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FARALATROSIDE AND FARATROSIDE, TWO FLAVONOL TRIGLYCOSIDES FROM *COLUBRINA FARALAOTRA*

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Key Word Index—*Colubrina faralaotra*; Rhamnaceae; leaf flavonoids; kaempferol and quercetin triglycosides.

Abstract—Two new flavonol triosides have been isolated from the leaves of *Colubrina faralaotra* (Rhamnaceae) and their structures elucidated as kaempferol-3-*O*-[β-D-glucopyranosyl-(1 → 3)-4‴-*O*-acetyl-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside] and the corresponding quercetin analogue mainly by ^{1}H and ^{13}C NMR spectroscopy (including T_1 measurements).

INTRODUCTION

Initial chemical investigations of *Colubrina faralaotra* var. *faralaotra*, a member of the Rhamnaceae from Madagascar, led to the isolation and structure elucidation of some aporphine alkaloids [1] as well as the detection of phenolic acids and flavonoids. The flavonoid aglycones were identified as kaempferol, quercetin and myricetin [2]. The present communication describes the isolation and structural elucidation of two new flavonol-*O*-triosides, faralatroside and faratroside, which are derived from kaempferol and quercetin, respectively.

RESULTS AND DISCUSSION

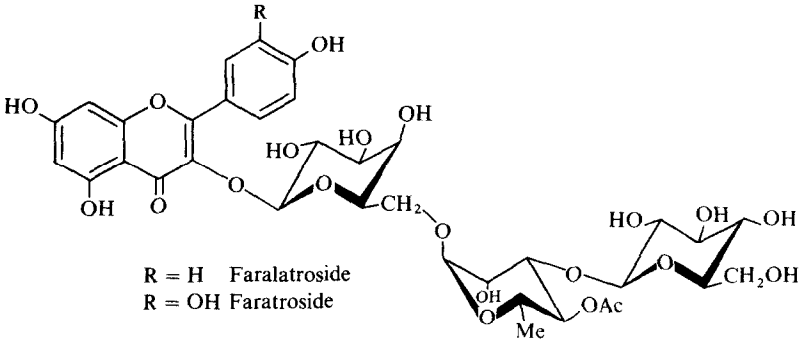
The residual methanol extract concentrate of the leaves was extracted successively with chloroform and butanol. The butanol phase was evaporated to dryness and the flavonoid and saponin fractions were separated on a Sephadex LH20 column using $CHCl_3$–MeOH (3:7). The two flavonoid glycosides were then separated on a silica gel H column using $CHCl_3$–MeOH–H_2O (64:36:8) as eluant. The first glycoside, faralatroside, was obtained in crystalline form from MeOH, mp 197–199°, whereas the second could only be isolated in amorphous form, mp 185°.

Structure of faralatroside

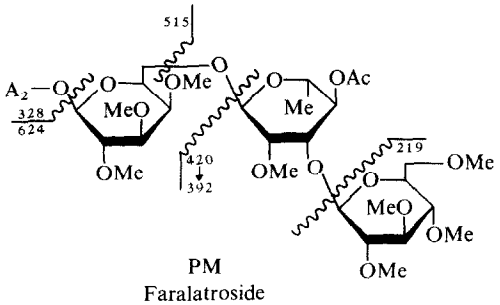
The acid hydrolysis products of faralatroside showed the presence of kaempferol and the three sugars glucose, galactose and rhamnose on chromatography. Kaempferol was also identified by UV and NMR spectroscopy. The ^{1}H NMR spectrum of faralatroside peracetate showed the presence of three phenolic and nine sugar acetoxyl signals indicating that the aglycone was linked to a trisaccharide moiety. The UV shift on the addition of NaOAc and $AlCl_3$ showed the position of linkage between sugar and aglycone as C-3 [3]. The appearance of a singlet at δ 2.06 ppm in the ^{1}H NMR spectrum of faralatroside and an ester carbonyl band at 1747 cm^{-1} in the IR spectrum was characteristic of an acetyl group located in the sugar moiety. The MS of faralatroside permethyl ether showed fragmentation at *m/z* 111, 155, 187, 219 and 515 showing that the terminal sugar in the oligosaccharide part was a hexose [4]. A fragment ion observed at *m/z* 420 indicated that this terminal hexose was linked to the acetylated rhamnose moiety. The second hexose must therefore be directly linked to the aglycone. Loss of the acetyl group during the methylation would have resulted in a fragment ion at *m/z* 392.

