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IDENTIFICATION OF NATURAL TRICETIN-DERIVED *C*-GLYCOSIDES THROUGH SYNTHESIS

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Key Word Index—5,7-dihydroxy-3′,4′,5′-trimethoxyflavone; *C*-glucosylation; *C*-galactosylation; *C*-xylosylation; *C*-arabinosylation; *C*-rhamnosylation; 6-*C*-glycosides; 6,8-di-*C*-glycosides; permethyl derivatives; EIMS.

Abstract—*C*-Glycosylation of 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone was carried out with acetobromo-α-D-glucose, -α-D-galactose, -α-D-xylose, -β-L-arabinose and -α-L-rhamnose. The respective 6-*C*-glycosides and 6,8-di-*C*-glycosides (excepted for galactose) were isolated and permethylated. MS and TLC comparison confirmed the proposed structures of five natural tricetin-derived *C*-glycosides.

INTRODUCTION

Owing to the resistance of *C*-glycosylflavonoids towards acid hydrolysis, the identification of the glycosyl residue still remains the most difficult problem in their structure determination when the amounts available preclude the use of ^{13}C NMR. Many years ago, we showed that 6-*C*-glycosyl and sometimes 6,8-di-*C*-glycosyl-5,7-dihydroxyflavones can be isolated in very low yield from the mixture of *O*-glycosides and *C*-glycosides resulting from the reaction of 5,7-dihydroxyflavone with acetobromo-α-D-glucose [1], -α-D-galactose [2], -α-D-xylose [3], -β-L-arabinose [4] and -α-L-rhamnose [5]. The apigenin *C*-glycosides obtained in this way and their permethyl derivatives have since been successfully used in the identification of the corresponding natural compounds. Until 1976, all known natural *C*-glycosylflavones were apigenin or luteolin derivatives, but the occurrence of natural tricetin-derived *C*-glycosylflavones has since been demonstrated in *Gentiana pyrenaica* [6], *Polygonum affine* [7], *Trichomanes venosum* [8] and mainly in Hepaticae: *Plagiochila asplenioides* [9], *Takakia lepidozioides* [10], *Apometzgeria pubescens* [11], *Metzgeria conjugata* and *M. leptoneura* [12]. In order to identify the glycosyl residue present in such compounds or to confirm proposed structures, *C*-glycosylation of 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone was carried out with acetobromo-α-D-glucose, -α-D-galactose, -α-D-xylose, -β-L-arabinose and -α-L-rhamnose and the resulting *C*-glycosides were permethylated. The standard compounds so obtained can be compared with the permethyl derivatives of natural tricetin, 3′-*O*-methyltricetin, tricin and apometzgerin (3′,4′-di-*O*-methyltricetin) *C*-glycosides. In the present paper, we confirm in this way the proposed structures of isoaffinetin (6-*C*-glucosyltricetin) from *Polygonum affine* [7, 8], 6,8-di-*C*-glucosyltricetin from *Plagiochila asplenioides* [9] and *Apometzgeria pubescens* [11], 6,8-di-*C*-glucosyltricin, 6,8-di-*C*-arabinosyltricin and 6,8-di-*C*-arabinosylapometzgerin from *Apometzgeria pubescens* [11, 13].

RESULTS AND DISCUSSION

In order to minimize the formation of *O*-glycosides, 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone (4′-*O*-methyltricin) **1** was chosen because it only contains the 5,7-dihydroxy system required for *C*-glycosylation to take place. Moreover it could be easily prepared from phloracetophenone tris-3,4,5-trimethoxybenzoate by Baker–Venkataraman rearrangement according to Hauteville's method [14, 15]. Glycosylation of **1** was carried out in the presence of LiOMe in methanol with a very large excess of acetobromosugar (molar ratio 50:1) in order to get some 6,8-di-*C*-glycosylflavone along with 6-*C*-glycosylflavone, the major product of *C*-glycosylation. No appreciable Wessely–Moser isomerization was observed during one hour on acid hydrolysis of the complex reaction mixture of *O*- and *C*-glycosides, in agreement with the usual reluctance of 4′-*O*-methyl-*C*-glycosylflavones to isomerization [1]. The hydrolysate was then extracted with ether and butanol and *C*-glycosides were isolated from the butanol extract by chromatography on a cellulose column in a gradient water–acetic acid followed by PC in BAW and 15% acetic acid. The compounds were characterized by their migrations and colour reactions with bis-diazotized benzidine: red for 6-*C*-glycosides, brown for 8-*C*-glycosides, pale yellow for 6,8-di-*C*-glycosides, orange yellow for 7-*O*-glycosides and violet for the starting flavone. They were identified by UV spectra and diagnostic shifts with aluminium chloride + hydrochloric acid (5-hydroxyl) and sodium acetate (7-hydroxyl) [16], *C*-glycosides by the mass spectra of the permethylated (PM) derivatives [17] and *O*-glycosides by acid hydrolysis.

