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Unprecedented olefin-dependent histidine–kinase inhibitory of zerumbone ring-opening material

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Abstract—Zerumbone ring-opening derivative, **4** (10*E*/10*Z* = 3/2), inhibited autophosphorylation of the essential histidine–kinase YycG existing in *Bacillus subtilis* constituting a two-component system (TCS). Generation of 4*E*-form could be regulated chemically using the difference from the ring-opening reactivity of the precursor forming of **4** and pure **4*E*** was isolated. The stereoisomer, **4*E***, showed main inhibition activity of autophosphorylation of YycG (IC_{50} = 63.5 μM).

1. Introduction

Zerumbone **1**, determined structure by Dev,[1] is a monocyclic sesquiterpene found as the major component of the essential oil of the wild ginger *Zingiber zerumbet* Smith. Not only does zerumbone show attractive reactivity,[2] but also zerumbone[3] and its derivatives[4] have a broad array of biological activities.

In our previous reports, one of the ring-cleavage compounds of zerumbone, **4**, obtained by treatment of dibromozerumbone with potassium hydroxide, showed the inhibition of the autophosphorylation of the essential histidine protein kinase (HPKs), YycG[4] and the growth of Gram-positive bacteria specifically.[3d] HPKs, one of the major components in the two-component regulatory system, plays a key role in prokaryotic signal transduction to various environmental stresses. One of the two-component signal transduction systems consists of a histidine kinase YycG and its cognate response regulator YycF. Recently, the YycG–YycF system was found in *Bacillus subtilis*,[5] *Staphylococcus aureus*,[6] and *Streptococcus pneumoniae*[7] YycG–YycF is also conserved among other Gram-positive pathogen bacteria and is considered a novel target for antibacterial agents. Zerumbone derivative **4** has a geometrical isomer of *E*- and *Z*-forms (the ratio of 3:2 by ^{1}H NMR) at C10 and inhibited the incorporation of phosphate from ATP into YycG at 500 μg/mL, with a one-half maximal inhibitory concentration (IC_{50}) of 750 μM. However, **4** had antibacterial activity in *B. subtilis* with MIC 62.5 μg/mL. Interestingly, **4** showed growth inhibition of Gram-positive bacterium, *B. subtilis*, but did not show any effect on Gram-negative bacterium, *E. coli* MC4100. The substantial result might show the relationship of inhibition effect between the two-component system and the growth of bacteria. Moreover, a good inhibitor of those might be developed as a quite substantial medicine against drug resistant bacteria. Therefore, it is a very important research task to explore this field for the development of new medicines, in addition to establish a green chemistry design for development of zerumbone chemistry.

If it will have a certain influence on TCS regulation, even if the structural changes of inhibitors are few, even the smallest phenomenon must not be overlooked. We believe that this result serves as a driving force of development of a brand-new next generation type antibiotics.

We report here the isolation method for only the **4*E*** isomer, which was established using different reactivities of

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dibromozerumbone **2** with potassium hydroxide. In addition, the **4*E*** isomer showed the main potential inhibition of the autophosphorylation of YycG.

2. Results and discussion

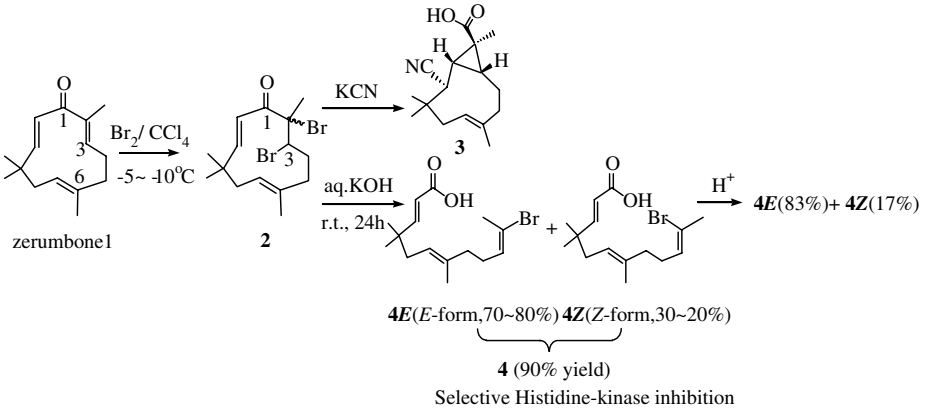
As shown in Scheme 1, we have discovered a remarkable double Favorskii rearrangement. The intramolecular displacement sequence was initiated by a conjugate addition of cyanide to dibromozerumbone **2**. This was followed by a ring cleavage for 24 h to give cyclopropanecarboxylic acid **3**.[3b] Surprisingly, regioselective fragmentation at the C1–C2 bond in **1** was achieved by treatment of **2** with aqueous potassium hydroxide. This afforded **4** in 90% yield without generating **3** regardless of their approximate equivalent nucleophilicities.[3d] Although **4*E*** and **4*Z*** have three olefins in the structure and C2 and C6 positions showed the *E*-form having same geometrical structure as starting material, the double bond at C10 showed the geometrically mixtures (10*E* (**4*E***)/10*Z* (**4*Z***) = 3/2) using the previous reaction condition.

