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Structures of the radical (DPPH) oxidation products of dihydrocapsaicin

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Abstract—Dihydrocapsaicin was oxidized with 2,2-diphenyl-1-picrylhydrazyl (DPPH), a free radical, and the products were analyzed by spectroscopic means. © 2002 Elsevier Science Ltd. All rights reserved.

The pungent principal of hot pepper has long been used as an ingredient of spices all over the world. Capsaicin (**1**) and dihydrocapsaicin (**2**) are the main components of hot pepper, constituting more than 90% of the total pungent principle.[1] The capsaicins are known to possess potent antioxidative activity. It has been reported that the oxidation of oleic acid at cooking temperatures was inhibited by the presence of capsaicin.[2] The antioxidation effect of capsaicin is also discussed.[3] Recently, Kogure et al. exhibited that the radical scavenging activity of capsaicins was more potent[4] than that of tocopherol by the experiments using 2,2-diphenyl-1-picrylhydrazyl [DPPH: (Ph_2N-N(·)-2,4,6-trinitrophenyl)], a free radical, although the oxidation course of capsaicins was not clarified. Detection of a capsaicin dimer by HPLC and MS/MS in the oxidation product of **1** has been reported.[3] The objective of the present study is to identify the radical oxidation products of dihydrocapsaicin by means of spectroscopic analyses and to deduce the antioxidation process of capsaicinoid on the structural basis of the products (Fig. 1). Dihydrocapsaicin (**2**) was preferred to capsaicin (**1**), because (i) **1** and **2** have the same biological activity[5] and (ii) the absence of the olefinic bond, which might be attacked by a radical, on the side-chain of **2** will simplify the composition of the oxidation product.

Dihydrocapsaicin (**2**) was treated with 2 equivalents[4] of DPPH in chloroform at room temperature for 1 h. After the reaction was completed, the solvent was evaporated to dryness. The residue was then subjected to ^{1}H NMR analysis. The spectrum showed that the signals due to the aromatic (δ 6.75–6.85), benzyl (δ 4.34), and methoxy (δ 3.87) protons of **2** remarkably decreased in comparison with the side chain proton signals. This indicates that the 4-hydroxy-3-methoxyphenyl-

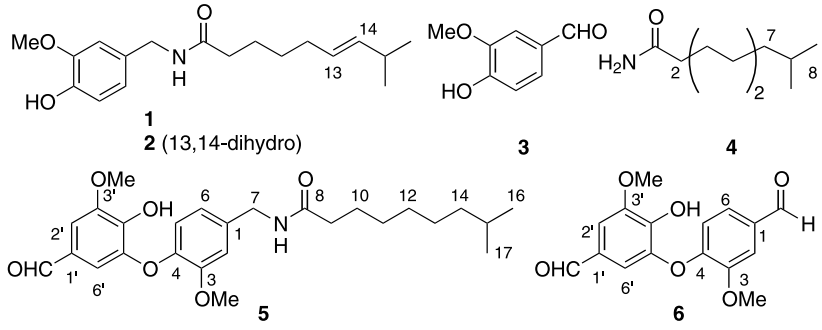

Figure 1. Chemical structures of capsaicin (**1**), dihydrocapsaicin (**2**) and the radical oxidation products (**3**, **4**, **5** and **6**) isolated in the present experiments.

Keywords: capsaicin; dihydrocapsaicin; oxidation; radical scavenger.

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methylene moiety of **2** underwent decomposition and the product possessing the 8-methylnonyl group might be the main radical oxidation product. The major signals apparently resemble those of 8-methylnonanamide (**4**), which was actually isolated from the reaction mixture (vide infra), although the recovery of **4** by silica gel chromatography was poorer than expected possibly because of its high polarity and partial adsorption on silica gel.

Chromatographic separation of the reaction mixture afforded unreacted **2** (13%) and compounds **3** (10% based on consumed **2**), **4** (26%), **5** (10%) and **6** (2%), together with DPPH-H (~100%).

