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7-*O*-METHYL-(2*R*:3*R*)-DIHYDROQUERCETIN 5-*O*-β-D-GLUCOSIDE AND OTHER FLAVONOIDS FROM *PODOCARPUS NIVALIS*

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Key Word Index—*Podocarpus nivalis*; Podocarpaceae; snow totara; gymnosperm; flavonoid glycosides, 7-*O*-methyl-(2*R*:3*R*)-dihydroquercetin 5-*O*-β-D-glucoside; 7-*O*-methylquercetin 5-*O*-β-D-glucoside; luteolin 3′-*O*-β-D-xyloside; luteolin 7-*O*-β-D-glucoside-3′-*O*-β-D-xyloside, FAB-MS; ^{13}C NMR.

Abstract—In the course of a chemotaxonomic survey of New Zealand *Podocarpus* species, a number of new flavonoid glycosides have been isolated from *P. nivalis*. These are: luteolin 3′-*O*-β-D-xyloside, luteolin 7-*O*-β-D-glucoside-3′-*O*-β-D-xyloside, dihydroquercetin 7-*O*-β-D-glucoside, 7-*O*-methyl-(2*R*:3*R*)-dihydrokaempferol 5-*O*-β-D-glucopyranoside, 7-*O*-methyl-(2*R*:3*R*)-dihydroquercetin 5-*O*-β-D-glucopyranoside, 7-*O*-methylkaempferol 5-*O*-β-D-glucopyranoside and 7-*O*-methylquercetin 5-*O*-β-D-glucopyranoside. Diagnostically useful physical techniques for distinguishing substitution patterns in dihydroflavonols are discussed and summarized. Glucosylation of the 5-hydroxyl group in (+)-dihydroflavonols is shown to reverse the sign of rotation at 589 nm.

INTRODUCTION

It has been proposed by De Laubenfels [1] that the classical gymnosperm genus *Podocarpus* be subdivided into five different genera. The seven New Zealand representatives of *Podocarpus* would fall into three of these genera, *Dacrycarpus*, *Prumnopitys* and *Podocarpus*. In the course of a phytochemical survey of the flavonoids of the New Zealand species, which was designed to either support or refute this proposal, a number of new flavonoid glycosides have been encountered. Unique flavonoid glycosides found in one of these species, namely *Podocarpus* (*Dacrycarpus*) *dacrydioides* were the subject of an earlier communication [2]. The purpose of the present paper is to discuss the structure elucidation of the remaining new flavonoid glycosides encountered in this survey, the majority of which were isolated from *Podocarpus nivalis*†.

RESULTS AND DISCUSSION

Apart from the biflavone aglycones (which appear to be ubiquitous in the genus *Podocarpus*) previously known flavonoids isolated from *P. nivalis* in this study were: (+)-dihydrokaempferol and (+)-dihydroquercetin and their 3-*O*-glucosides, naringenin 7-*O*-glucoside, vitexin and orientin and their 2″-*O*-glucosides, kaempferol and quercetin 3-*O*-glucosides and 7-*O*-glucosides, apigenin 7-*O*-glucoside, and luteolin 7- and 3′-*O*-glucosides These were identified by established procedures [3, 4] and the structures confirmed by direct chromatographic and spectrophotometric comparison with authentic samples. Authentic vitexin 2″-*O*-glucoside was obtained from *Silene alba* petals but the orientin glucoside was not available. The structures of both were confirmed by direct comparison with the same compounds from *Podocarpus totara*, the ^{13}C NMR spectra of which clearly defined the nature of the sugar, the linkage point and the identity of the flavone *C*-glycoside (see Experimental). In addition to the above, several new flavonoid glycosides were isolated and these are discussed in detail below.

