Pergamon

Tetrahedron Letters 43 (2002) 4797–4800

TETRAHEDRON LETTERS

Lipase-catalyzed synthesis of geranyl methacrylate by transesterification: study of reaction parameters

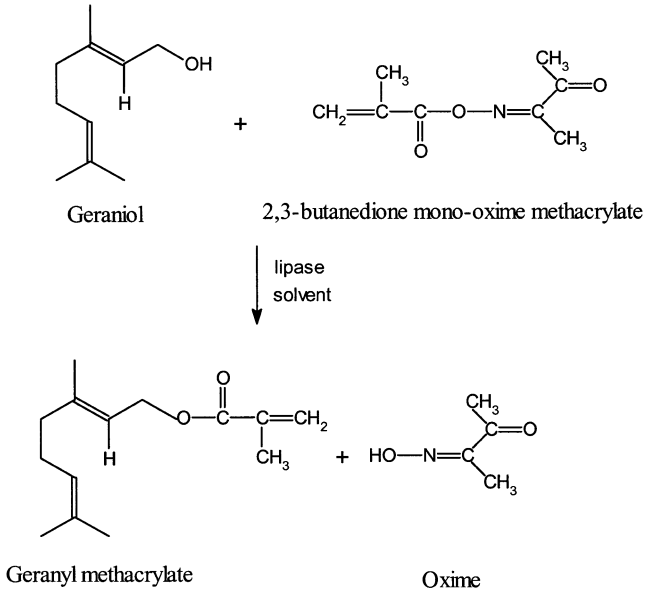
Vilas Athawale,* Narendra Manjrekar and Manoj Athawale

Department of Chemistry, University of Mumbai, Vidyanagari, Santacruz (E), Mumbai 400 098, India

Received 10 January 2002; revised 3 April 2002; accepted 13 May 2002

Abstract—The methacrylate ester of geraniol was synthesized using various lipases, such as porcine pancreatic lipase (PPL), *Candida rugosa* lipase (CRL) and *Pseudomonas cepacia* lipase (Amano-PS) as catalysts. The effects of reaction parameters such as type and amount of lipase, solvent, temperature and acylating agent on the conversion of geraniol to geranyl methacrylate was studied. The monomer obtained could be used in the synthesis of controlled release perfume.

Terpene esters are found in many essential oils and are commonly used as flavor and fragrance compounds in a variety of foods and beverages for creating fruity aromas. Conventionally, the terpene esters are synthesized using chemical reagents. However, owing to a number of drawbacks in the chemical synthesis, the recent trend is more towards the use of biocatalysts, especially lipases, which can be used in organic solvents, to synthesize terpene esters. It has been reported that during the synthesis of terpene esters by direct esterification with acetic acid, the catalytic activity of the enzyme was badly affected by acetic acid.[1,2] Therefore, the preferred route is transesterification in which various acyl donors such as propyl acetates,[3] isopropenyl acetates[4] and fatty acid anhydrides[5] are used.



Scheme 1.

From a commercial point of view, perfume compounds, when used as additives in consumable products, should be released gradually over a long period of time. Hence, many sustained release carriers such as fixatives, microcapsules and cyclodextrins have been developed from which the perfume compounds were released in a controlled manner.[6] It is well known that surfactants and enzymes have been used in the controlled release of the perfume compounds.[7–9] Currently, synthesis of polymer supported catalysts is gaining importance, wherein the molecule with catalytic activity is anchored on the polymer backbone. This prompted us to explore the potential of the polymer supported perfumery compounds as sustained release perfumes. Terpene esters are prone to hydrolysis in the presence of moisture, thereby releasing the corresponding terpene alcohol. Therefore, attaching these terpene esters to the polymer backbone could lead to their possible application as sustained release perfumes.

Geraniol, an important member of the terpene class, is widely used in perfumes. Lipase-catalyzed syntheses of geranyl esters as well as of methacrylate monomers has

Keywords: geraniol; lipase; methacrylate; controlled release perfume; oxime.

* Corresponding author. Tel.: 91.22.652 7957-61, ext: 567; fax: 91.22.652 85 47; e-mail: vilasda@yahoo.com

0040-4039/02/$ - see front matter
PII: S0040-4039(02)00926-7

been studied.[10,11] Although chemical synthesis of geranyl methacrylate[12] has been reported, there is no report available on its synthesis catalyzed by lipases. Therefore, in the present work we planned to study the influence of various reaction parameters such as type and amount of lipase, solvent, temperature and acyl donor on the synthesis of geranyl methacrylate from geraniol (Scheme 1).

