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GLUCOSE-CONJUGATION OF THE FLAVONES OF *PSIADIA ARABICA* BY *CUNNINGHAMELLA ELEGANS*

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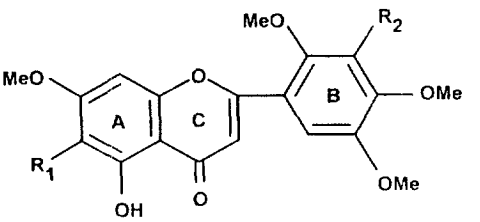
Key Word Index—*Psiadia arabica*; Compositae; *Cunninghamella elegans*; psiadiarabin; 5,3′-dihydroxy-7,2′,4′,5′-tetramethoxyflavone; glucoside conjugates; biotransformation.

Abstract—Microbial transformation of psiadiarabin and its 6-desmethoxy analogue 5,3′ dihydroxy-7,2′,4′,5′-tetramethoxyflavone by *Cunninghamella elegans* NRRL 1392 gave the 3′-glucoside conjugates of the two flavones. Structural elucidation of these two new metabolites was achieved using 1D and 2D NMR spectroscopy and CIMS. © 1997 Elsevier Science Ltd

INTRODUCTION

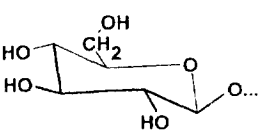
Flavones of *Psiadia arabica* Jaub. et Spach (Compositae) are characterized by having an unusual tetraoxygenation pattern of ring B, which is seldom encountered [1]. As a part of an on going programme to study the bioconversion of natural products by micro-organisms, the microbial metabolism of two of these flavones was investigated. Thus, psiadiarabin (**1**) and its 6-desmethoxy analogue 5,3′dihydroxy-7,2′,4′,5′-tetramethoxyflavone (**2**) were found to be metabolized by *Cunninghamella elegans* NRRL 1392 to yield two glucose-conjugates (**3** and **4**). The site of glucosidation, namely C-3′, was validated by 1D and 2D NMR, besides CIMS.



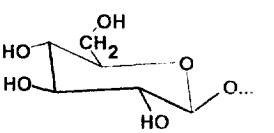
1 R_1=OMe, R_2 = OH

2 R_1=H, R_2 = OH

3 R_1=OMe, R_2 =



4 R_1=H, R_2 =



RESULTS AND DISCUSSION

The microbial screening experiments showed that only *C. elegans* NRRL 1392 was able to convert **1** and **2** into the highly polar metabolites **3** and **4**, respectively. Other micro-organisms used in this study (see Experimental) did not yield any metabolites. The CIMS (methane) of **3** and **4** had $[M+H]^+$ peaks at 567 and 537, respectively, suggesting conjugation of both compounds with a hexose residue. The ^{13}C NMR showed six additional carbon signals, with chemical shift values not observed in the spectra of the substrates. These carbon signals had close resonances to those reported [2, 3] for the β-D-glucose signals of luteolin 3′-glucoside, and were almost identical in both **3** and **4** (see Table 1).

The ^1H NMR spectra of the two metabolites were similar to those of the respective substrates [1], except for the additional proton signals for the sugar moiety. These signals were similar to those of glucose with the anomeric proton doublet resonating at δ 5.07 in both **3** and **4**, with J = 7.2 Hz and 6.7 Hz for **3** and **4**, respectively. These J values agreed with a β-D configuration for the glucose moiety in both metabolites [4, 5]. The glucosidation of the 5-hydroxyl group was excluded, since the heavily hydrogen-bonded 5-OH proton signal was still present in the ^1H NMR of the metabolites at δ 12.79 and 12.81 for **3** and **4**,

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Table 1. ^{13}C NMR signals for Compounds **1**–**4***

