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ANTHRAQUINONE, ANTHRONE AND PHENYLPYRONE COMPONENTS OF *ALOE NYERIENSIS* VAR. *KEDONGENSIS* LEAF EXUDATE

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Key Word Index—*Aloe nyeriensis* var *kedongensis*; Liliaceae; leaf exudate; anthraquinones; nataloe-emodin; 2-*O*-β-D-glucopyranosylnataloe-emodin; anthrones; nataloin; 6-phenyl-4-methoxypyran-2-ones; aloenin; aloenin-2″-*p*-coumaroyl ester; aloenin aglycone; chemotaxonomy.

Abstract—The leaf exudate of *Aloe nyeriensis* var. *kedongensis* yielded six compounds which were identified on the basis of spectral data and inter-conversions as two groups of three allied compounds. These were (a) 4-methoxy-6-(2′,4′-dihydroxy-6′-methylphenyl)-pyran-2-one, its 2′-*O*-β-D-glucopyranoside (aloenin) and the 2″-*O*-*p*-coumaroyl ester of aloenin, (b) the anthracene derivatives 1,2,8-trihydroxy-6-methylanthraquinone (nataloe-emodin), its 2-*O*-β-D-glucopyranosyl ester and the corresponding 10-*C*-β-D-glucopyranoside nataloin.

INTRODUCTION

In a two-dimensional TLC analysis of the leaf exudates of shrubby *Aloe* species from east Africa [1] the two subspecies of *A. nyeriensis* Christian were reported to give four distinct patterns of phenolic compounds when chromatoplates were sprayed with Fast Blue B salt. Of these one was characterised by the presence of barbaloin and another two by containing homonataloin, with or without aloenin. The remaining pattern, which lacked both barbaloin and homonataloin but appeared to contain another anthraquinone *C*-glycoside as a blue-staining spot [1], was found in individuals assigned to *A. nyeriensis* var. *kedongensis* (Reynolds) S. Carter and collected around and to the west of the town of Gilgil in Kenya. We have now examined plants with this pattern and have isolated a series of anthracene derivatives based on 1,2,8-trihydroxy-6-methylanthraquinone (nataloe-emodin). In addition the phenylpyrone-*O*-glucoside aloenin and two related compounds were also obtained. Four of the compounds appear to be novel.

RESULTS AND DISCUSSION

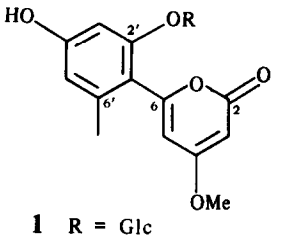
Six compounds were isolated by column chromatography over silica gel and labelled A-F in order of their elution. The major compound (F) was, on the basis of UV, ^{1}H NMR and EIMS analysis, identified as aloenin (**1**) which has previously been reported from *Aloe arborescens* var. *natalensis* [2]. The minor compound B gave a similar UV spectrum and an EIMS fragmentation and ^{1}H NMR spectrum comparable to that of the 2-phenylpyran nucleus of **1**, suggesting that it was aloenin aglycone (**2**). This was confirmed by hydrolysis of **1** which yielded glucose and a product identical in all respects to **2** (UV, ^{1}H NMR, EIMS). In both **1** and **2** the assignment of H-5′ was established by ^{1}H NMR spin-decoupling of the 6′-methyl resonance. Hydrolysis of **1** led to a much greater shift in H-3′ than in H-5′ so giving additional evidence for the placement of the sugar unit at C-2′ rather than C-4′.

Compound E also had spectral characteristics similar to **1**. Fragments at *m/z* 164 and 119 in the EIMS suggested the presence of a hydroxycinnamoyl moiety and the presence of a *p*-hydroxy-*trans*-coumaroyl residue was confirmed by the ^{1}H NMR spectrum. Placement of the coumaroyl ester at C-2″ of the glucose followed from the ^{1}H NMR spectrum which revealed the anomeric proton H-1″ as a doublet at δ5.05 and an oxymethine triplet, deshielded because of the coumaroyl attachment, at δ4.80. Decoupling the latter collapsed H-1″ to a singlet, thereby placing it at C-2″. Compound E must therefore be assigned structure **3**.

