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FLAVONOL GLYCOSIDES FROM LEAVES OF *STRYCHNOS VARIABILIS*

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Key Word Index—*Strychnos variabilis*; Loganiaceae; leaves; flavonoids; quercetin 3-*O*-galactoside; quercetin 3-*O*-robinobioside; kaempferol 3-*O*-robinobioside.

Abstract—Quercetin 3-*O*-galactoside, quercetin 3-*O*-robinobioside and kaempferol 3-*O*-robinobioside were obtained from the leaves of *Strychnos variabilis*.

INTRODUCTION

Strychnos variabilis is an endemic African species (Kinshasa province, Zaire) whose root bark is a violent poison; it contains indole alkaloids [1]. The leaves contain a very small amount of indoline alkaloids [2]. In this paper, we report the isolation by DCCC and characterization of quercetin 3-*O*-galactoside and two rare diglycosides quercetin and kaempferol 3-*O*-rhamnosyl-(1 → 6)galactoside [3].

RESULTS AND DISCUSSION

Monoglycoside

The crude ethyl acetate extract contained quercetin 3-*O*-galactoside. The structure assignments were determined by TLC (co-chromatography), UV [4], ^{13}C NMR [5], FAB-MS, hydrolytic and chemical methods. FAB-MS showed signals at *m/z* 465 and 303 corresponding to the molecular ion [M + 1] and to the loss of the sugar unit respectively. The ^{13}C NMR spectrum showed that galactose was in the β-D-galactopyranose form.

Diglycoside

The crude butanol extract contained quercetin 3-*O*-robinobioside. The structure assignments were determined by TLC, UV [4], ^{13}C NMR [5], FAB-MS and hydrolytic methods. It is worth noting that this compound has exactly the same chromatographic behaviour as rutin in the usual systems. However, acidic hydrolysis with 6% aqueous hydrochloric acid afforded quercetin, galactose and rhamnose. Mild acidic hydrolysis with formic acid in cyclohexanol [6] afforded quercetin-3-*O*-galactoside (co-chromatography with authentic sample). The failure of the glycoside to give a positive test with aniline phthalate reagent indicated that both the sugars are linked through their respective reducing group. FAB-MS showed signals at *m/z* 611, 465 and 303 respectively corresponding to the molecular ion [M + 1], the loss of rhamnose and the loss of the rhamnosylgalactose unit. The ^{13}C NMR spectrum showed the sugars to be in the β-D-galactopyranose and α-L-rhamnopyranose forms.

Maksyutina isolated a quercetin 3-*O*-rhamnogalactoside, bioquercetin, from the unripe fruits of *Robinia pseudacacia* and postulated that the disaccharide was 6-*O*-β-L-rhamnopyranosyl-β-D-galactofuranose [7]. Farkas *et al.* synthesized a quercetin-3-*O*-β-robinobioside but it had a different optical rotation [8]. Lakhman *et al.* isolated quercetin 3-*O*-β-robinobioside from the epigeal part of *Lespedeza hedysaroides* but the disaccharide moiety was not fully characterized [9]. Williams and Harborne [10] isolated a quercetin 3-rhamnosylgalactoside from leaves of *Costus sanguineus*, but its R_f value appears to be different from our substance. Similarly, quercetin 3-rhamnosylgalactosides reported in flowers of *Crataegus pinnatifida* [11] and in leaves of *Brickellia chlorolepis* [12] have yet to be fully characterized.

Acidic hydrolysis with 6% aqueous hydrochloric acid of kaempferol 3-*O*-robinobioside afforded kaempferol, galactose and rhamnose. UV spectra showed it was a kaempferol 3-*O*-glycoside [4]. Mild acidic hydrolysis with formic acid in cyclohexanol [6] yielded a monoglycoside having a lower R_f value than kaempferol 3-*O*-glucoside (astragalin); this indicated that galactose was linked to the aglycone. The failure of the glycoside to give a positive test with aniline phthalate indicated that both sugars are linked through their respective reducing group. FAB-MS showed signals at 595, 449 and 287 respectively corresponding to the molecular ion [M + 1], the loss of rhamnose and the loss of rhamnosylgalactose unit. ^{13}C NMR showed that α-L-rhamnopyranose was linked to C-6 of β-D-galactopyranose [5]. Thus, the new compound is kaempferol 3-*O*-robinobioside. The only similar compound cited by Harborne [13] is a kaempferol 3-rhamnogalactoside isolated by Steinegger from *Atropa belladonna* leaves [14]. Without complete structure assignments, this compound should not be called kaempferol 3-robinobioside.

Although *S. variabilis* leaves contain quercetin 3-*O*-robinobioside and the corresponding monoglycoside, quercetin 3-*O*-galactoside, we have not found any trace of kaempferol monoglycoside.

EXPERIMENTAL

Plant material. Leaves of *Strychnos variabilis* were collected in 1951 at the Botanical Garden of Kisantu and well stored, sheltered from light, in the laboratorium of Pharmacognosy (Liege University). Herbarium specimens are kept in the

Botanical Garden of Belgium at Meise and in the University of Liege (Duvigneaud, 147 et 725). An extract from this sample has the same chromatographic behaviour as fresh leaves collected in 1980 at Kinshasa by Professor Kambu and stored in the University of Liege (Kambu, s.n.).

