Three New Oleanane Saponins from *Zanha africana*

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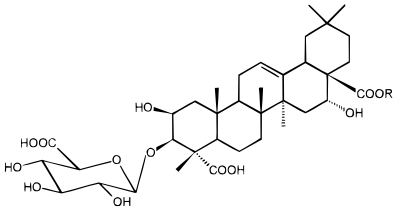
Three new saponins, zanhasaponins A, B, and C, were isolated from the MeOH extract of the root bark of *Zanha africana* and were, respectively, identified by spectroscopic methods as 3-*O*-*β*-D-glucuronopyranosyl-2*β*,16α-dihydroxyolean-12-ene-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl(1 → 2)-α-L-rhamnopyranoside (**1**); 3-*O*-*β*-D-glucuronopyranosyl-2*β*,16α-dihydroxyolean-12-ene-23,28-dioic acid 28-*O*-*β*-D-xylopyranosyl(1 → 2)-α-L-rhamnopyranosyl(1 → 2)-α-L-rhamnopyranoside (**2**); and 3-*O*-*β*-D-glucuronopyranosyl-2*β*,16α-dihydroxyolean-12-ene-23,28-dioic acid 28-*O*-*β*-D-xylopyranosyl(1 → 3)-*β*-D-xylopyranosyl(1 → 2)-α-L-rhamnopyranosyl(1 → 2)-α-L-rhamnopyranoside (**3**). These saponins proved to be effective in a model of topical inflammation induced by phorbol ester. The cyclitols quebrachitol, pinitol, and bornesitol were also identified.

Zanha africana (Radlk.) Exell (Sapindaceae) is a tree of medium height growing in Southern Africa. It is known that the aqueous extract of the roots is used in traditional medicine as a remedy for disentery.[1] After acid hydrolysis of the MeOH extract of the root bark, Kapundu *et al.* isolated and identified the aglycons of the triterpene glycosides from this plant as medicagenic and zanhic acids.[1] Zanhagenic acid and zanhic acid *γ*-lactone were described as artifact products of this hydrolysis.[1,2] These last three compounds had been previously isolated from another species of the same genus, *Zanha golungensis*. From the perchloric hydrolysis of the root bark of this species two novel prosapogenins, medicagenin and zanhin, were also identified.[3] Referring to the pharmacological activity, we previously reported that the MeOH extract of the root bark of *Z. africana* significantly reduced carrageenan-induced paw edema and tetradecanoylphorbol acetate (TPA)-induced ear edema in mice.[4] The aim of the present study is to isolate, identify, and evaluate the topical antiinflammatory activity of the main compounds of this extract.

The MeOH extract of *Z. africana* was fractionated by Si gel column chromatography to obtain nine fractions, two of which were further purified by low-pressure column chromatography, DCCC, and preparative TLC and yielded three saponins (**1**−**3**) and three cyclitols. Alkaline hydrolysis of the saponin mixture gave two prosapogenins, which were identified as 3-*O*-*β*-D-glucuronopyranosyl-2*β*,16α-dihydroxyolean-12-ene-23,28-dioic acid (zanhic acid 3-glucuronide) and 3-*O*-*β*-D-glucuronopyranosyl-2*β*-hydroxyolean-12-ene-23,28-dioic acid (medicagenic acid 3-glucuronide), respectively, by comparison of their ^{13}C-NMR data with those of the corresponding aglycons.[1,5] Zanhic acid 3-glucuronide is now reported as a new prosapogenin. Each of its carbon resonances were coincident with those of zanhic acid with the exception of C-3, which was shifted downfield from the aglycon value (74 ppm to 86 ppm) due to the glycosidic linkage with the glucuronic moiety. This sugar residue gave its characteristic signals for one carboxyl group, four carbinols, and one hemiacetalic carbon.[6]



