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LEAF FLAVONOIDS OF *ZIZIPHUS SPINA-CHRISTI*

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Key Word Index—*Ziziphus spina-christi*; Rhamnaceae; flavonol glycosides; quercetin 3-xylosyl(1 → 2)rhamnoside-4′-rhamnoside.

Abstract—From the leaves of *Ziziphus spina-christi*, the new flavonoid quercetin 3-xylosyl(1 → 2)rhamnoside-4′-rhamnoside as well as rutin, hyperin and quercitrin were characterized. The structures were established by chromatography, chemical degradation and UV spectroscopy, and confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy.

INTRODUCTION

Ziziphus species are used in traditional medicine, especially for treating insomnia [1]. Chemical studies have shown the presence of cyclopeptide alkaloids in the bark of *Z. sativa* [2], of saponins in the stem of *Z. mauritania* [3] and of *C*-glucosylflavones in the seeds of *Z. vulgaris* [1]. In a previous analysis *Z. spina-christi* was shown to contain betulic and ceanothic acid [4], but there are no reports on other constituents.

The present paper describes the isolation of the new natural product quercetin 3-*O*-β-D-xylopyranosyl(1 → 2)-α-L-rhamnopyranoside-4′-*O*-α-L-rhamnopyranoside in addition to rutin, hyperin and quercitrin from the aqueous ethanolic extract of the leaves of *Z. spina-christi* Miller. Similar flavonol triglycosides are known to occur in *Rhamnus petiolaris* [5] (Rhamnaceae).

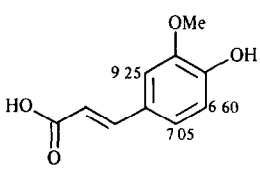
RESULTS AND DISCUSSION

Two-dimensional paper chromatography (PC) of the aqueous ethanolic extract of the fresh leaves of *Z. spina-christi* revealed an oligoglycosylated flavonoid (high mobility in aqueous solvents; dull brown gradually changing to yellow in UV + NH_3), together with a complicated mixture of flavonol 3-glycosides (dark brown changing to yellow in UV + NH_3). Application of polyamide column chromatography (CC) followed by PCC afforded four pure compounds, **1**–**4**.

Acid hydrolysis of compound **1** gave quercetin, rhamnose and xylose. UV spectral analysis of **1** in methanol and in the presence of diagnostic reagents [6, 7] suggested that the sugar moieties are bonded to the quercetin moiety at positions 3 and 4′ (positive shift with NaOAc, small shift with $AlCl_3$, small and moderately intense shift with NaOMe, and no shift with NaOAc–H_3PO_3).

Enzymatic hydrolysis of **1** with α-rhamnosidase [8] gave an intermediate, **1a** (dark brown on PC changing to yellow in UV + NH_3), which gave UV spectral data similar to those reported for quercetin 3-glycosides [9]. **1a** yielded quercetin, rhamnose and xylose (coPC) on acid hydrolysis; on controlled acid hydrolysis, it yielded quercetin 3-rhamnoside (quercitrin) (coPC). **1a** is therefore quercetin 3-xylosylrhamnoside and **1** is quercetin 3-xylosylrhamnoside-4′-rhamnoside. The suggested structure of **1** was confirmed by NMR spectroscopy. From the ^{13}C NMR spectra of **1**, the presence of two rhamnose moieties followed from two signals in the methyl region. The positions of these signals at δ17.84 and 18.08 indicated that the sugars were attached directly to the quercetin hydroxyls, because attachment to sugar hydroxyls would shift the signals downfield to *ca* δ21 [10]. The sugar moieties (rhamnose or xylose) must be attached to positions 3 and 4′ of quercetin, because these carbon signals were shifted upfield and the corresponding *ortho* and *para*-carbon signals were shifted downfield (see Experimental). Similar shifts are well-known from the work of Markham *et al.* [10]. The β-configuration of the xylose moiety was derived from the C-1 chemical shift at δ106.9 [11]; the α-configuration of the two rhamnose moieties followed from the C-1 chemical shift values at δ101.0 and 99.2 [10]. Attachment of the xylose moiety to C-2 of rhamnose was indicated by the shift of the rhamnose C-1 signal to δ99.2 (γ-upfield shift caused by C-1 of xylose) and of the rhamnose C-2 signal to δ80.4 (β-downfield shift caused by C-1 of xylose). The chemical shift values of all the sugar carbons confirmed the pyranose form of the three sugar moieties [12].

The ^{1}H NMR spectrum of **1** was also in accordance with the proposed structure. The small chemical shift difference ($\Delta\delta$ = 0.23 ppm) between the signals of the C-5′ and C-6′ protons indicated substitution (methylation,



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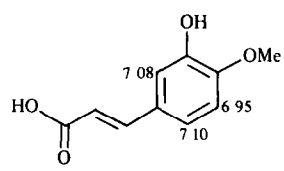

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acetylation, glycosylation, etc.) on the hydroxyl group at C-4′ and not at C-3′. A reversed substitution would have caused a larger shift difference, as can be derived from the ^{1}H NMR data (obtained in the same solvent, DMSO) of ferulic acid **A** and isoferulic acid **B**.

The two rhamnose anomeric protons gave rise to two signals, the positions of which (δ5.34 and 5.37) indicated the attachment of the anomeric carbons of each rhamnose to the quercetin hydroxyls [6], and the half-width of which (*ca* 4 Hz) proved the α-configuration at the anomeric carbons. The anomeric proton of the xylose moiety was hidden by a broad hydroxyl signal at δ4–4.5.

