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KAEMPFEROL 3-α-D-GLUCOPYRANOSIDE-7-α-L-RHAMNOPYRANOSIDE FROM *ERYTHROXYLON CUNEIFOLIUM*

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Abstract—Kaempferol 3-α-D-glucopyranoside-7-α-L-rhamnoside, a novel glycoside with the rare α-D-glucopyranosyl moiety was identified in the aerial parts of *Erythroxylon cuneifolium* Kaempferol 3,7-dirhamnoside, ombuin 3-rutinoside and ombuin 3-rutinoside-5-glucoside were also characterized

INTRODUCTION

In continuation of our work on Argentine *Erythroxylon* species [1, 2] we now report the isolation of four flavonoid glycosides from *Erythroxylon cuneifolium* (Mart) Schulz, one of which was identified as kaempferol 3-α-D-glucopyranoside-7-α-rhamnoside (**1**), a rare example of an α-D-glucopyranose-containing flavonoid

The aqueous subextract from the defatted methanolic extract of *E. cuneifolium* showed four major spots on TLC (Si gel, $CHCl_3$–MeOH–HOAc 40 14 3) with R_f 0 07, 0 47, 0 59 and 0 75 Separation on Sephadex LH20 and Si gel columns afforded pure compounds The glycosides with R_f 0 75 and 0 07 were identified as 7,4′-dimethylquercetin (ombuin)-3-rutinoside (**2**) and ombuin 3-rutinoside-5-glucoside (**3**), respectively by comparison of their physical and spectroscopic data with those of authentic material previously isolated from *E argentinum* [1].

Upon acid hydrolysis the compound with R_f 0 47 (**4**) gave kaempferol and two equivalents of rhamnose The glycoside was identified as kaempferol 3,7-α-L-dirhamnoside (**4**) by UV spectroscopy [3] and ^{1}H NMR of the free glycoside as well as of its TMSi derivative

Acid hydrolysis of the compound with R_f 0 59 (**1**) yielded an equimolecular mixture of glucose, rhamnose and kaempferol. UV analysis using shift reagents [3] showed that **1** was a 3,7-di-*O*-glycoside Accordingly, the ^{1}H NMR spectrum ($CDCl_3$) of the TMSi derivative displayed two doublets at 6 36 and 6 78 with $J = 2$ Hz assigned to H-6 and H-8 respectively The 4′-monosubstitution on the B-ring was indicated by two doublets ($J = 9$ Hz) of two protons each at 6.89 (H-3′ and H-5′) and 8 06 (H-2′ and H-6′) The rhamnosyl anomeric proton appeared as a doublet ($J = 1$ 5 Hz) at 5 32 ppm while the rhamnosyl-methyl group appeared as an ill-shaped 3H doublet ($J = 4$ Hz) at 1 23 ppm, a characteristic feature [3] of 7-*O*-α-rhamnopyranosides The glucosyl anomeric proton appeared as a broadened singlet ($J \simeq 1$ Hz) at 5 65 ppm where the small value for the splitting of H-1″ indicated that the glucose was α-linked [3-5] to the aglycone (β-glucosides typically show the anomeric proton as a doublet with $J \simeq 7$ Hz) A broadened doublet ($J = 2$ 4 Hz) at 4 37 ppm was assigned to H-2″ by DR experiments The remaining sugar protons appeared as a complex multiplet between 3 2-4 1 ppm This glycoside was not affected by β-glucosidase but it was rapidly hydrolysed with α-glucosidase† giving kaempferol 7-rhamnoside and glucose Therefore, **1** was characterized as kaempferol 3-α-D-glucopyranoside-7-α-L-rhamnopyranoside

Chemical studies on the genus *Erythroxylon* have been mostly directed towards the alkaloids [6, 7] with only a few flavonoid investigations [1, 8 11] Our results on *E cuneifolium* and *E argentinum* [1] are in line with the finding [8] that 3-*O*-glycosides of kaempferol and quercetin (or their *O*-methylated derivatives) are typical for the genus The present report of an α-glucopyranosyl-containing flavonoid suggests that a search for this kind of glycoside should be carried out in order to evaluate its possible chemotaxonomical significance in the genus

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Brucker FT 80 (80 MHZ) in the solvents stated For sugar identification a Waters HPLC equipment (M 45 pump, U6K injector and R-401 differential refractometer) with a Waters Carbohydrate Analysis column and acetonitrile–water 4 1 at a flow rate of 1 2 ml min was used

Plant material Aerial parts of *E cuneifolium* were collected by Mr P R Legname on the margins of 'Piray-Guazu' rivulet between Eldorado and San Pedro, Misiones Province, Argen-

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†α-Glucosidase Type III (Sigma) kindly supplied by Dr M Dankert (Fundación Campomar, Buenos Aires) was used

tina A voucher specimen has been deposited at the Miguel Lillo Institute (Tucumán, Argentina) under No 7390

