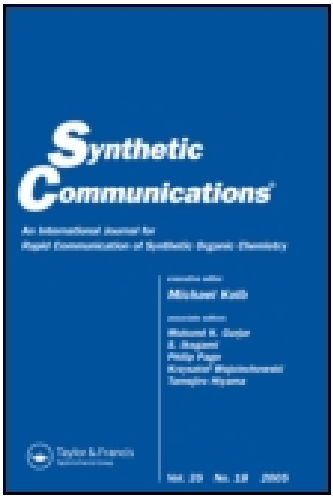

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Geotrichum candidum Assisted Synthesis of Sitophilate, Male Aggregation Pheromone of Granary Weevil

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GEOTRICHUM CANDIDUM ASSISTED SYNTHESIS OF SITOPHILATE, MALE AGGREGATION PHEROMONE OF GRANARY WEEVIL

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ABSTRACT : The syntheses of optically inactive Sitophilate and the optically active isomers (2S, 3S) and (2S, 3R) were completed by a simple route using easily avilable starting compounds.

The cosmopolitan granary weevil, *Sitophilus granarius* (L) is a major pest of stored products[1]. Existence of a male produced granary weevil aggregation pheromone, Sitophilate was first reported by Faustini *et al.*[2] Its isolation, purification and synthesis was carried out by Phillips et al.[3] Synthesis of the chiral isomer (2S, 3R) was achieved by J.M. Chong [4].

Retrosynthetic analysis of the pheromone Sitophilate indicates that 2- methyl-3-oxopentanoic acid or its ester (1) prepared by Claisen condensation of ethyl propionate [5] would be the required simple intermediate. Accordingly reduction of the keto ester with sodium borohydride, hydrolysis of the

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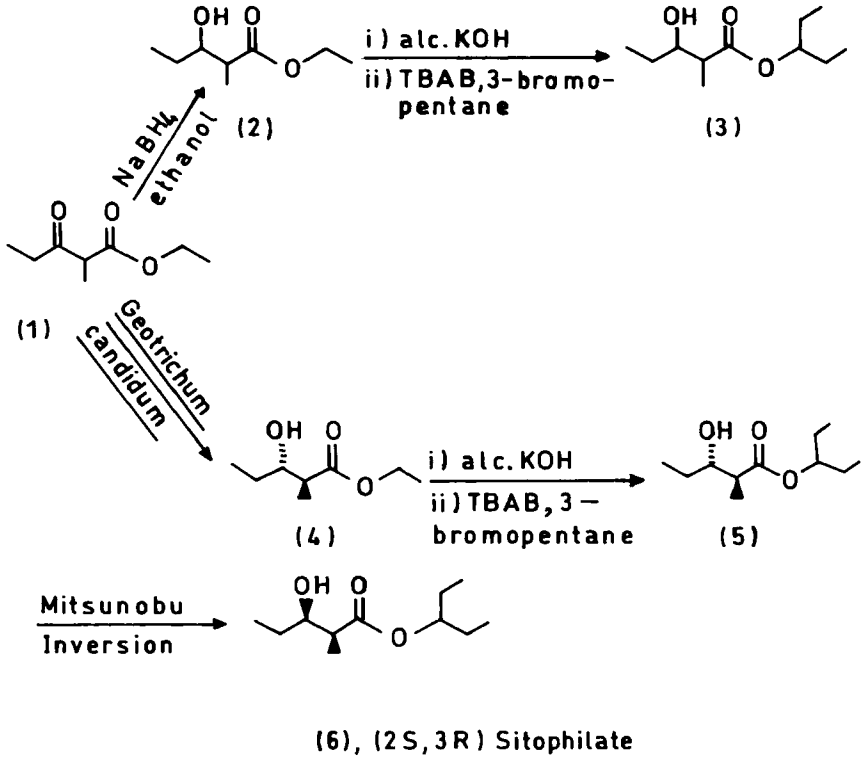
hydroxyester (2) with alcoholic potassium hydroxide and esterfication of the generated carboxylate salt with 3-bromopentane under phase transfer catalysis[6] gave the optically inactive Sitophilate (3, Scheme 1).



SCHEME 1: Synthesis of optically inactive and active Sitophilate.