The conformation of the three sugar moieties is $^1C_4$ for the two rhamnose moieties and $^4C_1$ for the xylose moiety. This follows from the α- and β-configurations discussed above. The attachment of the disaccharide moiety to C-3 and not to C-4′ could not be derived from ^{1}H NMR or from ^{13}C NMR data, but only from the results of enzymatic degradation as discussed above.

Compound **2** was identified as rutin by acid hydrolysis, UV spectral analysis and coPC. The structure was confirmed by ^{1}H NMR spectroscopy, which gave data identical to those reported for rutin [6]. Further confirmation of the structure was achieved through ^{13}C NMR spectroscopy.

Compounds **3** and **4** were identified as hyperin and quercitrin, respectively, by acid hydrolysis, UV spectral analysis, coPC, ^{1}H NMR and ^{13}C NMR spectroscopy [6, 10].

EXPERIMENTAL

NMR: JEOL FX 100, δ values, solvent DMSO-d_6; reference: signal of DMSO-d_6 set at δ39.5 which is the chemical shift in relation to $\delta_{TMS} = 0$. Atomic absorption: Varian 1000 spectrometer. PC was carried out on Whatman No. 1 paper using solvent systems: (1) HOAc (HOAc–H_2O, 3:17); (2) BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, top layer); (3) iPW (*i*-PrOH–H_2O, 11:39); (4) forestal (conc. HCl–H_2O–HOAc, 3:10:30); (5) BPOH (C_6H_6–*n*-BuOH–pyridine–H_2O, 1:5:3:3, top layer). In addition, solvent systems 1, 2 and 3 were used in PPC on Whatman No. 3 paper.

Leaves were extracted with EtOH–H_2O, 1:3. The dried extract was transferred to a Polyamide column and eluted with H_2O followed by H_2O–MeOH (9:1, 3:2 and 7:3), successively. Pure **1** was isolated from the 9:1 fraction by PPC using solvent 3. Pure **2** and **3** were isolated from the 3:2 fraction by PPC using solvents 1 and 2. Crystals of **4** were separated from the concentrate of the 7:3 fraction.

Quercetin 3-xylosyl(1 → 2)*rhamnoside-4′-rhamnoside* (**1**). R_f values: 0.58 (HOAc); 0.50 (BAW); 0.61 (iBW). UV λ_{max}^{MeOH} nm: 256, 266′, 353; $\Delta\lambda$ (nm) on addition of: NaOAc = 6, $AlCl_3$ = 52, $AlCl_3$–HCl = 50, NaOMe = 45. Acid hydrolysis (1.5 M aq. HCl, 100°, 45 min) of **1** gave xylose, rhamnose (coPC), and quercetin (mp, mmp, coPC and UV data). Hydrolysis with α-rhamnosidase (pectinase, from Koch and Light) yielded the intermediate quercetin 3-xylosyl(1 → 2)rhamnoside (**1a**). R_f values: 0.52 (HOAc); 0.60 (BAW); 0.55 (iPW). UV λ_{max}^{MeOH} nm: 256, 266′, 295′, 352; $\Delta\lambda$ (nm) on addition of: NaOAc = 6, NaOAc–H_3PO_3 = 15, $AlCl_3$ = 68, $AlCl_3$/HCl = 56, NaOMe = 48.

Acid hydrolysis of **1a** yielded xylose, rhamnose and quercetin (coPC), while controlled acid hydrolysis (1.5 M HCl, 100°, 5 min) yielded quercitrin (coPC and UV spectral data).

^{13}C NMR: aglycone: δ156.84 (C-2), 135.24 (C-3), 178.60 (C-4), 161.00 (C-5), 99.20* (C-6), 164.82 (C-7), 93.26 (C-8), 156.84 (C-9), 104.20 (C-10), 124.02 (C-1′), 116.08† (C-2′), 147.90 (C-3′), 146.82 (C-4′), 117.10† (C-5′), 120.04 (C-6′); 3-*O*-α-L-rhamnoside: δ98.82* (C-1), 80 40 (C-2), 70.46‡ (C-3), 71.98§ (C-4), 70.02‡ (C-5), 17.84 (Me); 4′-*O*-α-L-rhamnoside: δ101.06 (C-1), 70.22‡ (C-2), 70.46‡ (C-3), 71.98§ (C-4), 70.02† (C-5), 18 08 (Me); 3-*O*-β-D-xyloside: δ106.40 (C-1), 73.66 (C-2), 76.02 (C-3), 70.46‡ (C-4), 65.02 (C-5). (*,†,‡,§: Assignments bearing the same superscript may be reversed).

^{1}H NMR: aglycone moiety. δ6.2 (*d*, *J* = 2.5 Hz, 6-H), 6.4 (*d*, *J* = 2 5 Hz, 8-H), 7.15 (*d*, *J* = 8 Hz, 5′-H), 7.23 (*d*, *J* = 8 Hz, 6′-H); the two lines were each broadened by 4 Hz due to coupling to 2′-H; 7.36 (*s*, $W_{1/2}$ = 4 Hz, 2′-H); sugar moieties· δ5.27 (*s*, $W_{1/2}$ = 3 5 Hz, 1-H of rhamnoside), 5.34 (*s*, $W_{1/2}$ = 4 Hz, 1-H of rhamnoside), 2.8–4.1 (*m*, 12 sugar protons), 0 85 (*d*, *J* = 6 Hz, Me of rhamnoside), 1.08 (*d*, *J* = 6 Hz, Me of rhamnoside).

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