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Baker's-Yeast-Catalyzed Metabolite Synthesis of Drugs Carrying a Ketone Group: Microbial Reduction of Nabumetone and Pentoxifylline

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Baker's-Yeast-Catalyzed Metabolite Synthesis of Drugs Carrying a Ketone Group: Microbial Reduction of Nabumetone and Pentoxifylline

Summary : *Baker's yeast was screened for its ability to metabolize drugs carrying a ketone group such as nabumetone and pentoxifylline. The corresponding alcohol metabolites were obtained as enantiomerically enriched mixtures with a yield of between 35-40 % and 65– 72 % ee. In addition, esters of these alcohols were prepared and lipase resolution was applied in order to obtain enantiomerically pure metabolites, and stereo chemisty of these metabolites were determined.*

Keywords : *Nabumetone, pentoxifylline, metabolite, baker's yeast, lipase, microbial reduction*

Hamur Mayası Kullanılarak Keton Grubu Taşıyan İlaçların Metabolitlerinin Sentezleri: Nabumeton ve Pentoksifilinin Mikrobiyolojik Redüksiyonu

Özet : *Hamur mayası, nabumeton ve pentoksifilin gibi bir keton grubu taşıyan ilaçları metabolize edebilme özelliği açısından incelendi. İlgili alkol metabolitleri %35-40 verimle ve % 65-72 enantiyomerik fazlalık içeren karışımlar halinde elde edildi. Ayrıca bu alkollerin esterleri hazırlanarak saf enantiyomerik metabolitlerinin elde edilmesi için lipaz çözümlemesi uygulandı. Bu yolla elde edilen saf metabolitlerin stereokimyası tayin edildi.*

Anahtar kelimeler : *Nabumeton, pentoksifilin, metabolit, hamur mayası, lipaz, mikrobiyolojik redüksiyon*

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INTRODUCTION

In recent years, the advantages of using enzymatic catalysis in preparative organic chemistry have become apparent, in particular transformations mediated by baker's yeast. One of the earlier reports in 1970's that used baker's-yeast-mediated transformation was published on the synthesis of enantiomerically pure compounds[1]. Since then the amount of scientific work devoted to baker's yeast transformation of small organic molecules has increased significantly[2]. Asymmetric reduction of carbonyl containing compounds by baker's yeast constitutes one of the most widely applicable reactions. Ketones with varying substituents were reduced by baker's yeast and the secondary alcohols obtained[3-5].

Nabumetone [4-(6-methoxy-2-naphtyl)-butan-2-one] (1), a nonacidic nonsteroidal anti-inflammatory drug, appears to possess no analgesic, prostaglandin synthesis inhibiting activity in the body. Nabumetone undergoes hepatic metabolism and gives to mainly 6-methoxy-2-naphtyl acetic acid (2) and 6-methoxy-2-naphtyl-2-butanol(3) (Scheme 1). These compounds exhibit analgesic, antipyretic, antiinflammatory and significant prostaglandin synthesis inhibitory activity[6,7].

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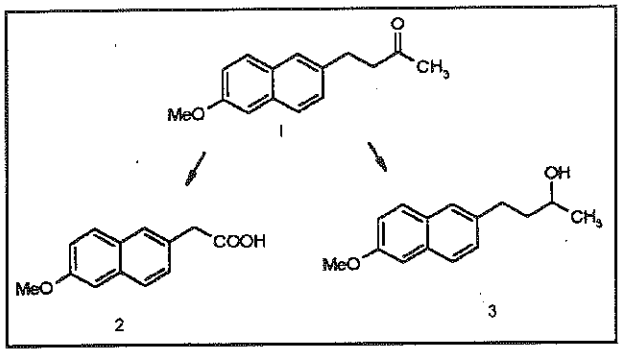
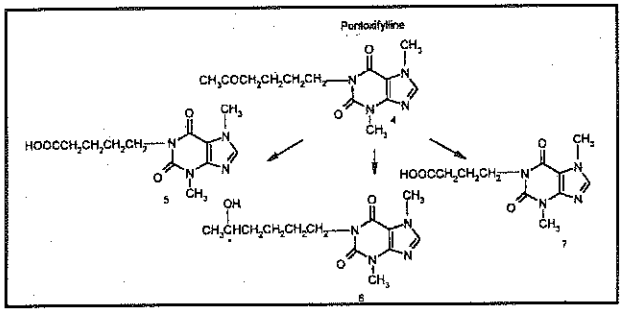


Scheme 1. Nabumetone biotransformation

To the authors' knowledge there is no study on enzymatic reduction of nabumetone in the literature, therefore stereochemistry of the related alcohol is not specified.

