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AN UNUSUALLY LIPOPHILIC FLAVONOL GLYCOSIDE FROM *RANUNCULUS SARDOUS* POLLEN

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Key Word Index—*Ranunculus sardous*; Ranunculaceae; pollen; 7-*O*-methylherbacetin-3-*O*-[2-*O*-*E*-feruloyl-β-D-glucoside].

Abstract—A lipophilic flavonoid detected by HPLC in earlier work and assumed to be a flavone aglycone from preliminary screening has been isolated from *Ranunculus sardous* pollen. It is shown, principally by NMR techniques, to be a new flavonoid glycoside, 7-*O*-methylherbacetin 3-*O*-[2-*O*-*E*-feruloyl-β-D-glucoside].

INTRODUCTION

In the course of developing a method for the characterization of bee pollens via the HPLC profiles of their EtOH/H_2O extracts [1], a more intensive study of the glycosides from *Ranunculus sardous* Crantz. pollen was carried out [2]. The major components with the spectra and HPLC retention times (30.5–31.6 min) of flavonoid glycosides were isolated and identified and shown to be 7- and 8-*O*-methylherbacetin 3-*O*-diglycosides, with the new compound 7-*O*-methylherbacetin 3-*O*-sophoroside being the major component. One other major flavonoid (**1**) with a flavone-like absorption spectrum and a retention time of 43.5 min was initially thought to be a flavone aglycone and was not identified. The present paper describes the isolation and structure identification of this compound.

RESULTS AND DISCUSSION

Compound **1** was isolated from an EtOH/H_2O extract of *R. sardous* pollen by successive column chromatography over reversed phase silica (RP-18) and LH-20. Its absorption spectrum with λ_{max}^{MeOH} nm: 280, 310 sh, 325 (and a slight shoulder at 380) was initially interpreted as that of a flavone (e.g. see ref. [3]), and coupled with the aglycone-like HPLC retention time of 43.5 min, suggested that **1** was a flavone aglycone. Its ^{1}H NMR spectrum however, indicated a much more complex structure. A sugar H-1 doublet (J = 7.8 Hz) was evident at δ5.76, accompanying the signals associated with the flavone nucleus (δ8.10 *d*, J = 8.9 Hz; 6.91 *d*, J = 8.9 Hz; 6.51 *s*). Additionally, the presence of a hydroxylated *trans*-cinnamoyl moiety and two methoxyl groups were revealed.

Acid hydrolysis of **1** gave glucose plus an aglycone with the same retention time and distinctive absorption spectrum [2] as 7-*O*-methylherbacetin. This aglycone is also indicated by the proton resonances of the flavonoid nucleus of **1** which match closely with those of 7-*O*-methylherbacetin 3-*O*-sophoroside [2]. Alkaline treatment of **1** yielded ferulic acid which was identified by HPLC, thereby accounting for the second methoxyl indicated by the ^{1}H NMR spectrum.

The site of attachment of the glucose to 7-*O*-methylherbacetin was considered to be the 3- or 4′-hydroxyl group as the ^{1}H NMR resonance of the 5-hydroxyl (δ12.1) proved that this was unsubstituted. A 3-*O*-glycosylated structure was favoured by the ^{1}H NMR spectrum in which the glucose H-1 chemical shift at δ5.76 was more like that of a 3-linked glucose (*ca* δ5.44) than that of a 4′-linked glucose (*ca* δ4.85 [4]). Furthermore, in the HMBC spectrum, the H-2′,6′ signal showed connectivity to a carbon signal at 156.5 ppm. This must be the signal of C-2 [5], as the alternative signal at 145.9 ppm shows connectivity (HMQC) to the β-proton of the ferulic acid residue and therefore must represent the β-carbon. The C-2 signal should only appear at *ca* 156 ppm if the 3-hydroxyl is glycosylated [5]. The feruloyl residue is shown to be attached to the 2-hydroxyl of the glucose by the ^{1}H NMR spectrum in which both the glucose H-1 doublet at δ5.76 and the coupled (^{1}H,^{1}H-COSY) H-2 triplet at δ4.88 appear markedly downfield from those in flavonol-3-*O*-glucosides [4]. Such shifts are

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in accord with those previously observed for 3-*O*-glucosides acylated at the 2-hydroxyl [6]. The ^{13}C NMR spectrum is also consistent with this in that both C-1 and C-3 signals appear downfield by about 3 ppm, from signals of the equivalent carbons in quercetin-3-*O*-glucoside [5].

