storage of the lyophilized peptide in vacuo at +4 °C for several months produces faint traces of a decomposition product which is detectable by HPLC.

1 S.I. Said and V. Mutt, Science *169,* 1217 (1970).
2 V. Mutt and S.I. Said, Eur. J. Biochem. *42,* 581 (1974).
3 A. Nilsson, FEBS Lett. *60,* 322 (1975).
4 P.L. Rayford, T.A. Miller and J.C. Thompson, New. Engl. J. Med. *294,* 1093 (1976).
5 J.M. Polak, A.G.E. Pearse, J.C. Garaud and S.R. Bloom, Gut *15,* 720 (1974).
6 a) M.G. Bryant, J.M. Polak, I. Modlin, S.R. Bloom, R.H. Albuquerque, and A.G.E. Pearse, Lancet *1,* 991 (1976); b) L.J. Larsson, J. Faharenkrug, O.S. de Muckadell, F. Sundler, R. Hakanson and J.F. Rehfield, Proc. natl Acad. Sci. USA *73,* 3197 (1976).
7 S.I. Said and J.C. Porter, Life Sci. *24,* 227 (1979).
8 R.B. Merrifield, J. Am. chem. Soc. *85,* 2149 (1963).
9 D.H. Coy and J. Gardner, Int. J. Peptide Prot. Res. *15,* 73 (1980).
10 C. Tzougraki, R.C. Makofske, T.G. Gabriel, J. Michalewsky, J. Meienhofer and C.H. Li, Int. J. Peptide Prot. Res. *15,* 377 (1980).
11 R. Colombo, Int. J. Peptide Prot. Res. *19,* 71 (1982).
12 L.A. Carpino and G.Y. Han, J. Am. chem. Soc. *92,* 5748 (1970).
13 a) E. Atherton, H. Fox, D. Harkiss, and R.C. Sheppard, J. chem. Soc. chem. Commun. *1978,* 539; b) C.-D. Chang and J. Meienhofer, Int. J. Peptide Prot. Res. *11,* 246 (1978); c) C.D. Chang, M. Waki, M. Ahmad, J. Meienhofer, E.O. Lundell and J.D. Haug, Int. J. Peptide Prot. Res. *15, 59* (1980).
14 a) R. Colombo, Experientia *37,* 798 (1981); b) R. Colombo, J. chem. Soc. chem. Commun. *1981,* 1012.
15 P.G. Pietta and O. Brenna, J. org. Chem. *40,* 2995 (1975).
16 L.A. Carpino and G.Y. Han, J. org. Chem. *38,* 4218 (1973).
17 C.-D. Chang, A.M. Felix, M.H. Jimenez and J. Meienhofer, Int. J. Peptide Prot. Res. *15,* 485 (1980).
18 E. Schnabel, J. Stoltefuss, H.A. Offe and E. Klauke, Justus Liebigs Annln Chem. *743,* 57 (1971).
19 E. Kaiser, L.R. Colescott, C.D. Bossinger, and P.I. Cook, Analyt. Biochem. *52,* 377 (1973).
20 A.M. Felix and M.H. Jimenez, Analyt. Biochem. *52,* 377 (1973).
21 B.S. Hartley, Biochem. J. *119,* 805 (1970).
22 G.O. Barbezat and M.I. Grossman, Science *174,* 422 (1971).

Stereospecific reduction of geraniol to (*R*)-(+)-citronellol by *Saccharomyces cerevisiae*

Paola Gramatica, P. Manitto, B. Maria Ranzi, A. Delbianco and Maria Francavilla

Istituto di Chimica Organica, Facoltà di Scienze, Università degli Studi di Milano, Via Venezian 21, I-20133 Milano (Italy), 11 November 1981

Summary. (*R*)-(+)-citronellol, a useful C_{10} chiral synthon for natural terpenoid products, can be obtained in enantiomerically pure form and satisfactory yield by yeast reduction of geraniol.

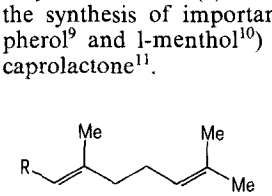
Although it is well recognized that yeasts are capable of reducing stereospecifically cinnamyl alcohols to the corresponding dihydroderivative[1], to our knowledge no analogous transformation has been reported for aliphatic allylic alcohols. We report here a simple route which allows (*R*)-(+)-citronellol (**2**) to be prepared in enantiomerically pure form[2] and satisfactory yield from geraniol (**1**), an achiral readily-available starting material, via microbiological *(Saccharomyces cerevisiae)* reduction.

A highly dense suspension of resting cells of *S. cerevisiae*[3] was incubated with geraniol (**1a**, 100 mg) and shaken at 27 °C for 24 h. The ether extract of the fermentation medium and the acetone extract of centrifuged cells were evaporated at room temperature under vacuum and chromatographed on preparative silica gel (TLC; benzene: ethyl acetate 8:2) to give (*R*)-(+)-citronellol (**2**) (yield 25%)[4] ($[\alpha]_D^{20} = +4.87°$ (MeOH); lit. +4.97° (MeOH)[5a], +2.32° to +2.7° (from Java citronella oil)[5b], +4.17°[5c], +5.2[6]) pure by NMR and GLC-MS (FFAP 10%, T = 160 °C).

