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Microbial physiology of sidechain degradation of sterols

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SUMMARY

A large number of valuable starting materials for steroid synthesis (e.g. 4-androstene-3,17-dione, 1,4-androstadiene-3,17-dione, 9α-hydroxy-4-androsten-17-one) have been produced by microbial transformation methods. This review helps to evaluate the microbial physiological interest of the widely used sterol sidechain degradation processes. Four inducible groups of the catabolic enzymes are involved in the sterol sidechain degradation pathway; the fatty acid β-oxidation system, the ω-oxidase reaction, a methyl-crotonyl-CoA carboxylation system and the propionyl-CoA carboylase system.

Altogether nine catabolic enzymes are involved in the β-sitosterol sidechain degradation pathway. They work in 14 consecutive enzymatic steps. Summing up: three molecules of $FADH_2$, three molecules of propionyl-SCoA, three of NADH and one molecule of acetic acid are formed, while the sidechain of one mole of sitosterol is removed selectively. The metabolism of the propionates and the acetate yield 18 molecules of NADH and 7 molecules of $FADH_2$. Taking into consideration the whole process more than 80 molecules of ATP could be formed during the sitosterol sidechain degradation process.

INTRODUCTION

The transformation of sterols by microorganisms has been reviewed frequently [18,22,24,25,28]. We focus our discussion in this paper on the microbial physiology of sidechain cleavage of sterols, summarizing some publications, patent specifications and our own research results. Experiments have been carried out on a few *Mycobacterium* mutants blocked at different conversion steps. The steroid drugs represent only 2.5% of the value of pharmaceuticals presently on the world market. However, the worldwide pharmaceutical industry needs more than 2000 tons per year of steroid raw materials. Year by year the natural sterol compounds gain increasing importance. For a long time sitosterol was a waste product of stigmasterol production. It is known that stigmasterol has been a suitable raw material for chemical synthesis of pregnane derivatives on an industrial scale because the 24,25 double-bound promotes the chemical degradation of the sidechain of the steroid skeleton. At the same time a large amount of sitosterol accumulated as a waste material during the production of stigmasterol. Sitosterol now represents one of the most economical, inexpensive raw materials.

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DEGRADATION OF STEROLS

Several well-known teams worked diligently to elucidate in detail the microbial degradation pathways of sterols [3,8,9,11,29,30,32,34,35,36,38]. Many papers and patent specifications were published describing some methods of selective sidechain cleavage of sterols. More than 60% of the raw material for steroid drugs are produced by this route and the bioconversion process is the most economical way to obtain steroid compounds as primary products for chemical synthesis producing sexual hormones, anticoncipients, antiphlogistics and blood pressure regulating agents.

The catabolic biochemical pathway of the microbial degradation of phytosterols was elucidated effectively by working teams of applied research. Now we know that the oxidation of sterols could start in two different parts of the molecule. On the one hand the sidechain of sterols could be split off independently of the chemical structure of ring-A of the steroid skeleton and on the other hand, the 3-β-ol-5,6-dehydro structure of the steroid nucleus could be transformed to a 3-keto-4-ene system: either a hydroxy group is introduced in the 9α-position, or 1,2-dehydro-

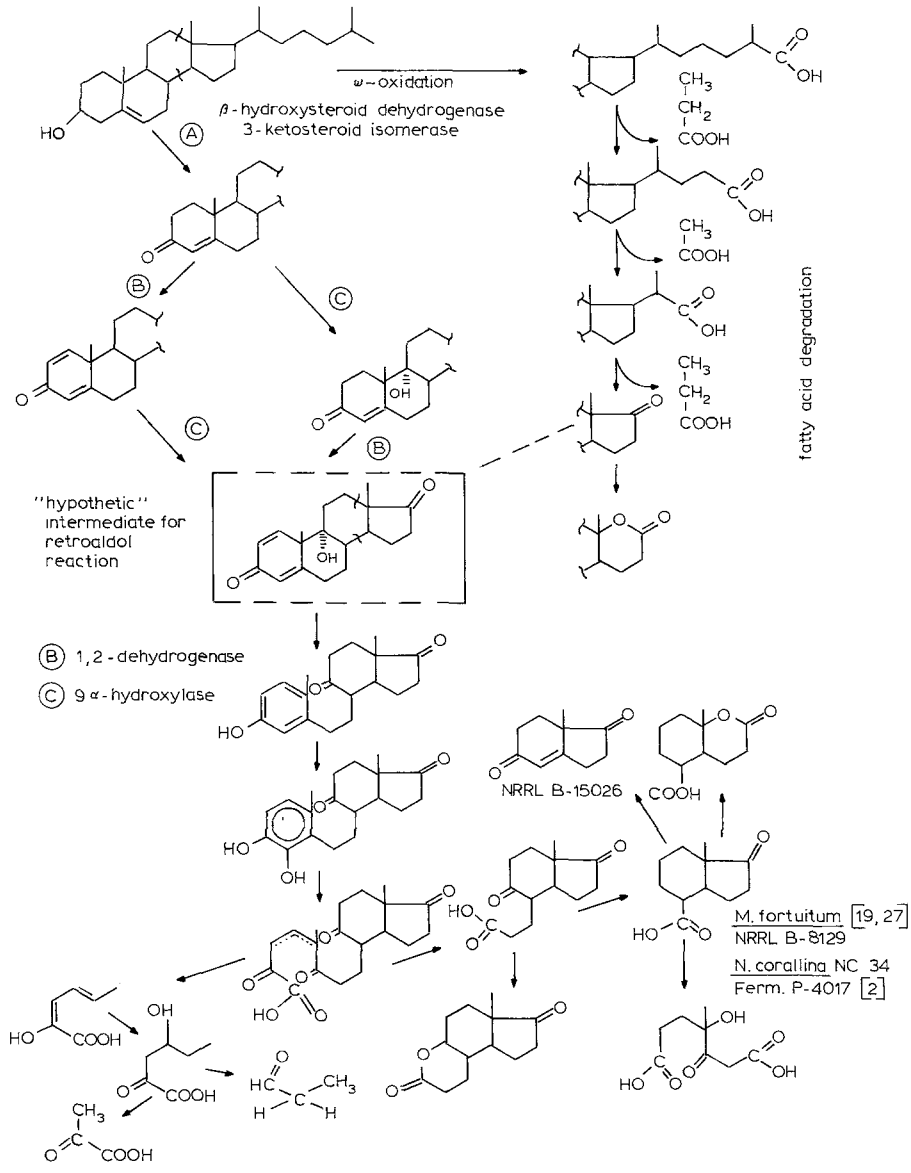


Fig. 1. Microbial degradation of cholesterol.

genated derivatives are formed. The combination of both reactions causes a simultaneous aromatization with the cleavage of ring-B. The 9,10-seco-1,3,5(10)-androstatriene-3-ol-9,17-dione intermediate product could be degraded by a well-known oxidative route to carbon dioxide (Fig. 1). However a number of mutants were isolated for the production of hexahydroindan derivatives [2,19,27].

HOW TO SAVE THE STEROID NUCLEUS

Considerable efforts have been made by applied microbiologists to save the steroid skeleton using chemically modified substrates or inhibiting the 9α-hydroxylase activity of the microbes (Fig. 2). At first estrone was achieved from 19-hydroxy-sitosterol-3-acetate using *Nocardia restrictus* by modification of the substrate (Sih et al.

