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3-*O*-METHYLQUERCETIN 7-*O*-DIGLUCOSIDE 4'-*O*-GLUCOSIDE FROM THE FERN, *OPHIOGLOSSUM VULGATUM*

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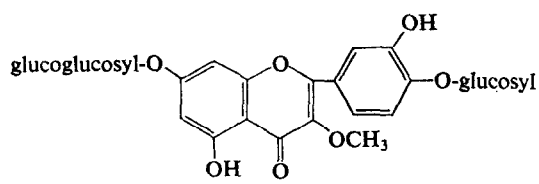
Abstract—A new flavonol triglycoside which was isolated from the fern *Ophioglossum vulgatum* L. is shown to be 3-*O*-methylquercetin 7-*O*-diglucoside 4'-*O*-glucoside.

INTRODUCTION

METHANOL extracts of dried fronds from the fern *Ophioglossum vulgatum* L. afforded, in 0·4 per cent yield, a new flavonoid triglycoside which was shown to be 3-*O*-methylquercetin 7-*O*-diglucoside 4'-*O*-glucoside (I). Although the aglycone 3-*O*-methylquercetin is a known natural product,[1] to our knowledge this is only the second report[1a] of a 3-*O*-methylquercetin glucoside from nature.



(I)

STRUCTURE DETERMINATION

The u.v. spectrum of the new flavonoid in methanol showed maxima at 349 (Band I), 269 and 254 nm, typical of flavones and 3-*O*-substituted flavonols with a 5,7,3',4'-oxygenation pattern.[2,3] The presence of a free 5-hydroxyl group was indicated by the 51 nm bathochromic

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[1] For example, see J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, pp. 53–56 and p. 143, Academic Press, London (1967).

[1a] J. B. HARBORNE and E. HALL, *Phytochem.* 3, 453 (1964).

[2] T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *Systematic Identification of Flavonoids*, Springer-Verlag, New York (1969).

[3] L. JURD, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), Chapter 5, Macmillan, New York (1962).

shift of Band I on addition of $AlCl_3$/HCl. Free hydroxyl groups at the 3-, 7-, and 4′-positions were not detected by addition of the usual diagnostic reagents,[2,3] NaOMe and NaOAc.

The NMR spectrum of the trimethylsilyl ether derivative indicated that the compound was a triglycoside, contained a methoxyl group and had the substitution pattern of quercetin.[4]

Complete hydrolysis of the glycoside with either acid or β-glucosidase yielded an aglycone identical with an authentic sample of 3-*O*-methylquercetin[5] (u.v., i.r., NMR, and two-dimensional paper chromatography). The hydrolysate also contained a single sugar identified by paper chromatography as glucose. Elemental analysis did not distinguish between a diglucoside.$2H_2O$ or a triglucoside.$1H_2O$, but quantitative acid hydrolysis (giving 37·5 per cent of aglycone) and the high R_f value on polyamide TLC showed that the latter formulation was correct. Complete methylation of the original glycoside, followed by acid hydrolysis, yielded a trimethyl ether (as determined by NMR spectroscopy) which on the

TABLE 1. R_f VALUES OF 3-*O*-METHYLQUERCETIN 7-*O*-DIGLUCOSIDE 4′-*O*-GLUCOSIDE (I), ITS ACID HYDROLYSIS PRODUCTS AND REFERENCE COMPOUNDS

Flavonoid	R_f (paper)				R_f (polyamide, TLC)	
	BAW*	TBA†	15% HOAc	60% HOAc	WEMA‡	MAW§
3-*O*-Methylquercetin	0·88	0·80	0·14	0·65	0·05	—
3-*O*-Methylquercetin 4′-*O*-glucoside	0·62	0·63	0·31	—	0·18	0·31
3-*O*-Methylquercetin 7-*O*-diglucoside	0·48	0·47	0·30	0·71	0·32	0·44
3-*O*-Methylquercetin 7-*O*-diglucoside 4′-*O*-glucoside	0·30	0·29	0·52	0·79	0·74	—
Quercetin 3-*O*-rhamnoside (quercitrin)**	0·70	0·61	0·52	0·70	0·18	—
Quercetin 3-*O*-rhamnoglucoside (rutin)**	0·40	0·44	0·51	0·70	0·32	—

* BAW = *n*-butanol:acetic acid:water, 4:1:5.
† TBA = t-butanol:acetic acid:water, 3:1:1.
‡ WEMA = water:methanol:methyl ether ketone:acetyl acetone, 13:3:3:1.
§ MAW = methanol:acetic acid:water, 18:1:1.
** Reference compounds.

basis of u.v. spectra contained hydroxyl groups at the 7- and 4′-positions and was therefore 3,5, 3′-tri-*O*-methyl quercetin.

