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A 4-METHYL-7-HYDROXYPHTHALIDE GLYCOSIDE AND OTHER CONSTITUENTS FROM *QUILLAJA SAPONARIA MOLINA*

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Key Word Index—*Quillaja saponaria molina*; Rosaceae; bark; glycosides; phthalides.

Abstract—A so far unknown 4-methyl-7-hydroxyphthalideglycoside has been isolated from the methanol extract of the bark of *Quillaja saponaria molina*. Its structure has been established from NMR experiments as 7-*O*-[β-glucopyranosyl-(1 → 6)-β-arabinopyranosyl]-7-hydroxy-4-methyl-1[3H]-isobenzofuranone. Two known compounds, 3,4,5-trimethoxyphenyl-β-D-glucopyranoside and lyoniresinol-3α-*O*-β-D-glycopyranoside were also identified.

INTRODUCTION

Quillaja saponaria molina is a tree indigenous to Chile. Its bark is used for the production of quillaja saponin, which causes sneezing as a powder and strongly foams in aqueous solution. In some countries, for example, the U.S.A. and the U.K., the addition of this extract to foods is legal [1, 2].

We now report the isolation and structural determination of three compounds from the methanol extract of the bark of *Q. saponaria molina*, one of which is so far unknown. Isolation was performed by a series of column chromatographic separations with further purification by HPLC.

RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{20}H_{26}O_{12}$ by the fast bombardment (FAB) mass spectrum (*m/z* 476 $[M + NH_4]^+$ and 459 $[M + H]^+$) and that (*m/z* 711.2, $[M + H]^+$) of the acetyl derivative, $C_{32}H_{38}O_{18}$. Two methine carbon NMR signals at $\delta_C = 104.8$ and $\delta_C = 100.8$, typical for acetalic carbon atoms, and a group of ten carbon NMR signals between $\delta_C = 76.8$ and $\delta_C = 67.3$ (one of which belongs to the aglycone part) provided evidence for the existence of one hexose and one pentose in the molecule. They were identified as β-glucosyl and β-arabinosyl by means of the HH COSY contour plot upon acetylation of the sample, which gave sufficient resolution between the formerly extensively overlapping cross-signals of the sugar moiety, and HH coupling constants ($^3J_{HH} = 7.67$ Hz for the anomeric proton of arabinose, $^3J_{HH} = 7.15$ Hz for the anomeric proton of the glucose). This assignment could be established by hydrolysis and comparison with authentic samples on TLC. The absolute configuration of the arabinose and glucose (D or L) could not be determined from the sample available for hydrolysis (6 mg).

Concerning the aglycone part, a ^{13}C NMR signal ($\delta_C = 173.87$) indicated the presence of a carboxyl group, which could be classified as a lactone function by the IR absorption at $\nu = 1740\ cm^{-1}$. Furthermore, a methylene group ($\delta_C = 71.16$), a methyl group ($\delta_C = 16.80$), as well as four aromatic quaternary carbon atoms ($\delta_C = 154.30$, 149.75, 127.86 and 113.47) and two aromatic methine groups ($\delta_C = 139.11$ and 115.66) could be recognized. The two methine groups form an AB system ($^3J_{HH} = 8.5$ Hz) in the ^1H NMR spectrum, which reveals a 1,2,3,4-*tetra*-substituted benzene ring. NOE-difference spectroscopy indicated that both the aromatic 5-H proton and the 2-H methylene protons are in the neighbourhood of the 9-H methyl protons. The HMBC cross-signals between C-3, C-4 and C-5 to 9-H clearly localize C-9 at the aromatic carbon C-4, as is supported by the ^{13}C chemical shift of C-4 ($\delta_C = 127.8$). The quaternary aromatic carbon atom at $\delta_C = 154.3$ also shows CH long-range coupling to the glucose anomeric proton, observable in the HMBC contour plot. Table 1 gives the HMBC cross-signals of the aglycone moiety of **1**. These observations and all NMR assignments are summarized in formula **1**.

EXPERIMENTAL

Dry and finely powdered bark (2 kg) were soaked in 5 × 6 l methanol at room temp. to give 420 g of crude extract. The extract (100 g) was chromatographed on silica gel with a gradient mixt. of $CHCl_3$–MeOH to give 6 frs (1–6). Fr. 2 was separated on silica gel RP18 (MeOH–H_2O, 1:1) into 4 frs, 21–24. Compound **2** recrys-

