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Asymmetric Reduction of Prochiral Ketones by Cell-Free Systems from *Alcaligenes eutrophus*

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Abstract: A strain of *Alcaligenes eutrophus* has been isolated from the soil by enrichment culture technique with nerolidol (**1**), a sesquiterpene alcohol, as the sole source of carbon and energy. Fermentation of nerolidol (**1**) by this bacterium in a mineral salts medium resulted in the formation of two major metabolites, viz. geranylacetone (**2**) and an optically active alcohol, (*S*)-(+)-geranylacetol (**3**). Nerolidol (**1**)-induced cells readily transformed 1,2-epoxynerolidol (**4**) and 1,2-dihydroxynerolidol (**5**) into geranylacetone (**2**). These cells also exhibited their ability to carry out stereospecific reduction of **2** into (*S*)-(+)-geranylacetol (**3**). Oxygen uptake studies clearly indicated that nerolidol-induced cells oxidized compounds **2**, **3**, **4**, **5** and ethyleneglycol (**7**). Based on the nature of the metabolites isolated, the ability of nerolidol-induced cells to convert compounds **4** and **5** into geranylacetone (**2**), and oxygen uptake studies, a pathway for the microbial degradation of nerolidol (**1**) has been proposed. The proposed pathway envisages the epoxidation of the terminal double bond, opening of the epoxide and cleavage between C-2 and C-3 in a manner similar to the periodate oxidation of *cis*-diol. The cell-free extract prepared from nerolidol-induced cells readily carried out the asymmetric reduction of compound **2** to an optically active alcohol (**3**) in the presence of NAD(P)H. The cell-free extract carried out both oxidation and reduction reactions at two different pH values and exhibited wide substrate specificity towards various steroids besides terpenes.

Key words: *Alcaligenes eutrophus*, biodegradation, nerolidol, new oxidative pathway, oxido-reductase, asymmetric reduction, terpenoids and steroids.

1 INTRODUCTION

Although biosynthesis of isoprenoid compounds has been studied to a significant extent, the bioconversion of these compounds by bacterial systems has received comparatively little attention. Microorganisms are known to convert the simple mono- and sesquiterpenes into hydroxylated, reduced, oxidized or degraded products.[1–5] Seubert and Fass in the course of their investigation on the biodegradation of isoprenoid compounds by *Pseudomonas citronellolis*, studied the metabolism of farnesol, a sesquiterpene alcohol and found that its degradative pathway proceeds through the same steps as that of geraniol, a monoterpene alcohol.[6] Previously, we carried out studies on the bacterial degradation of a monoterpene alcohol linalool, linalylacetate, and various structurally related terpenoids.[1,7,8] Although nerolidol (**1**) and linalool are acyclic sesquiterpene and monoterpene alcohols respectively, there exists remarkable structural similarities between these two terpenoids. The aim of the present study was to investigate whether or not the bacterial degradation of nerolidol (**1**) resembled that of linalool.

Nerolidol is one of the two most important acyclic sesquiterpenes, the other being farnesol. Nerolidol is a component of many essential oils such as neroli oil and cabreuva oil. It is used as a base note in many delicate flowery odour complexes.[9] It has been reported in the literature that both (*E*)- and (*Z*)-isomers of nerolidol are inhibitors of the production of a polyether antibiotic (monensin) in shake cultures of *Streptomyces cinnamonensis*.[10] Bacterial degradation of nerolidol has not been

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reported so far although an unsuccessful attempt was made by Yamada *et al.* using *Arthrobacter* species.[11]

A bacterial strain, identified as *Alcaligenes eutrophus*, has been isolated which is capable of utilizing nerolidol (**1**) as sole source of carbon and energy. Using this organism the mode of degradation of nerolidol was studied. The present paper describes the isolation and identification of metabolites derived from nerolidol. Based on the nature of the metabolites isolated and other supporting evidences, a new oxidative pathway for the degradation of nerolidol has been documented. In addition, the ability of the cell-free extract to carry out asymmetric reduction of geranylacetone, one of the metabolites derived from nerolidol, to an optically active alcohol, (*S*)-(+)-geranylacetol, in high enantiomeric excess is reported. The cell-free extract also carried out oxidation and reduction of various steroids besides terpenes.

