Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Molecular Catalysis B: Enzymatic 39 (2006) 18–23
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Microbial transformations of flavanone and 6-hydroxyflavanone by *Aspergillus niger* strains

Edyta Kostrzewa-Susłow [a,*], Jadwiga Dmochowska-Gładysz [a], Agata Białońska [b], Zbigniew Ciunik [b], Waldemar Rymowicz [c]

[a] *Department of Chemistry, Agricultural University, Norwida 25, 50-375 Wroclaw, Poland*
[b] *Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383 Wroclaw, Poland*
[c] *Department of Biotechnology and Food Microbiology, Agricultural University, Norwida 25, 50-375 Wroclaw, Poland*

Available online 2 March 2006

Abstract

Flavanone (**1**) and 6-hydroxyflavanone (**2**) were subjected to transformation by means of *Aspergillus niger* strains (one wild and three UV mutants). For both substrates the biotransformation resulted in reduction of the carbonyl group (products **5** and **7**) and dehydrogenation at C-2 and C-3 (**3** and **8**). Additionally, for flavanone (**1**) reduction of C-4 together with hydroxylation at C-7 (**6**) and dehydrogenation at C-2, C-3 along with hydroxylation at C-3 (**4**) were observed.

Keywords: Flavanone; 6-Hydroxyflavanone; Biotransformation; Reduction; Dehydrogenation; Hydroxylation; *Aspergillus niger*

1. Introduction

Flavonoids, which are the object of our research, are commonly found in plants [1,2]. There are great differences in both chemical structures and biological activity within this group [3,4].

Many of them have been accepted worldwide as medicines inhibiting permeability of capillaries, improving peripheral circulation, and having anti-inflammatory, antiatherogenic, antispasmodic, antiallergic and antihepatotoxic activity [5,6]. They display a wide spectrum of pharmacological activities of flavonoids, which strongly depend on the chemical structures. Especially important are hydroxyl groups—their presence and location [7,8]. Biotransformations of flavonoids by means of microorganisms that lead to modification of the substrate structure may be the source of valuable information about their metabolism in mammals [9–11].

Biotransformations of flavonoids by fungi have already been investigated. Ibrahim and Abul-Hajj reported the results of microbial transformation of flavanone by *Aspergillus niger*, among other microorganisms. Two hydroxylated dihydrochalcones, the product of dehydrogenation at C-2 and C-3 and the product of dehydrogenation at C-2, C-3 along with hydroxylation at C-3 were obtained [12]. In this paper, we wish to extend these studies by transforming flavanone (**1**) and 6-hydroxyflavanone (**2**) with various *A. niger* strains.

2. Materials and methods

2.1. Analysis

The course of microbial transformation was monitored by TLC (SiO_2, DC, Alufolien Kieselgel 60 F_{254}, Merck). Chromatograms were developed using the following developing systems—hexane:ethyl acetate 7:3, dichloromethane:ethyl acetate 1:1, toluene:diethyl ether 4:1. Column chromatography (SiO_2, Kieselgel 60, 230–400 mesh, 40–63 μm, Merck, Darmstadt) was performed using the same eluents.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 300 spectrometer. IR spectra were determined on a Specord M-80 infrared spectrophotometer (Carl Zeiss, Jena, Germany). Optical rotations were measured on an Autopol IV automatic polarymeter (Rudolph). HPLC analysis was performed on a Waters 2690 instrument with a Waters 996 Photodiode Array Detector, equipped with a ODS 2 column (4.6 mm × 250 mm, Waters) and a Guard-Pak Inserts μBondapak C18. precolumn.

* Corresponding author. Fax: +48 71 3284124.
E-mail address: kostrzew@ozi.ar.wroc.pl (E. Kostrzewa-Susłow).

1381-1177/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2006.01.020

Separation conditions: gradient elution using 80% of acetonitrile in 4.5% formic acid solution (eluent A) and 4.5% formic acid (eluent B); flow 1 ml/min; detection at 280 nm.

Crystallographic measurements were performed at 100 K using an Oxford Cryosystem device on a Kuma KM4CCD κ-axis diffractometer with a graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The data were corrected for Lorentz and polarization effects. No absorption correction was applied. Data reduction and analysis were carried out with the CrysAlis CCD and CrysAlis Red programs [13]. Structures were solved by direct methods (program SHELXS97) and refined by the full matrix least-squares method on all F^2 data using the SHELXL97 programs [14]. Non-hydrogen atoms were refined with anisotropic displacement parameters; hydrogen atoms were placed in calculated positions or found in $\Delta\rho$ maps. Before the last cycle of refinement all H atoms were fixed and were allowed to ride on their parent atoms. The Friedel pairs were merged before the final refinement.

