APPLIED MICROBIOLOGY, Jan. 1972, p. 72-77

Vol. 23, No. 1
Printed in U.S.A.

Microbial Degradation of Sterols

WILLIAM J. MARSHECK, STEPHEN KRAYCHY, AND ROBERT D. MUIR
Divisions of Biological and Biochemical Research, G. D. Searle and Co., Chicago, Illinois 60680

Received for publication 24 September 1971

A process is described for the microbial degradation of cholesterol and plant sterols, to produce androsta-1,4-diene-3,17-dione and androst-4-ene-3,17-dione, by two newly isolated bacteria designated *Mycobacterium* sp. NRRL B-3683 and *Mycobacterium* sp. NRRL B-3805. These myocbacteria produce substantial amounts of 17-ketonic compounds without appreciable degradation of the steroid nucleus. No ring degradation inhibitory agents are necessary. The first microbiological production of 20α-hydroxymethylpregna-1,4-dien-3-one is also reported.

Many microorganisms, including representatives of *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Microbacterium*, *Mycobacterium*, *Nocardia*, *Protaminobacter*, *Serratia*, and *Streptomyces*, are capable of completely decomposing cholesterol (2). Heretofore, attempts to effect the selective microbiological degradation of the 17-alkyl side chain of cholesterol and plant sterols without concomittant degradation of the steroid nucleus have centered on the use of certain inhibitory agents or on modification of the sterol structure itself.

When *Mycobacterium phlei* or *M. butyricum* is grown in a suitable medium in the presence of Ni, Co, Pb, or Se ions it produces significant amounts of androsta-1,4-diene-3,17-dione from cholesterol (9). Wix et al. (11) have successfully used various mycobacteria to remove selectively the side chain from cholesterol. The degradation of the steroid nucleus by these microorganisms was inhibited by the addition of 8-hydroxyquinoline to the fermentation at the time of the substrate addition. In another study, Arima et al. (U.S. Patent 3,388,042, 1968) identified androsta-1,4-diene-3,17-dione as a bioconversion product of cholesterol by many different bacteria in the presence of a variety of chelating agents.

Sih and Wang (8) and later Afonso et al. (1) employed 19-hydroxylated and 19-nor derivatives, respectively, to produce estrone via the microbial degradation of the cholesterol-type side chain and aromatization of the steroid A ring. Further modification of the basic sterol structure to 6,19-oxido (W. F. van der Waard, U.S. Patent 3,487,907, 1970; C. J. Sir, U.S. Patent 3,507,749, 1970) or 3α,5-cyclo-6β,19-oxido derivatives (M. Shirasaka et al., U.S. Patent 3,475,275, 1969) protects the ring system from decomposition while permitting side-chain degradation to the 17-ketone. *Mycobacterium*, *Arthrobacter*, and *Nocardia* are useful in the above processes.

This report describes a process for the microbial conversion of sterols to steroids of the androstane series in good yields without the necessity of adding metal ions or chelating agents or of modifying the substrate structure.

MATERIALS AND METHODS

Microorganisms. During a screening program to discover microorganisms capable of converting stigmast-4-en-3-one to androsta-1,4-diene-3,17-dione, a soil mycobacterium was isolated. Ultraviolet irradiation of this bacterium produced an isolate with an increased ability to effect the biotransformation, and it was later designated *Mycobacterium* sp. NRRL B-3683. Further ultraviolet irradiation produced an isolate, *Mycobacterium* sp. NRRL B-3805, which lacked the ability to 1-dehydrogenate steroids and was found to produce androst-4-ene-3,17-dione from sterols.

Culture media. In general, two different media were used in the sterol and steroid biotransformation studies. Medium A contained (per liter of tap water): nutrient broth (Difco), 8 g; and yeast extract (Amber BYF-300, Amber Laboratories), 1 g; *p*H 7.0. Medium B consisted of (per liter of tap water): enzymatic protein digest (Ferm-Amine I, Sheffield Chemical), 5 g; yeast extract (Amber BYF-300), 1 g; *myo*-inositol (General Biochemicals), 1 g; and $Na_2HPO_4 \cdot 7H_2O$, 2 g; *p*H 7.0.