The sugar sequence and anomeric configuration were determined by ^{13}C NMR spectroscopy. The chemical shift values of the three anomeric carbon atoms, 102 ppm ($^1J_{CH}$ = 165 Hz), 100.5 ppm ($^1J_{CH}$ = 171.5 Hz), and 103.9 ppm ($^1J_{CH}$ = 160 Hz), as well as their $^1J_{CH}$ values indicate that the signal at 100.5 is that of the α-L-rhamnose while the other two are the signals of β-linked hexoses [5]. The two signals at δ 66.5 and 60.7 ppm, exhibiting triplets in the off-resonance spectrum, must be assigned to the oxymethylene carbons C-6″ and C-6⁗ of the two hexose units. The signal at lower field (66.5 ppm) indicates that C-6″-OH-group of the hexose directly attached to the aglycone is linked to the mono-acetylated rhamnose unit (see Table 1).

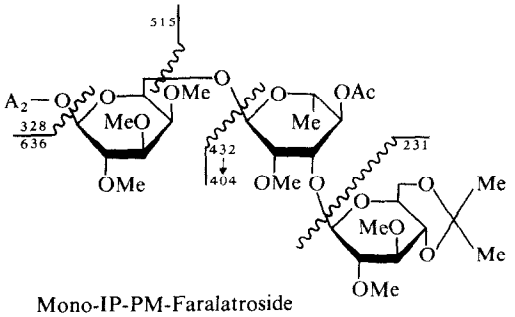
The sugar sequence and final assignments of all signals were determined by relaxation time (T_1) measurements. It has been reported [6] in the case of a cardiac glycoside with a linear oligosaccharide chain that the average NT_1 values for the sugar carbons in each sugar unit increase with increasing distance from the aglycone moiety. This is due to segmental motion in the oligosaccharide chain with the aglycone part exhibiting an anchoring effect. The signals at 76.5 and 76.3 ppm in the ^{13}C NMR spectrum of faralatroside, which can only be assigned to C-3 and C-5 in a β-D-glucoside moiety not carrying an acyl group at C-2 and C-4, correspond to carbon atoms having NT_1 values of 0.35 and 0.34 sec. These relatively larger values (see Table 2) and the relaxation-time gradient observed indicate that the glucose unit is terminal.