Mild acid hydrolysis of the triglucoside with methanolic HCl gave two new products which, on paper chromatographic evidence (Table 1), were considered to be 3-*O*-methylquercetin diglucoside and 3-*O*-methylquercetin monoglucoside. The diglucoside, which was the major product, was isolated by chromatography on paper and polyamide column, and the NMR spectrum of the trimethylsilyl ether derivative and its R_f value on polyamide TLC confirmed the presence of two glucosyl groups. The u.v. spectral results for the diglucoside showed that the compound was the 7-diglucoside (no shift on addition of sodium acetate; 40 nm bathochromic shift of Band I on addition of sodium methoxide; Band I shifts of 87 nm with anhydrous $AlCl_3$ and 20 nm with $NaOAc/H_3BO_3$). The u.v. data on the

[4] K. R. MARKHAM and T. J. MABRY, *Phytochem.* **7**, 1197 (1968).
[5] We are grateful to Dr. R. M. Horowitz of the Fruit and Vegetable Research Lab., U.S.D.A., Pasadena, for a sample of authentic 3-*O*-methylquercetin.

monoglucoside indicated that it was 3-*O*-methylquercetin 4′-*O*-glucoside (Band I shift of 53 nm with both $AlCl_3$ and $AlCl_3$/HCl and a 2 nm shift with $NaOAc/H_3BO_3$ indicating the absence of a 3′,4′-*ortho*-dihydroxy system).

On the basis of the above evidence the triglycoside from *Ophioglossum vulgatum* can be assigned structure I. The type of sugar–sugar linkage in the disaccharide portion of I is presently under investigation.

EXPERIMENTAL

The plant material was collected in May 1967 at the pond of Lavore, near Lyon, France. NMR spectra, unless otherwise stated, were measured in CCl_4 on a Varian A-60 spectrometer with tetramethylsilane as internal reference. U.v. spectra were measured on a Beckman DB-G spectrophotometer using standard procedures.[2,3] Paper and polyamide chromatography solvents are recorded in Table 1 unless otherwise noted.

Isolation of 3-O-*Methylquercetin* 7-O-*Diglucoside* 4′-O-*Glucoside* (*I*)

Dried, ground fronds of *Ophioglossum vulgatum* (430 g) were soxhlet extracted first with $CHCl_3$ then with MeOH. The MeOH extract was evaporated to dryness and the residue dissolved in hot water. This aqueous solution was then extracted successively with ether, ethyl acetate and *n*-butanol; the *n*-butanol extract containing most of the triglycoside (I). Purification of I was achieved by one-dimensional paper chromatography (on 500 sheets) of the *n*-butanol extract using BAW as solvent. Subsequent filtration of the product from the paper chromatograms through a polyamide column and recrystallization from MeOH gave 3-*O-methylquercetin* 7-*O-diglucosyl* 4′-*O-glucoside* (*I*) as fawn crystals (1·4 g), m.p. 189°, $[\alpha]_D$ (*c*, 5 mg/cm³ in 50% EtOH) = −112°. (Found: C, 50·52; H, 5·50. Calc. for $C_{34}H_{42}O_{22}.H_2O$: C, 49·77; H, 5·36 per cent. Calc. for $C_{28}H_{32}O_{17}.2H_2O$: C, 49·72; H, 5·50 per cent) R_f values for this compound are present in Table 1. U.v. spectra: λ_{max} (MeOH) 254, 269, 349 nm, log ε 4·39, 4·39, 4·37; λ_{max} (NaOMe) 268, 376 nm; λ_{max} ($AlCl_3$) 275, 298 sh, 355, 400 nm (essentially unchanged on addition of HCl); λ_{max} (NaOAc) 262, 350 nm; λ_{max} ($NaOAc/H_3BO_3$) 254, 266, 350 nm. NMR spectrum of the trimethylsilyl ether[1] of (I) (ppm): 7·68 br. singlet (H-2′); 7·62 quartet (H-6′) $J_{5'6'}$ = 2 cps, $J_{5'6'}$ = 9 cps; 6·98 doublet (H-5′) J = 9 cps; 6·58 doublet (H-8) J = 2 cps; 6·29 doublet (H-6) J = 2 cps; 5·0 multiplet (glucose H-1); 3·86 singlet ($-OCH_3$); 3·1–3·9 multiplet (sugar protons). Principal peaks in the i.r. spectrum: 3450, 1655, 1605, 1490, 1350, 1220, 1180, 1075 (broad), 915, 810, 722 cm^{-1}.

