Axial Conformation of 3-Methyl-2-butenoyl Group in Pyranocoumarin Ring Endows Biological Activity of (+)-Decursin

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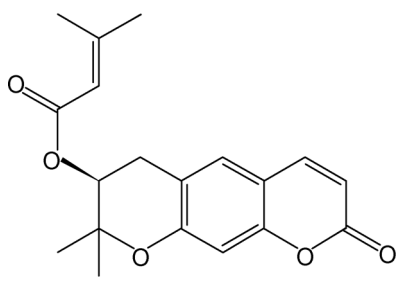
Angelica gigas Nakai "cham-dang-gui" is a monocarpic biennial or short lived perennial plant, and mainly cultivated in Korea. The root has been used as a traditional herbal medicine for treatment of anemia and as sedative, anodyne, or tonic agents. Recent comparison study of Korean angelica with others, including *A. sinensis* (Chinese origin) and *A. acutiloba* (Japanese origin), showed that only *A. gigas* Nakai contains significant amounts of (+)-decursin (Scheme 1).[1] Decursin shows anti-bacterial activity against *Bacillus subtilis*,[2] as well as *in vitro* and *in vivo* anti-tumor activities.[3,4] For examples, anticancer activities against human prostate carcinoma cells, human K562 erythroleukemia, and U937 myeloleukemia cells have been reported.[5,6] Besides, decursin is better platelet anti-aggregating agent than acetylsalicylic acid.[7] Acetylcholinesterase inhibition by decursin was also reported,[8] and it was suggested that decursin mitigates amnesia *in vivo* through inhibition of acetylcholinesterase activity in the hippocampus.[9] Due to the important biological activities, stereoselective syntheses of decursin have been reported, as well.[10-12] Although, two-dimensional structure of decursin has been established by the extensive NMR spectroscopy,[13,14] three-dimensional structure of decursin is not available yet. We have been studying absolute configurations of natural products with regard to the enzyme-substrate interactions.[15-17] Since decursin shows various biological activities, we have carried out single crystal X-ray crystallography of decursin to provide structural information (Table 1).

Overall structure of (+)-decursin is characterized by L-shaped geometry with a C11 hinge, and the distance between carbonyl oxygen and coumarin ring is found at about 10 Å.



Scheme 1. Molecular structure of (+)-decursin.

The coumarin ring moiety of (+)-decursin is planar and the adjacent dihydropyran ring forms distorted half-chair conformation with axial 3-methyl-2-butenoyl group (Figure 1). Generally, bulky substituent like 3-methyl-2-butenoyl is favored at equatorial position of the ring structure, but it is found at axial position and almost rectangular to the pyranocoumarin ring. The O4-C11-C10 and O4-C11-C12 angles are found at 109.87(18)^o and 106.06 (17)^o, respectively. Although short interaction of 2.453 Å between O5 and H9 is found in the packing diagram (data not shown), the major reason of the axial conformation by 3-methyl-2-butenoyl substituent appears to be the steric hindrance of 3-methyl-2-butenoyl group with two methyl groups of C13 and C14. Space filling model of (+)-decursin showed no room for O5 of 3-methyl-2-butenoyl group close to the two methyl groups, when it forms equatorial 3-methyl-2-butenoyl conformation (data not shown). The distance of O4-C11 single bond is found at 1.450(3) Å, and those of O3-C7 and O3-C12 single bonds are found at 1.373(3) Å and 1.464(3) Å, respectively. It seems the resonance structure of coumarin ring expands to O3 atom, based on the short distance and partial double bond character of O3-C7 bond.

For the acetylcholinesterase inhibitors, it is known that hydrophobic and electrostatic interactions are required at the active site.[18] The charged glutamate and polar serine and tyrosine residues form electrostatic site and the aromatic

Table 1. Crystal data and structure refinement for (+)-decursin.

Empirical formula	$C_{19}H_{19}O_5$
Formula weight	327.34
Temperature	293(2) K
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Unit cell dimensions	a = 9.519(4) Å, b = 10.694(4) Å, c = 17.043(7) Å
Volume	1747.9(12) Å^3
Z, Calculated density	4, 1.244 Mg/m^3
Absorption coefficient	0.090 mm^{-1}
F(000)	692
R(int)	0.0367
Data / restraints / parameters	4098 / 0 / 224
Goodness-of-fit on F^2	1.078
Final R indices [I>2sigma(I)]^a	R1 = 0.0592, wR2 = 0.1353
R indices (all data)	R1 = 0.0668, wR2 = 0.1384