Crystallographic data for crystal **5** in this paper, have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 275151. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

2.2. Materials

Racemic substrates for biotransformation – flavanone (**1**) and 6-hydroxyflavanone (**2**) – were purchased from Aldrich.

Peptobac used for the preparation of the growing media was bought in BTL (Poland), glucose was purchased from Chempur (Poland).

2.3. Microorganisms

For our research we used the wild strain of *A. niger KB* and three UV mutants of *A. niger* (13/5, *IBR* 6/2, *SBP*). The *KB* strain comes from culture collection of the Department of Biotechnology and Food Microbiology Agricultural University of Wrocław (Poland), the 13/5 strain was obtained from the Agricultural University of Lublin (Poland) and the strains *IBR* 6/2 and *SBP* come from Wrocław University of Economics (Poland).

Microorganisms were maintained on potato slants at 5 °C.

3. Biotransformations

3.1. Screening procedure

Cultivation media consisted of 3% glucose and 1% peptobac in water. The microorganisms were transferred from the slants to 250 ml Erlenmayer flasks, each containing 100 ml of the medium. Pre-incubation was performed at 25 °C for 24–48 h, until the proper growth of the microorganisms was achieved. Then portions of 1 ml of the culture solution were transferred to inoculate 250 ml flasks, each containing 100 ml of the medium. After cultivation at 25 °C for 48 h on a rotary shaker, 10 mg of a substrate, dissolved in 0.5 ml of acetone, was added to the grown culture. Control cultivation with no substrate was also performed. After 3, 6 and 12 days of incubation under the above conditions, portions of 5 ml of the transformation mixture were taken out and extracted with ethyl acetate (3 × 3 ml). The extracts were dried over $MgSO_4$, concentrated in vacuo and analyzed by TLC. Quantitative analysis of the mixtures was performed by means of HPLC, using calibration curve with an internal standard.

3.2. Preparative biotransformation

Portions of 1 ml of the pre-incubation culture solution were used to inoculate three 2000 ml flasks, each containing 500 ml of the cultivation medium. The cultures were incubated at 25 °C for 48 h on a rotary shaker. Then, 50 mg of a substrate dissolved in 2.5 ml of acetone was added to each flask (100 mg of the substrate per 1l of the cultivation mixture). After 12 days of incubation the mixtures were extracted with ethyl acetate (3 × 200 ml), dried ($MgSO_4$) and concentrated in vacuo. The transformation products were separated by column chromatography. Pure products were identified by means of spectral analyses (TLC, ^{1}H NMR, ^{13}H NMR, IR) and optical rotation measurements.

Physical and spectral data of the products obtained are presented below.

3.2.1. Flavone (*3*)

Melting point 96–97 °C, lit. mp 95–96 °C [12]; ^{1}H NMR see Table 1; ^{13}C NMR see Table 3; IR (KBr, ν_{max}, cm^{-1}): 1646, 1569, 1493, 1375, 1130, 768.

3.2.2. 3-Hydroxyflavone (*4*)

Melting point 170 °C, lit. mp 169–170 °C [12,15]; ^{1}H NMR see Table 1; ^{13}C NMR see Table 3; IR (KBr, ν_{max}, cm^{-1}): 3214, 1630, 1562, 1353, 1130, 759.

3.2.3. Flavan-4-ol (*5*)

Melting point 99 °C, lit. mp 98–99 °C [15]; $[\alpha]_{546}^{23} = 0$, ($c = 1$, CH_3OH); ^{1}H NMR see Table 1; ^{13}C NMR see Table 3; IR (KBr, ν_{max}, cm^{-1}): 3301, 1650, 1607, 1344, 1064, 758, 702.

Crystal data for (**5**): $C_{15}H_{14}O_2$, $M_w = 226.26$, colourless needle, crystal size 0.20 mm × 0.05 mm × 0.05 mm, monoclinic, space group Cc, $a = 30.176(4)$, $b = 8.7016(11)$, $c = 9.0773(13)$ Å, $\beta = 98.725(11)$, $V = 2355.9(5)$ Å^3, $Z = 8$, $D_c = 1.276$ Mg m^{-3}, $T = 100(2)$ K, $R = 0.052$, wR = 0.063 (for 1421 with $I > 2\sigma(I)$) for 307 variables. CCDC No. 275151.

