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STUDIES ON ALOE, PART 10.[1] FEROXINS A AND B, TWO *O*-GLUCOSYLATED 1-METHYLTETRALINS FROM CAPE ALOE

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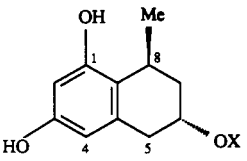
ABSTRACT.—Two new *O*-glucosylated 1-methyltetralins, feroxins A [**2**] and B [**3**], were isolated from a commercial sample of Cape aloe. Their structures and preferred conformations in solution were determined by spectroscopic methods and chemical transformations.

Aloe (or bitter aloes) is a natural drug well known since ancient times for its cathartic properties (2). It is the dried exudate from the cut leaves of *Aloe* species (Liliaceae) (3). The latex of *Aloe ferox* Miller and hybrids of this species with *Aloe africana* Miller and *Aloe spicata* Baker is known in commerce as Cape aloe (4).

Although aloe is also used as a bittering agent in alcoholic beverages (5), its chemical composition is far from being completely known (3). The main constituents appear to belong to the structural families of 2-acetonyl-5-methylchromones (6), 1,8-dihydroxy-9-anthrones (7) or 1,8-dihydroxy-9,10-anthraquinones (3). Recently, we reported the isolation and chemical characterization of a new constituent of Cape aloe having the 1-methyltetralin skeleton, i.e., feroxidin (8); its absolute stereochemistry was proven to be 6*S*,8*S*, as in formula **1**, by chemical correlaton (1). The present paper deals with the structural elucidation of two *O*-glucosylated derivatives of **1** which we isolated from commercial Cape aloe and named feroxin A [**2**] and feroxin B [**3**].



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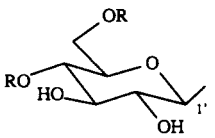
2 X= 

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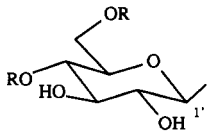
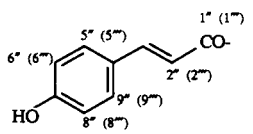
3 X= 

R= 

[1]For Part 9, see G. Speranza *et al.* (1).

RESULTS AND DISCUSSION

After addition of $CHCl_3$-hexane (10:3) to an Me_2CO extract of Cape aloe, a brown precipitate was formed. This insoluble material, when chromatographed on Si gel using gradient elution with $CHCl_3$ and EtOAc, gave crude feroxin A which was purified by preparative tlc. Similar flash chromatography of the mother liquor followed by dccc and semi-preparative hplc afforded feroxin B.

Feroxin A [**2**] was obtained from the drug in much smaller amounts than feroxin B. However, since feroxin A was shown to be identical in all respects with the product resulting from alkaline hydrolysis of feroxin B, we used feroxin B as a source of feroxin A. Feroxin A, $C_{17}H_{24}O_8$ (by elemental analysis and fabms), exhibited ^1H- and ^{13}C-nmr spectra (Tables 1 and 2) which appeared strongly reminiscent of those of feroxidin (8). Additional signals in the ranges 3.1–4.6 δ and 60–105 δ for ^1H and ^{13}C, respectively, suggested the presence of an *O*-linked hexose residue, presumably a β-D-glucopyranosyl group (9,10). This indication was strongly supported by a comparison with nmr data obtained for methyl β-D-glucopyranoside (Table 3).

Definitive proof that feroxin A [**2**] is a β-glucoside of feroxidin came from its enzymatic (β-glucosidase) hydrolysis, giving the expected aglycone **1** (1,8) and β-D-glucose. The linkage of the carbohydrate residue to the hydroxyl group in the 6 position

TABLE 1. ^1H-nmr (300 MHz) Assignments (δ) of Feroxins A [**2**] and B [**3**] in CD_3OD at 25°.[a]