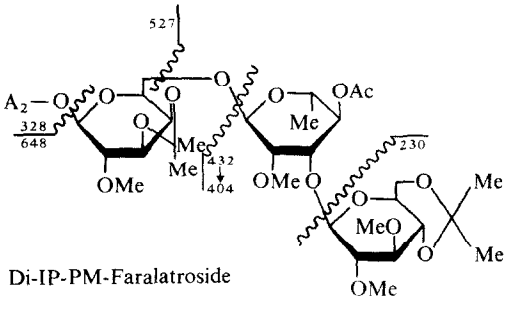
R = H Faralatroside
R = OH Faratroside



PM
Faralatroside



Mono-IP-PM-Faralatroside



Di-IP-PM-Faralatroside

The position of linkage between glucose and rhamnose as well as the site of acetylation was determined by a comparison of the rhamnose carbon chemical shifts in the spectra of faralatroside and its peracetate. The signal at 77.2 ppm in the spectrum of the former must be due to the carbon bearing the terminal *O*-β-D-glucosyl residue. In the spectrum of faralatroside peracetate the signal moves upfield to 74.5 ppm. At the same time, the signal for the anomeric carbon C-1‴, also moves upfield by 3.0 ppm (see Table 3) establishing that the hydroxy group on C-2‴ of the rhamnose is not substituted and that the terminal glucose must be linked to C-3‴ of the rhamnose unit, leaving position C-4‴ as the only possible site of the acetyl group in faralatroside. This is also consistent with the observation that the chemical shift for C-5‴ of the rhamnose moiety at 66.0 ppm is about 2.5 ppm upfield as compared with that of C-5 in methyl-rhamnose and other rhamnosides [7]. Comparison of the rhamnose carbon chemical shifts in the spectrum of faralatroside peracetate and those of synthetic methyl 3-*O*-glycosylated α-L-rhamnoside penta-*O*-acetate showed close similarity. Thus, the chemical shift for the carbon atom linked to the second sugar in the case of a 2-*O*-glycosylation is 76.9 ppm, that of a 3-*O*-glycosylation is at 75.1 ppm and in the case of a 4-*O*-glycosylation it is at 79.3 ppm [8]. The corresponding carbon atom in faralatroside peracetate resonates at 74.5 ppm indicating that the terminal glucose must be linked to the 3-OH group of the rhamnose moiety.

On the basis of these results, we propose the structure of faralatroside to be kaempferol-3-*O*-[β-D-glucopyranosyl-(1 → 3)-4‴-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside]. This structure is in full agreement with the fact that two different isopropylidene-permethyl ethers [9] of faralatroside could be prepared. Thus with copper sulfate and acetone, a mono-isopropylidene derivative was formed whose permethyl ether showed a M^+ peak in the MS at *m/z* 964 with fragment ions at *m/z* 231, 328, 432 and 624. The reaction in the presence of toluenesulfonic acid and subsequent permethylation led to a di-isopropylidene-permethyl derivative exhibiting a M^+ ion at *m/z* 976. The fragment ions at *m/z* 231 and 527 showed that the reactions must have occurred at the C-6 and C-4 OH groups of the terminal glucose moiety, as well as at the *cis*-C-3 and C-4 OH groups of the galactose unit.*

The spectral data of faratroside correspond with those of faralatroside except for the aglycone part. Therefore, faratroside can be assigned the structure of the homologous quercetin-3-*O*-triglycoside.

EXPERIMENTAL

Mps are not corr. The ^{1}H NMR spectra were recorded on a Varian A60 and T60; the MS spectra on an AEI MS 30 (EI 70 eV, 4 kV 100–300 μA, 200°, DE 190°; 3×10^{-7} Torr); and the ^{13}C NMR spectra on a Varian XL-100-FT-15 under the MOS-E-disk-operation system. Evaluation of the T_1 values by a three-parameter non-linear least-squares fit written by us in Fortran and used under the same operation system resulted in a precision of $\Delta T_1/T_1$ better than 4%, (pyridine-d_5, 85 mg/3 ml concn, temperature 80°).

Isolation. Leaf material (1 kg) was extracted for 12 hr with MeOH in a Soxhlet, the extract evapd to a syrup and the syrup dissolved in a small amount of water. The water extract was extracted several times with $CHCl_3$, followed by water-saturated BuOH until the water phase showed no further positive Shinoda test. The $CHCl_3$-phases were discarded. The butanol extract yielded 140 g of an extract-residue. The residue was digested with EtOH, the precipitated tannin was removed by filtration, and the filtrate evapd to dryness (85 g). The chromatographic separation of the extract (16 g) was performed on eight Sephadex LH 20 columns (2 g extract/100 g Sephadex) with a mixture of $CHCl_3$–MeOH (30:70). The fractions were monitored by TLC on Si gel (Merck) plates with the solvent $CHCl_3$–MeOH–H_2O (64:36:8). Fractions 21–30 resulted in a mixture of faralatroside and faratroside (1 g), which was subsequently separated on a Si gel H (Fa. Fluka) column with the same solvent mixture. Repeated chromatography yielded 0.720 g faralatroside and 0.18 g faratroside.

