Bull. Chem. Soc. Ethiop. 1998, 12(1), 45-48.
Printed in Ethiopia
ISSN 1011-3294

GLUCOFRANGULIN A DIACETATE FROM THE FRUITS OF *RHAMNUS PRINOIDES*

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(Received March 25, 1998)

Abstract. A new anthraquionone glycoside, emodin-1-O-β-D-glucopyranoside, 3-O-(1,2-di-O-acetyl-α-L-rhamnopyranoside) has been isolated from fruits of *R. prinoides* together with emodin-1-O-β-D-glycopyranoside, 3-O-α-L-rhamnopyranoside (glucofrangulin A) and emodin-1-O-β-D-glucoside (emodin glucoside B).

INTRODUCTION

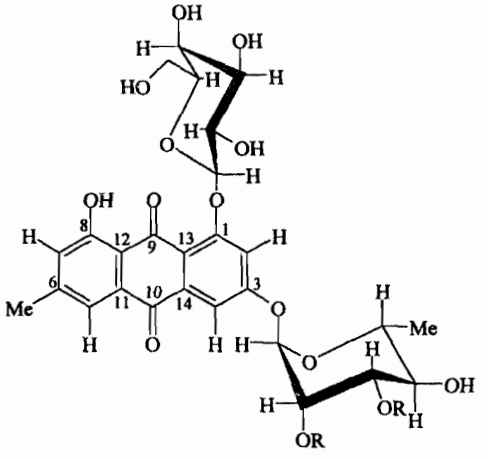
Rhamnus prinoides, L'Herit, family Rhamnaceae, and *Rhamnus staddo* represents the gneus *Rhamnus* in Africa [1]. In Ethiopia, known by the Amharic name *Gesho*, it is cultivated on commercial scale for its leaves and stems which are essential ingredients in the making of the domestic alcoholic beverages *Tella* and *Tej*. The fruits also have folk-lore medicinal applications in the treatment of ringworm infections. Previous phytochemical report[2] on the leaves of *R. prinoides* revealed the existence of eleven compounds: anthracene derivatives, naphthalene derivatives and flavonoids. In the same work the bitter principle of the leaves was identified to be β-sorignin-8-O-β-D-glucoside to which the trivial name geshoidin was given. Two papers [3, 4] on the fruits have also described the isolation of 10 compounds comprising anthraquinones, emodin anthrone, rhamnazin, and mono-, di- and tri- acetylated rhamnosyl glycosides of emodin and/or emodin anthrone. In this paper, we report the results of further investigation of the fruits of *R. prinoids* that led to the isolation and characterization of a new glycoside, glucofrangulin A diacetate and two known glycosides of emodin.

RESULTS AND DISCUSSION

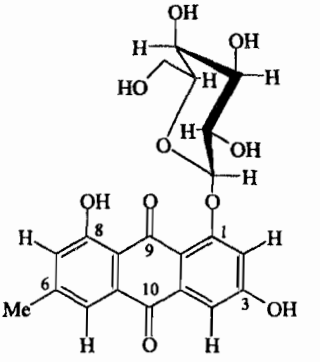
The polar fraction of the cold CH_2Cl_2-MeOH extract of the fruit of *R. prinoides* was subjected to column chromatorgraphy on silica gel and Sephadex LH-20 and preparative TLC to yield compounds **1-3.**

Compound **1**, a red solid, was characterized by IR bands at 3415, 1740, 1629 and 1596 cm^{-1} and a UV-VIS absorption maxima at 205, 220, 266 and 413 nm. It turned to pink when sprayed with 5% methanolic KOH on TLC. Compound **1** showed δH signals for two pairs of meta coupled aromaic protons at δ 7.56 (d, J = 2.52 Hz), 7.43 (d, J = 1.1 Hz), 7.39 (d, J = 2.55 Hz) and 7.02 *(br* s) and one sugar anomeric proton with di-equatorial coupling at δ 5.75 (d, J = 1.74 Hz). Another anomeric proton signal with a di-axial coupling was observed at δ 5.02 (d, J = 7.59 Hz). A three proton doublet at δ 1.30 (d, J = 6.12 Hz) and δC signal at δ 18.1 led to the identification of one of the sugars as rhamnose. On acid