3.2.4. 7-Hydroxyflavan-4-ol (*6*)

Oily liquid; $[\alpha]_{546}^{23} = 0$, ($c = 1$, CH_3OH); ^{1}H NMR see Table 1; ^{13}C NMR see Table 3; IR (CH_2Cl_2, ν_{max}, cm^{-1}): 3220, 1621, 1598.

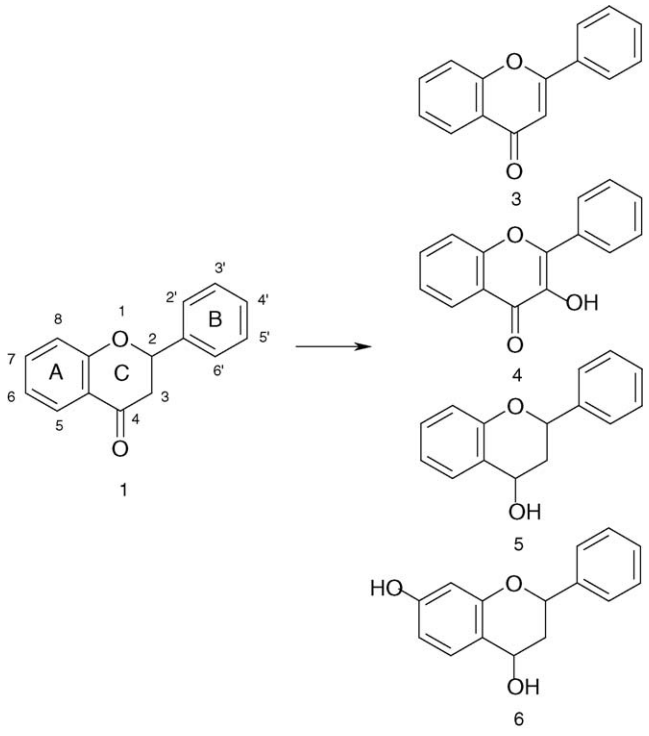
3.2.5. 6-Hydroxyflavan-4-ol (*7*)

Melting point 259–260 °C; $[\alpha]_{546}^{23} = 0$, ($c = 1$, CH_3OH); ^{1}H NMR see Table 2; ^{13}C NMR see Table 3; IR (KBr, ν_{max}, cm^{-1}): 3250, 1635, 1600, 1404, 1352, 752.

Table 1
^{1}H NMR chemical shifts (δ) of compounds **1**, **3**, **4**, **5**, **6**

Proton	Compound					
	1[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]
H-2	5.50(dd) (J = 13.2; 3.0)			5.17(dd) ($J_{2,3ax}$ = 11.6, $J_{2,3eq}$ = 1.8)	5.15(dd) ($J_{2,3ax}$ = 11.4, $J_{2,3eq}$ = 1.8)
H-3	H-3$_{ax}$ 3.11(dd) (J = 16.9; 13.2)	6.82(s)		H-3$_{ax}$ 2.13(ddd) ($J_{3ax,3eq}$ = 13.1, $J_{3ax,2}$ = 11.6, $J_{3ax,4}$ = 10.6)	H-3$_{ax}$ 2.09(ddd) ($J_{3ax,3eq}$ = 13.2, $J_{3ax,2}$ = 11.1, $J_{3ax,4}$ = 9.8)
	H-3$_{eq}$ 2.91(dd) (J = 16.9; 3.0)			H-3$_{eq}$ 2.51(ddd) ($J_{3eq,3ax}$ = 13.2, $J_{3eq,4}$ = 6.3, $J_{3eq,2}$ = 2.0)	H-3$_{eq}$ 2.49(ddd) ($J_{3eq,3ax}$ = 13.2, $J_{3eq,4}$ = 6.2, $J_{3eq,2}$ = 2.0)
H-4				5.08 (broad m)	5.0(dd) ($J_{4,3ax}$ = 9.7, $J_{4,3eq}$ = 6.2)
H-5	7.95(d) (J = 8.1)	8.22(dd) (J = 1.7; 8.0)	8.24(d) (J = 9.8)	7.51(d) (J = 8.1)	
H-6	7.47(m)	7.41(t)	7.47(m)	7.2(t)	6.47(dd) ($J_{6,5}$ = 8.4, $J_{6,8}$ = 2.4)
H-7	7.47(m)	7.69(t)	7.64(t)	6.98(t)	
H-8	7.47(m)	7.53(m)	7.47(m)	6.88(d) (J = 8.2)	6.36(d) ($J_{8,6}$ = 2.4)
H-2′	7.07(m)	7.92(m)	8.24(d) (J = 9.8)	7.39(m)	7.30–7.43(m)
H-3′	7.47(m)	7.53(m)	7.47(m)	7.39(m)	7.30–7.43(m)
H-4′	7.47(m)	7.53(m)	7.47(m)	7.39(m)	7.30–7.43(m)
H-5′	7.47(m)	7.53(m)	7.47(m)	7.39(m)	7.30–7.43(m)
H-6′	7.07(m)	7.92(m)	8.24(d) (J = 9.8)	7.39(m)	7.30–7.43(m)

