Pergamon

Tetrahedron Letters, Vol. 38, No. 42, pp. 7357-7360, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)10011-9

Colchicine is Formed by *para-para* Phenol Coupling from Autumnaline

Ulrich H. Maier and Meinhart H. Zenk*

Lehrstuhl für Pharmazeutische Biologie, Universität München,
Karlstrasse 29, D-80333 München, Germany

Abstract: In order to distinguish between *ortho-para* and *para-para* phenol coupling in colchicine biosynthesis, [3'-O^{14}CH$_3$]autumnaline was converted to [^{14}C]colchicine in seeds of *Colchicum autumnale* and the product was then selectively demethylated by a bacterium that exclusively demethylates C-3 of colchicine. The retention of the radioactive label in 3-demethylcolchicine indicates that biosynthesis proceeds through *para-para* coupling. © 1997 Elsevier Science Ltd.

The phenethylisoquinoline alkaloid *(S)*-autumnaline (**1**) was clearly proven to be the pivotal intermediate in the biosynthesis of colchicine (**2**),[1-3] the latter alkaloid possessing a unique tropolone ring system. Autumnaline (**1**) is also a precursor to a considerable number of alkaloidal metabolites other than isoandrocymbine (**3**),[2-4] the phenol coupling product *en route* to colchicine. A highly substrate-specific microsomal-bound cytochrome P-450 NADPH- and O_2-dependent enzyme system is responsible for the intramolecular phenol-oxidative coupling of (**1**) to (**3**) during colchicine (**2**) biosynthesis. It has been previously assumed that this phenol-oxidative coupling proceeds via *para-para* phenol coupling.[5] The structure of (**3**) was based on this assumption. Later, however, doubts were raised about the occurrence of *para-para* coupling during the further study of the biosynthesis of (**2**) and a statement was made that in principle the trioxygenated ring of (**1**) could be rotated 180° around its side chain axis to allow *ortho-para* coupling (Fig. 1) and this point was left for further experimentation.[6] Clearly, both coupling types are realized in alkaloid biosynthesis. An example for *p-p* coupling occurs in the biosynthesis of protostephanine[7] while *o-p* coupling takes place in the biosynthesis of salutaridine in morphine biosynthesis.[8,9]

In order to clarify this point and to verify the structure of (**3**)[3] we set out to solve this problem by synthesizing *(R,S)*-(**1**), selectively ^{14}C-labelled in the methoxy group of the 3'-position of ring C. This intermediate was fed to *Colchicum autumnale* seeds that convert (**1**) in high yield (up to 16%)[4] into (**2**). Since we had earlier discovered[10] a bacterium that exclusively demethylates the methyl group at position 3 of colchicine to yield 3-demethylcolchicine (**5**) (Fig. 2), this simple and mild specific degradation procedure would allow us to distinguish between the *p-p* or the *o-p* pathway in colchicine (**2**) biosynthesis. If the radioactivity in (**5**) is retained, the *p-p* pathway is operative. If the radioactivity is lost, the *o-p* pathway is prevalent. 7-Benzyloxy-1-(5'-benzyloxy-3'-methoxyethoxymethoxy-4'-methoxy-phenethyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (**6**)[11] was synthesized by standard procedures. After removal of the MEM group, the 3'-OH group was methylated by $^{14}CH_2N_2$ and the resulting labelled compound was deprotected (Pd/C; H_2) to