From the reaction of **1** with acetobromo-α-D-glucose were isolated 7-*O*-glucosyl-4′-*O*-methyltricin, 6-*C*-glucosyl-4′-*O*-methyltricin (**2**) and 6,8-di-*C*-glucosyl-4′-*O*-methyltricin (**3**). 8-*C*-Glucosyl-4′-*O*-methyltricin (**4**) could be isolated in very low yield after prolonged heating of **2** with acid. Co-TLC of PM **2** with PM isoaffinetin on the one hand, of PM **3** with the PM derivatives of natural 6,8-di-*C*-glucosyltricetin and 6,8-di-*C*-glucosyltricin confirmed that glucose was the sugar residue in these natural compounds, in agreement with ^{13}C NMR data [8, 11].

From the reaction of **1** with acetobromo-α-D-galactose was only isolated 6-*C*-galactosyl-4′-*O*-methyltricin, **5**. As expected, **5** and **2** could not be separated in the free state by PC in BAW and 15% acetic acid, but their PM

derivatives were easily separated by TLC. Reaction of **1** with acetobromo-α-D-xylose afforded 7-*O*-xylosyl-4′-*O*-methyltricin, 5-*O*-xylosyl-4′-*O*-methyltricin (**6**), 6-*C*-xylosyl-4′-*O*-methyltricin (**7**) and 6,8-di-*C*-xylosyl-4′-*O*-methyltricin (**8**). Compound **6** showed a blue fluorescence on paper under UV light and gave xylose and **1** on acid hydrolysis. However, its UV spectrum was unexpectedly shifted by aluminium chloride in methanol and the 5-*O*-xyloside structure was therefore demonstrated by permethylation and acid hydrolysis of the PM derivative. The UV spectrum of the resulting flavone was shifted by aluminium chloride and not by sodium acetate, showing the presence of a free 5-hydroxyl. Surprisingly, it was then observed that the UV spectrum of **6** was not shifted by aluminium chloride in 95% ethanol.

From the reaction of **1** with acetobromo-β-L-arabinose were isolated 7-*O*-arabinosyl-4′-*O*-methyltricin, 6-*C*-arabinosyl-4′-*O*-methyltricin (**9**) and 6,8-di-*C*-arabinosyl-4′-*O*-methyltricin (**10**). As expected, **9** and **7** on the one hand and **10** and **8** on the other hand, could not be separated in the free state by PC in BAW and 15% acetic acid, but their PM derivatives were again easily separated by TLC. As previously observed with symmetrical PM 6,8-di-*C*-pentosylapigenins [17], the mass spectra of PM **10** and PM **8** can be differentiated by the relative intensities of the ions [M − 119], [M − 131] and [M − 145]. Co-TLC of PM **10** with the PM derivatives of natural 6,8-di-*C*-arabinosyltricin and 6,8-di-*C*-arabinosylapometzgerin again confirmed that arabinose was the sugar residue in these natural compounds, in agreement with ^{13}C NMR data [11, 13]. Reaction of **1** with acetobromo-α-L-rhamnose afforded 7-*O*-rhamnosyl-4′-*O*-methyltricin, 6-*C*-rhamnosyl-4′-*O*-methyltricin (**11**), 6,8-di-*C*-rhamnosyl-4′-*O*-methyltricin (**12**) and traces of 7-*O*-rhamnosyl-6-*C*-rhamnosyl-4′-*O*-methyltricin (**13**).

The TLC behaviour of all these 4′-*O*-methyltricin *O*- and *C*-glycosides on cellulose in aqueous acetic acid and BAW (4:1:5), on ordinary (not activated) silica gel in ethylacetate–pyridine–water–methanol (80:20:10:5) (EPWM) and of their PM derivatives on silica gel in chloroform–ethylacetate–acetone (5:4:1) and (5:1:4) is shown in Table 1. Standard compounds are now available to the phytochemical community for the identification of the still unknown tricetin-derived *C*-xylosides, *C*-galactosides and *C*-rhamnosides.

