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Isolation of coniferyl esters from *Capsicum baccatum* L., and their enzymatic preparation and agonist activity for TRPV1

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Abstract

Coniferyl esters—capsiconiate and dihydrocapsiconiate—were isolated from the fruits of the pepper, *Capsicum baccatum* L. var. *praetermissum*. Their structures were determined by spectroscopic methods to be coniferyl (*E*)-8-methyl-6-nonenoate (capsiconiate) and coniferyl 8-methylnonanoate (dihydrocapsiconiate). This finding was further confirmed by the lipase-catalyzed condensation of coniferyl alcohol with its corresponding fatty acid derivative. The agonist activity of the esters for transient receptor potential vanilloid 1 (TRPV1) was evaluated by conducting an analysis of the intracellular calcium concentrations in TRPV1-expressing HEK293 cells. The EC_{50} values of capsiconiate and dihydrocapsiconiate were 3.2 and 4.2 μM, respectively.

Keywords: *Capsicum baccatum* L. var. *praetermissum*; Solanaceae; Coniferyl ester; Capsiconiate; Dihydrocapsiconiate; Spectroscopic analysis; Lipase-catalyzed preparation; Transient receptor potential vanilloid 1 (TRPV1); Calcium influx

1. Introduction

Hot red pepper (*Capsicum*) is widely used as a spicy seasoning and in medicinal materials worldwide. The pungency of the fruits of red pepper is caused by a lipophilic alkaloid capsaicin **4** (Fig. 1) and its analogs, termed capsaicinoids. The fundamental structure of capsaicinoids is that of an acid amide of vanillylamine with a fatty acid. Numerous reports on the chemical, biological, and physiological properties of capsaicinoids have been published; in particular, studies on the use of capsaicinoids as a medicinal target have increased tremendously since the cloning of a capsaicin receptor from mammalian sensory nerves (Caterina et al., 1997). Nowadays, the capsaicin receptor is termed as transient receptor potential vanilloid type 1 (TRPV1); it is a calcium permeable, non-selective cation channel. The activation of TRPV1 is considered to contribute to the various physiological activities of capsaicin (Szallasi, 2002).

We previously reported a novel compound group named capsinoids as an ingredient of the fruits of a non-pungent cultivar of *Capsicum annuum* L., 'CH-19 Sweet' (Kobata et al., 1998). The basic structure of capsinoids comprises an ester of vanillyl alcohol with a fatty acid, and the chemical structure of capsiate (**3**)—the major component of capsinoids—is as shown in Fig. 1. Interestingly, it was found that the TRPV1 agonist potential of capsiate was comparable to that of capsaicin despite its pungency being considerably lower than that of capsaicin (Iida et al., 2003). Recently, several reports on the TRPV1 related activities of capsiate (**3**) such as enhancement of thermogenesis and endurance capacity were published (Ohnuki et al., 2001; Haramizu et al., 2006). Furthermore, the potencies of cap-

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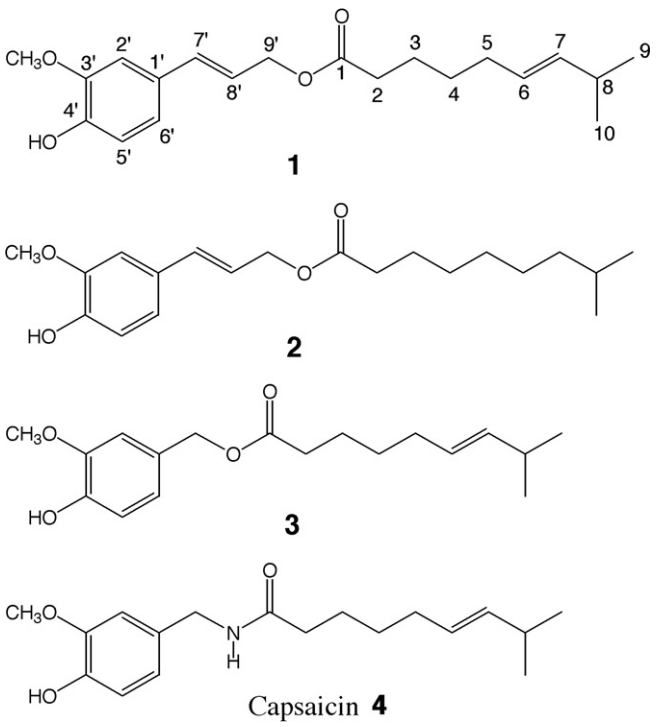

Fig. 1. Chemical structures of capsiconiate (**1**), dihydrocapsiconiate (**2**), capsiate (**3**) and capsaicin (**4**).

sinoids in apoptosis induction (Macho et al., 2003a) and as anticancer (Macho et al., 2003b), antioxidant (Rosa et al., 2002), immunosuppressive, and anti-inflammatory (Sancho et al., 2002) agents were reported. Therefore, "non-pungent esters" such as capsinoids and "amides" such as capsaicinoids will probably be attractive targets for pharmaceutical studies.

