Biotransformation of (–)-Caryophyllene Oxide by Cell Suspension Culture of *Catharanthus roseus*

Muhammad Iqbal Choudhary, Zafar A. Siddiqui, Saifullah Khan, Saifullah, Syed G. Musharraf, and Atta-ur-Rahman

H. E. J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi-75270, Pakistan

Reprint requests to Prof. M. I. Choudhary or Prof. S. Khan. E-mail: hej@cyber.net.pk

Z. Naturforsch. **61b,** 197 – 200 (2006); received August 8, 2005

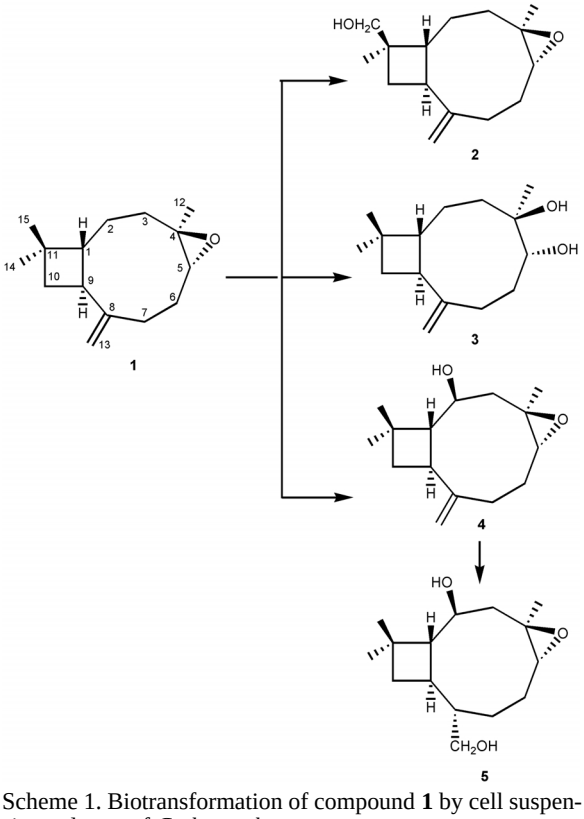
Catharanthus roseus cell suspension cultures were employed for the biotransformation of (–)-caryophyllene oxide (**1**), and four metabolites, 15-hydroxycaryophyllene oxide (**2**), 4β,5α-dihydroxycaryophyll-8(13)-ene (**3**), 2β-hydroxycaryophyllene oxide (**4**), and 2-hydroxy-4,5-epoxycaryophyllan-13-ol (**5**) were obtained. Metabolites **4** and **5** were found to be new compounds, and their structures were deduced by different spectroscopic techniques.

Key words: (–)-Caryophyllene Oxide, Biotransformation, *Catharanthus roseus*, Cell Suspension Cultures

Introduction

Plant cell cultures mediated biotransformation are now increasingly employed by synthetic chemists for the structural modifications of organic compounds. This exhibits a vast biochemical potential for the production of specific secondary metabolites. Plant cells, depending on the chosen conditions, can express enzymes which catalyse different types of transformations in chemical structures [1]. Cell suspension cultures of *Catharanthus roseus* have been commonly employed for the structural modifications of various natural and synthetic products [2 – 5]. Various chemical reactions, such as oxidation, hydroxylation, reduction, isomerization, esterification, and glycosylation [6, 7], were catalysed by *C. roseus* cell culture. In continuation of our studies on biotransformation of bioactive compounds [8 – 13], (–)-caryophyllene oxide (**1**) was incubated with *Catharanthus roseus* cell suspension cultures. This afforded four polar metabolites **2** – **5**, resulting from the *de novo* oxidation.

(–)-Caryophyllene oxide (**1**), a sesquiterpene, is a constituent of many essential oils of traditionally-used folk medicinal plants and spices [14, 15]. It also exhibits a variety of biological activities including potent antimutagenic property [16]. It is chemically synthesized from caryophyllene. Compound **1** has also been used as a flavoring agent. Biotransformation of compound **1** with various fungal strains [17, 18], yeasts [19], and mammals [20], and plant cell suspension cultures [21] have been reported earlier.