hydrolysis (2 N HCl-MeOH, 1:1), β-D-glucose was detected to be the second monosaccharide in **1** and the HCl-MeOH, 1:1), β-D-glucose was detected to be the second monosaccharide in **1** and the liberated aglycone was identified as emodin by comparison with an authentic specimen. Additional signal in the ^{1}H NMR spectrum of **1** was due to an aromatic methyl at δ 2.39 integrated for three protons. The presence of two acetate groups were deduced from the two acetyl methyl signals at δ 2.18 and 2.06 and δC signals at δ 172.2, 171.7 (-O-C=O(CH_3) x 2), 20.8 and 20.7 (-O-C=O(CH_3) x 2). The rhamnosyl anomeric proton signal at δ 5.75 was coupled to a proton signal (rha H-2) which appears at a relatively low field of δ 5.46 (dd, J = 1.74, 3.50 Hz) suggesting that one of the acetate groups in located at rha C-2, rha H-2 was further coupled to rha H-3 which appears at δ 5.25 (dd, J = 3.50, 9.74 Hz) which also occurs at a relatively low field suggesting attachment of the second acetate group to rha C-3. This pattern is similar to the pattern described for prinoidin and 10-oxoprinoidin [3, 4]. The glycosylation sites were confirmed through NOE experiments. Irradiation of the aromatic proton signal at δ 7.39 (H-2) enhanced the glucosyl anomeric proton signal at δ 5.02 and the rhamnosyl anomeric proton signal at δ 5.75 and irradiation of another aromatic proton signal at δ 7.56 (H-4) led to the enhancement of the signal for the rhamnosly anomeric proton. Similarly, enhancement of the aromatic proton signal at δ 7.39 was observed when the signal of the glucosyl anomeric proton was irradiated. These observations led to establish the glucose and the diacetate rhamonse monosaccharides are attached to the aglycone through C-1 and C-3 hydroxyls, respectively. ^{13}C NMR spectral data are given in Table 1. Assignments were made by comparison of generated data with those in the literature for emodin-1-O-β-D-glucoside [5], prinoidin [3], frangulin A [5], and physcion 1-O-rhamnosyl-(1→ 2)-glucoside [6].



1 R = Ac; **2** R = H



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Compound **2** was a red solid. It had similar IR and UV-Vis spectra to that of compound **1**. The major difference in the ^{1}H and ^{13}C NMR spectra of **1** and **2** is the presence of signals for two acetyl groups in compound **1**. From the spectroscopic data generated, compound **2** is found to be glucofrangulin A, earlier reported from *R. frangula* [7].

Compound **3** was identified as emodin 1-O-β-D-glucoside by comparison of spectroscopic data obtained for **3** with reported data [5]. It has been isolated from *R. fragula*

[8] and *R. libanoticus* [5].

There are 26 glycosides of emodin and physcion derivatives in the lieterature among which 10 are from the genus *Rhamnus*. The sugars in all cases are α-L-rhamnopyranside and β-D-glucopyransode with the exception of one instance where D-apiofuranoside is found as a glycoside. It is important to note that all acetylated glycosides are isolated only from R. *prinoides*.

Table 1. ^{13}C NMR spectral data for compounds 1-3 (75.5 MHz).

C	1*	2*	3**
1	162.0	162.0	161.3
2	110.5	110.7	108.6
3	163.7	163.6	166.0
4	112.1	112.2	109.3
5	121.0	121.1	119.1
6	149.2	149.1	146.6
7	125.5	125.5	124.1
8	162.5	163.1	161.7
9	188.7	188.6	185.9
10	183.0	183.0	182.4
11	133.6	133.6	132.1
12	118.0	117.1	114.5
13	115.8	115.8	112.2
14	138.1	137.9	136.4
Ar-CH_3	22.0	22.0	21.3
glc-1	103.7	104.1	101.0
glc-2	74.8	74.7	73.2
glc-3	77.6	77.5	76.3
glc-4	70.5	71.2	69.4
glc-5	78.8	78.8	77.3
glc-6	62.5	62.5	60.1
rha-1	97.2	100.0	
rha-2	71.5	72.0	
rha-3	71.2	71.6	
rha-4	72.1	73.5	
rha-5	71.0	71.4	
rha-6	18.1	18.2	
-O-$\underline{C}O(CH_3)$	172.2, 171.7	-	
-OC$\underline{O}(CH_3)$	20.8, 20.7	-	

*Measured in CD_3OD, **Measured in DMSO-d_6.

