Fagomine Isomers and Glycosides from *Xanthocercis zambesiaca*

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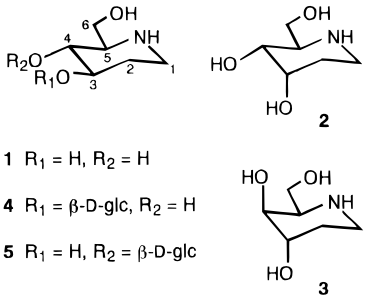
50% aqueous MeOH extracts from the leaves and roots of *Xanthocercis zambesiaca* (Leguminosae) were subjected to various ion-exchange column chromatographic steps to give fagomine (**1**), 3-*epi*-fagomine (**2**), 3,4-di-*epi*-fagomine (**3**), 3-*O*-*β*-D-glucopyranosylfagomine (**4**), and 4-*O*-*β*-D-glucopyranosylfagomine (**5**). Their structures were determined by spectroscopic analyses, particularly by extensive 1D and 2D NMR studies. Compounds **3** and **4** are new natural products. Compound **1** is a good inhibitor of isomaltase and certain α- and *β*-galactosidases. Whereas **2** is a more potent inhibitor of isomaltase and *β*-galactosidases than **1**, it does not inhibit α-galactosidase. Compounds **3**−**5** exhibited no significant inhibition against the glycosidases used.

Fagomine (**1**) was first isolated from buckwheat seeds (*Fagopyrum esculentum* Moench)[1] and subsequently isolated with the indolizidine alkaloid, castanospermine, from seeds of *Castanospermum australe* (Leguminosae).[2] Furthermore, a trace amount of **1** was present in seeds of *Xanthocercis zambesiaca* (Leguminosae), together with a 4-*O*-*β*-D-glucoside of **1**.[3] Recently, we reported the occurrence of **1** in the leaves and roots of *Morus* spp. (Moraceae).[4,5] Fagomine has been shown to have some activity against mammalian gut α-glucosidase and *β*-galactosidase, but no other biological activity has been reported.[2,3,6,7] More recently, compound **1** was found to have a potent antihyperglycemic effect in streptozocin-induced diabetic mice and to potentiate markedly immunoreactive insulin release.[8] We therefore searched for rich sources of **1** and its related compounds for further investigation. The occurrence in *Xanthocercis zambesiaca* (Bak.) Dunn. was reexamined, and it was found that **1** is abundantly present in this plant. The genus *Xanthocercis* Baill is grouped taxonomically with the genera *Castanospermum*, *Alexa*, and *Angylocalyx* as part of the Angylocalyx group of the Sophoreae tribe (Leguminosae).[9] Species in the genera *Alexa*[10] and *Angylocalyx*[11] also produce polyhydroxylated alkaloids that inhibit glycosidases. There are two species in the genus *Xanthocercis*, *X. zambesiaca* occurring in southern Africa in dry forest and woodland and *X. madagascariensis* in northern Madagascar. In this paper, we describe the isolation of five polyhydroxylated alkaloids from *X. zambesiaca*, their structure determination, and their glycosidase inhibitory activity.

A 50% MeOH extract of the dry leaves (450 g) of *X. zambesiaca* was chromatographed with various ion-exchange resins to give compounds **1** (501 mg), **2** (121 mg), **3** (23 mg), **4** (35 mg), and **5** (17 mg). A 50% MeOH extract of the root (700 g) of *X. zambesiaca* was also chromatographed in a similar manner to give compounds **1** (906 mg), **2** (53 mg), **4** (40 mg), and **5** (85 mg).



The optical rotation, FAB-MS, and NMR spectra of compounds **1** and **2** were completely in accord with those of fagomine and 3-*epi*-fagomine isolated from *Morus* spp., respectively.[4,5] The optical rotation, ^{1}H NMR, and ^{13}C (Table 1) NMR spectral data of compound **5** were consistent with those reported for 4-*O*-*β*-D-glucopyranosylfagomine isolated from the same plant.[3]

Compound **3** was found to be an isomer of **1** by HRFAB-MS (*m*/*z* 148.0972 [M + H]$^+$) and ^{13}C NMR spectral data (Table 1). The ^{1}H NMR spectral data, combined with extensive decoupling experiments and 2D ^{1}H−^{13}C COSY spectral data, defined the complete connectivity of carbon and hydrogen atoms. The definite NOE between H-1*ax* and H-5 indicates that these protons are 1,3-trans-diaxial, as illustrated in Figure

Table 1. ^{13}C NMR Data of Compounds **1**−**5** (in D_2O, 100 MHz)[a]

	compound				
carbon	**1**	**2**	**3**	**4**	**5**
1	45.4	41.2	41.4	45.1	44.8
2	35.6	33.8	29.9	32.5	34.0
3	76.1	70.7	70.0	83.6	74.0
4	76.1	72.4	71.0	73.7	85.3
5	63.7	58.6	58.2	63.6	62.5
6	64.5	64.9	63.3	64.0	62.9
1′				103.0	105.6
2′				75.7	76.1
3′				78.6	78.4
4′				72.5	72.3
5′				78.8	78.8
6′				63.6	63.4

[a] Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate.

