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Microbial models of animal drug metabolism Part 5. Microbial preparation of human hydroxylated metabolites of irbesartan☆

Vanessa Alexandre, Sophie Ladril, Michèle Maurs, Robert Azerad*

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR 8601, Université René Descartes-Paris V, 45 Rue des Saints-Pères, 75006 Paris, France

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Abstract

An exhaustive screening of fungal and microbial strains allowed to select the best microorganisms to produce in high yields some of the animal metabolites of an antihypertensive drug, irbesartan, in order to elucidate their structural and stereochemical characteristics. Among the microorganisms tested, bacteria, and especially *Streptomyces* strains, were the most active producers of the main metabolites which included diastereomeric derivatives hydroxylated in the spirocyclopentane ring, open rearranged hydroxylated derivatives, and a putative *N*-glycosidic conjugate of irbesartan.
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Keywords: Bacteria; Drug metabolism; Fungi; Glucoside; Hydroxylation; Whole-cell biocatalysis

1. Introduction

A family of non-peptide orally active angiotensin II receptor antagonists, based on a 5-(biphenyl)-2-tetrazolyl pattern, have been recently and successfully developed for the treatment of hypertension. The human metabolism of this drug family, as illustrated with Losartan [1,2], GR 117289 [3] and various other derivatives [4–6] mainly involves the *N*-glucuronidation of one of the tetrazole nitrogens and/or the C-hydroxylation of alkyl substituents. A systematic evaluation of these metabolic products, undertaken by the Merck group [7–15], using selected microorganisms for their preparation [16], has shown similar or increased antihypertensive activities.

Irbesartan (SR 47436, BMS 186295) (Scheme 1), a highly selective and potent drug of the same family, recently introduced in the market, is metabolized in animals and humans to give at least eight urinary metabolites [17–19]: (1) a tetrazole N^2-β-glucuronide conjugate, (2) a monohydroxylated metabolite at the ω-1 (C-3) position of the *n*-butyl side chain, and the corresponding oxidized keto derivative, (3) a carboxylic acid resulting from the oxidation of the terminal methyl group of the side chain, (4) two different monohydroxylated metabolites resulting from the oxidation of the spirocyclopentane ring, and (5) two additional metabolites combining the previous oxidations in two positions. However, probably because the metabolic products of irbesartan do not seem to play a significant role in the activity of this drug [18,20], the stereochemical features of the metabolites have not been fully elucidated, particularly those concerning the products hydroxylated in the spirocyclopentane ring: indeed, symmetrical positions (2b and 3b, see Scheme 2) on the cyclopentane ring are enantiotopic, due to a symmetry plane involving the substituted spiro-heterocyclic ring. Hydroxylation on each of these positions, on either face, should generate enantiomeric pairs of *cis*- or *trans*-hydroxylated derivatives.[1]

☆ Previous paper in this series: I. Lacroix, J. Biton, R. Azerad, Microbial models of mammalian metabolism. Microbial transformations of Trimegestone® (RU 27987), a 3-keto-$\Delta^{4,9(10)}$-norsteroid drug, Bioorg. Med. Chem. 7 (1999) 2329–2341.

* Corresponding author. Tel.: +33-1-42-86-21-71; fax: +33-1-42-86-83-87.
E-mail address: robert.azerad@univ-paris5.fr (R. Azerad).

[1] The *cis*-diastereomers have been defined as presenting the hydroxyl group on the cyclopentane face substituted by the spiro-nitrogen substituent. The *trans*-diastereomers present the hydroxyl group on the face substituted by the carbonyl group.

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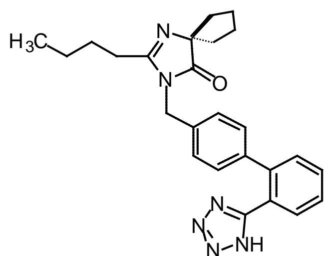


Scheme 1.

In addition, an open form of irbesartan, SR 49498, resulting from the hydrolytic cleavage of the imidazolone ring, was detected as a minor product [17] but was considered as an impurity in the radiolabeled administered irbesartan, apparently formed during storage at low temperature.

This report describes the biotransformation of irbesartan by selected microbial species in order to prepare some of its metabolites in sufficient amounts to complete the determination of their structural and stereochemical characteristics.

