0273-2289/86/1303-0249$02.80

PATENTS AND LITERATURE

ROBERT J. LINHARDT

Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, IA 52242

The objective of this section is to keep readers aware of significant inventions and trends in industrial research, as well as to highlight those areas of research that may lead to new biotechnological opportunities. The topics covered this year included enzymes and cells in organic solvents, applications of polysaccharides, protein engineering, DNA probes for clinical applications, and mammalian cell culture. The subject of this, the last Patents and Literature section of 1986, is microbial transformations and bioconversions.

Microbial Transformations and Bioconversions

Patents

This section identifies and gives a brief description of patents from the US patent literature from January 1982 to June 1986. The major search terms were: bio, enzyme, fungal, yeast, bact, and microb; with the cross terms: transformation(s), conversion(s), metabolism, and degradation. Several specific organisms and enzymes were also searched. Both US patent abstracts and titles were searched. Copies of US Patents can be obtained for $1.50 each from the Commissioner of Patents and Trademarks, Washington, DC 20231.

Cavazza, C.
PROCESS FOR ENZYMATICALLY PRODUCING L-CARNITINE
US 4,371,618, Feb. 1, 1983
Assignee: Sigma-Tau Industrie Farmaceutiche Riunite S.P.A.

An enzymatic process for producing L-carnitine is described in which a solution of gamma-butyrobetaine, sodium-2-oxoglutarate, a reducing agent and a ferrous salt as a hydroxylation catalyst, is contacted with a pure preparation of the mold *Neurospora crassa*.

Chibata, I., Tosa, T., Mori, T., and Fujimura, M.
IMMOBILIZED AMINOACYLASE
US 4,390,626, June 28, 1983
Assignee: Tanabe Seiyaku Co. Ltd.

An immobilized aminoacylase is prepared by binding aminoacylase to a water-insoluble, porous anion exchanger, such as trimethylammonium-substituted styrene resin and trimethylammonium-substituted silica.

Ensley, B. D., Jr.
MICROBIAL PRODUCTION OF INDIGO
US 4,520,103, May 28, 1985
Assignee: Amgen

Microbial synthesis of indigo dyestuff in indole-free media is described. Indigo productioin is accomplished by genetic transformation of selected host cells, having the capacity to produce and accumulate indole, to incorporate the capacity for synthesis of an aromatic dioxygenase enzyme. Growth of transformed cells under suitable conditions facilitates aromatic dioxygenase-catalyzed transformation of cellular indole, with formation of indigo from the oxidized reaction products. For example, *E. coli* having endogenous indole production is transformed with a DNA expression vector containing the structural gene for naphthalene dioxygenase, resulting in the microbial synthesis of indigo.

Fujiwara, M., Fujiwara, A., and Miyamoto, C.
PROCESS FOR THE MANUFACTURE OF HYDROXYLATED STEROIDS
US 4,336,332, June 22, 1982
Assignee: Hoffman-La Roche, Inc.

A process is described for producing hydroxylated steroids by fermenting or reacting a steroid with *Botryodiplodia* sp. or its enzymes. The invention process produces steroid compounds that are pharmacologically valuable substances.

Goldberg, I., and Stieglitz, B.
FERMENTATION PROCESS FOR PRODUCTION OF CARBOXYLIC ACIDS
US 4,564,594, Jan. 14, 1986
Assignee: E. I. Du Pont de Nemours and Co.

An improved fermentation process for producing carboxylic acids, especially fumaric acid, is described. The improvement uses growing fungi *Rhizopus* sp. in the presence of an effective amount of an additive selected from the group consisting of fatty acid (12–24 carbons), esters, and triglyceride mixtures.

Goodhue, C. T., Kydd, G. C., Foster, C. H., and McCombs, C. A.
METHOD OF PREPARING 11-BETA,17-ALPHA,20,21-TETRAHYDROXY STEROIDS AND CORRESPONDING 11-BETA,17-ALPHA,21-TRIHYDROXY-20-OXO STEROIDS
US 4,588,683, May 13, 1986
Assignee: Eastman Kodak Company

A method is described for preparing 11-beta,17-alpha,20,21-tetrahydroxy steroids of the pregnane class. The corresponding 17-alpha,20-alpha or -beta, 21-trihydroxy steroid is incubated in the a culture medium capable of effecting the 11-beta-hydroxylation by a fungal culture, such as the genus *Curvularia*. A method for preparing 11-beta,17-alpha,-21-trihydroxy-20-oxo steroids of the pregnane class is also described. The hydroxylation method described above is followed by conversion of the resulting tetrahydroxy steroid into the corresponding trihydroxy-20-oxo steroid.

