

*Tetrahedron*, 1991, 47, 5919

**ISOPENTENYL DIPHOSPHATE ISOMERASE. SITE-DIRECTED MUTAGENESIS OF Cys139 USING "COUNTER" PCR AMPLIFICATION OF AN EXPRESSION PLASMID**

Ian P. Street, Hazel R. Coffman, and C. Dale Poulter\* Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

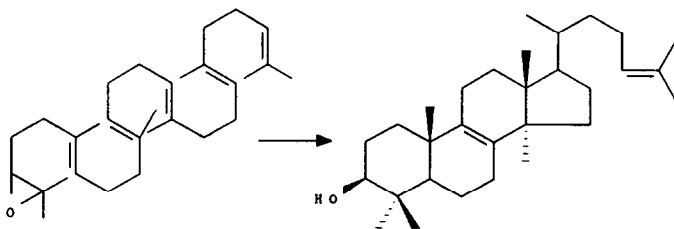
Cys139 is an active-site residue in isopentenyl diphosphate isomerase from *Saccharomyces cerevisiae*. Site-directed mutagenesis was conducted directly on pIPS241, the plasmid used to overproduce wt enzyme using a PCR technique based on two primers. Two mutants, C139A and C139V, were inactive, suggesting that Cys139 is an integral part of the catalytic machinery of isomerase.



**PARTIAL PURIFICATION AND CHARACTERIZATION OF OXIDOSQUALENE-LANOSTEROL CYCLASE FROM BAKER'S YEAST**

Tsutomo Hoshino, Howard J. Williams, Yongseog Chung, and A. Ian Scott  
Center for Biological NMR, Texas A&M University, College Station, TX 77843

Partial (120-fold) purification of oxidosqualene-lanosterol cyclase from yeast and a study of enzymatic properties are described.

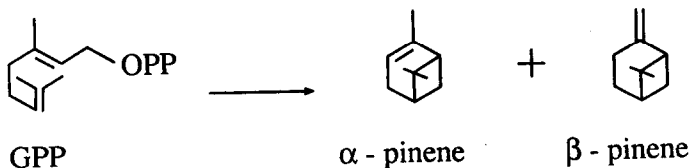


*Tetrahedron*, 1991, 47, 5925

**ISOTOPICALLY SENSITIVE BRANCHING AS A TOOL FOR EVALUATING MULTIPLE PRODUCT FORMATION BY MONOTERPENE CYCLASES**

Kurt C. Wagschal, Thomas J. Savage and Rodney Croteau\*  
Institute of Biological Chemistry, Washington State University,  
Pullman, WA 99164-6340 USA

Analysis of kinetic isotope effects, using deuterium-labeled geranyl pyrophosphate, demonstrated that  $\alpha$ - and  $\beta$ -pinene were synthesized by the same enzyme.



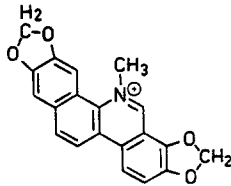
*Tetrahedron*, 1991, 47, 5933

**ENZYMOLGY AND MOLECULAR BIOLOGY OF ALKALOID BIOSYNTHESIS**

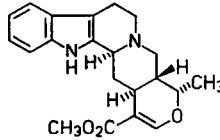
T.M. Kutchan\*, H. Dittrich, D. Bracher and M.H. Zenk\*

*Lehrstuhl für Pharmazeutische Biologie, Universität München, Karlstrasse 29, D-8000 München 2, Germany*

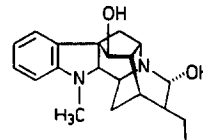
The enzymes (two heterologously expressed) involved in the biosynthetic pathways leading to the benzophenanthridine alkaloid, sanguinarine, and the indole alkaloids, ajmalicine and ajmaline, are presented.



