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QUERCETIN 3-GLYCOSIDES FROM THE LEAVES OF *SOLANUM NIGRUM*

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Key Word Index—*Solanum nigrum*; Solanaceae, quercetin 3-*O*-glycosides, NMR spectral analysis

Abstract—Two new quercetin glycosides have been identified from the leaves of *Solanum nigrum*, namely, quercetin 3-*O*-(2^{Gal}-α-rhamnosyl)-β-glucosyl(1→6)-β-galactoside and quercetin 3-*O*-α-rhamnosyl(1→2)-β-galactoside. The known compounds: quercetin 3-glucosyl(1→6)galactoside, 3-gentiobioside, 3-galactoside and 3-glucoside, were also found. All structures were determined by FAB MS, ^{1}H NMR and ^{13}C NMR analysis.

INTRODUCTION

The large genus Solanum contains some 2000 species [1]. In Egypt there are 10 native *Solanum* species, of which *S. nigrum* is widespread [2] and provides extracts used in folk medicine [3] for treating burns and infections.

Surveys of *Solanum* species have shown that the 3- and 3,7 glycosides of the flavonols quercetin and kaempferol are predominant, while quercetin and kaempferol 3-methyl ethers, 8-hydroxymyricetin 3,7,4′-trimethyl ether, 8-methoxymyricetin 3,7,4′-trimethyl ether, luteolin and 8-hydroxychrysoeriol 7-dimethyl ether are of restricted occurrence [4, 5]. From *Solanum nigrum* quercetin 3-glucoside and an unspecified quercetin 3-diglucoside have been reported [6].

The present work reports the isolation and structure elucidation of the new flavonoids, quercetin 3-*O*-(2^{Gal}-α-rhamnosyl)-β-glucosyl(1→6)-β-galactoside (**1**) and quercetin 3-*O*-α-rhamnosyl (1→2)-β-galactoside (**2**) from the leaves of *S. nigrum*. In addition, the known quercetin 3-β-glucosyl(1→6)-β-galactoside (**3**), quercetin 3-gentiobioside (**4**), quercetin 3-galactoside (**5**) and quercetin 3-glucoside (**6**) have been identified. It should be noted that this is only the second report of compound **3**, which was previously characterized on the basis of routine HPTLC and sugar acetate GC/MS analysis, from the leaves of *Astrantia major* (Umbellifereae) [7]. The kaempferol analogue of **3** was reported from *Eryngium planum* (Umbellifereae) [8], while quercetin 3-rhamnosyl(1→6) galactoside (quercetin 3-robinobioside), the positional isomer of **2** was recently characterized from *Strychnos variabilis* (Loganiaceae) [9].

RESULTS AND DISCUSSION

Compounds **1**–**6** were isolated from an aqueous ethanolic leaf extract of *S. nigrum* by applying a combination of CC on cellulose followed by polyamide and preparative paper chromatography. The known compounds **3**–**6** showed chromatographic, UV absorption and hydrolytic data identical with those of quercetin 3-β-glucosyl(1→6)-β-galactoside, quercetin 3-gentiobioside, quercetin 3-galactoside and quercetin 3-glucoside, respectively. These structures were confirmed by FAB MS, ^{1}H NMR and ^{13}C NMR spectral analysis [10].

Compound **1** was isolated as a brown amorphous powder with M_r of 772 as shown by negative FABMS. The chromatographic and UV spectral analysis [11, 12] of **1** suggested a quercetin 3-*O*-oligosaccharide, which was supported by acid hydrolysis of **1** to yield quercetin, rhamnose, glucose and galactose. On controlled acid hydrolysis **1** yielded **3** as an intermediate, which was identified as quercetin 3-β-glucosyl(1→6)-β-galactoside. Partial β-glucosidase hydrolysis of **1** gave compound **2**, which was identified as quercetin 3-α-rhamnosyl(1→2)-β-galactoside. Consequently, **1** must be quercetin substituted at its 3-position by the new branched sugar, 2^{Gal}-α-rhamnosyl-β-glucosyl(1→6)-β-galactoside. This proposed structure was confirmed by ^{1}H NMR analysis of **1**, which showed three anomeric sugar proton signals at δ5.57 (*d*, *J* = 8 Hz) assignable to the 1-H β-galactoside proton, 5.03 (*d*, *J* = 2 Hz) assignable to the 1-H α-rhamnosyl proton and at 4.02 (*d*, *J* = 7.5 Hz) assignable to the 1-H β-glucosyl proton. The remaining signals in this spectrum are in agreement with the proposed structure. Final confirmation of the structure of **1** as quercetin 3-*O*-(2^{Gal}-α-rhamnosyl)-β-glucosyl(1→6)-β-galactoside was obtained from the ^{13}C NMR. The spectrum, which showed three anomeric sugar carbon signals at δ99.2, 100.5 and 103.3, assignable to the C-1 carbons of β-galactoside, α-rhamnosyl and the β-glucosyl moieties of **1**, respectively. Also, it showed a methyl rhamnose carbon signal at 20.9. These and the remaining signals are in accordance with the proposed structure.