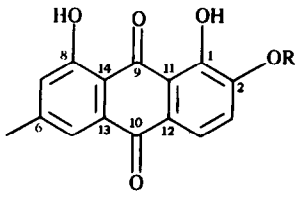
While both **2** and **3** have previously been recorded as derivatives of isolated compounds [3] this appears to be the first time that either has been reported as a natural product. The mp recorded here for **3** (192–194°) differs from that reported [3] for amorphous material (148–150°).

The EIMS of compound A showed an M^+ analysing for $C_{15}H_{10}O_5$ with a loss of 2 × CO, characteristic for an anthraquinone [4]. The ^{1}H NMR spectrum revealed resonances for all ten protons as an aromatic methyl, four aromatic protons (two *ortho*-coupled and two as broad singlets), and three phenolic hydroxyl protons (two hydrogen bonded). A spin decoupling experiment on the methyl resonance showed long-range interactions with both aromatic singlets thus requiring placement of the methyl between these two protons. On this basis A was tentatively identified as 1,2,8-trihydroxy-6-methylanthraquinone (**4**) although the alternative 1,2,5-trihydroxy-7-methyl structure could not be discounted.

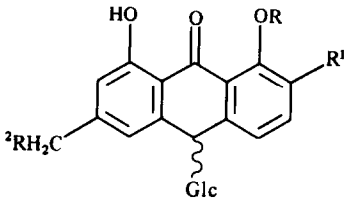
The identity of **4** was confirmed by an examination of compound D. This showed similar spectral characteristics but from both ^{1}H and ^{13}C NMR spectra was obviously an *O*-glycoside. The continuing presence of two H-bonded phenol protons and the pronounced shielding of H-3



1 R = Glc
2 R = H
3 R = 2-*p*-coumaroyl Glc



4 R = H
5 R = Glc



6 R = R^2 = H, R^1 = OH
7 R = Me, R^1 = OH, R^2 = H
8 R = R^1 = H, R^2 = OH

(from δ7.29 to 7.50) required placement of the sugar at C-2. Enzymic hydrolysis of D produced glucose and an aglycone identical with A. Peracetylation of D revealed H-1′ to H-4′ of the sugar to be axial confirming that it was β-D-glucose. A feature of the ^{13}C NMR spectrum of D was the non-equivalence of C-9 and C-10 (191.58 ppm and 180.17 ppm). This is comparable to emodin [5] and requires the association of both hydrogen-bonded hydroxyls with the same carbonyl. On this basis the glycoside must have structure **5** and the aglycone is confirmed as **4**.

The final compound (C) analysed for $C_{21}H_{22}O_9$ and gave a base peak ($C_{15}H_{12}O_4$) for loss of a hexose, suggesting an anthrone *C*-glycoside. This was substantiated by the UV and ^{13}C NMR spectra (194.02 *s* for C-9, 43.58 *d* for C-10, 78.33 ppm *d* for C-1′). The ^{1}H NMR spectrum revealed the same substitution pattern for the aglycone as found in **4** and **5** with an additional singlet at δ4.43 for H-10. C must therefore be **6** which has previously been reported as nataloin, a constituent of commercial Natal aloes [6]. However, a recent screening [7] of species that are cited as contributing to Natal aloes [8, 9] failed to reveal the presence of **6**.

Nataloin (**6**) would appear to represent the unidentified blue-staining band noted by Reynolds [1]. On this basis there seem to be three chemical varieties of *A. nyeriensis* which differ in their ability to produce anthracene derivatives based on **6** or on homonataloin (**7**) or barbaloin (**8**). The tetraploid *A. nyeriensis* is thought to have derived from a diploid ancestor close to *A. morijensis* Carter and Brandham [10]. This taxon and the most widely distributed chemical variety of *A. nyeriensis* produce **7** [1, 10] whilst varieties of the latter producing **6** and **8** are more localized. This suggests that in *A. nyeriensis* there may have been a loss of ability for methylation of hydroxy groups (**7** to **6** and **8**) and development of secondary oxidation at either C-2 (from **7** to **6**) or on the methyl substituent (from **7** to **8**).

