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MICROBIAL TRANSFORMATION OF *dl* 3-ACETYL-AZETIDINONE DERIVATIVES[1)]

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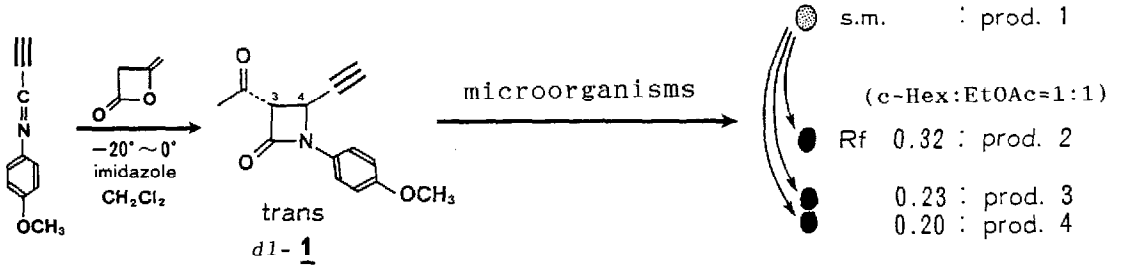
Summary: Monocyclic β-lactam, *dl*-1 prepared by the diketene cycloaddition method was subjected to microbial transformation to give 3,4-trans-(R),(S)-OH mixture 2 and 3,4-cis-(R)-OH 3 and 3,4-cis-(S)-OH 4 depending on the microorganisms.

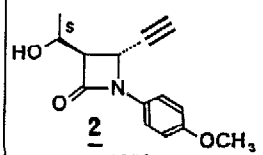
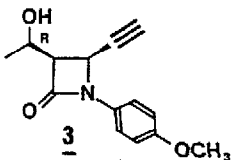
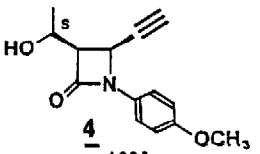
Current interest in the application of the microbial or enzymatic technique to the field of organic synthesis broaden the gate into the stereo- and regio-specific synthetic methodologies, particularly the enantio-specific syntheses of the chiral compounds[2)]. In the course of our study on the β-lactam chemistry the microbial transformation of the racemic β-lactam derivative such as 1 seemed to be attractive in the expectation for the specific reduction of the acetyl group on the azetidinone molecule. The starting material, *dl* 3,4-trans-1-(*p*-methoxyphenyl)-3-acetyl-4-ethynyl-2-azetidinone 1, mp 85°C was easily prepared by the method of Sumitomo group using diketene [2+2] cycloaddition[3)]: To a solution of propargyl aldehyde (4g) in benzene (50ml) was added *p*-anisidine (9g) and 4A-MS (4g) and the mixture was stirred for 30 min at 20°C. After filtration the solvent was removed *in vacuo* and the residue was dissolved in methylene chloride (60ml), and imidazole (4.3g) was added. The whole mixture was cooled to -20°C and added diketene (7.8g) slowly keeping the reaction temperature at -20° ~ -10°C. The reaction temperature was raised to 20°C (~ 2 hr). Methylene chloride (40ml) was added and the solution was washed with aquous dil.HCl and H_2O successively and dried over $MgSO_4$. After removal of the solvent *in vacuo* the desired compound was isolated by silica gel rapid chromatography



(c-Hex:EtOAc=3:1) to give 5.7 g (32%) of the 3-acetyl derivative 1. TLC Rf=0.5 (c-Hex:EtOAc=1:1). NMR ($CDCl_3$) δ: 2.35 (3H,s), 2.50 (1H,d,J=2 Hz), 3.75 (3H,s), 4.33 (1H,d,J=2 Hz), 4.91 (1H,t,J=2 Hz), 6.7-7.5 (4H,A_2B_2 type). IR (Nujol) cm-1: 1760, 1720, 2100.

The starting material in hand we examined at first the microbial transformation of this *dl* trans-3-acetyl derivative 1, and found mainly three products; prod.2,3 and 4 on silica gel TLC as illustrated. The products distribution was classified to the combination of prod.2, 2+3, 2+3+4 and 2+4 according to the microorganisms employed (about 40 species) and cultural conditions.