Hydrolysis of faralatroside. The glycoside (2 mg) was dissolved in some drops of MeOH and after addition of 0.5 ml 2 N CF_3COOH it was heated in a sealed ampoule for 1 hr at 110°. The identification of the aglycones and sugars was performed by TLC on Si gel. Solvent system: toluene–$CHCl_3$–Me_2CO (46:25:35) R_f 0.47 (kaempferol), R_f 0.40 (quercetin). Solvent system: $CHCl_3$, MeOH–H_2O (64:50:10) R_f 0.35 (glucose), R_f 0.30 (galactose), R_f 0.55 (rhamnose).

Faralatroside. Mp 197–199°; UV λ_{max} nm (log ε): (EtOH) 268 (4.25), 295 sh, 353 (4.30); (MeOH) 276, 329, 405; (+ NaOAc) 277, 309, 386; (+ $AlCl_3$) 277, 305, 396; (+ $AlCl_3$/HCl) 277, 305, 349, 396; IR cm^{-1}: 3400 (OH); 1747 (MeCO); 1530 (C=O); ^{1}H NMR (CD_3OD): δ 1.05 (*d*, Me rhamnose), 2.06 (*s*, MeCO), 6.26 (*d*, J = 2 Hz, H-6), 6.50 (*d*, J = 2 Hz, H-8), 6.93 (*dd*, J = 9 Hz, H-3′ and H-5′), 8.06 (*dd*, J = 9 Hz, H-2′ and H-6′); 3.0–5.2 (sugar protons). ^{13}C NMR (DMSO-d_6) kaempferol: δ 156.5 (C-2), 133.4 (C-3), 177.5 (C-4), 161.3 (C-5), 98.8 (C-6), 164.3 (C-7), 93.7 (C-8), 156.6 (C-9), 104.1 (C-10), 120.8 (C-1′), 131.0 (C-2′), 115.1 (C-3′), 161.0 (C-4′), 115.1 (C-5′), 131.0 (C-6′).

Faralatroside permethyl ether. Prepared according to ref. [9]. MS (rel. int.) 624 (0.5), 597 (0.4), 589 (0.5), 575 (0.4), 561 (0.8), 542 (0.6), 529 (0.8), 515 (6), 501 (0.8), 421 (4.3), 420 (3), 392 (10), 356 (8), 342 (30), 328 (100), 292 (17), 236 (13), 219 (5.8), 187 (43), 157 (24), 155 (15), 111 (38), 101 (67).

Mono-IP-PM-faralatroside monoisopropylidene permethyl ether). Prepared according to ref. [9]. MS (rel. int.): 976 M^+ (0.17), 961 (0.6), 947 (0.4), 933 (0.4), 729 (0.2), 648 (1), 620 (0.4), 601 (0.6), 587 (1.2), 573 (3), 527 (1.3), 433 (12), 432 (8, 6), 404 (13), 356 (13), 343 (30), 342 (98), 328 (100), 314 (99), 299 (40), 282 (28), 231 (28), 187 (49), 155 (60).

Table 1. ^{13}C signals for the trisaccharide moiety of faralatroside

^{13}C	Galactose	Rhamnose	Glucose
C-1	102.3	100.5	103.9
C-2	71.1	69.7	73.0
C-3	72.0	77.2	76.7
C-4	68.0	73.3	69.6
C-5	73.3	66.0	76.3
C-6	66.5	17.3	60.7
MeCO	—	20.8	—

Solvent: DMSO-d_6.

*Previous investigations [9] have reported the reverse reaction sequence during isopropylidenation. In the presence of $CuSO_4$ as catalyst, initially the *cis*-diol group reacted whilst a 4,6-OH group remained untouched and reacted only when toluenesulfonic acid was used as catalyst. This contradictory result may be explained as being due to steric hindrance and/or influence of the aglycone moiety.

Table 2. Relaxation-time-measurements for the trisaccharide moiety of faralatroside

	Galactose		Rhamnose		Glucose	
	ppm	T_1	ppm	T_1	ppm	T_1
C-1	103.7	0.21	100.7	0.25	104.3	0.32
C-2	71.7	0.21	70.4	0.25	73.4	0.34
C-3	72.4†	0.22	78.2	0.24	76.5†	0.35
C-4	68.6	0.18	73.7	0.23	70.0	0.36
C-5	73.9†	0.21	66.4	0.25	76.3†	0.34
C-6	67.3	0.12	16.7	0.59	61.2	0.15
MeCO	—	—	20.3	0.89	—	—

†Assignments are reversible.
T_1 is given in sec.