Extraction and isolation of the flavonoids Dried aerial parts of *E cuneifolium* were successively extracted with heptane and MeOH room temp The methanolic extract (7 5% rel to dry plant) was successively extracted with $CHCl_3$ (16 4% rel to MeOH extract), H_2O (64%) and MeOH (19 6%) CC on Sephadex LH 20 of the aqueous subextract using MeOH as eluent yielded three main (Shinoda positive test) fractions Fraction 1 showed a major constituent on TLC (Si gel, $CHCl_3$–MeOH–HOAc 40 14 3, R_f 0 07) which by further CC on Si gel using $CHCl_3$ and increasing amounts of MeOH (from 33 to 50%) yielded pure 7,4′-dimethylquercetin 3-rutinoside-5-glucoside (**3**) that was identified by its spectroscopic data and comparison with authentic material previously isolated from *E argentinum* [1] Fraction 2 from the Sephadex column showed two spots on TLC with R_f 0 47 and 0 59 corresponding to compounds **4** and **1**, respectively, which were purified by CC on Si gel and $CHCl_3$–MeOH 4 1 as solvent Fraction 3 from the Sephadex column yielded almost pure 7,4′-dimethylquercetin 3-rutinoside (**2**) (R_f on TLC = 0 75) that was further purified by CC on Si gel as before and identified by UV, ^{1}H NMR and comparison with a standard [1]

Kaempferol-3,7-α-L-dirhamnoside (**4**) was isolated as pale-yellow crystals, mp 188–189° (H_2O) (reported [13] 186–188°) ^{1}H NMR (TMSi deriv, $CDCl_3$). δ0 90 (*d*, 3H, J = 6 Hz, H-6″), 1.23 (*d*, 3H, J = 5 Hz, H-6‴), 3 0–3 9 (m, sugar protons), 3 94 and 4 19 (two *dd*, 1H each, $J_1 \simeq J_2 \simeq 2$ Hz, H-2″ and H-2‴), 5 28 and 5 31 (two partially superimposed *d*, 1H each, both with $J \simeq 2$ Hz, H-1″ and H-1‴), 6 44 (*d*, 1H, J = 2 Hz, H-6), 6 63 (*d*, 1H, J = 2 Hz, H-8), 6 94 (*d*, 2H, J = 9 5 Hz, H-3′ and H-5′), 7 80 (*d*, 2H, J = 9 5 Hz, H-2′ and H-6′) ^{1}H NMR (free glycoside, Me_2SO-d_6) 0 81 (*d*, 3H, J = 5 6 Hz, H-6″), 1 12 (*d*, 3H, J = 5 Hz, H-6‴), 5 30 (*d*, 1H, J = 1 6 Hz, H-1″), 5 53 (*br s*, 1H, H-1‴), 6.43 (*d*, 1H, J = 2 Hz, H-6), 6 75 (*d*, 1H, J = 2 Hz, H-8), 6 91 (*d*, 2H, J = 8.8 Hz, H-3′ and H-5′), 7 78 (*d*, 2H, J = 8 8 Hz, H-2′ and H-6′)

Kaempferol 3-α-D-*glucopyranoside*-7-α-L-*rhamnoside* (**1**). Pale-yellow crystals, mp 177–178° (H_2O–Me_2CO) UV λ_{max} MeOH 266, 318 (sh), 348, +NaOMe 256, 266, 296, 394; +$AlCl_3$ 274, 300 (sh), 350, 398, $AlCl_3$/HCl 274, 300 (sh), 350, 398, +NaOAc 266, 390, NaOAc/H_3BO_3 266, 350 ^{1}H NMR (TMSi deriv, $CDCl_3$) discussed in the text ^{1}H NMR (free glycoside, Me_2SO-d_6) 1 19 (ill-shaped *d*, 3H, J = 6 Hz, H-6‴), 3 0–4.1 (*m*, sugar protons), 5 55 and 5 63 (broadened singlets, 1H each, H-1‴ and H-1″, respectively), 6.45 (*d*, 1H, J = 1 7 Hz, H-6), 6.83 (*d*, 1H, J = 1 7 Hz, H-8), 6 90 (*d*, 2H, J = 8 Hz, H-3′ and H-5′), 8 07 (*d*, 2H, J = 8 Hz, H-2′ and H-6′), 12 6 (*br s*, 1H, 5-OH)

Acid hydrolysis of glycosides The glycoside dissolved in a minimum of 7% aq. H_2SO_4 was refluxed 1 hr and the aglycone extracted with EtOAc The aq soln was neutralized with powdered $BaCO_3$ (magnetic stirring overnight), filtered, the water distilled off *in vacuo* and the residue examined by TLC on cellulose For HPLC the residue was dried under vacuum, dissolved in acetonitrile–H_2O 4 1 and analysed using a Waters Carbohydrate Analysis column and sugar standards

Hydrolysis of **1** *with α-glucosidase.* To the glycoside **1** (6 mg) dissolved in Pi buffer pH 6 8 (8 ml), 4 drops of α-glucosidase (Type III Sigma) suspension were added and the mixture incubated 4 hr at 37° After concentration the residue dissolved MeOH was chromatographed on a Sephadex LH20 column, to give Kaempferol-7-rhamnoside, characterized by UV spectroscopy and acid hydrolysis to aglycone and sugar (identified as above)

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