The representative results are shown in the next Table.

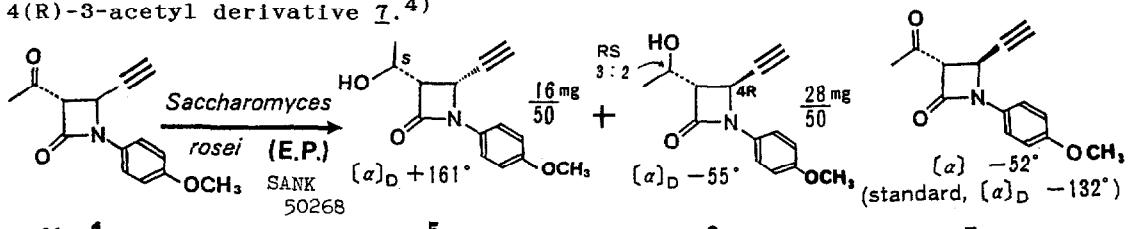
	prod. 2 3,4-trans R,S-OH mix.	prod. 3 3,4-cis R-OH	prod. 4 3,4-cis S-OH
S.P.: Stationary phase culture E.P.: Exponential phase culture	 2 mp 135° $[\alpha]_D$ +210°	 3 mp 155° $[\alpha]_D$ −197°	 4 mp 160° $[\alpha]_D$ −170°
Pichia terricola SANK 51684 (S.P.)	$\frac{49\ mg}{100}$ $[\alpha]_D$ +184° S:R=3:1		$\frac{14\ mg}{100}$ $[\alpha]_D$ −144° ee 85%
Saccharomyces cerrevisiae SANK 50161 (E.P.)	$\frac{90\ mg}{115}$ $[\alpha]_D$ +85° S only ee 40%		
Schizosaccharomyces pombe SANK 57362 (E.P.)	$\frac{30\ mg}{100}$ $[\alpha]_D$ +189° S only ee 90%	$\frac{37\ mg}{100}$ $[\alpha]_D$ −180° ee 91%	
Trichosporon penicillutum BY-356 (S.P.)	$\frac{35\ mg}{90}$ $[\alpha]_D$ +144° S only ee 69%	$\frac{25\ mg}{90}$ $[\alpha]_D$ −197° ee 100%	
Streptomyces cattleya SANK 63876 (S.P.)	$\frac{21\ mg}{100}$ $[\alpha]_D$ +144° S:R=15:1		$\frac{15\ mg}{100}$ $[\alpha]_D$ −169° ee 100%

$[\alpha]_D$ means $[\alpha]_D^{24}$ (c=1, $CHCl_3$). $\frac{B}{A}$ mg means that A is the mg weight which was feeded and B mg is the mg weight obtained after silica gel TLC.

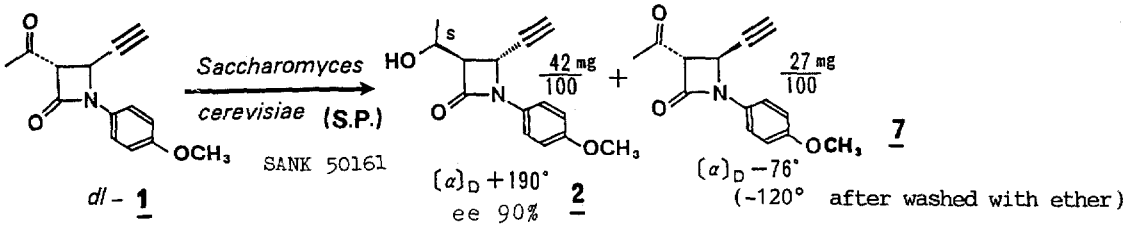
The structures of the each product were determined by the usual way: Product 2 was a mixture of 3,4-trans (R) and (S) hydroxyethyl azetidinones, but (S)-hydroxyethyl derivative 2 was predominant in every case. Recrystalization from ethylacetate and ether gave the pure prod.2, whose NMR is coincident with the structure 2 in which the coupling constant between C_3-H (δ 3.38,dd,J=2 and 4 Hz) and C_4-H (δ 4.60,t,J=2Hz) is 2 Hz. The absolute configuration was determined by the comparison of the chemically converted derivative, 3(R)-[1(S)-[(*tert*-butyldimethylsilyl)oxy]ethyl]-4(S)-[[(phenylthio)carbony]methyl]azetidin-2-one $[\alpha]_D^{24}$ -42°, with the standard enantiomer, $[\alpha]_D^{24}$+42° derived from penicillin[5]. Prod. 3 has