EXPERIMENTAL

General. Mps: uncorr; FT-IR: KBR disks; ^1H NMR: 300 MHz; ^{13}C NMR: 75.5 MHz; chemical shifts were referenced to the residual solvent peaks (CD_3OD: DMSO-d_6). Flash CC: silica gel (particle size 40-63 μm) impregnated with 5% aq. oxalic acid. Prep. TLC: 0.5 mm thick layer silica gel.

Plant material. The fruit of *Rhamnus prinoides* were purchased from the central market, Markato in Addis Ababa, Ethiopia.

Extraction and isolation. Dried and powdered fruits of *R. prinoides* (400 g) were successively extracted with 1.5 L CH_2Cl_2-MeOH (1:1) overnight and 1 L MeOH (20 min). A portion of the residue obtained (30 g) by reduced pressure distillation (at $T \leq 40$ °C) of the combined extracts was subjected to silica gel flash CC and six fractions of each *ca.* 500

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Glucofrangulin A di-acetate (**1**). Red solid, mp 201-213° $[\alpha]_D^{26}$ - 120 (MeOH, c 0.02); UV-VIS λ_{max}^{MeOH} nm: 205, 220, 266, 413; IR_ν^{KBr} cm^{-1}: 3415, 2920, 1740, 1629, 1596, 1374, 1257, 1065, 593; ^1H NMR (300 MHz, CD_3OD): δ 7.56 (1H, d, J, = 2.52 Hz, H-4), 7.43 (1H, d, J = 1.11, H-5), 7.39 (1H, d, J = 2.55, H-2) 7.02 (1H, *br* s, H-7), 5.75 (1H, d, J = 1.74, rha-1), 5.46 (1H, dd, J = 1.74, 3.50, rha-2), 5.25 (1H, dd, J = 3.50, 9.74, rha-3), 5.02 (d, J = 7.59, glc-1), 3.50 - 4.00, m, sugar protons) 2.39 (3H, s, Ar-CH_3), 2.18, 2.06 (3H each, s, -O-$CO(CH_3)$), 1.30 (3H, d, J = 6.12 Hz, rha-6); ^{13}C NMR: Table 1.

Acid hydrolysis of compound **1**. 10 mg of compound **1** was dissolved in 10 ml 2 N HCl-MeOH (1:1) and refluxed for 2 h. The solution was cooled and extracted with EtOAc. The organic layer after dried over sodium sulphate and removal of the solvent gave emodin identical to an authentic specimen (TLC comparison). TLC analysis (EtOAc-MeOH-H_2O-AcOH, 6:2:1:1) of the aqueous phase also showed that the hydrolysates were β-D-glucose and α-L-rhamnose.

Glucofrangulin A (**2**). Red solid, mp 256°; $[\alpha]_D^{26}$ - 165 (MeOH, c 0.020; UV-VIS λ_{max}^{MeOH} nm: 205, 266, 356; IR_ν^{KBr} cm^{-1}: 3414, 2923, 1628, 1308, 1067, 614, 409; ^1H NMR (300 MHz, CD_3OD): δ 7.51 (1H, br, s, H-4), 7.39 (1H, br, s, H-5), 7.36 (1H, br s, H-2), 6.99 (1H, br, s, H-7), 5.64 (1H, br s, rha-1), 4.2 - 3.5 (m, sugar protons), 2.37 (3H, s, Ar-Me) 1.26 (3H, d, J = 6 Hz, rha-Me); ^{13}C NMR: Table 1.

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