In the course of the analyses of capsaicinoids and capsinoids in several varieties of pepper, we discovered the existence of two unknown compounds **1** and **2** in a kind of variety of *Capsicum baccatum*. In the present paper, we have described the isolation of **1** and **2** from the fruits of *C. baccatum* L. var. *praetermissum*, and the structural determination of these compounds was performed by spectroscopic methods such as HR-FABMS, IR, UV, and ^{1}H and ^{13}C NMR spectroscopy. Furthermore, the structure of **1** and **2** was confirmed by their enzymatic preparation and analyses. Finally, the agonist potency of **1** and **2** for TRPV1 was evaluated by measuring the intracellular calcium concentration in TRPV1-expressing mammalian cells.

2. Results and discussion

2.1. Structural determination of capsiconiate (1) and dihydrocapsiconiate (2)

The IR spectrum of **1** was similar to that of capsiate (**3**) (Kobata et al., 1998). Therefore, the structure of **1** was considered to be closely related to that of **3**. The coupling constants and patterns of the three aromatic protons in the ^{1}H NMR spectrum of **1** indicated presence of the typical 1-, 2-, and 4-substituted phenyl group. A methoxy group attached to the phenyl group was observed in the ^{1}H and ^{13}C NMR spectra. In the ^{1}H NMR spectroscopy data, characteristic signals for an isopropyl group were observed. The presence of an ethylenic moiety of *trans* configuration was also indicated from the ^{1}H NMR data. Thus, the NMR data of **1** was extremely similar to those of **3** (Kobata et al., 1998). These results suggested that the structure of **1** contains a partial structure of **3**.

The molecular formula of **1** was estimated by the HR-FABMS measurement to be $C_{20}H_{28}O_4$. This molecular formula indicated an additional C_2H_2 group in **3**. The ^{13}C NMR spectrum of **1** also suggested the presence of the additional two olefinic carbons when compared to that of **3**. The ^{1}H NMR spectrum of **1** exhibited 2 olefinic methine protons at δ 6.57 *d* and 6.14 *dt*; the coupling constant 15.6 Hz indicated the trans configuration of the protons. One of the olefinic methine protons (δ 6.14) was coupled with the protons of an oxygen-attached methylene group observed at δ 4.71. On the other hand, the former methine was considered to be neighboring an aromatic ring because its chemical shift values as observed in the ^{1}H and ^{13}C NMR spectroscopy were high. These ^{1}H NMR signals that indicated the typical AMX_2 pattern suggested the existence of the moiety, Ar–CH=CH–CH_2–O–, in the structure of **1**. Therefore, a part of the structure of **1** was considered to be an ester of 3-(4-hydroxy-3-methoxyphenyl)-2(*E*)-propenyl, i.e., a coniferyl ester. The existence of the coniferyl group in **1** was supported by UV absorption at 297 nm.

From the spectroscopic data, we estimated the structure of **1** to be coniferyl (*E*)-8-methyl-6-nonenoate as shown in Fig. 1.

The HR-FABMS analysis elucidated the molecular formula of **2** to be $C_{20}H_{30}O_4$, which indicated the dihydrogenation of **1**. The IR and UV spectra of **2** were similar to those of **1**. The NMR spectrum of **2** was also similar to that of **1**. However, 2 aliphatic methylene carbons (δ 27.2 and 38.9) instead of the 2 olefinic methine carbons detected in **1** (C-6 and C-7) were observed in the ^{13}C NMR spectrum. Further, the ^{1}H NMR spectrum of **2** exhibited none of the olefinic proton signals observed in **1**. These results suggested the structure of **2** to be a 6,7-dihydro derivative of **1**, that is, coniferyl 8-methylnonanoate, as shown in Fig. 1.

In order to confirm the structures of **1** and **2**, they were enzymatically prepared by the lipase-catalyzed esterification or transesterification of corresponding fatty acid derivatives with coniferyl alcohol. The transesterification of methyl (*E*)-8-methyl-6-nonenoate derived from the methanolysis of capsaicin with coniferyl alcohol yielded a compound with the estimated structure of **1** in 13.1% yield. The esterification of 8-methylnonanoic acid with coniferyl alcohol yielded a compound with the estimated structure of **2** in 22.5% yield. The spectral data of the enzymatically prepared compounds completely coincided with those of the naturally occurring compounds mentioned above. There-