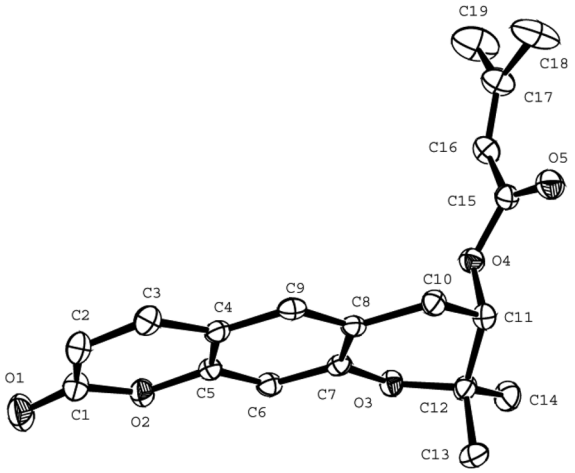


Figure 1. Crystallographic structure of (+)-decursin with thermal ellipsoids (50% probability). Hydrogen atoms were omitted for clarity. O(1)-C(1) 1.212(3) Å; O(2)-C(5) 1.385(3) Å; O(2)-C(1) 1.389(3) Å; O(3)-C(7) 1.373(3) Å; O(3)-C(12) 1.464(3) Å; O(4)-C(15) 1.359(3) Å; O(4)-C(11) 1.450(3) Å; C(10)-C(11)-C(12)-O(3) 61.0(3)°; O(4)-C(11)-C(12)-O(3)-58.6(3)°.

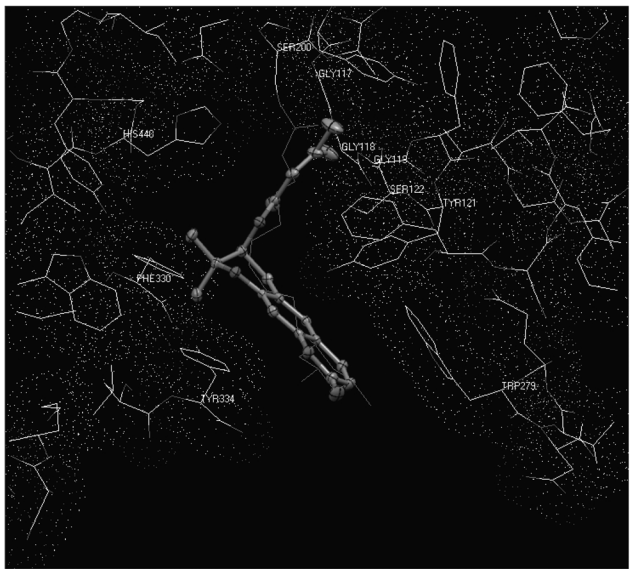


Figure 2. Docking model of (+)-decursin at the active site of acetylcholinesterase. The molecule in red is MF268 inhibitor.[24] The protein structure was obtained from RCSB protein databank (1OCE) and processed by Swiss-PdbViewer 3.7.[25]

rings of tryptophan and tyrosin residues form hydrophobic site, respectively.[19] From the crystallographic study of (+)-decursin, unusual axial conformation of 3-methyl-2-butenoyl group in pyranocoumarin ring moiety was formed, due to the steric hindrance to the dimethyl substitutents at neighboring C12 carbon center. The axial conformation of 3-methyl-2-butenoyl group at the half-chair dihydropyran ring moiety makes possible L-shaped structure of (+)-decursin, and which separates hydrophobic ring and polar carbonyl group. When the structure of (+)-decursin was positioned into the active site of acetylcholinesterase, excellent fitting was observed (Figure 2).[20] Possible hydrophobic interactions were found between (+)-decursin and the enzyme. The pyranocoumarin ring moiety can interact with Phe279 and Tyr334 at the opening of the substrate binding site. Besides, dimethyl group of pyranocoumarin ring appears to interact with Phe330. The polar residues of His440 and Ser200 are known to be very important for the catalysis and the inhibitor is covalently bound to Ser200 in the Figure 2. In case of (+)-decursin, 3-methyl-2-butenoyl group is positioned to flexible backbone of the active site, Gly117Gly118, and the carbonyl group may form hydrogen bonding with His440. As a result, the catalytic serine residue is blocked. Based on the docking model of (+)-decursin, the axial conformation of 3-methyl-2-butenoyl group appears to endow biological activity of acetylcholinesterase inhibition to (+)-decursin. This work will also provide important structural information on the other enzyme-ligand interactions, related to biological action of (+)-decursin.

Experimental Section

Decursin was isolated from the roots of *A. gigas* Nakai, according to the published method[21] and (+)-decursin was confirmed by optical rotation measurement.[22] The isolation yield was 0.1% (w/w), and it was crystallized from ethyl acetate/*n*-hexane (3:37) solution. Transparent rhombic-shaped crystals were isolated and used for structural determination. The crystal data and structural parameters are shown in Table 1 and X-ray crystallographic structure is shown at Figure 1. All calculations were performed using Bruker SHELXTL-97 software.[23]

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Supplementary data. Crystallographic data for the structure reported here have been deposited with Cambridge Crystallographic Data Center (Deposition No. CCDC 686759). The data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

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