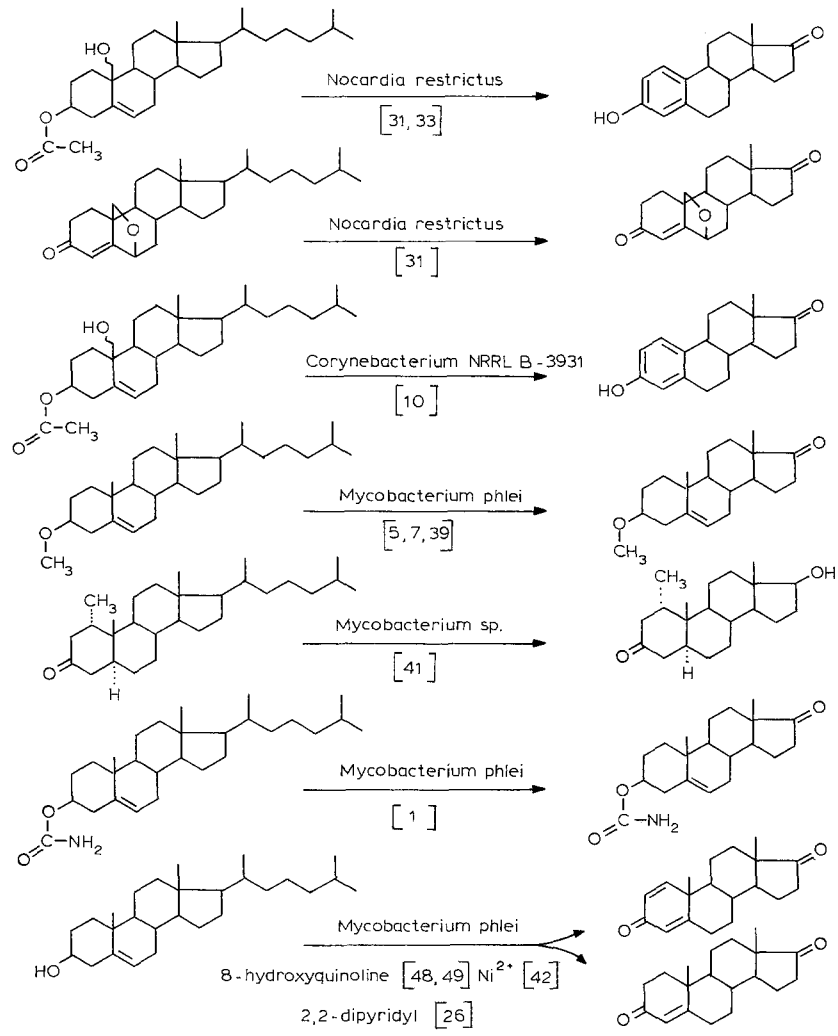


Fig. 2. Methods for preventing enzymic attack on the steroid nucleus.

[31,33]). A number of research teams were able to protect the steroid nucleus by the formation of 3β-alkyloxy-, or carbamoyloxy-derivatives (Büki et al. [5], Eder et al. [7], Weber et al. [39], Ambrus et al. [1]).

Thereafter 1,4-androsta-diene-3,17-dione was produced from cholesterol in industrial scale, by inhibiting the 9α-oxygenase of *Mycobacterium phlei* by a selective agent such as 8-hydroxyquinoline (Wix et al. [48,49]), Ni^{2+} (Willem F. van de Waard [42] or α,α-dipyridyl (Noda Institute [36]).

However, the most economical methods to produce 17-keto-steroids were elaborated by means of the mutation of suitable microbes: by blocking the 9α-hydroxylase, 1,4-androstadiene-3,17-dione was obtained; by blocking the 1,2-dehydrogenase, 9α-hydroxy-4-androstene-3,17-dione was accumulated, but by blocking both enzymes 4-androstene-3,17-dione was the major product (Fig. 3). A number of mutants were described in patent specifications and papers during the last two decades, which could produce different intermediates of the sidechain degradation route. However the production of those intermediate steroid compounds are connected with the fulfilment of the physiological requirements of the mutants [14,15,21,23,37,45,46]. One of the microbial products, namely 1,4-androstadiene-3,17-dione markedly inhibits the respiration of bacterial cells. However, hydrophobic

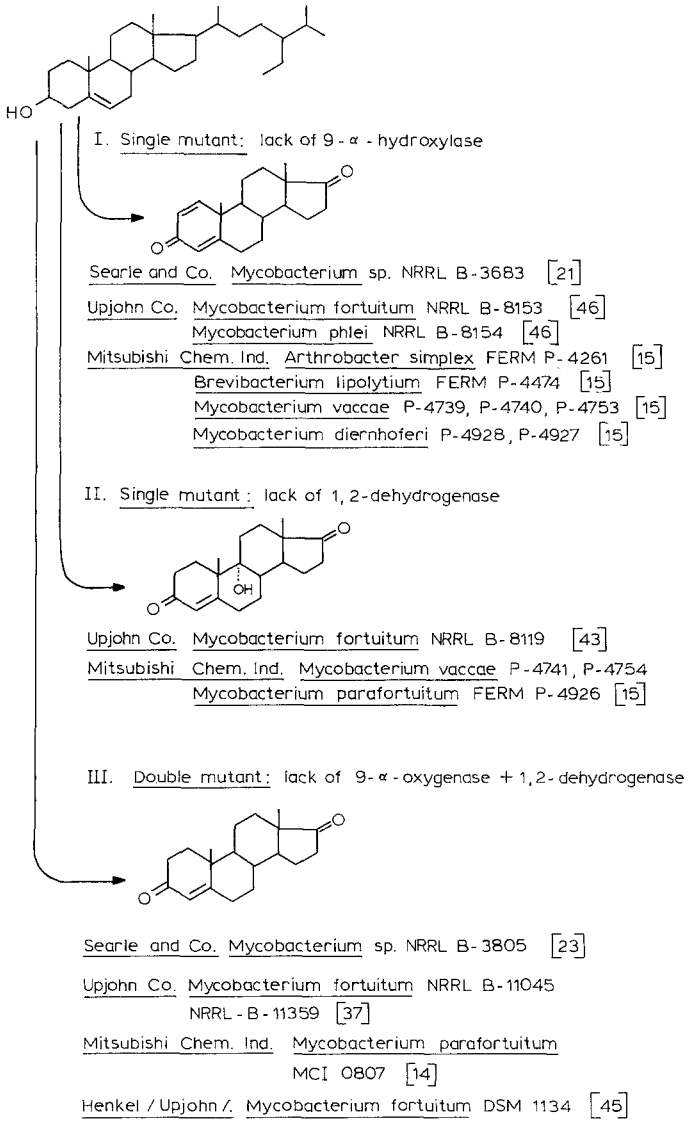


Fig. 3. Production of 17-keto-steroids from sitosterol by enzymeless mutants in industrial scale.

organic polymers, resins, etc., for example XAD-2, can be used to accumulate this toxic compound and enhance 1,4-androstadien-3,17-dione yields.

DEGRADATION OF THE SIDECHAIN

The first enzyme of the reaction pathway degrading the sidechain of sterols attacks the apolar end of sitosterol, introducing a hydroxyl at the terminal 27 methyl group, which is further oxidized to carboxyl (Fig. 4). The introduction of a hydroxyl group is catalyzed by a mixed function oxidase system and the reaction is performed in the presence of NADH. This enzyme system could be induced by appropriate substrates, such a cholesterol, sitosterol or pristane. The chemical structure of ring A does not influence significantly the degradation of the sterol sidechain. In the intact bacterial cells, the cholesterol, even as the cholesterol-ethylether-27-carboxy acid, is degraded by the classical route of the β-oxidation of fatty acids and one mol propionyl-SCoA and 24-carboxyl-SCoA derivatives of cholesterol are formed simultaneously. However in the case of campesterol or sitosterol the classical β-oxidation system alone is not effective. A methyl-crotonyl carboxylase is acting here. In the sterol conversion reaction mixture in the presence of C-24 methyl (campesterol), or ethyl group (β-sitosterol), 24-keto derivatives were detected as major products using a mutant strain [20] although nothing but this carboxylase is lacking (Fig. 5). In the sidechain degradation starting from sitosterol, the β-oxidation at C-24 is accompanied by the introduction of a carboxy group at C-28, similar to the carboxylation of the β-methyl-crotonyl-SCoA during the oxidation of leucine. Sih et al. [8] were able to detect the incorporation of carbon dioxide at the C-28 position of sitosterol, using ^{14}C-bicarbonate in the reaction mixture. The carboxylase is also an inducible enzyme and is formed in the presence of sitosterol or campesterol. We have found that a considerable amount of solubilized carbon dioxide is needed in the reaction mixture for a high rate of carboxylation because the carbon dioxide could be transported more easily through the bacterial membrane than the bicarbonate ion. Therefore the aeration and ventilation of the submerged fermentation depended on the size of the fermentor, as well as on the physiological condition of the microbes. It is absolutely necessary to provide for an optimal level of carbon dioxide and an adequate supply of oxygen during the fermentation process.