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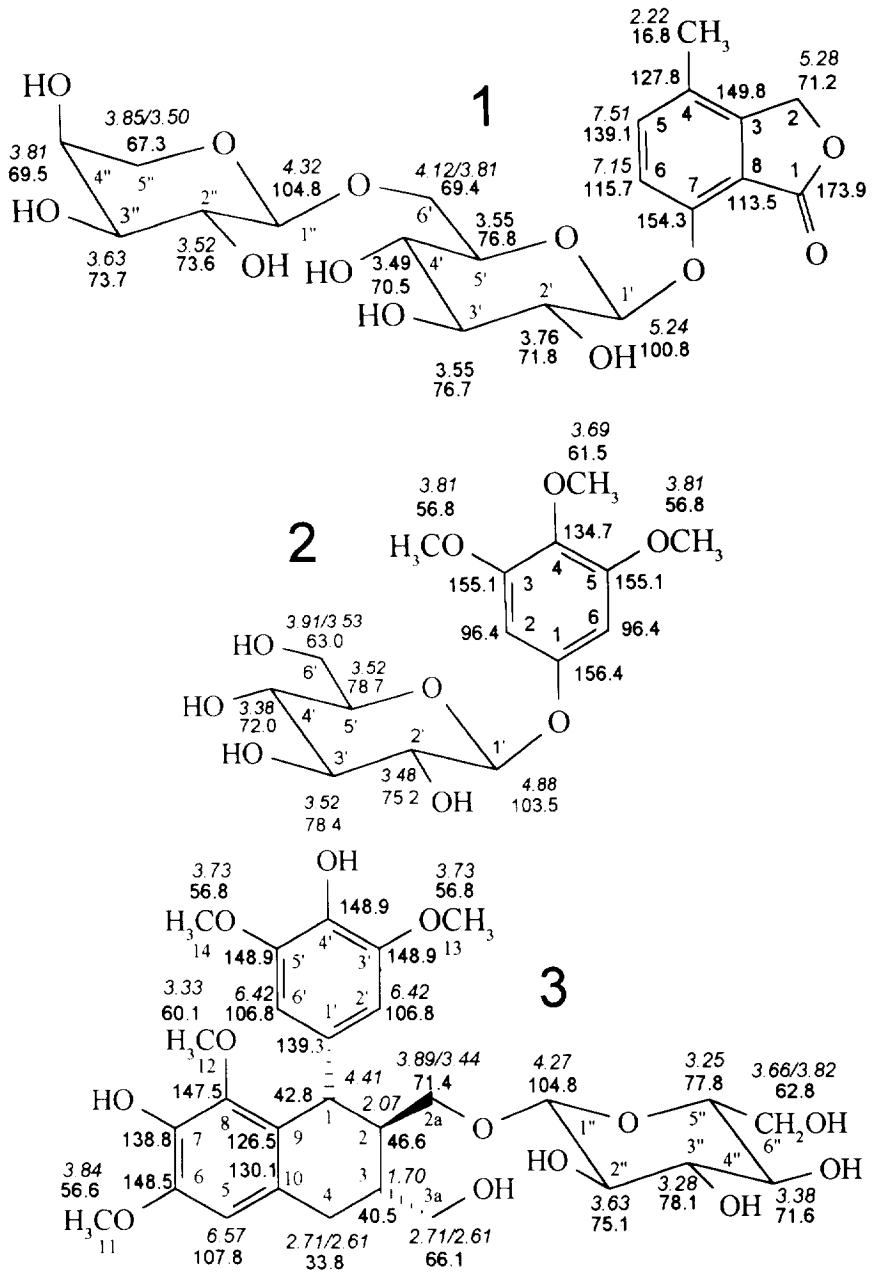

Table 1. Cross-signals in HMBC contour-plot of compound 1

	2-H	5-H	6-H	9-H
C-1	0		0	
C-2				
C-3	0	0		0
C-4			0	0
C-5				0
C-6				
C-7		0	0	
C-8	0		0	

tallized in a pure form from fr. 22. CC of fr. 4 on Sephadex LH-20 (MeOH) gave frs 41–43 Fr. 43 was combined with fr. 3 and purified on LichroPrep. RP18 (MeOH–H_2O, 1:1) and silica gel ($CHCl_3$–MeOH, 5:2). Finally, HPLC on Nucleosil RP_{18} gave 19 mg of **1** (R_f = 0.5, $CHCl_3$–MeOH, 5:2, on silica gel 60). Compound **3** was isolated from another portion of the crude extract, using a similar procedure.

Compound **1**. White crystalline substance (19 mg, 0.00095% yield, dry wt) $[\alpha]_D^{20} = +88.5$ (*c* 0.003 g ml^{-1}, H_2O). Mp 223°. $C_{20}H_{26}O_{12}$: *m/z* found 458 (FAB-MS), calcd 458.1424. Acetylated **1** $C_{32}H_{38}O_{18}$: *m/z* found 710.2 (FAB-MS), calcd 710.2058. NMR data

D_2O–CD_3OD, 500 MHz for ^1H, 125 MHz for ^{13}C (assignments based on HH COSY, CH COSY, HMBC and NOE difference spectroscopy) are summarized in formula **1** (^1H chemical shifts in *italics*).

Compound **2** [3]. White needles (35 mg, 0.00175% yield dry wt). Mp 203°. $C_{15}H_{22}O_9$: *m/z* found 346.1 (FAB-MS), calcd 346.1263. NMR data (same conditions as **1**) see formula **2**.

Compound **3** [4]. Amorphous powder (50 mg, 0.0025% yield, dry wt). Mp 80°. $C_{28}H_{39}O_{13}$: *m/z* found 582 (FAB-MS), calcd 583.61. NMR data (CD_3OD, same conditions as **1**) see formula **3**.

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