Oxidative side-chain and ring fission of pregnanes by *Arthrobacter simplex*

Shashi B. MAHATO, Sukdeb BANERJEE and Sankar PODDER

Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Jadavpur, Calcutta-700 032, India

Metabolic processes involving side-chain and ring cleavage of progesterone, 17-hydroxyprogesterone, 11-deoxycortisol and 16-dehydropregnenolone by *Arthrobacter simplex* were studied. The formation of the metabolites from progesterone indicates a pathway somewhat different from normal in the enzymic reaction sequence, and the 17-hydroxyprogesterone metabolites reveal a non-enzymic rearrangement step. The presence of a hydroxy group at C-21, as in 11-deoxycortisol, induces reduction of the C-20 carbonyl group. The microbial preparation of a novel androstane analogue, 17 β -hydroxy-16 α -methoxyandrost-1,4-dien-3-one, by incubation of 16-dehydropregnenolone with the bacterial strain was achieved. The formation of this metabolite is a multistep process involving a novel microbial generation of a methoxy group from a double-bond transformation in a steroid skeleton.

Microbial scissions of carbon-carbon bonds are of much interest, as cleavage of side chains of widely available phytosterols and of the acetyl side chains of pregnanes to give C₁₉ steroids are commercial processes (Kieslich, 1980; Smith, 1984; Mahato & Mukherjee, 1984; Mahato & Banerjee, 1985). Whereas degradation of phytosterol side chains proceeds via β -oxidation (Martin, 1977), the cleavage of the acetyl side chain of progesterone has been shown to be via oxygen-insertion reactions of Baeyer-Villiger type (Rahim & Sih, 1966). Microbial side-chain and ring fission of phytosterols and pregnanes easily derivable from phytosterols and steroid sapogenins have received considerable attention for the preparation of useful precursors for convergent synthesis of pharmaceutically interesting retrosteroids (9 β ,10 α -configuration). A number of microbial strains, mainly of the genera *Nocardia*, *Mycobacterium*, *Arthrobacter* and *Pseudomonas*, have been developed for the synthesis of the precursors for retrosteroids from sitosterols (Schömer *et al.*, 1978; Knight & Wovcha, 1979, 1981), cholesterol (Nakamatsu *et al.*, 1980; Ferreira *et al.*, 1984) and bile acids (Leppik, 1981), and the mechanisms of formation of these products have been elucidated (Gibson *et al.*, 1966; Sih *et al.*, 1966; Schömer *et al.*, 1978). However, less work has been done on the microbial side-chain and ring fission of pregnanes. Moreover, production of the precursors for retrosteroids from pregnanes by any *Arthrobacter* species has not hitherto been reported. The present paper reports the preparation of these steroids from several pregnanes by *Arthrobacter simplex*, along with a unique efficient conversion of 16-dehydropregnenolone (XIII) into a new androstene analogue, 17 β -hydroxy-16 α -methoxyandrost-1,4-dien-3-one (XV), involving a novel microbial generation of a methoxy group from a double-bond transformation in a steroid skeleton.

EXPERIMENTAL

Materials

Inorganic salts were purchased from Sarabhai Chemicals, Bombay, India. Peptone, beef extract, yeast extract

and agar were purchased from Difco Laboratories, Detroit, MI, U.S.A. Silica gel for column chromatography and for t.l.c. (60HF₂₅₄) and organic solvents were supplied by E. Merck, Bombay, India. Steroid substrates and di-isopropyl phosphorofluoridate were the products of Sigma Chemical Co., St. Louis, MO, U.S.A. Reagents and solvents were of analytical grade.

Instrumental methods

Melting points were determined in open capillary tubes in an H₂SO₄ bath and are uncorrected. Optical rotations were measured in chloroform with a Perkin-Elmer automatic polarimeter. I.r. spectra in Nujol mulls were recorded with a Perkin-Elmer 177 spectrophotometer. Mass spectra were obtained with a Hitachi model RMU-6L mass spectrometer at an ionizing potential of 70 eV. ¹H- and ¹³C-n.m.r. spectra were obtained with a JEOL FT-100 spectrometer at 100 and 25.05 MHz respectively with tetramethylsilane as an internal standard. H.p.l.c. was performed in a Spectra Physics model SP 8000B apparatus controlled by a microprocessor that allows selection of constant pressure or constant flow for quantification, with an autoinjector, a dual-channel plotter/printer and model SP 8440 UV/Vis variable-wavelength detector. The column used for h.p.l.c. was a Spectra Physics 25 cm $\times$ 4 mm (internal diameter) stainless-steel column packed with octylsilane chemically bonded to silica gel (10 μ m particle size).