Asymmetric reduction of the keto ester, ethyl 2-methyl-3-oxopentanoate (1) was the key step in synthesis of optically active Sitophilate. Microbial reductions are useful in such situations. Of all microorganisms tried, a fungus *Geotrichum candidum*[7] was found to execute the reduction in the most effective way, yielding

an optically active compound (4). To determine the configuration of this compound, it was hydrolysed with alkali and the carboxylate salt generated was alkylated with 3-bromopentane under phase transfer catalysis. The spectral and physical data of 1'-ethylpropyl 3-hydroxy-2-methylpentanoate thus generated was identical[8] with (2S, 3S) 1'-ethylpropyl 3-hydroxy-2-methylpentanoate (5). This proves the configuration of the hydroxyester (4) to be (2S, 3S). Compound (5) on Mitsunobu inversion[8,9] yielded (2S,3R) 1'-ethylpropyl 3-hydroxy-2-methyl-pentanoate (6), which possessed the spectral and physical data identical with naturally occurring Sitophilate [4].

Thus the syntheses of optically inactive Sitophilate and the optically active (2S, 3R) isomer were completed by simple routes using easily available starting compounds.

EXPERIMENTAL

Gas chromatograms were recorded on a Packard gas chromatograph 437 A with flame ionisation detector using stainless steel column (2 m x 2 mm) packed with 10% FFAP on Chromosorb W(HP) 80/100 and Hewlett Packard 5890 series II with capillary column BP 20, SGE make (25 m x 0.53 mm x 1 m). Quantitative analysis was done using an internal standard on Shimadzu CR-3A integrator. IR spectra were recorded as thin films or as nujol mulls on Shimadzu IR 470 spectrophotometer. PMR spectra on Hitachi model-1200, 60 MHz spectrometer using TMS as internal standard (chemical shifts in δ, ppm). Mass spectra were recorded on a CEC model 21-1108 mass spectrometer at 70 eV, by direct inlet

system. Optical rotations were measured with Autopol API 589 polarimeter or Perkin-Elmer spectropolarimeter model 141. All chemicals used were of AR grade.

Synthesis of optically inactive Sitophilate

Ethyl 2-methyl-3-oxopentanoate [5], ethyl 3-hydroxy-2-methylpentanoate[10] and potassium 3-hydroxy-2-methylpentanoate[6] were synthesised by reported methods.

1'-Ethylpropyl 3-hydroxy-2-methylpentanoate (3):

1'-Ethylpropyl 3-hydroxy-2-methylpentanoate (3) was synthesised from potassium 3-hydroxy-2-methylpentanoate and 3-bromopentane using standard phase transfer catalysis condition[6]. It showed a single spot on tlc and its gas chromatogram showed two peaks of equal area indicating the presence of equal quantities of *syn* and *anti* isomers. Elemental Analysis of this product - found C, 65.4% and H, 10.93% (calculated for $C_{11}H_{22}O_3$ C, 65.31% and H, 10.96%). IR: (3450 cm^{-1} and 1720 cm^{-1}). PMR (δ): 0.96-1.00 (complex m, 9H), 1.2 (d, J = 7 Hz, 3H), 2.5 (m, 2H), 3.45 (m, 1H), 4.75 (quintet, J = 6 Hz, 1H). It matched with that reported in literature[3]. Mass Spectrum: M/Z 173 (2.1%), 144 (3.9%), 115 (48.7%), 103 (41.0%), 97(12.0%), 85(21%) and 74(100%, base peak). It is in agreement with that reported in literature[3].

Synthesis of optically active isomers of Sitophilate

(2S, 3S) Ethyl 3-hydroxy-2-methylpentanoate (4) :

Geotrichum candidum fungus was isolated from orange fruit (*Citrus sinensis*) using potato dextrose agar medium. Resting cells of this fungus were

used for reduction of the keto ester under aerobic conditions according to the reported method[11]. Wet mycelium of *Geotrichum candidum* (10 g.), each was taken in five 500 ml conical flask and tap water (100 ml) was added to each flask. Ethyl 2-methyl-3-oxopentanoate (1, 0.2 g., 0.0013 mole) and five drops of distilled ethanol were added to every flask. The flasks were shaken for 72 hrs at 150 rpm. Usual workup gave crude product (4, 0.538 g., 53.1%) which was purified by using a short column of silica gel. The pure product (4, 0.45 g.) showed a single spot on tlc and single peak on GC, which indicates that only one diastereoisomer has been produced exclusively during the reduction. Gas chromatography on polar column (FFAP) indicated, it to be an *anti* isomer. IR: (3450, 1720 and 1380 cm^{-1}). PMR (δ): 0.95(t, J = 7 Hz, 3 H), 1.15 (t, J = 4 Hz, 3 H), 1.25 (d, J = 4 Hz, 3 H), 2.35 (m, 2 H), 3.45 (m, 1 H) and 4.1 (q, J = 7 Hz, 2 H). $[\alpha]_D^{25}$ + 6° (C = 1.00, $CHCl_3$).