Fermentation conditions: shaken flask studies. One-liter Erlenmeyer flasks containing 250 ml of medium A were inoculated with a 48-hr liquid culture of *Mycobacterium* sp. NRRL B-3683 and incubated in a controlled environment rotary shaker (New Brunswick Scientific Co.) at 250 rev/min (1-inch orbit). After 48 hr at 31 C, 100 mg of powdered sterol, prepared by pulverizing with a mortar and pestle, was added to each of two flasks, and the in-

cubation was continued for 72 to 96 hr.

Fermentation conditions: stirred-jar fermentor studies. A 7.5-liter fermentor (New Brunswick Scientific Co.) with a working volume of 5 liters was charged with medium B unless specified otherwise. The sterol substrate, added to the medium before sterilization, was prepared by sonically treating pulverized material for 10 min in a warm aqueous Tween 80 solution (Atlas Chemicals; 0.1%, v/v). The substrate concentration in the fermentor was routinely 1 g/liter. A 48-hr 0.5% inoculum was added, and the fermentation was allowed to proceed for 168 to 240 hr. The temperature was maintained at 31 C at 200 rev/min, and aeration was at 3 liters/min. Foaming was controlled by the addition of silicone Antifoam compound (Dow-Corning).

Fermentation conditions: 250-liter study. To a 350-liter fermentor, charged with 250 liters of medium B, we added a suspension of 250 g of sitosterols, N.F. [The Upjohn Co.; containing 55% β-sitosterol and 45% campesterol as determined by gas-liquid chromatography (GLC)] in aqueous Tween 80 (0.1%) prepared by sonic treatment. After sterilization, a 48-hr 0.4% inoculum of *Mycobacterium* sp. NRRL B-3683 was introduced, and the fermentation was continued for 116 hr at 31 C with an airflow of 25 liters/min and agitation at 200 rev/min.

Extraction and analysis of products. The bioconversion beers were extracted twice with one-third volume of methylene chloride, and the extracts were combined. Preliminary analysis of the fermentations was performed by thin-layer chromatography on Silica Gel G with ethanol-benzene (4:6) as the developing solvent. The progress of the biotransformation was also monitored by GLC on a Varian 2100 equipped with hydrogen flame ionization detector and a 5-ft (1.5 m) glass column packed with 1.5% OV 17 (Ohio Valley Chemical) on Chromosorb W 80/100 mesh (Johns-Manville). The oven temperature was 240 C.

Isolation and identification of products. After removal of the methylene chloride under reduced pressure, the residue from a 5-g fermentation was subjected to column chromatography on 500 g of Silica Gel CC-7 (J. T. Baker). Elution with benzene containing successively increasing proportions of ethyl acetate afforded the following products: androst-4-ene-3,17-dione (15 to 20% ethyl acetate); androsta-1,4-diene-3,17-dione (20 to 25% ethyl acetate); and 20α-hydroxymethylpregna-1,4-dien-3-one (30 to 50% ethyl acetate; I, Fig. 1). The fractions containing each of the aforementioned products were combined and recrystallized: androst-4-ene-3,17-dione from ethyl acetate-hexane, melting point (mp) 170 to 172 C; androsta-1,4-diene-3,17-dione from ether, mp 139 to 141 C; and 20α-hydroxymethylpregna-1,4-dien-3-one from acetone-ether, mp 179 to 182 C.

Androst-4-ene-3,17-dione and androsta-1,4-diene-3,17-dione were identified by comparison of their physical properties, including mp, mixed mp, optical rotation, thin-layer chromatography, and GLC, and ultraviolet, infrared, and nuclear magnetic resonance (NMR) spectra, with those of authentic samples.