Compound **3** was identified as vanillin by comparison of its NMR properties and R_f value on the TLC with those of the authentic sample.

The ^1H and ^{13}C NMR properties of compound **4**[6] (HRMS: calcd for $C_{10}H_{21}NO$ 171.1623, found m/z 171.1628) were very close to those of **2** except for the signals of vanillyl moiety. The ^1H NMR spectrum showed a broad signal (2H) at δ 5.38 due to -$CONH_2$ and the ^{13}C NMR spectrum showed a singlet at δ 175.3 ascribable to an amide carbonyl group. These data indicated that **4** was 8-methylnonanamide, the side-chain part of **2**.

The molecular formula of compound **5**[6] (HRMS: calcd for $C_{26}H_{35}NO_6$ 457.2464, found m/z 457.2491) indicated that **5** was an oxidative coupling product of dihydrocapsaicin with vanillin. It's ^1H NMR spectrum appeared to be the combination of the spectra of **2** and **3**, except for the signals of aromatic region. To confirm the oxidative coupling site, the coupling patterns of the aromatic proton signals were analyzed (Fig. 2(a)) with the help of 2D NMR techniques. Signals **a** and **c** were assigned as 2′ and 6′, respectively, on the basis of the facts that they were *meta*-coupled by 1.6 Hz and that C-2′ and 6′ [assignable by HSQC] showed correlation peaks ($^3J_{H,C}$) with the aldehyde proton in the HMBC spectrum. Proton **a** (H-2′) was distinguishable from **c** (H-6′) because the former showed an NOE [NOESY] with the adjacent methoxy protons. Protons **e** (H-6) and **d** (H-2) are *meta*-coupled by 1.6 Hz, the former at the same time being coupled with **b** (H-5) by 8.0 Hz. In the HMBC spectrum, the carbons (C-3 and C-3′) have correlations with adjacent methoxy protons, and **d** (H-2) and **e** (H-6) show cross peaks with the benzyl protons. Two methoxy protons were distinguished by the NOE with protons **a** (H-2′) and **d** (H-2), respectively. An NOE was observed between protons **b** (C-5) and **c** (C-6′) (NOESY spectrum), which ensures that dihydrocapsaicin and vanillin were coupled at 4 and 5′ positions, as shown in structure **5**.

The molecular formula of compound **6**[6] (HRMS: calcd for $C_{16}H_{14}O_6$ 302.0790, found m/z 302.0766) suggested that **6** was an oxidative self-coupling product of vanillin ($C_8H_8O_3$). It's ^1H NMR spectrum showed two aldehyde signals at δ 9.75 (1H, s) and 9.90 (1H, s), two methoxy signals at δ 3.96 (3H, s) and 4.01 (3H, s), a phenolic hydroxyl signal at δ 6.33 (1H, s), and aromatic proton signals exhibited in Fig. 2(b). Signals **c** and **d** were assigned as H-2′ and 6′, respectively, by their mutual *meta*-coupling (1.6 Hz) and the HMBC correlations of C-2′ and 6′ (assignable by HSQC) from an aldehyde proton (δ 9.75). Proton **c** was distinguished from **d** by an NOE from the adjacent methoxy group (δ 4.01).