Scheme 1. Biotransformation of compound **1** by cell suspension cultures of *Catharanthus roseus*.

Table 1. ^{1}H NMR data[#] of substrate **1** and transformed products **4** and **5** (400 MHz; $CDCl_3$).

H	1	4	5
1β	1.74, t (10)	1.91, t (9.7)	1.96, t (9.9)
2	1.42, m;	3.86, td (10.4, 4.8)	3.85, td (10.0, 5.1)
	1.65, m		
3	2.09, m;	2.45, dd (12.2, 4.8);	2.52, dd (12.0, 5.6);
	0.93, m	1.11, m	1.10, m
5β	2.85, dd (10.6, 4.2)	2.86, dd (10.4, 4.4)	2.93, dd (11.4, 3.6)
6	2.23, ddt (12.0, 8.0,	2.23, m;	2.22, m;
	4.1); 1.32, m	1.32, m	1.17, m
7	2.32, ddd, (12.6, 8.0,	2.34, ddd(12.1, 8.2,	1.48, m;
	4.3); 2.11, m	4.1); 2.10, m	1.22, m
8β	–	–	1.55, m
9α	2.59, dt (9.6, 9.6)	2.60, dt (10.0, 9.4)	2.39, m
10	1.69, m;	1.68, m;	1.45, dd (10.0, 7.7);
	1.62, m	1.59, m	1.22, m
12α	1.18, s	1.21, s	1.26, s
13	4.95, brs;	5.03, brs;	3.33, m (2H)
	4.83, brs	4.91, brs	
14α	0.98, s	1.25, s	1.25, s
15β	0.96, s	1.13, s	1.10, s

[#] Values are given in ppm (coupling constant, J = Hz).

Table 2. ^{13}C NMR data[#] of substrate **1** and transformed products **4** and **5** (100 MHz; $CDCl_3$).

C	1	4	5	C	1	4	5
1	50.8, d	57.1, d	52.3, d	2	27.2, t	72.1, d	72.7, d
3	39.2, t	49.2, t	49.6, t	4	59.8, s	56.7, s	56.4, s
5	63.7, d	63.8, d	65.6, d	6	30.3, t	30.4, t	26.7, t
7	29.8, t	28.9, t	21.5, t	8	151.8, s	151.3, s	39.5, d
9	48.7, d	43.4, d	41.4, d	10	39.8, t	40.8, t	40.1, t
11	34.1, s	34.4, s	34.6, s	12	17.0, q	18.2, q	17.7, q
13	112.8, t	113.4, t	66.8, t	14	21.7, q	22.7, q	22.4, q
15	29.9, q	31.9, q	31.7, q				

[#] Carbon multiplicities were determined by DEPT experiments; s = quaternary, d = methine, t = methylene, q = methyl carbons.

Results and Discussion

(–)-Caryophyllene oxide (**1**), $C_{15}H_{24}O$, was incubated with the cell suspension culture of *Catharanthus roseus* during the screening experiments. It was observed that *C. roseus* cell culture was able to convert compound **1** into several metabolites after 10 days of incubation. Scale-up of this experiment afforded metabolites **2**–**5**. Metabolites **2** ($C_{15}H_{24}O_2$) and **3** ($C_{15}H_{26}O_2$) were identified as known compounds, 15-hydroxycaryophyllene oxide, and 4β,5α-dihydroxycaryophyll-8(13)-ene, respectively. Compound **2** was previously reported as a biotansformed product of compound **1** by *Botrytis cinerea* [17], while compound **3** was reported as a synthetic derivative of 5α-hydroxycaryophyll-8(13)-ene 3, 4-epoxide [22]. Metabolites **4** and **5** were found to be new metabolites. The structures of known metabolites were elucidated through comparison of their reported data [17, 22].