Proton	Compound	
	2	**3**
H-2	6.10 (d, 2.3)[b]	6.10 (d, 2.3)[b]
H-4	6.03 (d, 2.3)[b]	6.04 (d, 2.3)[b]
$H_{e'}$-5	3.09 (ddd, 16.1, 5.5, 1.6)	3.10 (ddd, 16.0, 6.2, 1.8)
$H_{a'}$-5	2.58 (dd, 16.1, 10.2)	2.61 (dd, 16.0, 10.3)
H_a-6	4.23–4.34 (m)	4.18–4.36 (m)
H_e-7	2.05 (dm, 12.3)	2.05 (dm, 11.6)
H_a-7	1.78 (ddd, 12.3, 12.1, 5.9)	1.79 (ddd, 11.6, 11.6, 5.8)
$H_{e'}$-8	3.14–3.22 (m)[c]	3.18 (ddq, 5.8, 1.6, 6.9)
$Me_{a'}$-8	1.19 (d, 7.0)	1.16 (d, 6.9)
H-1′	4.50 (d, 7.9)	4.61 (d, 7.8)
H-2′	3.18 (dd, 7.9, 8.9)	3.34 (dd, 7.8, 9.3)
H-3′	3.38 (dd, 8.9)	3.69 (dd, 9.3, 9.2)
H-4′	3.28–3.33 (m)[d]	4.96 (dd, 9.2, 9.6)
H-5′	3.28–3.33 (m)[d]	3.80–3.89 (m)
H_a-6′	3.87 (dd, 11.9, 1.7)	4.18–4.36 (m)
H_b-6′	3.68 (dd, 11.9, 5.1)	4.18–4.36 (m)
H-2″		6.24 (d, 15.9)
H-3″		7.57 (d, 15.9)
H-5″, H-9″		7.29 (d, 8.7)
H-6″, H-8″		6.71 (d, 8.7)
H-2‴		6.37 (d, 15.9)[e]
H-3‴		7.67 (d, 15.9)
H-5‴, H-9‴		7.45 (d, 8.7)[e]
H-6‴, H-8‴		6.79 (d, 8.7)

[a]Splitting patterns and *J* values (Hz) are given in parentheses; assignments were supported by homonuclear experiments; a = axial; e = equatorial; a′ = pseudoaxial; e′ = pseudoequatorial.

[b]Based on nOe experiments. See Figure 1.

[c]Obscured by the signal of H-2′.

[d]Buried under the solvent signal.

[e]Related by nOe enhancements. See text.

TABLE 2. ^{13}C-nmr (75.47 MHz) Assignments (δ) of Feroxins A [**2**] and B [**3**] in CD_3OD at 25°.[a]

Carbon	Compound	
	2	**3**
C-1	156.96[b]	156.97[b]
C-2	101.46	101.55
C-3	156.86[b]	156.85[b]
C-4	107.64	107.63
C-4a	136.96	136.90
C-5	37.32	37.34
C-6	72.71	73.37
C-7	39.00	39.12
C-8	29.29	29.31
C-8a	120.74	120.72
8-Me	21.93	22.03
C-1′	102.58	102.97
C-2′	75.18	75.26
C-3′	78.09	75.70
C-4′	71.60	73.14
C-5′	77.88	73.00
C-6′	62.69	64.52
C-1″		166.44[c]
C-2″		114.74[d]
C-3″		146.96[e]
C-4″		127.00[f]
C-5″, -9″		131.17[g]
C-6″, -8″		116.63[h]
C-7″		161.21[i]
C-1‴		166.71[c]
C-2‴		114.84[d]
C-3‴		147.31[e]
C-4‴		127.11[f]
C-5‴, -9‴		131.30[g]
C-6‴, -8‴		116.79[h]
C-7‴		161.39[i]

[a]Multiplicities were obtained from DEPT spectra. Assignments are based on ^{1}H-^{13}C COSY and comparison with those of feroxidin [**1**] (8), of methyl (*E*)-*p*-coumarate, and of methyl β-D-glucopyranoside (Table 3).