On the basis of the above data the structure 7-*O*-methylherbacetin 3-*O*-[2-*E*-feruloyl-β-D-glucopyranoside] is assigned to **1**. This is a new flavonoid glycoside [7] and its structure accounts for its deceptive HPLC behaviour and absorption spectrum.

EXPERIMENTAL

Plant material. Ranunculus sardous plant material was collected from the property of Dr K. Hammett, Massey, Auckland, NZ and identified by Prof. P. Garnock-Jones, Dept of Biological Sciences, Victoria Univ., Wellington, NZ (Voucher specimen: WELTU 16841). The HPLC profile of the EtOH/H_2O extract of the pollen from this plant material was used to identify *R. sardous* pollen in a NZ bee pollen mix (see ref. [2]). *R. sardous* pollen hand selected from this bee pollen was used for the present work.

Extraction and isolation. Pollen was extracted as described in ref. [2] and the extract applied in aqueous solution to an RP-18 column. Elution with a gradually increasing proportion of MeOH yielded compound **1** in the last eluted fraction. Compound **1** was further purified on an LH-20 column eluted with MeOH.

HPLC analysis. See ref. [2].

Hydrolyses. Acid hydrolyses were carried out in MeOH–3M HCl (1:1) at 100 for 10 min. HPLC analysis of the products revealed the aglycone with a retention time of 45.6 min and an absorption spectrum with maxima at 276, 310 sh, 329, 387 nm. The sugar was separated from phenolic products by passing the vacuum-dried hydrolysis mix, in water, through an RP-18 column. Sugar identification was by paper chromatography [3]. Alkaline hydrolysis of **1** involved treatment at R.T. with 2 N NaOH in the absence of air, followed by neutralisation with HCl. The acid liberated was identified as ferulic acid by HPLC comparison with an authentic sample.

7-O-Methylherbacetin 3-O-[2-O-E-*feruloyl*-β-D-*glucopyranoside*] (**1**). HPLC R_t = 43.5 min, aglycone R_t = 45.6 min. UV λ_{max}^{MeOH} nm: 280, 310 sh, 325, 380 sh; +NaOMe: 250 sh, 270 sh, 304 sh, 374 (inc); +NaOAc: 280, 310, 325 sh, 380 sh; +$AlCl_3$ or $AlCl_3$/HCl: 284, 323, 394 sh. NMR spectra: ^1H NMR (300 MHz, DMSO-d_6): 12.1 (*s*, 5-OH), 8.10 (*d*, H-2′6′, *J* = 8.9 Hz), 7.59 (*d*, H-β-fer, *J* = 15.8 Hz), 7.30 (*s*, H-2-fer), 7.09 (*dd*, H-6-fer, *J* = 8.1, *ca* 1 Hz), 6.91 (*d*, H-3′5′, *J* = 8.9 Hz), 6.78 (*d*, H-5-fer, *J* = 8.1 Hz), 6.51 s (H-6), 6.48 (*d*, H-α-fer, *J* = 15.8 Hz), 5.76 (*d*, H-1-glu, *J* = 8.1 Hz), 4.88 (*t* H-2-glu, *J* = *ca* 8.1 Hz), 3.87 (*s*) and 3.82 (s, OMe); ^{13}C NMR (75.5 MHz, DMSO-d_6): *flavonoid nucleus*: 175.1 (C-4), 160.4 (C-4′), 156.5 (C-2), 146–160 (quaternaries, indistinct), 131.3 (C-2′6′), 125.8 (C-8), 123.3 (C-1′?), 115.7 (C-3′5′), 95.7 (C-6), 56.6 (OMe): *sugar carbons*: 98.3 (C-1), 78.0 (C-5), 74.4 (C-2, C-3), 70.3 (C-4), 60.8 (C-6): *feruloyl carbons*: 149.5 (C-3), 148 (C-4), 145.9 (C-β,), 123.3 (C-1?, C-6), 115.3/114.8 (C-α, C-5), 111.3 (C-2), 55.9 (OMe).

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