C	1†	2†	3‡	4‡
2	161.7(0)	161.7(0)	162.2(0)	161.0(0)
3	109.6(1)	109.9(1)	108.8(1)	109.0(1)
4	182.9(0)	182.7(0)	182.2(0)	181.8(0)
4a	106.2(0)	105.6(0)	105.2(0)	104.6(0)
5	153.0(0)	162.2(0)	152.9(0)	162.1(0)
6	132.7(0)	98.0(1)	131.9(0)	97.9(1)
7	158.9(0)	165.6(0)	158.9(0)	165.2(0)
8	90.6(1)	92.6(1)	91.7(1)	92.7(1)
8a	153.5(0)	157.9(0)	151.9(0)	157.3(0)
1′	119.7(0)	119.6(0)	119.5(0)	119.4(0)
2′	141.3(0)	141.2(0)	145.7(0)	145.6(0)
3′	143.5(0)	143.4(0)	146.3(0)	146.2(0)
4′	139.5(0)	139.4(0)	143.4(0)	143.2(0)
5′	148.9(0)	148.9(0)	149.5(0)	149.4(0
6′	102.6(1)	102.6(1)	107.2(1)	107.0(1)
OMe	61.2(3)	61.2(3)	61.6(3)	61.4(3)
	61.1(3)	61.1(3)	60.6(3)	60.7(3)
	60.8(3)	56.3(3)	60.1(3)	56.0(3)
	56.4(3)	55.8(3)	56.6(3)	56.7(3)
	56.4(3)		56.2(3)	
1″			102.4(1)	102.2(1)
2″			74.0(1)	73.8(1)
3″			76.4(1)	76.2(1)
4″			69.9(1)	69.8(1)
5″			77.4(1)	77.2(1)
6″			60.8(2)	60.4(2)

* Number between parentheses denotes multiplicity.
† solvent is $CDCl_3$.
‡ solvent is DMSO-d_6.

respectively, and disappeared upon deuteration. Furthermore, the 3′-OH proton signal at 6.12 ppm in ^1H NMR of **1** and **2** was not observed in the spectra of either metabolite **3** or **4**.

The ^{13}C NMR lent further support to conjugation of the 3′-hydroxyl as no significant differences in chemical shift value were observed between ring A carbons of the metabolites and those of the substrates. However, differences in ^{13}C NMR chemical shifts between ring B carbons in the substrates and metabolites were observed, especially at the *ipso* carbon as well as at the carbons *ortho* or *para* to the glucosylated position (Table 1). It was found that the most reliable indication of glycosylation was the downfield shift of the *para*-carbon signal which was more deshielded [2] (see Table 1). Further substantiation of the site of glycosylation at C-3′ came from the chemical shift value of the anomeric carbon of the glucose residue, which depends on the environment at the phenolic hydroxyl group. In 3′-*O*-glucosides, the anomeric carbon has been reported [2] to resonate at 102.4 ppm while in 5-*O*-glucosides it resonates at 104.3 ppm. Since the anomeric carbons of the glucose residue in **3** and **4** resonated at 102.4 and 102.2, respectively, the glucosidation of the C-3′ hydroxyl group was confirmed.

Most microbial transformations of flavonoids have been found to occur in ring B, particularly at C-4′ and to a lesser extent C-3′. These transformations may include hydroxylation [6,7] and sulphation [8]. Previous substrate specificity studies have indicated the chromone ring system of the flavonoid nucleus (ring A and ring C) is attached to the binding site of the enzyme [9], thus exposing ring B to the active site of the enzyme. Since most ring B positions of psiadiarabin (**1**) and its 6-demethoxy analogue (**2**) are already substituted, no transformation of these compounds was anticipated. However, it does seem that the ring B substituents have allowed exposure of the C-3′ hydroxyl group to the active glucosidating site of the microbial enzyme, leading to the formation of 3′-glucosides, from the substrates.

Conjugation of natural products, including flavonoids, and xenobiotics is a well documented metabolic pathway in mammalian species [10]. Although glycosylation by micro-organisms is not a particularly common pathway [11], glucosidation of phenolic hydroxyl groups by *Cunninghamella* species, presumably as a detoxification mechanism, has been previously reported [4, 12]. The results mentioned herein are interesting because this is one of a growing number of reports which demonstrate parallels between microbial and mammalian systems not only in phase I but also in phase II metabolism [12, 13].

EXPERIMENTAL

General. Psiadiarabin (**1**) and 5,3′-dihydroxy-7,2′,4′,5′ tetra methoxyflavone (**2**) were purified from aerial parts of *Psiadia arabica* Jaub. et Spach (Compositae) using literature procedures [1]. Mps were uncorr. NMR spectra were determined on a Varian XL300 spectrometer and chemical shift values are given in δ (ppm). DEPT, APT and COSY spectra were determined using standard Varian pulse sequences.