[b–i]Signals with the same superscript are interchangeable.

could then be inferred by nmr data. Glycosylation shifts [$\Delta\delta = \delta$(glycoside) − δ(aglycone) (8)] were found to be typical of a secondary alcoholic function flanked by two methylene groups (11–17), i.e., in CD_3OD Δδ C-6 +7.88, Δδ C-5 −3.07, Δδ C-7 −1.80, Δδ H_a-6 +0.21, Δδ H_e′-5 +0.18, Δδ H_a′-5 +0.10, Δδ H_e-7 +0.17, Δδ H_a-7 +0.11. On the other hand, no significant differences ($\Delta\delta < 0.1$) appeared for ^{1}H and ^{13}C signals corresponding to the other nuclei of the tetralin moiety.

In addition, significant nOe associations were observed between the anomeric proton (H-1′) and both H_a-6 and H_e′-5 (Figure 1). Thus, the structure of (6*S*,8*S*)-6-*O*-β-D-glucopyranosyl-1,3-dihydroxy-8-methyl-5,6,7,8-tetrahydronaphthalene [**2**] is to be assigned to feroxin A.

TABLE 3. ^{1}H- (300 MHz) and ^{13}C-Nmr (75.47 MHz) Data of Methyl β-D-Glucopyranoside in CD_3OD at 25°.[a]

Proton		Carbon	
H-1	4.17 (d, 7.78)	C-1	105.32
H-2	3.15 (dd, 7.78, 9.13)	C-2	74.99
H-3	3.35 (dd, 9.13)	C-3	77.97
H-4	3.26–3.31 (m)	C-4	71.56
H-5	3.26–3.31 (m)	C-5	77.86
H_a-6	3.87 (dd, 11.92, 1.60)	C-6	62.60
H_b-6	3.66 (dd, 11.92, 5.24)		
OMe	3.52 (s)	OMe	57.29

[a]Splitting patterns and J values (Hz) are given in parentheses.

Comparative inspection of ^{1}H- and ^{13}C-nmr spectra of feroxin B [**3**] and feroxin A [**2**] (Tables 1 and 2) showed strong similarities between the two compounds and the presence of two additional coumaroyl groups (6) in the former. In agreement with this observation, the negative ion fabms spectrum of feroxin B exhibited ions at *m/z* 647 $[M-H]^-$, 501 $[M-p\text{-coumaroyl}]^-$, while the base peak at *m/z* 455 in the positive ion fabms spectrum was consistent with a hexose residue bearing two *p*-coumaroyl groups.

Alkaline hydrolysis of feroxin B afforded approximately 2 mol of *p*-coumaric acid per mol of feroxin A. Remarkably, the acylated compound appeared to be resistant to enzymatic (β-glucosidase) as well as mild acid hydrolysis.

The location of the two *p*-coumaroyl groups at the 4′-*O* and 6′-*O* positions of the sugar moiety of feroxin B [**3**] rested on the following spectroscopic evidence. A marked "acylation" effect on ^{13}C chemical shifts of C-3′, C-4′, C-5′, and C-6′ was observed going from feroxin A [**2**] to feroxin B [**3**] ($\Delta\delta$ −2.39, +1.54, −4.88, and +1.83, re-

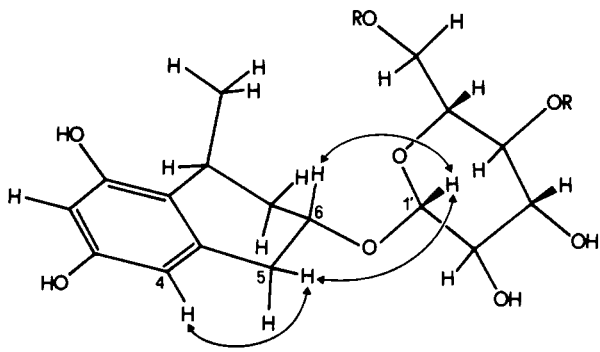


FIGURE 1. Preferred conformation of feroxin A (R=H) [**2**] showing selected ^{1}H nOe associations (MeOH). The same conformation is typical of all related tetralins mentioned in this paper, i.e., feroxidin [**1**] (1) and feroxin B [R=(*E*)-*p*-coumarate] [**3**]. NOe enhancements in feroxin A [**2**] and B [**3**] (%, respectively): from H-1′ to H_a-6 (6.0, not observed) and to H_e′-5 (4.8, 2.4); from H_a-6 to H-1′ (4.4, 2.4); from H_e′-5 to H-1′ (not observed, 1.4) and to H-4 (4.2, 5.1); from H-4 to H_e′-5 (5.1, 3.1). Additional nOe associations are reported in the text.