Higgins, I. J.
BIOTRANSFORMATIONS USING METHANE-UTILIZING BACTERIA
US 4,323,649, Apr. 6, 1982
Assignee: Imperial Chemical Industries Limited

The partial degradation of complex cyclicorganic compounds, such as 1-phenylheptane and *m*-chlorotoluene is performed using methane-utilizing microorganisms, such as *Methylosinus trichosporium* OB3b (NCIB 11131), or enzyme extracts containing methane mono-oxygenase and/or a dehalogenase.

Hill, F. F., Schindler, J., Schmid, R., Preuss, W., and Struve, A.
PROCESS FOR THE PREPARATION OF DEFECTIVE MUTANTS OF MICROORGANISMS
US 4,362,815, Dec. 7, 1982
Assignee: Henkel Kommanditgesellschaft auf Aktien

The production of 17-C-steroid-alpha-propionic acid compounds by microbial side chain degradation on 17-C-side chain steroid substrates is described. Mutant strains are capable of supplying the desired compounds, e.g., delta-4 BNC and/or delta-1,4 BNC in high yields even in the absence of inhibitors that arrest the growth and degradation of the steroid ring. Suitable wild strains and mutants and their choice, production, and use, are described.

Hsieh, J. H.
CONTINUOUS FERMENTATION PROCESS AND BIOCONVERSION-PRODUCT RECOVERY
US 4,480,034, Oct. 30, 1984
Assignee: Celanese Corporation

A continuous bioconversion process is described, which employs a cross-flow membrane filtration zone to recover the whole cell-containing retentate stream and the cell-free bioconversion product-containing permeate stream. The retentate stream is recycled to the fermentation zone. In a specific embodiment, toluene is bio-oxidized to muconic acid with *Pseudomonas putida* Biotype A (ATCC 31,916). The muconic acid is recovered as a precipitate from the cell-free permeate fermentation broth, and the fermentation broth is recycled in the process.

Hsieh, J. H., Barer, S. J., and Maxwell, P. C.
MUCONIC ACID PRODUCTIVITY BY A STABILIZED MUTANT MICROORGANISM POPULATION
US, 4,535,059, Aug. 13, 1985
Assignee: Celanese Corp.

An improved fermentation process for the bioconversion of toluene to muconic acid is described. The bioconversion system is operated under phosphate-limiting conditions to achieve an increase in specific muconic acid productivity by a stabilized population of *Pseudomonas putida* Biotype A (ATCC 31,916).

Imada, Y., Osozawa, T., Morimoto, Y., and Kinoshita, M.
PROCESS FOR PREPARING ANDROSTANE STEROIDS
US 4,397,946, Aug. 9, 1983
Assignee: Mitsubishi Chemical Industries Ltd.

Androstane steroids are prepared by the microbiological conversion of a sterol substrate with *Mycobacterium* sp., in which the medium used contains at least 0.1 wt% egg yoke.

Knight, J. C., and Wovcha, M. G.
MYCOBACTERIUM FORTUITUM STRAIN
US 4,329,432, May 11, 1982
Assignee The Upjohn Co.

Novel compounds prepared by microbial transformation using mutants to selectively degrade steroids, some with 17-alkyl side chains (2–10 carbon atoms). These compounds can be used as intermediates to make useful steroids.

Knight, J. C., and Wovcha, M. G.
PROCESS FOR PREPARING AN INDENEDIONE AND A MYCOBACTERIUM CULTURE THEREFOR
US 4,443,541, Apr. 17, 1984
Assignee: The Upjohn Co.

The microbiological conversion of steroids to (2+)-(7aS) 2,3,7,7-tetrahydro-7a-methyl-(1-H)-indene-1,5(6-H)-dione (indenedione) is described.

Kominek, L. A., and Wolf, H. J.
PROCESS FOR PREPARING 1,2-DEHYDRO STEROIDS
US 4,524,134, June 18, 1985
Assignee: The Upjohn Co.

An improved microbial bioconversion to produce 1,2-dehydro steroids from their corresponding 1,2-saturated derivatives is described.