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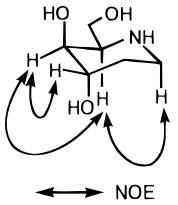

Figure 1. Selected NOE effects for 3,4-di-*epi*-fagomine (**3**).

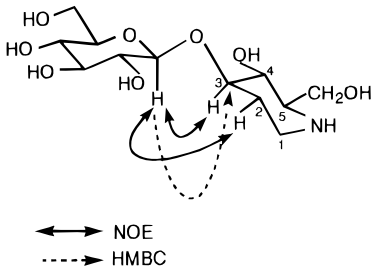

Figure 2. Selected NOE effects and an HMBC correlation for 3-*O*-*β*-D-glucopyranosylfagomine (**4**).

1. The coupling patterns of H-5 (δ 3.16, ddd, $J_{4,5} = 2.7$ Hz, $J_{5,6a} = 7.3$ Hz, $J_{5,6b} = 6.1$ Hz) and H-2*ax* (δ 1.62, ddt, $J_{1ax,2ax} = 9.0$ Hz, $J_{1eq,2ax} = J_{2ax,3} = 3.4$ Hz, $J_{2ax,2eq} = 13.0$ Hz) indicate equatorial orientations of H-4 and H-3. Furthermore, no significant NOE effect between H-3 and H-5 and definite NOE effects between H-4 and H-3 or H-5 support that both H-3 and H-4 are equatorial. Thus, compound **3** was determined to be 3,4-di-*epi*-fagomine.

Compound **4** was found to be a glycoside of fagomine (**1**) or its isomer by the HRFAB-MS (*m*/*z* 310.1500 [M + H]$^+$) and ^{13}C NMR spectral data. After acid hydrolysis of this glycoside using Dowex 50W × 2 [H$^+$ form], the glycon part in the filtrate of the resin was determined to be D-glucose by the D-glucose-oxidase peroxidase method, and the aglycon part was eluted with 0.5 M ammonia solution from the resin, concentrated to dryness, and confirmed as **1** by direct comparison of its optical rotation and ^{13}C NMR spectrum with those of an authentic sample. The ^{1}H NMR spectral data, together with information from extensive decoupling experiments, ^{13}C NMR, and 2D ^{1}H-^{13}C COSY spectral data, defined the complete connectivity of the carbon and hydrogen atoms. The coupling constant of the anomeric proton (δ 4.58, $J_{1',2'} = 8.0$ Hz) showed that this glycoside was the *β*-D-glucoside of **1**. As illustrated in Figure 2, a correlation peak between the anomeric proton of the glycon and the aglycon C-3 carbon in the HMBC spectrum and a definite NOE effect between the anomeric proton and H-3 defined the linkage site of the glycon as C-3. Thus, the structure of **4** was determined to be 3-*O*-*β*-D-glucopyranosylfagomine.

While, as reported previously,[2,3,6,7] compound **1** is a good inhibitor of rat isomaltase and α-(green coffee beans) and *β*-galactosidases (rat and bovine), **2** is a more potent inhibitor of the isomaltase and *β*-galactosidase than **1**, but this compound did not inhibit α-galactosidase. Compounds **3**−**5** exhibited no significant inhibition of the glycosidases tested (Table 2).

Table 2. Concentration of Fagomine Isomers and Glucosides Giving 50% Inhibition of Various Glycosidases

	IC$_{50}$ (μM)				
enzyme	**1**	**2**	**3**	**4**	**5**
α-glucosidase					
rice	320	120	NI	NI	NI
rat intestinal isomaltase	46	6	NI	NI	NI
β-glucosidase					
rat liver lysosomal	NI[a]	740	NI	NI	NI
rat intestinal cellobiase	NI	100	NI	NI	NI
almond	NI	120	NI	NI	NI
α-galactosidase					
rat liver lysosomal	NI	NI	NI	NI	NI
green coffee bean	56	NI	NI	NI	NI
β-galactosidase					
rat intestinal lactase	15	4	NI	NI	NI
bovine liver	38	3	NI	NI	NI

[a] NI = less than 50% inhibition at 1000 μM.