Compound **2** was obtained as a brown amorphous powder, which exhibited a M_r of 610 (−ve FABMS). It was recognized as a quercetin 3-*O*-diglycoside from acid hydrolysis, its chromatographic behaviour and UV spectral analysis. Thus, acid hydrolysis of **2** gave quercetin, rhamnose and galactose. Partial acid hydrolysis gave the intermediate quercetin 3-*O*-β-galactoside suggesting that **2** is a quercetin 3-*O*-rhamnosylgalactoside. In the ^{1}H NMR spectrum of **2**, the presence of two sugar moieties was evidented by the two proton signals at δ5.03 (*d*, *J* = 2 Hz) assignable to the anomeric α-rhamnose proton and at 5.62 (*d*, *J* = 8 Hz) assignable to the anomeric β-galactoside proton. The recognizable down-field shift (Δδ = 0.3 ppm) which was detected on comparing the chemical shifts of the anomeric galactoside proton signals

in the spectrum of **2** with those of quercetin 3-galactoside proved that the terminal α-rhamnosyl moiety is attached to C-2 of the inner β-galactoside moiety. Consequently **2** is identified as quercetin 3-*O*-α-rhamnosyl(1→2)-β-galactoside Finally, the ^{13}C NMR spectrum of **2** confirmed the (1→2) linkage between the sugar moieties, whereby two signals have revealed their presence at δ100.5 and 98.8 and were assigned to the C-1 of the α-rhamnosyl and the C-1 of the β-galactoside moieties of **2**, respectively. The remaining signals, in this spectrum are in close agreement with the proposed structure of **2**

Compound **3** was isolated as yellow crystals with a M_r of 626 (−ve FABMS). It showed chromatographic properties and UV spectral data identical with those reported for quercetin 3-*O*-β-glucosyl(1→6)-β-galactoside [7]

The ^{1}H NMR spectrum of **3** was in accordance with the proposed structure and revealed two anomeric sugar proton signals at δ5 32 (*d*, *J* = 8 Hz) assignable to the 1-H-β-galactoside proton and at 4.08 (*d*, *J* = 7.5 Hz) assignable to an anomeric β-glucosyl proton. The chemical shift values of these two anomeric protons proved that the linkage between them was of the 1→6 type. ^{13}C NMR analysis of **3** finally confirmed its structure. Thus in the ^{13}C NMR spectrum, two anomeric sugar carbon signals were revealed at δ102.2 and 103 2, which were assigned to the C-1 of the β-galactoside and the C-1 of the β-glucosyl moieties, respectively In this spectrum, the recorded quercetin carbon signals possess chemical shift values which were closely similar to those reported for quercetin with an *O*-substitution at the 3-position [13]. Assignment of the eight sugar carbon signals, in the region from 61.2 to 77.0 was achieved by applying the substituent additive rules on the sugar carbon signals recorded in the spectrum of quercetin 3-*O*-β-galactoside.

EXPERIMENTAL

NMR spectra were measured in DMSO-d_6, using TMS as an int standard. Solvent systems for PC (Whatman paper No. 1). 1. H_2O, 2 HOAc (HOAc–H_2O, 3 17); 3 BAW (*n*-BuOH–HOAc–H_2O, 4 1·5, top layer), 4 HOAc* (HOAc–H_2O, 3·2), 5 *n*-BuPOH (C_6H_6–*n*-BuOH–pyridine–H_2O, 1·5 3 3, top layer). Solvent systems 2 and 3 were used for PPC on Whatman paper No 3MM Solvent systems 3 and 5 were used in sugar analysis.