Krasnobajew, V.
MICROBIOLOGICAL TRANSFORMATIONS OF IONONE COMPOUNDS
US 4,311,860, Jan. 19, 1982
and
US 4,390,556, June 28, 1983
and
US 4,390,557, June 28, 1983
and
US 4,402,989, Sept. 6, 1983
Assignee: Givaudan Corp.

Novel odorant and/or flavorant mixtures are prepared by fermentation of ionone type compounds with microorganisms of the genera *Botryodiplodia, Botryosphaeria,* or *Lasiodiplodia.*

Kula, M. R., Hummel, W., Schutte, H., and Leuchtenberger, W.
MICROBIOLOGICALLY PRODUCED L-PHENYLALANINE-DEHYDROGENASE, PROCESS FOR ITS RECOVERY AND USE
US 4,590,161, May 20, 1986
Assignee: Degussa Aktiengesellschaft; Gesellschaft fur biotechnologisch Forschung

A microbiologically produced L-phenylalanine-dehydrogenase and a process for its recovery from *Brevibacterium* sp. (DSM 2448) is described. The new enzyme can be used for the enzymatic conversion of phenyl pyruvic acid *p*-hydroxyphenyl pyruvic acid, indolyl pyruvic acid, or 2-keto-4-(methylmercapto)-butyric acid into the corresponding L-alpha-aminocarboxylic acids.

Kurtzman, C. P., Bothast, R. J., and VanCauwenberge, J. E.
CONVERSION OF DXYLOSE TO ETHANOL BY THE YEAST *PACHYSOLEN TANNOPHILUS*
US 4,359,534, Nov. 16, 1982
Assignee: The United States of America as represented by the Secretary of Agriculture

A method is described for converting D-xylose to ethanol relying on the unique ability of the yeast *Pachysolen tannophilus* to ferment this 5-carbon sugar without the use of added enzymes. This process will be particularly useful in the production of ethanolic fuel from plant biomass.

Manecke, G., and Klussman, U.
POLYMER-CONTAINING BIOCATALYST
US 4,546,078, Oct. 8, 1985
Assignee: Schering Aktiengesellschaft

A biocatalyst is described, which contains microorganisms, such as *Arthrobacter simplex, Aspergillus ochraceus, Bacillus sphaericus, Curvularia lunata, Flavobacterium dehydrogenans, Mycobacterium* sp., or *Saccharaomyces uvarum,* immobilized on a copolymer of acrolein and 1-vinyl-2-pyrrolidone, cross-linked by reaction with an alkylenedioxydiamine. The preparation of steroids is also described.

Marsheck, W. J., Jiu, J., and Wang, P. T.
MICROBIAL PROCESS FOR 9-ALPHA-HYDROXYLATION OF STEROIDS
US 4,397,947, Aug. 9, 1983
Assignee: G. D. Searle & Co.

Valuable 9-alpha-hydroxy steroids are prepared via microbial enzymatic oxidation by fermentation utilizing *Nocardia canicruria* (ATCC 31548) without the need for the delta-(1)dehydrogenation inhibitor. A method using any delta-(1)dehydrogenase producing organism and a novel bioreactor technique is also described for preparing these steroids.

Maxwell, P. C.
PRODUCTION OF MUCONIC ACID
US 4,355,107, Oct. 19, 1982
Assignee: Celanese Corp.

The microbiological oxidation of toluene to muconic acid is achieved with strains of *Pseudomonas putida* Biotype A, which are capable of converting toluene to muconic acid quantitatively by the *ortho* (beta-ketoadipate) pathway. Munonate lactonizing enzyme is not induced in the microorganism, permitting the muconic acid to be produced and accumulated at a concentration of over 1 g/L.

Maxwell, P. C.
PROCESS FOR THE PRODUCTION OF MUCONIC ACID
US 4,588,688, May 13, 1986
Assignee: Celanese Corp.

A process is described for bioconversion of an organic substrate, such as ethylbenzene or catechol, to muconic acid. A procedure is provided for constructing novel strains of microorganisms, such as *Pseudomonas putida* (Biotype A), capable of converting an organic substrate to muconic acid quantitatively by the *ortho* (catechol 1,2-oxygenase) pathway. Muconate lactonizing enzyme is not induced in the microorganisms, permitting the muconic acid to be produced and accumulated at a concentration of over 1 g/L.

McCullough, J. E.
METHOD OF INCREASED PRODUCTION OF PENICILLIN ACYLASE AND PLASMID EMPLOYED THEREIN
US 4,554,250, Nov. 19, 1985
Assignee: E. R. Squibb & Sons, Inc.