2. Experimental

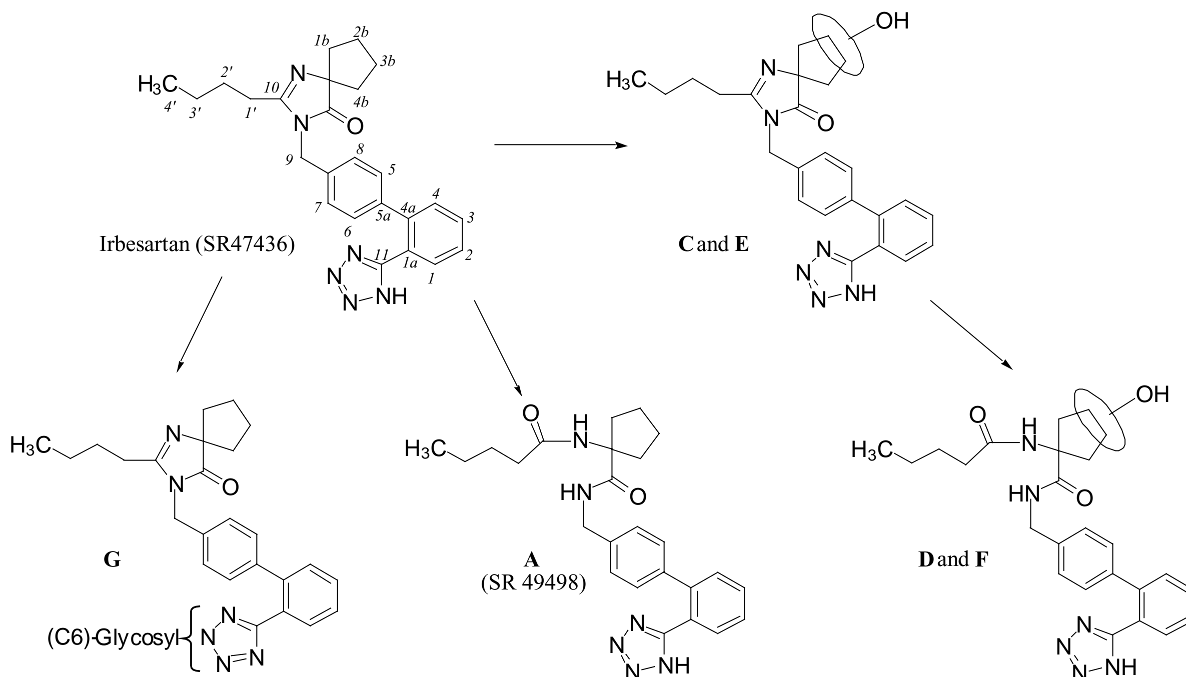
2.1. General

^1H and ^{13}C NMR (1D and 2D) spectra were performed in $CDCl_3$, 21 °C, at 500.13 and 125.77 MHz respectively on a Bruker AMX500-2 instrument, using standard pulse sequences. Positive-ion FAB high resolution mass spectrometric analyses were performed on a Jeol MS-700 Instrument using calibration with Ultramark 2005, xenon gas and a Magic Bullet matrix (1,4-dithioerythritol/1,4-dithio-L-threitol, 1:4) at the Mass Spectrometry Service of Ecole Normale Supérieure, Paris. Positive-ion ESI-LC/MS analyses were run on a LCQ Advantage Finnigan Instrument at 270 °C and 4.5 kV, using the reverse-phase chromatographic method described under chromatographic procedures, and substituting 0.5% trifluoroacetic acid by 1% formic acid. Irbesartan was kindly donated by Dr. Bertrand Castro (SANOFI-Synthelabo, France). All other chemicals were obtained from commercial sources and used without further purification.

2.2. Microorganisms

All fungal cultures were maintained on agar slants containing (l^{-1}), yeast extract (Difco, 5 g), malt extract (Difco, 5 g), glucose (20 g) and Bacto-agar (Difco, 20 g), stored at 4 °C and subcultured at 26 °C before use. Fungi were purchased from the American Type Culture Collection (ATCC strains), Rockville, MD, USA, the Northern Regional Research Laboratories (NRRL strains), Peoria, IL, USA, the Centralbureau voor Schimmelcultures (CBS strains), Baarn, The Netherlands, or the Laboratoire de Cryptogamie of the Museum d'Histoire Naturelle (LCP strains), Paris, France.

Bacterial strains were purchased from the same collections, the Deutsche Sammlung von Mikroorganismen und Zellkulture (DSM strains), Braunschweig, Germany, the National Collection of Industrial and Marine Bacteria (NCIMB strains) Aberdeen, Scotland, the Belgian Coordinated Collection of Microorganisms (LMG strains),



Scheme 2.

Louvain-la-Neuve, Belgium, and the Institut Pasteur Collection (CIP strains), Paris, France. They were maintained on agar slants containing (l^{-1}), ISP medium 2 (Difco, 38 g) for Streptomycetes or Nutrient Agar (Difco, 23 g) for other bacteria. Agar slants were stored at 4 °C and subcultured at 26 °C before use.

