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Microbial transformation of papaveraldine

Khalid A. El Sayed

Medicinal, Aromatic and Poisonous Plants Research Center, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

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Abstract

Preparative-scale fermentation of papaveraldine (**1**), the known benzylisoquinoline alkaloid, with *Mucor ramannianus* 1839 (sih) has resulted in a stereoselective reduction of the ketone group and the isolation of *S*-papaverinol (**2**) and *S*-papaverinol *N*-oxide (**3**). The structure elucidation of both metabolites was based primarily on 1D-, 2D-NMR analyses and chemical transformations. The absolute configuration of **2** was determined using Horeau's method of asymmetric esterification. These metabolism results were consistent with the previous plant cell transformation studies on papaverine and isopapaverine. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Microbial metabolism; Benzylisoquinoline alkaloids; *Mucor ramannianus* 1839 (sih); Papaveraldine; Absolute configuration

1. Introduction

Papaveraldine (**1**) is one of the minor benzylisoquinoline alkaloids of poppy capsules (*Papaver somniferum L.*) (Hodkova, Vesely, Koblicova, Holubec & Trojanek, 1972). The *N*-methyl derivative of **1**, *N*-methylpapaveraldinium cation, is also reported to be a natural product of *Stephania sasakii*, Family Menispermaceae, (Dictionary of Alkaloids, 1989; Dictionary of Natural Products, 1994). Papaveraldine (**1**) and papaverinol (**2**) are somewhat similar to papaverine in biological activity, i.e., they show antispasmodic and protective activity against histamine-induced bronchospasm but no analgesic activity was reported after oral administration of papaveraldine in rats (Weisbach, Kirkpatrick, Macko & Douglas, 1968). In carragenine edema test in mice, several papaveraldine oximes had analgesic and antiinflammatory activities comparable to phenylbutazone at 60 mg/kg oral dose (Buzas, 1974). Papaveraldine was one of the active ingredients of a hair tonic preparation which promotes melanin formation for grey hair (Sugiyama, Takada & Fukushima, 1988).

Several attempts to biotransform papaverine and isopapaverine were reported. Rosazza et al. (1977) reported *O*-demethylation of papaverine at C-4′, C-6 and C-7 using the microbes *Aspergillus*, *Cunninghamella* and *Streptomyces* species. The cell culture of *Glycyrrhiza glabra* L. var. *typica* Reg. and Hed. was able to biotransform papaverine to papaverinol in a 31.5% yield (Dorisse, Gleye, Loiseau, Puig, Edy & Henry, 1988). The same biotransformation reaction was also reported using *Thevetia nereifolia* Juss. (Apocynaceae) cell culture (Rideau, Morard, Gansser, Chenieux & Viel 1988). In both cases, the optical activity of papaverinol remained uncertain. Papaverine was biotransformed to papaveraldine using cell cultures of *Silene alba* Miller (Bister-Miel, Agier, Bury, Postaire, Guignard & Viel, 1986), *Ptelea trifoliata* L. (Rutaceae), *Phaseolus vulgaris* L. (Papilionaceae) and *Parthenocissus tricuspidata* Planch. (Vitaceae) (Rideau et al., 1988). *Ochrosia elliptica* Labill. (Apocynaceae) cell culture was able to biotransform papaverine to its *N*-oxide (Rideau et al., 1988). *Silene alba* Miller E. H. L. Krause cell suspension also transformed papaverine to 6- and 4′-monodemethylpapaverine (Verdeil, Bister-

E-mail address: abdelaty@ksu.edu.sa (K.A. El Sayed).

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Miel, Guignard & Viel, 1986). *Silene alba* cell suspension was able to biotransform isopapaverine to *O*-demethylisopapaverine derivative, isopapaveraldine and isopapaverine *N*-oxide (Christinaki, Bister-Miel, Hammoumi, Bury, Guignard & Viel, 1987).

Microbial metabolism studies have been used successfully as model systems to predict metabolic pathways of drugs in humans or to increase the efficacy of drugs by metabolic activation (Lee, 1990). The current report represents the first microbial metabolism study of papaveraldine and the first enzyme mediated stereoselective reduction of the ketone group of this class of compounds.