A method is described for enhancing penicillin acylase production. A *Bacillus subtilis* plasmid containing an insert of a chromosomal DNA fragment of *Bacillus megaterium,* which includes the appropriate gene for enhancing penicillin acylase production, is cloned into *B. megaterium.* The *B. megaterium* is then employed in the production of penicillin acylase.

Mitchell, T. G., Barnes, A. G., Jackson, J. S., and Bevan, P. C.
SMOKE FLAVOR ENHANCING AGENTS
US 4,441,514, Apr. 10, 1984
Assignee: British-American Tobacco Co. Ltd.

A method for enhancing the smoke flavor of a smoking material by treating it with an agent comprising 3-hydroxysclareol, particularly with the compound 3-beta-hydroxysclareol. The smoke-enhancing agent is prepared by microbial transformation of sclareol.

Oki, T., Yoshimoto, A., Matsuzawa, Y., Inui, T., Takeuchi, T., and Umezawa, H.
RHODAMYCIN ANTIBIOTICS
US 4,316,011, Feb. 16, 1982
Assignee: Sanraku-Ocean Co. Ltd.

New anthracycline glycoside derivatives of rhodomycin-group, epsilon-rhodomycin RDC, epsilon-isorhodomycin RDC, beta-rhodomycin RDC, gamma-rhodomycin RDC, gamma-rhodomycin RDRs, and beta-pyrromycin RDC having potent anticancer activities and reduced toxicities and

a process for their production by microbiological conversion are described.

Oki, T., Yoshimoto, A., Kouno, K., Inui, T., Takeuchi, T., and Umezawa, H.
PROCESS FOR PRODUCING ANTHRACYCLINE GLYCOSIDES
US 4,337,312, June 29, 1982
Assignee: Sanraku-Ocean Co. Ltd.

A process for producing daunomycin and baumycins having potent antitumor activity and low toxicity by the microbial conversion of anthracyclinones, such as aklavinone and epsilon-rhodomycinone, is described.

Oki, T., Yoshimoto, A., Kouno, K., Inui, T., Takeuchi, T., and Umezawa, H.
2-HYDROXYACLACINOMYCIN A AND 2-HYDROXYAKLAVINONE AND PROCESS FOR PREPARING SAME
US 4,386,198, May 31, 1983
Assignee: Sanraku-Ocean Co. Ltd.

The production of new anthracycline compounds, such as, 2-hydroxyaclacinomycin A, having potent antitumor activity and reduced toxicity, and 2-hydroxyaklavinone as a useful precursor for producing anthracycline glycosides by microbial conversion are described.

Sawada, Haruji, and Taguchi, H.
METHOD FOR PRODUCTION OF URSODEOXYCHOLIC ACID BY MEANS OF MICROBIAL TRANSFORMATION
US 4,579,819, Apr. 1, 1986
Assignee: Kabushiki Kaisha Yakult Honsha

A one-step method of producing ursodeoxycholic acid by microbial transformation is described in which lithocholic acid is subjected to the action of a ursodeoxycholic acid-producing microorganism.

Seely, R. J.
PRODUCT AND PROCESS FOR STIMULATING BACTERIAL ACTION IN AN ANAEROBIC DIGESTION SYSTEM
US 4,529,701, July 16, 1985
Assignee: American Genetics International, Inc.

A method for stimulating bacterial action in an anaerobic digestion system is described. The product includes an inhibitory-ion-regulating component and an inorganic-pyrophosphate-containing compound. The process includes forming and then adding a mixture of this product to the anaerobic digestion system to stimulate and enhance bacterial growth and metabolism.

Sih, C. J.
PROCESS FOR PREPARING STEROIDS
US 4,444,884, Apr. 24, 1984
Assignee: Wisconsin Alumni Research Foundation

A method for enhancing the degradation of the side chain of sterols with branched chains at C-24 utilizing microbiological means is described, in which an exogenous source of bicarbonate ion is included in the medium in which the degradation is carried out.

Sonoyama, T., Kageyama, B., and Honjo, T.
PROCESS FOR PRODUCING 2-KETO-L-GULONIC ACID
US REISSUE 30,872, Feb. 23, 1982
Assignee: Shionogi & Co. Ltd.