In the enzymatic syntheses of geranyl esters, it has been reported that the inhibitory effect of geraniol depends on the structure of the lipase as well as on the concentration of geraniol.[3,4] In the present study, geraniol was transesterified[13] (Scheme 1) with 2,3-butanedione mono-oxime methacrylate.[14] It was found that an increase in the amount of geraniol had a detrimental effect on the activity of Amano-PS and CRL, whereas, the activity of PPL was almost unaffected and hence it was selected as a catalyst for optimization of other reaction parameters. It was also found that there was a gradual increase in the conversion of geraniol to geranyl methacrylate with the increase in the amount of lipase from 50 to 300 units (91.4%). However, with further increase in the amount of enzyme to 400 units, only a marginal increase in the conversion (98.4%) was observed.

In biocatalytic reactions, proper selection of organic solvent is essential.[15] The polarity of the organic solvents can be quantitatively measured by the value of log P, the logarithm of the partition coefficient of a given solvent between water and 1-octanol. Organic solvents with a log P value >2.0 are generally preferred for biocatalysis. The highest conversion (98.3%) was obtained with diisopropyl ether (DIPE) as solvent, even though its log P value is only 2. Diethyl ether with log P value (0.85), gave moderate conversion (87.6%). n-Hexane also proved to be a good solvent with 94.9% conversion. Cyclohexane and toluene when used as reaction medium did not give encouraging results. The use of chloroform as a solvent led to poor conversion. From the above results it can be concluded that the log P value cannot be considered as the only parameter responsible for the extent of conversion.

The reaction temperature being a crucial parameter in biocatalysis,[16] we selected six different temperatures in the range of 15–55°C for the synthesis of geranyl methacrylate from geraniol. It is clear from Table 1 that initially the conversion increased with the rise in the reaction temperature and reached a maximum at 35°C. However, a decrease in the conversion was observed with a further rise in the temperature from 35 to 55°C. This could be attributed to the deactivation of the lipase at these temperatures. The results of the detailed kinetic study at 25, 30 and 35°C (Table 2) indicated that the reaction followed first order kinetics. The rate constants, k for the conversion of geraniol into geranyl methacrylate, showed that there was a marginal rise in the rate of the reaction from 25 to 30°C, whereas at 35°C the rate was doubled as compared to that at 30°C. There was a negligible change observed in the rate at 37°C, which is considered to be an ideal temperature for enzymatic transformation. However, a decrease in the rate was observed above 37°C. Therefore, 35°C seemed to be the optimum temperature for our reaction system.

Table 1. Effect of temperature on lipase-catalyzed synthesis of geranyl methacrylate[a]

Temperature (°C)	% Conversion
15	51.3
25	74.8
30	93.4
35	98.3
37	98.0
40	97.1
45	85.4
55	74.2

[a] Using a 1:1 mol ratio of geraniol (0.001 mol) and 2,3-butanedione mono-oxime methacrylate (0.001 mol) in DIPE as a solvent and PPL (400 units) as a catalyst for 16 h.

Table 2. Rate constants for the transesterification of geraniol to geranyl methacrylate at various temperatures[a]

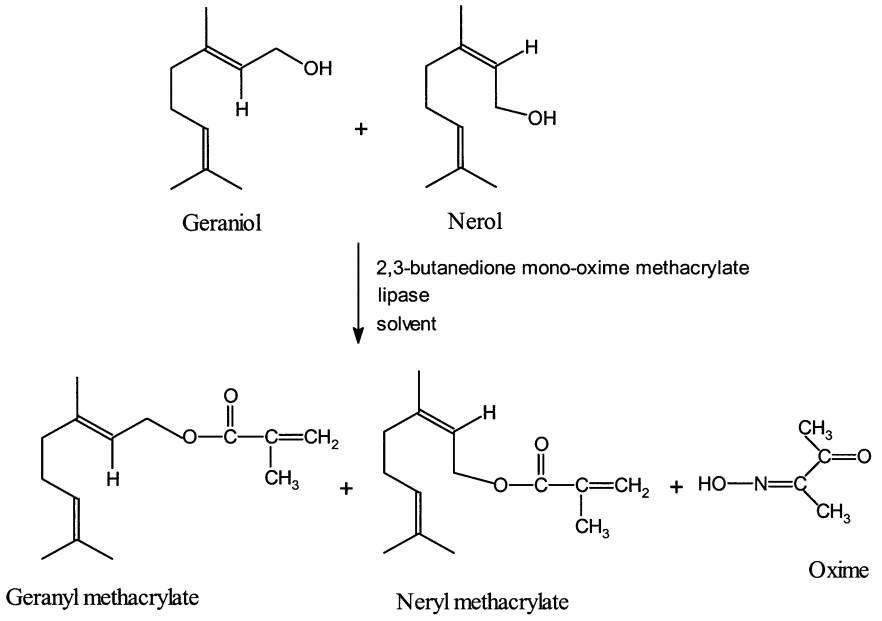
Temperature (°C)	Rate constant (k) $\times 10^3$/min^{-1}
25	1.38
30[b]	1.82
35	3.67
37	3.69
40	3.33
45	1.85

[a] Reactions were performed in DIPE at a 1:1 mole ratio of geraniol (0.001 mol) to 2,3-butanedione mono-oxime methacrylate (0.001 mol) using 400 units of PPL for 16 h.