[a] *Solvent*: $CDCl_3$.



Scheme 1.

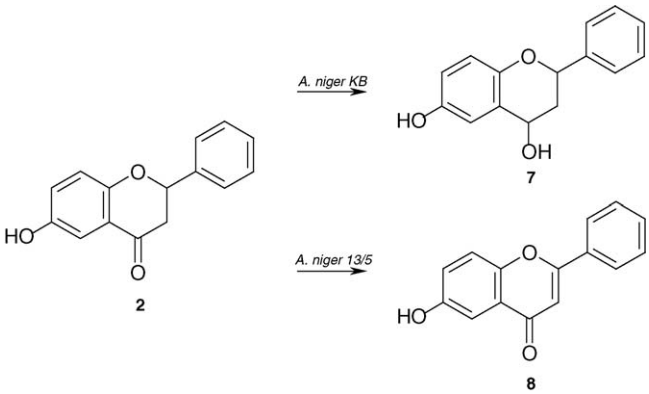
3.2.6. 6-Hydroxyflavone (**8**)

Melting point 231–232 °C, lit. mp 231–232 °C [16]; ^{1}H NMR see Table 2; ^{13}C NMR see Table 3; IR (KBr, ν_{max}, cm^{-1}): 3476, 1627, 1404, 773.

4. Results and discussion

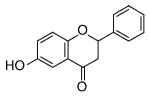
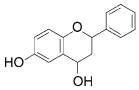
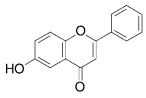
Biotransformations of flavanone (**1**) Scheme 1 and 6-hydroxyflavanone (**2**) Scheme 2 were performed using four strains of *Aspergillus niger*, three of which were obtained as a result of UV mutations.

Transformation of both substrates – flavanone (**1**) and 6-hydroxyflavanone (**2**) – by *A. niger KB* culture led to reduction of the C-4 carbonyl groups. No other biotransformation products were isolated. The products obtained – flavan-4-ol (**5**) and 6-hydroxyflavan-4-ol (**7**) – were racemic compounds. Screening



Scheme 2.

Table 2
^{1}H NMR chemical shifts (δ) of compounds **2**, **7**, **8**

Proton	Compound		
	 **2**[a]	 **7**[b]	 **8**[c]
H-2	5.51(dd) (J = 2.8; 13.0)	5.09(d) (J = 11.9)	
H-3	H-3$_{ax}$ 3.13(dd) (J = 13.0; 16.9) H-3$_{eq}$ 2.75(dd) (J = 16.9; 2.9)	H-3$_{ax}$ 1.98(q) H-3$_{eq}$ 2.35(ddd) ($J_{3eq,3ax}$ = 12.9, $J_{3eq,4}$ = 6.3, $J_{3eq,2}$ = 1.6)	6.92(s)
H-4		4.98(dd) ($J_{4,3ax}$ = 10.8, $J_{4,3eq}$ = 6.4)	
H-5	7.09(d) (J = 2.9)	6.93(d) (J = 2.5)	7.30(d) (J = 2.9)
H-7	7.00(dd) (J = 8.8; 3.0)	6.62(m)	7.22(dd) (J = 9.0; 3.0)
H-8	6.92(d) (J = 8.8)	6.62(m)	7.61(d) (J = 9.0)
H-2′	7.49(d)	7.35(m)	8.03(m)
H-3′	7.36(m)	7.35(m)	7.54(m)
H-4′	7.36(m)	7.35(m)	7.54(m)
H-5′	7.36(m)	7.35(m)	7.54(m)
H-6′	7.49(d)	7.35(m)	8.03(m)

[a] *Solvent*: $CDCl_3$.
[b] *Solvent*: DMSO-d_6.
[c] *Solvent*: CD_3OD.