Experimental Section

General Experimental Procedures. Alkaloids were chromatographed on HPTLC silica gel-60F$_{254}$ (E. Merck) using the solvent system *n*-PrOH−AcOH−H$_2$O (4:1:1), and a chlorine−*o*-tolidine spray reagent[12,13] was used for detection. Optical rotation was measured with a JASCO DIP-370 digital polarimeter. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL JNM-GX 400 spectrometer as indicated in D$_2$O using sodium 3-(trimethylsilyl)propionate (TSP) as internal reference. Mass spectra were measured on a JEOL JMS-SX 102A spectrometer.

Plant Material. *X. zambesiaca* (Bak.) Dunn. was grown in the Institute of Grassland and Environmental Research. The specimen used was grown from seeds collected in Zimbabwe (Zhou National Area) and has a herbarium specimen at the Herbarium Botanic Garden in Harare (Lundi K. Gona 2131 B4 1982).

Extraction and Isolation. The leaves (450 g dry weight) of *X. zambesiaca* were homogenized in 50% aqueous MeOH. The filtrate was applied to a column of Amberlite IR-120B [H$^+$ form, 200 mL] prepared in 50% aqueous MeOH. A 0.5 M NH$_4$OH eluate was concentrated to give a brown oil (5.8 g). This oil was chromatographed over a Dowex 1 × 2 column [3.8 × 90 cm, OH$^-$ form] with H$_2$O as an eluant (fraction size 15 mL). The H$_2$O eluate was divided into four pools A (fractions 23−30, 501 mg), B (fractions 31−43, 273 mg), C (fractions 44−54, 49 mg), and D (fractions 58−68, 26 mg). Pool A was concentrated and lyophilized to give fagomine (**1**, 501 mg). Pool B was further chromatographed on a column of CM Sephadex C-25 [1.5 × 42 cm, NH$_4^+$ form] and eluted with 0.01 M NH$_4$OH to give 4-*O*-*β*-D-glucopyranosylfagomine (**5**, 17 mg) and 3-*epi*-fagomine (**2**, 121 mg), in order of elution. Pools C and D were rechromatographed on a Dowex 1 × 2 column [1.9 × 92 cm, OH$^-$ form] to give 3-*O*-*β*-D-glucopyranosylfagomine (**4**, 35 mg) and 3,4-di-*epi*-fagomine (**3**, 23 mg), respectively.

The roots (700 g) of *X. zambesiaca* were extracted in a similar manner and treated with Amberlite IR-120B [H$^+$ form, 200 mL] to give a brown oil (8.1 g). This oil was treated with a Dowex 1 × 2 resin [OH$^-$ form, 100 mL] and washed with H$_2$O to give a colorless solid (2.4 g). This solid was chromatographed over a column of Amberlite CG-50 [3.8 × 90 cm, NH$_4^+$ form] with H$_2$O as eluant (fraction size 15 mL), and the eluate was divided into two pools, A (fractions 38−50, 754 mg) and B (fractions 56−70, 60 mg). The 0.5 M NH$_4$OH eluate from the same column was designated pool C (1.07 g). Pool A was further chromatographed on a column of CM Sephadex C-25 [1.5 × 42 cm, NH$_4^+$ form] and eluted with 0.01 M NH$_4$OH to give **5** (85 mg) and **4** (40 mg) in

order of elution. Pools B and C were also further chromatographed on a Dowex 1 × 2 column [1.9 × 92 cm, OH^- form] to give **2** (53 mg) and **1** (906 mg), respectively.

Fagomine (1): $[\alpha]_D$ +19.5° (*c* 1.0, H_2O); ^{13}C NMR in Table 1; HRFAB-MS *m/z* 148.0977 $[M + H]^+$ ($C_6H_{14}O_3N$ requires 148.0974).

3-*epi*-Fagomine (2): $[\alpha]_D$ +69° (*c* 0.5, H_2O); ^{13}C NMR in Table 1; HRFAB-MS *m/z* 148.0973 $[M + H]^+$ ($C_6H_{14}O_3N$ requires 148.0974).