Compound **4** was isolated as a colorless crystalline solid. The HREI-MS exhibited an M^+ at *m/z* 236.1761, corresponding to the formula $C_{15}H_{24}O_2$ (calcd. 236.1776), 16 a.m.u. greater than the parent compound **1**. The IR spectrum exhibited an absorption at 3423 cm^{-1}, indicating the presence of a hydroxyl group. The ^{1}H NMR spectrum of compound **4** showed an additional downfield signal at $\delta = 3.86$ (td, $J_{2\alpha,1\beta;2\alpha,3\beta} = 10.4$ Hz, $J_{2\alpha,3\alpha} = 4.8$ Hz), indicated the presence of an additional OH-group, as compared to substrate **1**. The downfield shifts of H-1 ($\delta = 1.91$ brt, $J_{1\beta=2\alpha,9\alpha} = 9.7$ Hz) and H-3 ($\delta = 2.45$, dd, $J_{3\alpha,3\beta} = 12.2$ Hz, $J_{3\alpha,2\alpha} = 4.8$ Hz) signals, as compared to substrate **1** (Table 1), indicated that the newly introduced hydroxyl group was present at C-2. The presence of an OH at C-2 was also inferred from the COSY 45 ° interactions between H-2 ($\delta = 3.86$), C-3 methylene ($\delta =$ 2.45, 1.11) and C-1 methine ($\delta = 1.91$) protons. The ^{13}C NMR spectra showed the presences of 15 carbons, including four methine, five methylene, three methyl and three quaternary carbons. The new methine carbon was appeared at $\delta = 72.1$ (C-2). The HMBC spectrum showed interactions of H-2 ($\delta = 3.86$) with C-1 ($\delta =$ 57.1), C-3 ($\delta = 49.2$), C-4 ($\delta = 56.7$), and C-9 ($\delta =$ 43.4), while the H-9 ($\delta = 2.60$), H-1 ($\delta = 1.91$), and CH_2-3 ($\delta = 2.45$; 1.11) also showed interactions with C-2 ($\delta = 72.1$), further supporting the position of an OH group at C-2. The configuration of the newly introduced hydroxyl group was assigned to be β on the basis of NOESY correlations between H-2 with H-9α, Me-12α and Me-14α. Thus the structure of the new metabolite was deduced as 2β-hydroxycaryophyllene oxide, resulting from an stereospecific hydroxylation at C-2.

Compound **5** was isolated as colorless crystals. The HREI-MS of compound **5** showed an M^+ at *m/z* at 254.1681 ($C_{15}H_{26}O_3$, calcd. 254.1637). The IR spectrum of **5** did not show any olefinic absorption but showed an absorption for a hydroxyl functionality at 3382 cm^{-1}, in comparison of substrate **1**. The ^{1}H NMR spectrum of compound **5** was found to be substantially different from **1** in many aspects. First, the disappearance of the C-13 exo-methylene olefinic signals, secondly the appearance of an additional oxy-bearing methylene protons as a multiplet at $\delta = 3.33$, and thirdly the appearance of an oxymethine signal at $\delta = 3.85$ (td, $J_{2\alpha,1\beta;2\alpha,3\beta} = 10.0$ Hz, $J_{2\alpha,3\alpha} =$ 5.1 Hz). This indicated an anti-Markonikov hydration of the C-8/C-13 bond and introduction of an OH at C-2,

like compound **4**. The ^{13}C NMR spectrum of **5** (Table 2) also showed a new methine carbon resonance at $\delta = 72.7$, and the disappearance of the signals for exocyclic double bond carbons. Two new carbon signals at $\delta = 39.5$ (C-8) and 66.8 (C-13), have further indicated the hydration of the aforementioned double bond. The 2D NMR interactions (COSY-45° and HMBC) of H-2 were similar as in compound **4**, while the H_2-13 ($\delta = 3.33$) showed homonuclear couplings with H-8 ($\delta = 1.55$), and heteronuclear interactions with C-8 ($\delta = 39.5$), C-7 ($\delta = 21.5$), and C-9 ($\delta = 41.4$). The configuration of the C-2 OH was also found to be same (β) as in compound **4**, based on NOESY correlations of H-2α with H-9α and Me-12α. The H_2-13 showed NOESY correlations with H-9α and Me-14α, which indicated an α-orientation of the hydroxy methylene group. Thus the structure of compound **5** was deduced as $(2S,4R,5R,8S)$-2-hydroxy-4,5-epoxycaryophyllan-13-ol.