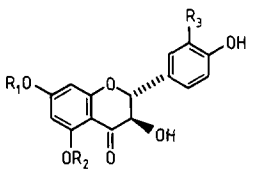
Flavone O-glycosides

P. nivalis was found to accumulate two flavone *O*-glycosides in addition to the known apigenin and luteolin 7-*O*-glucosides and luteolin 3′-*O*-glucoside. One, a monoglycoside (**1**), which ran slightly ahead of luteolin 3′-*O*-glucoside in both TBA and acetic acid and produced luteolin and xylose on acid or β-glucosidase hydrolysis, was identified as luteolin 3′-*O*-β-xyloside. Absorption spectroscopy using standard shift reagents defined the 5,7 and 4′-hydroxyl groups as free and the B-ring *ortho*-dihydroxyl system as absent. Related to this glycoside was another (**2**) with higher mobility in acetic acid and lower mobility in TBA on paper chromatograms. On acid (or β-glucosidase) hydrolysis, **2** gave luteolin plus xylose and glucose, and its absorption spectra defined the 7- and 3′-hydroxyls as being substituted. Its chromatographic mobility and colour reactions (UV, UV/ammonia and NA) are consistent with it being a luteolin 3′,7-diglycoside [5]. Mild acid hydrolysis produced luteolin 7-glucoside with the liberation of xylose, thereby defining the structure as luteolin 7-*O*-β-glucoside-3′-*O*-β-xyloside. Both **1** and **2** are new luteolin glycosides [6].

Dihydroflavonol glycosides

Dark UV-absorbing spots which turned blue–green fluorescent in ammonia vapour were observed in the same region of the two dimensional paper chromatogram

†A more botanically oriented taxonomic paper based in part on the total survey is to be submitted to the New Zealand Journal of Botany in the near future.



3 R_1 = glucopyranosyl, R_2 = H, R_3 = OH

4 R_1 = glucopyranosyl, R_2 = H, R_3 = H

5 R_1 = Me, R_2 = glucopyranosyl, R_3 = OH

6 R_1 = Me, R_2 = glucopyranosyl, R_3 = H

as were the dihydroflavonol 3-*O*-monoglucosides (the spots of which were unaffected by ammonia vapour). The major compound of this type (**3**) in *P. nivalis* gave dihydroquercetin and glucose on acid hydrolysis, together with small amounts of a flavonol glycoside (see discussion below) which appeared as a yellow fluorescent spot on a two dimensional paper chromatogram (in UV). β-Glucosidase hydrolysis produced only dihydroquercetin and glucose. In the absorption spectrum of **3**, the main 283 nm band was unaffected by alkali indicating that the 7-hydroxyl is glucosylated. Compound **3** is therefore assigned the structure, dihydroquercetin 7-*O*-β-D-glucopyranoside, a previously unknown compound [7]. It is also considered that small amounts of dihydrokaempferol 7-*O*-β-D-glucopyranoside (**4**) occur in *P. nivalis*, since acid treatment of the mixture of components which run near **3** on the paper chromatogram, produces kaempferol 7-glucoside as well as quercetin 7-glucoside. Dihydrokaempferol 7-*O*-glucoside has been isolated also from *Podocarpus hallii* in the present survey, and was identified by the methods outlined above for **3**. It has also been isolated previously from several other plant sources [7].

A *P. nivalis* variant, possibly of hybrid origin, gave a new dihydroflavonol glycoside, **5**, together with smaller amounts of a related co-occurring glycoside, **6**. These compounds ran in the same general region of the two dimensional paper chromatogram as did **3** and **4** but gave adjoining dark spots which distinctively, (i) turned blue–green fluorescent in ammonia vapour and unlike **3** and **4** remained blue–green fluorescent, and (ii) changed to yellow fluorescent within days (but within 15 min in the presence of ammonia). When these yellow fluorescent spots were extracted and rechromatographed, two yellow fluorescent (PC/UV) compounds, **7** and **8**, of much lower mobility were evident.