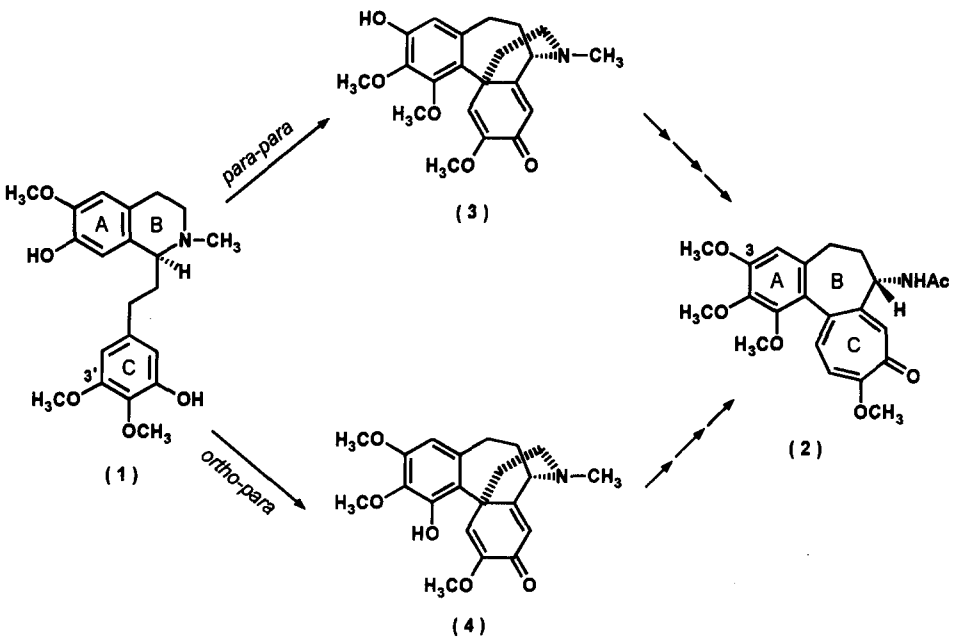


Fig. 1 The biosynthetic formation of colchicine (**2**) from (*S*)-autumnaline (**1**) alternatively via *para-para* coupling involving isoandrocymbine (**3**) or via *ortho-para* with the isomer (**4**) as intermediate.

yield *(R,S)*-[3'-O^{14}CH$_3$]autumnaline (**7**) (spec. act. 12.8x10^6 cpm/μmol) (Fig. 3). This compound was fed to immature *C. autumnale* seeds, having an average weight of about 16 mg per seed.[3,4] The resulting colchicine (**2**) was isolated, purified to constant specific activity and diluted with 1 mg unlabelled (**2**) to yield a final specific activity of 8.42x10^5 cpm/μmol.

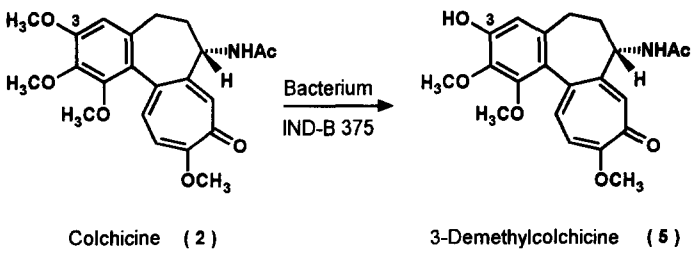
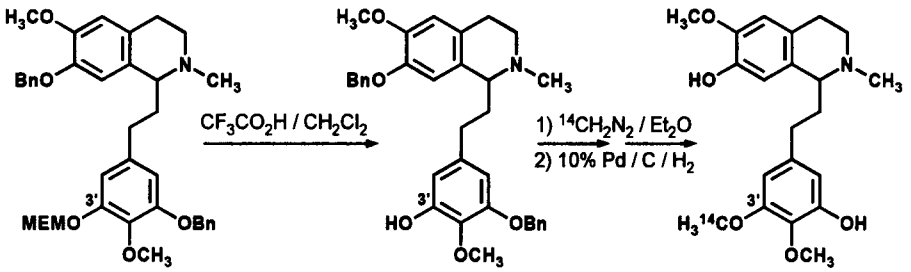


Fig. 2 Regioselective 3-demethylation of colchicine (**2**) to yield 3-demethylcolchicine (**5**) by a bacterial strain.

This biosynthetically labelled (2) was after sterile filtration applied to a growing *Bacillus* IND-B 375 culture in medium 1153.[10] After 24 hrs of incubation about half of the supplied (2) was demethylated to (5) and both alkaloids were separated, purified and their specific activities determined. The reisolated unmetabolized (2) had a specific activity of 8.32×10^5 cpm/μmol while the selectively demethylated product, 3-demethylcolchicine (5), had a specific activity of 8.20×10^5 cpm/μmol, within experimental error the same specific activity as the starting material.