the coupling constant of 6 Hz (C_3-H and C_4-H) and C_3-H appears at δ 3.50 as a triplet(J=6 Hz), and the prod.4 has the coupling constant of 6 Hz and C_3-H appears at 3.43 as a doublet of doublet (J=6 and 9 Hz).

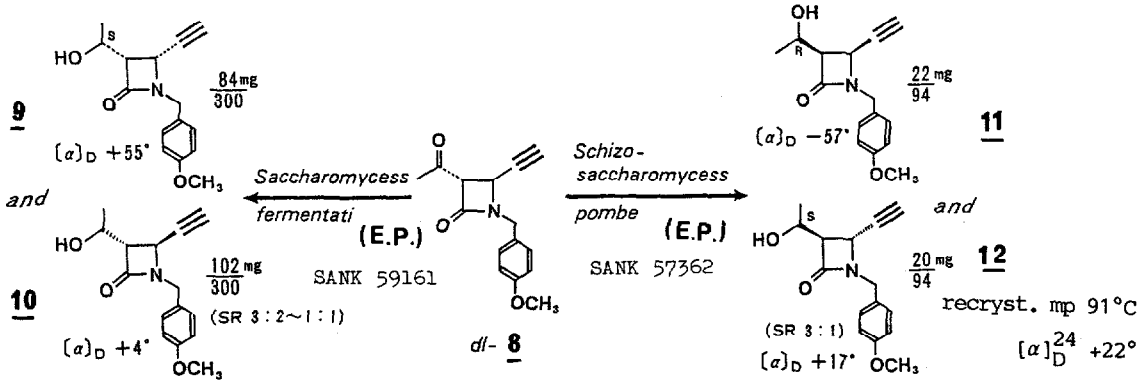
From these date and the other chemical criteria, and Dreiding model consideration we could determine the structure of the prod.3 and 4 as 3 and 4 which have (R) and (S) hydroxyethyl side chain with 3,4-cis configuration on the azetidinone molecule. Contrary to the above results in the case of *Saccharomyces rosei* two products, 5 and 6 were isolated, and one of which was proved to be 3,4-trans 4(R) derivative 6 (RS 3:2 from NMR) whose structure was confirmed by the comparison of the oxidation product of 6 with standard 4(R)-3-acetyl derivative 7.[4]