2-Keto-L-gulonic acid is prepared from 2,5-diketo-D-gluconic acid through microbial conversion. The 2-Keto-L-gulonic acid-producing microorganism used for this microbial conversion includes strains belonging to genera of *Brevibacterium, Arthrobacter, Micrococcus, Staphylococcus, Pseudomonas,* and *Bacillus.* Both the incubation of the microorganism in a medium containing 2,5-diketo-L-gluconic acid and the direct contact of any products obtained from the cells with 2,5-diketo-D-gluconic acid may be used.

Teichmuller, G., Rabe, J., and Henkel, H.
PROCESS FOR THE SEPARATION OF 4-ANDROSTEN-3,17-DIONE AND 1,4-ANDROSTADIEN-3,17-DIONE
US 4,474,701, Oct. 2, 1984
Assignee: Veb Jenapharm Jena

The separation of 4-androsten-3,17-dione and 1,4-androstadien-3,17-dione mixtures, which are produced, upon microbiological sterol-side chain decomposition, is described. The separation of the products results in high yields and in sufficient purity for further work up in the synthesis of androstane and pregnane derivatives, as well as estratrienes.

Terahara, A., and Tanaka, M.
ML-236B DERIVATIVES AND THEIR PREPARATION
US 4,346,227, Aug. 24, 1982
and
US 4,410,629, Oct. 18, 1983
Assignee: Sankyo Co. Ltd.

Hydroxyacids and the corresponding ring-closed lactones, salts, and esters may be prepared by subjecting ML-236B or ML-236B carboxylic acid or a salt or ester to enzymatic hydroxylation, effected by microorganisms of the genera *Mucor, Rhizopus, Zygorynchus, Circinella, Actinomucor, Gongronella, Phycomyces, Martierella, Pycnoporus, Rhizoctonia, Absidia, Cunning-*

hamella, Syncephalastrum, and *Streptomyces,* or their cell-free, enzyme-containing extracts. The compounds are capable of inhibiting biosynthesis of cholesterol and are thus useful in the treatment of hypercholesteraemia.

Terahara, A., and Tanaka, M.
ML-236B DERIVATIVES
US 4,447,626, May 8, 1984
Assignee: Sankyo Company Ltd.

Assignee: Sankyo Company Ltd.

6-Methoxy-IsoML-236B lactone and its corresponding free hydroxy-carboxylic acid, salts, and esters may be prepared by the enzymatic alkoxylation of ML-236B, using a microorganism of the genus *Syncephalastrum, Absidia (Absidia coeruleaor),* or *Cunninghamella,* or a cell-free enzyme-containing extract. If desired, the lactone or carboxylic acid may be converted by conventional salification or esterification techniques to the desired salt or ester. These compounds have the ability to inhibit the biosynthesis of cholesterol and are of value in the treatment of hypercholesteraemia.

Terahara, A., and Tanaka, M.
ML-236B DERIVATIVES
US 4,448,979, May 15, 1984
Assignee: Sankyo Co. Ltd.

Hydroxyacids and the corresponding ring-closed lactones, salts, and esters may be prepared by subjecting ML-236B or ML-236B carboxylic acid or a salt of ester to enzymatic hydroxylation, effected by means of microorganisms of the genera *Mucor, Rhizopus, Zygorynchus, Circinella, Actinomucor, Gongronella, Phycomyces, Martierella, Pycnoporus, Rhizoctonia, Absidia, Cunninghamella, Syncephalastrum,* and *Streptomyces,* or cell-free, enzyme-containing extracts. The compounds are capable of inhibiting biosynthesis of cholesterol and are useful in the treatment of hypercholesteraemia.

Turner, J. R., Krupinski, V. M., Fukuda, D. S., and Baltz, R. H.
PROCESS FOR PREPARING MACROCIN DERIVATIVES
US 4,559,301, Dec. 17, 1985
Assignee: Eli Lilly and Co.

New ester derivatives are prepared by bioconversion of macrocin or lactenocin with an acylating enzyme system produced by *Streptomyces thermotolerans* strains, have improved activity against *Mycoplasma* species.

Udvardy, N., Cserey, P., Bartho, I., Hantos, G., Trinn, M., Vida, Z., Szejtli, J., Stadler, E., Szoke, A., Habon, I., Bal, E., and Czurda, M. E.