Following the carboxylation reaction both propionyl-SCoAs are split off to form cholenyl-SCoA derivatives (Fig. 4). The first propionyl-SCoA is removed by retro-aldol reaction but the formation of the second propionyl-SCoA is catalyzed by acyl-SCoA thiophorase and β-keto-thiolase respectively. The next acetyl-SCoA is easily formed by the well-known fatty acid β-oxidation route. The final splitting reaction of the sidechain degradation process, which can remove the last propionyl-SCoA by retroaldole reaction from the steroid skeleton apparently does not differ from the first splitting reaction (Fig. 5). In

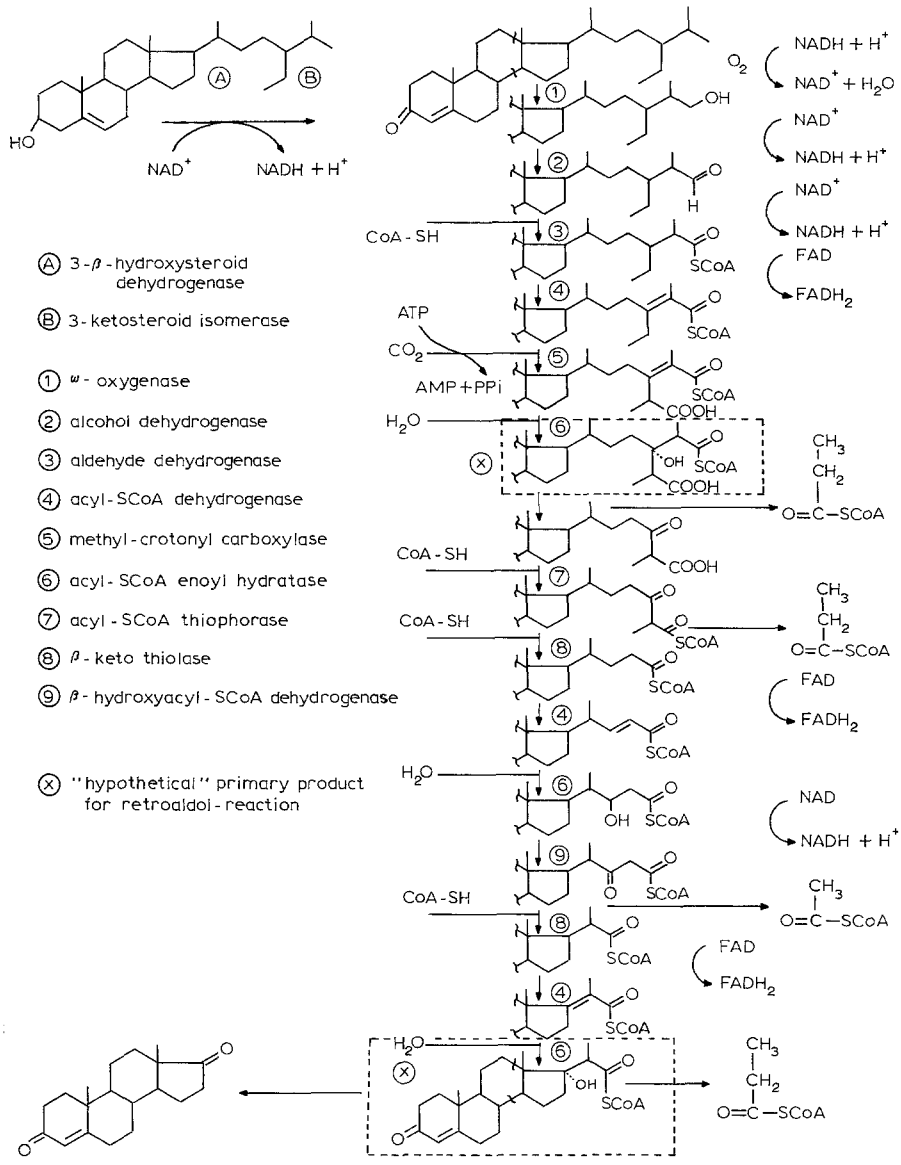


Fig. 4. Transformation of β-sitosterol to 4-androstene-3, 17-dione by *Mycobacterium* sp. NRRL B-3805.

both cases, the α-carbon atom of the propionic acid is covalently bound to a secondary carbon atom of a saturated hydrocarbon chain. However, the structural position of the two secondary carbon atoms are significantly different. In the case of the last reaction, this secondary carbon atom is part of a five membered ring, which is attached to a saturated phenanthrene, but in the case of the first splitting reaction the biscarbonate structure promotes the retroaldol reaction.

The activity of the enzymes of the fatty acid β-oxidation system is influenced by the chemical structure of the substrate; for example the reaction rate of acyl-SCoA dehydrogenase or enoyl hydratase is lower right near to the steroid skeleton, than at the terminal part of the sidechain of sterol.

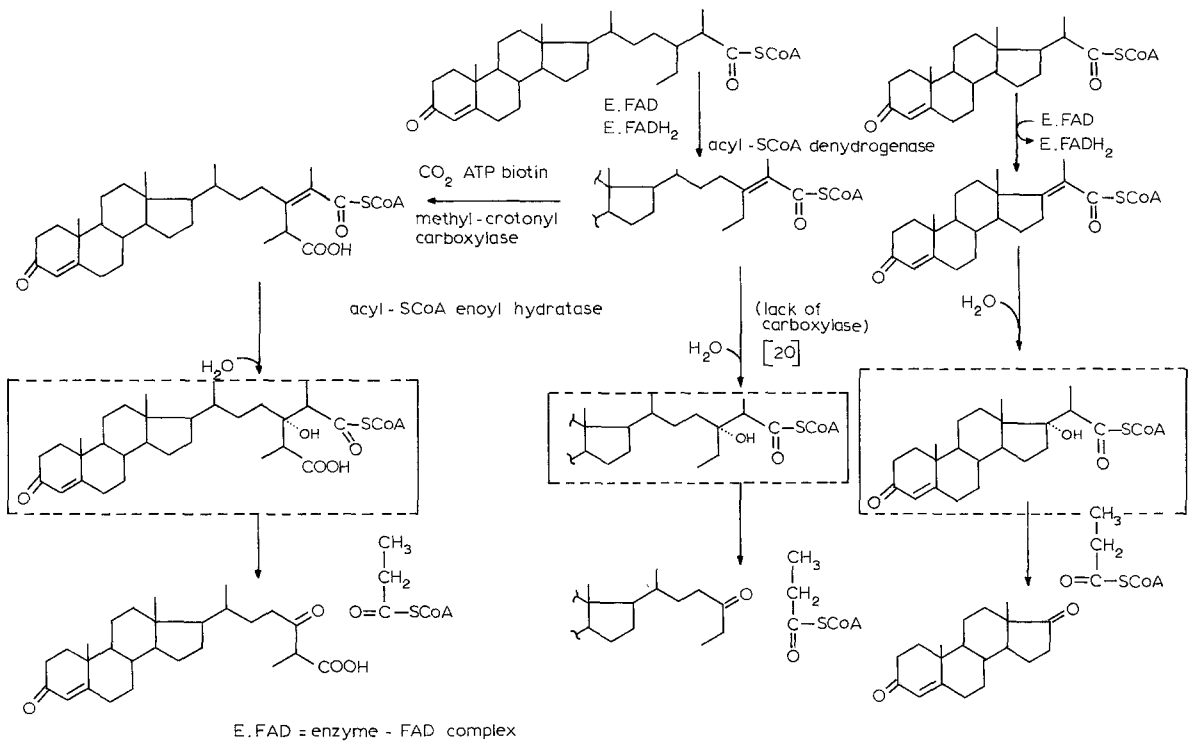


Fig. 5. Retroaldol reactions of sitosterol sidechain degradation process splitting off the first and the last propionic acids.