2. Results and discussion

Twenty-eight growing cultures were screened for their ability to bioconvert **1**. Few cultures were observed to transform **1** partially or completely to metabolites of higher polarity. *Mucor ramannianus* 1839 (obtained from Dr. Charles Sih, Department of Pharmaceutical Biochemistry, University of Wisconsin, Madison, USA) was chosen for preparative-scale fermentation of **1** because it entirely depleted and converted **1** to two more polar metabolites **2** and **3**.

The high resolution FT-ICR MS of **2** displayed a molecular ion peak $(M+H)^+$ at m/z 356.1443, suggesting the molecular formula $C_{20}H_{22}O_5N$ and 11 degrees of unsaturation. The FT-IR spectrum of **2** ($CHCl_3$) showed a strong absorption band at 3514–3327 cm^{-1}, suggesting the presence of a hydroxy functionality. The ^{13}C- and ^1H-NMR spectra of **2** (Table 1) suggested that it is papaverinol (Dorisse et al., 1988). The proton singlet resonated at δ 6.10 which correlated to the methine carbon resonating at δ 72.4 is assigned as the benzylic C-α. Papaverinol is previously reported as a synthetic derivative or detected as a biotransformation product without any reported optical activity (Weisbach et al., 1968, Dorisse et al., 1988; Rideau et al., 1988). The X-ray crystallographic analysis of setigerine (α-*O*-methylpapaverinol), isolated from *Papaver setigerum DC*. confirmed its racemic nature (Slavik and Slavikova, 1996; Mahboobi, Pongratz & Wiegrebe, 1997). Since metabolite **2** displayed an $[\alpha]_D^{25} - 54.9$, hence this is the first report of an optically active papaverinol enantiomer, presumably because the reduction of the ketone group was enzyme mediated. The absolute stereochemistry of the secondary hydroxyl group of **2** was determined by the application of Horeau's method of asymmetric esterification (Herz & Kagan, 1967; Horeau, 1977; Capon & Macleod, 1985). The results of this experiment suggested that C-α in **2** has an *S* configuration, as suggested by the value of $[\alpha]_D^{25} - 3.2$ of the partially resolved α-phenylbutyric acid. Hence, **2** was proved to be *S*-papaverinol.

The high resolution FT-ICR MS of **3** displayed a molecular ion peak $(M+H)^+$ at m/z 372.1448, suggesting the same molecular formula of **2** with one additional oxygen atom ($C_{20}H_{22}O_6N$). The ^{13}C- and ^1H-NMR spectra of **3** (Table 1) were closely similar to those of **2** and suggested that **3** is the *N*-oxide derivative of **2**. The proton doublet resonated at δ 8.40 ($J = 5.7$), which correlated with the methine carbon at δ 138.0 and was assigned H-3 (Table 1). This proton displayed a ^3J-HMBC coupling (Fig. 1) with the quaternary carbon resonating at δ 156.0, which was assigned C-1. The upfield shifting of C-1 and C-3 in **3** (−0.5 and −0.7 ppm, respectively) as compared to those of **2** was consistent with the fact that **3** is the *N*-oxide derivative of **2**. This assumption was supported by detailed analysis of the HMBC spectra of **3** (Fig. 1). This is also further confirmed by the reduction of **3**

Table 1
^{13}C- and ^1H-NMR spectral data of metabolites **2** and **3**[a]

Position	**2**		**3**	
	δ_C	δ_H	δ_C	δ_H
1	156.5, *s*	–	156.0, *s*	–
3	138.7, *d*	8.34, *d* (5.5)	138.0, *d*	8.40, *d* (5.7)
4	119.6, *d*	7.51 *d* (5.7)	119.6, *d*	7.51 *d* (5.7)
4a	133.3, *s*	–	133.3, *s*	–
5	105.0, *d*	7.01, *s*	105.1, *d*	7.07, *s*
6	152.4, *s*	–	152.1, *s*	–
7	149.1, *s*	–	149.6, *s*	–
8	103.0, *d*	7.07, *s*	103.0, *d*	7.12, *s*
8a	120.7, *s*	–	120.8, *s*	–
1′	136.0, *s*	–	136.0, *s*	–
2′	110.8, *d*	6.79, brs	110.8, *d*	6.81, *d* (1.5)
3′	148.5, *s*	–	148.5, *s*	–
4′	149.6, *s*	–	149.1, *s*	–
5′	110.4, *d*	6.74, *d* (8.2)	110.4, *d*	6.78, *d* (8.2)
6′	120.0, *d*	6.87, *dd* (8.2, 1.1)	120.0, *d*	6.90, *dd* (8.2, 1.5)
α	72.4, *d*	6.10, *s*	72.4, *d*	6.14, *s*
6-OMe	55.7, *q*	3.92, 3H, *s*	55.6, *q*	3.99, 3H, *s*
7-OMe	55.7, *q*	3.71, 3H, *s*	55.6, *q*	3.83, 3H, *s*
3′-OMe	55.8, *q*	3.77, 3H, *s*	55.7, *q*	3.81, 3H, *s*
4′-OMe	55.6, *q*	3.76, 3H, *s*	55.8, *q*	3.76, 3H, *s*