Plant material Samples of *S nigrum* were collected from the Delta region, near Cairo, during February 1987 and classified by Dr Loutfy Boulos (Prof. of Botany, National Research Centre, Cairo) Leaves were extracted with EtOH–H_2O, 3 1 and the dried extract was applied to a microcrystalline cellulose (Merck) column Elution was started with *n*-BuOH saturated with H_2O to eliminate the non-flavonoid materials The flavonoids were eluted with MeOH and the conc methanolic eluate fractionated on a polyamide (6S for CC, Riedel-De Haen AG, Germany) column using H_2O–EtOH mixtures of decreasing polarities for elution PPC of the 7 3, 3.2 and 1 1 fractions afforded pure samples of **1** and **2** from the first two fractions, respectively and of **3** and **4** from the latter Compounds **5** and **6** were isolated pure from fraction 3.7, also by PPC

General hydrolytic procedures Normal acid hydrolysis—1 5 M aq HCl, 100°, 2 hr Controlled acid hydrolysis—0 1 M aq HCl, 100°, 30 min Partial β-glucosidase hydrolysis-0 5 ml of β-glucosidase in 0 05 M acetate buffer, pH 5 1, 37°, 4 hr

Quercetin 3-O-(2^{Gal}-α-rhamnosyl)-β-glucosyl(1→6)-β-galactoside (**1**) M_r 772, −ve FABMS (M^{-1} 771) R_f-values 0 72 (H_2O), 0 76 (HOAc), 0.44 (BAW) UV λ_{max}^{MeOH} nm. 255, 266$^+$, 362, +NaOAc 257$^+$, 272, 378, +NaOAc–H_3BO_3 262, 379, $AlCl_3$ 265, 300$^+$, 362$^+$, 418, +NaOMe 270, 327, 409 (+ = inflection) Normal acid hydrolysis gave rhamnose, glucose, galactose (CoPC) and quercetin (CoPC, UV absorption and ^{1}H NMR). Controlled acid hydrolysis gave quercetin 3-glucosyl (1→6)-galactoside (CoPC against **3**, -ve FABMS M_r 626, M^{-1} 625, ^{1}H NMR identical with that recorded for **3**) Partial β-glucosidase hydrolysis gave quercetin 3-*O*-α-rhamnosyl (1→2)-β-galactoside (CoPC against **2**, −ve FABMS M_r 610, M^{-1}· 609, ^{1}H NMR identical with that recorded for **2**) ^{1}H NMR of the aglycone of **3**· δ6 15 (*d*, *J* = 2 5 Hz, 6-H), 6 38 (*d*, *J* = 2 5 Hz, 8-H), 6 88 (*d*, *J* = 7 5 Hz, 5′-H), 7.73 (*m*, 2′-H and 6′-H), sugar moieties 5.57 (*d*, *J* = 8 Hz, 1-H galactoside), 5 03 (*d*, *J* = 2 Hz, 1-H rhamnosyl), 4 02 (*d*, *J* = 7 5 Hz, 1-H glucosyl), 3 3–3 85 (*m*, sugar protons overlapped by hydroxyl protons), 1 12 (*d*, *J* = 6 Hz, Me rhamnosyl) ^{13}C NMR of the aglycone of **3** δ156 1 (C-2), 133 3 (C-3), 177 4 (C-4), 161 2 (C-5), 97 7 (C-6), 163.9 (C-7), 94 0 (C-8), 156 4 (C-9), 103 0 (C-10), 121 1 (C-1′), 115 3 (C-2′), 144 8 (C-3′), 148.5 (C-4′), 116 2 (C-5′), 121 6 (C-6′); galactosyl moiety· 99 0 (C-1), 74 0 (C-2), 73 4 (C-3), 68 0 (C-4), 73.1 (C-5), 67 4 (C-6); rhamnosyl moiety 100 5 (C-1), 69.8 (C-2), 70 6 (C-3), 71 8 (C-4), 68 0 (C-5), 20 9 (C–Me), glucosyl moiety. 103 0 (C-1), 74 9 (C-2), 76 5 (C-3), 69 8 (C-4), 76 5 (C-5), 60 8 (C-6)