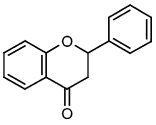
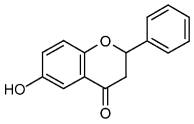
tests showed that the amount of flavan-4-ol (**5**) was constantly increasing in time. After 12 days of incubation flavan-4-ol (**5**) was isolated in 61% yield (91.5 mg per 150 mg of the substrate), and the substrate was fully consumed (Table 4). The product was identified by means of ^{1}H NMR and ^{13}C NMR. In the ^{1}H NMR spectrum there is a broad signal of 4-H at δ = 5.08 ppm, which indicates reduction of the carbonyl group. In the ^{13}C NMR spectra the C-4 signal of flavanone (**1**) at 191.9 ppm was replaced by the signal at 65.9 ppm in the spectrum of product **5**. The final confirmation of a hydroxyl group presence is the absorption band at 3301 cm^{-1} in the IR spectrum. X-ray analysis of product **5** proved the structure of 2,4-*cis*-flavan-4-ol as a racemic mixture of isomers (2*R*, 4*R*) and (2*S*, 4*S*) (Fig. 1). Two crystallographically unrelated enantiomers of **5**, presented in Fig. 1a and b, indicate pseudo-equatorial orientation of the phenyl groups and pseudo-axial position of 2-H atoms. This is confirmed by the torsion angles values: H-2–C-2–C-3–H3A (67.11), H-2–C-2–C-3–H3B (−174.21), H2A–C2A–C3A–H3D (−67.80) and H2A–C2A–C3A–H3C (73.04) (Fig. 1), which are consistent with the respective coupling constants: J_{2-3eq} = 1.83 Hz and J_{2-3ax} = 11.62 Hz (Table 5). In both molecules 4-OH groups are pseudo-equatorial, whereas 4-H are pseudo-axial. This is proved by the coupling constants: J_{4-3eq} = 6.27 Hz and J_{4-3ax} = 10.59 Hz, and also by the torsion angles values: H-4–C-4–C-3–H3A (−46.61), H-4–C-4–C-3–H3B (−165.28), H4A–C4A–C3A–H3D (47.18) and

Table 3
^{13}C NMR chemical shifts (δ) of compounds **1**–**8**

Carbon	Compound							
	1[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[c]	**8**[b]
C-2	79.6	79.2	163.4	144.9	77.0	77.0	78.2	162.8
C-3	44.7	44.2	115.5	138.4	40.1	40.3	41.4	108.1
C-4	191.9	192.2	178.5	173.4	65.9	65.7	66.5	177.6
C-5	127.1	110.4	107.4	125.4	128.7	128.3	109.6	106.5
C-6	121.6	152.1	119.8	124.5	121.0	108.8	149.2	150.0
C-7	136.2	124.9	125.2	133.6	130.0	156.3	122.9	124.9
C-8	118.2	119.5	118.0	118.2	117.5	103.0	114.1	120.4
C-9	161.6	154.9	156.2	155.4	154.5	155.4	165.7	155.5
C-10	121.0	121.4	123.8	120.6	126.1	118.7	117.9	123.7
C-1′	138.8	139.7	131.6	131.0	140.6	140.4	138.0	132.0
C-2′	126.2	127.0	126.2	127.7	126.2	126.0	127.1	126.8
C-3′	128.9	129.0	128.9	128.6	128.2	128.7	130.2	129.7
C-4′	128.8	128.9	133.8	130.2	127.0	128.3	128.9	132.2
C-5′	128.9	129.0	129.4	128.6	128.2	128.7	130.2	129.7
C-6′	126.2	127.0	126.2	127.7	126.2	126.0	127.1	126.8

[a] *Solvent*: $CDCl_3$.
[b] *Solvent*: DMSO-d_6.
[c] *Solvent*: CD_3OD.

