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Structure–activity relationship study on α_1 adrenergic receptor antagonists from beer

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ABSTRACT

Hordatine A and aperidine have been previously isolated from beer as active ingredients, which bind to muscarinic M_3 receptor. In addition, these compounds have exhibited antagonist activity against the α_{1A} adrenoceptor. Although the relative structures of these two molecules have previously been determined, the absolute stereochemistry was unclear. Hence, to elucidate the absolute stereochemistry of natural hordatine A, we synthesized each enantiomer of hordatine A and aperidine from optically pure dehydrodi-*p*-coumaric acid. Several additional related compounds were also synthesized for structure–activity relationship studies. Chiral column HPLC analysis demonstrated that the absolute stereochemistry of natural hordatine A is (2*S*,3*S*), while based on the isomerization mechanism, the stereochemistry of aperidine is (2*R*,3*S*). The α_{1A} adrenoceptor binding activity of (2*R*,3*R*)-hordatine A is the most potent among the enantiomeric pairs of hordatines and aperidines. Furthermore, the related, synthetic compound, (2*R*,3*R*)-methyl benzofurancarboxylate exhibits antagonist activity against the α_{1A} adrenoceptor at a lower concentration than that of hordatine A.

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Hordatine A (**1**) was initially discovered in 1996 from barley as an antifungal compound, which was predominantly distributed in the shoots of seedlings.[1–3] Subsequent biochemical and genetic studies have revealed that **1** can act as a phytoalexin for pathogen defense.[4–6] Stoessl has originally reported that **1** (diacetate) can be obtained in the optically active form ($[\alpha]_D^{23} = +69$), and the relative stereochemistry was tentatively proposed to be *cis*.[1,2] Later, Yoshihara et al. reported that the relative stereochemistry of **1** was *trans* as well as grossamide based on the ^{1}H NMR data.[7] However, in both cases, the absolute chemistry of **1** has yet to be reported.

In 2004, we reported the isolation of two active ingredients from beer in the guidance of muscarinic M_3 receptor binding activity,[8] and the relative structures of these two active compounds, **1** and aperidine (**2**), were disclosed (Fig. 1).[9] Furthermore, we found that these compounds exhibited antagonist activity against the α_1 adrenoceptor.

Adrenergic receptors, which belong to the seven transmembrane spanning, G-protein-coupled receptor super family, mediate the actions of the endogenous catecholamines, adrenaline, and noradrenaline. Adrenergic receptors are broadly categorized into α_1, α_2, and β subtypes.[10] Of these, α_1 adrenoceptors, which are located in both the central and peripheral nervous systems, are of great therapeutic relevance due to their important roles in the contraction of smooth muscle tissue, especially in the cardiovascular system and lower urinary tract.[11,12] Moreover, the α_1 adrenoceptor subtype can be further divided into α_{1A}, α_{1B}, and α_{1D}. Selective ligands acting at these subtypes have therapeutic potential in the treatment of hypertension, stress urinary incontinence, benign prostatic hyperplasia, and lower urinary tract symptoms.

Thus, determining the absolute stereochemistry of **1** should be important not only for predicting the binding mode to α_1 adrenoceptors, but also for developing subtype-selective antagonists.[13–15] Herein, we report the complete structure of natural **1** as well as the α_{1A} adrenoceptor antagonist activities of all stereoisomers of **1** and related synthetic compounds.

First, the enantiomeric purity of **1** from beer has been analyzed by chiral column HPLC, which revealed natural **1** is a single enantiomer.[16] In order to determine the absolute stereochemistry of **1**, we attempted the following methodology: (1) optical resolution and absolute stereochemical assignment of dehydrodi-*p*-coumaric acid (**4**), (2) synthesis of **1** from optically pure **4**, (3) comparison of retention time using chiral column HPLC and the optical rotation between natural and synthetic **1**. Racemic **4** was synthesized by single electron oxidation with horseradish peroxidase and H_2O_2. The optical resolution of **4** was performed by chiral column HPLC

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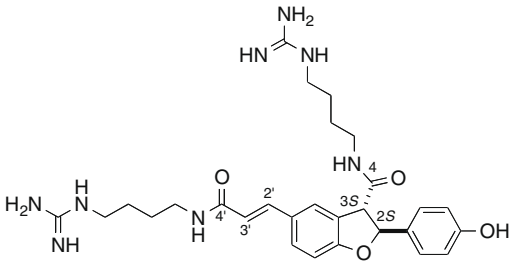

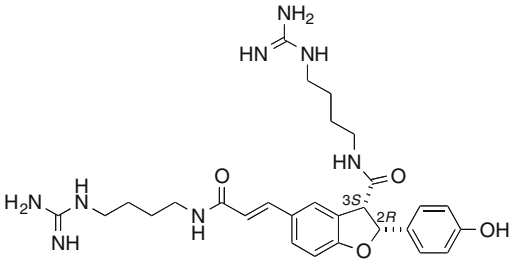

Figure 1. Structures of hordatine A (**1**) and aperidine (**2**).