The absorption spectra of **5** were typically those of a dihydroflavonol with both the 7- and 5-hydroxyl groups substituted. In particular, the 24 nm shift observed with aluminium chloride was instantly reversed on the addition of HCl, a property usually associated with dihydroflavonols lacking free 5-hydroxyl groups [4, 8]. β-Glucosidase or acid hydrolysis of **5** yielded, together with glucose, an aglycone with a substituted 7-hydroxyl and a free 5-hydroxyl, thus defining the 5-hydroxyl-linked substituent in **5** as glucose. The MS of the aglycone showed $[M]^+$ at 318 mu, $[A_1+H]^+$ at 167 mu and $[B_3]^+$ at 152 mu. These data require that the aglycone be a tetraoxygenated dihydroflavonol with a 7-methoxyl group. MS(FAB) of the glycoside itself, confirmed these findings and, exhibiting ions at 503 $[M+Na]^+$, 481 $[M+H]^+$ and 319 [aglycone + H]$^+$, defined **5** as a monoglucoside. Accordingly **5** is the 5-*O*-monoglucoside of the above aglycone.

The ^{1}H NMR spectrum of **5** revealed, in addition to sugar proton signals at δ 3–4 (6H) and 4.9 (1H), a methoxyl at δ 3.79, H-2 at 4.9, H-3 at 4.37 and a clear dihydroquercetin-type pattern of aromatic proton signals [9]. Some signal shifts were observed due to the effects of 5-*O*-glycosylation and 7-*O*-methylation but these are in accord with shifts previously reported [9] for similar substitution of naringenin, i.e. in sakuranin. The ^{13}C NMR spectrum also supported the structure assignments made above and in particular confirmed the presence of the methoxyl group and a β-D-glucopyranosyl substituent. On the basis of the above data, **5** is assigned the structure 7-*O*-methyldihydroquercetin 5-*O*-β-D-glucopyranoside.

The minor co-occurring glycoside, **6**, is considered to be 7-*O*-methyldihydrokaempferol 5-*O*-β-D-glucopyranoside on the basis of evidence exactly paralleling that described for **5** above (e.g. hydrolyses, absorption spectra, MS, FAB-MS, ^{1}H NMR and ^{13}C NMR—see Experimental). A related compound, 7,4′-di-*O*-methyldihydrokaempferol 5-*O*-glucoside has previously been isolated from another *Podocarpus* species, *P. neriifolius* [11].

The absolute stereochemistry of **5** (and by analogy, **6**) was shown to be 2*R*.3*R* by ^{1}H NMR and optical rotation measurements. A *trans*-diaxial relationship between the protons at C-2 and C-3 in **5** was evident from the 11 Hz coupling constant and it has been established [12] that in dihydroflavonol aglycones of this type, positive rotation at 589 nm relates to the 2*R*:3*R* absolute configuration. Although **5** itself exhibited a strong negative rotation, $[\alpha]_D^{21}$ (MeOH) = −84.3°, its aglycone (7-methyldihydroquercetin) had a rotation of +41.5° under the same conditions. The 5-*O*-linked β-glucopyranose in **5** is clearly having a marked and unexpected effect on the optical rotation. In contrast glucosylation of the 3-hydroxyl was shown to have no effect on the sign of rotation at 589 nm in the 3-*O*-β-glucopyranosides of (+)-dihydroquercetin and (+)-dihydrokaempferol isolated from the same plant. Rhamnosylation of the 3-hydroxyl however does reverse the sign of rotation [12, 13].

In the standard two dimensional paper chromatogram (see Experimental) of the flavonoid constituents of the *P. nivalis* variant, two bright yellow fluorescent spots were evident (in UV). The flavonoids from these spots were chromatographically identical with compounds **7** and **8** above. Being yellow fluorescent and readily derived from the dihydroflavonol glucosides **5** and **6** by oxidation in air or with sodium metabisulphite, these compounds were considered to be the flavonol equivalents of **5** and **6**. Additional support for these structures was obtained from (i) acid hydrolyses which produced rhamnetin and 7-*O*-methylkaempferol from **7** and **8** respectively, and (ii) the chromatographic appearance/mobility and absorption spectra which defined the 3,4′- (and 3′- in **7**) hydroxyl groups as free but the 7-hydroxyl as substituted. Like the 5-*O*-glucosides of quercetin and kaempferol [14] they were very difficult to elute from paper chromatograms Accordingly, compounds **7** and **8** are assigned the structures, 7-*O*-methylquercetin 5-*O*-β-D-glucopyranoside and 7-*O*-methylkaempferol 5-*O*-β-D-glucopyranoside, respectively. Both compounds are new natural products.

