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Enzymatic Synthesis of Capsaicin Analogs with Liver Acetone Powder

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Abstract: The enzymatic synthesis of capsaicin analogs with saturated or unsaturated acyl moieties has been achieved by using liver acetone powder as a catalyst. Copyright © 1996 Elsevier Science Ltd

Capsaicin (CAP) is a major pungent principle of capsicum fruits and has many bioactivities.^{1,2} However, the strong pungency and nociceptive activity of CAP seem to inhibit its usage as a food additive or a drug. Several non-pungent CAP analogs (including 4 and 5) cause adrenal catecholamine secretion.³ A (Z)-9-octadecenoyl vanillylamide named olvanil (6) is known to be an antinociceptive and antiinflammatory analgesic without initial nociceptive reaction.⁴⁻⁶

To establish the facile supply of CAP analys, we studied an enzymatic synthesis proceeding under mild conditions and without poisonous agents.

Applications of crude enzyme have gained importance in organic transformations because they are a cheap and easily available biocatalyst. Basavaiah et al. reported enzymatic hydrolysis using bovine liver acetone powder as a catalyst.^{7,8} On the other hand, Park et al. recently reported that the CAP hydrolase was extracted from rat liver microsome.⁹ We therefore used liver acetone powder as an enzyme source.

Vanillylamine hydrochloride could be condensed with a fatty acid methyl ester by using liver acetone powder. This reaction proceeded in a two-phase system in which an oil phase itself consists of the fatty acid methyl ester used as a substrate.

Reactive conditions were examined with methyl tetradecanoate (methyl myristate: $C_{14:0}$ -Me) as a model substrate, and the yields were analyzed by HPLC method.¹⁰

The CAP analog with a tetradecanoyl moiety (myristoyl: $C_{14:0}$, 4) was obtained under the following conditions. Ten µmol of vanillylamine hydrochloride (1.9 mg) and 1000 µmol of methyl tetradecanoate (275 µl) were dissolved in 500 µl of 10 mM borate buffer, pH 9.0, and 10 µmol of diisopropylethylamine (1.7 µl) was added. Further, 10 mg of chicken liver acetone powder (Sigma Chem. Co., St. Louis, MO) was added, and the solution was stirred with a magnetic stirrer at 37 °C in a water bath. The yield after the 24 h reaction

under this condition was 14.8%. No product was obtained when deactivated enzyme, or no enzyme, was used. The reaction was then performed on a twenty times larger scale, and the structure of the product was confirmed by 'H-NMR method.¹¹

Various CAP analogs, octanoyl ($C_{8:0}$, 1), decanoyl (caproyl: $C_{10:0}$, 2), dodecanoyl (lauroyl: $C_{12:0}$, 3), hexadecanoyl (palmitoyl: $C_{16:0}$, 5) and (Z)-9-octadecenoyl (oleoyl: $C_{18:1}$, 6) vanillylamides could be synthesized under the same conditions as mentioned above. The yields of each analog were analyzed by HPLC method and are shown in Table 1.

Capsaicin: R=		D	Reaction time and yield (%)	
CO(CH2)4CH [£] CHCH(CH3)2		n	24 h	48 h
	1	CO(CH2)6CH3	7.4	8.3
H₂CO	2	CO(CH2)8CH3	25.0	27.6
HO-CH2NHR	3	CO(CH2)10CH3	21.5	17.5
	4	CO(CH2)12CH3	14.8	17.2
	5	CO(CH2)14CH3	9.3	12.9
	6	CO(CH2)7CH ^Z CH(CH2)7CH3	8.4	13.4

Table	1 The yields of	f various CAP	analogs (1-6)	synthesized by	enzymatic synthesis
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In summary, CAP analogs with acyl moieties of various chain lengths could be synthesized by the use of a cheap and easily available biocatalyst, *i.e.*, liver acetone powder.

References and Notes

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- 10. Preparation of the HPLC sample: after centrifuging of the reaction mixture at 3000 rpm for 5 min, one µl of the upper layer was injected. HPLC conditions: column, Wakopak 5C18 AR, 48 x 150 mm (Wako Pure Chem. Ind., Osaka, Japan); eluent, methanol; flow rate, 0.5 ml/min; detection, UV 280 nm.
- 11. ¹H-NMR spectrum of 4: δ (CDCl₃): 6.81 (1H, d, J= 8.4 Hz), 6.76 (1H, s), 6.71 (1H, d, J= 8.4 Hz), 5.60 (1H, bt), 5.56 (1H, s), 4.31 (2H, d, J= 6.0 Hz), 3.83 (3H, s), 2.14 (2H, t, J= 7.6 Hz), 1.60 (2H, quint, J= 7.6 Hz), 1.20 (20H, s), 0.83 (3H, t, J= 7.6 Hz).

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