PRODUCTION OF BISNORCHOLENIC DERIVATIVES

In the last decade, a number of mutants have been isolated and described by different research teams; with large amounts of bisnorcholene derivatives, e.g. bisnorcholene alcohol accumulated as major products during the bioconversion process (Fig. 6). These compounds, 20-carboxy-pregnene, 20-hydroxy methyl-pregnene, 20-carboxy-17,(20)-dehydro-pregnene, could not be used as substrates in the bioconversion processes. Earlier they were considered as waste products of the sidechain cleavage procedures for the production of androstene derivatives. Borate increases the overproduction of the 17(20)-dehydrobisnorcholenic derivatives formed by the acyl-SCoA dehydrogenase, which is not able to transform the free bisnorcholenic acid [40]. The formation of bisnorcholenic acid could be repressed by vegetable oil acting as a source of acetyl-SCoA. Not only were the free acids formed but often some reduced derivatives of the bisnorcholenic compounds and 20-methoxycarbonyl progesterone, or 9α-hydroxy derivatives also accumulated in the reaction mixture. These bisnorcholenyl-SCoA derivatives could be hydrolyzed in the cells by thiolesterase or thiophorase when the intermediary metabolism needs CoA-SH for some essential catabolic reactions and at the same time bisnorcholenic acid and more or less reduced derivatives thereof are secreted into the environment. The formation of the reduced compounds is an easy way to reoxidize NADH to NAD^+ when the activity of the respiratory chain is not high enough for the microbe (Fig. 7) (Table 1).

The question is: Why are bisnorcholene derivatives accumulated in the culture medium? It has been found that the sidechain degradation activity of mutant strains producing bisnorcholenic derivatives is much lower than the activity of the wild strains. Possibly the bisnorcholenyl-SCoA is not a proper substrate for the (genetically) slightly modified acyl-SCoA dehydrogenase or the enoylhydratase of mutant strains. This reaction has been performed on the bisnorcholenyl-SCoA in the cytoplasm, and the thiophorase, which catalyzes the secretion of the free acids, is bound into the membrane of *Mycobacterium* cells. However bisnorcholenyl derivatives could be pro-

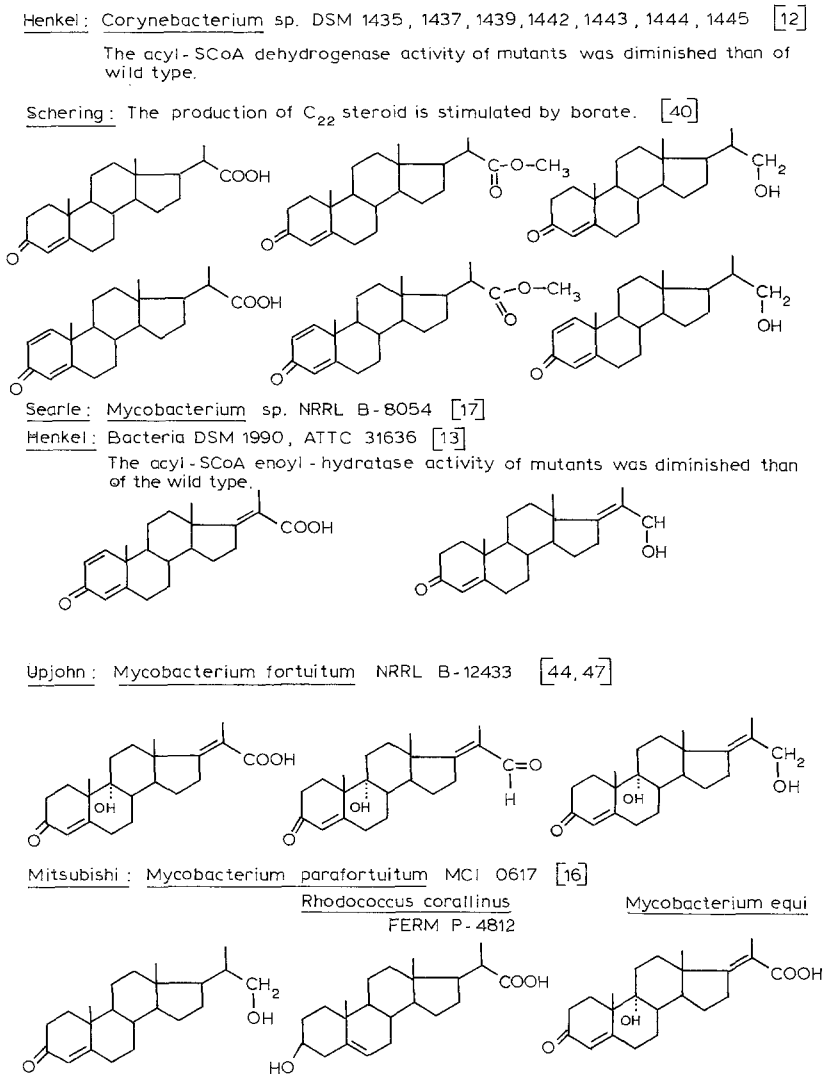


Fig. 6. 20-Carboxy pregnane derivatives formed by mutant bacteria have been described by different pharmaceutical firms.

duced by the chemical modification of sterol substrate too. Büki et al. [4,5] found that the sidechain of sterol methylether was removed much more slowly than that of the free sterol by a wild strain of *Mycobacterium*, but simultaneously a number of different, partially degraded intermediates, namely 3β-methoxy-bisnorcholest-5-en-22-oic acid, were secreted into the culture medium (Fig. 8).

PHYSIOLOGICAL EFFECTS

One may raise the question: how does the metabolism of the bacterial cells influence the intermediates of the sidechain degradation route? Three molecules of propionate, one of acetic acid, three of NADH and three of $FADH_2$ are formed when a sidechain of sitosterol is removed. The regeneration activity of cofactors might be a rate limiting factor. However several physiological problems originate from the formation of propionic acid. This toxic compound could be eliminated only by the formation of propionyl-SCoA which is carboxylated to methyl-malonyl-SCoA. Thereafter the consecutive activities of the epimerase and of the mutase are capable of converting this compound to succinyl-SCoA (Fig. 9).

This metabolic pathway could be induced by propio-

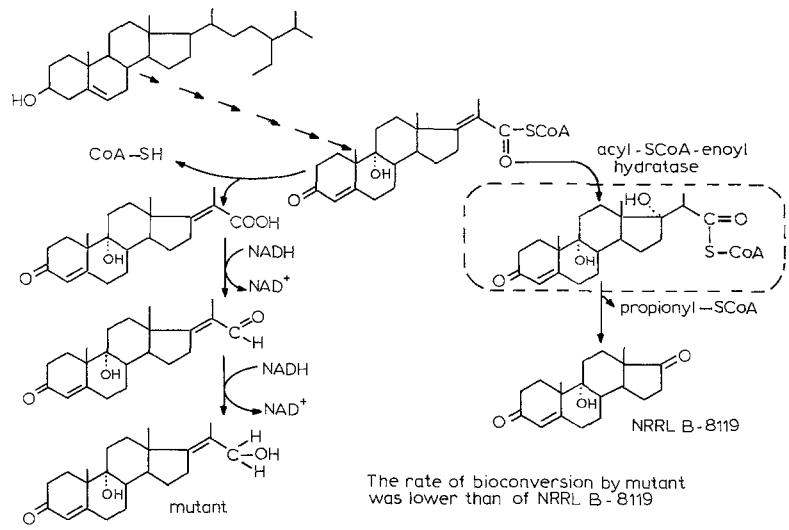


Fig. 7. Formation of 3-oxo-23, 24-bisnorcholene derivatives by a mutant, which was obtained from 1,2-dehydrogenase less *Mycobacterium fortuitum* NRRL B-8119.

nate, but probably to a much greater extent by propionyl-SCoA. A limited amount of propionate in the culture medium results in an increase of the rate of the sidechain degradation. The formation of methyl-malonyl-SCoA is increased by the presence of carbon dioxide in the reaction mixture. That means that 1% carbon dioxide in the air-outlet is advantageous for the metabolism of propionyl-SCoA.