In the course of this study, several diagnostically useful

characteristics of substituted dihydroflavonols have become apparent which have not been noted in major review texts on flavonoids (e.g. [3, 4 and 6]). These include the following:

1. *Paper chromatographic appearance (in ultra violet light).* 7-*O*-Substituted 5-hydroxydihydroflavonols appear as dark absorbing spots which turn blue–green fluorescent in ammonia vapour. However when the 7-hydroxyl is free (e.g. in 3-*O*-glycosides or aglycones) the spots are unaffected by ammonia. Dihydroflavonols with the 5-hydroxyl substituted also appear initially as dark absorbing spots, but these change irreversibly to blue–green fluorescent in ammonia and ultimately (after 15 min in ammonia) turn yellow fluorescent as oxidation to the flavonol occurs.

2. *Oxidation.* Dihydroflavonols with a free 3-hydroxyl oxidise more readily than those with the 3-hydroxyl substituted. Thus, hot acid treatment ('hydrolysis') of 7-*O*-glucosides produces significant quantities of the equivalent flavonol glucosides. This is not observed with the 3-*O*-glycosides although some flavonol aglycone is often produced in addition to the dihydroflavonol aglycone. Oxidation with sodium metabisulphite similarly only occurs when the 3-hydroxyl is free (see Experimental).

3. *Ultra violet-visible spectrophotometry.* When the 5-hydroxyl is substituted (or absent), the free 3-hydroxyl may be detected by the acid labile nature of the aluminium chloride complex formed in routine absorption spectra measurements (see refs [4, 8]). Although the complex formed with 3,5-dihydroxydihydroflavonols is also acid labile, it is degraded only slowly compared with the instant decomposition observed with 5-*O*-substituted dihydroflavonols.

4. *Optical rotation.* β-D-Glucosylation of the 5-hydroxyl group is evidenced by reversal of the sign of rotation measured (at 589 nm) for the aglycone. This is not true for α-L-rhamnosylation (cf. [12]).

EXPERIMENTAL

Plant material. Podocarpus nivalis samples supplied and identified by Dr B. P. J. Molloy, Botany Division, DSIR, were collected from Mt Cook National Park, Island Pass (Nelson), Mt Ruapehu, Kaweka Range and Porters Saddle. Voucher specimens are held in the herbarium, Botany Division, D.S.I.R. CHR98379, CHR215951, CHR258445, CHR298070, CHR388238. The *P. nivalis* variant was collected from the Christchurch Botanical Gardens (voucher: CHR388216).

Sample extraction and work-up. A total of 100 g dry wt of *P. nivalis* foliage was extracted with EtOH–H_2O (1·1). Standard reference 2D-PCs were obtained by running the extract from 100 mg of plant material on Toyo No 514A filter paper (60 cm × 60 cm) in *t*-BuOH–HOAc–H_2O, 3:1:1 (TBA) and 15% HOAc (HOAc). R_f values quoted here were measured from this 2D-PC The bulk of the extract was chromatographed on a column of Merck microcrystalline cellulose using 2% HOAc as solvent. Analysis of fractions was by 2D-PC and individual compounds were isolated from these fractions by 1D- or 2D-PC. H_2O was used as solvent for the chromatographic separation of flavonoids from fluorescent impurities and for the separation of dihydroflavonol and flavanone glycosides 30% HOAc was used as solvent for the purification of low R_f glycosides. *P. nivalis* variant (100 g, dry wt) gave 33 g of extract after thorough extraction with MeOH–H_2O (1:1) at room temp Extract (13 g) was applied to a polyamide (Macherey Nagel SC-6), column (37 × 5 cm) in H_2O and the eluting solvent gradually changed by adding MeOH in increasing amounts The fractions eluted with 5–15% MeOH contained **5** (and **6**) which was further purified by 1D-PC in 5% HOAc and finally by chromatography on Sephadex LH-20 in MeOH. The crystalline mixture so obtained was predominantly **5**.