2 EXPERIMENTAL

2.1 Chemicals

Nerolidol, farnesol, NADPH, NADH, NAD^+, $NADP^+$ and all steroids were purchased from Sigma (St Louis, MO, USA). Geranylacetone, methylheptenol and methylheptenone were obtained from Aldrich (Milwaukee, WI, USA). Geranylacetol was prepared by reducing geranylacetone using $NaBH_4$. 1,2-Epoxynerolidol (**4**) was prepared by adopting the Sharpless epoxidation procedure.[12] [**4**; ^{1}H NMR ($CDCl_3$) (90 MHz, 1:1 diastereomers)δ:1·2 and 1·3 (3H, s, H-15), 1·6–1·7 (9H, 2s, H-12, H-13, H-14), 1·8 (1H, s, 1OH), 1·9–2·3 (8H, m, H-4, H-5, H-8, H-9), 2·6–3·0 (3H, m, H-1, H-2), 5·0–5·3 (2H, m, H-6, H-10); MS *m/z* 238(M^+), 220(M^+-H_2O); HRMS $C_{15}H_{26}O_2$ requires 238·1933, found 238·1909]. 1,2-Dihydroxynerolidol (**5**) was prepared by acid hydrolysis of 1,2-epoxynerolidol (**4**) [**5**; ^{1}H NMR ($CDCl_3$)δ: 1·23 (3H, s, H-15), 1·6–1·7 (9H, 2s, H-12, H-13, H-14), 1·9–2·2 (11H, m, H-4, H-5, H-8, H-9, 3OH), 3·3 (1H, dd, J = 2·5 and 12·5 Hz, H-2), 4·7(2H, d, H-1), 5·0–5·2 (2H, br s, H-6, H-10); MS *m/z* 238(M^+-H_2O)]. All the terpene substrates were purified by silica gel column chromatography using 5–10% ethylacetate in hexane as the eluting solvent before use.

2.2 Analytical methods

Thin layer chromatographic (TLC) analyses were performed on silica gel G plates (0·5 mm) developed with ethylacetate–hexane (1:4, system I) and ethylacetate–hexane (1:1, system II) as the solvent systems. Spectroscopic and gas chromatographic (GC) analyses were carried out as described earlier.[13] Analyses of the neutral compounds were carried out on a 10% QF_1 on a Chromosorb-W column (180 cm × 0·3 cm) maintained at 180°C.

High-performance liquid chromatographic (HPLC) analyses were carried out on a Waters model 440 using a μ-porasil normal phase column with chloroform–methanol (95:5) as solvent system (0·8 cm^3 min^{-1}). The compounds were detected either by using UV or a refractive index (RI) detector as reported earlier.[14]

2.3 Microbiological methods

The organism used in this study was identified as a strain of *A. eutrophus* that utilizes nerolidol as sole source of carbon. The organism was maintained in the liquid mineral salts medum[15] containing 0·3% nerolidol and incubated aerobically at 28–30°C. Whenever a starter culture was required, a portion (5 cm^3) of the maintenance culture was transferred to 100 cm^3 sterile liquid mineral salts medium containing 0·3% nerolidol and incubated on a rotary shaker (220 rev min^{-1}) at 28–30°C for 24 h.

2.4 Fermentation conditions

Degradation experiments were conducted in 500 cm^3 Erlenmeyer flasks containing 100 cm^3 sterile salts medium (pH 7·0) to which 5% of 24 h inoculum (A_{660} = 1·5) and 0·3% nerolidol were added. The flasks were incubated on a rotary shaker (220 rev min^{-1}) at 28–30°C for 72 h. Fermentation was not prolonged beyond 72 h to minimize the loss due to evaporation and formation of air oxidation products. A control experiment was also run with the substrate but without organism. At the end of the fermentation period, the contents from all the flasks were pooled, acidified to pH 3–4 using 2 mol dm^{-3} HCl, and extracted with distilled diethylether. The organic layer was then separated into acidic and neutral fractions as described earlier.[1]

2.5 Resting cell experiment

A. eutrophus cells grown on nerolidol for 48 h were harvested by centrifugation (5000 × *g*, 20 min) and the cells were washed well with Tris–HCl buffer (25 mmol dm^{-3}, pH 7·3). The bacterial paste was then suspended in the same buffer to give a final A_{660} of 1·5. The washed bacterial suspension (100 cm^3) was incubated at 28–30°C with 100 mg of the substrate for 12 h on a rotary shaker (220 rev min^{-1}). After the incubation period, the contents of the flasks were acidified to pH 3–4 with 2 mol dm^{-3} HCl and then extracted with diethylether as described in Section 2.4.