REGULATION OF THE DEGRADATION PATHWAY

Altogether nine catabolic enzymes act in the sitosterol sidechain degradation pathway (Fig. 4). They work in 14 consecutive enzymatic steps. Summing up: three molecules of propionyl-SCoA, three molecules of $FADH_2$, three of NADH and one molecule of acetic acid are

TABLE 1

Bioconversion activity of B-8119 and the mutant[a]

Strain + addition 10 mmol	β-sitosterol	Bisnorcholenic acid derivatives	17(20)-Dehydro-bisnorcholenic acid derivatives	9α-hydroxy-4-androstene-3,17-dione
8119	200	50	5	700
mutant	400	50	450	30
8119 + glucose	200	1–2	1–2	700
mutant + glucose	450	30	200	150
8119 + linseed oil	300	1–2	1–2	600
mutant + linseed oil	400	10	250	200

In the beginning each shake-flask contained 1.2 mmol β-sitosterol in 80 ml minimal medium (2.5 mmol ammonium phosphate, 8 mmol glycerol. 16 mg magnesium sulfate, 100 mg polyoxyethylenesorbitan monooleate). The acyl-SCoA enoyl hydratase activity of the mutant strain may be less than that of the parent strain (NRRL B-8119).

[a] Steroid content in μmol of whole culture after 3 days incubation.

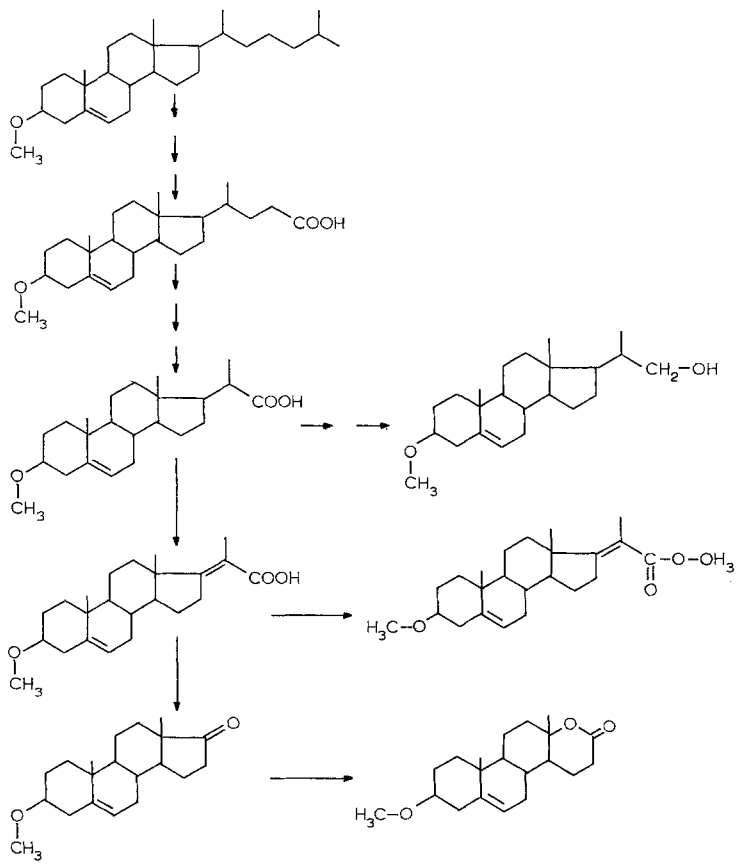


Fig. 8. Metabolic intermediate products of sterol sidechain degradation route from sterol methyl ether in *Mycobacterium phlei* MNG 0029.

formed while the sidechain of one mole of sitosterol is removed selectively (Fig. 9). The metabolism of the propionates and of the acetate yield also 18 molecules of NADH and seven molecules of $FADH_2$.

Total net reaction (summing-up Fig. 4 and Fig. 9):

β-sitosterol → 4-androstene-3,17-dione

21 NAD^+ 21 NADH + H^+

10 FAD 10 $FADH_2$

4 ATP 4 AMP + 4 PPi

7 GDP 7 GTP.

Taking into consideration the whole process more than 80 molecules of ATP could be formed during the sitosterol sidechain degradation process by the transport of electrons through the carrier chain to oxygen. However, a number of NADH molecules could be reoxidized by the production of reduced metabolic intermediates which decrease markedly the formation of ATP (Fig. 10).

INDUCIBLE ENZYME REACTIONS

Three inducible groups of the catabolic enzymes act in the β-sitosterol sidechain degradation pathway.

The first step is the C-27 hydroxylation catalyzed by a mixed function oxidase system. The actual enzyme level depends on the content of the appropriate substrate in the growth medium [50]. This enzyme system could be induced not only by sitosterol but some kinds of saturated oligoisopren derivatives are suitable inducers too. A relatively low partial oxygen tension during the induction period is necessary for the formation of the oxygenase system.

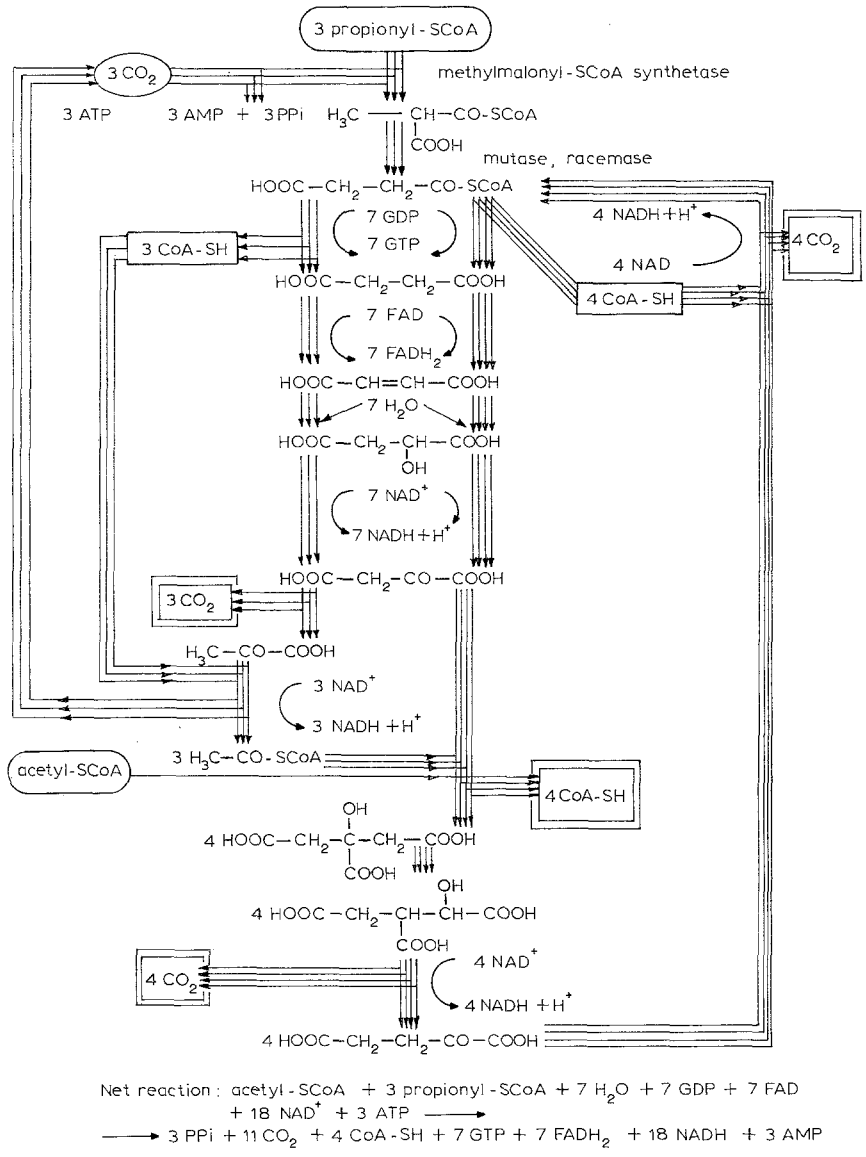


Fig. 9. Fate of the active fragments of sitosterol sidechain.

The second inducible enzymatic step is the carboxylation of C-28. Sitosterol could induce this enzyme system, but cholesterol could not. However, the rate of carboxylation is influenced by the actual concentration of the dissolved carbon dioxide.

The metabolism of propionyl-SCoA is also an inducible route. The levels of the propionyl-SCoA carboxylase, epimerase and mutase changed simultaneously. Propionic acid is an inducer. However the real inducer might be the propionyl-SCoA. The carboxylation of the propionyl-SCoA needs high solubilized carbon dioxide level. Carbon dioxide could probably be transferred more easily through the cell membrane than the bicarbonate ion.

