スタチン類緑体合成の中間体Butyl 1-p-Menthene-3-Azido-4-Hydroxy-7-Carboxylateの合成
Synthesis of Butyl 1-p-Menthene-3-Azido-4-Hydroxy-7-Carboxylate, the Synthetic Intermediate of Statine Analogue

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Summary

Protease inhibitor, pepstatin is a pentapeptide which involves two unusual amino acid, statine (3S-hydroxy-4S-amino-6-methylheptanoic acid (I)). Pepstatin is known to inhibit renin in the hypertensive renin-angiotensin system and shown to suppress blood pressure strongly.

The present paper deals with the simple and convenient synthesis of butyl 1-p-menthen-3-azido-4-hydroxy-7-carboxylate (III) in which III is one of the important intermediate leading to the isosteric isomer of statin, namely, p-menthan-3-amino-2-hydroxy-7-carboxylic acid (II). Further, the acid-catalysed isomerization is also discussed.

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The proteolytic enzyme renin cleaves the angiotensinogen to yield angiotensin I which is transformed by angiotensin converting enzyme (ACE) into the octapeptide pressor substance angiotensin II. Interruption of this proteolytic cascade by inhibition of renin or ACE could provide a novel approach to the treatment of hypertension. Specifically, inhibitors have been developed of ACE which may be a major advance. On the other hand, two different approaches have been followed by several investigators in the design of renin inhibitors. The first approach is based on chemical modification of the natural substrate of the enzyme either by replacement of some constitutive amino acids or by introduction of peptidase-resistant groups at the level of the peptide bond to be cleaved. An alternative approach in the design of renin inhibitors is based on appropriate modification of natural inhibitors of aspartyl proteinases such as pepstatin.

Pepstatin\textsuperscript{b)}, isovaleryl-Val-Val-Sta-Ala-Sta, a naturally occuring pentapeptide containing two units of statine (3S-hydroxy-4S-amino-6-methylheptanoic acid (I)) is a good inhibitor of renin \((K_i = 3.9-130 \times 10^{-7} \text{M})\).

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The structure of I has provided us with isosteric replacement for monoterpene skeleton unit from the comparison between I and p-menthan-3-amino-2-hydroxy-7-carboxylic acid (II).

Apparently, II mimics one of the constrained conformations of I in the reaction pathway intermediate for the enzyme catalyzed reaction. Pauling\(^2\) and later Wolfenden\(^3\) and Liehard\(^4\) recognized that an analogue that closely approximates the structure and geometry of the transition-state should be bound exceedingly tightly by the enzyme.

In the present paper, we describe the titled intermediate (III) in synthesizing statine analogue. III may be of synthetic interest from the viewpoint of its possessing the possibility of further transformations leading to II and it is fascinating that III can be obtained by a simple procedure.

The synthetic scheme of III is shown in Fig. 1. \(\text{I-Perillaldehyde (IV)}\) was used as a starting material. IV was oxidized with \(\text{Ag}_2\text{O}\) to perfolic acid (V) in 67.2% yield. The IR and NMR data of V is consistent with those of lit\(^5\).
which \( V \) had been obtained by the microbiological conversion of \( IV \) in 32% yield. \( V \) was treated with 10% \( H_2SO_4 \) in \( R-OH \) (\( R = \text{ethyl, propyl and butyl} \)) by the modified method of the lit\(^6,7\). In the case of using ethylalcohol and propylalcohol, two or three peaks were detected in GLC, the structure of which are shown in Fig 1. (\( IX \sim XIII \)), respectively. Each structure of \( IX \sim XIII \) was assigned via epoxidation. The details of them will be published elsewhere.

On the other hand, by using butylalcohol, the desired isomerized compound \( \text{(VI)} \) was obtained predominantly concomitant with minor aromatic compound \( \text{(VII)} \). The structures of \( VI \) and \( VII \) were determined by the IR and NMR analysis. Subsequent selective epoxidation of \( VII \) was carried out using m-chloroperbenzoic acid in \( \text{CH}_2\text{Cl}_2 \) and epoxide \( \text{(VIII)} \) was obtained quantitatively. The structure of \( VIII \) was also determined by IR and NMR analysis. In NMR spectrum, typical epoxy proton at C-3 appeared at 3.18 ppm(d), and the double-doublet at 7.04 ppm was easily assigned to C-2 methine proton.

Finally, epoxide \( \text{(VIII)} \) was treated with \( \text{NaN}_3 \) and deesterified azide \( \text{(III)} \) was obtained. The IR and NMR spectra were consistent with those of \( \text{III} \). The configuration of \( \text{III} \) was assumed as \( \text{III-c} \) in Fig. 2 from the viewpoint of reaction mechanism.

**Experimental Section**

Melting points were taken on a Thomas-hoover capillary melting point apparatus and are uncorrected. Spectra were obtained on the following instruments: IR, Hitachi EPI-G; MS, Shimadzu GCMS-7000; \(^1\)HNMR, BRUKER AC 250.
1, 8-p-Menthadien-7-carboxylic acid (V)

AgNO₃(12g) was dissolved in H₂O(70ml) and aq. NaOH solution (6.4g/30ml) was added with stirring for 5 min. The resulting crystalline Ag₂O was washed with H₂O until HNO₃ ion was fully removed. Ag₂O(8.1g) was dissolved in aq. NaOH solution(29g/140ml) and the reaction temperature was kept at 55-60°C. Then, (−)-perillaldehyde(10g) was added and the reaction mixture was stirred for 10-20 min. After filtration the residue was washed with hot water. The combined solution was acidified with 6N HCl(pH 1) and extracted with AcOEt (300ml×2). The AcOEt layer was treated with 2N NaOH(300ml×2) and the aq. layer was again acidified with 6N HCl to yield crystalline V (7.49g) (67.2%) which was recrystallized from petroleum ether. m. p. 125°C (lit. m. p. 125°C).