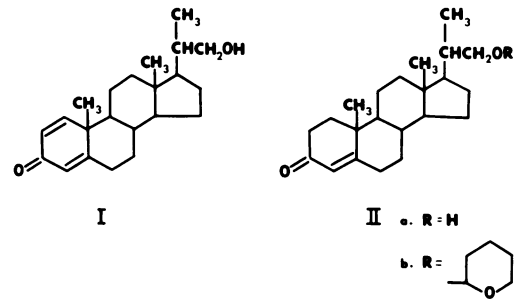

FIG. 1. *Structure of 20α-hydroxymethylpregna-1,4-dien-3-one (I) and synthetic intermediates (II).*

Synthesis and structural proof of 20α-hydroxymethylpregna-1,4-dien-3-one. A sample of 20α-hydroxymethylpregna-1,4-dien-3-one obtained by column chromatography and recrystallization was further recrystallized twice from acetone-ethyl acetate, giving pure material: mp, 181 to 183.5 C; $[\alpha]_D^{28}$ + 28.17° (*c* 1, chloroform); ultraviolet maxima (methanol), 245 nm (ϵ 16,100); infared maxima (KBr), 3438 (OH), 1669, 1621, 1601, and 887 cm^{-1} ($\Delta^{1,4}$-3-ketone); NMR (Table 5); molecular weight, 328 (M.S.). Analysis: $C_{22}H_{32}O_2$ (328.48); calculated: C, 80.44; H, 9.83; found: C, 80.09; H, 9.99.

Synthesis of 22-(2′-tetrahydropyranyl)-20α-hydroxymethyl-pregn-4-en-3-one (IIb). A solution of 2 g of 20α-hydroxymethylpregn-4-en-3-one (IIa) (4) in 100 ml of benzene was distilled until 20 ml of distilled material was collected and then was treated with 0.05 g of *p*-toluenesulfonic acid. After the vigorous reaction subsided, 5 ml of saturated potassium carbonate was added and the mixture was vigorously shaken. The organic solution was washed four times with water, dried over anhydrous sodium sulfate, and evaporated. Crystallization of the residue from methanol containing a trace of pyridine afforded 1.6 g of IIb: mp, 114 to 118.5 C; ultraviolet maxima (methanol), 240 nm (ϵ 15,550); infrared maxima (chloroform), 1662 and 1612 cm^{-1} (Δ^4-3-ketone); NMR consistent with structure IIb. Analysis: $C_{27}H_{42}O_3$ (414.61); calculated: C, 78.21; H, 10.21; found: C, 78.41; H, 10.26.

20α-Hydroxymethylpregna-1,4-dien-3-one (I). A solution of 1.0 g of IIb in 50 ml of benzene was dried by distillation of 10 ml of benzene, treated with 0.60 g of dichlorodicyanoquinone, and heated under reflux for 20 hr. The mixture was cooled and filtered through a small amount of Woehlm basic alumina, and the filtrate was evaporated. The residue was crystallized from aqueous ethanol containing a trace of hydrochloric acid and gave 20α-hydroxymethylpregna-1,4-dien-3-one (I), identical in all respects with the material isolated from sterol fermentation.

RESULTS

The ability of *Mycobacterium* sp. NRRL B-3683 to degrade the 17-alkyl side chain of sev-

eral sterols to produce androsta-1,4-diene-3,17-dione is shown in Table 1. This bacterium displayed a definite preference for substrates possessing the 3β-hydroxy-Δ^5 system rather than the corresponding 3-keto-Δ^4 structure. In the 5-liter fermentor studies, a 78% yield of recrystallized androsta-1,4-diene-3,17-dione was obtained from cholesterol in 144 hr of fermentation time. In 168 hr, only 44% of cholest-4-en-3-one was converted. Similar results were obtained with other sterols. Both lanosterol and ergosterol were resistant to side-chain degradation.