Experimental Section

IR Spectra were recorded in $CHCl_3$ on FTIR-8900 spectrophotometer. MPs were determined on Buchi 535 melting point apparatus. Optical rotations were measured on Jasco DIP-360 digital polarimeter. UV Spectra were recorded in MeOH on Hitachi U-3200 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on Bruker Avance- 400 NMR at 400 and 100 MHz, respectively. Chemical shifts (δ) were recorded in ppm, relative to the $SiMe_4$ as an internal standard, while the coupling constants (J) were measured in Hz. The EI-MS and HREI-MS were measured on a Jeol JMS-600H mass spectrometer. TLC were performed on Si gel precoated plates (PF_{254}, 20×20, 0.25 mm, Merck). Compound **1** was purchased from Sigma Aldrich. The compounds were detected on TLC with the help of vanillin spray reagent.

Callus culture

The callus cultures of the plant were derived from young shoot tips cultivated in 300 ml jars, each having 25 ml of Murashige and Skoog media [23], supplemented with sucrose (30 g/l), 2,4-D (1 mg/l), and kinetin (0.5 mg/l), and solidified by agar (8 g/l) at 25 ± 1 °C under complete darkness.

Biotransformation protocol

Cell suspension cultures were derived from static cultured calli in Erlenmeyer flasks (500 ml), each containing 200 ml of the Murashige and Skoog media, supplemented with ingredients as mentioned above, except kinetin and agar. After 15 days of preculturing on a gyratory platform shaker at 100 rpm and 25 ± 1 °C with a 16 h photoperiod, a solution of substrate (40 mg in 1 ml of acetone) was added to each flask through a 0.2 μM membrane filter and the flasks were placed on a shaker for 8 days. The time course study was carried out by taking aliquots from culture on daily basis and the content of transformation was analyzed by TLC. A negative control containing only plant cell suspension cultures and a positive control containing compound **1** in the media were also prepared in order to check the presence of plant metabolites in the cell culture and the chemical changes as a result of chemical reaction (if any) due to media components, respectively.

Extraction and isolation procedure

After 10 days of incubation, the cells and the media were separated by filtration. The filtrate was extracted with CH_2Cl_2 (3×1.5 l) and the cells were extracted in an ultrasonic bath with CH_2Cl_2 (3×500 ml) at r. t. The combined extract were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure which afforded a brown residue (1.2 g). The transformed metabolites were isolated from this gummy crude by column chromatography (silica gel) with petroleum ether/EtOAC gradient, afforded metabolites **2** (8 mg, 4% yield, with petroleum ether/EtOAc 81 : 19), **3** (12 mg, 6% yield, with pet. ether-EtOAc, 75 : 25), **4** (15 mg, 7.5% yield, with pet. ether-EtOAc, 74 : 26), and **5** (9.2 mg, 4.6% yield, with pet. ether-EtOAc, 64 : 36). Some quantities of substrate **1** was also recovered unchanged (51 mg).

15-Hydroxycaryophyllene oxide (**2**). – $[\alpha]_D^{25} = -41.3$ ($c = 0.7$, $CHCl_3$). – IR ($CHCl_3$): $\nu_{max} = 3361$, 2947, 2868, 1447 cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.99$ (br s, 1H, 13-H), 4.87 (br s, 1H, 13'-H), 3.33 (br s, 2H, 15-H), 2.88 (dd, $J = 10.0$ Hz, $J = 4.4$ Hz, 1H, 5-H), 2.65 (dd, $J = 9.4$ Hz, $J = 9.2$ Hz, 1H, 9-H), 2.05 (m, 1H, 1-H), 1.19 (s, 3H, 12-H), 1.04 (s, 3H, 14-H). – ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): $\delta = 47.4$ (C-1), 27.1 (C-2), 36.5 (C-3), 59.8 (C-4), 63.7 (C-5), 30.4 (C-6), 30.0 (C-7), 152.8 (C-8), 48.9 (C-9), 34.5 (C-10), 38.5 (C-11), 16.7 (C-12), 110.3 (C-13), 26.3 (C-14), 71.0 (C-15). – MS (EI, 70 eV): *m/z* (%) = 236(12) [M$^+$], 218(21) [M-H_2O]$^+$, 205(18) [M-CH_2OH]$^+$, 193(26), 187(16), 177(5), 161(31), 121(24), 107(42), 93(89), 79(100), 55(90). – MS (HREI): *m/z* = 236.1731 ($C_{15}H_{24}O_2$, calcd. 236.1776).