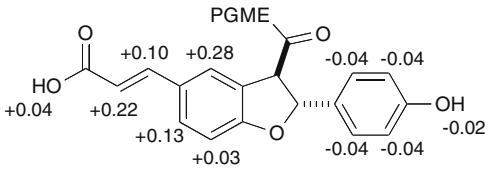

Figure 2. Distribution of $Dd_{S\text{-}R}$ values for PGME derivatives (**5**) in DMSO-d_6.

with chiracel OJ-RH. Taking advantage of the carboxylic acid on the chiral center, a modified Mosher's method with PGME amide was applied to each enantiomer.[17] The $\Delta\delta$ values were clearly distributed (Fig. 2), which led to the assignment of the absolute stereochemistry of each enantiomer. Consequently, the elution order of the two peaks were (*S*,*S*)-(+) and then (*R*,*R*)-(−)-**4**.

With optically active **4** in hand, each enantiomer of **1** was synthesized according to a modified Stossel's method (Fig. 3). Protected agmatine was prepared by condensation between *N*,*N*′-diBoc-imidazoyl guanidine and *N*-Cbz-diaminobutane in good yield. Subsequent hydrogenolysis of the Cbz group afforded *N*,*N*′-diBoc-agmatine. Coupling **4** with *N*,*N*′-diBoc-agmatine using PyBOP in the presence of HOBt gave the *N*,*N*′,*N*″,*N*‴-tetra-Boc-hordatine A (**10**). Finally, deprotection of the Boc group with TFA yielded **1**. Chromatographic comparison using chiral column HPLC demonstrated that the retention time of natural **1** was identical to that of synthetic (2*S*,3*S*)-**1**. We therefore concluded that the absolute stereochemistry of natural **1** is (2*S*,3*S*). However it was unexpected that this configuration was the opposite to that of (−)-ephedradine A, which was a macrocyclic spermine alkaloid having similar dihydrobenzofuran skeleton.[18]

To confirm our assignment using the modified Mosher's method, chiral column HPLC analysis was applied to synthetic (2*R*,3*R*)-dimethyl-dehydrodi-*p*-coumarate (**12**), which was prepared from the intermediate (**11**) for total synthesis of (−)-ephedradine A.[19] Consequently, **1** had antipodal stereochemistry to (−)-ephedradine A (Fig. 4), which is consistent with the result from the modified Mosher's method. It has been known that some natural lignans have been isolated as non-uniform stereochemical isomers, including the enantiomeric form or a racemic mixture[6] and lignans with a dihydrobenzofuran skeleton, such as **1** and ephedradine as well.[20]

Next our attention turned to determination of absolute stereochemistry of **2**. During the final step of the total synthesis of **1**, **2** was obtained as a minor product. We assumed that **2** was formed by an acid catalyzed isomerization during the deprotection step, and the most favorable condition to form **2** was a pH of 8.0 in an aqueous buffer, which yielded a product ratio *cis*:*trans* = 1:3. Moreover, based on the chiral column HPLC analysis, natural **2** was so optically pure that racemization did not occur during the isomerization step. Hence, we proposed that isomerization mechanism from **1** to **2** could be via a *p*-quinone methide intermediate because this isomerization route retained the C-2 stereochemistry.[21,22] Treatment of *N*,*N*′,*N*″,*N*‴-tetra-Boc-hordatine A with TFA-*d* did not lead to a H/D exchange in ^{1}H NMR spectrum, which supports an isomerization mechanism via *p*-quinone methide. Therefore, the absolute stereochemistry of natural **2** was determined as (2*R*,3*S*).