CONCLUSION

It follows from this that it is not too easy to define the optimal degree of aeration and also of the scale of ventila-

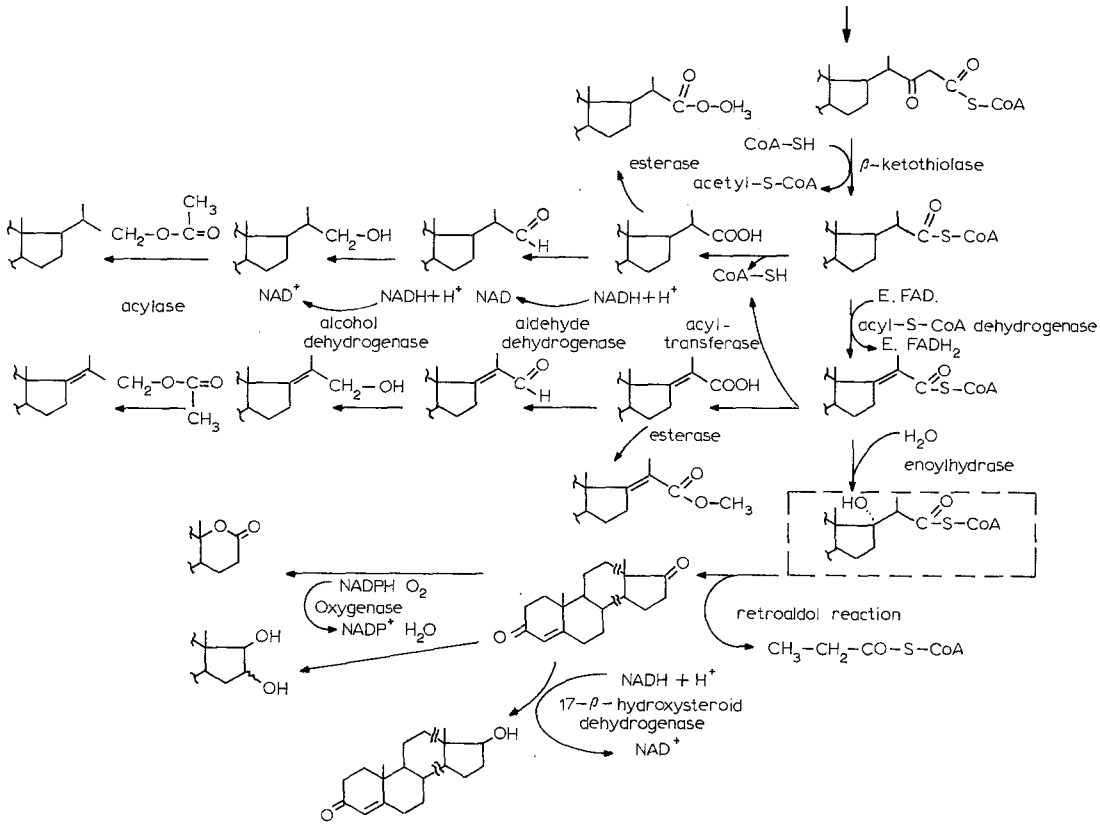
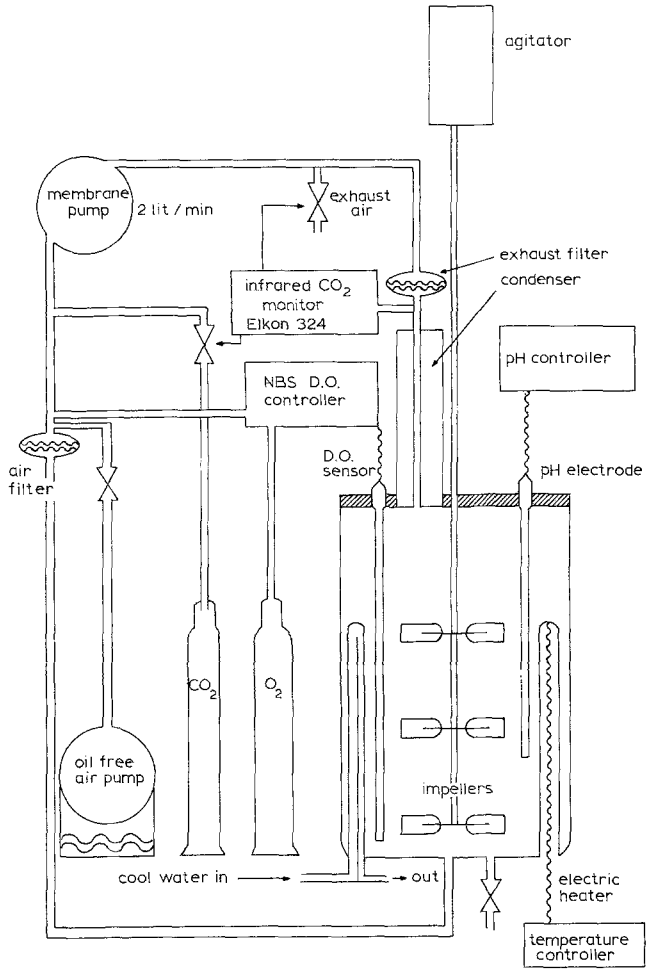


Fig. 10. Production of reduced (oxidized) intermediates, bisnorcholenyl derivatives.

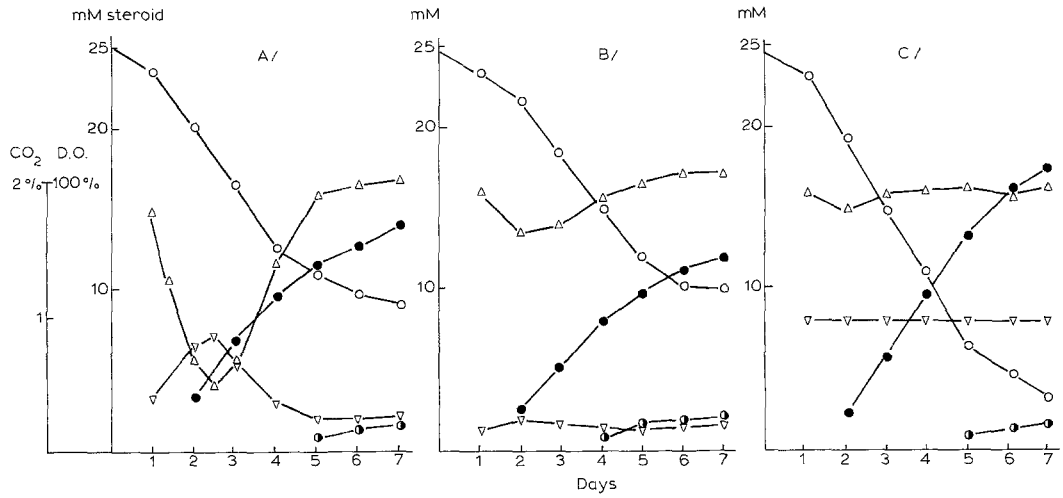
tion. The yield of bioconversion process is depressed by the overaeration of the reaction mixture. It can be shown that a well-controlled bioreactor system is required for the elaboration of a biotransformation process with high yield, using mutants for the production of partially degraded sterol derivatives. The optimal parameters of the process might differ when using different kinds of mutants with genetically determined metabolic activities. It seems to be a fundamental condition that the optimal dissolved O_2 and CO_2 level could be controlled independently one from another. For this special pupose we have installed a laboratory-scale (10 liter) stainless steel stirred fermentor in which the exhausted air could be recirculated by a diaphragm-pump. The mass transfer is influenced by the size of air-bubbles and the number of air bubbles in the whole volume of the fermentation source. The increasing influence of power input on K_La is widely known, but in this case is impracticable. The optimum values of air-flow and rpm are connected with the rheological feature of the fermentation source. Both parameters can be controlled manually; however, the optimum level of rmp and air-flow were never changed during the whole bioconversion process.