Micro-organisms and culture conditions. Micro-organisms were obtained from either American Type Culture Collection (ATCC) or Northern Regional Research Laboratories (NRRL). Organisms were maintained on Sabouraud dextrose agar (Oxoid) slants at 4 and were used to inoculate the autoclaved culture medium. Twenty micro-organisms were used for the preliminary screening as follows:

Aspergillus alliaceous NRRL 315, *A. flavipes* ATCC 11013, *A. niger* NRRL 599, *A. niger* NRRL 2295, *A. ochraceus* NRRL 398, *A. ochraceous* NRRL 405, *Candida albicans*, lab isolate, *Cunninghamella blackesleeana* MR 198, *C. echinulata* NRRL 1382 (ATCC 42616), *C. elegans* NRRL 1392 (ATCC 10028a), *Gymnascella citrina* NRRL 6050 (ATCC 16956), *Lindera pinnespora* NRRL 2237, *Penicillium chrysogenum* ATCC 10002, *P. chrysogenum*, ATCC 10002-K, *P. purpureus* UI 193, *P. vermiculatum* NRRL 1009, *Rhizopus nigricans* NRRL 1477, *Rhodotorula rubra* NRRL y1592, *Saccharomyces cerevisiae* (Baker's yeast) and *Streptomyces fulvissimus* NRRL 1453B.

Components of culture medium. Glucose 10 g, glycerol 10 ml, yeast extract 5 g, peptone 5 g, K_2HPO_4 5

g, NaCl 5 g, H_2O 1 l. The pH was adjusted to 6.0 before autoclaving at 121° for 15 mins.

Cultivation of micro-organisms. Cells of micro-organisms were transferred from two-week old slants into sterile culture medium and kept on a gyratory shaker for 72 hr to give stage I culture. 5 ml of stage I cultures were used as inoculum for stage II cultures (50 ml 250 ml flask^{-1}). After 24 hr incubation of stage II cultures, psiadiarabin (**1**), or its 6-demethoxy analogue **2**, were added as a soln in EtOH or Me_2CO (10 mg 0.25 ml^{-1}). Both substrate and organism controls were made. Fermentations were sampled by extracting 5 ml of culture with 5 ml $CHCl_3$ or EtOAc. After evapn of the solvent, the residue was chromatographed on silica gel plates using $CHCl_3$–MeOH (9:1) as mobile phase.

Fermentation of psiadiarabin (**1**) *with* C. elegans. Psiadiarabin [**1**, 450 mg], dissolved in EtOH, was evenly distributed among 45 flasks containing stage II cultures. Fermentation was stopped after 3 days, the mixt. was filtered and fermentation broth was extracted ×3 with equal vols of $CHCl_3$. Silica gel CC using $CHCl_3$–MeOH gradient, followed by crystn from MeOH, gave 100 mg of pure **3**; mp 234–235°; $[\alpha]_D$ −19.5°; CIMS (methane) *m/z* (rel. intensity): 567 $(M+H)^+$, 405 $(M+H-\text{glucose})^+$ 100%; ^{1}H NMR (DMSO-d_6) δ: 12.79 (1H, *s*, 5-OH, exchangeable), 7.20 (1H, *s*, H-6′), 6.92 (1H, *s*, H-8), 6.82 (1H, *s*, H-3), 5.07 (1H, *d*, J = 7.2 Hz, H-1″), 3.64 (1H, *m*, H-6″), 3.27 (2H, *m*, H-2″ and H-3″), 3.12 (2H, *m*, H-4″, H-5″), 3.75–3.94 (15H, 5*s*, $5OCH_3$), ^{13}C NMR (DMSO-d_6) δ: see Table 1.

Fermentation of 5,3′-dihydroxy-7,2′,4′,5′-tetramethoxy flavone (**2**) *by* C. elegans. Compound **2** (360 mg) dissolved in Me_2CO, was evenly distributed among 36 flasks each containing 50 ml stage II culture. After 6 days, fermentation was stopped and the broth extracted ×3 with equal vols of $CHCl_3$. Evapn of the solvent gave 250 mg of residue. Sephadex LH-20 CC, using MeOH as eluent, gave 70 mg of pure **4**; mp 226–227°; $[\alpha]_D$ 0°; CIMS (methane) *m/z* (rel. intensity): 537 $(M+H)$, 375 $(M+H-\text{glucose})^+$ 100%; ^{1}H NMR (DMSO-d_6) δ: 12.81 (1H, *s*, 5-OH, exchangeable), 7.18 (1H, *s*, H-6′), 6.77 (2H, *m*, H-3 and H-8), 6.41 (1H, *d*, J = 1.8 Hz, H-6), 5.08 (1H, *d*, J = 6.7 Hz, H-1″), 3.64 (1H, *m*, H-6″), 3.26 (2H, *m*, H-2″ and H-3″), 3.12 (2H, *m*, H-4″, 5″), 3.78–3.87 (12H, 4*s*, 4 OCH_3): ^{13}C NMR (DMSO-d_6) δ: see Table 1.

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