2.3. Chromatographic procedures

Analytical reverse-phase HPLC was carried out using the following conditions: column, Nucleosil 5C18 (250 mm × 4.6 mm); solvent, water–MeCN–TFA (from 950:50:5 to 400:600:5) at 1 ml/min (total gradient time: 20 min), using Gilson pumps, a Gilson 231 sample injector equipped with a 20 μl loop and a Shimadzu SPD-6A LC-UV detector set at 250 nm. Calibration was performed using external standard solutions of irbesartan. TLC was carried out with the ascending method using silica gel $60F_{254}$ precoated plates (Merck, Germany) and CH_2Cl_2–EtOH–AcOH (89:10:1) as solvent. Spots were detected under UV light.

2.4. Microbial transformations

2.4.1. Culture and screening procedures

Fungi were grown at 27 °C in a liquid medium containing (l^{-1}), corn steep liquor (Roquette, France, 10 g), glucose (30 g), KH_2PO_4 (1 g), K_2HPO_4 (2 g), $NaNO_3$ (2 g), KCl (0.5 g), $MgSO_4{\cdot}7H_2O$ (0.5 g), $FeSO_4{\cdot}7H_2O$ (0.02 g). Bacterial and *Streptomyces* strains were grown at 27 °C in a liquid medium containing (l^{-1}) yeast extract (Difco, 5 g), soy peptone (Organotechnie, France, 5 g), glucose (20 g), NaCl (5 g), K_2HPO_4 (5 g) and adjusted to pH 7.0 with HCl.

For screening experiments, 250 ml conical flasks containing 100 ml (fungi) or 50 ml (bacteria) of sterile liquid medium were inoculated with a few drops of a spore suspension obtained from freshly grown agar slants, then orbitally shaken (200 rpm) at 27 °C for 60–65 h. Irbesartan was then added as an ethanol solution (2 ml) to yield a final concentration of $0.25\,g\,l^{-1}$. Samples (1–2 ml) were aseptically withdrawn every day, centrifuged and the supernatants were microfiltered (0.45 μm). Aliquots of the filtrates were analyzed by reverse-phase HPLC, and the remaining solutions were saturated with sodium chloride and extracted with ethyl acetate for TLC analysis. Transformations were continued up to 7 days or until no further increase of metabolite(s) was observed. Control experiments performed by incubating fungi or bacterial strains in the absence of substrate were currently run to exclude excreted microbial products possibly detected by HPLC.

2.4.2. General preparative biotransformation procedure

Extrapolation of screening methods to a larger number of flasks or larger volume incubations was performed for the preparation of metabolites, using the strains previously selected. The bioconversions were monitored by HPLC determination of the desired metabolites in the incubation supernatant, as described above, and stopped by filtration of the biomass, saturation of the filtrates with sodium chloride, acidification to pH 5.0 and repeated extraction with ethyl acetate. After drying with $MgSO_4$, the organic extracts were evaporated in vacuo and the oily crude products were flash-chromatographed on a silica gel column to give pure or enriched fractions. Final purifications were achieved by crystallization or multiplate thin layer chromatography.

2.4.3. Preparation of main metabolites

Ten Erlenmeyer flasks (500 ml) each containing 100 ml of a grown culture of *Streptomyces griseus* NRRL B150 were added with a solution of 25 mg of irbesartan in ethanol (2 ml) and incubated with orbital shaking (200 rpm) at 27 °C during 24 h. The incubation medium was filtered with celite, the filtrate was adjusted to pH 5, saturated with sodium chloride and extracted three times with ethyl acetate to give 361.5 mg of a crude extract which was chromatographed on a silica gel column (25 g) eluted with CH_2Cl_2–EtOH–acetic acid (95:4:1). Pure metabolite **C** (75.2 mg) was recovered as a white powder in fractions 19–27 and pure metabolite **E** (58.7 mg) in fractions 33–46; for ^1H and ^{13}C NMR, see Tables 1 and 2. Metabolite **C**, MS–ESI, *m/z*: 445.3 ($M + H^+$). MS–MS, *m/z*: 207 (biphenyltetrazole), 417 (M + H-18). HRMS-FAB, *m/z*: found 445.2348, calculated for $C_{25}H_{31}O_2N_6$: 447.2352; 467.2160; calculated for $C_{25}H_{28}O_2N_6Na$: 467.2171. Metabolite **E**, MS–ESI, *m/z*: 445.3 ($M + H^+$). MS–MS, *m/z*: 207 (biphenyltetrazole), 417 (M + H-18). HRMS-FAB, *m/z*: found 445.2347, calculated for $C_{25}H_{29}O_2N_6$: 447.2352.