Table 4
Yield of biotransformation products determined by HPLC (with an internal standard)

Substrate	Microorganism	Time of incubation (days)	Biotransformation products (%)						Unreacted substrate (%)
			3	**4**	**5**	**6**	**7**	**8**	
 Flavanone	*Aspergillus niger KB*	3	–	–	31	–	–	–	51
		6	–	–	42	–	–	–	22
		12	–	–	64	–	–	–	0
	Aspergillus niger 13/5	3	14	–	0	0	–	–	64
		6	31	–	22	6	–	–	12
		12	33	–	15	25	–	–	0
	Aspergillus niger IBR 6/2	3	–	39	–	–	–	–	53
		6	–	73	–	–	–	–	9
		12	–	75	–	–	–	–	0
	Aspergillus niger SBP	3	12	0	7	0	–	–	62
		6	26	0	34	11	–	–	13
		12	25	9	27	13	–	–	0
 6-Hydroxyflavanone	*Aspergillus niger KB*	3	–	–	–	–	0	–	97
		6	–	–	–	–	13	–	70
		12	–	–	–	–	59	–	11
	Aspergillus niger 13/5	3	–	–	–	–	–	5	91
		6	–	–	–	–	–	32	59
		12	–	–	–	–	–	71	0
	Aspergillus niger IBR 6/2	12	–	–	–	–	–	–	100
	Aspergillus niger SBP	12	–	–	–	–	–	–	100

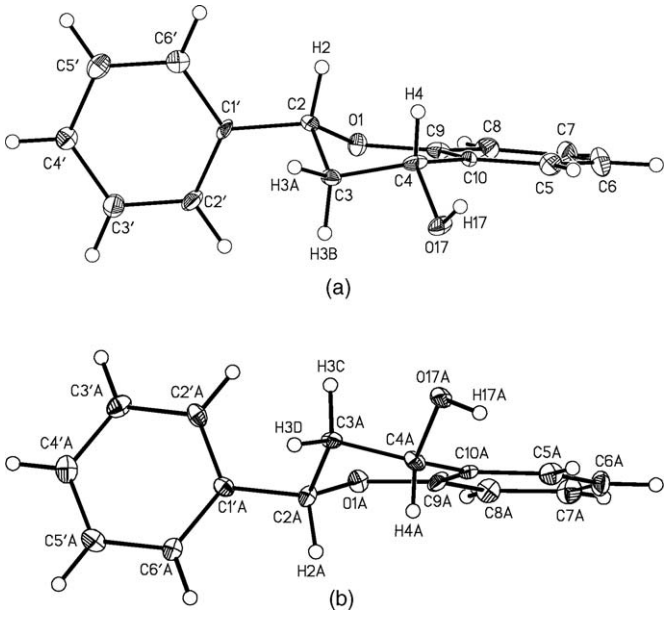


Fig. 1. Two crystallographically independent isomers of flavan-4-ol (**5**); (a) (2*R*,4*R*) and (b) (2*S*,4*S*).

H4A–C4A–C3A–H3C (166.34). Flavan-4-ol (**5**), which is the product of flavanone (**1**) reduction, was also identified as one of the products of the biotransformation by means of *A. niger* 13/5 and *A. niger SBP*, however in much smaller yields (Table 4).

Transformation of 6-hydroxyflavanone (**2**) by means of *A. niger KB* (Scheme 2) proceeds in a similar way to flavanone (**1**). After 12 days of the biotransformation the only product observed was 6-hydroxyflavan-4-ol (**7**), isolated in 57% yield (85.5 mg) along with 12% of the unreacted starting material. In the ^{1}H NMR spectrum reduction of the carbonyl group is visible by the presence of a new, characteristic signal—doublet of doublets at $\delta = 4.98$ ppm, which is ascribed to 4-H. In the ^{13}C NMR the chemical shift of C-4 moved from 192.2 ppm in the spectrum of substrate **2** to 66.5 ppm in the spectrum of 6-hydroxyflavan-4-ol (**7**). The stereochemistry of product **7** was established by means of NMR analysis. Comparison of the chemical shifts of 2-H, 4-H, 3-H_{ax} and 3-H_{eq} and the respective coupling constants in the spectra of flavan-4-ol (**5**) and compound **7** (Table 5) confirms the structure of 2,4-*cis*-6-hydroxyflavan-4-ol as the product, formed in a stereospecific reduction of the carbonyl group.