2.6 Manometric experiments

Manometric studies were performed with a Gilson 5/6 Oxygraph at 30°C. The reaction mixture contained

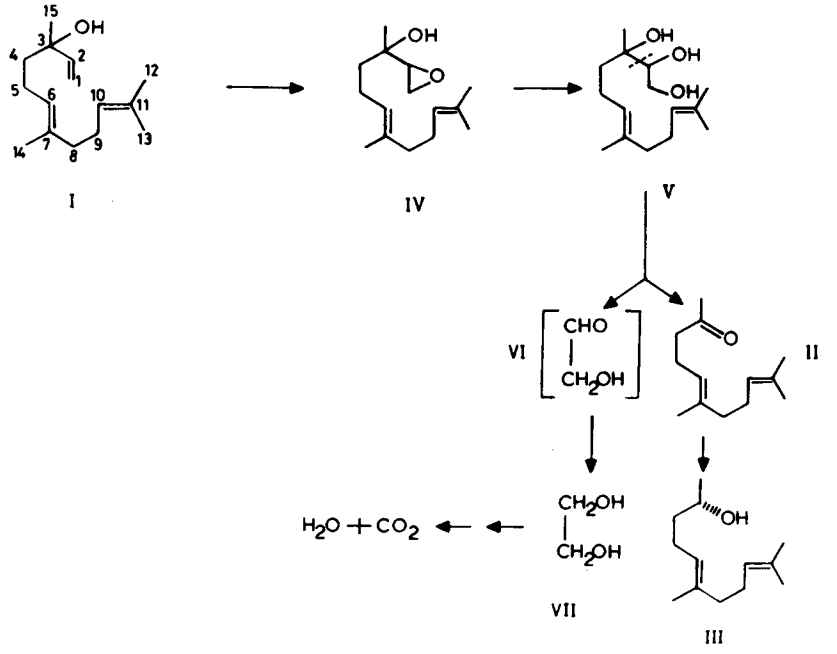

Fig. 1. Proposed pathway for the degradation of nerolidol by *Alcaligenes eutrophus.*

freshly washed cells (2 mg dry weight), Tris–HCl buffer (25 mmol dm^{-3}, pH 7·3) and the substrate (0·5 µmol in 10 µmol acetone) in a total volume of 2 cm^3. Oxygen consumption was monitored for at least 15 min.

2.7 Preparation of cell-free extract

All operations described were carried out between 0 and 4°C unless otherwise specified. Washed cells obtained as described in Section 2.3 were resuspended in Tris–HCl buffer (25 mmol dm^{-3}, pH 7·3) containing 10% glycerol (5 cm^3 mg^{-1} wet weight of cells). The cell suspension was sonicated using a Branson B-30 sonicator for 5 min with intermittent cooling for every 30 s at maximum output, the sonicate was centrifuged (10 000 × *g*, 30 min) to remove the cell debris. The supernatant so obtained was further centrifuged at 105 000 × *g* for 60 min. The 105 000 × *g* supernatant obtained was designated as crude cell-free extract. Protein estimations were performed using the method of Lowry *et al.*[16]

2.8 *In-vitro* assay conditions

The cell-free extract (2 mg protein) was incubated with the substrate (0·2 µmol in 10 µmol acetone) in the presence of an exogenous cofactor, nicotinamide nucleotide (0·2 µmol) in a total volume of 1 cm^3. The oxidation reaction was carried out using Tris–NaOH buffer (25 mmol dm^{-3}, pH 9·5) and the reduction reaction was carried out using Tris–HCl buffer (25 mmol dm^{-3}, pH 5·5). The assay mixture was incubated aerobically for 1 h at 30°C on a rotary shaker. A boiled enzyme control was also run simultaneously. At the end of the incubation period, the reaction mixture was extracted with $CHCl_3$:MeOH (5:1). Analysis of the enzymatic product formed was performed either by HPLC or GC methods as described in Section 2.2. Quantification was made by measuring the area under the peak.