Five Erlenmeyer flasks (2 l) each containing 400 ml of a grown culture of *Streptomyces griseolus* ATCC 3325 were added with a solution of 100 mg of irbesartan in ethanol (8 ml) and incubated with orbital shaking (200 rpm) at 27 °C during 48 h. After centrifugation and three extractions of the supernatant with EtOAc, the crude extract (864 mg) was chromatographed on a silica gel column (40 g). The separated fractions were further purified by TLC to give metabolites **C** (88.9 mg) and **E** (58.8 mg). A similar incubation with *S. griseolus* during 96 h gave after purification metabolites **C** (77.3 mg), **E** (94.7 mg), **D** (33.5 mg), **F** (30 mg) and **G** (2.5 mg). Metabolite **D**, MS–ESI, *m/z*: 463 ($M + H^+$). MS–MS, *m/z*: 445 (M + H-18), 252, 235, 212. Metabolite **F**, MS–ESI, *m/z*: 463 ($M + H^+$). MS–MS, *m/z*: 445 (M+H-18), 252, 235, 212. Metabolite **G**, MS–ESI, *m/z*: 591 ($M + H^+$). MS–MS, *m/z*: 563 (M+H-18), 429 (M+H-162), 369 (207 + 162).

Pooled supernatants (4 × 100 ml) of *Rhodococcus rhodochrous* DSM 43198, *R. erythropolis* DSM 743, *R. erythropolis* DSM 43066, and *R. erythropolis* ATCC 4277 incubated during 194 h with 25 mg each of irbesartan were acidified to pH 5 and extracted three times with ethyl ac-

Table 1
^{1}H NMR spectral data of the main irbesartan metabolites ($CDCl_3$, δ (ppm), J (Hz))

	Irbesartan	**A**	**C**	**E**
H-1	7.92 (d, J = 7.7)	7.66 (d, J = 7.7)	7.78 (d, J = 7.6)	7.81 (d, J = 7.7)
H-3	7.58 (dd, J = 7.6)	7.48 (dd, J = 7.6)	7.54 (dd, J = 7.5)	7.56 (dd, J = 7.6)
H-2	7.53 (dd, J = 7.6)	7.39 (dd, J = 7.6)	7.45 (dd, J = 7.5)	7.48 (dd, J = 7.6)
H-4	7.43 (d, J = 7.7)	7.35 (d, J = 7.7)	7.38 (d, J = 7.6)	7.41 (d, J = 7.7)
H-7, H-8, H-5 and H-6	AA′BB′ system at 7.06–7.15	AA′BB′ system at 6.94–7.07	AA′BB′ system at 7.00–7.07	AA′BB′ system at 7.06–7.11
H_2-9	4.64 (s)	4.25 (s)	AB system at 4.59–4.68	AB system at 4.66
H_2-1′	2.17 (t, J = 7.5)	2.06 (t, J = 7.5)	2.28 (t, J = 7.5)	2.30 (t, J = 7.7)
H-2′	1.47 (m)	1.44 (m)	1.52 (m)	1.52 (m)
H-3′	1.26 (m)	1.20 (m)	1.27 (m)	1.26 (m)
H-4′	0.81 (t, J = 7.4)	0.77 (t, J = 7.3)	0.80 (t, J = 7.3)	0.80 (t, J = 7.3)
CHOH (2b/3b)	–	–	4.42 (br, t, J = 4.6)	4.38 (br, s)
H-1b to 4b	1.83 (m)	2.10 (m)	2.17 (m)	2.15 (dd, J = 15, 5.4)
	1.74 (m)	1.88 (m)	2.09 (dd, J = 13.5, 5)	2.10 (dd, J = 17.5, 9.8)
	1.66 (m)	1.64 (m)	1.98 (m) and 1.90 (m)	1.90 (dd, J = 11.9, 8)
			1.76 (br, d, J = 13.5)	1.87 (t, J = 15.1)

etate. The crude extract (151.8 mg) was chromatographed on several TLC plates with CH_2Cl_2–EtOH–AcOH (92:7:1) as solvent, and the main bands eluted with EtOAc–MeOH (8:2) to give irbesartan (18 mg) and metabolite **A** as a colorless oil (19.1 mg). MS–ESI, m/z: 447 ($M + H^+$). MS–MS, m/z: 235 and 252. HRMS-FAB, m/z: found 447.2515, calculated for $C_{25}H_{31}O_2N_6$: 447.2508; 469.2331; calculated for $C_{25}H_{30}O_2N_6Na$: 469.2328.