EXPERIMENTAL

5,7-Dihydroxy-3′,4′,5′-trimethoxyflavone (**1**) mp 275–277°, was prepared in 43% yield from phloracetophenone according to [14] and [15]: α-bromo-2,3,4,6-tetra-*O*-acetyl-D-glucopyranose and galactopyranose, α-bromo-2,3,4-tri-*O*-acetyl-D-xylopyranose and L-rhamnopyranose, β-bromo-2,3,4-tri-*O*-acetyl-L-arabinopyranose were prepared from D-glucose, D-galactose, D-xylose, L-rhamnose and L-arabinose according to standard procedures.

C-Glycosylation. To a stirred soln of lithium (600 mg, 86 mmol) in MeOH (40 ml) are added **1** (433 mg, 1.25 mmol) and a soln of acetobromosugar (50–60 mmol) in $CHCl_3$ (70 ml). After 1.5 hr at room temp. the reaction mixture was neutralized with aq. 4 N HCl, concd and extracted with Et_2O and *n*-BuOH. The BuOH extract, after separation of eventual ppts of *O*-glycosides, was dissolved in MeOH–4 N HCl (1:1) and the soln refluxed during 45 min, neutralized with 4 N NaOH and extracted with Et_2O and *n*-BuOH. The BuOH extract was chromatographed on a cellulose column eluted with a gradient H_2O–HOAc and the fractions analysed by PC in BAW (4:1:5) and 15% HOAc.

6-C-β-D-glucopyranosyl-4′-O-methyltricin (**2**). UV λ_{max}^{MeOH} nm: 271, 331; + $AlCl_3$ 281, 301, 339, 388 sh; + NaOAc 278, 299 sh, 355; + N/10 NaOH 284, 299 sh, 375. *PM derivative*: EIMS (70 eV) *m/z* (rel. int.): 590 $[M]^+$ (28), 575 $[M-15]^+$ (25), 559 $[M-31]^+$ (88), 545 $[M-45]^+$ (16), 487 $[M-103]^+$ (18), 427 $[M-163]^+$ (20), 415 $[M-175]^+$ (100), 401 $[M-189]^+$ (14), 385 $[M-205]^+$ (17), 371 $[M-219]^+$ (20).

6,8-di-C-β-D-glucopyranosyl-4′-O-methyltricin (**3**). UV λ_{max}^{MeOH} nm: 272, 329; + $AlCl_3$ 277, 302, 335, 385 sh; + NaOAc 277, 315 sh, 353 sh; + N/10 NaOH 285, 300 sh, 376. *PM derivative*: EIMS (70 eV) *m/z* (rel. int.): 808 $[M]^+$ (20), 793 $[M-15]^+$ (31),

Table 1. TLC: R_f values of 4′-*O*-methyltricin *O*- and *C*-glycosides

	Cellulose					Silica gel		
	aq. HOAc			BAW		EPWM	$CHCl_3$–EtOAc–Me_2CO*	
	5%	15%	30%	27%	(4:1:5)	(80:20:10:5)	(5:4:1)	(5:1:4)
6-*C*-glucoside	0.38	0.57	0.84	0.81	0.70	0.73	0.27	0.64
8-*C*-glucoside	0.19	0.36	0.63	—	0.56	—	0.11	0.35
6-*C*-galactoside	0.37	0.55	0.84	0.80	0.71	0.75	0.17	0.54
6-*C*-arabinoside	0.24	0.44	0.76	0.83	0.71	0.80	0.15	0.49
6-*C*-xyloside	0.27	0.47	0.76	0.80	0.70	0.78	0.30	0.63
6-*C*-rhamnoside	0.15	0.38	0.76	0.92	0.83	0.90	0.13	0.40
6,8-di-*C*-glucoside	0.61	0.71	0.87	0.61	0.44	0.49	0.30	0.70
6,8-di-*C*-arabinoside	0.40	0.54	0.78	0.63	0.46	0.54	0.12	0.45
6,8-di-*C*-xyloside	0.40	0.55	0.78	0.64	0.46	0.61	0.33	0.67
6,8-di-*C*-rhamnoside	0.60	0.68	0.85	0.84	0.71	0.74	0.21	0.62
6,7-dirhamnoside	0.50	0.60	0.86	0.92	0.80	0.83	—	—
7-*O*-glucoside	0.10	0.28	0.64	0.78	—	0.84	—	—
7-*O*-arabinoside	—	—	0.63	0.80	0.70	0.84	—	—
7-*O*-xyloside	0.07	0.23	0.62	0.84	0.73	0.87	—	—
7-*O*-rhamnoside	—	0.30	0.79	0.74	0.74	0.90	—	—
5-*O*-xyloside	0.11	0.26	0.59	0.80	0.77	0.87	—	—

*PM derivatives.