The yield of androst-4-ene-3,17-dione in the 5-liter runs was minimal, ranging from 1 to 4% of the substrate consumed when the initial substrate concentration was 1 g/liter. GLC monitoring of the progress of the fermentations indicated that androst-4-en-3,17-dione was formed at a rapid rate early in the fermentation but was apparently converted to the diene compound by the 1-dehydrogenating ability of the organism.

Degradation of sitosterols, N.F., in a 250-liter fermentation for 116 hr afforded a 30% yield (recrystallized) of androsta-1,4-diene-3,17-dione and a 13% yield (recrystallized) of androst-4-ene-3,17-dione. Incomplete conversion was indicated by a 15% recovery of the substrate.

TABLE 1. *Degradation of various sterols by Mycobacterium sp. NRRL B-3683*[a]

Substrate (1 g/liter)	Crude substrate recovered		Androsta-1,4-diene-3,17-dione	
	Amt (g)	Per cent	Amt (g)[b]	Per cent[c]
Cholesterol[d]	1.88	38	1.80	78
Cholest-4-en-3-one	2.99	60	0.66	44
Sitosterols, N. F.	0.98	20	1.34	48
Stigmasterol	2.23	45	0.70	37
Stigmast-4-en-3-one	1.91	38	0.18	4
Stigmasta-1,4-dien-3-one	0.49	12	0.83	34
Stigmasta-4,22-dien-3-one[e]	3.13	63	0.40	31
Soya sterol residues[f]	7.80	39	1.65	20

[a] A 5-liter scale in medium A; fermentation time, 168 to 192 hr.

[b] Recrystallized.

[c] Per cent yield based on substrate consumed: % yield = [(weight of androsta-1,4-diene-3,17-dione)/(weight of substrate − weight substrate recovered)] × [(molecular weight of substrate)/(molecular weight of androsta-1,4-diene-3,17-dione)] × 100%.

[d] Fermentation time, 144 hr.

[e] Fermentation time, 240 hr.

[f] The Upjohn Co., 4 g/liter; fermentation time, 240 hr.

The effect of various media on the degradation of sitosterols, N.F., to androsta-1,4-diene-3,17-dione by this strain is shown in Table 2. Medium A, supplemented with *myo*-inositol and Tween 40, and medium B gave comparable product steroid yields. Addition of *myo*-inositol to other nutrients appeared to stimulate growth of the bacterium. Its inclusion as the sole source of carbon in a minimal salts solution produced a product yield as high as did several of the more complete media.

A new compound, 20α-hydroxymethylpregna-1,4-dien-3-one, was repeatedly isolated from the biotransformations. The yields, however, were consistently low (approximately 0.1 g from 5-liter fermentations). Variations in the concentration of the substrate, sitosterols, N.F., from 1 to 10 g/liter and increased aeration had little effect on its production. Attempts to convert this compound to androsta-1,4-diene-3,17-dione were unsuccessful.

The ability of this mycobacterial strain (B-3683) to degrade the 17-alkyl side chain of a number of sterol derivatives in shaken flasks was also examined. 3α,5-Cyclo-6β-hydroxystigmastane, 3α,5-cyclo-6β-hydroxycholestane, 5α-acetoxy-6β,19-epoxystigmastan-3-one, 5α-acetoxy-6β,19-epoxystigmastan-3β-ol, 6β,19-epoxystigmastan-3-one, stigmastane, γ-sitosterol, and 27-norcholesterol were resistant to degradation. Furthermore, lithocholic acid, 3β-hydroxy-5-cholenic acid, 3β-hydroxy-bisnor-5-cholenic acid, and progesterone were also resistant to side-chain degradation. Biotransformation of a variety of steroids revealed that this organism carries out (i) Δ^1-dehydrogenation; (ii) reduction of 1–2 double bond (*see below*); (iii) oxidation of 3β-hydroxy-Δ^5 to 3-keto-Δ^4; and (iv) 17-oxidation-reduction.

