312, 400; $AlCl_3$/HCl: 262, 280, 310, 377, 412sh; NaOAc: 270, 320, 418; NaOAc/$H_3BO_3$: 275, 365. NMR: $C_6D_6$: 3.76 and 3.66 (OMe groups at 3 and 6); 3.52 (OMe groups at 3',5'); 7.45 (H-2', H-6'); 6.7 (H-8); data for $CCl_4$ given in text. MS (values in parenthesis represent relative intensities): $M^+$ 390 (100); $M + H^+$ 391 (51); $M - H^+$ 389 (44); 376 (35); $M - Me^+$ 375 (81); 372 (28), 357 (26), 347 (50), 329 (32), 181 (24), 165 (20).

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# PHENOLIC GLYCOSIDES FROM *SOLANUM GLAUCOPHYLLUM*: A NEW QUERCETIN TRIGLYCOSIDE CONTAINING D-APIOSE

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**Key Word Index**—*Solanum glaucophyllum*; Solanaceae; phenolic and flavonoid glycosides.

**Abstract**—Eight phenolic glycosides have been isolated from the leaves of *S. glaucophyllum*, one of them being quercetin-3-*O*-(2$^G$-$\beta$-D-apiosylrutinoside).

## INTRODUCTION

It has been established that cattle feeding on leaves of *S. glaucophyllum* Desf. (*S. malacoxylon* Sendt.) [1] contract a disease named 'enteque seco' in Argentine and 'espichamiento' in Brazil [2]. The active principle has been isolated and tentatively characterized as a complex glycoside of 1,25-dihydroxy-vitamin $D_3$ [3]. We now report on the phenolic constituents in the leaves of the plant.

## RESULTS

The fraction extracted by butanol, from an initial water extract from the leaves, yielded hydroquinone, kaempferol and quercetin and the following known glycosides: arbutin, *O*-methylarbutin, isoquercitrin, avicularin, rutin, kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside. A new amorphous quercetin trioside was isolated and identified as quercetin 3-*O*-(2$^G$-$\beta$-D-apiosylrutinoside). It is a new example of a flavonoid trioside which has a monosaccharide linked to carbon 2 of the D-glucose moiety of rutinose [4]. This compound 1, on mild hydrolysis produced D-apiose and rutin. Methylation with diazomethane in methanol–ether and subsequent hydrolysis with 2N HCl, afforded 5,7,3',4'-tetra-*O*-methylquercetin. Hydrolysis of the permethylated glycoside gave permethylated L-rhamnose and 3,4-di-*O*-methyl-D-glucose showing that the apiose residue was attached at carbon 2 of glucose. Preparation of the 1,2:3,5-di-*O*-isopropylidene derivative showed that the apiose belonged to the D-series. By applying Klyne's rule to the new glycoside as used by Hulyalkar *et al.* [5] in the case of apiin, it is clear that the apiose must be linked as the $\beta$ anomer. The difference between the molecular rotation of apiin ($-754°$) and 7-*O*-(D-glucopyranosyl)-apigenin ($-349°$) was $-405°$. In our case, the molecular rotations in methanol of the triglycoside ($-461°$) and rutin ($+10°$) gave a difference value very close to that reported by the above authors.

## EXPERIMENTAL

Mps are uncorr. All known compounds were obtained in crystalline condition and identified by mp, mmp, $[\alpha]_D$. UV

spectra and TLC under indicated conditions. The leaves of *S. glaucophyllum* Desf. were collected in the outskirts of Buenos Aires (SI 26.583, on deposit in herbarium of Instituto Darwinion, Acassuso, Buenos Aires, Argentina) and dried at room temp. Dry leaves (4 kg) were extracted with $H_2O$ (5 × 36 l) and the extract was concnd to 45 l. The ppt. produced by addition of EtOH was discarded. The filtrate was concnd and the remaining aq. soln extracted with *n*-BuOH. Evaporation of the solvent yielded 183 g of a syrupy residue which was dissolved in $H_2O$ and extracted with $Et_2O$. The solid residue (45 g) obtained by evaporation of the solvent was chromatographed on a polyamide column (1 kg). It was eluted first with $H_2O$, followed by $H_2O$–EtOH (1:1) and then thoroughly washed with EtOH. The first $H_2O$ fractions yielded *O*-methylarbutin: TLC on Avicel $R_f$ 0.64, *n*-BuOH–EtOH–$H_2O$ (19:1:4) followed by arbutin: TLC on Avicel $R_f$ 0.45, *n*-BuOH–EtOH–$H_2O$ (19:1:4); $R_f$ 0.70, $CHCl_3$–MeOH (7:3). MS (*m/e*): 272, M, and two important peaks at *m/e* 162 (M-$HOC_6H_4OH$) and 110 ($HOC_6H_4OH$). From the next fractions hydroquinone was isolated. The last $H_2O$ fractions contained 3 flavonoids glycosides which by TLC on polyamide; $CHCl_3$–MeOH–MeCOEt (9:4:1) gave $R_f$ 0.43, 0.33 and 0.20 respectively. Chromatography on a polyamide column with the same solvent, allowed the separation of the 3 compounds. The compound with $R_f$ 0.33 was crystallized and identified as kaempferol-3-*O*-rutinoside. The glycoside with $R_f$ 0.43 was purified further on a cellulose powder column eluted with HOAc–$H_2O$ (3:17), affording crystalline isorhamnetin-3-*O*-rutinoside. The glycoside with $R_f$ 0.20 was a new quercetin triglycoside and its structure is considered below. Rutin was isolated from the initial fractions eluted with $H_2O$–EtOH, followed by fractions containing avicularin and isoquercitrin, all of them crystallizing easily. Final elution with EtOH afforded quercetin and kaempferol.