EXPERIMENTAL

Mp's uncorr. UV: MeOH. ^{1}H NMR and ^{13}C NMR spectra run with TMS as int. standard. EIMS obtained by direct probe insert at 70 eV and at elevated temp.

Plant material. Aloe nyeriensis var. *kedongensis* was grown under glass at the Royal Botanic Gardens, Kew. A voucher specimen has been retained at the Herbarium (voucher BIOC-1).

Extraction and isolation of compounds. Fresh segmented leaves were soaked in MeOH overnight and the resulting extract filtered, conc. under red. pres. and freeze-dried (105 g). The freeze-dried exudate was dissolved in H_2O (1 l) and partitioned with 10 × 700 ml EtOAc. Concn of the EtOAc fraction gave a solid (35 g) which was chromatographed over silica gel (400 g) that had been previously washed with 5 M HCl and then water until neutral and dried at 105°. The column was developed with toluene (5 l), then toluene–$CHCl_3$ (5 l), $CHCl_3$ (5 l) and then by a $CHCl_3$–MeOH (0–100%) gradient. Column fractions were screened by silica gel TLC (solvent, EtOAc–MeOH–H_2O 100:16.5:13.5). Fractions 31–60, eluted with $CHCl_3$–toluene yielded **4** (12 mg). Fractions 142–152, eluted with 2% MeOH in $CHCl_3$, were bulked and rechromatographed over polyamide eluting with 20% aq. Me_2CO to give **2** (60 mg). Fractions 224–235, eluted with 7.5% MeOH in $CHCl_3$, were bulked and recrystallized from Me_2CO to give **6** (120 mg). Elution with 10% MeOH in $CHCl_3$ gave three compounds, each of which was recrystallized from MeOH; **5** (160 mg) from fractions 239–251, **3** (500 mg) from fractions 252–255, and **1** (3.5 g) from fractions 261–265.

Aloenin (**1**). Prisms from MeOH, mp 200–202°. UV λ_{max} nm: 230, 303; (+NaOH) 247, 346. ^{1}H NMR (Me_2SO-d_6, 250 MHz) δ:2.11 (3H, *s*, 6′-Me), 3.04–3.69 (6H, *m*, CHOH, CH_2OH), 3.83 (3H, *s*, 4-OMe), 4.79 (1H, *d*, J = 7.4 Hz, H-1″), 5.59, 6.25 (2H, *ABq*, J = 2.2 Hz, H-3, H-5), 6.34 (1H, *br s*, H-5′), 6.48 (1H, *d*, J = 2.0 Hz, H-3′). EIMS (*m/z*, rel. int.): 262 $[C_{14}H_{14}O_5]^+$ (65), 248 (100), 151 (29), 150 (23), 125 (2), 69 (17).

Aloenin aglycone (**2**). Obtained as an amorphous solid. Found: M^+ 248.0690; $C_{13}H_{12}O_5$ requires 248.0685. UV λ_{max} nm: 247,

300; (+NaOH) 250, 340. ^{1}H NMR (Me_2SO-d_6, 250 MHz) δ:2.08 (3H, *s*, 6′-Me), 3.82 (3H, *s*, 4-OMe), 5.59, 6.11 (2H, AB*q*, J = 2.2 Hz, H-3, H-5), 6.16 (1H, *br s*, H-5′), 6.23 (1H, *d*, J = 2.0 Hz, H-3′), 9.59, 9.65 (2 × 1H, 2 × *br s*, 2 × OH). EIMS (*m/z*, rel. int.): 248 (100), 151 (40), 150 (40), 125 (8), 69 (9). **2** (20 mg) was acetylated with Ac_2O (2 ml) and 4-dimethylaminopyridine (30 mg) in pyridine (1 ml) at 50°. The normal work-up gave 2-diacetate as a colourless oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 2.20, 2.29 (2 × 3H, 2 × *s*, 2 × Ac), 2.34 (3H, *s*, 6′-Me), 3.86 (3H, *s*, 4-OMe), 5.54, 6.00 (2H, AB*q*, J = 2.2 Hz, H-3, H-5), 6.85 (1H, *br s*, H-5′), 6.94 (1H, *d*, J = 2.4 Hz, H-3′).