4β, 5α-Dihydroxycaryophyll-8(13)-ene (**3**). – M. P. 43 – 44 °C. – $[\alpha]_D^{25} = -71.4$ ($c = 0.5$, $CHCl_3$). – IR ($CHCl_3$): $\nu_{max} = 3127$, 2971, 2861, 1637, 1443 cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.91$ (brs, 1H, 13-H), 4.90 (brs, 1H, 13'-H), 3.57 (t, $J = 5.3$ Hz, 1H, 5-H), 2.35 (m, 1H, 9-H), 1.61 (m, 1H, 1-H), 1.12 (s, 3H, 12-H), 0.98 (s, 3H, 15-H), 0.96 (s, 3H, 14-H). – ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): $\delta = 57.0$ (C-1), 23.2 (C-2), 40.8 (C-3), 75.1 (C-4), 73.4 (C-5), 32.5 (C-6), 34.7 (C-7), 151.8 (C-8),

42.3 (C-9), 36.0 (C-10), 34.1 (C-11), 21.4 (C-12), 110.4 (C-13), 23.2 (C-14), 22.1 (C-15). – MS (EI, 70 eV): *m/z* (%) = 238(4) $[M^+]$, 223(17) $[M\text{-}Me]^+$, 221(34), 205(17), 203(41), 195(12), 177(21), 162(24), 149(31), 147(21), 123(21), 121(54), 109(94), 55(100). – MS (HREI): *m/z* = 238.1921 ($C_{15}H_{26}O_2$, calcd. 238.1934).

2β-Hydroxycaryophyllene oxide (**4**). – M. P. 69 – 70 °C. – $[\alpha]_D^{25} = -57.6$ (c = 0.41, $CHCl_3$). – IR ($CHCl_3$): ν_{max} = 3423, 2941, 2856, 1439 cm^{-1}. – UV/vis ($CHCl_3$): $\lambda_{max}(\log\varepsilon) = 207$ nm (2.4). – ^{1}H NMR (400 MHz, $CDCl_3$): see Table 1. – ^{13}C $\{^1H\}$ NMR (100 MHz, $CDCl_3$): see Table 2. – MS (EI, 70 eV): *m/z* (%) = 236(11) $[M^+]$, 221(16) $[M\text{-}Me]^+$, 203(17) $[M\text{-}Me\text{-}H_2O]^+$, 192(21), 177(27), 159(35), 147(29), 133(30), 119(43), 105(47), 93(100), 79(71), 55(52). – MS (HREI): *m/z* = 236.1761 ($C_{15}H_{24}O_2$, calcd. 236.1766).

2-Hydroxy-4,5-epoxycaryophyllan-13-ol (**5**). – M. P. 94 – 95 °C. – $[\alpha]_D^{25} = -33.7$ (c = 0.4, $CHCl_3$). – IR ($CHCl_3$): ν_{max} = 3382, 2931, 2865 cm^{-1}. – UV/vis ($CHCl_3$): $\lambda_{max}(\log\varepsilon)$ = 193 nm (1.8). – ^{1}H NMR (400 MHz, $CDCl_3$): see Table 1. – ^{13}C $\{^1H\}$ NMR (100 MHz, $CDCl_3$): see Table 2. – MS (EI, 70 eV): *m/z* (%) = 254(2) $[M^+]$, 239(6) $[M\text{-}Me]^+$, 231(11) $[M\text{-}Me\text{-}H_2O]^+$, 223(14) $[M\text{-}CH_2OH]^+$, 207(21), 157(9), 121(12), 107(17), 43(100). – MS (HREI): *m/z* = 254.1681 ($C_{15}H_{26}O_3$, calcd. 254.1637).