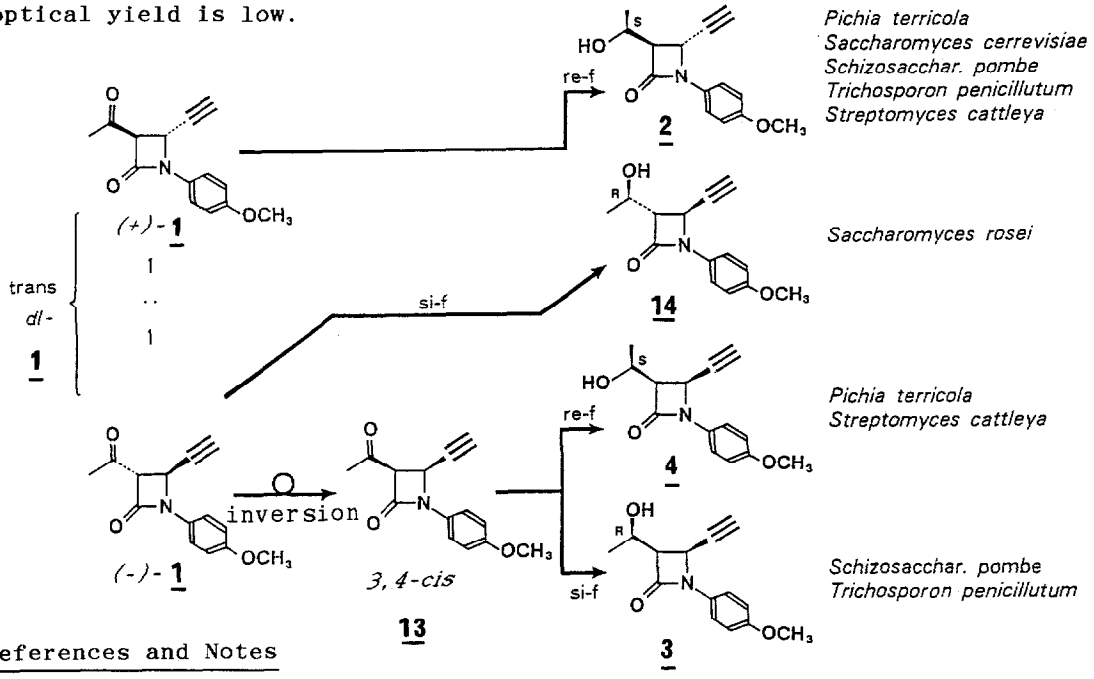
In the stationary phase (S.P.) culture we got (S)-alcohol 2 and the optically active 3-acetyl derivative 7(=(-)-1), which means exponential phase (E.P.) is more active than S.P. in our microbial reduction.



In the case of N-anisyl derivative(*dl*-8) the parallel results were obtained, and one of the products 12 was recrystalized (mp 91°C, $[\alpha]_D^{24}$+22°) and silylated to give silyl derivative $[\alpha]_D^{24}$+17.5°, which is opposite signal and value of the standard one from 6-APA[5].



The summary of the microbial transformation products of the *dl* monocyclic β-lactam 1 is shown below. The acetyl group of the one enantiomer (+)-1 was directly reduced to the (S)-hydroxyethyl product 2. In the other enantiomer (-) -1 the acetyl group was first converted to 3,4-cis intermediate 13 which was reduced (S)- or (R)-hydroxyethyl derivative 4 or 3 depending on the microorganisms. *Saccharomyces rosei* is different from other microorganisms so far examined because of the production of 3,4-trans with 3-(S), 4-(S) configuration, but the ratio of the R-hydroxyethyl to S-isomer is 3:2 and the optical yield is low.



References and Notes

1) A part of this work was presented at 106th Ann. Meeting Pharm. Soc. Japan 1986 and 16th IUPAC Int. Symp. Chem. Natural Prod. Kyoto, 1988.

2) 5th IUPAC Symp. Org. Syn. Freiburg, 1984. K. Mori and S. Senda, Tetrahedron 41,541 (1985). J.B. Jones, Tetrahedron 42,3351 (1986).

3) M. Sunagawa, K. Goda, M. Enomoto and A. Sasaki, Abst. 9th Int. Cong. Heterocyclic Chem., p484 (1983,Tokyo). T. Kawabata, Y. Kimura, Y. Ito, S. Terashima, A. Sasaki and M. Sunagawa, Tetra. Lett. 27 6241 (1986)

4) Obtained by PCC oxidation of prod.3.

5) From 6-APA by the following procedures the standard (-) silyl protected azetidinone derivative, $[\alpha]_D^{24}$ -17.5° was obtained.
cf. T.Kobayashi, N.Ishida and T.Hiraoka, Chem. Commun., 737 (1980).
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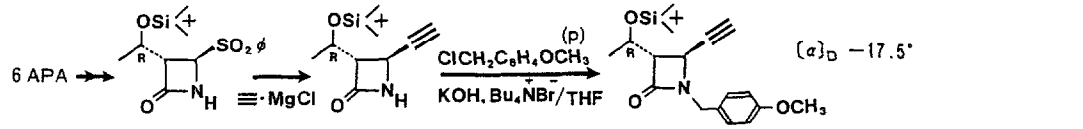

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