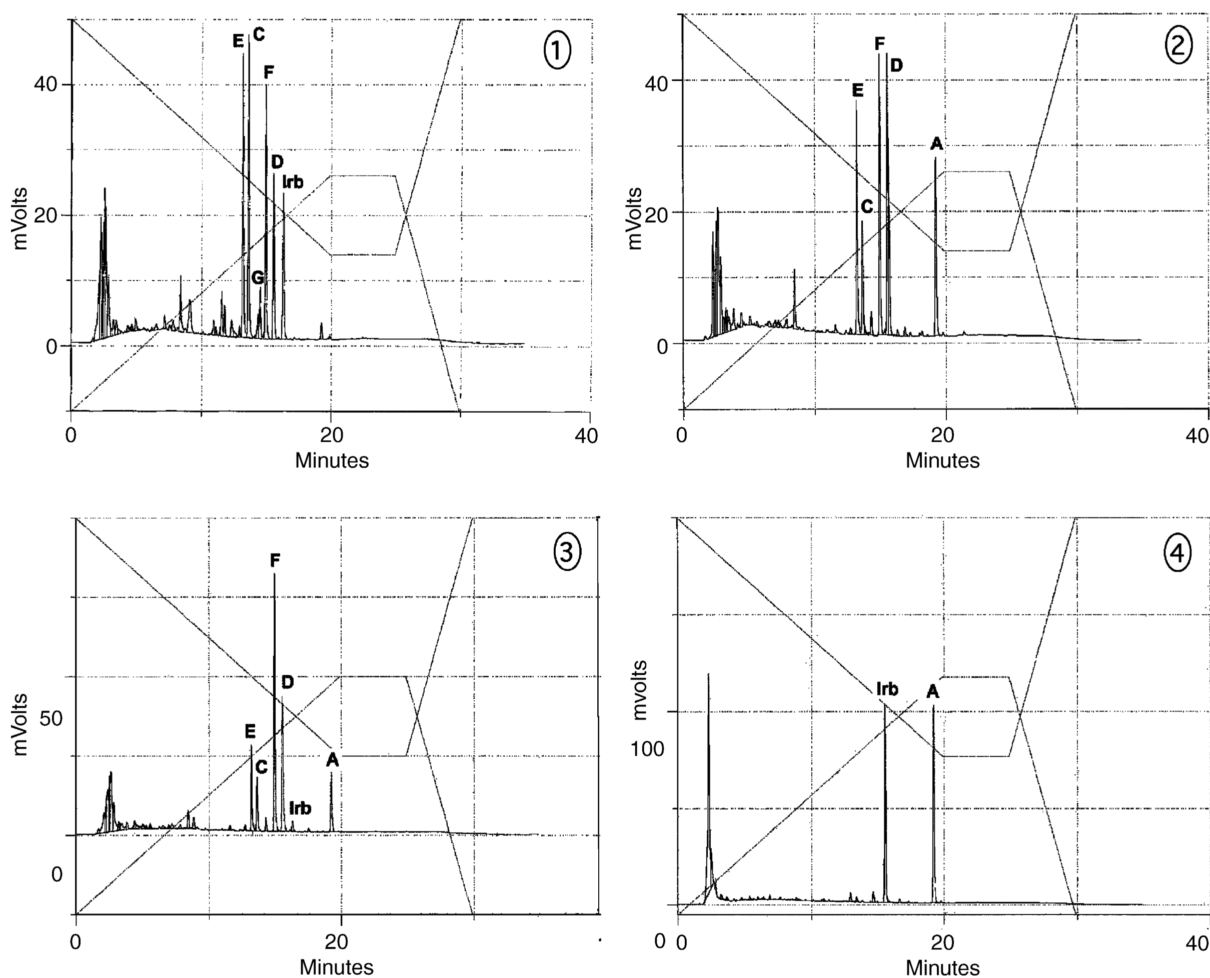


Fig. 1. HPLC profiles of incubation supernatants from *S. griseus* NRRL B150 (**1**, 96 h), *S. vinaceus* ATCC 11861 (**2**, 170 h), *S. griseolus* ATCC 3325 (**3**, 170 h), or *R. erythropolis* DSM 43066 (**4**, 170 h) with irbesartan (0.25 g l^{-1}). Irb: irbesartan; **A** and **C**–**G**: products described in Scheme 2.