3 RESULTS

Based on the various morphological, cultural (thin and long rods occur singly or in pairs, actively motile, peritrichous, Gram negative) and biochemical characteristics, the organism has been identified as *Alcaligenes eutrophus* as per *Bergey's Manual of Determinative Bacteriology.*[17]

From 50 flasks, 6·83 g of neutral fraction and 0·293 g of acidic fraction were obtained. The neutral fraction after removing the unmetabolized substrate (1·283 g) was shown by TLC examination (system I) to contain two major components (R_f: 0·53 and 0·49) and two minor components (R_f: 0·38 and 0·24). The two major components were separated and purified on a silica gel column with 5–10% ethylacetate in hexane. Among the two major components, the less polar component (R_f: 0·53, system I; GC R_t: 3·9 min; 57%) had the following spectral characteristics: IR spectrum (liquid film) ν_{max} 1715 cm^{-1} (carbonyl group), 1378 cm^{-1} (*gem* dimethyl group); ^1H NMR($CDCl_3$) δ: 1·6–1·7 (9H, m, H-11, H-12, H-13), 1·9–2·1 (7H, m, H-1, H-7, H-8), 2·2–2·4 (4H, m, H-3, H-4), 5·1 (2H, m, H-5, H-9); MS *m/z* 194 (M^+). Based on the spectral data, this component was identified as geranylacetone (Fig. 1, **2**). The spectral data are in excellent agreement with an earlier report[11] and also with that of authentic geranylacetone. The medium polar compound (R_f: 0·49, system I; GC R_t: 2·8 min; 17%) had the following

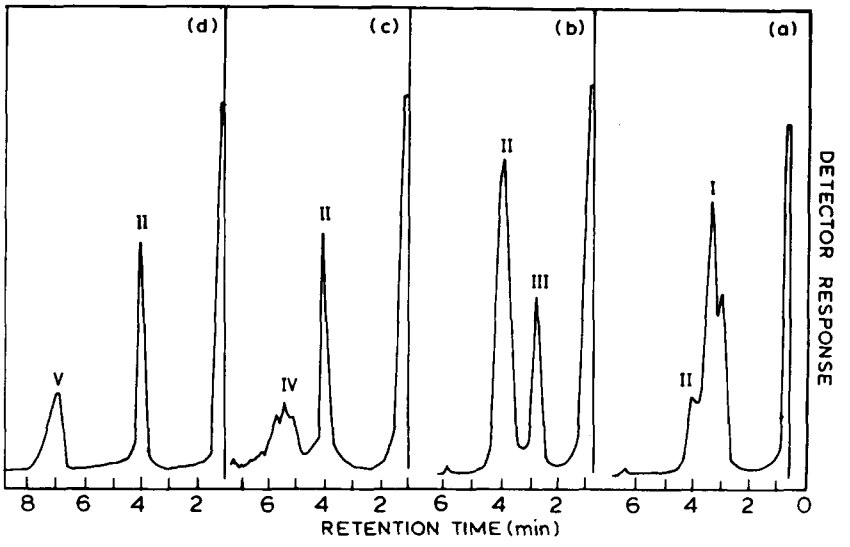

Fig. 2. GC analyses of the reaction products resulting from the incubation of compounds **1**/**2**/**4**/**5** with nerolidol-induced *Alcaligenes eutrophus* cells. (a)–(d) are the product profiles of compunds **1**, **2**, **4** and **5** respectively. Control did not show the presence of a peak corresponding to R_t: 3·9 min (geranylacetone, **2**).

spectral features: IR spectrum (liquid film) ν_{max} 3400–3600 cm^{-1} (hydroxyl group), 1378 cm^{-1} (*gem* dimethyl group); ^1H NMR($CDCl_3$) δ: 1·23 (3H, d, H-1), 1·5 (2H, m, H-3), 1·6 (3H, s, H-12), 1·66 (6H, s, H-11, H-13), 1·7–1·75 (1H, br s, 1 OH), 1·9–2·2 (6H, m, H-4, H-7, H-8), 3·8 (1H, m, H-2), 5·0–5·2 (2H, t, H-5, H-9); MS *m/z* 196 (M^+), 178 (M^+-H_2O); HRMS, $C_{13}H_{24}O$ requires 196·1827, found 196·1824; observed $[\alpha]_D = +3·4°$ (c = 5·0, $CHCl_3$), reported $[\alpha]_D = +4·0°$ (c = 6·7, EtOH). Based on the spectral data, the compound was identified as (*S*)-(+)-geranylacetol (Fig. 1, **3**). The spectral data matched well with that of an earlier report.[18] However, the two minor components could not be obtained in pure form, and hence were not characterized. A significant proportion of the acidic fraction contained cellular material and the amount of the acidic metabolites derived from nerolidol was extremely low and could not be processed further.