Aloenin-2″-p-coumaroyl ester (**3**). Needles from Me_2CO, mp 192–194°. UV λ_{max} nm: 225, 310; (+NaOH) 244, 358. ^{1}H NMR (Me_2SO-d_6, 250 MHz) δ: 2.03 (3H, *s*, 6′-Me), 3.30–3.80 (5H, *m*, CHOH, CH_2OH), 3.71 (3H, *s*, 4-OMe), 4.71 (1H, *s*, CH_2OH), 4.80 (1H, *t*, J = 8.8 Hz, H-2″), 5.05 (1H, *d*, J = 8.1 Hz, H-1″), 5.26–5.34 (2H, *m*, CHOH), 5.53, 5.83 (2H, AB*q*, J = 2.2 Hz, H-3, H-5), 6.20, 7.43 (2H, AB*q*, J = 15.9 Hz, H-2‴, H-3‴), 6.35 (1H, *br s*, H-5′), 6.49 (1H, *d*, J = 2.0 Hz, H-3′), 6.79 (2H, *d*, J = 8.5 Hz, H-6‴, H-8‴), 7.50 (2H, *d*, J = 8.5 Hz, H-5‴, H-9‴), 9.92 (2H, *s*, 2 × OH). EIMS (*m/z*, rel. int.): 308 $[C_{15}H_{16}O_7]^+$ (11), 248 (100), 164 $[C_9H_8O_3]^+$ (59), 151 (83), 150 (81), 147 $[C_9H_7O_2]^+$ (99), 125 (31), 119 (51), 69 (46).

1,2,8-*Trihydroxy-6-methylanthraquinone* (*nataloe-emodin*) (**4**). Orange needles from MeOH, mp 140–142°. Found: M^+ 270.0457; $C_{15}H_{10}O_5$ requires 270.0528. UV λ_{max} nm: 299, 402, 545; (+$AlCl_3$) 299, 480; (+NaOH) 245, 355, 525). ^{1}H NMR (Me_2CO-d_6, 250 MHz) δ: 2.47 (3H, *br s*, 6-Me), 7.14 (1H, *br s*, H-7), 7.29, 7.74 (2H, AB*q*, J = 8.1 Hz, H-3, H-4), 7.59 (1H, *br s*, H-5), 9.59 (1H, *br s*, 2-OH), 11.93, 12.18 (2 × 1H, 2 × *s*, 1-OH and 8-OH). EIMS (*m/z*, rel. int.): 270 (100), 242 (33), 214 (8), 139 (36).

