Microbial Hydroxylation of Yohimbine

Hanan G. Sary and Khaled Y. Orabi*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Health Sciences Center, Kuwait University, Safat 13110, Kuwait

Abstract: Yohimbine (**1**), an indole alkaloid, was isolated from the dried bark of the tropical West African tree *Pausinystalia yohimbe.* It was approved by the US FDA for erectile dysfunction. Microbial systems serve as *in vitro* convenient, reliable, and predictive models for drug metabolism study in mammals, including man. Microbial bioconversion studies conducted on yohimbine have revealed that it was metabolized by *Bacillus cereus var. fluorescens* ATCC 13824 to give 18β-hydroxyyohimbine (**2**) and 21α-hydroxyyohimbine (**3**). Their structures were established on the basis of their spectral data, and in comparison with those of the substrate yohimbine.

Keywords: Yohimbine, microbial transformation, *Bacillus cereus var. fluorescens*, 18β-hydroxyyohimbine, 21α-hydroxyyohimbine.

One of the main recurrent ingredients of the commercialized aphrodisiac products is yohimbine (**1**), an indole alkaloid extracted from the dried bark of the tropical West African tree *Pausinystalia yohimbe* (Rubiaceae). This bark has long been considered as aphrodisiac and stimulant and extensively used by local populations as part of folk medicine [1,2]. In many societies, crude yohimbe bark and ready-to-use yohimbe preparations are promoted as health food products with aphrodisiac properties [3]. Commercial yohimbe bark obtained from American Herbal Pharmacopeia was reported to contain up to 1.4% w/w yohimbine [4].

Yohimbine hydrochloride was approved by the US Food and Drug Administration for erectile dysfunction [5] and is commonly prescribed for functional impotence and marketed under several trade names. Yohimbine was found to be a promising therapy for erectile dysfunction in type II diabetic patients [6]. Yohimbine is a selective α_2-adrenergic receptor antagonist, raising the blood levels of the neurotransmitter norepinephrine, which stimulates areas controlling sexual response in the brain, and is known to be effective in instances of nonorganic erectile dysfunction. Moreover, it engorges blood vessels in the genitals of both men and women [7].

Some side effects have been reported from the clinical use of yohimbine. Among these, elevated blood pressure, anxiogenic action, increased frequency of urination [8], manic symptoms and several drug-yohimbine interactions [9,10].

These side effects may be parent drug-related or metabolites-related. In order to rule out the first possibility, metabolites in quantities enough to test their pharmacological effects are needed.

One important method used to furnish enough metabolites is the use of microorganisms, because they can serve as convenient, reliable, and predictive models for mammalian drug metabolism [11]. Microbial systems have the advantages of being efficient catalysts, act under mild conditions, catalyze a wide range of reactions, not restricted to their natural substrates, and chemo-, regio- and stereoselective [12]. This method produces significant quantities of metabolites that would be difficult to obtain from either animal system or chemical synthesis. The result from this method often parallel these obtained from human biotransformation and, thus, can be predictive.

Yohimbine aromatic ring hydroxylation was reported to happen in man and microorganisms, as well. 9-hydroxy, 10-hydroxy, 11-hydroxy and 12-hydroxyyohimbine were reported previously as human [13] and microbial metabolites [14-16].

It is anticipated that the microbial metabolism of yohimbine would also produce some new analogues that may serve as prospective candidates for anti-erectile dysfunction evaluation or as a starting leads for the semisynthesis of other derivatives. This note reports on the microbial transformation of yohimbine and the subsequent isolation and characterization of its metabolites.

MATERIALS AND METHODS

The IR spectra were recorded using Jasco FTIR-4100 spectrophotometer (Easton, MD, USA). UV

*Address corresponding to this author at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Health Sciences Center, Kuwait University, Safat 13110, Kuwait; Tel: (965)-2-498-6048, Fax: (965)-2-498-6898, E-mail: kyorabi@hsc.edu.kw

ISSN: 1927-3037/12

spectra were measured in methanol using Shimadzu UV-1601 UV-visible spectrophotometer (Kyoto, Japan). The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II spectrometer (Fallanden, Switzerland)) operating at 600 and 150 MHz, respectively. Both ^{1}H and ^{13}C NMR spectra of **2** and **3** were recorded in DMSO-d_6 and in $CDCl_3$, respectively, and the chemical shift values were expressed in δ (ppm) relative to the internal standard TMS. For the ^{13}C NMR spectra, the number of attached protons was determined by DEPT 135°. 2D NMR spectroscopic data were obtained using the standard pulse sequence of the Bruker Avance II for COSY, HSQC, HMBC and NOESY. The HRMS spectra were measured on Thermo Finnigan mass spectrophotometer (Bermen, Germany) with electrospray ionization. CC used silica gel 60 (230-400 mesh ASTM, Merck, Darmstadt, Germany) for purifications. Precoated TLC plates (silica gel) were used to check the purity of compounds, and Dragendorff's spraying reagent was used for staining of the compounds on TLC. A commercial sample of Yohimbine HCl was purchased from Sigma and used in this work. All reagents used were of analytical grades.