Growth and fermentation conditions

The strain of *Arthrobacter simplex* (Registry no. IICB-321) was isolated from soil by an enrichment culture technique (Pelczar *et al.*, 1977) with 11-deoxycortisol as the sole source of carbon. It is being maintained in nutrient agar slants at the Culture Collection Unit of the Steroid and Terpenoid division of this Institute. Fermentations of different steroid substrates were carried out as follows. *A. simplex* was grown for 24 h in 20 flasks (500 ml) each containing 100 ml of a sterile medium of either of the two following compositions: (a) peptone, 5 g; yeast extract, 2.5 g; beef extract, 2.5 g; NaCl, 2.5 g; distilled water, 1 litre (pH adjusted with NaOH to 7.0); or (b) K₂HPO₄, 0.7%; KH₂PO₄, 0.3%; (NH₄)₂SO₄,

0.1%; MgSO_4 , 0.01%; glucose, 1%; pH 7.0. The mycelia of each flask were then harvested by centrifuging, washed with 0.5% NaCl solution and suspended in a 500 ml Erlenmeyer flask containing 100 ml of 50 mM-Tris/HCl buffer, pH 7.5. A total of 1 g of progesterone dissolved in acetone (60 ml) was evenly distributed among the 20 flasks containing the buffer, the enzymic inhibitors were added at the required concentrations, the flasks were plugged with cottonwool and the fermentation was continued for 40 h at 37 °C in aerobic conditions with shaking. The course of the incubation was monitored by t.l.c. The other steroid substrates were also incubated in the same experimental conditions used for progesterone.

Extraction and isolation of metabolites

After the end of the fermentation the fermentation mixture was harvested and exhaustively extracted thrice with equal volumes of chloroform. The organic layer was washed with distilled water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. After complete evaporation of solvent a semi-solid residue was obtained. A little of the residue was dissolved in 1 ml of chloroform and analysed by t.l.c. on silica-gel plates (Merck 60HF₂₅₄) with chloroform/ethyl acetate/methanol in different proportions as the developing solvent system. The individual metabolites were separated and purified by column chromatography over silica gel and preparative t.l.c. followed by crystallization. The characterization of the metabolites was carried out by the application of various spectroscopic techniques, e.g. u.v., i.r., ^1H n.m.r., ^{13}C n.m.r. and m.s. The very-well-known steroids were identified by t.l.c. comparison with authentic samples.

Transformation of 16-dehydropregnenolone (XIII). Fermentation of 1 g of 16-dehydropregnenolone and isolation of the metabolites, pregna-1,4,16-triene-3,20-dione (XIV) and 17 β -hydroxy-16 α -methoxyandrost-1,4-dien-3-one (XV), were done as described above. The yields of the two metabolites (XIV) and (XV) were 20 and 35% respectively.

Pregna-1,4,16-triene-3,20-dione (XIV). This crystallized from ethyl acetate as needles, m.p. 208–210 °C and $[\alpha]_{\text{D}}^{25} + 113^\circ$ (*c* 0.35 in chloroform) [Shull (1957) gives m.p. 208–211 °C and $[\alpha]_{\text{D}}^{25} + 120^\circ$]; i.r. (Nujol) 1660, 1650, 1615, 1595 and 1575 cm^{-1} ; m.s. *m/z* 310 (M^+ , 55%), 295 ($M^+ - \text{CH}_3$, 15%), 267 ($M^+ - \text{COCH}_3$, 12%) and 122 (100%); ^1H n.m.r. ($[\text{H}]$ chloroform) 0.96 (s, 3H, 18- CH_3), 1.25 (s, 3H, 19- CH_3), 2.25 (s, 3H, 21- CH_3), 6.08 (br s, 1H, 4-*H*), 6.24 (dd, 1H, *J* = 2 and 10 Hz, 2-*H*), 6.68 (t-like, 1H, 16-*H*) and 7.07 p.p.m. (d, 1H, *J* = 10 Hz, 1-*H*).