Acknowledgements

We gratefully acknowledge financial support of the Higher Education Commission. S. G. Musharraf acknowledges the financial support of the Medics Laboratories, Karachi, Pakistan.

[1] J. Dia, H. Guo, D. Lu, W. Zhu, D. Zhang, J. Zheng, D. Guo, Tettrahedron Lett. **42**, 4677 (2001).
[2] H. Hamada, H. Yasumune, Y. Fuchikami, T. Hirata, I. Sattler, H. J. Williams, A. I. Scott, Phytochemistry **44**, 615 (1997).
[3] J. Balsevich, Planta Medica 128 (1985).
[4] A. Kergomard, M. F. Renard, H. Veschambre, D. Courtois, V. Petiard, Phytochemisty **27**, 407 (1988).
[5] H. Hamada, T. Tanaka, T. Furuya, H. Takahata, H. Nemoto, Tetrahedron Lett. **42**, 909 (2001).
[6] Y. Min, D. Jungui, G. Hongzhu, C. Yajun, G. Dean, J. Zheng, D. Guo, Tetrahedron Lett. **43**, 8535 (2002).
[7] A. Giri, V. Dhingra, C. C. Giri, A. Singh, O. P. Ward, M. L. Narasu, Biotechnol. Adv. **19**, 175 (2001).
[8] M. I. Choudhary, Z. A. Siddiqui, S. G. Musharaf, S. A. Nawaz, Atta-ur-Rahman, Nat. Prod. Res. **19**, 311 (2005).
[9] M. I. Choudhary, S. G. Musharraf, A. Sami, Atta-ur-Rahman, Helv. Chim. Acta **87**, 2685 (2004).
[10] M. I. Choudhary, S. G. Musharraf, R. A. Ali, M. Atif, Atta-ur-Rahman, Z. Naturforsch. **59b**, 319 (2004).
[11] M. I. Choudhary, S. G. Musharraf, M. T. H. Khan, D. Abdelrahman, M. Parvaz, F. Shaheen, Atta-ur-Rahman, Helv. Chim. Acta **86**, 3450 (2003).
[12] M. I. Choudhary, S. G. Musharraf, F. Shaheen, Atta-ur-Rahman, Nat. Prod. Lett. **16**, 417 (2002).
[13] S. G. Musharraf, Atta-ur-Rahman, M. I. Choudhary, S. Sultan, Nat. Prod. Lett. **16**, 345 (2002).
[14] H. Heymann, Y. Tezuka, T. Kikuchi, S. Supriyatna, Chem. Pharm. Bull. **42**, 138 (1994)
[15] R. Tressl, L. Friese, F. Fendesack, H. Koppler, J. Agri. Food. Chem. **26**, 1422 (1978)
[16] J. M. Lee, E. J. Lee, K. N. Bahn, N. Kyeong, J. O. Kim, Y. L. Ha, Han'guk Nonghwa Hakhoechi **38**, 468 (1995).
[17] R. Duran, E. Corrales, R. Hernandez-Galan, I. G. Collado, J. Nat. Prod. **62**, 41 (1999).
[18] W. R. Abraham, L. Ernst, B. Stumpf, Phytochemistry **29**, 115 (1990).
[19] A. J. King, J. R. Dickinson, FEMS Yeast Research **3**, 53 (2003).
[20] Y. Asakawa, T. Ishida, M. Toyota, T. Takemoto, Xenobiotica **16**, 753 (1986).
[21] Y. Orihara, K. Saiki, T. Furuya, Phytochemistry **35**, 635 (1994).
[22] I. G. Collado, J. R. Hanson, P. B. Hitchcock, A. J. Macias-Sanchez, J. Org. Chem. **62**, 1965 (1997)
[23] T. Murashige, F. Skoog, Physiologia Plantarum **15**, 473 (1962).