The resistance of the androsta-1,4-diene-3,17-dione nucleus to further degradation was examined. A 5-liter fermentation containing 2 g of steroid substrate was performed under the above conditions for 144 hr. Recovery and recrystallization of the steroids afforded 1.24 g of a mixture of androsta-1,4-diene-3,17-dione and androst-4-ene-3,17-dione, 0.05 g of Δ^1-testosterone, and a trace of what appeared to be 5α-androstane-3,17-dione.

Mycobacterium sp. NRRL B-3805 produced good yields of androst-4-ene-3,17-dione from various sterols (Table 3) and in a variety of media (Table 4), although the yields were somewhat lower than those of the diene compound isolated from bioconversions carried out

TABLE 2. *Effect of media on the degradation of sitosterols, N.F., by Mycobacterium sp. NRRL B-3683*[a]

Medium	Crude sitosterol recovered		Androsta-1,4-diene-3,17-dione		Fermentation time (hr)
	Amt (g)	Per cent	Amt (g)[b]	Per cent[c]	
Medium A	1.59	32	0.94	40	168
A + inositol	0.67	13	0.84	28	192
A + inositol + Tween 20 (0.5%)	0.34	7	0.60	19	240
A + inositol + Tween 40 (0.5%)	0.03	1	1.56	46	240
Ferm-Amine I (FA)	0.68	14	1.03	35	216
FA + yeast extract	0.08	2	0.44	13	172
FA + NZ-Amine[d]	1.80	36	0.91	41	192
Yeast extract (YE)	0.97	19	0.92	33	172
YE + meat peptone[e]	1.79	36	1.03	47	172
Medium B	0.36	7	1.51	48	216
MS[f] + inositol	1.56	31	0.62	26	240
MS + mannitol	1.70	34	0.17	7	240

[a] A 5-liter scale; substrate concentration, 1 g/liter.
[b] Recrystallized product.
[c] Per cent yield based on substrate consumed: % yield = [(weight of androsta-1,4-diene-3,17-dione)/(weight of sitosterol − weight of sitosterol recovered)] × [(molecular weight sitosterol)/(molecular weight of androsta-1,4-diene-3,17-dione)] × 100%.
[d] Sheffield Chemical, enzymatic digest of casein.
[e] Amber Laboratories.
[f] Minimal salts solution (grams/liter: K_2HPO_4, 1.8; KH_2PO_4, 0.75; $(NH_4)_2SO_4$, 0.25; $MgSO_4$, 0.02).

TABLE 3. *Degradation of various sterols by Mycobacterium sp. NRRL B-3805*[a]

Substrate (1 g/liter)	Crude substrate recovered		Androst-4-ene-3,17-dione	
	Amt (g)	Per cent	Amt (g)[b]	Per cent[c]
Cholesterol	0.57	11	1.00	31
Sitosterols, N.F.	0.48	10	1.20	39
Stigmasterol	0.84	17	0.29	10
Stigmast-4-en-3-one	2.25	45	0.29	16
Stigmasta-4,22-dien-3-one	2.08	42	0.24	12
Soya sterol residues[d]	0.48	10	0.69	22

[a] A 5-liter scale in medium B; fermentation time, 168 to 171 hr.
[b] Recrystallized product.
[c] Per cent yield based on substrate consumed: % yield = [(weight of androst-4-ene-3,17-dione)/(weight of substrate − weight of substrate recovered)] × [(molecular weight of substrate)/(molecular weight of androst-4-ene-3,17-dione)] × 100%.
[d] The Upjohn Company; fermentation time, 216 hr.