17 β -Hydroxy-16 α -methoxyandrost-1,4-dien-3-one (XV). This crystallized from ethyl acetate as micro-needles, m.p. 212–214 °C and $[\alpha]_{\text{D}}^{25} - 45.5^\circ$ (*c* 0.2 in chloroform); i.r. (Nujol) 3400, 1650–1640, 1610, 1585, 1085, 1060 and 885 cm^{-1} ; m.s. *m/z* 316 (M^+ , 14%), 269 ($M^+ - \text{CH}_3\text{OH} - \text{CH}_3$, 2%), 266 ($M^+ - \text{CH}_3\text{OH} - \text{H}_2\text{O}$, 4%), 257 ($M^+ - \text{CO} - \text{OCH}_3$, 5%), 223 ($M^+ - \text{CH}_3\text{OH} - \text{CO} - \text{H}_2\text{O} - \text{CH}_3$, 10%) and 122 (100%); ^1H n.m.r. ($[\text{H}]$ chloroform) 0.84 (s, 3H, 18- CH_3), 1.22 (s, 3H, 19- CH_3), 3.34 (s, 1H; 16- OCH_3), 3.54 (m, 2H, 16-*H*, 17-*H*), 6.08 (br s, 1H, 4-*H*), 6.23 (dd, 1H, *J* = 2 and 10 Hz,

2-*H*) and 7.03 p.p.m. (d, 1H, *J* = 10 Hz, 1-*H*) (Found: C, 75.8; H, 8.8; $\text{C}_{20}\text{H}_{28}\text{O}_3$ requires C, 75.9; H, 8.9%).

Preparation of the acetate (XVI) of metabolite (XV).

The metabolite (XV) (30 mg) was acetylated with acetic anhydride (6 ml) and pyridine (1 ml) at 80 °C for 1 h. The reaction mixture was worked up in the usual manner and the acetate (XVI) was crystallized from methanol as needles. The yield was 26 mg, m.p. 230–232 °C and $[\alpha]_{\text{D}}^{25} - 18.66^\circ$ (*c* 0.15 in chloroform); m.s. *m/z* 358 (M^+ , 33%) 326 ($M^+ - \text{CH}_3\text{OH}$, 16%), 298 ($M^+ - \text{CH}_3\text{CO}_2\text{H}$, 6%) 283 ($M^+ - \text{CH}_3\text{CO}_2\text{H} - \text{CH}_3$, 26%), 266 ($M^+ - \text{CH}_3\text{CO}_2\text{H} - \text{CH}_3\text{OH}$, 12%), 251 ($M^+ - \text{CH}_3\text{CO}_2\text{H} - \text{CH}_3\text{OH} - \text{CH}_3$, 9%) and 122 (100%); ^1H n.m.r. ($[\text{H}]$ -chloroform) 0.82 (s, 3H, 18- CH_3), 1.2 (s, 3H, 19- CH_3), 2.06 (s, 3H, 17- OCOCH_3), 3.24 (s, 1H, 16- OCH_3), 3.84 (q, 1H, 16-*H*), 4.8 (d, 1H, *J* = 7 Hz, 17-*H*), 6.08 (br s, 1H, 4-*H*), 6.23 (dd, 1H, *J* = 2 and 10 Hz, 2-*H*) and 7.03 p.p.m. (d, 1H, *J* = 10 Hz, 1-*H*) (Found: C, 73.8; H, 8.5; $\text{C}_{22}\text{H}_{30}\text{O}_4$ requires C, 73.7; H, 8.4%).

RESULTS AND DISCUSSION

The transformed products of different substrates with their yields under different conditions are shown in Table 1. It is apparent that the yields of the metabolites are better when the substrates are incubated in nutrient medium.

Metabolism of progesterone (I), 17-hydroxyprogesterone (VIII) and 11-deoxycortisol (XI)