Faralatroside-PA (peracetyl derivative). Mp 128–130°. MS (rel. int.): 599 (0.8), 561 (20), 501 (1.9), 412 (5), 370 (16), 331 (30), 328 (30), 317 (5.4), 299 (3.5), 186 (100). ^{1}H NMR ($CDCl_3$): δ 1.08 (*d*, Me rhamnose), 2.0 (9 H), 2.05 (12 H), 2.13 (6 H) (9 OCOMe trisaccharide), 2.31 (6 H) and 2.45 (3 H) (3 OCOMe at 5,7,4′), 6.82 (*d*, J = 2 Hz, H-6), 7.22 (*dd*, J = 9 Hz, H-3′ and H-5′), 7.3 (*d*, J = 2 Hz, H-8), 8.05 (*dd*, J = 9 Hz, H-2′ and H-6′), 3.1–5.50 (sugar protons). ^{13}C NMR (pyridine-d_5) aglycone: δ 155.6 (C-2), 136.7 (C-3), 172.2 (C-4), 150.4 (C-5), 113.5 (C-6), 152.7 (C-7), 115.2 (C-8), 154.1 (C-9), 109.0 (C-10), 121.7 (C-1′), 130.5 (C-2′), 121.4 (C-3′), 156.8 (C-4′), 111.4 (C-5′), 130.6 (C-6′).

Faratroside. Mp 185°. ^{1}H NMR (CD_3OD): δ 1.0 (*d*, Me rhamnose), 2.04 (*s*, OCOMe), 6.27 (*d*, J = 1.5 Hz, H-6), 6.47 (*d*, J = 1.5 Hz, H-8), 6.92 (*d*, J = 9, H-5′), 7.16 (*dd*, J = 9, H-2′ and H-6′). ^{13}C NMR (pyridine-d_5) aglycone (quercetin): 157.8 (C-2), 135.2 (C-3), 178.6 (C-4), 162.5 (C-5), 99.8 (C-6), 165.9 (C-7), 94.5 (C-8), 157.4 (C-9), 106.1 (C-10), 122.1 (C-1′), 116.1 (C-2′), 146.6 (C-3′), 147.7 (C-4′), 117.6 (C-5′), 121.7 (C-6′).

Faratroside peracetate. ^{1}H NMR ($CDCl_3$): 1.08 (*d*, Me rhamnose), 2.0 (*s*, 9 H), 2.08 (*s*, 12 H), 2.13 (*s*, 3 H), 2.15 (*s*, 3 H), (9 OCOMe), 2.33 (*s*, 9 H) and 2.43 (*s*, 3 H) (OCOMe, 5, 7, 3′, 4′), 6.83 (*d*, J = 1.5 Hz, H-6), 7.32 (*d*, J = 1.5 Hz, H-8), 7.34 (*d*, J = 9.5 Hz, H-5′), 7.9 (*dd*, J = 9.5 Hz, H-6′ and H-2′), 3.1–5.8 (sugar protons).

Table 3. ^{13}C signals for the trisaccharide moiety of faralatroside-PA

^{13}C	Galactose	Rhamnose	Glucose
C-1	101.1	97.5	99.7
C-2	69.3	71.3	71.3
C-3	71.0	74.5	73.2
C-4	67.1	71.8	68.2
C-5	71.3	66.8	72.3
C-6	64.7	17.3	61.2
MeCO	—	20.4	—

Solvent: pyridine-d_5.

Table 4. ^{13}C signals for the trisaccharide moiety of faralatroside

^{13}C	Galactose	Rhamnose	Glucose
C-1	104.9	101.8	104.9
C-2	73.0	73.3	74.8
C-3	73.3	79.3	78.2
C-4	71.1	75.7	72.4
C-5	74.5	67.3	77.6
C-6	67.3	17.8	62.1
MeCO	—	21.3	—

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