777 [M − 31]$^+$ (100), 761 [M − 47]$^+$ (11), 705 [M − 103]$^+$ (16), 645 [M − 163]$^+$ (32), 633 [M − 175]$^+$ (46), 619 [M − 189]$^+$ (10), 603 [M − 205]$^+$ (11).

8-C-β-D-*glucopyranosyl*-4′-O-*methyltricin* (**4**). UV λ_{max}^{MeOH} nm: 269, 326; + $AlCl_3$ 277, 301 sh, 334, 380 sh; + NaOAc 275, 296 sh, 354; + N/10 NaOH 284, 301 sh, 376. *PM derivative*: EIMS (70 eV) *m/z* (rel. int.): 590 [M]$^+$ (100), 429 [M − 161]$^+$ (6), 415 [M − 175]$^+$ (81) 401 [M − 189]$^+$ (14), 385 [M − 205]$^+$ (6), 371 [M − 219]$^+$ (5).

7-O-*glucosyl*-4′-O-*methyltricin*. UV λ_{max}^{MeOH} nm: 269, 330; + $AlCl_3$ 277, 299 sh, 335, 383 sh; + NaOAc 269, 328; + N/10 NaOH 287, 362.

6-C-β-D-*galactopyranosyl*-4′-O-*methyltricin* (**5**). UV λ_{max}^{MeOH} nm: 277, 335; $AlCl_3$ 285, 304, 342, 375 sh; + NaOAc 283, 296 sh, 362; + N/10 NaOH 284, 298 sh, 375. *PM derivative*: EIMS (70 eV) *m/z* (rel. int.): 590 [M]$^+$ (20), 575 [M − 15]$^+$ (33), 559 [M − 31]$^+$ (53), 543 [M − 47]$^+$ (7), 487 [M − 103]$^+$ (11), 427 [M − 163]$^+$ (17), 415 [M − 175]$^+$ (100), 401 [M − 189]$^+$ (22), 385 [M − 205]$^+$ (19), 371 [M − 219]$^+$ (25).

5-O-*xylosyl*-4′-O-*methyltricin* (**6**). UV λ_{max}^{EtOH} nm: 266, 326; + $AlCl_3$ 266, 328; + NaOAc 274, 300 sh, 352. *PM derivative*: UV λ_{max}^{MeOH} nm: 256, 266, 320; + $AlCl_3$ 256, 267, 321; + NaOAc 256, 266, 320; + N/10 NaOH 256, 265, 320. Acid hydrolysis of **6** gave xylose and 4′-*O*-methyltricin. Acid hydrolysis of PM (**6**) gave a flavone: UV λ_{max}^{MeOH} nm: 270, 304 sh, 324; + $AlCl_3$ 278, 294, 345, 376 sh; + NaOAc 270, 300, 324; + N/10 NaOH 286, 348.

7-O-*xylosyl*-4′-O-*methyltricin*. UV λ_{max}^{MeOH} nm: 275, 315; + $AlCl_3$ 283, 301 sh, 332, 345 sh, 383; + NaOAc 276, 302 sh; + N/10 NaOH 289, 340 sh.

6-C-β-D-*xylopyranosyl*-4′-O-*methyltricin* (**7**). UV λ_{max}^{MeOH} nm: 276, 326; + $AlCl_3$ 284, 296 sh, 351, 378 sh; + NaOAc 282, 296 sh, 336 sh, 365 sh; + N/10 NaOH 283, 298 sh, 375. *PM derivative*: EIMS (70 eV) *m/z* (rel. int.): 546 [M]$^+$ (22), 531 [M − 15]$^+$ (20), 515 [M − 31]$^+$ (100), 499 [M − 47]$^+$ (24), 487 [M − 59]$^+$ (12), 427 [M − 119]$^+$ (14). 415 [M − 131]$^+$ (44), 401 [M − 145]$^+$ (24), 385 [M − 161]$^+$ (10), 371 [M − 175]$^+$ (19).

6,8-*di*-C-β-D-*xylopyranosyl*-4′-O-*methyltricin* (**8**). UV λ_{max}^{MeOH} nm: 277, 328; + $AlCl_3$ 281, 305, 341, 375 sh; + NaOAc 286, 298 sh, 375; + N/10 NaOH 286, 296 sh, 380. *PM derivative*: EIMS (70 eV) *m/z* (rel. int.): 720 [M]$^+$ (20), 705 [M − 15]$^+$ (20), 689 [M − 31]$^+$ (100), 673 [M − 47]$^+$ (14), 659 [M − 61]$^+$ (12), 601 [M − 119]$^+$ (20), 589 [M − 131]$^+$ (21), 575 [M − 145]$^+$ (8).