The pathways of formation of metabolites of the substrates (I), (VIII) and (XI) are shown in Schemes 1, 2 and 3. The metabolite (II) was identified by its mass and ^1H -n.m.r. spectra and by comparison of its m.p. and $[\alpha]_{\text{D}}$ with those reported in the literature (Charney *et al.*, 1962). The metabolites, Δ^1 -testosterone acetate (III), Δ^1 -testosterone (IV) and androstadienedione (V) were characterized by direct comparison (m.p., co-t.l.c. and i.r.) with authentic samples. The physical and spectral data of the metabolites 3-hydroxy-9,10-secoandrost-1,3,5(10)-triene-9,17-dione (VI) and hexahydro-5 α -hydroxy-7 $\alpha\beta$ -methyl-3 $\alpha\alpha$ -*H*-4 α -(3'-propionic acid)-indan-1-one δ -lactone (VII) were in good agreement with the literature values (Schömer *et al.*, 1978; Owen *et al.*, 1984). The ^{13}C -n.m.r. chemical shifts of these compounds were compatible with their structures and could be completely assigned by comparison with those of compounds with similar carbon atoms. It was observed by Dodson & Muir (1961) that incubation of androst-4-ene-3,17-dione with different species of micro-organisms involved 9 α -hydroxylation followed by Δ^1 -dehydrogenation (or vice versa) with the formation of metabolite (VI). Subsequently Wang & Sih (1963), Gibson *et al.* (1966) and Lee & Sih (1967) demonstrated that these reactions are essential for further metabolism of the steroid nucleus. Although *A. simplex* is known for its property of Δ^1 -dehydrogenation of steroids and cleavage of the side chain of phytosterols involving β -oxidation, degradation of the side chain that is induced by Baeyer-Villiger-type oxygenation and of the nucleus of the pregnanes by any *Arthrobacter* species has not hitherto been reported. The normal pathway in the degradation of the side chain of progesterone has been shown to be via the sequence: progesterone $\rightarrow$ testosterone acetate $\rightarrow$ testosterone $\rightarrow$ androstenedione $\rightarrow$ androstadienedione $\rightarrow$ further degrad-

Table 1. Different substrates and their transformed products with yields

The cells of *A. simplex* were grown in two culture media: (a) peptone, 0.5 %; yeast extract, 0.25 %; beef extract, 0.25 %; NaCl, 0.25 %; pH 7.0; and (b) K_2HPO_4 , 0.7 %; KH_2PO_4 , 0.3 %; $(NH_4)_2SO_4$, 0.1 %; $MgSO_4$, 0.01 %; glucose, 1 %; pH 7.0. The cells from both media were harvested by centrifuging, washed with 0.5 % NaCl solution and suspended in 50 mM-Tris/HCl buffer, pH 7.5. The substrates, progesterone (I), 17-hydroxyprogesterone (VIII), 11-deoxycortisol (XI) and 16-dehydropregnenolone (XIII) (500 mg each), were then incubated with the cells in the buffer for 40 h. The yields (in parentheses) of the metabolites, Δ^1 -progesterone (II), Δ^1 -testosterone acetate (III), Δ^1 -testosterone (IV), androstadienedione (V), the 9,10-seco compound (VI), the indanone derivative (VII), androstenedione (X), 17,20,21-trihydroxypregna-1,4-dien-3-one (XII), Δ^1 -16-dehydropregnenolone (XIV) and 17 β -hydroxy-16 α -methoxyandrost-1,4-dien-3-one (XV) were determined by h.p.l.c. The enzymic inhibitors, namely $\alpha\alpha'$ -bipyridyl and di-isopropyl phosphorofluoridate (Pr'_2 -P-F), were incubated with the substrates at the concentrations indicated.

Substrate	Growth medium (inhibitor)	Amount incubated (mg)	Amount isolated (mg)	Products isolated (% yield)
(I)	(a) (0.5 mM- $\alpha\alpha'$ -bipyridyl)	500	478	(II)(8) + (V)(12)
(I)	(b)	500	471	(IV)(8) + (V)(26) + (VII)(9)
(I)	(b) (0.5 mM- $\alpha\alpha'$ -bipyridyl)	500	462	(II)(9) + (IV)(11) + (V)(16) + (VI)(1)
(I)	(b) (1.5 mM-Pr ^I ₂ -P-F)	500	482	(III)(2) + (II)(12) + (IV)(10) + (V)(7)
(VIII)	(a)	500	460	(X)(6) + (V)(8)
(VIII)	(a) (0.5 mM- $\alpha\alpha'$ -bipyridyl)	500	485	(X)(8) + (V)(32)
(XI)	(b) (0.5 mM- $\alpha\alpha'$ -bipyridyl)	500	456	(X)(2) + (V)(12) + (VII)(4) + (XII)(34)
(XIII)	(a)	500	465	(XIV)(210) + (XV)(35)