The profile of the metabolites formed from the incubation of **1**/**2**/**4**/**5** with nerolidol-induced cells is given in Fig. 2. Incubation of **1**/**4**/**5** with whole cells resulted in the formation of **2** which on reduction gave **3**. Formation of **2** from **4** and **5** was shown by GC analysis (Fig. 2) and conclusively established by co-injection of the authentic geranylacetone with the reaction product where the peak corresponding to geranylacetone was enhanced. Furthermore, oxygen uptake studies carried out with compounds **1**–**5** and ethyleneglycol suggested the possible involvement of compounds **2**–**5** and ethyleneglycol as intermediates in the nerolidol degradative pathway since they showed comparable oxygen uptake rates (Table 1). In fact, ethyleneglycol (**7**) which could have been possibly derived from compound **5**, showed better oxygen uptake in contrast to other compounds.

Incubation of **2** with the cell-free extract in the presence of NAD(P)H gave **3** suggesting the presence of an active

TABLE 1
Oxygen Uptake Measurements[a]

Substrates	*Oxygen consumed*[b] *(nmol min^{-1} mg^{-1} dry weight)*
Nerolidol(**1**)	8·12
Geranylacetone(**2**)	4·11
Geranylacetol(**3**)	3·52
1,2-Epoxynerolidol(**4**)	4·69
1,2-Dihydroxynerolidol(**5**)	3·52
Ethyleneglycol(**7**)	5·86
Glycolic acid	—
Farnesol	—

[a] Experimental details are given in the text.
[b] All values were corrected for endogenous respiration.

oxido-reductase. Although bacteria were grown on the terpenoid substrate, viz. nerolidol, the oxido-reductase(s) present in the cell-free extract was found to accept various C_{19}-steroids besides terpenoids as substrates. Among the steroids tested, androst-4-ene-3,17-dione and testosterone were found to be the best substrates for this enzyme. Table 2 shows the percentage of both androstenedione and testosterone formed when the reaction was carried out at different pH values ranging from 5 to 10. Maximum conversion of androst-4-end-3,17-dione to testosterone was noticed at pH 5·5 and at this pH reverse reaction was negligible. However, the reverse reaction (oxidation of secondary alcohol to ketone) was maximal at pH 9·5 and the reduction at this pH was negligible. It was found that for the reduction and oxidation reactions, NADPH and $NADP^+$ were preferred to NADH and NAD^+ respectively.

The cell-free extract showed its ability to carry out

TABLE 2
Effect of pH on Oxidation–Reduction Reaction[a]

pH	*Reduction*[b] *Testosterone formed (%)*	*Oxidation*[c] *Androstenedione formed (%)*
5·0	96	3
5·5	97	3
6·0	95	3
6·5	92·5	6
7·0	83·7	26·3
7·5	79	44
8·0	72	49·5
8·5	61	58·5
9·0	39·7	61·3
9·5	19·1	64·8
10·0	12·6	61·1

[a] Assays were conducted as described in Section 2.8 using 2 mg of crude protein.
[b] Reduction assays were carried out using androstenedione as substrate and NADPH as cofactor.
[c] Oxidation assays were carried out using testosterone as substrate and $NADP^+$ as cofactor. Phosphate buffer (pH range 5–7), Tris–HCl buffer (pH range 7·5–9) and Tris–NaOH (pH range 9·5–10) of 25 mM concentration containing 10% glycerol were used.

the oxidation/reduction of various C_{19} steroids and terpenoids (Table 3). Surprisingly C_{21} steroids and C_{27} sterols were not accepted as substrates (Table 3). When 17α-hydroxyandrost-4-ene-3-one (epitestosterone) was used as the substrate, the cell-free extract in the presence of $NADP^+$ failed to catalyse its conversion to androst-4-ene-3,17-dione. However, testosterone was readily converted into androstenedione in the presence of $NADP^+$ at pH 9·5 (Table 3). This shows conclusively that the reduction was stereospecific. Furthermore, reduction of geranylacetone afforded an optically active alcohol, (*S*)-(+)-geranylacetol, in high enantiomeric excess (above 96%) which also supported the stereospecificity of the reaction carried out by the enzyme. The cell-free extract retained its activity for at least 6 months when stored at −20°C without appreciable loss in activity. It lost activity, however, on frequent freezing and thawing.