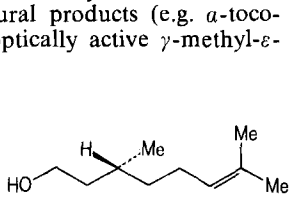
Taking into account that, in the case of citronellol, optical rotation cannot be used as a criteria for enantiomeric purity (see above $[\alpha]_D$ data and Valentine et al[7]), citronellol from yeast reduction was examined by ^{1}H-NMR-spectrum in the presence of a chiral lanthanide shift reagent. In the NMR-spectrum ($CDCl_3$), registered in the presence of Eu(tfe)$_3$ (molar ratio = 1:1), only a doublet due to 3-methyl group was observed, thus indicating that this material was essentially enantiomerically homogeneous[7]. As a further confirmation, mixtures of commercial (*S*)-(−)-citronellol and (*R*)-(+)-citronellol, derived from fermentation, showed 2 well resolved (1 Hz) doublets, that at higher fields being assignable to the (*R*)-(+)-isomer.

Geranial (**1b**) was found to give (*R*)-(+)-citronellol, as expected[1], whilst nerol (**4a**) and neral (**4b**), reduced in analogous way, afforded a mixture of the 2 enantiomers of (**2**). The enantiomeric ratio, determined by NMR as described, was (*R*):(*S*) = 6:4. This result is consistent with a partial cis-trans isomerisation of neral (**4b**) which appear to be an obligatory intermediate in the microbiological conversion of nerol into citronellol[1].

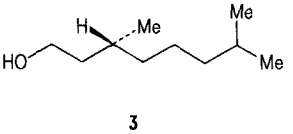
These findings, together with the ready availability of the diastereomerically pure geraniol (**1a**) appear to be of relevant synthetic value. (*R*)-(+)-citronellol (**2**) and its 6,7-dihydroderivative (**3**)[8] are known as key intermediates in the synthesis of important natural products (e.g. α-tocopherol[9] and l-menthol[10]) and optically active γ-methyl-ε-caprolactone[11].



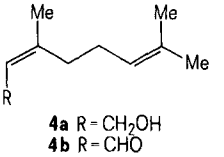
1a R = CH_2OH
1b R = CHO



2



3



4a R = CH_2OH
4b R = CHO

1 a) C. Fuganti, D. Ghiringhelli and P. Grasselli, J. chem. Soc. chem. Commun. *1975*, 846; b) P. Gramatica, B.M. Ranzi and P. Manitto, Bioorg. Chem. *10*, 22 (1981).
2 Natural (*R*)-(+)-citronellal, from which (**2**) can be obtained, has been shown to be only 75-80% optically pure: a) H.E. Eschinazi, J. Org. Chem. *26*, 3072 (1961); b) G.W. O'Donnell and M.D. Sutherland, Aust. J. Chem. *19*, 525 (1966); c) F. Ciardelli and P. Pino, Ric. Sci. Rc. A *4*, 694 (1964); B.D. Sully and P.L. Williams, Perfum. essent. Oil Rec. *59*, 365 (1968).
3 1×10^8 cell ml^{-1}. The growth conditions of *Saccharomyces cerevisiae* have been described in ref. 1b).
4 Average of 6 independent experiments.
5 a) C.G. Overberger and J.K. Weise, J. Am. chem. Soc. *90*, 3525 (1968); b) J.L. Simonsen, in: The terpenes, vol. I, p. 28. Cambridge Press 1953; c) W. Karrer, in: Konstitution und Vorkommen der Organischen Pflanzenstoffe, p. 52. Eds H. Hürlimann and E. Cherbuliez. Birkhäuser, Basel 1958.
6 R. Rienacker and G. Ohloff, Angew. Chem. *77*, 240 (1961).
7 D. Valentine, K.K. Chan, C.G. Scott, K.K. Johnson, K. Toth and G. Saucy, J. org. Chem. *41*, 62 (1976).
8 Compund (**3**) can be obtained from (**2**) by Ni-Raney hydrogenation without concomitant racemization[9a].
9 a) K.K. Chan, N. Cohen, J.P. DeNoble, A.C. Specian and G. Saucy, J. org. Chem. *41*, 3497 (1976); b) N. Cohen, W.F. Eichel, R.J. Lopresti, C. Neukom and G. Saucy, J. org. Chem. *41*, 3505 (1976); C. Fuganti and P. Grasselli, J. chem. Soc. chem. Commun. *1979*, 995.
10 J. Kulesza, J. Gora, K. Kowalska, Z. Dogielska and M. Druri, Przem. chem. *50* (9), 571 (1971); chem. Abstr. *76*, 14722t; T.P. Dang, P. Aviron-Violet, Y. Colleuille and J. Varagnat, 8th Congr. int. Huiles Essentielles, Cannes-Grasse 1980, abstract p. 158.
11 C.G. Overberger and H. Kaye, J. Am. chem. Soc. *89*, 5640 (1967).