1 R = α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl

2 R = β-D-xylopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl

3 R = β-D-xylopyranosyl (1→3)-β-D-xylopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl

The polarity of compounds **1**−**3** renders them insoluble in the majority of the usual NMR solvents, with the exception of the mixture CD_3OD−AcOD (9:1). Alkaline hydrolysis of **1** gave zanhic acid 3-glucuronide and a sugar identified by TLC as rhamnose. ^{13}C NMR

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Table 1. ^{13}C-NMR Spectral Data Values of Compounds **1**−**3** (CD_3OD−AcOD)

Aglycon [δ(ppm)]					Sugar [δ(ppm)]				
C	**1**	**2**	**3**	DEPT	C	**1**	**2**	**3**	DEPT
1	44.2	45.0	44.4	CH_2	glucuronic acid				
2	70.9	70.8	70.9	CH	1	104.8	104.6	104.8	CH
3	86.5	87.5	86.7	CH	2	74.4	74.0	74.0	CH
4	53.2	53.2	53.3	C	3	75.7	75.9	75.6	CH
5	53.0	53.1	53.0	CH	4	72.7	72.5	72.5	CH
6	21.3	21.4	21.2	CH_2	5	76.8	76.7	76.6	CH
7	33.8	33.4	33.8	CH_2	6	174.0	173.8	173.8	C
8	41.2	41.2	42.3	C	rhamnose I				
9	48.5	48.4	49.0	CH	1	95.1	95.2	95.2	CH
10	36.3	36.4	36.3	C	2	79.6	79.9	81.4	CH
11	24.6	25.1	24.6	CH_2	3	72.1	72.0	72.0[b]	CH
12	123.5	123.4	123.4	CH	4	73.7	73.8	74.0	CH
13	144.2	144.7	144.3	C	5	70.8	70.0	70.2[c]	CH
14	42.3	42.4	42.3	C	6	18.9	18.4[a]	18.3[a]	CH_3
15	36.3	36.7	36.3	CH_2	rhamnose II				
16	75.7	75.0	76.6	CH	1	101.6	102.0	100.9	CH
17	n.o.	n.o.	n.o.	n.o.	2	71.7	80.9	81.4	CH
18	41.2	41.6	42.8	CH	3	72.7	72.0	72.1[b]	CH
19	47.8	47.1	47.7	CH_2	4	73.7	73.8	73.8	CH
20	31.2	31.2	31.1	C	5	70.2	70.5	70.4[c]	CH
21	37.3	37.2	37.3	CH_2	6	18.3	18.0[a]	18.0[a]	CH_3
22	31.2	31.3	31.4	CH_2	xylose I				
23	181.4	180.1	182.2	C	1		105.5	105.5	CH
24	13.6	14.0	13.6	CH_3	2		75.0	74.9	CH
25	16.7	16.7	16.7	CH_3	3		77.6	86.7	CH
26	17.9	17.5	18.0	CH_3	4		70.5	69.7	CH
27	27.3	27.2	27.3	CH_3	5		67.0	66.7	CH_2
28	177.2	176.2	175.8	C	xylose II				
29	33.3	33.4	33.2	CH_3	1			106.9	CH
30	25.2	25.1	25.3	CH_3	2			75.6	CH
					3			77.3	CH
					4			70.9	CH
					5			67.1	CH_2

[a,b,c] Values with same superscript can be reversed.

of **1** (Table 1) showed two more anomeric signals with respect to zanhic acid 3-glucuronide. One of these was linked to a −COOH moiety due to the shift at 95 ppm (esteral linkage), while the chemical shift of the other anomeric carbon (102 ppm) suggested the linkage of a second sugar unit to a sugar or a −OH of the aglycon, namely 2β-OH or 16α-OH. However, none of the shifts lent support to this last hypothesis when these signals were compared with those of zanhic acid 3-glucuronide. Furthermore, the products of the alkaline hydrolysis were in accordance with a disaccharide moiety, namely rhamnose I−rhamnose II. The linkage of rhamnose I to 28−COOH derived from the upfield shift (about 5 ppm), attributable to −COOH, and the absence of characteristic shifts of C-23, C-24, and C-4 indicates no linkages with 23−COOH. The interglycosidic linkage was evident from the fact that the site of glycosylation of rhamnose I has to be shifted downfield by 7−9 ppm with respect to the free sugar. Due to the absence of signals between 80 and 86 ppm in the ^{13}C-NMR spectrum, the glycosidic linkage of rhamnose II with rhamnose I could only be located at C-2 of rhamnose I.[7] In the case of a linkage at C-3 or C-4, their chemical shifts would be more than 80 ppm. Compound **1** was therefore identified as 3-*O*-β-D-glucuronopyranosyl-2β,16α-dihydroxyolean-12-ene-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl(1 → 2)-α-L-rhamnopyranoside (zanhasaponin A).