(6) (7)

Fig. 3 Late steps of the synthesis of (*R,S*)-[3'-O^{14}CH$_3$]autumnaline (7).

If the *o-p* oxidative phenolic coupling mechanism (Fig. 1) would have been operating in this plant, all of the radioactivity would have been lost. Since the radioactive label has, however, fully been retained in the demethylated product (5) this clearly demonstrates that the *p-p* coupling pathway is realized in colchicine (2) biosynthesis in *Colchicum* plants. This experiment also establishes that the phenol-coupled intermediate isoandrocymbine (3)[3] must have the structure as depicted in Fig. 1, implying in turn that the recently discovered cytochrome P-450 enzyme from *Colchicum* microsomes is responsible for the *p-p* coupling of (1) to (3).

Acknowledgements: We thank Dr. T. M. Kutchan for linguistic help in the preparation of this manuscript. This work was supported by SFB 369 of the Deutsche Forschungsgemeinschaft, Bonn, and by the Fonds der Chemischen Industrie, Frankfurt/Main.

REFERENCES AND NOTES

1. Battersby, A. R. *Pure and Applied Chemistry* **1967**, 14, 117-136.
2. Herbert, R. B. In *The Chemistry and Biology of Isoquinoline Alkaloids*; Phillipson, J. D.; Roberts, M. F.; Zenk, M. H. Eds.; Springer: Berlin, 1985; pp 213-228.

3. Nasreen, A.; Rueffer, M.; Zenk, M. H. *Tetrahedron Lett.* **1996**, 45, 8161-8164.
4. Nasreen, A.; Gundlach, H.; Zenk, M. H. *Phytochemistry* **1997**, 46, 107-115.
5. Barker, A. C.; Battersby, A. R.; McDonald, E.; Ramage, R.; Clements, J. H. *Chem. Commun.* **1967**, 390-392.
6. Battersby, A. R.; Herbert, R. B.; McDonald, E.; Ramage, R.; Clements, J. H. *J. Chem. Soc. Perkin I* **1972**, 1741-1746.
7. Battersby A. R.; Jones R. C. F.; Minta A.; Ottridge A. P.; Staunton J. *J. Chem. Soc. Perkin I*, **1981**, 2030-2039.
8. Barton, D. H. R.; Kirby, G. W.; Steglich, W.; Thomas, G. M.; Battersby, A. R.; Dobson, T. A.; Ramuz, H. *J. Chem. Soc.* **1965**, 2423-2438.
9. Gerardy, R. and Zenk, M. H. *Phytochemistry* **1993**, 32, 79-86.
10. Poulev, A.; Bombardelli, E.; Ponzone, E.; Zenk, M. H. *J. Fermentation & Bioengineering* **1995**, 79, 33-38.
11. Mp.: 54-55°C (Et_2O); ^1H NMR (360 MHz, $CDCl_3$): δ = 2.29-2.37 (2H, m, H-10), 2.67-2.82 (2H, m, H-3), 2.92-3.05 (2H, m, H-4), 3.12 (3H, s, N-Me), 3.23 (-OMe), 3.26-3.39 (2H, m, H-9), 3.52-3.85 (4H, m, $-OCH_2CH_2O-$), 3.83 (3H, s, 4'-OMe), 3.89 (3H, s, 6-OMe), 4.22 (1H, q, H-1), 4.75 (2H, s, $-OCH_2O-$), 5.09 (2H, s, $ArCH_2O-$), 5.12 (2H, s, $ArCH_2O-$), 6.33 (2H, s, H-5, H-8), 6.62 (1H, d, J = 1.6 Hz, H-2'), 6.89 (1H, d, J = 1.6 Hz, H-6'), 7.20-7.48 (10H, Ar); CI-MS (70 eV): m/z = 628 $(M+H)^+$.

(*Received in Germany* 26 *August* 1997; *accepted* 29 *August* 1997)