TABLE 4. *Effect of media on the degradation of sitosterols, N.F., by Mycobacterium sp. NRRL B-3805*[a]

Medium	Crude sitosterol recovered		Androst-4-ene-3,17-dione	
	Amt (g)	Per cent	Amt (g)[b]	Per cent[c]
Medium A	2.17	43	0.70	36
Medium A + inositol	2.26	45	0.34	18
Yeast extract (0.5%)[d] + inositol	0.56	11	0.65	21
Medium B	0.26	5	1.14	35
Soy flour[e] + inositol	0.25	5	0.40	12

[a] A 5-liter scale; fermentation time, 168 hr.
[b] Recrystallized product.
[c] Per cent yield based on substrate consumed: % yield = [(weight of androst-4-ene-3,17-dione)/(weight of sitosterol − weight sitosterol recovered)] × (molecular weight of sitosterol)/(molecular weight of androst-4-ene-3,17-dione)] × 100%.
[d] Amber Milbrew DBY series I, *p*H adjusted to 7 with Na_2HPO_4.
[e] Staley Hi-Fat soy flour, *p*H adjusted to 7 with Na_2HPO_4.

by strain B-3683. The Δ^1-dehydrogenating activity was not completely absent, however, as evidenced by frequent isolation of trace quantities of androsta-1,4-diene-3,17-dione. Moreover, 20α-hydroxymethylpregna-1,4-dien-3-one, in addition to 20α-hydroxymethylpregn-4-en-3-one, was isolated in trace amounts (Table 5).

Other products occasionally isolated in minute amounts from 5-liter, 1-g/liter sterol

TABLE 5. *NMR spectrum (60 mHz) of 20α-hydroxymethylpregna-1,4-dien-3-one*[a]

T	Type[b]	Assignment
2.94	d	C_1—H, $J_{1,2} = 10$
3.80	dd	C_2—H, $J_{1,2} = 10$, $J_{2,4} = 2$
3.93	bd.s	C_4—H
6.37	dd	C_{22}—H_A, $J_{20,22A} = 3.0$, $J_{22A,22B} = 10$
6.67	dd	C_{22}—H_B, $J_{22A,22B} = 10$, $J_{20,22B} = 6.6$
8.05	s	C_{22}—OH
8.77	s	C_{19}—H_3
8.95	d	C_{21}—H_3, $J_{20,21} = 5.5$
9.25	s	C_{18}—H_3

[a] $CDCl_3$ solution, tetramethylsilane reference; T = tau value.

[b] Abbreviations: d, doublet; dd, doublet of doublets; s, singlet; bd.s, broad singlet.

biotransformations gave evidence for the ability of the bacterium to introduce an oxygen function at carbons 6 and 9 and for reduction of the 4–5 double bond of androst-4-ene-3,17-dione.

Androst-4-ene-3,17-dione proved to be resistant to further degradation. In a 5-liter study, 3 g of the steroid was incubated for 74 hr. About 80% of the starting material was recovered, and it contained a small amount of what appeared to be 6α-hydroxyandrost-4-ene-3,17-dione (by infrared and ultraviolet spectroscopy).

A comparison of the transformations of 19-hydroxystigmast-4-en-3-one by both mycobacteria indicated the basic difference between these two organisms. Both bacteria produced 19-hydroxyandrost-4-en-3,17-dione in approximately 18% yield in a 5-liter study, in addition to which only strain B-3683 yielded estrone.

DISCUSSION

Utilization of the soybean sterols β-sitosterol, campesterol, and stigmasterol as an economical source of intermediates for steroid manufacture has been considered for supplementing or supplanting the current commercial processes from diosgenin, obtained from the Barbasco root and other sources (10). The chemical production of pregnenolone and progesterone from stigmasterol is the only process that has employed these materials and has resulted in the accumulation of a substantial quantity of sitosterol residues (6).

The microbiological conversion of the phytosterols to the androstane steroids offers an attractive approach, in principle, for the use of the sterol residues. Heretofore, problems of ring degradation of the fermentation products and low substrate concentrations have negated the use of the microbial approach on a production basis.

Mycobacterium sp. NRRL B-3683 and NRRL B-3805 convert plant sterols in good yield to useful 17-ketone compounds. These bacteria have advantages over others, which also degrade the 17-alkyl side chain, in that the sterol nucleus need not be modified nor inhibitory agents be added to the fermentation in order to minimize ring degradation. In fact, modification of the 3β-hydroxy-Δ^5 sterol structure inhibits or completely prevents the desired side-chain degradation by these two organisms.