4 DISCUSSION

Degradation of nerolidol (**1**) by *A. eutrophus* yielded two major and two minor metabolites. The major metabolites formed were identified as geranylacetone (**2**) and geranylacetol (**3**). In fact both these metabolites accumulated in the broth suggesting that either they are not further metabolized or that further metabolism may take place only at a low rate. Geranylacetone was previously reported as being one of the metabolites of the degradation of squalene by *Arthrobacter* species.[19] Biotransformations of various acyclic monoterpene alcohols in bacterial,[1,7,8] fungal,[4] mammalian[20,21] and higher plant[22] systems have previously been studied in this laboratory. One of the striking similarities in all these living systems is their ability to carry out specific ω-methyl hydroxylation of some of the acyclic monoterpenes. However, the bacterial systems tested so far showed a rigid substrate specificity—while it readily carried out the ω-hydroxylation of tertiary acyclic monoterpene alcohols such as linalool, 1,2-dihydrolinalool and their corresponding acetates, it failed to accept acyclic primary monoterpene alcohols such as geraniol, nerol, citronellol as substrates for the ω-hydroxylation. Contrary to these observations, the fungal systems studied showed a broad substrate specificity in their ability to carry out ω-methyl hydroxylation of both primary and tertiary acyclic mono- and sesquiterpene alcohols.[3,4] Besides, fungal systems are also known to carry out the oxidation of the remote double bond in some of the acyclic sesquiterpene alcohols.[3,23] Seubert[15] showed that the degradation of farnesol by *Pseudomonas citronellolis* proceeds through the oxidation of C-1 to give farnesic acid, followed by carboxylation of the β-methyl group. Subsequent to this, the 2,3-double bond of the dicarboxylic acid is hydrated to give a 3-hydroxy acid which is then acted upon by a lyase resulting in the formation of a β-keto acid and acetic acid. The β-keto acid readily enters the fatty acid oxidation pathway.[6] However, it is interesting to note that *A. eutrophus* used in the present investigation carries out the biotransformation of nerolidol (**1**) following a pathway which appears to be hitherto unknown. The nature of the metabolites isolated and characterized clearly suggests the inability of the organism to bring about the oxidation of the ω-methyl group or the remote double bond in nerolidol (**1**). On the other hand, it appears that the organism initiates the degradative sequence through epoxidation of the terminal 1,2-double bond in nerolidol (**1**) and then opening of the epoxide (**4**) to the corresponding diol (**5**) which may be cleaved between C-2 and C-3 to generate geranylacetone (**2**) and a glycolaldehyde (**6**). It is quite possible that the opening of the 1,2-epoxide may take place in such a way that the hydroxyls at C-2 and C-3 may be *cis* to each other which would facilitate the cleavage of the C-2 and C-3 bond in a manner similar to the periodate oxidative cleavage of *cis*-diols. Glycolaldehyde may be converted either to ethyleneglycol (**7**) or glycolic acid which further gets metabolized to CO_2 and H_2O. However, both growth and oxygen uptake studies clearly revealed that glycolic acid is not further metabolized (Table 1). It is quite possible that the organism derives most of its carbon requirement from the terminal C_1–C_2 unit of nerolidol (**1**).

The proposed pathway (Fig. 1) is substantiated based on two important pieces of evidence: (i) cells adapted to nerolidol convert both 1,2-epoxynerolidol (**4**)