6-C-α-L-*arabinopyranosyl*-4′-O-*methyltricin* (**9**). UV λ_{max}^{MeOH} nm: 275, 335; + $AlCl_3$ 282, 306 sh, 342, 399 sh; + NaOAc 282, 305 sh, 366; + N/10 NaOH 283, 301 sh, 376. *PM derivative*: EIMS (70 eV) *m/z* (rel. int.): 546 [M]$^+$ (33), 531 [M − 15]$^+$ (28), 515 [M − 31]$^+$ (100), 499 [M − 47]$^+$ (17), 487 [M − 59]$^+$ (10), 427 [M − 119]$^+$ (25), 415 [M − 131]$^+$ (90), 401 [M − 145]$^+$ (67), 385 [M − 161]$^+$ (15), 371 [M − 175]$^+$ (13).

6,8-*di*-C-α-L-*arabinopyranosyl*-4′-O-*methyltricin* (**10**). UV λ_{max}^{MeOH} nm: 277, 325; + $AlCl_3$ 280, 302 sh, 339, 380 sh, 339, 380 sh; + NaOAc 284, 295 sh, 370; + N/10 NaOH 285, 306 sh, 378. *PM derivative*: EIMS (70 eV) *m/z* (rel. int.): 720 [M]$^+$ (28), 705 [M − 15]$^+$ (25), 689 [M − 31]$^+$ (100), 673 [M − 47]$^+$ (46), 659 [M − 61]$^+$ (22), 601 [M − 119]$^+$ (36), 589 [M − 131]$^+$ (44), 575 [M − 145]$^+$ (33).

7-O-*arabinosyl*-4′-*O*-*methyltricin*. UV λ_{max}^{MeOH} nm: 272, 330; + $AlCl_3$ 281, 301, 344, 375 sh; + NaOAc 272, 330; + N/10 NaOH 289, 371.

6-C-α-L-*rhamnopyranosyl*-4′-O-*methyltricin* (**11**). UV λ_{max}^{MeOH} nm: 277, 335; + $AlCl_3$ 285, 300 sh, 343, 380 sh; + NaOAc 285, 300 sh, 371; + N/10 NaOH 283, 295 sh, 375. *PM derivative*: EIMS (70 eV) *m/z* (rel. int.): 560 [M]$^+$ (21), 545 [M − 15]$^+$ (11), 529 [M − 31]$^+$ (72), 487 [M − 73]$^+$ (14), 427 [M − 133]$^+$ (14), 415 [M − 145]$^+$ (100), 401 [M − 159]$^+$ (34), 385 [M − 175]$^+$ (24), 371 [M − 189]$^+$ (40).

6,8-*di*-C-α-L-*rhamnopyranosyl*-4′-O-*methyltricin* (**12**). UV λ_{max}^{MeOH} nm: 279, 330; + $AlCl_3$ 287, 307, 335, 342 sh, 380 sh; + NaOAc 285, 300 sh, 375; + N/10 NaOH 286, 300 sh, 376. *PM derivative*: EIMS (70 eV) *m/z* (rel. int.): 748 [M]$^+$ (22), 733 [M − 15]$^+$ (25), 717 [M − 31]$^+$ (100), 701 [M − 47]$^+$ (11), 675 [M − 73]$^+$ (17), 615 [M − 133]$^+$ (22), 603 [M − 145]$^+$ (51), 589 [M − 159]$^+$ (17).

7-O-*rhamnosyl*-6-C-*rhamnosyl*-4′-O-*methyltricin*. UV λ_{max}^{MeOH} nm: 277, 328; + $AlCl_3$ 284, 298 sh, 355, 378 sh; + NaOAc 277, 316 sh; + N/10 NaOH 284, 297 sh, 368.

7-O-*rhamnosyl*-4′-O-*methyltricin*. UV λ_{max}^{MeOH} nm: 274, 331; + $AlCl_3$ 282, 307, 342, 384 sh; + NaOAc 274, 333; + N/10 NaOH 291, 357.

Acid hydrolyses. The *O*-glycosides were heated with MeOH–4 N HCl (1:1) in a sealed tube at 100° during 1 hr and the hydrolysis products identified by TLC.

Acid isomerization of **2**. The compound was heated with MeOH–4 N HCl (1:1) in a sealed tube at 100° during 8 hr and the isomers **2** and **4** separated by prep. PC.

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