Techniques such as absorption spectroscopy with added shift reagents, acid and enzyme hydrolyses, sugar analyses etc. were carried out as described in ref. [4] unless otherwise stated

Vitexin and orientin 2″-O-β-glucopyranosides—physical data. R_f values (TBA, HOAc), vitexin 2″-*O*-glucoside· 0.6, 0.65; orientin 2″-glucoside: 0.45, 0.58. Absorption spectra, as for vitexin and orientin [3] ^{13}C NMR spectra (DMSO-d_6), vitexin 2″-*O*-glucoside· δ 181.9 (C-4), 163 7 (C-2), 162.6 (C-7), 161.1 (C-4′), 160.5 (C-5), 155.8 (C-9), 128.7 (C-2′,6′), 121.5 (C-1′), 115.9 (C-3′,5′), 105.1, 103.5/103.7 (C-8, C-10, C-1‴), 102.5 (C-3), 98.2 (C-6), 81 7 (C-2″), 81.1 (C-5″), 78 2 (C-3″), 76.1 (C-3‴, 5‴), 74.3 (C-2‴), 71.4 (C-1″) 70.0/69.3 (C-4″, C-4‴), 60 9/60.3 (C-6″, C-6‴); orientin 2″-*O*-glucoside· δ 181.8 (C-4), 163.9/163.5 (C-2,7), 160.6 (C-5), 156 (C-9), 150.1 (C-4′), 146.0 (C-3′), 121.9 (C-1′), 119.3 (C-6′), 115.9 (C-5′), 114.0 (C-2′), 105 2, 103 7 (C-8, C-10, C-1‴), 102 5 (C-3), 98.5 (C-6), 82 0 (C-2″), 81 4 (C-5″), 78 6 (C-3″), 76.3/76.1 (C-3‴, C-5‴), 74.4 (C-2‴), 71.6 (C-1″), 70.5/69.5 (C-4″, C-4‴), 61.5/60.5 (C-6″, C-6‴).

Luteolin 3′-O-*β-xyloside* (**1**) R_f 0.73 (TBA), 0 1 (HOAc); spot appearance, dark (UV), dull green (UV/NH_3). λ_{max}^{MeOH} nm: 269, 340; (NaOMe) 274, 330, 394↑, (NaOAc) 272, 330sh, 392↑; (NaOAc–H_3BO_3) 270, 340 β-Glucosidase hydrolysis [4] yielded luteolin, and xylose was the only sugar detected (PC) after standard [4] acid hydrolysis

Luteolin 7-*O-glucoside*-3′-O-*xyloside* (**2**). R_f 0.25 (TBA), 0.2 (HOAc); spot appearance, dark (UV), green fl. (UV/NH_3). λ_{max}^{MeOH} nm 270, 340; (NaOMe) 260, 274 sh, 394↑, (NaOAc) 258, 268 sh, 400, (NaOAc–H_3BO_3) 270, 342. β-Glucosidase hydrolysis [4] yielded luteolin, as did acid hydrolysis Both xylose and glucose were detected (PC) in the acid hydrolysate Treatment of **2** with 0 1 N TFA for 30 min at 100° yielded a mixture of **2** and a mono-glycoside which cochromatographed with luteolin 7-*O*-glucoside on TLC (TBA, HOAc, 50% HOAc).