spectively) (Table 2). In addition H-4′, H_a-6′ and H_b-6′ resonances in **3** appeared downfield-shifted with respect to the corresponding signals in feroxin A [**2**] in the ^{1}H-nmr spectrum (Table 1).

It can be noted that nOe enhancements were clearly observed for protons of one of the two *p*-coumaroyl groups, i.e., from H-2‴ to H-5‴, H-9‴ (2.1%), and from H-5‴, H-9‴ to H-3‴ (2.3%) and H-2‴ (6.1%). This fact allowed unequivocal chemical shift assignments to both acyl residues (Table 1).

The ^{13}C glucosylation shifts observed in these feroxins are in agreement with empirical rules (11–16) relating frequency differences to the chirality of glycosylated secondary alcohols.

In glucopyranoside, the exo-anomeric effect (18) offers an important resistance to rotation about the glycosidic bond (19). Referring to the segment O-5′–C-1′–O-1′–C_α in the general formula **4** (Figure 2), experimental data (18) as well as hard-sphere calculations (19) indicate a strong preference of the aglycone carbon (C_α) for the synclinal position at the C-1′–O-1′ bond rotational potential specified by a torsional angle Φ ranging from -60 to $-90°$ (the latter value being due to a possible sp^2 hybridization of the glucosidic oxygen atom) (19). Thus, the predominant conformation of an alkyl β-D-glucopyranoside relates to the rotational preference of the central bond in the segment H_α–C_α–OΔ1′–C-1′ (specified by the torsional angle Ψ^H in **4**). In the case of β-D-glucopyranosides of sterically unhindered (13,14) secondary alcohols, the most stable conformation is characterized by $\Psi^H \cong 0°$ (19). However, when one or two substituents are located at the β (pro-*R*) carbon of the aglyconic moiety, the conformation of the glucosidic linkage changes as the alcoholic moiety rotates in order to avoid steric nonbonded interactions. Such rotamers having $\Psi^H > 0$ are defined as corresponding to a sterically hindered case I (13,14). An opposite conformational change occurs when one or two substituents are located at the β (pro-*S*) carbon of the aglycone moiety, and the corresponding rotamers ($\Psi^H < 0$) are referred to as a sterically hindered case II (13,14). It has been found the β-D-glucosidation shifts (in pyridine-d_5) are characteristic of each case and are of diagnostic value for determining the absolute configuration of secondary alcohols.

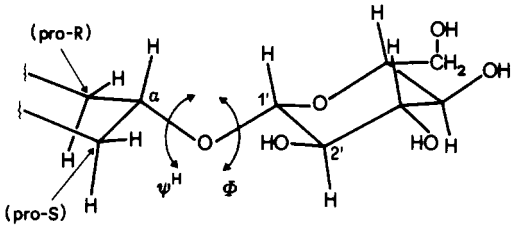


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FIGURE 2. Schematized representation of a secondary alcohol β-D-glucoside showing conventional symbols (for definitions, see text).

The negative $\Delta\delta$ (C_β) values are larger for the β-carbon *anti* to the pyranose-ring oxygen (pro-*S* carbon in **4**) than for the *syn*-carbon (pro-*R*) in alcohols corresponding to the sterically unhindered (S.U.) case or to the sterically hindered case I (S.H.I.). Opposite relationships are displayed by alcohols belonging to the sterically hindered case II (S.H.II) (13,14). In addition, typical $\Delta\delta_S$ (C-1′) [defined as δ (alcoholic glycoside) − δ (methylglycoside)] were found to be -2.61 ± 1, -4.2 ± 1, 0 ± 1.5 ppm (in pyridine-d_5) for β-D-glucosides of the S.U., S.H.I, or S.H.II family, respectively (13,14).