Table 2
^{13}C NMR spectral data of main irbesartan metabolites ($CDCl_3$, δ (ppm))

Carbon no.	Irbesartan	**A**	**C**	**E**
C-13 (CO)	187.09	175.01	184.76	187.49
C-10	155.77	155.28	155.78	155.66
C-12	163.04	174.89	162.78	164.10
C-1a	123.18	123.03	123.26	123.30
C-4a	140.59	141.29	140.79	140.76
C-5a	139.20	137.89	139.27	139.35
C-8a	136.15	138.10	135.51	135.33
CH-1	131.28	130.59	130.74[a]	131.14
CH-2	128.33	127.72	128.14	128.17
CH-3	130.89	131.02	131.11	130.84
CH-4	130.67	130.52	130.67[a]	130.63
CH-7, CH-8	129.69	127.31	129.71	129.80
CH-5, CH-6	126.79	129.02	126.86	127.05
C-11	76.41	67.22	75.80	75.60
CH_2-9	43.19	43.07	43.42	43.62
CH_2-1′	28.55	36.00	28.27	28.49
CH_2-2′	27.79	27.74	27.08	27.23
CH_2-3′	22.26	22.13	22.07	22.12
CH_3-4′	13.61	13.50	13.62	13.58
CHOH 2b/3b	–	–	74.40	74.17
CH_2-1b to 4b	37.35	36.23	34.57	35.55
	25.99	23.66	46.13	44.31
			35.22	34.45

For carbon numbering, see Scheme 2.
[a] Attributed values may be reversed.

3. Results and discussion

3.1. Preliminary screening

In a limited screening of 10 fungal strains and 28 bacterial strains currently used in our laboratory for drug hydroxylations [16], a number of strains were found to be able to generate several metabolites **A**–**G**, sometimes in substantial amounts, from irbesartan (at $0.25\,g\,l^{-1}$ concentration), within a 1–4-day period. The main products were separated by TLC and HPLC as shown in Fig. 1 and Table 3. Although small amounts of metabolites were detected in fungal incubations, some bacteria, and particularly *Streptomyces* strains, were found more productive, both on quantitative and qualitative respects.

3.2. Identification of main products

Some of the bacterial strains thus identified were selected to transform irbesartan into a single or a small number of metabolites (Scheme 2) in preparative incubations in order to obtain, in 20–100 mg amounts, the major metabolites. TLC and HPLC monitoring of incubation course (Fig. 2)