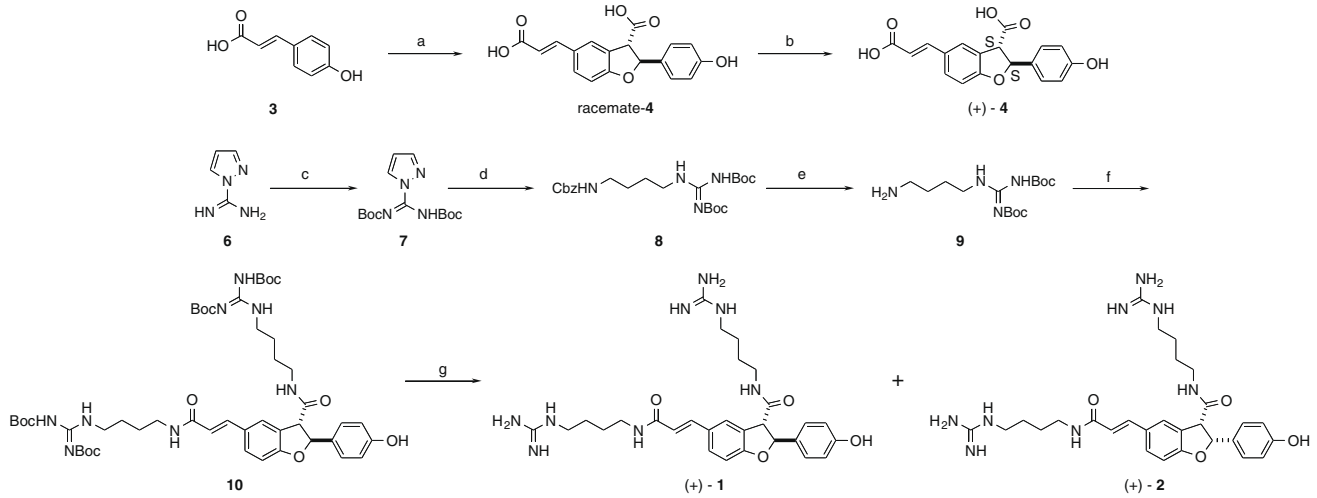

Figure 3. Synthesis of hordatine A (**1**) and aperidine (**2**). Reagents and conditions: (a) horseradish peroxidase, H_2O_2, phosphate buffer (pH 7.4) (34%); (b) ChiralPak OJ-RH; (c) $(Boc)_2O$, LiH, THF (70%); (d) *N*-Cbz-diaminobutane, Et_3N, CH_3CN, H_2O (86%); (e) H_2, Pd-C, AcOEt (85%); (f) (+)-**4**, EDCI, HOBt, DMF (53%); (g) (1)TFA, (2) Sephadex G-15 column chromatography with 0.01 N HCl (92%).

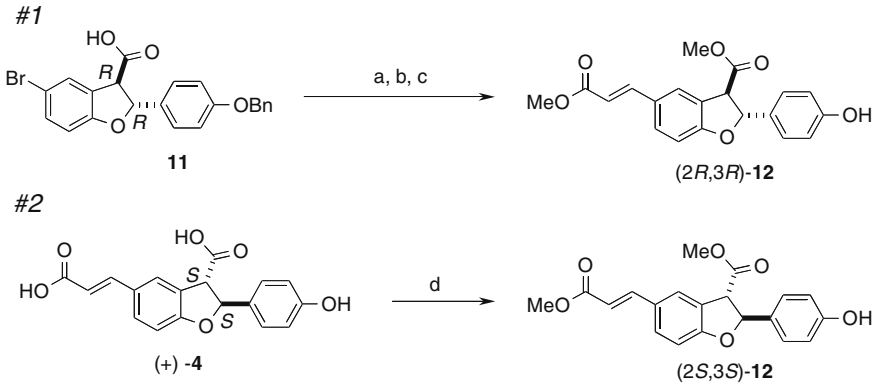

Figure 4. Synthesis of optically active **12**. Reagents and conditions: (a) CH_2N_2, Et_2O; (b) methyl acrylate, tri(*o*-tolyl)phosphine, $Pd(OAc)_2$, NEt_3, DMF, 100 °C (36% in 2 steps); (c) BCl_3, CH_2Cl_2, −78 °C (89%); (d) CH_2N_2, Et_2O (93%).

To examine binding[23] and antagonist[24] activities of optically active hordatines and aperidines, all stereoisomers of **1** and **2** were synthesized utilizing optically active **4**. In regards to binding activities to the α_{1A} adrenoceptor, (2*S*,3*S*)-(+)-**1** and (2*R*,3*R*)-(−)-**1** exhibited IC_{50} values of 0.31 μg/mL and 0.29 μg/mL, respectively. Hence, the stereochemistry on the dihydrobenzofuran ring of **1** does not seem to be essential for binding to the receptor. On the other hand, the relationship between optical isomers of **2** was different. Synthetic (2*S*,3*R*)-(−)-**2** and (2*R*,3*S*)-(+)-**2** exhibited IC_{50} values of 2.44 μg/mL and 0.42 μg/mL, respectively. Interestingly, these findings indicate unnatural isomers of **1** and **2** are more potent than the corresponding natural isomers. Among all four stereoisomers of **1**, (2*R*,3*S*)-**2** exhibits the most potent antagonistic activity at the α_{1A} adrenoceptor. It is likely that the *R* configuration at the C-2 might be suitable for binding to receptors (Table 1).