Cultures and Fermentation Screening Procedure

The microbial cultures were originally obtained from the American Type Culture Collection (ATCC), Rockville, Maryland, and are maintained at Kuwait University, Department of Pharmaceutical Chemistry Culture Collection. Stock cultures were maintained on agar slants of media recommended by the ATCC and were stored at 4 °C.

All the preliminary screening and preparative-scale experiments were carried as reported before [11] and according to the standard two-stage protocol [17]. Twenty microbial cultures (Table **1**) were screened using twenty (250 mL) Delong culture flasks. The culture flasks held one fifth of their volume the universal medium α containing per liter, 20 g glucose, 5 g yeast extract, 5 g peptone extract, 5 g NaCl, and 5 g K_2HPO_4 in distilled water. This composition provided a pH of 7.2. The medium was autoclaved for 20 min at 121°C. The culture flasks were inoculated with twenty different microorganisms and incubated on a Lab-Line orbital shaker (SHKE3000-ICE, Mumbai, India) operating at 170 rpm and 25±1 ºC (stage I). After 72 h, 5 mL of stage I cultures were used to inoculate another twenty (250 mL) sterile medium flasks and left for 24h (stage II). Yohimbine was prepared as a 10 % solution in *N,N*-dimethylformamide (DMF) and added to the 24-h-old stage II culture flasks at a concentration of 0.1 mg/mL of medium. Substrate control was composed of sterile medium to which the substrate (5 mg/50 µL DMF) was added and incubated without microorganisms. Culture controls consisted of fermentation blanks in which the microorganisms were grown under identical conditions but without the substrate addition. After 14 days of incubation, test and controls were harvested by extraction with $CHCl_3$ and analyzed by TLC.

Table 1: Microorganisms Screened for their Ability to Metabolize Yohimbine[a]

***Absidia glauca* ATCC 22752**
***Acinetobacter calcoaceticus* ATCC 14987**
Agrobacterium tumefaciens ATCC15955
Alcaligenes eutrophus ATCC 17697
Aspergillus fumigatus ATCC 1022
***Aspergillus niger* ATCC 16888**
***Bacillus cereus var. fluorescens* ATCC 13824**
Beauveria bassiana ATCC 7159
Cellulomonas flavigena ATCC 482
Cunninghamella blakesleeana ATCC 9245
***Cunninghamella echinulata var. echinulata* ATCC 9244**
Cunninghamella homothallica ATCC 16161
Cylindrocarpon radicicola ATCC 11011
Flavobacterium oxydans ATCC 21245
***Mucor circinelloides f. griseocyanus* 1207a**
***Mucor griseocyanus* ATCC 1207b**
Nocardia coralline ATCC 19148
Rhizopus arrhizus ATCC 11145
Rhizopus stolonifer ATCC 24795
Sphingobacterium heparinum ATCC 13125

[a]Microorganisms in bold represent those gave positive results.

Preparative Scale Fermentation by *Bacillus cereus var. fluorescens* ATCC 13824

Under similar culture conditions (temperature and shaking speed) to the screening stage, *Bacillus cereus var. fluorescens* ATCC 13824 was grown in five 1-L culture flasks each containing 200 mL of medium α. After 72 h, 20 mL of stage I culture were used to inoculate another fifty 1-L sterile medium flasks and left for 24h (stage II), then a total of 1 gm of yohimbine (in 2.5 mL DMF) was evenly distributed among these flasks. After 14 days, the incubation mixtures were checked by TLC. TLC revealed that most of yohimbine was transformed and two more polar metabolites were produced.