Potassium salt of this optically active hydroxy acid was obtained by the same method that is used for hydrolysis of optically inactive hydroxy ester (2).

(2S, 3S) 1'-Ethylpropyl 3-hydroxy-2-methylpentanoate (5) :

Conversion of (2S, 3S) ethyl 3-hydroxy-2-methylpentanoate (4) into (2S,3S) 1'-ethylpropyl 3-hydroxy-2-methylpentanoate (5) was carried out as in the case of optically inactive pheromone. The pure product showed single peak on GC. IR: (3450 , 1720 and 1380 cm^{-1}). PMR (δ) : 0.9-1.2 (m, 12 H) , 2.45 (m, 2 H), 3.40 (m, 1 H) and 4.7 (quintet, J = 6 Hz, 1 H). $[\alpha]_D^{25}$ + 5.5° (C = 1.00, $CHCl_3$).

All these data including rotation are comparable with reported data of (2S, 3S) 1'-ethylpropyl 3 - hydroxy - methylpentanoate[8].

(2S,3R) 1'-Ethylpropyl 3-(3,5-dinitrobenzoyloxy)- 2-methylpentanoate :

A mixture of (2S,3S) 1'-ethylpropyl 3- hydroxy - 2 - methyl- pentanoate (5, 110 mg, 0.0005 mole), triphenylphosphine (454 mg, 0.0017 mole) and 3,5-dinitrobenzoic acid (367 mg, 0.0017 mole) in dry tetrahydrofuran (2 ml) was stirred and cooled to 0°C. Diethyl azodicarboxylate (DEAD, 302 mg, 0.0017 mole) was added to the reaction mixture at 0°C. The reaction mixture was stirred for five days at room temperature. n-Hexane (3 ml) and diethyl ether (1 ml) were added to the reaction mixture and stirring was continued for one hour. After filtration and concentration of the filtrate *in vacuo*, the residue was chromatographed on silica gel. Elution with n-hexane-ether (90:10) gave the pure product (103 mg, 48%). UV maxima at 204 nm ($\epsilon = 1.324 \times 10^7$). IR: (1730, 1380, 1550 and 1345 cm^{-1}). PMR (δ): 2.8 (dq, J=2 Hz and J=6Hz, 1H), 4.7 (quintet, J = 6 Hz, 1 H), 5.4 (q, J = 5 Hz, 1 H) and 9.1-9.2 (m, 3 H). These values matched with the literature values[8] . $[\alpha]_D^{26}$ - 6° (C = 1.00, $CHCl_3$) Lit.[8] $[\alpha]_D^{23}$ - 6.52° (C = 0.97, $CHCl_3$).

(2S,3R) 1'-Ethylpropyl 3-hydroxy-2-methylpentanoate (6) :

A catalytic amount of potassium carbonate (5 mg) was added to a solution of (2S,3R) 1'-ethylpropyl 3-(3,5-dinitrobenzoyloxy) -2-methylpentanoate (100 mg, 0.00025 mole) in dry methanol (2 ml) and dry tetrahydrofuran (0.15 ml). The

reaction mixture was stirred for one hour at room temperature and then cooled to 0°C and neutralised with p-toluenesulfonic acid. The stirring was continued for five minutes. After filtration and concentration of the filtrate *in vacuo* the residue was purified by column chromatography which yielded pure product (22 mg, 43%). Elemental Analysis - Found C, 65.2% and H, 10.9% (calculated for $C_{11}H_{22}O_3$, C, 65.31% and H, 10.96%). The pure product showed a single spot on tlc and single peak in the gas chromatogram. IR:(3480, 1720 and 1380 cm^{-1}). PMR (δ): 0.9-1.15 (complex m, 12 H), 1.2 - 1.8 (m, 6H), 2.4 (m, 2 H), 3.65 (m, 1 H) and 4.75 (quintet, J = 6.5 Hz, 1 H). It matched with the reported data[8]. $[\alpha]_D^{25}$ - 3.6° (C = 1.00, $CHCl_3$). Lit.[8] $[\alpha]_D^{24}$ - 3.9° (C = 1.7, $CHCl_3$). Mass spectrum showed the peaks at M/Z 173 (21.8%), 144 (18.3%), 115 (96.5%), 103 (92.7%), 97 (23%), 85 (25.6%) and 74 (100%, base peak). It matched with the mass spectrum reported in literature[8].

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