[a] In $CDCl_3$, at 400 MHz for ^1H and 100 MHz for ^{13}C. Carbon multiplicities were determined by DEPT 135 experiments; *s*: quaternary, *d*: methine, *q*: methyl carbons, coupling constants (J) are in Hz.

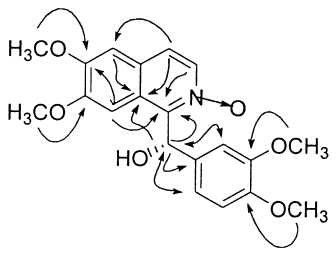
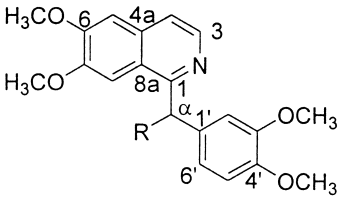


Fig. 1. Important ^1H–^{13}C-HMBC correlations of **3**.

into **2** using Zinc dust and HCl. The configuration of C-α of compound **3** was assigned *S* because both **2** and **3** have similar optical rotation sign ($[\alpha]_D^{25}$ − 54.9 and −22.4, respectively). Hence, metabolite **3** was proved to be *S*-papaverinol *N*-oxide.

These microbial metabolism results of papaveraldine were consistent with the previous plant cell transformation studies on papaverine and isopapaverine (Dorisse et al., 1988; Rideau et al., 1988; Bister-Miel et al., 1986; Christinaki et al., 1987).

S-Papaverinol did not show antimicrobial (against *Candida albicans*, *Staphyllococcus aureus* and *Pseudomonas aeruginosa*), antiviral (against herpes simplex type 1) or antimalarial (against *Plasmodium falciparium* D6 and W2 clones) activities.



	R
Papaveraldine (**1**)	=O
S-Papaverinol (**2**)	α-OH
S-Papaverinol *N*-oxide (**3**)	α-OH, *N*-oxide

3. Experimental

3.1. General experimental procedure

The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$, on an NMR spectrometer operating at 400 for ^{1}H-NMR, and 100 MHz for ^{13}C-NMR. The HRMS spectra were measured on a FT-ICR MS with electrospray ionization. TLC analyses were carried out on precoated Silica gel G_{254} 1000 μm, with the following developing system: $CHCl_3$–MeOH–NH_4OH (90:10:0.01). For column chromatography, Si gel 60, 40 μm was used.

3.2. Chemicals

Papaveraldine (**1**) was purchased from a commercial source.

3.3. Organisms

Preliminary microbial metabolism studies were conducted as previously reported (Lee, ElSohly & Hufford, 1990; El Sayed, 1998). Twenty-eight microbial cultures, obtained from the University of Mississippi, Department of Pharmacognosy culture collection, were used for screening. The microbes utilized were reported earlier (El Sayed, 1998), in addition to: *Gongronella butleri* ATCC 22822, *Mucor mucedo* UI 4605 and *Mucor ramannianus* 1839, which obtained from Dr. Charles Sih, Department of Pharmaceutical Biochemistry, University of Wisconsin, Madison, Wisconsin, USA, (Orabi, Li, Clark & Hufford, 1999). Stock cultures were maintained on agar slants of media recommended by the ATCC and were stored at 4°C.