Pentoxifylline [1-(5'-oxohexyl)-3,7-dimethylxanthine] (4) is used in the treatment of cerebrovascular and peripheral vascular diseases. In mammals, pentoxifylline is principally reduced to the related alcohol (6) and oxidatively metabolized to the homologous carboxylic acids(5,7)[8]. (R)-1-(5'-Hydroxyhexyl)-3,7-dimethylxanthine (4a), lisofylline, is a novel anti-inflammatory compound that inhibits stress-activated lipid metabolic pathways. It is being developed to decrease morbidity and mortality associated with dose-intensive cancer treatment and for the prevention of acute lung injury following severe trauma[9].



Scheme 2. Pentoxifylline biotransformation

In this study, baker's yeast was chosen for nabumetone and pentoxifylline reduction in order to obtain alcohol metabolites, 6-methoxy-2-naphtyl-2-butanol (3) and 1-(5'-hydroxyhexyl)-3,7-dimethylxanthine (6), respectively. These metabolites are not only metabolites but also pharmacologically active compounds[6,7,9].

MATERIALS AND METHODS

Materials

Nabumetone and pentoxifylline were provided from Fako and Abdi İbrahim Drug Companies in Turkey. Baker's yeast, SYSC-1 yeast type 2, and lipase from porcine pancreas were purchased from the Aldrich Chemical Company. Analytical TLC was performed on silica gel 60-F 254 plates. Metal hydride reduction of the nabumetone and pentoxifylline were prepared in order to use as standards for TLC studies. All melting points were measured on a Thomas-Hoover melting point apparatus and the melting points are uncorrected. IR spectra were recorded as neat samples with a Perkin-Elmer FT-IR spectrometer 1720X. ^{1}H-NMR spectra were recorded in $CDCl_3$ with a Bruker AC 400 MHz FT NMR. Proton shifts were reported in ppm. Optical rotations were measured with an Autopol IV automatic polarimeter with a 0.5 dm cell.

Methods

Procedure for asymmetric reduction of nabumetone and pentoxifylline

3.6 Mmol of nabumetone or pentoxyfilline was dissolved in 50 mL of methanol and 172 mg (4.7 mmol) of sodium borohydride was added to this solution. The mixture was stirred at room temperature for about 4 h. 15 mL of water was then added to the mixture in order to end the reaction. The water layer was extracted three times with 15 mL of chloroform and the organic layer was dried over Na_2SO_4 and reduced under pressure.

Procedure for baker's yeast reduction of nabumetone and pentoxifylline

A mixture of 10 g of baker 's yeast, 22.5 g of glucose and 150 mL of distilled water was incubated at 37 °C for about 48 h. Then 5 g of baker's yeast, 4 g of glucose and 0.005 mol of ketone in 3 mL of alcohol were added to this mixture and incubated for another 72 h. The mixture was centrifuged. The water layer was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and reduced under pressure. The residue was purified by preparative chromatography

using ethyl acetate-hexane 1:1 and benzene: acetone 1:1 mixtures as solvents.

Procedure for acetylation of 4-(6-methoxy-2-naphtyl)-2-butanol (1a) and 1-(5'-hydroxyhexyl)-3,7-dimethylxanthine (2a)

0.005 Mmol of corresponding alcohol was dissolved in 5 mL of pyridine and 5 mL of acetic anhydride was added to this solution. The mixture was stirred at room temperature for about 4 h. 15 mL of water was added to this solution and extracted 4 times with ethyl acetate . Then the organic layer was dried over Na_2SO_4 and reduced under vacuum. The yield was 90 %.