\[ \text{IR (cm}^{-1}: \text{f} \beta \text{2500 (C=O)}, \text{f} \gamma \text{1720 (C=O-C)}, \text{f} \lambda \text{1290 (C-O)}; \text{lHNMR (ppm): 0.95 (t. C-4 CH₃), 1.06 (d. C-9, 10 CH₃), 1.41-1.65 (m. C-2', C-3' CH₃), 2.45 (t. C-6 CH₃, m. C-8 CH), 4.15 (t. C-1' CH₂), 7.01 (dt C-2 CH); l¹CNMR (ppm): 13.6 (C-4'), 1.93 (C-9, 10), 20.9 (C-5), 22.0 (C-6), 25.6 (C-3'), 30.9 (C-2'), 35.1 (C-8), 64.1 (C-1'), 116.4 (C-3), 125.1 (C-4), 134.3 (C-2), 154.2 (C-1), 167.6 (C-7); MS: MW=222, m/z=166, 149, 123 (base), 121, 105, 91, 79, 77, 57, 43, 41. \]

By-product, butyl cuminate (VII) was also purified by silica gel chromatography.

IR (cm⁻¹): 1720 (C=O), 1620 (m), 1580 (w) (benzene ring), 1290, 1110 (C-O-C); l¹HNMR (ppm): 0.89 (t. C-4 CH₃), 1.16 (d. C-9, 10 CH₃), 1.38-1.66 (m. C-2', C-3' CH₃), 2.86 (m. C-8 CH), 4.22 (t. e-1' CH₂), 7.17 (d. C-3, 5 CH), 7.88 (d. C-2, 6 CH); l¹CNMR (ppm): 13.6 (C-4'), 19.3 (C-9, 10), 23.7 (C-3'), 30.1 (C-2'), 34.3 (C-8), 64.6 (C-1'), 126.5 (C-3, 5), 128.4 (C-4), 129.7 (C-2, 6), 154.2 (C-1), 166.8 (C-7); MS: MW=222, m/z=205, 165, 164, 149, 147 (base), 119, 105, 91, 77, 43, 41.

Butyl 1-p-menthene-3, 4-epoxy-7-carboxylate (VIII)

VI (1.72g) was dissolved in anhydrous CH₂Cl₂ (50ml) and m-chloroperbenzoic acid (2.0g) in anhydrous CH₂Cl₂ (300ml) was added with stirring at -5~0°C. After the reaction mixture was kept stirring for 1.5 h, the solution was
treated with 2N NaOH (200ml×2). CH₂Cl₂ layer was washed with H₂O and evaporated to yield light yellow syrup (1.77g). Purification was conducted with silica gel chromatography (hexane/AcOEt=30:1) and 1.38g of pure oil VIII was obtained (71.7%). IR (cm⁻¹): 1710 (C=O), 1640 (C=C), 1270, 1100 (C-O-C); ¹H NMR (ppm): 0.94 (t. C-4' CH₃), 0.98, 1.06 (d. ×2 C-9, 10 CH₃), 1.35-1.80 (m. C-2', C-3' CH₂; C-5, C-8 CH), 2.05-2.20 (m. C-6 CH₂), 3.18 (d. C-3 CH), 4.14 t. C-1' CH₂), 7.04 (dd. C-2 C1'-1); ¹³C NMR (ppm): 13.7 C-4', 16.3, 16.4 (C-9, 10), 19.2 (C-3'), 20.5 (C-5), 20.7 (C-6), 30.7 (C-8), 52.5 (C-3), 64.6 (C-1'), 66.4 (C-4), 133.9 (C-2), 134.7 (C-1), 166.3 (C-7); MS: MW=238, m/z=209, 195, 164, 147, 119, 107, 91, 77, 69, 57, 43, 41 (base).

Butyl 1-p-menthene-3-azido-4-hydroxy-7-carboxylate (III)

To the mixture of sodium azide (200mg), NaCl (100mg) and 90% ethanol (12ml), VIII (340mg) was added and refluxed for 5 h. Then, H₂O (70ml) was added and the reaction solution was acidified with 6N HCl. The product was extracted with AcOEt and yellow syrup (283mg) was obtained. Purification was carried out by silica gel chromatography. 160mg of pure oil (III) was obtained (84.2%).

IR (cm⁻¹): 3500-2500 (COOH), 3350 (OH), 2200 (N₃), 1690 (C=O), 1660 (C=C); ¹H NMR (ppm): 0.97-1.01 (d. ×2, C-9, 10 CH₃), 1.40-1.53 (m. C-8 CH), 1.90-2.05 (m. C-6 CH₂), 2.20-2.57 (m. C-5 CH₂), 3.78 (d. C-3 CH), 7.03 (d. C-2 CH).

References
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プロテアーゼ阻害剤、ベプスタチンはペプテプチドであるが、その構成成分に2個の異常アミノ酸であるスタチン（3S-hydroxy-4S-amino-6-methylheptanoic acid (I)）を含み、昇圧系の酵素レニン－アンジオテンシン系に作用して酵素レニンを阻害し強い血圧下降作用を示す。今回、我々はスタチンと構造が酷似している isoetric isomer とみなされる p-menthan-3-amino-2-hydroxy-7-carboxylic acid (II) をデザインし、II を合成する際の中間体、butyl 1-p-menthen-3-azido-4-hydroxy-7-carboxylate (III) を簡便かつ収率よく合成したので報告する。なおその際、酸による異性化の段階で興味ある知見も得られたので少し触れたい。