforded a crude product which was purified by semi-preparative hplc (Rt 15 min). After lyophilization, compound **3** was obtained as an amorphous powder (25 mg).

Mp 148-150° $[\alpha]^{25}D$ −17.7° (*c* 0.21, MeOH); ir νmax (KBr) 3400, 1695, 1690, 1635, 1605, 1555 cm^{-1}, uv λmax (MeOH) 205 (log ε), 224sh (4.33), 298 (4.39), 308 (4.40); ^1H nmr see Table 1; ^{13}C nmr see Table 2; fabms *m/z* 579 $(M+Na)^+$, 557 $(M+H)^+$. Calcd for $C_{28}H_{28}O_{12}\cdot 1.5\ H_2O$: C,57.63; H,5.35% Found: C,57.48; H,5.51%. The identification of glucose, which was present in the aqueous mother liquor, was carried out by glc analysis after evaporation and silylation (16).

ENZYMATIC HYDROLYSIS OF ALOENIN B (**1**) TO **3**.—β-Glucosidase (almond emulsin, Sigma) was added to a solution of aloenin B (**1**) (200 mg) in H_2O (60 ml), and the mixture incubated at 39° for 2 days. After adding MeOH, the solution was filtered and concentrated under reduced pressure. Semi-preparative hplc of the aqueous residue gave a product which was found to be identical in all respects with compound **3** obtained as described above.

METHYLATION OF **3** TO **4**.—A slow stream of CH_2N_2 prepared from Diazald (Aldrich, 1.6 g), (17) (N_2 as carrier) was bubbled for 2 h into a solution of **3** (100 mg) in MeOH (10 ml) at 25°. Evaporation of the solvent left a residue showing a single spot (Rf 0.4) in tlc (EtOAc-C_6H_6-MeOH, 80:20:5). Purification by semi-preparative hplc (eluent MeOH/H_2O, linear gradient from 60 to 90% MeOH in 20 min, Rt 11.5 min), gave pure **4** (59 mg). Mp 128-131°; $[\alpha]^{25}D$ −11.4° (*c* 0.21, MeOH); uv λmax (MeOH) 205 (log ε 4.60), 224 sh (4.43), 308 nm (4.45); ^1H nmr see Table 1; ^{13}C nmr see Table 2; fabms *m/z* 607 $(M+Na)^+$, 585 $(M+H)^+$. Calcd for $C_{30}H_{32}O_{12}\cdot 0.5\ H_2O$: C,60.70; H, 5.60. Found: C,60.92; H,5.67%.

PREPARATION OF **5**.—A solution of aloenin B (**1**) (100 mg) in MeOH (25 ml) was treated with a large excess of gaseous CH_2N_2 (N_2 as carrier) (17). The disappearance of **1** was monitored by tlc and hplc. After 4 h the reaction mixture was evaporated to dryness in vacuo and the residue, showing two peaks in hplc (Rt 6.0 and 23.0 min), was separated by semi-preparative hplc. Lyophilization of the eluate containing the more polar compound (Rt 6.0 min) gave pure **5** (45% yield). Mp 136-138°; $[\alpha]^{25}D$ −53.1° (*c* 0.2, MeOH); ir νmax (KBr) 3400, 1690, 1645, 1610, 1560 cm^{-1}; uv λmax (MeOH) 205 (log ε 4.55), 224 (4.16), 232 sh (4.10), 297 nm (4.00); ^1H nmr see Table 1; ^{13}C nmr see Table 2; fabms *m/z* 595 $(M+Na)^+$, 573 $(M+H)^+$. Calcd for $C_{25}H_{32}O_5\cdot 1.5\ H_2O$: C,50.08; H,5.88%. Found: C,50.24; H,5.53%.

The less polar compound (Rt 23.0 min) was also isolated by semi-preparative hplc and shown to be pure methyl *p*-methoxycinnamate by comparison (tlc, hplc, glc, ^1H nmr) with an authentic sample.

TREATMENT OF **4** WITH CH_2N_2 TO OBTAIN **6**.—A solution of **4** (40 mg) in MeOH (10 ml) was treated with CH_2N_2 as described for the preparation of **5**. After evaporation of reaction mixture in vacuo, compound **6** was isolated by semi-preparative hplc (eluent MeOH/H_2O; linear gradient from 60 to 75% MeOH in 15 min, Rt 12 min) and crystallized from EtOAc/Et_2O. Its physical properties (mp, ir, ^1H nmr, elemental analysis) were found to be identical with those reported for the same compound derived from aloenin A (**2**) (5). ^{13}C nmr, see Table 2.

ENZYMATIC HYDROLYSIS OF **5**.—By treatment of **5** (90 mg) in H_2O (20 ml) with β-glucosidase (72 h at 39°) followed by semi-preparative hplc, compound **2** was obtained in pure form (42 mg, Rf 0.47, Rt 12 min in analytical hplc). Its physical properties (mp, $[\alpha]D$, ir, uv, ^1H nmr, ^{13}C nmr, elemental analysis) were shown to be identical with those reported for aloenin A (5).

When compound **5** was incubated with β-glucosidase for 5 days a less polar (Rf 0.86, Rt 15.4 min) compound was obtained along with **2**. Extraction of the reaction mixture with Et_2O followed by evaporation in vacuo gave a product (60% yield) whose physical properties (mp, ir, ^1H nmr, elemental analysis) were in close agreement with those reported for the aloenin A aglycone (**7**) (5).

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