The ^{13}C-NMR spectrum of compound **2** (Table 1) differed from that of **1** only in its extra anomeric carbon signal and four carbinolic ones. Because alkaline hydrolysis of **2** led again to zanhic acid 3-glucuronide, we had to consider the possibility that this third sugar could be linked to a carboxyl moiety. The absence of shifts to C-23, C-24, or C-4 meant that this sugar was linked to

the chain at C-28. It was characterized as xylose by TLC analysis of the aqueous layers after alkaline hydrolysis. Xylose can only be terminal because it lacks signals between 80 and 86 ppm in the ^{13}C-NMR spectrum, and rhamnose II was substituted at C-2 by the xylose moiety.[7] The structure of **2** proved to be 3-*O*-*β*-D-glucuronopyranosyl-2*β*,16α-dihydroxyolean-12-ene-23,28-dioic acid 28-*O*-*β*-D-xylopyranosyl(1 → 2)-α-L-rhamnopyranosyl(1 → 2)-α-L- rhamnopyranoside (zanhasaponin B).

The results of alkaline hydrolysis of **3** indicated a tetrasaccharide moiety, namely rhamnose I−rhamnose II−xylose I−xylose II. The ^{13}C-NMR spectrum of **3** (Table 1) revealed the same signals of the aglycon as those of **1** and **2**. In the sugar region, the most outstanding signal was 86.09 ppm (CH), which represents the sugar carbon that has been glycosylated. In view of the value of the very downfield chemical shift, and taking into account the value of the glycosylation shift of about 8−9 ppm, we can affirm that this second unit of xylose should be linked to C-3 of xylose I.[8] Compound **3** was assigned the structure 3-*O*-*β*-D-glucuronopyranosyl-2*β*,16α-dihydroxyolean-12-ene-23,28-dioic acid 28-*O*-*β*-D-xylopyranosyl(1 → 3)-*β*-D-xylopyranosyl(1 → 2)-α-L-rhamnopyranosyl(1 → 2)-α-L-rhamnopyranoside (zanhasaponin C).

The three cyclitols (polyhydroxycyclohexanes), quebrachitol, pinitol, and bornesitol, were identified by comparison of spectroscopic data with literature reports.[9]

The saponins **1**−**3** were active against TPA-induced ear edema, exhibiting ID_{50} of 14, 20, and 79 *μ*g/ear, respectively. Thus, these saponins may be considered as responsible, at least partially, for the activity exhibited by the plant extract.

This is the first time that these saponins (**1**−**3**) are described in nature. Our report revealed the structures of *Zanha* saponins that have remained unknown for years. Zanhic acid, their aglycon, has been identified in the genus *Zanha* and *Ganophyllum giganteum* from the family Sapindaceae,[3,10] and also from *Herniaria* species of the Caryophyllaceae.[10] A tridesmosidic glycoside of zanhic acid has been found in *Medicago sativa* (Fabaceae). This compound, endowed with weakly hemolytic activity, contains no glucuronic moiety, a three-glucose chain at 3-OH, a four-unit sugar chain esterifying the 28−COOH, and, notably, an arabinosyl ester moiety at 23−COOH.[5,11]