Transformation of 6-hydroxyflavanone (**2**) in the culture of *A. niger* 13/5 gave 6-hydroxyflavone (**8**), isolated in high yield (114 mg, 76%). The substrate was fully consumed. The

Table 5
Selected ^{1}H NMR data of flavan-4-ol (**5**), 7-hydroxyflavan-4-ol (**6**) and 6-hydroxyflavan-4-ol (**7**)

	δ 2-H	δ 4-H	δ 3_{ax}-H	δ 3_{eq}-H	$J_{3ax–3eq}$	$J_{2–3ax}$	$J_{2–3eq}$	$J_{4–3ax}$	$J_{4–3eq}$
(**5**)	5.17	5.08	2.13	2.51	13.06	11.62	1.83	10.59	6.27
(**6**)	5.15	5.00	2.09	2.49	13.19	11.39	1.75	9.72	6.22
(**7**)	5.09	4.98	1.98	2.35	12.90	11.86	1.90	10.77	6.36

structure of 6-hydroxyflavone (**8**), which was the only product of the transformation of 6-hydroxyflavanone (**2**), was confirmed by ^{1}H NMR. In the spectrum of **2** the signal of 2-H appears at $\delta = 5.51$ ppm as a doublet of doublets (coupling constants $J = 2.79$ and 12.95 Hz), the signal of 3-H_{ax} is found at $\delta = 3.13$ ppm (dd, $J = 12.98$ and 16.86 Hz) and 3-H_{eq} at $\delta = 2.75$ ppm (dd, $J = 16.87$ and 2.93 Hz). There are no such signals in the ^{1}H NMR of 6-hydroxyflavone (**8**). Instead, there is a singlet of one proton at $\delta = 6.92$ ppm, which has been ascribed to 3-H. These unequivocally indicate the presence of a double bond between C-2 and C-3 in the C ring of **8**.

Transformations of flavanone (**1**) by means of *A. niger* 13/5 led to three products. The major one was flavone (**3**), which was formed independently, and there were also flavan-4-ol (**5**) and 7-hydroxyflavan-4-ol (**6**), the contents of which were correlated to each other. It was observed that in the 12th day of the biotransformation a significant increase in the amount of 7-hydroxyflavan-4-ol (**6**) occurred, along with a drop in the flavan-4-ol (**5**) yield. These indicate that reduction of the carbonyl group occurs prior to hydroxylation at C-7 (Table 4).

In the ^{1}H NMR spectrum of flavone (**3**), which is the product of dehydrogenation of **1**, there is no signal at $\delta = 5.5$ ppm, analogous to 2-H in the spectrum of **1** (dd, $J = 13.18$ and 2.95 Hz). Also, there is a signal of a single proton at C-3, observed at $\delta = 6.82$ ppm. These changes in the spectra suggest the presence of a double bond between C-2 and C-3 in the molecule of flavone (**3**).

In the ^{1}H NMR spectrum of 7-hydroxyflavan-4-ol (**6**) there is a doublet of doublets at $\delta = 5.0$ ppm ($J_{4,3ax} = 9.72$ Hz, $J_{4,3eq} = 6.22$ Hz). In the ^{13}C NMR spectrum the signal of C-4 moved from $\delta = 191.9$ ppm for flavanone (**1**) to $\delta = 65.7$ ppm for 7-hydroxyflavan-4-ol (**6**), whereas the signal of C-7 was shifted from $\delta = 136.2$ to 156.3 ppm.

7-Hydroxyflavan-4-ol (**6**), similarly to 6-hydroxyflavan-4-ol (**7**) and flavan-4-ol (**5**), was racemic compound. Being an oily liquid, compound **6** was not suitable for X-ray analysis and the assignment of the structure was based on the spectral analysis. Comparison of the chemical shifts and coupling constants of 2-H, 4-H, 3-H_{ax} and 3-H_{eq} with the respective data in the spectra of flavan-4-ol (**5**) and 6-hydroxyflavan-4-ol (**7**) (Table 5) suggest a 2,4-*cis* configuration of product **6**.

Transformations of flavanone (**1**) may proceed in many different ways, leading to diversity of products. A good example of that is biotransformation of **1** by *A. niger SBP* (Scheme 1), which gave four products: flavone (**3**), 3-hydroxyflavone (**4**), flavan-4-ol (**5**) and 7-hydroxyflavan-4-ol (**6**).

The course of the reaction was monitored by TLC and HPLC. When the transformation was carried out for longer time the ratio of products changed (Table 4). The amount of 3-hydroxyflavone (**4**) and 7-hydroxyflavan-4-ol (**6**) significantly increased, whereas the yields of flavone (**3**) and flavan-4-ol (**5**) dropped. These suggest that the dehydrogenation at C-2, C-3 occurs prior to the hydroxylation at C-3, and that the reduction of the carbonyl group is followed by the hydroxylation at C-7 (Table 4). 6-Hydroxyflavanone (**2**) was not transformed by the strain *A. niger SBP*.