Past structure–activity relationship studies of ligands for the α_{1A} adrenoceptor supports the hypothesis that the cationic functional group is essential for binding to this receptor.[25] Hordatine A also has a guanidino group as a cationic function. However, there are two identical functionalities on the molecule, one is located on the C-4 agmatine side chain and another is C-4′ agmatine. We hypothesized that only one moiety is critical for binding, and the other is unnecessary. In order to clarify this, we prepared the two types of hordatine-related compounds by a selective reaction on each carboxylic acid of dehydrodi-*p*-coumaric acid. Methyl benzofurancarboxylate derivative (**13**), which lacked the C-4 agmatine side chain of **1**, was synthesized from monomethylester prepared by selective methylation of the C-4 carboxylic acid. On the other hand, benzofurancarboxamide derivative (**14**), which lacked a C-4′ agmatine side chain, was prepared from **4**. Moreover, *p*-coumaroylagmatine (**15**) was synthesized by condensation between *p*-coumaric acid and *N*,*N*′,*N*″,*N*‴-tetra-Boc-agmatine (Fig. 5).

Table 1
Activities of hordatine derivatives against α_1 receptor (IC_{50})

	Binding activity (mM)	Antagonistic activity (mM)
(2*S*,3*S*)-**1**	0.31	19.3
(2*R*,3*R*)-**1**	0.29	14.3
(2*R*,3*S*)-**2**	0.42	4.90
(2*S*,3*R*)-**2**	2.44	17.7
(2*S*,3*S*)-**13**	2.16	42.2
(2*R*,3*R*)-**13**	0.56	9.72
(2*S*,3*S*)-**14**	>100	>100
(2*R*,3*R*)-**14**	>100	>100
15	57.6	>100

Compound **13** exhibited binding activity to the α_{1A} adrenoceptor. The IC_{50} values for (2*S*,3*S*)-**13** and (2*R*,3*R*)-**13** were 2.16 and 0.56 μM, respectively. On the other hand, compound **14** was inactive, while **15** showed only weak activity. The antagonistic activity of each compound was clearly associated with its binding ability to the α_{1A} adrenoceptor. In particular, (2*R*,3*R*)-**13** exhibited a more potent antagonist activity than that of **1** at a lower concentration (IC_{50} = 9.72 μM). The assay results for these molecules are clear-cut as only compound **13** is active for both binding and the antagonist test, while **14** is inactive. These data indicate that the C-4′ agmatine unit is essential for activity against the α_{1A} adrenoceptor. Moreover, upon comparing optical isomers, (2*R*,3*R*)-**13** exhibits the most potent activity. Interestingly, the antagonistic activity of (2*R*,3*R*)-**13** is more potent than natural **1**. Furthermore, the weak activity of coumaroylagmatine (**15**) suggests that the dihydrobenzofuran ring of **1** is also important Table 1.

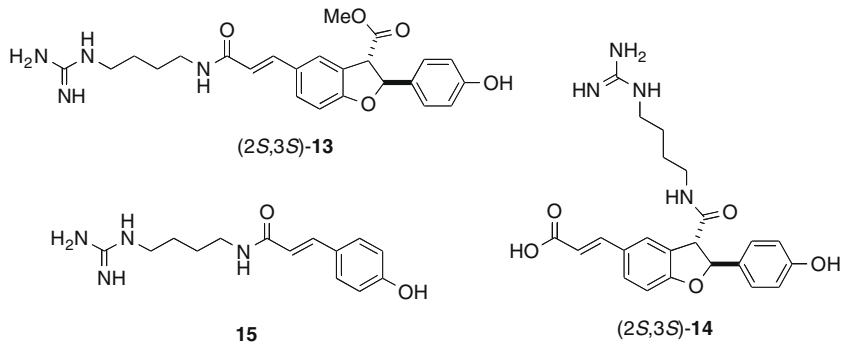

Figure 5. Synthetic hordatin derivatives.