Procedure for lipase resolution

0.007 Mmol of corresponding acetylated products and lipase from porcine pancreas (PPL) was suspended in 50 mL of pH 7 phosphate buffer solution. The mixture was stirred for about 3 h at room temperature by controlling pH value. 1 N NaOH solution was used to adjust the pH of the solution. Then the mixture was filtered and extracted three times with 20 mL of ethyl acetate. The organic layer was dried over Na_2SO_4 and reduced under vacuum. The residue was purified by preparative chromatography using ethyl acetate-hexane 1:1, and benzene: acetone 1:1 mixtures as solvents.

(-)-4-(6-Methoxy-2-naphthyl)-2-butanol (1a). Rf :0.33 (Ethyl acetate : hexane 1:1) yield : 40 %, m.p.: 93-94° C, m.p.: 94-95(C (lit[10]) $[\alpha]_D^{25}$ – 7.9 (c, 5.0, EtOH).
IR : (cm^{-1}) 3315, 3002, 2870, 1032.
^{1}H-NMR ($CDCl_3$) (δ) 1.2 (d, J=6 Hz, 3H, CH_3), 1.8 (m, 2H, CH_2), 2.8 (m, 2H, CH_2), 3.8 (m, 1H, CH), 3.9 (s, 3H, OCH_3), 7-7.8 (m, 6H, Ar).

(+)-4-(6-Methoxy-2-naphthyl)-2-acetyloxybutane (1e): Rf :0.6 (Benzene:acetone 1:1) yield: 40 % . m.p.: viscous liquid. $[\alpha]_D^{25}$ + 6.35 (c, 5.0, EtOH).
IR : (cm^{-1}) 3005, 2870, 1690, 1035.
^{1}H-NMR ($CDCl_3$) (δ) 1.35 (d, J=6 Hz, 3H, CH_3), 1.98 (m, 2H, CH_2), 2.05 (s, 3H, $COCH_3$) 2.78 (m, 2H, CH_2), 4.0 (s, 3H, OCH_3), 5.01(m, 1H, CH), 7-7.8 (m, 6H, Ar).

(R)-1-(5'-Hydroxyhexyl)-3,7-dimethylxanthine (4a): Rf :0.28 (Benzene:acetone 1:1) yield: 35 % m.p.:121-123°C, m.p.: 123-124°C (lit.[11]). $[\alpha]_D^{25}$ – 6.55 (c, 5.0, EtOH).
IR : (cm^{-1}) 3345, 3010, 2870,1734.
^{1}H-NMR ($CDCl_3$) (δ) 0.8 (d, J=6 Hz, 3H, CH_3), 1.1-1.5 (m, 4H, CH_2-CH_2), 2.50 (m, 2H, CH_2) 3.20 (s, 3H, N-CH_3), 3.70 (m, 2H, CH_2), 3.74 (s, 3H, N-CH_3), 5.01(m, 1H, CH), 7.4 (s, 1H, Ar).

(S)-1-(5'-Acetyloxyhexyl)-3,7-dimethylxanthine (4e): Rf :0.63(Benzene:acetone 1:1) yield: 35 % m.p.: 80-82°C, m.p.: 81-83° C (lit.[12]). $[\alpha]_D^{25}$ + 4.45 (c, 5.0, EtOH).
IR : (cm^{-1}) 3010, 2885, 1680.
^{1}H-NMR ($CDCl_3$) (δ) 0.87 (d, J=6 Hz, 3H, CH_3), 1.1-1.4(m, 4H, CH_2-CH_2), 2.08 (s, 3H, $COCH_3$) 2.55 (m, 2H, CH_3) 3.25 (s, 3H, N-CH_3), 3.73 (m, 2H, CH_3), 3.74 (s, 3H, N-CH_3), 5.03 (m, 1H, CH), 7.5 (s, 1H, Ar).