Complete Hydrolysis of (*I*)

(*a*) *Enzymatic hydrolysis.* The triglycoside (150 mg) was hydrolysed at pH 5 and 37° with β-glucosidase (300 mg, N.B.C., 350 units/mg) for 17 hr. The precipitated aglycone was recrystallized from methanol. It was identical (R_f, u.v., i.r.) with authentic[5] 3-*O*-methylquercetin. U.v. spectra: λ_{max} (MeOH) 256, 268 infl., 292 sh, 359 nm; λ_{max} (NaOMe) 272, 326, 405 nm; λ_{max} ($AlCl_3$) 276, 305 sh, 333, 439 nm; λ_{max} ($AlCl_3$/HCl) 268, 301, 367 sh, 406 nm; λ_{max} (NaOAc) 266, 303 sh, 322 sh, 386 nm; λ_{max} ($NaOAc/H_3BO_3$) 261, 306 sh, 378 nm.

(*b*) *Quantitative acid hydrolysis.* The triglycoside (150 mg) was hydrolysed with 2 N HCl for 40 min at 100°, and the aglycone (56·3 mg) recovered by filtration. Treatment of the aglycone (30 mg) with pyridine chloride at 170° for 1 hr yielded quercetin, identified chromatographically. The aqueous phase from the hydrolysis, evaporated to dryness after neutralization on an anion exchange column, showed only one sugar on paper chromatography and this was identical with glucose (R_f 0·22 in BAW and R_f 0·54 in *n*-BuOH:Pyr:H_2O (3:4:2)).

Methylation of (*I*) *and Hydrolysis of the Product*

The triglycoside (20 mg) was methylated in methanol (6 ml) with diazomethane; subsequent acid hydrolysis yielded an aglycone (R_f 0·14 in 15% HOAc) with the following spectral characteristics. U.v. spectra: λ_{max} (MeOH) 250, 262 infl., 345 nm; λ_{max} (NaOMe) 264, 318, 396 (increased intensity) nm; λ_{max} (NaOAc) 270, 312, 365 nm; λ_{max} ($NaOAc/H_3BO_3$) 250 sh, 264 sh, 347 nm; λ_{max} ($AlCl_3$) 250, 266 infl., 345 nm (unchanged on addition of HCl). NMR spectrum: In d_5-pyridine, the methoxyl protons appeared as three, three-proton singlets at 4·02, 3·86 and 3·84 ppm. In d_6-acetone these signals appeared at 3·95, 3·89 and 3·83 ppm.

Partial Hydrolysis of (*I*)

The triglycoside (90 mg) was hydrolysed in a 1:1 mixture of ethanol and 2 N HCl at 100° for 10 min. The hydrolysate was then successively extracted with ether, ethyl acetate and *n*-butanol. Paper chromatography showed that the ether extract contained the aglycone, the *n*-butanol extract contained most of the

unhydrolysed triglycoside and the ethyl acetate extract contained largely a mixture of two partially hydrolysed products. Separation of these latter compounds on paper in BAW, followed by polyamide column chromatography using water–ethanol mixtures, gave 28 mg of the major product (u.v. light, purple, brown yellow with NH_3; R_f 0·48 in BAW) and trace amounts of the minor (u.v. light, purple, no change with NH_3, R_f 0·63 in BAW).

The major compound (3-*O*-methylquercetin 7-*O*-diglucoside) had the following spectra (the chromatographic properties are presented in Table 1): u.v. spectra: λ_{max} (MeOH) 256, 268 infl., 357 nm; λ_{max} (NaOMe) 267, 397 (increased intensity) nm; λ_{max} (NaOAc) 259, 372 nm; λ_{max} (NaOAc/H_3BO_3) 260, 377 nm; λ_{max} ($AlCl_3$) 276, 444 nm; λ_{max} ($AlCl_3$/HCl) 269, 297 sh, 362, 402 nm. The NMR spectrum of the TMS ether was similar to that observed for the TMS ether of the triglycoside: (7·55 (H-2′), 7·46 (H-6′), 6·80 (H-5′), 6·52 (H-8), 6·23 (H-6), 4·8–5·0 (glucose H-1), 3·82 (—OCH_3), 3·1–3·9 (sugar protons). Integration revealed a ratio of 17:78 for —OCH_3:sugar protons.

The minor compound (3-*O*-methylquercetin 4′-*O*-glucoside) gave the following u.v. spectra: λ_{max} (MeOH) 253, 269, 346 nm; λ_{max} (NaOMe) 274, 379 (reduced intensity) nm; λ_{max} (NaOAc) 275, 365 nm; λ_{max} (NaOAc/H_3BO_3) 269, 348 nm; λ_{max} ($AlCl_3$) 277, 297 sh, 348, 399 nm (unchanged on the addition of HCl). Chromatographic properties are presented in Table 1.

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