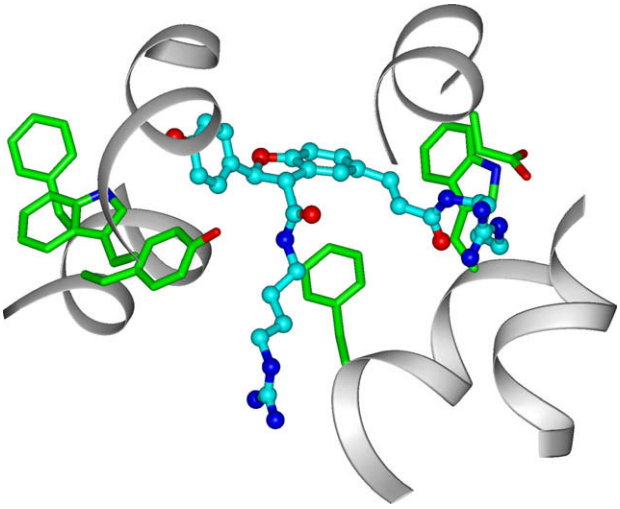

Figure 6. Complex model of (*R*,*R*)-hordatine A at the binding cleft of α_1 receptor models.

To confirm the above results, hordatine A was docked into a α_{1A} adrenoceptor model (Fig. 6), which was constructed by homology modeling using an antagonist-bound form of GPCR as the template.[26] We assumed the cationic guanidino group interacted with the carboxylate group of Asp148 in TM3 through an ionic bond. Hence, the conformation of **1** was minimized and subjected to manual docking in order to yield reasonable interactions with the cavity of the receptor binding site calculated and visualized by the Binding-Site module installed in Insight II. The docking experiment assumed a salt bridge between the carboxylate group of Asp148 and C-4 agmatine side chain was not a reasonable interaction due to the results of structural optimization with molecular dynamics and energy minimization using Discover 3. On the other hand, the docking model considering the C-4′ agmatine side chain as a cationic center displayed a reasonable conformation of **1** in the receptor model. In this model, the other agmatine side chain did not significantly interact with any residues of the receptor. These results are in good agreement with our SAR data.

In summary, we disclosed the absolute structures of **1** and **2** utilizing the synthesis of all possible stereoisomers of **1**. Moreover, five additional related compounds were also prepared for the structure–activity relationship study. The resulting assay data demonstrated that the guanidino group on C-4′ agmatine side chain is critical for the affinity to the receptor, which was also supported by the docking study. The asymmetric total synthesis and development of a subtype-selective antagonist based on these molecules are currently under investigation in our laboratory.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.068.

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23. *Assay of α_{1A} adrenoceptor binding activity*: Test samples were added to a suspension of a homogenate of rat salivary gland containing the α_1 adrenoceptor in the presence of 0.06 nM [^{3}H]-prazosin, and the mixture was incubated at 22 °C for 60 min. The reaction was terminated by vacuum filtering through glass-fiber filters (GF/B; Perkin Elmer, Wellesley, MA), which were individually washed several times with 50 mM Tris–HCl. The radioactivity remaining on the filter was counted in a liquid scintillation cocktail (MicroScint-O, Perkin Elmer) using a TopCount (Perkin Elmer) liquid scintillation counter. The specific binding of [^{3}H]-prazosin was calculated by subtracting the nonspecific binding in the presence of 10 μM of phentolamine from the total binding of [^{3}H]-prazosin. IC_{50} values were obtained as the concentration that competitively inhibited the binding of [^{3}H]-prazosin to the α_1 adrenoceptor at 50% of maximal binding.
24. *Assay of α_{1A} adrenoceptor antagonist activity*: Rings of rat caudal artery denuded of endothelium were suspended in 20 mL organ baths containing an oxygenated (95% O_2 and 5% of CO_2) and pre-warmed (37 °C) physiological salt solution with the following composition: 118.0 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 25.0 mM $NaHCO_3$, and 11.0 mM glucose (pH 7.4). Yohimbine (1 μM), propranolol (1 μM), pyrilamine (1 μM), atropine (1 μM), and methysergide (1 μM) were also present in assay incubates to block the α_2 adrenergic, β-adrenergic, histamine H_1, muscarinic and 5-HT_2 receptors, respectively. The tissues were connected to force transducers for isometric tension recordings, stretched to a resting tension of 1 g, and then allowed to equilibrate for 30 to 60 min. During the equilibration, the tissues were washed repeatedly and the tension readjusted. The tissues were exposed to a submaximal concentration of the reference agonist phenylephrine (10 μM) to obtain a control contractile response. After stabilization of the phenylephrine-induced response, the concentration of the test sample was increased or the reference antagonist prazosin was added cumulatively. At each concentration, the tissues remained in contact with the solution until a stable response was obtained or for a maximum of 15 min. Inhibition of the phenylephrine-induced response by a test sample indicated antagonist activity at the α_{1A} adrenergic receptors.
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