3,4-Di-*epi*-fagomine (3): $[\alpha]_D$ −8.7° (*c* 0.3, H_2O); ^1H NMR (400 MHz, D_2O) δ 1.62 (1H, ddt, *J* = 3.4, 9.0, 13.0 Hz, H-2*ax*), 2.04 (1H, m, H-2*eq*), 2.91 (1H, m, H-1*ax*), 2.92 (1H, m, H-1*eq*), 3.16 (1H, ddd, *J* = 2.7, 6.1, 7.3 Hz, H-5), 3.68 (1H, dd, *J* = 7.3, 11.5 Hz, H-6a), 3.71 (1H, dd, *J* = 6.1, 11.5 Hz, H-6b), 3.75 (1H, dd, *J* = 2.7, 4.6 Hz, H-4), 3.93 (1H, dt, *J* = 3.4, 4.6 Hz, H-3); ^{13}C NMR in Table 1; HRFAB-MS *m/z* 148.0972 $[M + H]^+$ ($C_6H_{14}O_3N$ requires 148.0974).

3-*O*-β-D-Glucopyranosylfagomine (4): $[\alpha]_D$ −18.2° (*c* 0.48, H_2O); ^1H NMR (400 MHz, D_2O) δ 1.51 (1H, m, H-2*ax*), 2.19 (1H, m, H-2*eq*), 2.63 (1H, m, H-5), 2.65 (1H, dt, *J* = 2.7, 13.0 Hz, H-1*ax*), 3.11 (1H, ddd, *J* = 2.2, 4.4, 13.0 Hz, H-1*eq*), 3.30 (1H, dd, *J* = 8.0, 9.3 Hz, H-2′), 3.38 (1H, dd, *J* = 9.3, 9.5 Hz, H-4), 3.41 (1H, dd, *J* = 8.8, 9.5 Hz, H-4′), 3.47 (1H, ddd, *J* = 2.2, 5.9, 9.5 Hz, H-5′), 3.51 (1H, dd, *J* = 8.8, 9.3 Hz, H-3′), 3.71 (1H, dd, *J* = 6.1, 11.7 Hz, H-6a), 3.73 (1H, dd, *J* = 5.9, 12.5 Hz, H-6′a), 3.79 (1H, ddd, *J* = 4.9, 9.3, 11.2 Hz, H-3), 3.87 (1H, dd, *J* = 3.2, 11.7 Hz, H-6b), 3.92 (1H, dd, *J* = 2.2, 12.5 Hz, H-6′b), 4.58 (1H, d, *J* = 8.0 Hz, H-1′); ^{13}C NMR in Table 1; HRFAB-MS *m/z* 310.1500 $[M + H]^+$ ($C_{12}H_{24}O_8N$ requires 310.1502).

Hydrolysis of 4. Compound **4** (12 mg) was heated at 100 °C with Dowex 50W × 2 (1 g dry weight, H^+ form) in H_2O for 8 h. The resin was filtered off and packed into a short column. After the filtrate was neutralized, the released D-glucose was determined to be 4.7 mg (82%) by the D-glucose oxidase−peroxidase method using commercially available Glucose B-test (Wako Pure Chemical Industries). The alkaloid moiety was eluted with 0.5 M NH_4OH from the short column and concentrated. The residue was applied to a Dowex 1 × 2 column [0.8 × 12 cm, OH^- form] and eluted with water to give 6.1 mg (87%) of crystalline free base. Comparison of the optical rotation and ^{13}C NMR spectrum of this alkaloid with those of **1** showed that they were identical. Consequently, acidic hydrolysis of **4** gave equimolar amounts of D-glucose and **1**.

4-*O*-β-D-Glucopyranosylfagomine (5): $[\alpha]_D$ −3.0° (*c* 0.82, H_2O) [lit.[3] $[\alpha]_D$ −3.1° (*c* 1.2, H_2O)]; ^{13}C NMR in Table 1; HRFAB-MS *m/z* 310.1504 $[M + H]^+$ ($C_{12}H_{24}O_8N$ requires 310.1502).

Glycosidase Inhibitory Activities. Rice α-glucosidase (EC 3.2.1.20), almond β-glucosidase (EC 3.2.1.21), green coffee bean α-galactosidase (EC 3.2.1.22), bovine liver β-galactosidase (EC 3.2.1.23), *p*-nitrophenyl glycosides, and disaccharides were purchased from Sigma Chemical Co. Brush border membranes, prepared from the intestine of male Wister rats by the method of Kessler et al.,[14] were used as the source of rat digestive glycosidases. The partially purified lysosomal fraction prepared by the procedures of Tsuji et al.[15] was used as a source of lysosomal β-glucosidase and α-galactosidase.

The activities of rice α-glucosidase and rat digestive glycosidases were determined using the appropriate disaccharides as substrates at the optimum pH of each enzyme. The released D-glucose was determined colorimetrically using the glucose B-test. Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as a substrate at the optimum pH of each enzyme. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

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