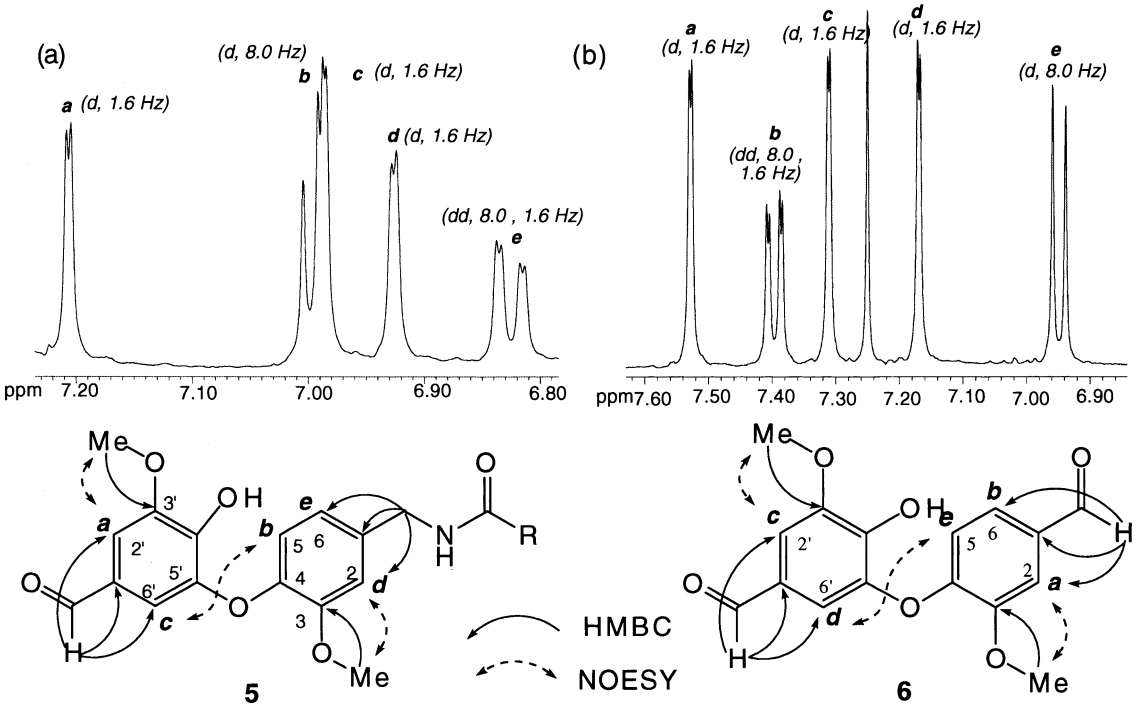

Figure 2. The patterns of the aromatic proton signals and 2D correlations (HMBC and NOESY) found for **5** (a) and **6** (b) ($CDCl_3$, 400 MHz). Multiplicities and coupling constants (J in Hz) in parentheses.

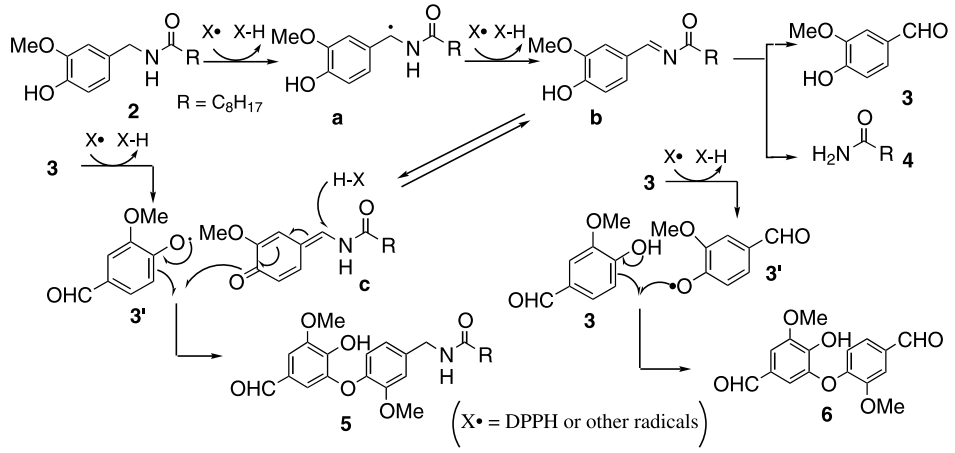

Figure 3. A presumed mechanism of oxidation of dihydrocapsaicin producing **3**, **4**, **5** and **6**.