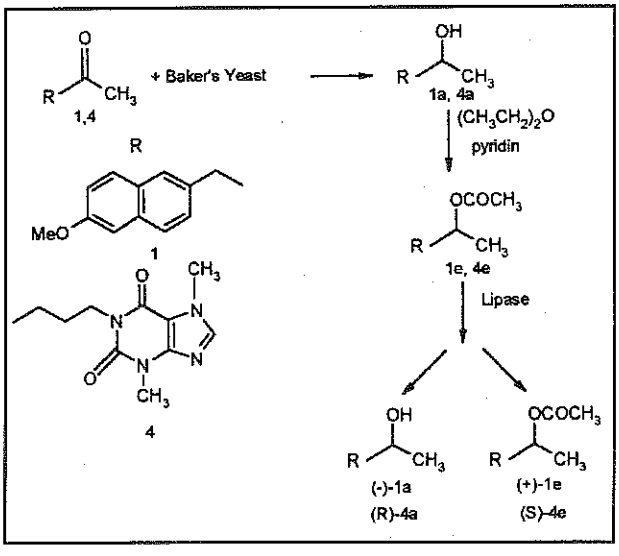
RESULTS AND DISCUSSION

In this paper, we reported baker's yeast reduction of nabumetone and pentoxifylline to their alcohol metabolites. The studies described support the concept of microbial models of mammalian metabolism, in that several of the same major metabolites seen in mammalians are produced in microbial cultures. Researchers continue to explore the stereo chemical course of ketone reduction as well as other parallel reactions between the mammalian and microbial metabolisms. In the literature, different cultures were chosen and microbial reduction of pentoxifylline was studied. Incubation durations were extended to 144 h. Results indicated that conversion of pentoxifylline to alcohol ranged from 6 to 90 % and the most active cultures were *Cryptococcus macerans, Curvularia falcato* and *Rhodotoluna mucilaginase*. In the case of baker's yeast (NNRL NY2034) in soybean meal dextrose medium, conversion was 8 % with the 72h. incubation time[8]. The stereo chemical studies showed that (-)-1-(5'-hydroxyhexyl)-3,7-dimethylxanthine has an R configuration[11]. On the other hand, Lillibridge et al.,[9] reported that lisofylline, (R)-1-(5'-hydroxyhexyl)-3,7-dimethylxanthine, is a minor metabolite of pentoxifylline in human liver. In cytosol, reduction of pentoxifylline to the alcohol was exclusively (S)-1-(5'-hydroxyhexyl)-3,7-dimethylxanthine.

In this study, nabumetone and pentoxifylline were re-

duced by baker's yeast SYSC-1 yeast type 2 in a glucose medium to obtain optically enriched mixture of 4-(6-methoxy-2-naphthyl)-2-butanol (3) and 1-(5'-hydroxyhexyl)-3,7-dimethylxanthine(6) with a yield of 40 % (72 % ee) and 35 %, (65 % ee) respectively. Although incubation time was 72 h, the yield was higher than that in the literature[8]. Esters of these alcohols were prepared in high yield (90 %), using acetic anhydride-pyridine by starting with an already optically enriched mixture instead of raceme alcohol derivatives. Then the lipase resolution method was used to obtain pure enantiomers. In this way, we combined baker's yeast and lipase resolution methods in order to prepare enantiomerically pure alcohol metabolites. 4-(6-Methoxy-2-naphthyl)-2-butanol (1a) is indicated as (-), crystal structure of this compound is not suitable for X-ray analysis. Optical rotation of 1-(5'-hydroxyhexyl)-3,7-dimethylxanthine (4a) was (-). According to the literature 11, compound (-)-4a had (R) configuration.

The overall reaction procedure is shown in scheme 3.



Scheme 3. The overall reaction procedure

(R)-1-(5'-Hydroxyhexyl)-3,7-dimethylxanthine, lisofylline, is a minor metabolite of pentoxifylline in the human liver, but it is a novel anti-inflammatory compound that inhibits stress-activated lipid metabolic pathways[9]. 4-(6-Methoxy-2-naphthyl)-2-butanol and its esters also possess anti-inflammatory activity[6,7]. Using baker's yeast, an alternative synthetic method was introduced for these compounds.

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