Scheme 1. Possible pathway in the metabolic conversion of progesterone (I)

Scheme 2. Possible pathway in the metabolic conversion of 17-hydroxyprogesterone (VIII)

Scheme 3. Possible pathway in the metabolic conversion of 11-deoxycortisol (XI)

ation (Rahim & Sih, 1966). In the present study it appears that the first step in the transformation of progesterone by the *A. simplex* strain is the introduction of a Δ^1 -double bond followed by fission of the side chain by enzymic Baeyer–Villiger oxygenation. This is indicated by the fact that Δ^1 -progesterone (II) has been isolated in 8–12% yield. However, the occurrence of 1,2-dehydrogenation of testosterone acetate cannot be excluded. It may be mentioned that fermentation of androst-4-ene-3,17-dione, which is generally obtained as an intermediate metabolite in the progesterone side-chain degradation process, could not be detected by h.p.l.c. The other metabolites of progesterone (Scheme 1) are formed according to the usual reaction sequence. However, it is noteworthy that when 17-hydroxyprogesterone (VIII) was used as a substrate in the fermentation with the strain under identical conditions androstenedione could indeed be isolated as an intermediate metabolite and no Δ^1 -dehydrogenated product of compound (VIII) was detected. The use of the esterase inhibitor di-isopropyl phosphorofluoridate did not induce any effect on the rate of formation of the metabolites of 17-hydroxyprogesterone (VIII). This anomaly in the metabolic pathway is attributed to the presence of the 17-hydroxy group, which stimulates the activity of the enzyme responsible for the Baeyer–Villiger oxygenation generating the ester (IX). This ester, presumably on non-enzymic rearrangement, yields metabolite (X) as shown (Scheme 2). This assumption was supported by the observation that the employment of di-isopropyl phosphorofluoridate during fermentation did not have any effect on the rate of metabolism. The presence of a hydroxy function at C-21,

as in 11-deoxycortisol (XI), induced the reduction of the 20-carbonyl group, as it was observed that metabolism of 11-deoxycortisol (XI) with this strain under similar conditions produced 17,20 β ,21-trihydroxypregna-1,4-dien-3-one (XII) as the major metabolite besides the other expected metabolites, (V), (VII) and (X) (Scheme 3). The metabolite (XII) was characterized by comparison of its m.p., $[\alpha]_D$ and i.r. spectrum with those of reported data (Herzog *et al.*, 1962) and also by determination and assignment of its ^{13}C -n.m.r. values (Table 2). The results demonstrated that the strain of *A. simplex* possesses altered specificity with respect to the steroid side chain.

Metabolism of 16-dehydropregnenolone (XIII)

A novel microbial conversion of 16-dehydropregnenolone by the strain of *A. simplex* into a new androstane analogue, 17 β -hydroxy-16 α -methoxyandrost-1,4-dien-3-one (XV) along with the isolation of an intermediate, pregna-1,4,16-triene-3,20-dione (XIV), was achieved. The microbial preparation of compound (XIV) from 16-dehydropregnenolone is the subject of a U.S. patent (Shull, 1957). The structure determination of compound (XV) was accomplished by the analytical and spectral data of the compound and also of its 17-acetate.

The presence of a hydroxy group in compound (XV) was evident from its i.r. spectrum, and a singlet integrated for three protons at 3.34 p.p.m. revealed the presence of a methoxy group. The ^1H -n.m.r. spectrum also displayed peaks at 6.08 (br s, 1H), 6.23 (dd, 1H, $J = 2$ and 10 Hz) and 7.03 p.p.m. (d, 1H, $J = 10$ Hz) assigned to C-4-H, C-2-H and C-1-H respectively. The ^{13}C -n.m.r. data (Table 2) of compound (XV) suggested the orientations of the 17-hydroxy and 16-methoxy groups as shown, taking into consideration their substituent effects (Eggert *et al.*, 1976) on the 18-methyl group and C-14 respectively. Assignments of the ^{13}C -n.m.r. signals of compound (XV) were made on the basis of their chemical shifts, off-resonance studies and comparison of the shift data with those reported for 17 β -hydroxyandrost-1,4-dien-3-one (Hickey *et al.*, 1980). It is noteworthy that the C-12 resonance of compound (XV) is shifted upfield by 6.5 p.p.m. upon introduction of the 16 α -methoxy group. The ^1H -n.m.r. spectrum of the acetate of compound (XV) showed a doublet of 4.8 p.p.m. ($J = 7$ Hz), which established the position of the hydroxy group at C-17 in compound (XV).