Sanguinarine



Ajmalicine



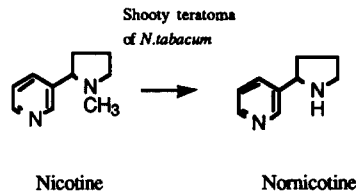
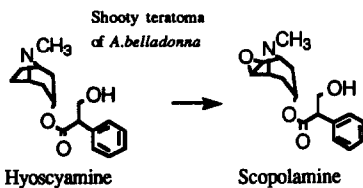
Ajmaline

**METABOLISM OF SOLANACEOUS ALKALOIDS IN TRANSGENIC PLANT TERATOMAS INTEGRATED WITH GENETICALLY ENGINEERED GENES**

Kazuki Saito\*, Mami Yamazaki, Akihiko Kawaguchi<sup>†</sup> and Isamu Murakoshi

Department of Plant Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Chiba University, Chiba 260, Japan, and <sup>†</sup>Department of Biology, The University of Tokyo, Meguro-ku, Tokyo 153, Japan

Transgenic shooty teratomas of *Atropa belladonna* and *Nicotiana tabacum* have ability for oxidative conversion of the alkaloids.

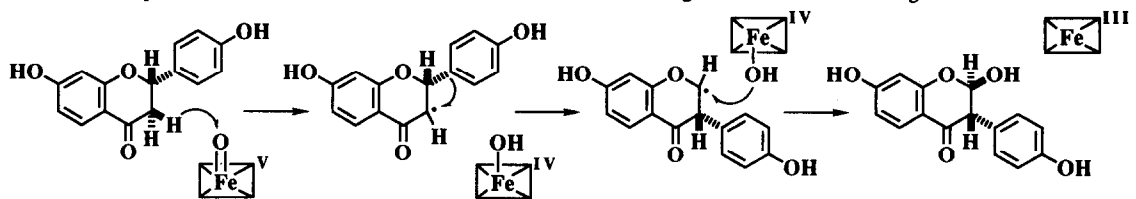


**P-450-DEPENDENT OXIDATIVE REARRANGEMENT IN ISOFLAVONE BIOSYNTHESIS: RECONSTITUTION OF P-450 AND NADPH:P-450 REDUCTASE**

Takashi Hakamatsuka, Muhammed Faisal Hashim, Yutaka Ebizuka and Ushio Sankawa\*

Faculty of Pharmaceutical Sciences, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

The conversion of flavanone into 2-hydroxyisoflavanone and various biosynthetic reactions of natural products can be interpreted as P-450-mediated reactions associated with migration or bond cleavage.

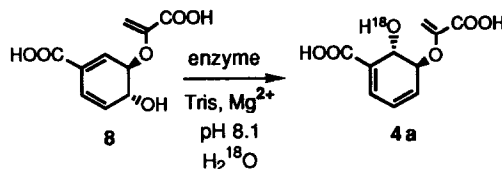


### The Origin of the C-2 Hydroxyl in the Isochorismate Synthase Reaction

Steven J. Gould\* and Rodney L. Eisenberg

Department of Chemistry, Oregon State University, Corvallis, OR 97331-4003

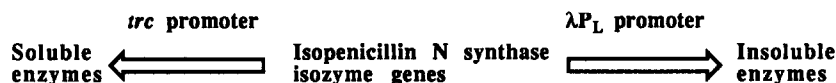
Isochorismate synthase, isolated from *Enterobacter aerogenes* 62-1, converts chorismic acid, **8**, to isochorismic acid, **4a**, with the introduction of the C-2 hydroxyl from water, as revealed by  $^{13}\text{C}$  NMR spectroscopy.



### HIGH-LEVEL SOLUBLE EXPRESSION OF ISOPENICILLIN N SYNTHASE ISOZYMES IN E. COLI.

J.E. Baldwin\*, J.M. Blackburn, J.D. Sutherland and M.C. Wright.

The Dyson Perrins Laboratory and The Oxford Centre for Molecular Sciences,  
South Parks Road, Oxford OX1 3QY, U.K..

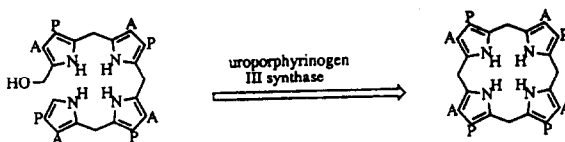


A comparative method for assessing the contribution of enzyme geometry to the product ratios of this occasionally multi-product enzyme is outlined. Genes for various isozymes of Isopenicillin N synthase are expressed to high-level in soluble form in *E. coli*. A novel, *in vivo*, deletion of a promoter from a tandem promoter expression vector is described. A discussion of the effect of differing promoters on enzyme expression is presented.