*Quercetin-3-O-(2$^G$-β-D-apiosylrutinoside).* The glycoside with $R_f$ 0.20 was obtained (by evaporation at low temp.) as a powder which could not be crystallized. By TLC on Avicel it had $R_f$ 0.80, HOAc–$H_2O$ (1:3) and $R_f$ 0.30, *n*-BuOH–EtOH–$H_2O$ (19:1:4). It had $[\alpha]_D^{20°}$ − 62.2° (*c* 1.43; MeOH); UV: $\lambda_{max}^{MeOH}$ nm (log ε): 356 (3.4), 294 *sh* (3.2), 264 *sh* (3.5), 257 (3.5); + NaOMe: 400, 328, 272; + $AlCl_3$: 435, 337, 303 *sh* 276; + $AlCl_3$ + HCl: 400, 363, 297 *sh* 270; + NaOAc: 405, 291, 272; + NaOAc + $BO_3H_3$: 375, 298, 262. Hydrolysis in a sealed tube with 4% $H_2SO_4$ at 100° for 2 hr gave quercetin; pentaacetate mp 199–200° (lit. [6] mp 188–190°). PC in *n*-BuOH–HOAc–$H_2O$ (4:1:5) of the remaining acid soln showed 3 sugars in equimolecular concentrations (reagent aniline-phthalate): with $R_g$ 1 (glucose), $R_g$ 2.61 (rhamnose) and $R_g$ 2.33. The sugar with $R_g$ 2.33 was the only one obtained in soln when the glycoside was dissolved in 1N HCl and left at room temp. for 12 hr. When benzidine–TCA was used as chromogenic reagent, a yellow colour appeared with strong yellow fluorescence when irradiated with UV light, a reaction typical of apiose [7]. A ppt. produced in this mild hydrolysis was identified as rutin. Identification of apiose was confirmed by comparison with a sample prepared from apiin. Both samples submitted to TLC on $H_3BO_3$ Si gel gave $R_f$ 0.27, *n*-BuOH–HOAc–$H_2O$ (4:1:5). Permethylation of the glycoside (150 mg) according to the method of Hakomori *et al.* [8] produced a residue which was treated with 10 ml MeOH 20 ml 4% $H_2SO_4$ and refluxed for 4 hr. The soln was extracted with $CH_2Cl_2$ and the residue obtained for evaporation of the solvent was chromatographed on a Si gel column, eluted first with $CH_2Cl_2$ and then with $CH_2Cl_2$–MeOH (9:1). The first fractions gave permethylated D-apiose and L-rhamnose. The next fractions gave a product with positive reaction to aniline–phthalate which was identified as 3,4-di-*O*-methyl-D-glucose by PC with an authentic sample; both gave $R_f$ 0.28 when eluted with the azeotrope MeCOEt–$H_2O$ and $R_f$ 0.63 with *n*-BuOH–EtOH–$H_2O$ (4:1:5) and had the same mobility (Mg 0.42) on paper electrophoresis on Whatman No 3 using borax buffer 0.05 M (pH 9.95), 900 V.

*Apiose phenyl osotriazole.* Mp 91–92° (lit. [9], mp 95–96°), identified by mmp, UV and IR.

*1,2:3,5-di-O-isopropylidene-α-D-apiofuranose* Needles, mp 79–80°, $[\alpha]_D^{20}$ + 61° (*c* 0.15, EtOH), identical to a sample prepared from apiose of apiin (lit. [10], mp 78°; $[\alpha]_D^{25°}$ + 54° (*c* 0.55; EtOH).

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