Similarly, signals **a** and **b** were assigned as H-2 (d, 1.6 Hz) and 6 (dd, 8.0, 1.6 Hz), respectively, by their coupling patterns and the HMBC correlations of C-2 and 6 from the aldehyde proton (δ 9.90). Proton **e** was *ortho*-coupled with **b** by 8.0 Hz. Two methoxy protons were distinguished by the NOE with protons **a** and **c**. An NOE was observed between **d** (H-6′) and **e** (H-5) in the NOESY spectrum.

Based on the structures of the products, **3**, **4**, **5**, and **6**, formed by the DPPH oxidation of **2**, we assumed a plausible mechanism leading to the products, as shown in Fig. 3. The DPPH radical attacks the benzyl hydrogen of **2** forming a benzyl radical (**a**). By the action of another DPPH that abstracts a hydrogen of NH, an immino ketone (**b**), which tautomerizes with a methylene quinone (**c**) may be formed. The C–N bond is hydrolytically cleaved by water present in the solvent to give **3** and **4**. The resulting vanillin (**3**) is converted to radical **3′** by DPPH. This radical reacts with **c** and **3** to afford **5** and **6**, respectively.

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6. ^{1}H (400 MHz: *J* in Hz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ solutions. **4**: ^{1}H δ 0.85 (6H, d, *J* 6.8; Me′s on C-8), 1.09–1.19 (2H, m), 1.19–1.39 (6H, m), 1.50 (1H, m; H-8), 1.63 (2H, quint, *J* 7.6; 3), 2.21 (2H, t, *J* 7.6; 2), 5.38 (2H, brs; -NH_2). ^{13}C δ 22.5 (q×2), 25.4 (t), 27.1 (t), 27.8 (d), 29.2 (t), 29.5 (t), 35.8 (t), 38.8 (t), 175.3 (s). **5**: ^{1}H δ 0.84 (6H, d, *J* 6.4; H-16, 17), 1.06–1.18 (m; 14), 1.18–1.39 (m; 11, 12, 13), 1.49 (m; 15), 1.66 (quint, *J* 7.6; 10), 2.22 (t, *J* 7.6; 9), 3.82 (s, 3-OMe), 3.98 (s, 3′-OMe), 4.43 (d, *J* 6.0; 7), 5.73 (brs; NH), 6.48 (s; OH), 6.83 (dd, *J* 8.0, 1.6; 6), 6.93 (d, *J* 1.6; 2), 6.99 (d, *J* 1.6; 6′), 7.00 (d, *J* 8.0; 5), 7.20 (d, *J* 1.6; 2′), 9.70 (s; CHO). ^{13}C δ 22.5 (C-16, 17), 25.7 (10), 27.1 (12 or 13), 29.3 (11), 27.8 (15), 29.5 (13 or 12), 36.7 (9), 38.8 (14), 43.2 (7), 55.9 (3-OMe), 56.4 (3′-OMe), 106.5 (2′), 112.4 (2), 113.6 (6′), 120.3 (6), 120.8 (5), 128.3 (1′), 136.1 (1), 142.3 (4′), 143.8 (4), 145.2 (5′), 148.2 (3′), 151.0 (3), 172.9 (8), 190.3 (CHO). **6**: ^{1}H δ 3.96 (s; 3-OMe), 4.01 (s; 3′-OMe), 6.33 (s; OH), 6.94 (d, *J* 8.0; 5), 7.17 (d, *J* 1.6; 6′), 7.31 (d, *J* 1.6; 2′), 7.40 (dd, *J* 8.0, 1.6; 6), 7.53 (d, *J* 1.6; 2), 9.75 (s; 1′-CHO), 9.90 (s; 1-CHO). ^{13}C δ 56.1 (3-OMe), 56.5 (3′-OMe), 106.6 (2′), 110.7 (2), 116.8 (6′), 117.3 (5), 125.5 (6), 128.6 (1′), 132.7 (1), 142.4 (5′), 143.0 (4′), 148.3 (3′), 150.5 (3), 150.9 (4), 189.8 (1′-CHO), 190.5 (1-CHO).