Isolation and Purification of the Metabolites

After two weeks of incubation, cultures were combined and exhaustively extracted with 3x4 liters of $CHCl_3$ after alkalinizing the media with concentrated NH_4OH solution (pH=9). The combined $CHCl_3$ layers were concentrated down to 250 mL and, then, subjected to an acid/base shake up using equal volumes of acidified (6M HCl) and alkalinized (concentrated NH_4OH) water. The water layers were re-extracted with $CHCl_3$. Finally, the $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford brown syrupy residue. This residue (2.29 g) was chromatographed over silica gel column (250 g, 5 x 60 cm) using a mixture of $CHCl_3$: CH_3OH: concentrated NH_4OH (8:1:0.8 v/v) as a mobile phase. Fractions of 25 mL each were collected and analyzed by TLC. Similar fractions were pooled together. This afforded three main fractions; A, B and C. Fraction A gave 595.8 mg of the recovered substrate. Fraction B and C afforded impure metabolites **2** and **3** in 39.5 mg and 21.7 mg, respectively.

Fraction B was subjected to another column chromatographic separation using CH_3CN: CH_3OH: $C_2H_5NH_2$ in the ratio of (8.3:1.7:0.5 v/v) as a mobile phase to afford the semi-pure metabolite **2** which yielded 21.5 mg of pure metabolite **2** after crystallization from a mixture of 1% of $CHCl_3$ in CH_3CN.

Fraction C was purified using preparative TLC eluted with a mixture of $CHCl_3$: CH_3OH: concentrated NH_4OH (8.5:1.5:0.08 v/v) to afford 14.4 mg of pure metabolite **3**.

Metabolite 2

Colorless powder; IR neat υ_{max} 3300 (OH), 2919 (C-H), 1735 (C=O) cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 282 (3.29) nm; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz): see Table **2**. HRESIMS: *m*/*z* $[M]^+$ calcd for $C_{21}H_{26}N_2O_4$: 370.4421; found: 370.1862.

Metabolite 3

Colorless powder; IR neat υ_{max} 3309 (OH), 2920 (C-H), 1735 (C=O) cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 289 (2.78) nm; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz): see Table **2**. HRESIMS: *m*/*z* $[M]^+$ calcd for $C_{21}H_{26}N_2O_4$: 370.4421; found: 370.1881.

RESULTS AND DISCUSSION

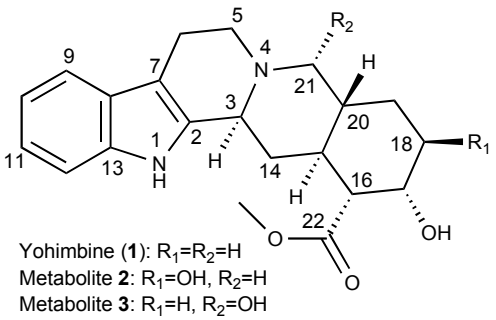
Out of 20 microbial cultures screened for their ability to metabolize yohimbine, only *Absidia glauca* ATCC 22752, *Acinetobacter calcoaceticus* ATCC 14987, *Bacillus cereus var. fluorescens* ATCC 13824, *Aspergillus niger* ATCC 16888, *Cunninghamella echinulata var. echinulata* ATCC 9244, *Mucor circinelloides f. griseocyanus* ATCC 1207a, and *Mucor griseocyanus* ATCC 1207b showed definite biotransformation of **1** (Table **1**). All of them produced one common nitrogen containing metabolite (**2**). *Bacillus cereus var. fluorescens* ATCC 13824 produced **2** in a much higher yield, in addition to another nitrogen-containing metabolite (**3**). Therefore, *Bacillus cereus var. fluorescens* ATCC 13824 was selected for preparative scale fermentation to produce these metabolites in quantities enough for structural elucidation and other potential biological studies.

Preparative scale biotransformation of 1 g of yohimbine using *Bacillus cereus var. fluorescens* ATCC 13824 afforded 21.5 mg of metabolite **2** (2.06 % yield, colorless powder) and 14.4 mg of metabolite **3** (1.38 % yield, colorless powder).

ESIMS of **2** indicated that its molecular weight is *m*/*z* 370.1862, which is consistent with the molecular formula $C_{21}H_{26}N_2O_4$. The IR spectrum showed a broad absorption band at 3300 cm^{-1} which suggested the presence of at least one hydroxyl group. The strong absorption band at 2919 cm^{-1} was attributed to saturated C-H stretch, while the presence of a strong absorption band at 1735 cm^{-1} indicated the presence of an ester carbonyl group. ^{13}C NMR spectrum (Table **2**) of **2** showed 21 carbon resonances distributed as five singlets, ten doublets, five triplets and one quartet. Carbon multiplicities showed that **2** has one less triplet (δ_C 30.9) and, on the other hand, one more oxygenated doublet (C-18, δ_C 68.8), when compared to yohimbine, suggesting the bioconversion of yohimbine into hydroxyyohimbine.