The mechanism of formation of compound (XV) from 16-dehydropregnenolone is a subject of speculation. However, a reasonable though not obligatory mechanism may be envisaged as shown in Scheme 4. The compound

Table 2. ^{13}C -n.m.r. chemical shifts (δ) of 11-deoxycortisol (XI), 17,20 β ,21-trihydroxypregna-1,4-dien-3-one (XII), pregna-4,16-diene-3,20-dione (XVIII), pregna-1,4,16-triene-3,20-dione (XIV), 17 β -hydroxyandrosta-1,4-dien-3-one (IV), 16 α -methoxy-17 β -hydroxyandrosta-1,4-dien-3-one (XV), 3-hydroxyoestra-1,3,5(10)-trien-17-one (XVII) and 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (VI)

The spectra were obtained at 25.05 MHz in $[\text{H}]$ chloroform and the values are given in p.p.m. with respect to the tetramethylsilane used as an internal standard. Values marked ^a and ^b within a vertical column may be interchanged.

Carbon atom	Chemical shift (p.p.m.)							
	(XI)*	(XII)	(XVIII)*	(XIV)	(IV) [†]	(XV)	(XVII)*	(VI)
1	35.2	156.6	36.0	155.4	155.2	155.7	126.9	127.3
2	33.4	127.0	34.1	127.3	128.0	127.3	113.5	112.7
3	197.4	186.6	197.6	185.9	185.1	186.2	155.8	154.2
4	123.0	123.3	124.4	123.7	124.3	123.7	115.9	115.6
5	170.4	170.3	169.7	168.5	168.2	169.0	138.2	141.6
6	31.9	32.8 ^a	32.8	32.5 ^a	33.8	32.5 ^a	30.2	30.2
7	32.1	33.1 ^a	32.5	33.2 ^a	32.9	32.9 ^a	27.4	26.6
8	35.2	35.5	34.4	33.7 ^b	36.2	35.0	39.3	49.0
9	53.0	52.0	54.8	52.6	53.2	52.3	45.0	211.3
10	38.1	43.7	39.0	43.5	43.6	43.4	131.9	130.9
11	20.3	22.5	21.2	22.6	22.8	22.0	26.4	37.2
12	30.1	31.5	36.2	34.3 ^b	37.2	30.7	32.5	30.8
13	47.0	47.5	46.6	46.1	43.7	43.5	48.3	47.5
14	49.9	48.5	56.3	55.1	50.8	47.9	51.1	49.6
15	23.2	23.9	32.3	32.0	24.0	36.2	22.2	22.3
16	33.4	33.6	155.9	154.75	30.8	87.2	35.9	36.0
17	88.4	84.8	143.5	143.7	81.2	87.2	219.3	218.6
18	14.5	14.9	16.0	15.7	11.4	12.3	13.9	13.4
19	17.0	18.5	17.1	18.5	18.8	18.6		18.1
20	211.3	73.6	195.7	196.2				
21	65.9	64.2	26.7	26.8				
OCH ₃						57.2		

* Blunt & Stothers (1977).

[†] Hickey *et al.* (1980).

Scheme 4. Postulated pathway in the metabolic conversion of 16-dehydropregnenolone (XIII)

(XIII) is transformed by the enzyme system produced by the *A. simplex* strain to the metabolite (XIV) by dehydrogenation of the 3 β -hydroxy group to a 3-oxo group, isomerization of the 5,6-double bond and introduction of the 1,2-unsaturation. The occurrence of these

microbial reactions are well documented (Smith, 1984). The intermediate formation of metabolite (XIV) was demonstrated by its transformation into compound (XV) on fermentation with the bacterial strain. Methanol can add on at the 16,17-double bond of metabolite

(XIV), which is followed by degradation of the acetyl side chain involving Baeyer–Villiger-type oxygenation (Rahim & Sih, 1966) and hydrolysis by an esterase to yield compound (XV). Although methanol was not used at any stage in the work-up, its generation by degradation of 16-dehydropregnenolone by the bacterium may be assumed, as an *A. simplex* strain is known to produce methanol in a similar way (Donnelly *et al.*, 1981). It may be mentioned that, although the micro-organism is capable of metabolizing Δ^1 -testosterone, the metabolite (XV) was not degraded further even after incubation with the strain for 96 h. The presence of the 16 α -methoxy group perhaps inhibits enzymic 9 α -hydroxylation of compound (XV), which is essential for the generation of ring-cleavage products. The microbial preparation of the biologically potential compound (XV) is of much interest, and the generation of a methoxy group involving a double-bond transformation in the steroid skeleton has here been observed for the first time. Moreover, the generation of enzymes of such diverse character by the same organism is a novel observation that should be useful in further biochemical investigations.