In attempt to evaluate the autophosphorylated inhibition of YycG with isolated **4*E*** or **4*Z***, reaction condition to isolate either or both of the compounds were studied since the isolation using chromatographical analysis was very hard. Since the ratio of the mixture was changed to 83/17 (**4*E*/4*Z***) by equilibration of C10 olefin under acidic condition, it is impossible to isolate **4*E*** using thermodynamic equilibration.

As shown in Table 1, the conditions of bromination and its ring-cleavage reactions were studied. The ratio of geometrical isomer of **4** is directly reflected by the ratio of diastereomers of **2** from the viewpoint of the stereochemistry. As shown in run 5, **2** (*trans*, 85%) was treated with potassium hydroxide at room temperature. The ratio of **4*E*** and **4*Z*** resulted in the same ratio with that of **2**. Under the condition using aqueous potassium bromide system as the bromine source, the proportion of *trans*-form of **2** was hardly changed even if the reaction temperature was changed. Since the diastereomeric ratio of **2** was maintained with bromine addition to zerumbone at various temperature conditions in the heterogeneous aqueous system, the relationship between bromine addition of **2** and the ratio of geometric isomers of **4** was then investigated under cool conditions in various organic solvents. Interestingly, **4** of 92% *E*-form was obtained in high stereoselectively using a hydrophobic solvent such as only CCl_4 in run 4. This suggests that stereoselectivity of bromination to **1** depends on the circumstances of solvent and temperature. However, the stereochemistry was not regulated perfectly.

As shown in Scheme 2, the ring cleavage of **2** was investigated to regulate the stereochemistry. *trans*-Form (82%) of **2** was treated with potassium hydroxide at room temperature for short time. After 5 min, **4a** constituted by **4*Z*** as a main component was slightly generated.

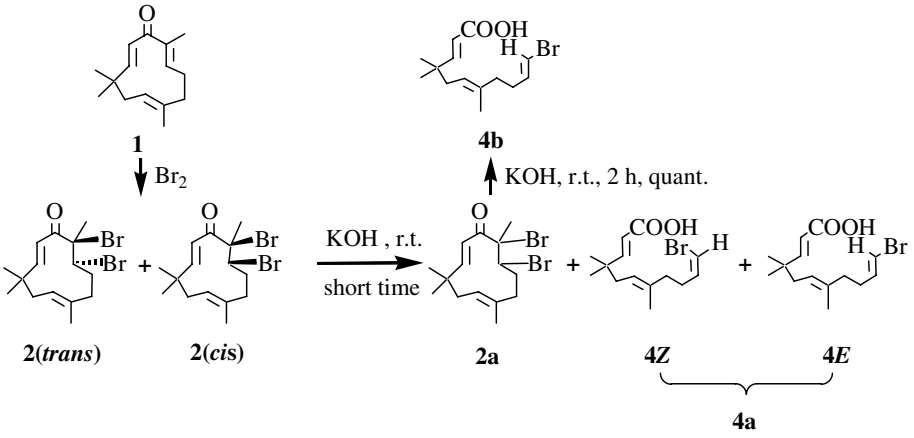
As shown in Table 2, the *trans*-ratio of remained **2a** increased following ring cleavage with potassium hydroxide to afford **4b** of 94% *E*-form. When *trans*-form (82%) of **2** was reacted with potassium hydroxide at room temperature for 60 min, **2a** of over 99% *trans*-form was obtained. Furthermore, after ring cleavage of **2a** with potassium hydroxide **4b** of over 99% *E*-form was



Scheme 1.