Dihydroquercetin 7-O-*β-glucoside* (**3**). R_f 0.5 (TBA), 0.7 (HOAc); spot appearance, dark (UV), blue–green fluorescence (UV/NH_3). λ_{max}^{MeOH} nm· 287, 340 sh; (NaOMe) 288, 362; (NaOAc) 285, 340 sh (NaOAc–H_3BO_3) 287, 340 sh ($AlCl_3$) 310, 382 ($AlCl_3$–HCl) 308, 381 initially, changing to 284, 340 sh in 5 min. Standard [4] acid hydrolysis yielded taxifolin (MS. 304 $[M]^+$, 275 $[M-CHO]$, 153 $[A_1+H]$, 152 $[B_3]$ mu and co-TLC with authentic taxifolin) and glucose (PC), together with a flavonol glycoside which appeared as a yellow fluorescent spot (R_f 0.26; 0.07, TBA; HOAc) on a 2D-PC, which hydrolysed to give quercetin and glucose (0.5 N HCl, 30 min, 100°) This was chromatographically and spectrophotometrically identical with authentic quercetin 7-*O*-glucoside. Hydrolysis of **3** also occurred on standard [4] treatment with β-glucosidase *Dihydrokaempferol* 7-O-*β-glucoside* (**4**). Acid treatment of the crude dihydroquercetin 3-*O*-glucoside isolated from 2D-PCs of crude plant extract gave, in addition to taxifolin and glucose, kaempferol 7-glucoside (identified by the same procedures outlined above for quercetin 7-glucoside) Dihydroquercetin 3-*O*-glucoside is the major flavonoid constituent of *P. nivalis*.

7-O-*Methyldihydroquercetin* 5-O-*β-D-glucopyranoside* (**5**) *and* 7-O-*methyldihydrokaempferol* 5-O-*β-D-glucopyranoside* (**6**) Compound **5**: R_f 0 43 (TBA), 0.7 (HOAc); compound **6** R_f 0.56 (TBA), 0.7 (HOAc), spot appearance, dark (UV) changing to a persistent blue–green fluorescence in NH_3, and after a few days, to a yellow fluorescent spot λ_{max}^{MeOH} nm: 283, 317 sh, (NaOMe) 284, 317 sh; ($AlCl_3$) 286. 307, ($AlCl_3$–HCl) 283, 316 sh ^{1}H NMR (DMSO-d_6): δ6.88–6.73 *m* (3H, H-2′,5′,6′), 6 48 *d* (*J* = 2.5 Hz,

1H, H-8), 6.26 *d* (J = 2.5 Hz, 1H, H-6), 4.90 *m* (2H, H-2 and glucose H-1), 4.37 *d* (J = 11 Hz, 1H, H-3), 3.0–4.0 *m* (6H, glucose), 3.79 *s* (3H, OMe), also evidence of a dihydrokaempferol derivative (H-2′,6′ at 7 30, *d*, J = 8.5 Hz). ^{13}C NMR (DMSO-d_6), quaternaries poorly resolved, major signals at: δ 165.6, 163.5, 159.6 (C-5,7 and 9), 145/146 (C-3′,4′), 128 (C-1′), 119.2 (C-6′), 115.1 (C-2′,5′), 105.6 (C-10?), 101.7 (C-1″), 97.0 (C-6), 95.4 (C-8), 82.7 (C-2), 77.4/76.2 (C-3″,5″), 73.3 (C-2″), 72.7 (C-3), 69.8 (C-4″), 60.8 (C-6″), 55.7 (OMe) and additional signals due to a dihydrokaempferol contaminant at 129.3 (C-2′,6′), 114.8 (C-3′,5′) and duplicated sugar carbon signals. FAB-MS (in glycerol/tetraethylene glycol/DMSO) for **5** and **6**: $[M+Na]^+$ 503, 487; $[M+H]^+$ 481, 465; $[aglycone+H]^+$ 319, 303 mu. Acid hydrolysis (1 N TFA, 15 min, 100°) or β-glucosidase treatment yielded glucose, and aglycone R_f (SiO_2, $CHCl_3$–MeOH 4:1) 0.83, *cf* dihydrokaempferol 0 80, dihydroquercetin 0.70, naringenin 0.87 A lesser aglycone was evident at R_f 0 90. The aglycones had λ_{max}^{MeOH} nm. 289, 333 sh; (NaOMe) 290, 362, ($AlCl_3$) 314, 382; ($AlCl_3$–HCl) 289 sh, 312, 382, and an MS with key ions (*m/z*) as follows (**5, 6**): $[M]^+$ 318 (15%), 302 (15%), $[M-CHO]^+$ 289 (22%), 273 (28%), $[M-CHO-$ring B$]$ 179 (13%), 179 (13%), $[A_1+H]$ 167 (100%), 167 (100%) $[B_3]$ 152 (20%), 136 (21%), $[B_3-2H]$ 150 (32%), 134 (35%), $[B_3-CHO]$ 123 (23%), 107 (27%).