PROCESS FOR THE INTENSIFICATION OF MICROBIOLOGICAL CONVERSIONS OF STEROIDS USING CYCLODEXTRIN ADDITIVES

US 4,528,271, July 9, 1985

Assignee: Richter Gedeon Vegyeszeti Gyar Rt.; Chinoin Gyogyszer- Es Vegyeszeti Termekek Gyara Rt.

Microbiological conversions are intensified by adding to the reaction mixture 0.2–3 mol of alpha-, beta-, or gamma-cyclodextrin or an optical mixture per mole of steroid substrate. The cyclodextrin, if desired, can be removed after the reaction. In this way, the reaction velocity can be increased, the reaction time is reduced, the substrate concentration in the solution, i.e., its solubility, is increased, or the product inhibition may be avoided. In certain cases, a desired reaction can be catalyzed and in this manner selectivity is increased.

Weber, A., Kennecke, M., and Muller, R.

PROCESS FOR THE PREPARATION OF 11-BETA,21-DIHYDROXY-2'-METHYL-5'BETA H-1,4-PREGNADIENO(16,17-D)-OXAZ OLE-3,20-DIONE

US 4,431,732, Feb. 14, 1984

Assignee: Schering Aktiengesellschaft

A process is described for preparing 11-beta,21-dihydroxy-2'-methyl-5'beta H-1,4-pregnadieno(16,17-D)-oxazole-3,20-di one, by fermenting 11-BETA,21-dihydroxy-2'-methyl-5'beta H-4-pregneno (16,17-d)-oxazole-3,20-dione with a living culture of *Arthrobacter simplex.*

Wovcha, M. G., and Biggs, C. B.

MICROORGANISM MUTANT CONVERSION OF STEROLS TO ANDROSTA-4-ENE-3,17-DIONE

US 4,345,030, Aug. 17, 1982

Assignee: The Upjohn Co.

A mutant is used in a microbiological process to selectively degrade steroids having 17-alkyl side chains (2–10 carbon atoms) to yield predominantly androst-4-ene-3,17-dione (AD) and small amounts of androsta-1,4-diene-3,17-dione. AD is a valuable intermediate to make useful steroids.

Zeikus, J. G., and Lamed, R. J.

PREPARATION OF A NOVEL NADP-LINKED ALCOHOL-ALDEHYDE/KETONE OXIDOREDUCTASE FROM THERMOPHILIC ANAEROBIC BACTERIA FOR ANALYTICAL AND COMMERCIAL USE

US 4,352,885, Oct. 5, 1982

Assignee: Wisconsin Alumni Research Foundation

A partially purified NADP-specific thermostable oxidoreductase is prepared, which can react with a wide range of alcohols, ketones, and aldehydes. The enzyme has a unique preference for secondary over primary alcohols. The thermostability, broad range of operating temperatures, and lack of sensitivity to metal ions and complexing agents, in addition to the absolute specificity for the coenzyme, increases the utility of the enzyme in asymmetric organic synthesis and NADPH regeneration.

Literature

This section surveys the literature in the area of microbial transformations and bioconversions published from January 1984–July 1986. This section lists all major articles and reviews that appeared during this time period.

REFERENCES

1. Abraham, W. R., Hoffmann, H. M., Kieslich, K., Reng, G., and Stumpf, B. (1985), Microbial transformations of some monoterpenoids and sesquiterpenoids. *Ciba. Found. Symp.* **111,** 146–160.
2. Aly, A., Johansson, C., Slezak, P., and Green, K. (1984), Bioconversion of arachidonic acid in the human gastrointestinal tract. *Biochem. Med.* **31,** 319–331.
3. Anderson, B. D., Conradi, R. A., Spilman, C. H., and Forbes, A. D. (1985), Strategies in the design of solution-stable, water-soluble prodrugs III: influence of the pro-moiety on the bioconversion of 21-esters of corticosteroids. *J. Pharmacol. Sci.* **74,** 382–387.
4. Anderson, B. D., Conradi, R. A., and Knuth, K. E. (1985), Strategies in the design of solution-stable, water-soluble prodrugs I: a physical-organic approach to pro-moiety selection for 21-esters of corticosteroids. *J. Pharmacol. Sci.* **74,** 365–374.
5. Beale, J. M., Jr., Chapman, R. L., and Rosazza, J. P. (1984), Studies on the biosynthesis of terrecyclic acid A, an antitumor antibiotic from *Aspergillus terreus*. *J. Antiobiot.* (Tokyo) **37,** 1376–1381.
6. Belic, I., Kastelic-Suhadolc, T., and Kralj, B. (1985), Bioconversion of steroid glycosides by *Nocardia restricta*. *J. Steroid Biochem.* **23,** 323–326.
7. Cerniglia, C. E., Miller, D. W., Yang, S. K., and Freeman, J. P. (1984), Effects of a fluoro substituent on the fungal metabolism of 1-fluoronaphthalene. *Appl. Environ. Microbiol.* **48,** 294–300.
8. Cerniglia, C. E., Freeman, J. P., White, G. L., Heflich, R. H., and Miller, D. W. (1985), Fungal metabolism and detoxification of the nitropolycyclic aromatichydrocarbon 1-nitropyrene. *Appl. Environ. Microbiol.* **50,** 649–655.
9. Cerniglia, C. E., White, G. L., and Heflich, R. H. (1985), Fungal metabolism and detoxification of polycyclic aromatic hydrocarbons. *Arch. Microbiol.* **143,** 105–110.
10. Cerniglia, C. E., Kelly, D. W., Freeman, J. P., and Miller D. W. (1986), Microbial metabolism of pyrene. *Chem. Biol. Interact.* **57,** 203–216.
11. Cerniglia, C. E., and Yang, S. K. (1984), Stereoselective metabolism of an-