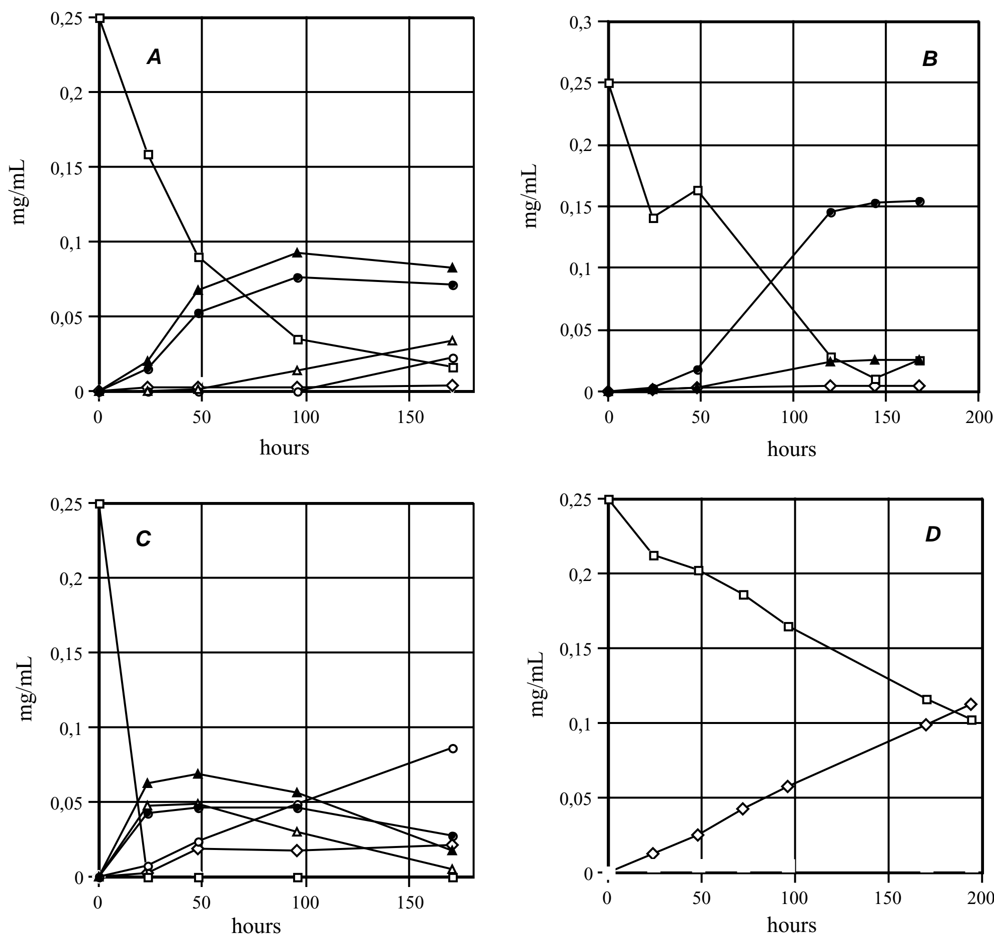


Fig. 2. Time course of the formation of irbesartan metabolites in incubations with *S. rimosus* NRRL 2234 (A), *S. paucisporogenes* LMG 5983 (B), *S. griseolus* ATCC 3325 (C), or *R. erythropolis* DSM 43066 (D). Irbesartan (□); compounds **C** (●), **E** (▲), **D** (○), **F** (△), **A** (◇).

were used to determine the best time to stop the incubations. Metabolites were extracted from incubation mixtures with ethyl acetate and purified by extensive chromatographic methods.

The ethyl acetate extract obtained from pooled 194 h incubations of irbesartan (100 mg) with *R. rhodochrous* DSM 43198, *R. erythropolis* DSM 743, *R. erythropolis* DSM 43066 and *R. erythropolis* ATCC 4277 was chromatographed on several TLC plates to give, after elution, about 20% of metabolite **A** as a colorless oil. This compound was easily identified by mass spectrometry (*m*/*z* 447.2715, calculated for $C_{25}H_{31}O_2N_6$: 447.2508) and NMR spectroscopy (see Table 2) as the hydrolyzed product of irbesartan described as SR 49498 by Chando et al. [17].

The ethyl acetate extracts obtained from a 24 h incubation of irbesartan (250 mg) with *S. griseus* NRRL B150 or *S. griseolus* ATCC 3325 were submitted to separation by silica gel column chromatography then preparative thin layer chromatography to give metabolites **C** and **E** (about 50% total yield). A similar extract obtained from a 96 h incubation of irbesartan (500 mg) with *S. griseolus* ATCC 3325 afforded in significant amounts metabolites **C**–**F**. In addition, a small amount of a new metabolite **G** was obtained.

Positive-ion ESI and FAB mass spectrometry of both metabolites **C** and **E** produced ($M + H^+$) molecular ions at *m*/*z* 445 with exact mass at *m*/*z* 445.2348 and 445.2347, respectively (*m*/*z* 445.2352 calculated for $C_{25}H_{29}O_2N_6$) corresponding to isomeric monohydroxylated derivatives of irbesartan. MS–MS of the *m*/*z* 445 parent ions resulted in product ions at *m*/*z* 207 indicative of an unchanged biphenyltetrazole unit [17]. Another product ion at *m*/*z* 211 was consistent with an additional hydroxyl group on the butyl

Table 3
Main metabolites formed from irbesartan (0.25 g l^{-1}) in fungal or microbial incubations