One of the best ways to control dissolved oxygen (D.O.) level is to add extra oxygen through the air-inlet using a New Brunswick D.O. controller. The dissolved CO_2 level could be influenced by the ventilation of the bioreactor or by the addition of extra CO_2 using an Elkon 324 infrared gas-monitoring system (Fig. 11). Constant pH is also necessary, because the dissolved CO_2, HCO_3^- ratio is influenced by the hydrogen ion concentration. A steam sterilizable Ingold pH electrode and controller was used. The whole fermentor can be sterilized in place by an electric heater. The temperature of the fermentor was regulated like an ultrathermostat. Using this fermentation system we were able to investigate the physiological effects of different technical parameters separately. (Figs. 12,13). The temperature of the reaction mixture and



◀ Fig. 11. Ten-litre capacity fermenter. Sterilizable in place. Dissolved oxygen level controlled. Constant CO_2 pressure.

Fig. 12. Effect of aeration on bioconversion activity of *Mycobacterium* sp. NRRL B-3805. -O-, C_{27-29}-steroids (β-sitosterol, stigmasterol, campesterol, cholesterol: 60, 6, 24, 3%); -◑-, C_{22}-steroids (20-carboxy or 20-hydroxy-methyl pregnene derivatives); -●-, C_{19}-steroid (4-androstene-3,17-dione). Bioconversion process performed at 32 °C in 10 liter fermentor. Controlled pH = 7. Impeller speed: 550 rpm. Aeration: (A) 0.1 vvm air-flow rate; (B) 1 vvm air-flow rate; (C) Dissolved oxygen level controlled by New Brunswick D.O. controller using aluminium-silver galvanic electrode (100% initial dissolved oxygen concentration ≈ 0.2 mmol O_2). Carbon dioxide concentration in the recirculated air controlled by Elkon infrared gas analyser. -△——△-, Dissolved oxygen level; -▽——▽-, CO_2 % in the outlet gas stream.



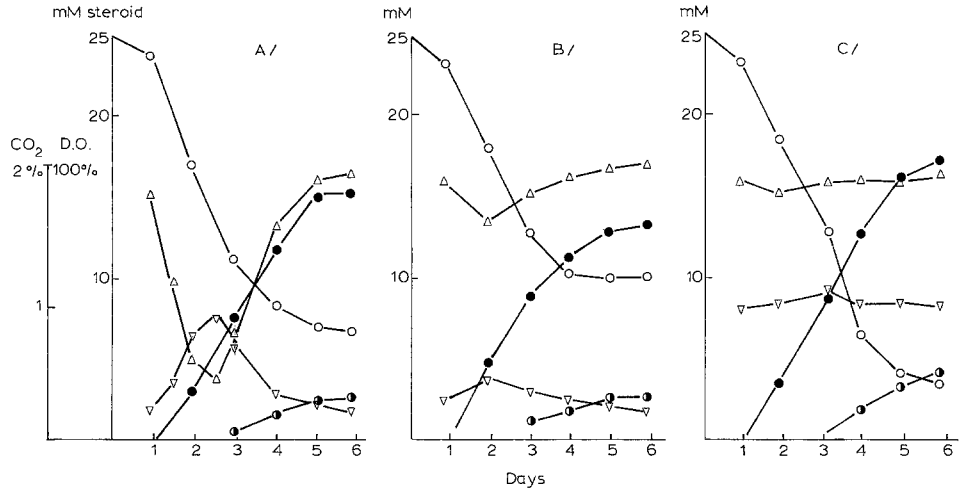


Fig. 13. Effect of aeration on bioconversion activity of *Mycobacterium fortuitum* NRRL B-8119. –O–, C_{27-29}-steroids (β-sitosterol, stigmasterol, campesterol, cholesterol: 60, 6, 24, 3%); –O–, C_{22}-steroids (20-carboxy or 20-hydroxy-methyl pregnene derivatives); –●–, C_{19}-steroid (9α-hydroxyandrost-4-ene-3,17-dione). Bioconversion process performed at 32 °C in 10 liter fermentor. Controlled pH = 7. Impeller speed: 550 rpm. Aeration: (A) 0.1 vvm air-flow rate; (B) 1 vvm air-flow rate; (C) Dissolved oxygen level controlled by New Brunswick D.O. controller using aluminium-silver galvanic electrode (100% initial dissolved oxygen concentration $\approx$ 0.2 mmol O_2). Carbon dioxide concentration in the recirculated air controlled by Elkon infrared gas analyser. –△——△–, Dissolved oxygen level; –▽——▽–, CO_2 % in the outlet gas stream.

the supply of energy sources are also important parameters for the optimization of the bioconversion process. For example, the elevation of temperature increases the formation of bisnorcholenic derivatives mostly in reduced form. However the formation of reduced product is also influenced by the dissolved oxygen level.

REFERENCES

1 Ambrus, G., K. Albrecht, I. Barta, M. Fábian and K. Köncöl. 1986. Hung. Pat. 190665 01 Oct. 1980. Bioconversion of 3β-carbamoyloxy-5-cholestene to 3β-carbamoyloxy-5-androsten-17-one. Chem. Abs. 105: 113608.

2 Arima, H., T. Beppu and W. Nakamatsu. 1979. Japan Kokai Tokkyo Kobo. 78130643. 17 Apr. 1977. 3a-αH-4α-acetyl-5-hydroxy-7a, β-methyl-hexahydro-1-indanone. Chem. Abs. 90: 150312.

3 Arima, K., H. Nagasawa, H. Bae and G. Tamura. 1969. Microbial transformation of sterols. I. Decomposition of cholesterol by microorganisms. Agr. Biol. Chem. 33: 1636–1643.

4 Büki, K., G. Ambrus and Gy. Horváth. 1975. Metabolic fate of cholesteryl methyl ether in Mycobacterium phlei. Acta Microbiol. Acad. Sci. Hung. 22: 447–451.

5 Büki, K., G. Ambrus, M. Halmos and J. Szabó. 1976. Hungarian Pat. 11189 16 Aug. 1974. 3β-alkoxycholest-5-ene is transformed to dehydroepiandrosterone. Chem. Abs. 85: 157962.

6 Dodson, R.H. and R.D. Muir. 1958. Microbial transformation II. The microbial aromatization of steroids. J. Am. Chem. Soc. 80: 5004.

7 Eder, U., G. Sauer, G. Haffer, G. Neef, R. Wiechert, A. Weber and A. Popper. 1977. Ger. Offen 25349111. 01 Aug. 1975. Formation of androst-5-en-17-one. Chem. Abs. 86: 187659.

8 Fujimoto, Y., C.S. Chen, A.S. Gopalan and C.J. Sih. 1982. Microbial degradation of the phytosterol side-chain. II. Incorporation of $NaH^{14}CO_3$ onto C-28 position. J. Am. Chem. Soc. 104: 4720–4722.

9 Fujimoto, Y., C.S. Chem, Z. Szeleczky, D. Di Tullio and C.J. Sih. 1982. Microbial degradation of the phytosterol side-chain. I. Enzymatic conversion of 3-oxo-24-ethylcholest-4-en-26-oic acid into 3-oxochol-4-en-24-oic-acid and androst-4-ene-3,17-dione. J. Am. Chem. Soc. 104: 4718–4720.

10 Gordon, E. Mallett. 1973. US Pat. 3741870 12 Oct. 1971. $\Delta^{9/11}$-Estrone is produced from 19-hydroxycholesterol-3-acetate. Chem. Abs. 79: 64555.

11 Hayakawa, S. 1973. Microbiological transformation of bile acids. Adv. Lipid Res. 11: 143.

12 Hill, F., W. Preuss, J. Schindler, R. Schmid and A. Struve. 1980. Euro. Pat. 04913 17 Apr. 1978. Production of 17-C-steroid-α-propionic acid derivatives by blockmutant microbes. Chem. Abs. 92: 126930.