2-O-*β*-D-*glucopyranosyl*-1,2,8-*trihydroxy*-6-*methylanthraquinone* (**5**). Yellow needles from MeOH, mp 236–238° (decomp). UV λ_{max} nm: 229, 259, 295, 428; (+$AlCl_3$) 234, 260, 300, 492; (+NaOH) 240, 288, 504. ^{1}H NMR (Me_2SO-d_6, 360 MHz) δ: 2.34 (3H, *br s*, 6-Me), 3.20–3.60 (6H, *m*, CHOH, CH_2OH), 5.16 (1H, *d*, J = 7.3 Hz, H-1″), 7.00 (1H, *dd*, J × 1.5, 0.9 Hz, H-7), 7.33 (1H, *br s*, H-5), 7.49, 7.56 (2H, AB*q*, J = 8.5 Hz, H-3, H-4), 11.71, 12.04 (2 × 1H, 2 × *s*, 2 × OH). ^{13}C NMR (Me_2SO-d_6, 90.56 MHz) ppm: *s* at 113.8 (C-11), 116.0 (C-14), 125.8 (C-13), 133.1 (C-12), 149.3 (C-6), 151.3, 151.7 (C-1, C-2), 161.6 (C-8), 180.2 (C-10), 191.6 (C-9), *d* at 69.7 (C-4′), 73.2 (C-2′), 76.8 (C-5′), 77.3 (C-3′), 99.8 (C-1′), 120.3, 120.3, 120.5 (C-3, C-4, C-5), 123.6 (C-7), *t* at 60.7 (C-6′), *q* at 21.7 (6-Me). EIMS (*m/z*, rel. int.): 270 (100), 242 (4), 213 (2), 139 (4). **5** (15 mg) dissolved in 50% aq. MeOH was incubated overnight at 37° with *β*-glucosidase. The resulting aglycone was partitioned into $CHCl_3$ and found to be identical (UV, TLC, ^{1}H NMR) to **4**. Acetylation of **5** (25 mg) as described under **2** yielded the hexa-acetate as a yellow oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ:2.05, 2.06, 2.08, 2.10 (4 × 3H, 4 × *s*, 2′-, 3′-, 4′-, 6′-OAc), 2.41, 2.43 (2 × 3H, 2 × *s*, 2 × Ar–O–Ac), 2.50 (3H, *br s*, 6-Me), 3.95 (1H, *m*, H-5′), 4.20 (1H, *dd*, J = 12.2, 2.2 Hz, H-6′), 4.30 (1H, *dd*, J = 12.2, 5.4 Hz, H-6′), 5.16 (2H, 2 × *t*, J = *ca* 9.6 Hz, H-2′, H-4′), 5.32 (1H, *t*, J = 8.9 Hz, H-3′), 5.36 (1H, *d*, J = 6.4 Hz, H-1′), 7.20 (1H, *br s*, H-5), 7.38, 8.18 (2H, AB*q*, J = 8.5 Hz, H-3, H-4), 8.01 (1H, *br s*, H-7).

Nataloin (**6**). Isolated as an amorphous solid, mp 184–188°. Found: M^+ 418.1235; $C_{21}H_{22}O_9$ 418.1264. UV λ_{max} nm: 263, 273, 306, 357; (+$AlCl_3$) 278, 340, 390. ^{1}H NMR (Me_2SO-d_6, 250 MHz) δ: 2.34 (3H, *br s*, 6-Me), 2.66–3.00 (5H, CHOH, CH_2OH), 3.22 (1H, *d*, J = 8.8 Hz, H-1′), 3.92 (1H, *t*, J = 5.3 Hz, CH_2OH), 4.43 (1H, *s*, H-10), 4.73–5.14 (3 × 1H, 3 × *d*, J = 4.6, 4.6, 5.6 Hz, 3 × CHOH), 6.69 (1H, *br s*, H-5), 6.86, 7.06 (2H, AB*q*, J = 8.3 Hz, H-4, H-3), 6.90 (1H, *br s*, H-7), 9.30 (1H, *s*, 2-OH), 11.80, 11.86 (2 × 1H, 2 × *s*, 2 × OH). ^{13}C NMR (Me_2SO-d_6, 90.56 MHz) ppm: *s* at 115.8 (C-11), 118.2 (C-14), 134.8 (C-13), 142.8 (C-12), 144.1 (C-2), 146.6 (C-1), 149.6 (C-6), 161.4 (C-8), 194.0 (C-9), *d* at 43.6 (C-10), 70.4, 70.5 (C-2′, C-4′), 78.3 (C-1′), 80.8 (C-3′), 85.5 (C-5′), 115.3 (C-5), 117.3 (C-4), 121.3, 121.8 (C-3, C-7), *t* at 61.6 (C-6′), *q* at 21.8 (6-Me). EIMS (*m/z*, rel. int.): 418 (6), 256 (100), 227 (15), 152 (14).

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