3.4. Microbial metabolism of papaveraldine (*1*) by mucor ramannianus

M. ramannianus 1839 (sih) was grown in 4 1l culture flasks, each containing 250 ml of compound medium α which consists of (per liter of distilled water): glucose, 20 g; NaCl, 5 g; K_2HPO_4, 5 g; yeast extract (BBL, Cockeysville, Maryland), 5 g; peptone (Difco, Detroit, Michigan), 5 g. A total of 200 mg of **1** was dissolved in 1 ml EtOH, equally divided between the four flasks and distributed among the 24 h old stage II cultures. After 7 days, the incubation mixtures were pooled and filtered. The filtrate (0.9l) was exhaustively extracted with EtOAc (3 × 300 ml), which was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue (720 mg) was flash chromatographed over 100 g Si gel 60, starting with (100%) cyclohexane and gradient eluted with increasing proportions of EtOAc and finally with (100%) MeOH. Alkaloid-containing fractions were subjected to repeated preparative TLC on Si gel G_{254} to give two metabolites: **2** (28.0 mg, R_f 0.45) and **3** (4.5 mg, R_f 0.32).

3.5. Horeau's method for determination of absolute configuration of *2*

A solution of 50 mg of α-phenylbutyric acid in 1 ml dry pyridine was added to 20 mg of **2**. The mixture was stirred for 24 h at room temperature. Excess anhydride was destroyed by adding 1 ml H_2O and allowing it to stand for 6 h at room temperature. The solution was then extracted with EtOAc (5 ml × 3). The EtOAc extracts were washed with 5% $NaHCO_3$ solution (5 ml × 2) and finally with H_2O (5 ml × 3). The residue (21 mg) contained no starting material as indicated by TLC, suggesting a 100% esterification. The combined aqueous extracts were washed with $CHCl_3$ (5 ml × 2) and then acidulated with an excess of 1 N H_2SO_4. The acidified solution was extracted with $CHCl_3$ (5 ml × 2), which was dried and evaporated. This afforded 22 mg of α-phenylbutyric acid ($[\alpha]_D^{25}$ − 3.2, from chloroform, enantiomeric excess 14.0%)

3.6. Reduction of **3**

A 2.0 mg sample of **3** was dissolved in 1 ml of 1 N HCl and about 2 mg of Zn dust was added. The reaction mixture was stirred for 6 h at room temperature. About 5 ml brine solution was then added and the solution was filtered. The filtrate was rendered alkaline to pH 9 using 25% NH_4OH and extracted with $CHCl_3$ (2 × 10 ml). The organic layers were washed with water (10 ml), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Repeated prep. TLC on Si gel G_{254} afforded **2** (1.2 mg).

3.7. S-papaverinol (**2**)

Yellow needles from MeOH, mp. 156–158°, $[\alpha]_D^{25}$ − 54.9 (*c* 0.2, $CHCl_3$); UV λ_{max} (log ε) (MeOH) 210 (4.51), 239 (4.75), 278 (3.85), 314 (3.66) nm; IR ν_{max} ($CHCl_3$) 3514–3327 (OH), 3024–2839, 1624, 1480, 1270, 1158, 1026, 860 cm^{-1}; ^{13}C- and ^{1}H-NMR, see Table 1; FT-ICR MS *m/z* calculated for $C_{20}H_{22}O_5N$ $[M+H]^+$ 356.1498, found 356.1443.

3.8. S-papaverinol N-oxide (**3**)

Yellow needles from MeOH, mp. 151–152°, $[\alpha]_D^{25}$ − 22.4 (*c* 0.05, CHCl3); UV λ_{max} (log ε) (MeOH) 210 (4.49), 237 (4.71), 275 (3.80), 312 (3.61) nm; IR ν_{max} ($CHCl_3$) 3574–3330 (OH), 3021–2840, 1622, 1480, 1271, 1158, 1026, 860 cm^{-1}; ^{13}C- and ^{1}H-NMR, see Table 1; FT-ICR MS *m/z* calculated for $C_{20}H_{22}O_6N$ $[M+H]^+$ 372.1488, found 372.1448.

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References

Bister-Miel, F., Agier, C., Bury, M., Postaire, E., Guignard, J. L., & Viel, C. (1986). Biotransformation of papaverine to papaveraldine by *Silene alba* cells. Effects of extraction techniques. *Plant Medicinal Phytotherapy*, *20*(1), 3–7.

Buzas, A. (1974). French Patent. Papaveraldine derivatives. FR 2199462 740412, Application: FR 72-33534 720921, Chemical abstract number, 82, 4448.