Transformation of flavanone (**1**) by *A. niger IBR* gave 3-hydroxyflavone (**4**) as a single product in 72% yield (108 mg). Analysis of the spectral data (^{1}H NMR, ^{13}H NMR, IR) led to the conclusion that the dehydrogenation of C-2 and C-3 took place along with the hydroxylation at C-3.

In the ^{1}H NMR spectrum of 3-hydroxyflavone (**4**) there are no signals at $\delta = 2.91$ and 3.11 ppm, which are observed in the spectrum of flavanone (**1**) due to 3-H_{eq} and 3-H_{ax} presence. Also, the signal of 2-H at $\delta = 5.50$ ppm (dd) disappeared. Instead, new singlet at $\delta = 7.02$ ppm appears, which comes from the OH group of 3-hydroxyflavone (**4**). The IR absorption band at 3214 cm^{-1} also confirms the presence of a hydroxyl group. In the ^{13}C NMR spectra the signal of C-3 moves from $\delta = 44.7$ ppm for flavanone (**1**) to $\delta = 138.4$ ppm for 3-hydroxyflavone (**4**).

The strain *A. niger IBR* did not promote transformation of 6-hydroxyflavanone (**2**).

5. Conclusions

1. Molds belonging to *A. niger* species are effective biocatalysts for transformations of flavanone, which is proved by the diversity of the transformation products.
2. Stereospecific reduction of the carbonyl groups of flavanone and 6-hydroxyflavanone by means of *A. niger* strain was observed. The racemic products were isolated in good yields.
3. Transformation of 6-hydroxyflavanone using enzymatic system of *A. niger* 13/5 resulted in the selective dehydrogenation at C-2 and C-3, leading to 6-hydroxyflavone (**8**) as a single product.
4. In the case of flavanone (**1**) the strain *A. niger* 6/2 promoted dehydrogenation at C-2 and C-3 along with simultaneous hydroxylation at C-3.
5. The hydroxyl group of 6-hydroxyflavanone (**2**) probably hinders the entrance of **2** in the active centers of *A. niger IBR* 6/2 and *A. niger SBP* enzymes.

References

[1] E. Wollenweber, V.H. Dietz, Phytochemistry 20 (1981) 869.
[2] E. Middleton, Trends Pharmacol. Sci. 5 (1984) 335.
[3] B. Havesteen, Biochem. Pharm. 32 (1983) 1141.
[4] M.E. Wall, M.C. Wani, G. Manikumar, P. Abraham, H. Taylor, T.J. Hughes, J. Warner, R. McGivney, J. Nat. Prod. 51 (1988) 1084.
[5] G. Di Carlo, N. Mascolo, A. Izzo, F. Capasso, Life Sci. 65 (1999) 337.
[6] T.N. Kaul, E. Middleton Jr., P.L. Ogra, J. Med. Virol. 15 (1985) 71.
[7] Z.Y. Chen, P.T. Chan, K.Y. Ho, K.P. Fung, J. Wang, Chem. Phys. Lipids 79 (1996) 157.
[8] R.A. Larson, Phytochemistry 27 (1988) 969.
[9] Y. Okuno, M. Miyazawa, J. Nat. Prod. 67 (2004) 1876.
[10] G.T. Maatooq, J.P.N. Rosazza, Phytochemistry 66 (2005) 1007.
[11] Y. Hyo-Kon Chun, K. Ohnishi, N. Shindo, K. Misawa, S. Furukawa, Horinouchi, J. Mol. Catal. B: Enzym. 21 (2003) 113.
[12] A.-R. Ibrahim, Y.J. Abul-Hajj, J. Nat. Prod. 53 (1990) 644.
[13] Oxford Diffraction, Poland Sp. z.o.o., CrysAlis CCD and CrysAlis Red, V166, 2001.
[14] Bruker AXS, SHELXTL V5.1, 1999.
[15] S.R. Udupa, M.S. Chadha, Indian J. Biochem. Biophys. 18 (1981) 142.
[16] A.-R. Ibrahim, Y.J. Abul-Hajj, J. Nat. Prod. 53 (1990) 1471.