Table 1. Various condition of bromination of zerumbone and its ring-cleavage reaction

Run	Br_2 Source	Solvent	Temperature (°C)	**2** (*trans*%)	**4*E*** (%)	**4*Z*** (%)
1	Br_2	CH_3CN	−35 to −40		81	19
2	Br_2	Toluene	−60		83	17
3	Br_2	CH_2C1_2	−25 to −30		81	19
4	Br_2	$CC1_4$	−20 to −25		92	8
5	$5KBr + KBrO_3 + 3H_2SO_4$	$CC1_4$	RT	85	86	14
6	$5KBr + KBrO_3 + 3H_2SO_4$	$CC1_4$	4	84		



Scheme 2.

Table 2. Stereochemistry of **2a**, **4a**, and **4b**

Time (min)	**2a**, Yield (%), *trans*/*cis*	**4a** (**4*E*/4*Z***)	**4b** (**4*E*/4*Z***)
5	80, 94/6	2/98	94/6
30	65, —	58/42	95/5
60	>47, 99/<1	64/36	>99/<1

achieved. The reason for this result might be that the *cis*-form of **2a**, which is more thermodynamically unstable was cleaved on the ring system faster the *trans*-form.

As shown in Figure 1, the ratio of **4*E*** to **4*Z*** was calculated by ^{1}H NMR using the integration value at the C10 position with the chemical shift around 5.6 ppm. The various stereoisomers of **4** were tested for the inhibition of the autophosphorylation of YycG (HPK). The purified YycG was contained in 50 mM Tris–HCl (pH 7.5), 50 mM KCl, and 10 mM $MgCl_2$ and the mixture was incubated for 10 min at room temperature.

The reaction was stopped by an equal volume of 2× sample buffer (120 mM Tris–HCl, pH 6.8, 20% glycerol, 4% SDS, 10% β-mercaptoethanol, 0.1% BPB), and then analyzed on an SDS–polyacrylamide gel. Consequently,

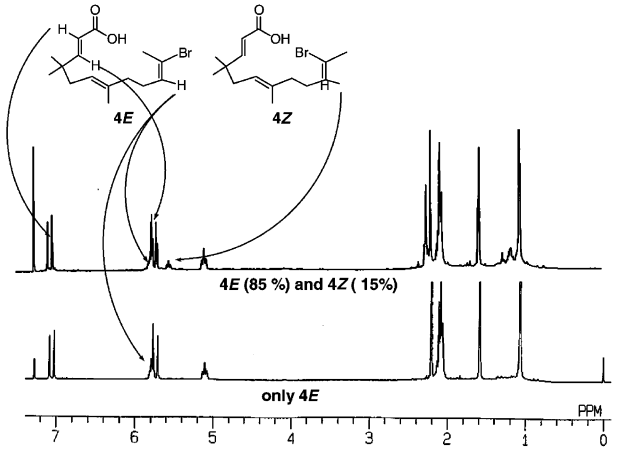


Figure 1. ^{1}H NMR spectrum of **4*E*** (85%) and **4*E*** (100%).

Table 3. Various ratio of **4*E*** and **4*Z***

4*Z*/4*Z*	IC_{50} (μM)[a]
100/0	63.5
95/5	159
94/6	270
93/7	270
84/16	508
65/35	730

[a] For autophosphorylation of YycG.

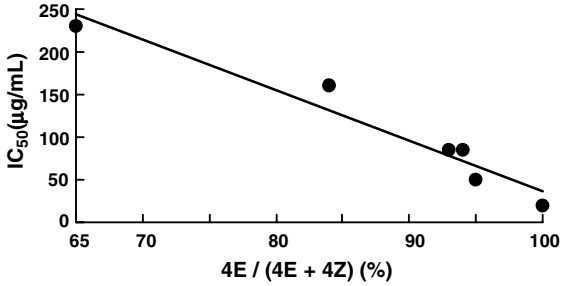


Figure 2. Relationship between histidine–kinase inhibition and the proportion of **4*E*** and **4*Z***.

the inhibition intensity of autophosphorylation of YycG was affected significantly according to the difference of stereochemistry at the C10 olefin. The concentration of the *E*-form was proportional to the IC_{50} for YycG with the maximum inhibition (IC_{50} = 63.5 μM) obtained from the *E*-form independently (Table 3 and Fig. 2). Excitingly, the slight difference of stereochemistry between geometrical isomers on the end olefin in the long carbon chain showed significant inhibition activity of autophosphorylation of essential HPK, YycG. This gives significant information for the search of inhibitors of HPK.

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