Experimental Section

General Experimental Procedures. NMR spectra were run in CD_3OD−AcOD (9:1) using a 200 MHz (Bruker AC) instrument. EIMS and FABMS were carried out in a VG Auto Spec (Fisons). Analytical TLC was carried out on Merck Si gel F_{254} aluminum sheets and Merck RP-8 plates visualized with 1% H_2SO_4−anisaldehyde. Alkaline hydrolysis was carried out by subjecting the solution of the sample (4 mg) in *n*-BuOH (0.4 mL) and NaOH (75 mg) to reflux at 100 °C for 24 h. TLC of sugars was performed with Si gel eluted with EtOAc−AcOH−MeOH−H_2O (13:4:3:3) and spraying with 0.5% thymol in H_2SO_4−EtOH (5:95), *p*-anisidine phtalate, and naphtoresorcinol reagents. Sugars were detected and identified by chromatographic comparisons with authentic samples of rhamnose and xylose. Indomethacin and TPA were purchased from Sigma (St. Louis, Mo.)

Plant Material. The root bark of *Z. africana* was collected on the Zomba Plateau in Malawi. A specimen has been deposited at the National Herbarium of Malawi, Zomba.

Extraction and Isolation. Air-dried and powdered root bark of *Z. africana* was extracted with MeOH at room temperature. The solvent was removed under reduced pressure. The MeOH extract (5 g) was chromatographed over a Si gel column eluting with $CHCl_3$−MeOH−H_2O−HCOOH (65:35:2:2 → 35:65:2:2) and MeOH−H_2O−HCOOH (65:2:2), and nine fractions (I−IX) were obtained. Fraction III (715 mg) was subjected to a DCCC with $CHCl_3$−MeOH−*n*-PrOH−H_2O (9:12:2:8) in a descending mode, yielding five fractions (III_1−III_5). Fraction III_2 (242 mg) was rechromatographed over a Si gel flash column eluted with $CHCl_3$−MeOH−H_2O−HCOOH (60:25:5:0.1) and yielded six fractions, two of which (III_2-3 and III_2-6) were single spots when analyzed by TLC and corresponded to compounds **1** (77.9 mg) and quebrachitol (49.5 mg), respectively. Fraction III_2-5 was further purified by preparative TLC over Si gel and EtOAc−AcOH−MeOH−H_2O (65:20:15:15), and compound **2** (30 mg) was obtained. Fraction III_3, fractionated in the same conditions as fraction III_2, yielded six fractions, the fourth of which was a pure compound identified as pinitol (23.8 mg). Purification of fraction III_4 by a Lobar LiChroprep RP-8 (Merck) column with MeOH−H_2O (1:1) yielded bornesitol (76 mg). Fraction V (480 mg) was rechromatographed by DCCC with $CHCl_3$−MeOH−*n*-PrOH−H_2O (7:13:1:8) in an ascending mode, and six fractions were obtained. TLC analysis of the fraction V_5 gave a single spot, corresponding to compound **3**. The same DCCC protocol made possible the purification of larger amounts (up to 128.7 mg) of compound **3** from fraction V_3.

Physical data: zanhasaponin A (**1**): crystalline scales, 245−247°, $[\alpha]_D$ −9 (CH_3OH; *c* 0.1); zanhasaponin B (**2**): amorphous, 258−260°, $[\alpha]_D$ +6 (CH_3OH; *c* 0.1); zanhasaponin C (**3**): amorphous, 260−262°, $[\alpha]_D$ +3 (CH_3OH; *c* 0.1). For ^{13}C-NMR data, see Table 1.

Topical Antiinflammatory Activity. TPA-Induced Mouse Ear Edema.[12] An edema was induced on the right ear by topical application of 2.5 *μ*g/ear of TPA in Me_2CO. The left ear (control) received vehicle (70% aqueous EtOH). The isolated compounds, dissolved in 70% aqueous EtOH, were applied topically (0.5 mg/ear), simultaneously with TPA. The standard drug indomethacin was administered at the same dose.

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