[b] $\Delta E = 74.16$ kJ mol^{-1}; $\Delta G = 89.84$ kJ mol^{-1}; $\Delta H = 71.59$ kJ mol^{-1}; $\Delta S = -59.25$ kJ mol^{-1}.

In lipase-catalyzed reactions, the type of acyl donor plays an important role in the reaction kinetics. We have studied three acyl donors viz. methyl methacrylate, vinyl methacrylate and 2,3-butanedione mono-oxime methacrylate using DIPE as solvent and PPL (400 units), as a catalyst at 35°C. It was observed that the rate of conversion was the fastest in the case of oxime methacrylate and the slowest for methyl methacrylate. The difference in the rate of conversion could be attributed to the following factors. Methanol, being a poor-leaving group as compared to the vinyl and the oxime group, and hence the rate of conversion was the slowest with methyl methacrylate. Further, it has been proved that when vinyl esters were used as acyl donors, the acetaldehyde formed during the transesterification has a detrimental effect on the lipase activity. On the contrary, in the case of oxime esters, the leaving group, oxime, being a weak nucleophile does not take part in the reverse reaction, thereby improving the conversion rate.[17]

Having optimized the reaction parameters with respect to the amount and type of lipase, temperature, solvent and acyl donor, it was decided to study the effect of



Scheme 2.

geometrical isomerism on the conversion. Therefore, an equimolar mixture of geraniol (*E* isomer, CH_3 on C-3 *trans* to H on C-2) and nerol (*Z* isomer, CH_3 on C-3 *cis* to H on C-2) was transesterified with the oxime methacrylate (Scheme 2). In this reaction, the rate constants for geraniol and nerol were calculated and found to be 8.069×10^{-4} min^{-1} and 5.853×10^{-3} min^{-1}, respectively. It was found that the rate of conversion of geraniol into geranyl methacrylate is higher when compared to the conversion of nerol to neryl methacrylate. The isomeric excess of 73.9 for geraniol could be achieved after 16 h.[18,19] The probable reason could be steric hindrance encountered at the active site of the enzyme, because of the *cis* orientation around the double bond in nerol.

The present work is the first comprehensive study of the reaction parameters governing the enzymatic synthesis of geranyl methacrylate. PPL was found to be the most suitable lipase for the synthesis of geranyl methacrylate. 2,3-Butanedione mono-oxime methacrylate and DIPE were the best acylating agent and an ideal solvent, respectively. During the transesterification of the equimolar mixture of geraniol and nerol with the oxime methacrylate, PPL was found to be more selective for the synthesis of geranyl methacrylate. The reaction conditions reported in the present study are mild and 'clean' as compared to chemical methods. The newly synthesized monomer, geranyl methacrylate, could be homo- or co-polymerized for its potential use as a sustained release perfume. By making suitable modifications in the optimized reaction conditions obtained in the present study, various other monomeric esters of different flavors can be produced commercially.

References

1. Iwai, M.; Okumura, S.; Tsujisaka, Y. *Agric. Biol. Chem.* **1980**, *44*, 2731–2732.
2. Langrand, G.; Rondot, N.; Triantaphylides, C.; Baratti, J. *Biotechnol. Lett.* **1990**, *12*, 581–586.
3. Chulalaksananukul, W.; Condoret, J. S.; Combes, D. *Enzyme Microb. Technol.* **1992**, *14*, 293–298.
4. Claon, P. A.; Akoh, C. C. *Biotechnol. Lett.* **1994**, *16*, 235–240.
5. Shaw, J.-F.; Lo, S. *J. Am. Oil Chem. Soc.* **1994**, *71*, 715–719.
6. Hosokawa, H. *Fragrance J.* **1991**, *19*, 63–67.
7. Abe, M.; Tokuoka, Y.; Uchiyama, H.; Ogino, K. *J. Jpn. Oil Chem. Soc.* **1990**, *39*, 565–571.
8. Oka, N.; Sakata, K.; Usui, T.; Watanabe, S. Jpn. Kokai Tokkyo Koho JP 10 139,793 (1998) CA 129; 71973n.
9. Zhu, W.; Lockwood, G. B. *Biotechnol. Lett.* **2000**, *22*, 659–662.
10. (a) Yee, L. N.; Akoh, C. C.; Philips, R. S. *J. Am. Oil Chem. Soc.* **1995**, *72*, 1407–1408; (b) Claon, P. A.; Akoh, C. C. *J. Agric. Food Chem.* **1994**, *42*, 2349–2352; (c) Akoh, C. C.; Yee, L. N. *J. Mol. Catal. B: Enzym.* **1998**, *4*, 149–153; (d) Shieh, C. J.; Akoh, C. C.; Yee, L. N. *Biotechnol. Bioeng.* **1996**, *51*, 371–374; (e) Gryglewicz, S.; Jadownicka, E.; Czerniak, A. *Biotechnol. Lett.* **2000**, *22*, 1379–1382.
11. (a) Margolin, A. L.; Fitzpatrick, P. A.; Dubin, P. L.; Klibanov, A. M. *J. Am. Chem. Soc.* **1991**, *113*, 4693–4694; (b) Potier, P.; Bouchu, A.; Gagnaire, J.; Queneau, Y. *Tetrahedron: Asymmetry* **2001**, *12*, 2409–2419; (c) Potier, P.; Bouchu, A.; Descotes, G.; Queneau, Y. *Synthesis* **2001**, 458–462.