On the basis of the rules quoted above, the glucosidation shifts in feroxins in CD_3OD [see above for $\Delta\delta$ (C_β) of feroxin A, and Tables 2 and 3] are consistent with the 6*S* configuration as shown by Speranza *et al.* (1), provided that these glucosides are referred to the S.U. (or S.H.I) case.

In fact, given the structure of C_α environment in the feroxins [-CH_2-C_αH(OR)-CH_2-], no significant steric interactions are expected to occur between sugar and aglyconic moieties. Thus, a time-averaged conformation characterized by $-90° < \Phi < -60°$ and Ψ^H 0° can be reasonably assumed. Two facts further support this assumption: (i) a significant nOe association between the anomeric proton and $H_{e'}$-5 in both feroxins A and B (Figure 1); (ii) additional nOe associations between the Me group at C-8 and protons belonging to the more deshielded coumarate group H-5‴, H-9‴, H-2‴. These nOe enhancements are best explained if the involved coumarate residue is linked to the hydroxymethyl group (C-6′) and Φ and Ψ^H torsional angles take on values as assumed above.

The esterification of the 2-hydroxyl group of a glucose residue by *p*-coumaric acid is very common in Cape aloe (6). However, the presence of two *p*-coumaroyl groups at the 4- and 6-positions of the same carbohydrate unit is a novelty in *A. ferox* metabolites and appears to be rare in nature (20).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter with a 10 cm microcell. Fabms spectra were recorded on a VG 7070 EQ mass spectrometer and uv spectra on a Perkin-Elmer 554 instrument. Microanalyses were obtained with a Perkin-Elmer 240 elemental analyser. ^{1}H- (300 MHz) and ^{13}C-nmr (75.47 MHz) spectra were recorded on a Bruker AC 300 spectrometer equipped with an ASPECT 3000 computer in CD_3OD, using the solvent signal as internal reference (3.30 and 49.00 ppm from TMS for ^{1}H and ^{13}C, respectively). NOe and ^{1}H-^{13}C COSY experiments were performed by using standard Bruker software (DISNMR version 88). A flip angle of 135° was used for DEPT experiments. Analytical and preparative tlc was performed on Si gel F_{254} pre-coated plates (0.2 and 1.0 mm layer, respectively); spots were visualized by spraying with 0.5% Fast Blue B salt, followed by heating to 140°. Commercial Merck Si gel 60 (230–400 mesh) was used for flash chromatography. Dccc was carried out on a Büchi Model 670 equipped with 300 standard glass tubes (40 cm × 2.7 cm i.d.). Analytical and semi-preparative hplc were performed on a Perkin-Elmer Series 3B liquid chromatograph connected to a variable wavelength uv detector (Perkin-Elmer LC 85 Spectrophotometric Detector). Analytical conditions: column 125 × 4 mm, LiChrospher 100 RP-18, 5 μm; flow rate 1 ml/min; detector λ 230 nm; eluent MeCN/H_2O, linear gradient from 10 to 30% MeCN in 15 min for feroxin A and from 30 to 50% MeCN in 15 min for feroxin B. Semi-preparative conditions: column 250 × 25 mm, LiChrosorb RP-18, 7 μm; flow rate 15 ml/min; detector λ 250; eluent MeCN/H_2O, linear gradient from 30 to 50% MeCN in 15 min.

PLANT MATERIAL.—Commercial Cape aloe used in this investigation was purchased from Pan-African Corporation (Cape Town, South Africa), and a voucher specimen is preserved in our laboratories.

EXTRACTION.—Powdered Cape aloe (1 kg) was extracted with Me_2CO (1 liter) for 3 h at 40° with vigorous mechanical stirring. The Me_2CO extract was then treated with $CHCl_3$ (1 liter) and hexane (300 ml), and the resulting mixture was left to stand overnight at room temperature. Filtration of insoluble material that had separated out gave residue I, 4.5 g, and removal of the solvent under reduced pressure gave a brown syrup (residue II, 12 g).