thracene and phenanthrene by the fungus *Cunninghamella elegans*. *Appl. Environ. Microbiol.* **47,** 119–124.
12. Cerniglia, C. E., Lambert, K. J., Miller, D. W., and Freeman, J. P. (1984), Transformation of 1- and 2-methylnaphthalene by *Cunninghamella elegans*. *Appl. Environ. Microbiol.* **47,** 111–118.
13. Clark, A. M., Hufford, C. D., Gupta, R. C., Puri, R. K., and McChesney, J. D. (1984), Microbial transformation of primaquine by *Candida tropicalis*. *Appl. Environ. Microbiol.* **47,** 537–539.
14. Clark, A. M., Hufford, C. D., Puri, R. K., and McChesney, J. D. (1984), Production of a novel dimeric metabolite of primaquine by *Streptomyces rimosus*. *Appl. Environ. Microbiol.* **47,** 540–543.
15. Davis, P. J., Abdel, M. H., Trainor, T. M., Vouros, P., and Neumeyer, J. L. (1985), Stereospecific microbiological 10-*O*-demethylation of R-(-)-10,11-dimethoxyaporphines. *Biochem. Biophys. Res. Commun.* **127,** 407–412.
16. Davis, P. J., Yang, S. K., and Smith, R. V. (1984), Microbial models of mammalian metabolism: microbial reduction and oxidation of pentoxifylline. *Appl. Environ. Microbiol.* **48,** 327–331.
17. Davis, P. J., Yang, S. K., and Smith, R. V. (1985), Microbial models of mammalian metabolism: Stereospecificity of ketone reduction with pentoxifylline. *Xenobiotica* **15,** 1001–1010.
18. Dugonski, J., Sedlaczek, L., and Jaworski, A. (1984), Protoplast release from fungi capable of steroid transformation. *Can. J. Microbiol.* **30,** 57–62.
19. Eckardt, K., Schumann, G., Grafe, U., Ihn, W., Wagner, C., Fleck, W. F., and Thrum, H. (1985), Preparation of labeled aklanonic acid and its bioconversion to anthracyclinones by mutants of *Streptomyces griseus*. *J. Antibiot.* (Tokyo) **38,** 1096–1097.
20. Ferdinandi, E. S., Sehgal, S. N., Demerson, C. A., Dubuc, J., Zilber, J., Dvornik, D., and Cayen, M. N. (1986), Disposition and biotransformation of ^{14}C-etodolac in man. *Xenobiotica* **16,** 153–166.
21. Goswami, P. C., Singh, H. D., and Baruah, J. N. (1984), Factors limiting the microbial conversion of sterols to 17-ketosteroids in the presence of metal chelate inhibitors. *Folia Microbiol.* (Praha) **29,** 209–216.
22. Heitkamp, M. A., Freeman, J. P., McMillan, D. C., and Cerniglia, C. E. (1985), Fungal metabolism of tert-butylphenyl diphenyl phosphate. *Appl. Environ. Microbiol.* **50,** 265–273.
23. Ipsen, J., and Rosazza, J. P. (1984), Microbial transformations of natural antitumor agents, 25. Conversions of 3-ketoaphidicolin. *J. Natl. Prod.* **7,** 497–503.
24. Itoh, S., Odakura, Y., Kase, H., Satoh, S., Takahashi, K., Iida, T., Shirahata, K., and Nakayama, K. (1984), Biosynthesis of astromicin and related antibiotics. I. Biosynthetic studies by bioconversion experiments. *J. Antibiot.* (Tokyo) **37,** 1664–1669.
25. Jones, J. B. (1985), An illustrative example of a synthetically useful enzyme: horse liver alcohol dehydrogenase. *Ciba Found. Symp.* **111,** 3–21.
26. Kieslich, K. (1986), The production of drugs by microbial biosynthesis and biotransformation. *Arzneim.-Forsch./Drug Res.* **36,** 774–778.
27. Kieslich, K. (1985), Microbial side-chain degradation of sterols. *J. Basic Microbiol.* **25,** 461–474. (REVIEW).
28. Magee, R. J., and Kosaric, N. (1985), Bioconversion of hemicellulosics. *Adv. Biochem. Eng. Biotechnol.* **32,** 61–93. (REVIEW).
29. Mahato, S. B., Banerjee, S., and Sahu, N. P. (1984), Metabolism of progesterone and testosterone by a *Bacillus* sp. *Steroids* **43,** 545–558.