TABLE 3
Substrates Tested for Oxidation–Reduction Reaction

Reduction			*Oxidation*		
Substrate	*Product*	*% Conversion*	*Substrate*	*Product*	*% Conversion*
Geranylacetone(**2**)	Geranylacetol(**3**)	50	**3**	**2**	48
Methylheptenone(**8**)	Methylheptenol(**8a**)	46	**8a**	**8**	42
Androst-4-ene-3,17-dione(**9**)	Testosterone(**9a**)	86	**9a**	**9**	72
5α-Androst-3,17-dione(**10**)	Dihydrotestosterone(**10a**)	44	**10a**	**10**	43
	3β,17β-Dihydroxy-5α-androstane(**10a**)	53	**10b**	**10**	84
Dihydrotestosterone(**10a**)	3β,17β-Dihydroxy-5α-androstane(**10b**)	92	**11**	**11a**	42
Androsterone(**11**)	3α,17β-Dihydroxy-5α-androstane(**11a**)	88	ND	ND	ND
Dehydroepiandrosterone(**12**)	3β,17β-Dihydroxy-5-androstene(**12a**)	36	**12a**	**12**	66
1α-Methyl-19nor-5α-androst-3,17-dione(**13**)	1α-Methyl-19nor-17β hydroxy-5α-androst-3-one(**13a**)	56	**13a**	**13**	61
Androst-1,4-diene-3,17-dione(**14**)	17β-hydroxy-androst-1,4-diene-3-one(**14a**)	63	**14a**	**14**	57
11α-Hydroxy-androst-4-ene 3,17-dione(**15**)	11α,17β-Dihydroxy-androst-4-eno-3-one(**15a**)	45	**15a**	**15**	22
Estrone(**16**)	Estradiol(**16a**)	23	**16a**	**16**	42
14α-Hydroxy-androst-4-ene 3,17-dione(**17**)	14α,17β-Dihydroxy-androst-4-ene-3-one(**17a**)	62	**17a**	**17**	75
19Nor-androst-4-ene-3,17-dione(**18**)	17β-Hydroxy-19nor-androst-4-ene-3-one(**18a**)	72	ND	ND	ND
9-Methyl-$\Delta^{5,(10)}$-octalin-1,6-dione(**19**)	1-Hydroxy-9-methyl-$\Delta^{5,(10)}$-octalin-6-one(**19a**)	ND	**19a**	**19**	17
6-Methyl-$\Delta^{4,(9)}$-heptalin-1,5-dione(**20**)	1-Hydroxy-8-methyl-$\Delta^{4,(9)}$-heptalin-5-one(**20a**)	ND	**20a**	**20**	20
Androstenedione(**9**)	Epitestosterone(**21**)	—	**21**	**9**	—
Cortisone(**22**)	Cortisol(**22a**)	ND	**22**	**22a**	—
Cholest-3-one(**23**)	Cholest-3-ol(**23a**)	—	**23a**	**23**	—
Progesterone	No product isolated	—	ND	ND	ND

The assays were conducted as described in Section 2.8. The products formed were identified by comparing their HPLC profiles (retention time) with that of authentic compounds. The % conversion of each substrate was determined from the peak height measurements as reported earlier.[14]
ND, Reaction was not carried out.

and 1,2-dihydroxynerolidol (**5**) into geranylacetone (**2**), (ii) cells adapted to nerolidol oxidize both compounds **4** and **5** (Table 1). One can also envisage the formation of geranylacetone (**2**) from farnesol since isomerization of nerolidol (**1**) to farnesol is a feasible process. However, such a process is ruled out since the organism failed to accept farnesol as the substrate.

The crude cell-free extract showed its ability to carry out the oxidation and reduction of geranylacetol (**3**) and geranylacetone (**2**) respectively at two different pH values. While the pH optimum for the forward (reduction) reaction was 5·5, the backward reaction (oxidation) has a pH optimum of 9·5. It is quite possible that a single enzyme, such as an oxido-reductase may carry out both forward and backward reactions and it is worthy of note that this enzyme system has a wide substrate specificity as it accepts various steroids besides terpenes as substrates. In fact steroids serve as better substrates than terpenes (Table 3). The significant aspect of this enzyme system is its ability to carry out the stereospecific reduction of a keto group. It was demonstrated that the cell-free extract converts androstenedione to testosterone in the presence of NADPH. Testosterone has a 17-β hydroxyl group. However, epitestosterone which has a 17α-hydroxyl group, is not oxidized to androstenedione in the presence of $NADP^+$, indicating the rigid stereospecificity. Stereospecific reduction was also shown in the case of geranylacetone which was converted to optically active (*S*)-(+)-geranylacetol (**3**). The enzyme system carries out the stereospecific reduction of isolated keto groups but failed to reduce α,β-unsaturated keto groups (Table 3). Formation of androstenedione from testosterone and (*S*)-(+)-geranylacetol from geranylacetone was conclusively established by performing a large scale incubation. Enzymatic products formed were purified by preparative TLC (system I for terpenes and system II for steroids) and characterized by conventional spectroscopic methods. Spectral data of androstenedione and

(*S*)-(+)-geranylacetol matched well with those of authentic samples.

Stereospecific reduction of keto groups is one of the most frequently encountered reactions in synthetic organic chemistry. Hence enzymes mediating such reactions may find several applications. In conclusion, this study demonstrates the pathway for the degradation of nerolidol by *A. eutrophus*. This organism can be used as a reagent to carry out stereospecific reductions of carbonyl compounds in high enantiomeric excess.

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