Capon, R. J., & Macleod, J. K. (1985). Structural and stereochemical studies on marine norsesterterpene cyclic peroxides. *Tetrahedron*, *41*(16), 3391–3404.

Christinaki, H., Bister-Miel, F., Hammoumi, A., Bury, M., Guignard, J. L., & Viel, C. (1987). Biotransformation of isopapaverine by *Silene alba* cell suspensions. *Phytochemistry*, *26*(11), 2991–2994.

Southon, I. W., & Buckingham, J. (1989). In *Dictionary of Alkaloids* (p. 820). London, New York: Chapman and Hall.

Ayres, D. C., Bycroft, B. W., Collins, P. M., Gunstone, F. D., Harborne, J. B., Haslam, E., Hill, R. A., Kelly, D. R., Leeper, F. J., Murray, R. D. H., & Southon, I. W. (1994). In *Dictionary of Natural Products*, *4* (p. 4440). London, New York: Chapman and Hall.

Dorisse, P., Gleye, J., Loiseau, P., Puig, P., Edy, A. M., & Henry, M. (1988). Papaverine biotransformation in plant cell suspension cultures. *Journal of Natural Products*, *51*(3), 532–536.

El Sayed, K. A. (1998). Microbial biotransformation of veratramine. *Journal of Natural Products*, *61*(1), 149–151.

Herz, W., & Kagan, H.B. (1967). Determination of the absolute configuration of hydroxylated sesquiterpene lactones by Horeau's method of asymmetric esterification. *Journal of Organic Chemistry*, 32(1), 216–218 and references therein.

Hodkova, J., Vesely, Z., Koblicova, Z., Holubek, J., & Trojanek, J. (1972). Alkaloids. Part XXV: Minor alkaloids of poppy capsules. *Lloydia*, *35*(1), 61–68.

Horeau, A. (1977). Determination of the Configuration of Secondary Alcohols by Partial Resolution. In H. B. Kagan, *Stereochemistry, Fundamentals and Methods*, *2* (p. 51). Stutgart: George Thieme.

Lee, I.-S. (1990). Utilization of microbial systems as predictors of mammalian metabolism of the antimalarial drugs arteether and artemisnin. Ph.D. Dissertation, University of Mississippi, University, Mississippi, USA, *Dissertation abstract International* B, 1991, 52(2), 770.

Lee, I.-S., ElSohly, H. N., & Hufford, C. D. (1990). Microbial metabolism studies of the antimalarial drug arteether. *Pharmaceutical Research*, *7*(2), 199–203.

Mahboobi, S., Pongratz, H., & Wiegrebe, W. (1997). Preparation of the alkaloid setigerine. *Pharmazie*, *52*(5), 399.

Orabi, K. Y., Li, E., Clark, A. M., & Hufford, C. D. (1999). Microbial transformation of sampangine. *Journal of Natural Products*, *62*(7), 988–992.

Rideau, M., Morard, P., Gansser, C., Chenieux, J. C., & Viel, C. (1988). Biotransformation and inductive behavior of papaverine in various plant cell lines. Structure of the compounds obtained. *Pharmazie*, *43*(5), 332–334.

Rosazza, J. P., Kammer, M., Youel, L., Smith, R. V., Erhardt, P. W., Truong, D. H., & Leslie, S. W. (1977). Microbial models of mammalian metabolism. *O*-demethylation of papaverine. *Xenobiotica*, *7*(3), 133–143.

Slavik, J., & Slavikova, L. (1996). Alkaloids from *Papaver setigerum* DC. *Collection of Czechoslovak Chemical Communications*, *61*, 1047–1052.

Sugiyama, K., Takada, K., & Fukushima, A., (1988). Japanese Patent. Hair preparations containing vasodilating agents and derivatives of fatty acids or alcohols, JP 63310813 A2 881219 Showa, Application: JP 87-146497 870612, Chemical abstract number 111:201379.

Verdeil, J. L., Bister-Miel, F., Guignard, J. L., & Viel, C. (1986). Papaverine biotransformation by *Silene alba* cell suspension. *Planta Medica*, *1*, 1–3.

Weisbach, J. A., Kirkpatrick, J. L., Macko, E., & Douglas, B. (1968). Synthesis and Pharmacology of some α-keto-,α-hydroxy-, and α-amino-1-benzylisoquinolines. *Journal of Medicinal Chemistry*, *11*(7), 760–764.