The degradation pathway of various sterols to androsta-1,4-diene-3,17-dione by *Mycobacterium* sp. NRRL B-3683 is unknown. Oxidized A-ring intermediates with the side chain intact, i.e., stigmast-4-en-3-one or stigmasta-1,4-dien-3-one, were not isolated from bioconversions either with strain NRRL B-3683 or with strain NRRL B-3805. Nagasawa et al. (5) identified such intermediates when cholesterol was fermented with a variety of microorganisms, but they could not observe these compounds when *M. avium*, *M. phlei*, or *M. smegmatis* was cultured in the presence of the ring degradation inhibitor α,α'-dipyridyl.

The production of 20α-hydroxymethylpregna-1,4-dien-3-one is believed to represent a side reaction and not an intermediate in the major pathway leading to production of 17-ketonic products. Although C-26 and C-27 hydroxylations have been reported for *M. smegmatis* (3) and a *Mycobacterium* sp. (12), respectively, C-20 oxygenation has been observed only in mammalian systems (7).

ACKNOWLEDGMENTS

The authors acknowledge the assistance of William Aksamit (GLC), Robert Nicholson (column chromatography), and Bruce Smith (thin-layer chromatography). We also thank Myra Greenberg and Jane Lee for their technical expertise.

LITERATURE CITED

1. Afonso, A., H. L. Herzog, C. Federbush, and W. Charney. 1966. Microbiol degradation of 19-nor-$\Delta^{1,3,5(10)}$-cholestatrien-3-ol. Steroids **7**:429–432.
2. Arima, K., M. Nagasawa, M. Bae, and G. Tamura. 1969. Microbial transformation of sterols. Part I. Decomposition of cholesterol by microorganisms. Agr. Biol. Chem. **33**:1636–1643.
3. Caspi, E., M. G. Kienle, K. R. Varma, and L. J. Mulheirn. 1970. *trans*-Reduction of Δ^{24} of lanosterol in the biosynthesis of cholesterol by rat liver enzymes. J. Amer. Chem. Soc. **92**:2161–2163.
4. Morita, K. 1959. Selective oxidation of allylic alcohols with active n-halogen compounds. Bull. Chem. Soc. Japan **32**:227–232.
5. Nagasawa, M., M. Bae, G. Tamura, and K. Arima. 1969. Microbial transformation of sterols. Part II. Cleavage of sterol side chains by microorganisms. Agr. Biol. Chem. **33**:1644–1650.

6. Petrow, V. 1969. Microbial transformations of sterols. Chem. Ind., Jan. 4, p. 18–20.
7. Shimizu, K., M. Hayano, M. Gut, and R. I. Dorfman. 1961. The transformation of 20α-hydroxycholesterol to isocaproic acid and C_{21} steroids. J. Biol. Chem. **236:** 695–699.
8. Sih, C. J., and K. C. Wang. 1965. A new route to estrone from sterols. J. Amer. Chem. Soc. **87:**1387–1388.
9. van der Waard, W. F., J. Doodewaard, J. de Flines, and S. van der Weele. 1969. Microbiological conversion of cholesterol into androsta-1,4-diene-3,17-dione. Abh. Deut. Akad. Wiss. Berlin Kl. Med. **2:**101–105.
10. Wiechert, R. 1970. Modern steroid problems. Angew. Chem. Int. Ed. Engl. **9:**321–388.
11. Wix, G., K. G. Buki, E. Tomorkeny, and G. Ambrus. 1968. Inhibition of steroid nucleus degradation in mycobacterial transformations. Steroids **11:**401–413.
12. Zaretskaya, I. I., L. M. Kogan, O. B. Tikhomirova, J. D. Sis, N. S. Wulfson, V. I. Zaretskii, V. G. Zaikin, G. K. Skryabin, and I. V. Torgov. 1968. Microbial hydroxylation of the cholesterol side chain. Tetrahedron **24:** 1595–1600.