Six carbon resonances were in the aliphatic deshielded region (δ_C 50-80). Three of these carbons were the ones attached to N-4 (C-3, δ_C 60.0, C-5, δ_C 52.5, and C-21, δ_C 63.8). HSQC spectra showed a correlation between the carbon resonated at δ_C 68.8 and a proton signal resonated as ddd (*J*= 5.3, 5.3, 5.3) at δ_H 4.85 (Table **2**). This proton was shown to possess a cross peak in the COSY spectrum with another proton resonating as a broad singlet at δ_H 4.13, assigned to H-17. H-17 showed ^1J-correlation, in HSQC spectrum, with a doublet carbon resonating at δ_C 66.3. This confirmed the conclusion that the new oxygenated carbon is C-18. It is worthnoting the downfield shift of C-19 (Δ 4.5 ppm). On the other

Table 2: NMR Data (600 MHz) for Yohimbine (1) and Metabolites 2 and 3[a]



Yohimbine (**1**): R_1=R_2=H
Metabolite **2**: R_1=OH, R_2=H
Metabolite **3**: R_1=H, R_2=OH

Yohimbine (1)			18β-Hydroxyyohimbine (2)		21α-Hydroxyyohimbine (3)	
#	δ_c, m[b]	δ_H (*J* in Hz)	δ_c, m	δ_H (*J* in Hz)	δ_c, m	δ_H (*J* in Hz)
2	129.2, s	-	126.6, s	-	130.0, s	-
3	60.2, d	4.78, m	60.0, d	4.75, br s	61.8, d	4.75, br s
5	51.8, t	3.65, m 3.39, dd (10.2, 10.2)	52.5, t	3.68, m 3.33, dd (10.1, 10.1)	56.8, t	3.78, m 3.25, dd (10.1, 10.1)
6	18.6, t	2.91, dd (12.8, 4.2) 3.24, dd (12.4, 12.4)	17.7, t	2.76, dd (12.0, 4.2) 3.17, m	18.4, t	2.80, dd (12.0, 4.2) 3.21, m
7	105.4, s	-	104.5, s	-	105.0, s	-
8	125.8, s	-	126.2, s	-	126.0, s	-
9	118.0, d	7.45, d (7.8)	117.6, d	7.39, d (7.8)	117.9, d	3.75, d (7.8)
10	119.1, d	7.02, dd (7.8, 7.8)	118.7, d	6.98, dd (7.8, 7.8)	119.4, d	6.99, dd (7.8, 7.8)
11	121.7, d	7.12, dd (7.8, 7.8)	120.7, d	7.04, dd (7.8, 7.8)	122.0, d	7.07, dd (7.8, 7.8)
12	111.5, d	7.35, d (8.1)	111. 2, d	7.29, d (7.7)	112.0,d	7.31, d (7.8)
13	136.5, s	-	136.0, s	-	136.7, s	-
14	31.9, t	1.79, dd (13.5, 3.0) 1.45, m	32.3, t	1.78, dd (13.5, 3.0) 1.51, m	31.9, t	1.77, dd (13.1, 3.0) 1.51, m
15	33.6, d	2.17, dddd (11.4, 11.4, 11.4, 2.4)	34.2, d	2.24, m	34.0, d	2.21, m
16	51.1, d	2.34, dd (11.4, 2.4)	51.0, d	2.50, m	51.2, d	2.35, dd (11.4, 2.4)
17	66.1, d	4.16, br s	66.3, d	4.13, s	66.3, d	4.14, s
18	30.9, t	1.58, dd (13.0, 13.0) 2.73, d (13.0)	68.8, d	4.85, ddd (5.3, 5.3, 5.3) -	31.1, t	1.58, m 2.72, d (13.0)
19	22.0, t	1.34, m 1.43, m	26.5, t	2.20, m 2.31, m	22.7, t	1.36, m 1.42, m
20	37.0, d	1.87, m	34.1, d	2.20, m	37.9, d	2.53, m
21	57.2, t	3.08, dd (12.4, 12.4) 3.39, dd (10.2, 10.2)	63. 8, t	3.55, dd (12.4, 12.4) 3.80, dd (10.2, 10.2)	72.3, d	4.15, d (6.0) -
22	172.2, s	-	172.4, s	-	174.2, s	-
23	51.4, q	3.69, s	51.2, q	3.69, s	52.3, q	3.65, s
OH-17	-	4.87, d (3.6)[c]	-	4.87, s[c]	-	4.86, s[c]
OH-18	-	-	-	5.45, s[c]	-	-
OH-21	-	-	-	-	-	4.20, s[c]
NH	-	11.28, br s[c]	-	10.91, br s[c]	-	10.55, br s[c]