General techniques. TLC of glycosides was carried out on silica gel 60-F254 precoated plastic sheets (Merck) with EtOAc–HCOOH–H_2O (6:1:1); TLC of aglycones on cellulose plastic sheets (Merck) with HOAc–H_2O (3:2) and $CHCl_3$–HOAc–H_2O (10:9:1); aglycones and glycosides are visualized with Naturstoffreagenz A-PEG 400; TLC of sugars on silica gel 60-F 254 pre-coated plastic sheets (Merck) with *n*-BuOH–Me_2CO–NaH_2PO_4 1.6% (4:5:1) (Eur. Ph.) and visualized with aniline phthalate reagent. Hydrolyses and recording of the UV spectra with the usual shift reagents were made according to standard procedures [4]. ^{13}C NMR spectra were recorded in DMSO-d_6 at 30° at 75.5 MHz. ^1H NMR spectra were recorded in DMSO-d_6 at 30° at 300 MHz.

Isolation. Leaves (100 g) were extracted with EtOH and the concd extract was taken up in boiling H_2O. The filtrate was successively extracted by Et_2O, EtOAc and *n*-BuOH. The crude EtOAc (2.5 g) extract was purified by CC (Lobar ^{R} LichroPrep ^{R} RP 8; 20–40% aq. Me_2CO). The purified extract was submitted to DCCC with $CHCl_3$–MeOH–PrOH–H_2O (5:6:1:4) in the descending mode (300 columns, 40 cm × 2 mm, instrument DCCA, Tokyo Rikakikai, Japan). The crude BuOH extract (4.6 g) purified by CC (Lobar ^{R} LichroPrep ^{R}RP 8; 20–30% aq. Me_2CO). The purified extract (1.5 g) was submitted to DCCC with $CHCl_3$–MeOH–PrOH–H_2O (10:12:3:8) in the descending mode (300 columns, 40 cm × 2 mm, instrument DCCA, Tokyo Rikakikai, Japan).

Quercetin 3-O-*galactoside.* UV, ^{13}C NMR in agreement with published data [4, 5].

Quercetin 3-O-*rhamnosyl*(1 → 6)*galactoside.* UV λ_{max}^{MeOH} nm: 360, 298 sh, 265 sh, 258; (NaOMe) 413, 329 sh, 273; ($AlCl_3$) 432, 333 sh, 303 sh, 276; ($AlCl_3$ + HCl) 402, 365 sh, 300 sh, 270; (NaOAc) 397, 326, 273 (NaOAc + H_3BO_3) 380, 296 sh, 262. ^{13}C NMR (75.5 MHz, DMSO-d_6): δ177.4 (C-4), 164.4 (C-7), 161.2 (C.5), 156.4 (C-2, C-9), 148.6 (C-4′), 144.9 (C-3′), 133.6 (C-3), 121.9 (C-1′), 121.1 (C-6′), 116 (C-5′), 155.2 (C-2′), 103.8 (C-10), 102.2 (C-1″), 100.1 (C-1‴), 98.8 (C-6), 93.6 (C-8), 73.6 (C-5″), 73.1 (C-3″), 72 (C-4‴), 71.1 (C-2″), 70.7, 70.5 (C-2‴, C-3‴), 68.3 (C-5‴), 68.1 (C-4″), 65.2 (C-6″), 17.9 (C-6‴). ^1H NMR (300 MHz, DMSO-d_6): δ7.7 (1H, *dd*, *J* = 8.5 and 2 Hz, H-6′), 7.5 (1H, *d*, *J* = 2 Hz, H-2′), 6.8 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.4 (1H, *d*, *J* = 1.7 Hz, H-8), 6.2 (1H, *d*, *J* = 1.6 Hz, H-6), 5.3 (1H, *d*, *J* = 7.6 Hz, galactosyl H-1), 4.4 (1H, rhamnosyl H-1), 3.3 (*m*, sugar protons), 1.1 (3H, *d*, *J* = 6 Hz, rhamnosyl-Me).

Kaempferol 3-O-*rhamnosyl*(1 → 6)*galactoside.* UV λ_{max}^{MeOH} nm: 349, 301 sh, 264; (NaOMe) 402, 326, 275; ($AlCl_3$) 400, 353, 303 sh, 275, 229; ($AlCl_3$ + HCl) 395, 349, 303 sh, 275, 231; (NaOAc) 395, 309, 274; (NaOAc + H_3BO_3) 355, 267; ^1H NMR (300 MHz, DMSO-d_6): δ12.6 (1H, *s*, OH-5), 8.04 (2H, *d*, *J* = 8 Hz, H-2′ and H-6′) 6.85 (2H, *d*, *J* = 8 Hz, H-3′ and H-5′), 6.4 (1H, *s*, H-8), 6.18 (1H, *s*, H-6), 5.31 (1H, *d*, *J* = 7 Hz, galactosyl H-1), 4.39 (1H, *s*, rhamnosyl H-1), 1.05 (3H, *d*, *J* = 6 Hz, rhamnosyl-Me); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ177.5 (C-4), 164 (C-7), 161.2 (C-5), 160.0 (C-4′), 156.5 (C-9, C-2), 133.3 (C-3), 131 (C-6′), 120.9 (C-1′), 115.1 (C-3′, C-5′), 103.8 (C-10), 102.1 (C-1″), 100.1 (C-1‴), 98.8 (C-6); 93.8 (C-8), 73.6 (C-5″), 73.0 (C-3″), 72.0 (C-4‴), 71.1 (C-2″), 70.7, 70.4 (C-2‴, C-3‴), 68.3 (C-5‴), 68.1 (C-4″), 65.4 (C-6″), 17.9 (C-6‴).

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