AMP deaminase, 5′-nucleotidase and adenosine deaminase in rat myocardial tissue in myocardial infarction and hypothermia

Y. Saleem, T. Niveditha and B. Sadasivudu[1]

Departments of Biochemistry and Physiology, Osmania Medical College, Hyderabad-500001 (India), 25 August 1981

Summary. AMP deaminase, 5′-nucleotidase and adenosine deaminase have been estimated in skeletal muscle and myocardial tissue in normal rats and in rats subjected to experimental myocardial infarction or hypothermia. A difference in the enzyme distribution was found between the right and left ventricles in the normal rat. A decrease in the activity of 5′-nucleotidase and an increase in the activity of adenosine deaminase were observed in infarcted myocardial tissue. The activity of all 3 enzymes was found to be depressed in the myocardium in rats subjected to hypothermia. These results are discussed in relation to adenosine production and its beneficial effects.

Within the last few years a considerable amount of evidence has accumulated for a hormonal role for adenosine[2]. Adenosine has been implicated in a number of biological processes such as inhibition of cell proliferation[3], immuno suppression[4] and hormone liberation[5]. Adenosine has been shown to produce local vasodilatation[6]. This particular function has been exploited in the treatment of ischaemic heart disease by using drugs which maintain the adenosine levels in the tissues[7]. The immediate precursor of adenosine in the tissues is adenylic acid which is most quickly derived from the breakdown of ATP, though it is also synthesized de novo from simple metabolites. The production of adenosine from AMP in the tissues depends upon the relative activities of AMP deaminase and 5′-nucleotidase. While the former enzyme makes AMP unavailable for the production of adenosine, and AMP is diverted into the purine nucleotide cycle[8], the latter enzyme is specifically concerned with the production of adenosine. The preservation of the adenosine so liberated depends upon a number of factors, one of which is the activity of adenosine deaminase. In this paper we describe the activities of AMP deaminase, 5′-nucleotidase and adenosine deaminase in rat myocardium under 2 experimental conditions, namely myocardial infarction and hypothermia; the former requires more adenosine to maintain blood circulation in the tissue deprived of a blood supply, and the latter requires less adenosine due to depressed metabolic function and consequent low blood circulation. These enzymes have also been estimated in normal rat skeletal muscle tissue for purposes of comparison.

Materials and methods. Albino rats of the local strain were used for the experiments. Rats of both sexes were randomly used; they were killed by decapitation and exsanguinated, and the heart and a piece of skeletal muscle (gastrocnemius) were quickly removed and placed in ice-cold saline; they were then washed in the same solution to remove all traces of blood and a 10% homogenate of each tissue was prepared in 0.25 M sucrose using a Potter-Elvehjem type of homogenizer.

AMP deaminase activity was estimated by the method of Ogasawara et al.[9]. The assay mixture comprised 0.5 ml of 20 mM phosphate buffer, pH 7.0, 0.1 ml of 150 mM sodium chloride solution, 0.1 ml of 0.05% bovine serum albumin, 0.1 ml of 15 mM AMP solution and 0.1 ml of 10% tissue homogenate. Incubation was at 37 °C for 10 min. The ammonia liberated was estimated by Berthelot's method. The intensity of the indophenol-blue color developed was measured in a Spectronic-20 spectrophotometer at 640 nm against water. The activity of the enzyme was expressed as μmoles of ammonia liberated per h per g wet weight of tissue using ammonia standards processed in a like manner.

5′-Nucleotidase activity was determined by the method of Buniatian as described by Sadasivudu et al.[10]. The assay mixture consisted of 0.2 ml of 0.2 M Tris-HCl buffer, pH 7.5, 0.1 ml of 0.12 M magnesium sulphate solution, 0.5 ml of 0.01 M AMP solution, 0.2 ml of 10% tissue homogenate and 0.5 ml of distilled water. This was incubated at 37 °C for 2 h. The reaction was terminated by the addition of 1.5 ml of 10% TCA solution and inorganic phosphate liberated was determined in a 1 ml aliquot of the supernatant. The activity of the enzyme was calculated using phosphate standards and expressed as μmoles of Pi liberated per h per g wet weight of the tissue.

Adenosine deaminase activity was estimated by the method of Martinek[11]. The substrate solution comprised 0.5 ml of 0.675 mM adenosine in 0.2 M phosphate buffer pH 7.05. To this 0.05 ml of 5% tissue homogenate was added, and incubated at 37 °C for 1 h. A control tube was simultaneously prepared for each sample by adding phenol color reagent to the substrate prior to the addition of the tissue homogenate. At the end of 1 h the reaction was stopped by adding 2.5 ml of phenol color reagent (liquefied phenol