[a]NMR spectra were acquired in DMSO-d_6 for yohimbine and metabolite **2** and in $CDCl_3$ for metabolite **3**.
[b]Carbon multiplicities were determined by DEPT 135°.
[c]D_2O exchangeable protons.

hand, C-20 showed an upfield shift (Δ 2.9 ppm) when compared to the same carbons of yohimbine. HMBC spectrum showed ^3J-correlations between the methyl protons (δ_H 3.68, s) and the carbonyl carbon (δ_C 172.4), H-21 (δ_H 3.55 and 3.80, each as dd) and C-5 (δ_C 52.5), H-9 (δ_H 7.39, d) and C-11 (δ_C 120.7) and C-13 (δ_C 136.0), H-10 (δ_H 6.98, dd) and C-8 (δ_C 126.2) and C-12 (δ_C 111. 2), H-11 (δ_H 7.04, dd) and C-9 (δ_C 117.6) and C-13, H-12 (δ_H 7.29, d) and C-8 and C-10 (δ_C 118.7) and between N-H (δ_H 10.90, s) and C-7 (δ_C 104.5). Another ^2J-correlation existed between this proton and C-2 (δ_C 126.6). HSQC and HMBC aided the unambiguously assignment of other proton and carbon resonances.

Non-spinning mode NOESY spectrum showed a cross peak between H-18 and H_3-23 resonating as a sharp singlet at δ_H 3.68. This proves that those protons should be on the same side of the molecule, i.e., α-disposition. Consequently, the hydroxyl group on C-18 should adopt the β-orientation. Other NMR data were similar to those of yohimbine as shown in Table **2**. Hence, the identity of metabolite **2** was proposed to be the new metabolite 18β-hydroxyyohimbine. C-18 epimer, 18α-hydroxyyohimbine, has been reported before as a microbial metabolite [14,18].

Metabolite **3** was shown to possess the molecular formula $C_{21}H_{26}N_2O_4$, as was derived from the molecular ion peak at m/z 370.1881 and from NMR spectral data (Table **2**). The presence of at least one hydroxyl group was clearly shown in the IR spectrum from the broad absorption band at 3309 cm^{-1}. The strong absorption band at 2920 cm^{-1} was attributed to the presence of saturated C-H stretch. The introduction of another hydroxyl group was established from ^{13}C NMR data. ^{13}C NMR and DEPT spectra revealed the presence of one methyl, five methylene, ten methine, and five quaternary carbons. These resonances were similar to those of **2** and suggested that metabolite **3** is another hydroxyyohimbine derivative.

Five carbons, resonated between δ_C 50-80, were concluded to be aliphatic ones attached to electron-withdrawing atoms. C-3 resonated at δ_C 61.8 as a doublet, C-5 as a triplet at δ_C 56.8, C-17 as a doublet at δ 66.3, C-23 as a quartet at δ 52.3, while C-21 resonated more downfield, when compared to yohimbine C-21, as a doublet at δ_C 72.3. This carbon was suggested to have the newly introduced hydroxyl group, in addition to the original N atom. HSQC spectrum showed a correlation between this carbon and a proton resonated at δ_H 4.15 (d, J= 6.0 Hz, H-21). HMBC spectra showed a ^3J-correlation between the methyl protons (δ_H 3.65, s) and the carbonyl carbon (δ_C 174.2). Other important correlations existed in the aromatic region, between H-9 (δ_H 7.35, d) and C-11 (δ_C 122.0) and C-13 (δ_C 136.7), H-10 (δ_H 6.99, dd) and C-8 (δ_C 126.0) and C-12 (δ_C 112.0), H-11 (δ_H 7.07, dd) and C-9 (δ_C 117.9) and C-13 (δ_C 136.7). Other NMR data were similar to those of **2**. ^1H NMR coupling constant and pattern of H-21 (d, J= 6.0 Hz) indicated that this proton should be an equatorial one. Since the ring-junction proton, H-20, is adopting the axial β-disposition, therefore, H-21 should be adopting the equatorial β-disposition, as well, leaving the α-orientation for the coming hydroxyl group. Consequently, metabolite **3** was concluded to be the new 21α-hydroxyyohimbine.