Optical rotation measurements were carried out on methanolic solutions of **5** (2.42 mg/1.5 ml), and its aglycone (0 4 mg/1.5 ml, derived from **5** using 1.5 N HCl/100°/10 min) using a Perkin–Elmer 241 variable wavelength polarimeter. $[\alpha]_D^{21°}$ values obtained. **5**, −84.3°; **5** aglycone, +41.5° Values at 546 nm: **5**, −95.5°; **5** aglycone, + 52.8°; at 436 nm **5** aglycone had a rotation of +109.4°, dihydrokaempferol and dihydroquercetin 3-*O*-glucosides (1 . 1 mixture), $[\alpha]_D^{21°}$ +19.2°; aglycones (1 : 1) produced using 2 N HCl/100°/60 min, +17 5°.

7-O-Methylquercetin 5-O-β-D-glucopyranoside (**7**) *and 7-O-methylkaempferol 5-O-β-D-glucopyranoside* (**8**) Exposure of **5** and **6** (as a band on paper) to NH_3 vapour resulted in the ready conversion of these compounds to two yellow fluorescent (in UV) glycosides; **7**, R_f 0.23 (TBA), 0.09 (HOAc); and **8**, R_f 0.50 (TBA), 0.18 (HOAc). These compounds were chromatographically (PC; TBA, 15% HOAc, 50% HOAc) and spectrophotometrically identical with compounds **7** and **8** isolated by polyamide chromatography of the crude extract of *P. nivalis* 'variety'. Absorption spectra (v. weak solutions). **7**, λ_{max}^{MeOH} nm· 254, 269 sh, 370; (NaOMe) 452 (dec); (NaOAc) 255 sh, 266 sh, 380, (NaOAc–H_3BO_3) 255 sh, 268 sh, 386; ($AlCl_3$) 254, 266 sh, 445; ($AlCl_3$–HCl) 254 sh, 266, 424; **8**, λ_{max}^{MeOH} nm: 264 sh, 270, 350, (NaOMe) 402; (NaOAc) 264 sh, 270, 392; (NaOAc–H_3BO_3) 350 Acid hydrolysis (3 N HCl–MeOH, 1 . 1, 100°, 30 min) gave aglycones which were purified by PC (50% HOAc) and cochromatographed with authentic rhamnetin and 7-*O*-methylkaempferol on cellulose (TBA, 50% HOAc) and silica gel (toluene–Me_2CO–$CHCl_3$, 8·7:5 and C_6H_6–HOAc–H_2O, 125:72:3) TLC

Oxidations of dihydroflavonols with $Na_2S_2O_5$ *(based on the method of Kurth* [15]). To a 1 . 1 mixture of the dihydroflavonol glycosides **5** and **6** dissolved in H_2O (containing a few drops of EtOH) was added $Na_2S_2O_5$ (75 mg). This mixture in a spring-sealed tube was heated on a steam bath for 45 min, cooled and evaporated to dryness. The product was extracted out with MeOH, and TLC (cellulose, 50% HOAc) revealed about 50% conversion to the flavonol glycosides, **7** and **8**. The same experiment when carried out with a 1 : 1 mixture of dihydrokaempferol- and dihydroquercetin 3-*O*-glucosides produced only starting material whereas a 1 : 1 mixture of the aglycones yielded kaempferol and quercetin

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