Quercetin 3-O-α-rhamnosyl(1→2)-β-galactoside (**2**) M_r 610, −ve FABMS (M^{-1} 609) R_f-values 0.63 (H_2O), 0 55 (HOAc), 0 55 (BAW). UV λ_{max}^{MeOH} nm 255, 267$^+$, 360; +NaOAc 255$^+$, 270, 378, +NaOAc–H_3BO_3 261, 380, +$AlCl_3$ 266, 300$^+$, 364$^+$, 420; +NaOMe 270, 330, 403 (+ = inflection) Normal acid hydrolysis gave rhamnose, galactose and quercetin (CoPC) Controlled acid hydrolysis gave quercetin 3-galactoside (CoPC), −ve FABMS M_r 464 M^{-1}. 463, ^{1}H NMR of the aglycone δ6 24 (*d*, *J* = 2 5 Hz, 6-H); 6 44 (*d*, *J* = 2 5 Hz, 8-H), 6 84 (*d*, *J* = 8 Hz, 5′-H), 7 56 (*d*, *J* = 2 5 Hz, 2′-H), 7 64 (*d*, *d*, *J* = 2 5 and 8 Hz, 6′-H), galactose moiety 5.32 (*d*, *J* = 8 Hz, 1-H), 3 2–3 8 (*m*, galactose protons overlapped with hydroxyl protons) ^{1}H NMR of the aglycone of **2** δ6 19 (*d*, *J* = 2 5 Hz, 6-H); 6 4 (*d*, *J* = 2 5 Hz, 8-H), 6 8 (*d*, *J* = 7 5 Hz, 5′-H), 7 49 (*d*, *J* = 2 5 Hz, 2′-H), 7.7 (*d*, *d*, *J* = 2 5 and 7.5 Hz, 6′-H), sugar moieties 5.62 (*d*, *J* = 8 Hz, 1-H galactoside), 5 03 (*d*, *J* = 2 Hz, 1-H rhamnosyl), 3 35–3 8 (*m*, sugar protons overlapped by hydroxyl protons), 1 02 (*d*, *J* = 6 Hz, Me rhamnosyl) ^{13}C NMR of the aglycone of **2** δ155 9 (C-2), 132.9 (C-3), 177 3 (C-4), 161 1 (C-5), 98 6 (C-6), 163 9 (C-7), 93 4 (C-8), 156 2 (C-9), 103 8 (C-10), 121 1 (C-1′), 115 2 (C-2′), 144 8 (C-3′), 148 4 (C-4′), 115 6 (C-5′), 122 1 (C-6′), galactosyl moiety 98 8 (C-1), 75 0 (C-2), 74 0 (C-3), 68 1 (C-4), 75 6 (C-5), 60 0 (C-6), rhamnosyl moiety 100 5 (C-1), 70 6 (C-2), 70 7 (C-3), 71 9 (C-4), 68 3 (C-5), 19 9 (C-Me)

Quercetin 3-O-β-glucosyl(1→6)-β-galactoside (**3**). M_r 626, −ve FABMS (M^{-1} 625) ^{1}H NMR of the aglycone of **3**· δ6 2 (*d*, *J* = 2 5 Hz, 6-H), 6.4 (*d*, *J* = 2.5 Hz, 8-H), 6 82 (*d*, *J* = 7 5 Hz, 5′-H); 7 52 (*d*, *J* = 2 5 Hz, 2′-H), 7 68 (*d*, *d*, *J* = 2 5 and 7 5 Hz, 6′-H), sugar moieties. 5 32 (*d*, *J* = 8 Hz, 1-H galactoside), 4 08 (*d*, *J* = 7 5 Hz, 1-H glucosyl), 3.3–3 75 (*m*, sugar protons, hidden by hydroxyl signals) ^{13}C NMR of the aglycone of **3** δ156 3 (C-2), 133 5 (C-3), 177 4 (C-4), 161 2 (C-5), 98.6 (C-6), 164 1 (C-7), 93 5 (C-8), 156 3 (C-9), 104 1 (C-10), 121 0 (C-1′), 115 2 (C-2′), 144 8 (C-3′), 148 4 (C-4′), 115 9 (C-5′), 121 9 (C-6′), galactosyl moiety 102.2 (C-1), 71.3 (C-2), 73 5 (C-3), 68 3 (C-4), 73 9 (C-5), 67.3 (C-6), glucosyl moiety 103.2 (C-1), 74 2 (C-2), 76 8 (C-3), 70 0 (C-4), 76 8 (C-5), 61.1 (C-6)

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