30. Mahato, S. B., and Mukherjee, A. (1984), Microbial transformation of testosterone by *Aspergillus fumigatus*. *J. Steroid. Biochem.* **21,** 341–342.
31. Monticello, D. J., Bakker, D., and Finnerty, W. R. (1985), Plasmid-mediated degradation of dibenzothiophene by *Pseudomonas* species. *Appl. Environ. Microbiol.* **49,** 756–760.
32. Murphy, G. L., and Perry, J. J. (1984), Assimilation of chlorinated alkanes by hydrocarbon-utilizing fungi. *J. Bacteriol.* **160,** 1171–1174.
33. Nakagawa, M., Kawai, H., Hayakawa, Y., Seto, H., and Otake, N. (1985), 4-*O*-(beta-D-Glucopyranosyl)-epsilon-rhodomycinone, a new microbial transformation production of rhodomycinone. *J. Antibiot.* (Tokyo) **38,** 1622–1624.
34. Okuda, T., and Furumai, T. (1985), Penitricin, a new class of antibiotic produced by *Penicillium aculeatum*. IV. *Biosyn. J. Antibiot.* (Tokyo) **38,** 631–635.
35. Richardson, K. E., Hagler, W. M. Jr., and Hamilton, P. B. (1984), Bioconversion of alpha-[^{14}C]zearalenol and beta-[^{14}C]zearalenone into [^{14}C]zearalenone by *Fusarium roseum* 'Gibbosum,' *Appl. Environ. Microbiol.* **47,** 1206–1209.
36. Sariaslani, F. S., and Rosazza, J. P. (1985), Biotransformations of 1',2'-dihydrorotenone by *Streptomyces griseus*. *Appl. Environ. Microbiol.* **49,** 451–452.
37. Sariaslani, F. S., Beale, J. M., Jr., and Rosazza, J. P. (1984), Oxidation of rotenone by *Polyporus anceps* laccase. *J. Natl. Prod.* **47,** 692–697.
38. Wagner, C., Eckhardt, K., Schumann, G., Ihn, W., and Tresselt, D. (1984), Microbial transformation of aklanonic acid, a potential early intermediate in the biosynthesis of anthracyclines. *J. Antibiot.* (Tokyo) **37,** 691–692.
39. Wang, Y. F., Chen, C. S., Girdaukas, G., and Sih, C. J. (1985), Extending the applicability of esterases of low enantioselectivity in asymmetric synthesis. *Ciba Found. Symp.* **111,** 128–145.
40. Williamson, J., Van-Orden, D., and Rosazza, J. P. (1985), Microbiological hydroxylation of estradiol: formation of 2- and 4-hydroxyestradiol by *Aspergillus alliaceus*. *Appl. Environ. Microbiol.* **49,** 563–567.
41. Yu, E. K., Deschatelets, L., Louis-Seize, G., and Saddler, J. N. (1985), Butanediol production from cellulose and hemicellulose by *Klebsiella pneumoniae* grown in sequential coculture with *Trichoderma harzianum*. *Appl. Environ. Microbiol.* **50,** 924–929.