13 Hill, F., W. Preuss, J. Schindler, R. Schmid, A. Struve. 1980. Euro. Pat. 15308 07 March 1979. Blockmutants for the pro-

duction of 17-C-steroid-α-proponic acid derivatives. Chem. Abs. 93: 236940.
14 Imada, Y., Takahashi, K. and S. Katogy. 1979. Japan Kokai Tokkyo Kobo 7959395 17 Oct. 1977. Microbial production of androstanes. Chem. Abs. 91: 122153.
15 Imada, Y. and T. Yutaka. 1979. Japan Kokai Tokkyo Kobo 79 73191 24 Nov. 1977. Preparation of androstanes by Arthrobacter mutants. Chem. Abs. 91: 156036.
16 Imada, Y. and K. Takahashi. 1980. Eur. Pat. 01622 14 Oct. 1977. 20α-hydroxy-methylpregnane derivatives. Chem. Abs. 92: 4699.
17 Jiu, J. and W.J. Marsheck. 1977. US. Pat. 4032408 30 Apr. 1975. Process of preparing 3-oxo-pregna-4,17/20/-diene-20-carboxylic acid and esters with Mycobacterium. Chem. Abs. 87: 132321.
18 Kieslich, K. 1985. Microbial side-chain degradation of Sterols. J. Basic Microbiol. 25: 461–474.
19 Knight, J.C. and M.G. Wovcha. 1977. Ger. Offen 2652377. 17 Nov. 1975. 7a, β-methylhexahydro-1/5/-indanedione compounds. Chem. Abs. 87: 100688.
20 Knight, J.C. and M.G. Wovcha. 1980. Microbial degradation of the phytosterol sidechain to 24-oxo products. Steroids 36: 723–730.
21 Kraychy, S., W.J. Marsheck and R.D. Muir. 1972. US. Pat. 3684657 11 May 1970. Selective microbiological degradation of steroidal 17-alkyls. Chem. Abs. 77: 124777.
22 Marsheck, W.J. 1971. Current trends in the microbiological transformation of steroids. Prog. Ind. Microbiol. 10: 49–103.
23 Marsheck, W.J. and S. Kraychy. 1973. Brit. Pat. 1329387 Dec. 10 1970. Selective microbiological preparation of androst-4-ene-3,17-dione. Chem. Abs. 79: 144867.
24 Martin C.K.A. 1977. Microbial cleavage of sterol side chains. Adv. Appl. Microbiol. 22: 29–58.
25 Martin, C.K.A. 1984. Sterols. In: Biotechnology, A Comprehensive Treatise in 8 Volumes, Vol. 6a: Biotransformations (Kieslich, K., ed.), p. 79–96, Verlag Chemie, Weinheim.
26 Noda Institute for Sci. Res. Netherlands. 1966. Pat. 6502883 3 Dec. 1965. (Prior Japanese Pat. 30915 02 June 1964) Androst-4-en-3,17-dione and androsta-1,4-dien-3,17-dione production from sterols. Chem. Abs. 65: 6262g.
27 Pyke, T.R. and M.P. Salmond. 1979. Ger. Offen. 2802524. 14 Feb. 1977. 3a-αH-4α-/3-hydroxypropyl/-7a,β-methylhexahydro-1,5-indandione hemiketal. Chem. Abs. 90: 53151.
28 Schoemer, U. and C.K.A. Martin. 1980. Microbial transformation of sterols. Biotechnol. Bioeng. 22: 11–25.
29 Schubert, K., K.H. Böhme and C. Hörhold. 1961. Bildung einer Ketosäure durch mikrobiellen Abbau von Progesteron. Hoppe Seyler's Z. physiol. Chem. 325: 260–262.
30 Shaw, D.A., L.F. Berkenhagen and P. Talalay. 1963. Enzymatic oxidation of steroids by cell free extracts of Pseudomonas testosteroni. Isolation of cleavage of ring A. Proc. Natl. Acad. Sci. U.S.A. 54: 837.
31 Sih, C.J., S.S. Lee, Y.Y. Tsong, K.C. Wang and F.M. Chang. 1965. An efficient synthesis of estrone and 19-norsteroids from cholesterol. J. Am. Chem. Soc. 87: 2765.
32 Sih, C.J., H.H. Tai, Y.Y. Tsong and R.G. Coombe. 1986. Mechanism of steroid oxidation by microorganisms. XIV. Pathway of cholesterol side-chain degradation. Biochemistry 7: 808–818.
33 Sih, C.J. and K.C. Wang. 1965. A new route to estrone from sterols. J. Am. Chem. Soc. 87: 1387.
34 Sih, C.J., K.C. Wang, D.T. Gibson and H.W. Whitlock. 1965. On the mechanism of ring cleavage in the degradation of 9,10-seco steroids by microorganisms. J. Am. Chem. Soc. 87: 1386.
35 Sih, C.J., K.C. Wang and H.H. Tai. 1968. Mechanism of steroid oxidation by microorganisms XIII. C_{22} acid intermediates in the degradation of the cholesterol side chain. Biochemistry 7: 796–807.
36 Stadtman, T.C., A. Cherkes and C.N. Anfinsen. 1954. Studies on the microbial degradation of cholesterol. J. Biol. Chem. 206: 511–523.
37 Upjohn Co. 1979. US Pat. 4293644 09 Oct. 77 and 4345033 08 Sept. 1980. Androst-4-ene-3,17-dione production by selective degradation of 17-alkyl-steroids with M. fortuitum NRRL B-11045 (DSM 1134). Chem. Abs. 89: 40889.
38 Turfitt, G.E. 1944. Microbiological agencies in the degradation of steroids. I-III. J. Bacteriol. 47: 487; Biochem. J. 40: 79 (1946); Biochem. J. 42: 3376 (1948).
39 Weber, A. and M. Kennecke. 1978. H. Dahl. Ger. Offen. 2632677. 16 July 1976. Formation of androstan-17-one derivatives from 3β-methoxycholestene. Chem. Abs. 88: 150626.
40 Weber, A., M. Kennecke and R. Mueller. 1979. Ger, Offen. 2757156 19 Dec. 1977. 21-Hydroxy-20-methylpregnane derivatives production. Chem. Abs. 91: 173386.
41 Weber, A., R. Mueller, M. Kennecke, U. Eder and R. Wiechert. 1977. Ger. Offen. 2558089. 19 Dec. 1975. 17-β-hydroxy-1-α-methyl-5-α-androstan-3-one production from 1α-methyl-5α-cholestan-3-one. Chem. Abs. 87: 165995.
42 van der Waard, W.F. 1968. Netherlands Pat. 6513718 22 Oct. 1965. Process for the microbiological preparation of steroids. Chem. Abs. 68: 114844.
43 Wovcha, M.G. 1977. Ger. Offen. 2647895 (US Prior. 24 Oct. 1975) Process for the production of 9α-hydroxyandrost-4-ene-3,17-dione. Chem. Abs. 87: 66595.
44 Wovcha, M.G., F.J. Antosz, J.M. Beaton, A.B. Garcia and L.A. Kominek. 1980. US. Pat. 4175006 21 Oct. 1977. 9α-hydroxy-3-keto-bisnorchol-4-en-22-oic acid methyl ester formation. Chem. Abs. 92: 126929.
45 Wovcha, M.G. and C.B. Biggs. 1979. Ger. Offen. 2746383 22 Oct. 1976. Mycobacterium mutant (DSM 1134) and uses in the production of androst-4-ene-3,17 dione. Chem. Abs. 89: 40889.
46 Wovcha, M.G., C.B. Biggs and T.R. Pyke. 1979. FR Pat. 2408621 9 Nov. 1977. Mutant microorganisms for selectively

degrading sterols to androstane-3,17-dione derivatives. Chem. Abs. 91: 173395.

47 Wovcha, M.G., J.C. Knight and A.B. Garcia. 1983. Fr. Pat. 2505360 11 May 1981. 9α-hydroxy-3-oxo-4,17/20/-pregnadiene-20-carboxylic acid by mutant. Chem. Abs. 98: 105712.

48 Wix, G., K. Büki and E. Tömörkény. 1967. Hung. Pat. 153173 05 March 1965. Microbiological preparation of 1,4-androstadien-3,17-dione. Chem. Abs. 66: 114594.

49 Wix, G., K. Büki, E. Tömörkény and G. Ambrus. 1967. Hung. Pat. 153831 23 Sept. 1965. Microbiological preparation of androsta-1,4-diene-3,17-dione derivatives. Chem. Abs. 67: 107385.

50 Zajaczkowska, Ewa, L. Sedlaczek. 1988. Microbiological degradation of sterols. I. Selective induction of enzymes of the cholesterol sidechain cleavage in Rhodococcus sp. IM 58. Acta Microbiol. Polon. 37: 27–44.