ISOLATION OF FEROXIN A [**2**].—Residue I (4.5 g) was flash chromatographed (Si gel, 1 kg) eluting with solvent of increasing polarity from $CHCl_3$ through EtOAc and increasing amounts of MeOH in EtOAc. Similar fractions were combined on the basis of tlc analysis [eluent EtOAc-MeOH (8:2)] and further purified by preparative tlc (eluent as above) to give 8 mg of feroxin A [**2**], pure on tlc (R_f 0.29) and analytical hplc (Rt 4.7 min): mp 133–136°; $[\alpha]^{20}_D$ −35.3 (c = 0.096, MeOH); uv λ max (MeOH) (log ε) 228 sh (3.77), 278–286 nm (3.31); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; positive ion fabms *m/z* $[M + Na]^+$ 379, $[M + H]^+$ 357. Found C 57.12, H 6.54; calcd for $C_{17}H_{24}O_8$, C 57.29, H 6.79.

ISOLATION OF FEROXIN B [**3**].—Residue II (6 g) was subjected to flash chromatography (Si gel, 1.2 kg) using $CHCl_3$ containing increasing amounts of EtOAc as eluent. Separation was monitored by tlc [eluent EtOAc-$CHCl_3$-HOAc (100:30:5)], and fractions containing feroxin B (R_f 0.4) were combined

(900 mg) and further purified by dccc [$CHCl_3$-MeOH-H_2O (4:4:3), ascending mode, 15 ml/h]. Final isolation by semi-preparative hplc (eluent MeCN/H_2O, linear gradient from 35 to 90% in 30 min; Rt 15.6 min) afforded **3** (52 mg) as an amorphous solid, which was found to be pure on tlc (eluent as above) and analytical hplc (Rt 4.8 min): mp 125–126°; $[\alpha]^{20}D$ −14.0 (c = 0.05, MeOH); uv λ max (MeOH) (log ε) 225 (4.53), 286 sh (4.40), 297 sh (4.45), 310 nm (4.52); ^1H nmr see Table 1; ^{13}C nmr see Table 2; positive ion fabms *m/z* $[M + Na]^+$ 671, $[M + H]^+$ 649, $[C_{24}H_{23}O_9]^+$ 455; negative ion fabms *m/z* $[M - H]^-$ 647, $[M - C_9H_7O_2]$ 501. Found C 64.49, H 5.23; calcd for $C_{35}H_{36}O_{12}$, C 64.81, H 5.59.

HYDROLYSIS OF FEROXIN B [3] TO FEROXIN A [2].—Feroxin B (30 mg) dissolved in 1 N NaOH (3 ml) was kept at room temperature overnight under N_2. After neutralization with concentrated HCl, the reaction mixture was desalted by passing through a column of Amberlite XAD-7 (wet volume, 50 ml). Elution with MeOH, evaporation of the solvent, and separation by semi-preparative hplc (eluent MeCN/H_2O, linear gradient from 10 to 40% MeCN in 20 min) yielded a compound (Rt 8 min, 13.2 mg) that was found chromatographically (tlc, hplc) and spectrally (fabms, ^1H and ^{13}C nmr) identical with feroxin A [2] isolated as described above. Besides **2**, 12 mg of *p*-coumaric acid (Rt 14 min) was obtained.

ENZYMATIC HYDROLYSIS OF FEROXIN A [2] TO FEROXIDIN [1].—β-Glucosidase (almond emulsin, Boehringer, 5 mg) was added to a solution of feroxin A (10 mg) in H_2O (5 ml), and the mixture was incubated at 37° overnight. After adding MeOH, the solution was filtered and concentrated under reduced pressure. Semi-preparative hplc (8) of the aqueous residue gave a product (4 mg) which was found to be identical in all respects with feroxidin [1] isolated from Cape aloe (1,8). D-Glucose was identified by tlc comparison with an authentic sample [eluent HOAc-$CHCl_3$-H_2O (35:30:5), detection by aniline-diphenylamine reagent] (21).

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