Metabolites **2** and **3** might represent new leads as anti-erectile drugs.

ACKNOWLEDGEMENTS

NMR analyses were carried out at Science Analytical Facility, Faculty of Science, Kuwait University, supported by Grants number GS01/01 and GS01/03.

REFERENCES

[1] Zanolari B, Ndjoko K, Ioset J-R, Marston A, Hostettmann K. Qualitative and quantitative determination of yohimbine in authentic yohimbe bark and in commercial aphrodisiacs by HPLC-UV-API/MS methods. Phytochem Anal 2003; 14: 193-201. http://dx.doi.org/10.1002/pca.699

[2] HO CC, Tan HM. Rise of herbal and traditional medicine in erectile dysfunction management. Curr Urol Rep 2011; 12: 470-8. http://dx.doi.org/10.1007/s11934-011-0217-x

[3] De Smet PAGM, Keller K, Hänsel R, Chandler RF. Adverse Effects of Herbal Drugs, vol 3. Berlin: Springer; 1997. http://dx.doi.org/10.1007/978-3-642-60367-9

[4] Sun J, Chen P. Chromatographic fingerprint analysis of yohimbe bark and related dietary supplements using UHPLC/UV/MS. J Pharm Biomed Anal 2012; 5: 142-9. http://dx.doi.org/10.1016/j.jpba.2011.11.013

[5] Mittal S, Alexander KS, Dollimore D. A high-performance liquid chromatography assay for yohimbine HCl analysis. Drug Dev Ind Pharm 2000; 26: 1059-65. http://dx.doi.org/10.1081/DDC-100100269

[6] Tanweer MS, Fatima A, Rahimnajjad MK. Yohimbine can be the new promising therapy for erectile dysfunction in type 2 diabetics. J Pak Med Assoc 2010; 60: 980.

[7] Morales A. Yohimbine in erectile dysfunction: would an orphan drug be properly assessed? World J Urol 2001; 19: 251-5. http://dx.doi.org/10.1007/s003450000182

[8] Shamloul R. Natural aphrodisiacs. J Sex Med 2010; 7: 39-49. http://dx.doi.org/10.1111/j.1743-6109.2009.01521.x

[9] Tam SW, Worcel M, Wyllie M. Yohimbine: a clinical review. Pharmacol Therapeut 2001; 91: 215-43. http://dx.doi.org/10.1016/S0163-7258(01)00156-5

[10] Guirguis WR. Oral treatment of erectile-dysfunction: from herbal remedies to designer drugs. J Sex Marital Ther 1998; 24: 69-73. http://dx.doi.org/10.1080/00926239808404920

[11] Orabi KY. Microbial models of mammalian metabolism. Sampangines. In: Atta-Ur-Rahman, editor. Studies in natural products chemistry-Bioactive natural products, Part D. New York: Elsevier 2000; pp. 3-49.

[12] Loughlin WA. Biotransformation in organic synthesis. Biores Technol 2000; 74: 49-62. http://dx.doi.org/10.1016/S0960-8524(99)00145-5

[13] Duflos A, Redoules F, Fahy J, Jacquesy JC, Jouannetaud MP. Hydroxylation of yohimbine in superacidic media: one-step access to human metabolites 10 and 11-hydroxyyohimbine. J Nat Prod 2001; 64: 193-5. http://dx.doi.org/10.1021/np000425z

[14] Patterson EL, Andres WW, Krause EF, Hartman RE, Mitscher LA. Microbiological transformation of some yohimbine-type alkaloids. Arch Biochem Biophys 1963; 103: 117-23. http://dx.doi.org/10.1016/0003-9861(63)90017-1

[15] Adam JM, Fonzes L, Winternitz F. Biohydroxylations in the yohimbine series. Annales de Chimie 1973; 8: 71-8.

[16] Meyers E, Pan SC, Samuel C. Microbial transformation of Rauwolfia alkaloids. II. 10-Hydroxyyohimbine. J Bacteriol 1961; 81: 504-5.

[17] Orabi KY, Li E, Clark AM, Hufford CD. Microbial Transformation of Sampangine. J Nat Prod 1999; 62: 988-92. http://dx.doi.org/10.1021/np980457a

[18] Pan SC, Weisenborn FL. Microbiological transformations of Rauwolfia alkaloids. JACS 1958; 80: 4749. http://dx.doi.org/10.1021/ja01550a092

Received on 18-01-2012 Accepted on 08-03-2012 Published on 06-04-2012

DOI: http://dx.doi.org/10.6000/1927-3037.2012.01.01.08