We thank Dr. S. Sengupta of this Institute for his help in identification of the strain and Mr. P. Ghosh Dastider for recording the n.m.r. spectra.

REFERENCES

- Blunt, J. W. & Stothers, J. B. (1977) *Org. Magn. Reson.* **9**, 439–464
- Charney, W., Nobile, A., Federbush, C., Sutter, D., Perlman, P. L., Herzog, H. L., Payne, C. C., Tully, M. E., Jevnik Gentles, M. & Hershberg, E. B. (1962) *Tetrahedron* **18**, 591–596
- Dodson, R. M. & Muir, R. D. (1961) *J. Am. Chem. Soc.* **83**, 4627–4631
- Donnelly, M. I., Chapman, P. J. & Dagley, S. (1981) *J. Bacteriol.* **147**, 477–481
- Eggert, H., VanAntwerp, C. L., Bhacca, N. S. & Djerassi, C. (1976) *J. Org. Chem.* **41**, 71–78
- Ferreira, N. P., Robson, P. M., Bull, J. R. & Vander Walt, W. H. (1984) *Biotechnol. Lett.* **6**, 517–522
- Gibson, D. T., Wang, K. C., Sih, C. J. & Whitlock, H., Jr. (1966) *J. Biol. Chem.* **241**, 551–559
- Herzog, H. L., Payne, C. C., Tully Hughes, M., Jevnik Gentles, M., Hershberg, E. B., Nobile, A., Charney, W., Federbush, C., Sutter, D. & Perlman, P. L. (1962) *Tetrahedron* **18**, 581–589
- Hickey, J. P., Butler, I. S. & Pouskouleli, G. (1980) *J. Magn. Reson.* **38**, 501–506
- Kieslich, K. (1980) *Econ. Microbiol.* **5**, 369–465
- Knight, J. C. & Wovcha, M. G. (1979) U.S. Patent 4176123
- Knight, J. C. & Wovcha, M. G. (1981) U.S. Patent 4304860
- Lee, S. S. & Sih, C. J. (1967) *Biochemistry* **6**, 1395–1403
- Leppik, R. A. (1981) *Tetrahedron* **37**, 1747–1751
- Mahato, S. B. & Banerjee, S. (1985) *Phytochemistry* **24**, 1403–1421
- Mahato, S. B. & Mukherjee, A. (1984) *Phytochemistry* **23**, 2131–2154
- Martin, C. K. A. (1977) *Adv. Appl. Microbiol.* **22**, 29–57
- Nakamatsu, T., Beppu, T. & Arima, K. (1980) *Agric. Biol. Chem.* **44**, 1469–1474
- Owen, R. W., Mason, A. N. & Bilton, R. F. (1984) *Tetrahedron* **40**, 421–426
- Pelczar, M. J., Jr., Reid, R. D. & Chan, E. C. S. (1977) *Microbiology*, pp. 138–139, Tata McGraw-Hill Publishing Co., New Delhi
- Rahim, M. A. & Sih, C. J. (1966) *J. Biol. Chem.* **241**, 3615–3623
- Schömer, U., Sheldrick, W. S. & Wagner, F. (1978) *J. Chem. Soc. Perkin Trans. 1* 336–340
- Shull, G. M. (1957) U.S. Patent 2776927
- Sih, C. J., Lee, S. S., Tsong, Y. Y. & Wang, K. C. (1966) *J. Biol. Chem.* **241**, 540–550
- Smith, L. L. (1984) in *Biotechnology* (Rehm, H.-J. & Reed, G., eds.), vol. 6a, pp. 31–78, Verlag Chemie, Weinheim
- Wang, K. C. & Sih, C. J. (1963) *Biochemistry* **2**, 1238–1243

Received 29 February 1988/20 April 1988; accepted 25 April 1988