Strains	Incubation time (h)	Metabolites							
		Irb (15.8)	**A** (19.0)	**C** (13.2)	**E** (13.7)	**D**(*y*) (14.9)	**F**(*x*) (15.4)	**G** (14.8)	Other products
Absidia blakesleeana ATCC 6811	144	+	−	−	−	−	−	−	+/−
Absidia corymbifera LCP 86-3480	144	+++	+/−	+	+/−	−	−	−	−
Absidia cylindrospora LCP 57-1569	144	++	−	−	−	−	−	−	+
Actinomucor elegans LCP 76-3122	144	+++	+/−	−	−	−	−	−	−
Aspergillus alliaceus NRRL 315	96	+	−	−	−	−	−	−	++
Aspergillus ochraceus ATCC 1008	144	++	++	−	+/−	+/−	+/−	−	+
Beauveria bassiana ATCC 7159	144	+++	−	−	−	−	−	−	+/−
Curvularia lunata NRRL 2380	144	+++	+/−	−	−	−	−	−	−
Mortierella isabellina NRRL 1757	144	+++	−	−	−	−	−	−	−
Mucor plumbeus CBS 110-16	144	++	+	−	−	−	−	−	−
Agrobacterium tumefaciens CIP 67-1	170	++	+	−	−	−	−	−	−
Bacillus brevis CIP 52-86	170	++	+	−	−	−	−	−	+
Pseudomonas chlororaphis CIP 63-22	170	++	+++	−	−	−	−	−	+
Pseudomonas fluorescens CIP 69-13	48	+	−	−	−	−	−	−	++
Pseudomonas putida ATCC 29607	170	+	−	−	−	−	−	−	−
Pseudomonas sp. ATCC 43648	170	+++	+++	−	−	−	−	−	
Rhodococcus erythropolis DSM 743	170	+++	+++		−	+/−	−	−	+/−
R. erythropolis ATCC 4277	170	+++	+++	−	−	+/−	−	−	−
R. erythropolis DSM 43066	170	+++	+++	−	−	+/−	+/−	−	+/−
R. rhodochrous DSM 43198	170	+++	+++		−	−	−	−	−
Rhodococcus sp. NCIMB 112-15	170	+++	+	−	−	−	−	−	−
Rhodococcus sp. NCIMB 112-16	170	+++	+++	−	−	−	−	−	−
Streptomyces antibioticus NRRL 8167	170	+++	−	−	−	−	−	−	−
Streptomyces argenteolus LMG 5967	170	+++	−	−	−	−	−	−	+/−
Streptomyces aureofaciens ATCC 10762	170	++	+	++	++	+	+	−	−
Streptomyces cinnamonensis ATCC 15413	170	+++			−	−	−	−	−
Streptomyces fradiae NRRL B1195	96	+++	+	+/−	−	−	−	−	+/−
S. griseolus ATCC 3325	170	+/−	+	+	+	++	+	+/−	+/−
S. griseus NRRL B150	170	−	+/−	+	+	++	++	+/−	++
Streptomyces hygroscopicus NRRL 3444	170	+++	+/−	+	+	−	−	−	−
Streptomyces lavendulae ATCC 8664	170	+++	−	−	−	−	−	−	−
Streptomyces olivaceus NRRL 1125	170	++	++	+	+	+	+	−	+/−
Streptomyces paucisporogenes LMG 5983	170	++	+/−	++	+	−	−	−	−
Streptomyces platensis NRRL 2364	170	+++	−	−	−	−	−	−	−
Streptomyces punipalus NRRL 3529	170	+++	+	−	−	−	−	−	+
Streptomyces rimosus NRRL 2234	170	+	+/−	++	++	+	+	+/−	+
Streptomyces sp. ATCC 3351	170	++	+	+	+	+	−	−	−
Streptomyces vinaceus ATCC 11861	170	++	++	++	+	++	++	+/−	+

spiropentane-imidazolone group [17]. ^{1}H NMR data of both metabolites (Table 1) were in agreement with an unchanged butyl side chain and a monohydroxylated cyclopentane ring with a broad triplet signal at δ 4.4 ppm, associated with a disrupted pattern of the remaining cyclopentane six protons signals from 1.75 to 2.15 ppm: the COSY 2D spectrum analysis, decoupling experiments and HMBC and HMQC spectra are in agreement with an OH group in a β-position to the spiro carbon atom and indicate an hydroxylation in one of the external 2b- or 3b-CH_2 groups, as confirmed by a complete attribution of ^{13}C NMR signals (Table 2). NOESY studies confirmed this structural assignment but as previously pointed [17] do not allow to attribute a *cis*- or *trans*-stereochemistry to each of the **C** or **E** isomers.

Positive-ion ESI mass spectrometry of metabolites **D** and **F**, which were apparently formed from **C** and **E** during prolonged incubations, produced ($M + H^+$) molecular ions at *m/z* 463 corresponding to the further addition of water to the primary monohydroxylated metabolites. MS–MS of the parent ion at *m/z* 463 resulted in product ions at *m/z* 445, 252 and 235, respectively expected for dehydration ion and product ions arising from the cleavage of the biphenyltetrazol-methylene group without or with the imidazolone nitrogen included. Metabolites **D** and **F** thus correspond, respectively, to the hydrolyzed cleavage products of the initially formed metabolites **C** and **E**.

Positive-ion ESI mass spectrometry of metabolite **G** produced a ($M + H^+$) molecular ion at *m/z* 591 and MS–MS product ions at *m/z* 563, 429 (M + H-162) and 369 (207 + 162). The last two ions are strongly indicative of a conjugated metabolite with a (C_6)-glycosidic moiety localized at one of the nitrogen groups of the tetrazolyl ring and analogous to the *N*-glucuronide derivative previously noticed among the human metabolites of irbesartan [17].

4. Conclusion

The metabolic potential and the versatility of microorganism-catalyzed transformations has been useful to obtain easily, in substantial amounts, several of the hydroxylated metabolites of irbesartan. Those metabolites will be used shortly for the determination of the yet unknown stereochemistry of the animal and human metabolites of this drug.

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