12. Ratusky, J.; Sorm, F. *Ger Offen*. 2,022,363 (1970), CA 74: 88173v.
13. The reactions were carried out in screw-capped vials, wherein 0.154 g (0.001 mol) of geraniol, 0.169 g (0.001 mol) 2,3-butanedione mono-oxime methacrylate and lipase catalyst were added to 5 mL of solvent and the reaction mixture was stirred at 200 r.p.m. Progress of the reaction was monitored by removing aliquots of the reaction mixture and subsequent analysis with a Hewlett–Packard 5890 gas chromatograph using a DB-23 capillary column (conditions: Injector = 300°C; Detector(FID) = 350°C, Programme = 90°C-3-10°C/min-105°C-7min, 5°C/min-110°C-0min-30°C/min-250°C-5min, R_T(min): oxime = 6.553, nerol = 8.586, geraniol = 9.860, 2,3-butanedione mono-oxime methacrylate = 10.455, neryl methacrylate = 13.032, geranyl methacrylate = 13.419). The absence of any undesired competing chemical acyl transfer reaction was verified by a controlled experiment in the absence of enzyme. Geranyl methacrylate: IR (neat) 2960, 2920, 2850, 1720, 1670, 1638, 1450, 1370, 1305, 1291,1160, 1008, 955, 932, 809 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 6.09 (1H, s, =C$\underline{H}_2$), 5.53 (1H, m, =C$\underline{H}_2$), 5.29–5.38 (1H, br.t, =C$\underline{H}$-CH_2O), 5.08 (1H, br.s, $(CH_3)_2$=C$\underline{H}$-), 4.61–4.70 (2H, d, -C$\underline{H}_2O$), 2.05 (4H, s, C$\underline{H}_2$-C$\underline{H}_2$), 1.93 (3H, s, CH_2=C-C$\underline{H}_3$,-CO), 1.67–1.70 (6H, d, C$\underline{H}_3$=CH-CH_2O, $(C\underline{H}_3)_2$C=CH), 1.59 (3H, s, $(C\underline{H}_3)_2$C=CH). GCMS *m/e* 222, 207, 193, 136, 121, 107, 93, 80, 69 (100), 53. Neryl methacrylate: IR (neat) 2960, 2920, 2850, 1720, 1670, 1640, 1450, 1375, 1320, 1290, 1160, 1005, 960, 935, 810. ^1H NMR ($CDCl_3$, 80 MHz) δ 6.09 (1H, s, =C$\underline{H}_2$), 5.53 (1H, m, =C$\underline{H}_2$), 5.31–5.40 (1H, br.t, =C$\underline{H}$-CH_2O), 5.1 (1H, br.s, $(CH_3)_2$=C$\underline{H}$-, 4.58–4.67 (2H, d, -C$\underline{H}_2O$), 2.12 (4H, s, C$\underline{H}_2$-C$\underline{H}_2$), 1.93 (3H, s, CH_2=C-C$\underline{H}_3$,-CO), 1.76 (3H, s, C$\underline{H}_3$=CH-CH_2O), 1.67 (3H, s, $(C\underline{H}_3)_2$C=CH), 1.59 (3H, s, $(C\underline{H}_3)_2$C=CH). GCMS *m/e* values for neryl methacrylate are the same as for geranyl methacrylate.
14. Athawale, V.; Manjrekar, N. *J. Mol. Catal. B: Enzym*. **2000**, *10*, 551–554.
15. Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev*. **1971**, *71*, 525–616.
16. Phillips, R. S. *Enzyme Microb. Technol*. **1992**, *14*, 417–419.
17. Ghogare, A.; Sudesh Kumar, G. *J. Chem. Soc., Chem. Commun*. **1989**, *20*, 1533–1535.
18. Chen, C-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc*. **1982**, *104*, 7294–7299.
19. Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl*. **1989**, *28*, 695–707.