### UROPORPHYRINOGEN III SYNTHASE: STUDIES ON ITS MECHANISM OF ACTION, MOLECULAR BIOLOGY AND BIOCHEMISTRY

Nigel Crockett, Peter R Alefounder, Alan R Battersby, and Chris Abell.\*

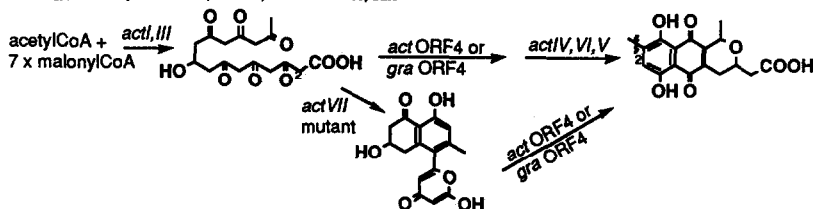
University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, England.



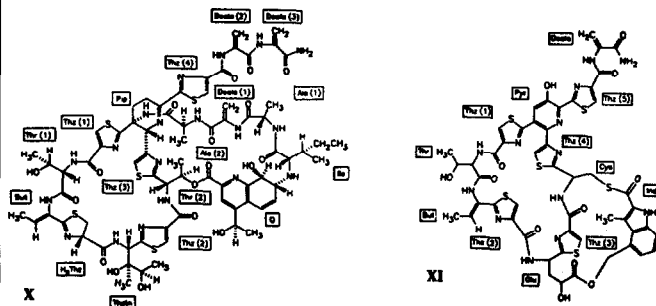
**Molecular Genetic Analysis Reveals a Putative Bifunctional Polyketide Cyclase/Dehydrase Gene from *Streptomyces coelicolor* and *Streptomyces violaceoruber*, and a Cyclase/O-methyltransferase from *Streptomyces glaucescens*.**

David H. Sherman<sup>1,2,\*</sup>, Maureen J. Bibb<sup>1</sup>, Thomas J. Simpson<sup>3</sup>, Darrin Johnson<sup>4</sup>, Francisco Malpartida<sup>5</sup>, Miguel Fernandez-Moreno<sup>5</sup>, Eduardo Martinez<sup>6</sup>, C. Richard Hutchinson<sup>4</sup>, and David A. Hopwood<sup>1</sup>

<sup>1</sup>John Innes Institute, John Innes Centre for Plant Science Research, Norwich NR4 7UH, England, <sup>2</sup>Institute for Advanced Studies in Biological Process Technology, and Department of Microbiology, University of Minnesota, 1479 Gortner Avenue, St. Paul, Minnesota 55108, USA, <sup>3</sup>Department of Chemistry, University of Bristol, Bristol BS81TS, England, <sup>4</sup>Molecular Biology Computing Center, University of Minnesota, 1479 Gortner Avenue, St. Paul, Minnesota, 55108, USA, <sup>5</sup>Centro Nacional de Biotecnología, Serrano 115, 28006 Madrid, Spain, <sup>6</sup>School of Pharmacy and Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706, USA



**GENETIC ENGINEERING OF HYBRID ANTIBIOTICS - A PROGRESS REPORT.** Heinz G. Floss and William R. Strohl, Department of Chemistry, University of Washington, Seattle, WA 98195 and Department of Microbiology, The Ohio State University, Columbus, OH 43210.



Progress in the interspecies cloning of antibiotic biosynthesis genes to produce new hybrid antibiotics is reviewed, and initial efforts to apply this approach to the thiopeptide antibiotics thiostrepton (X) and nosiheptide (XI) are reported.

**THE BIOSYNTHESIS OF SINEFUNGIN: INVESTIGATIONS USING A CELL-FREE SYSTEM**

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Department of Chemistry, Rice University, P.O. Box 1892  
Houston, TX 77251

The origin of the adenylyl moiety of the antifungal antibiotic sinefungin (1) has been investigated by administration of doubly-labeled forms of ATP and adenosine to cell-free extracts of *Streptomyces griseolus*.

