Synthesis and elucidation of deuterated vanillylamine hydrochloride and capsaicin

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Capsaicin is the major pungent component of hot peppers, which belong to the plant genus *Capsicum*. Although the biosynthesis of capsaicin is known to involve the condensation of vanillylamine and 8-methylnonenoic acid by capsaicin synthase, the mechanism of biosynthesis is still not fully understood. In this study, deuterium labelled versions of capsaicin and the precursor vanillylamine were synthesized in order to investigate the biosynthesis of capsaicin in hot peppers.

Keywords: [^{2}H$_3$]Capsaicin; synthesis; biosynthetic pathway; hot pepper

Introduction

Capsaicin, *trans*-8-methyl-*N*-vanillyl-6-nonenamide, is the major pungent component in hot peppers. It is an irritant for mammals, including humans, and produces a burning sensation in tissue with which it comes into contact. Capsaicin and several related compounds are called capsaicinoids and are produced as secondary metabolites by the plant genus *Capsicum*. Hot peppers are widely used as a food additive in many East Asian and Latin-American countries and are known to be rich in vitamin C.[1] Besides its use as a food additive, the medicinal and pharmacological properties of capsaicin have been the focus of recent attention. It has been reported that capsaicin has anticancer effects on several cancer cell lines and can provide relief in arthritis and respiratory ailments.[1,2] Capsaicin is currently used as an ingredient of a muscle pain relieving drug and is also used for the treatment of gastric ulcers and rheumatism.[2] As interest in the biological activity of capsaicin has increased, the need for an understanding of its biosynthesis has become evident. It has been reported that capsaicin synthase (CS) is responsible for the condensation reaction between vanillylamine and 8-methyl-nonenoic acid, leading to capsaicin production.[2–4] However, the biosynthetic pathway is not fully understood. To investigate this pathway, methods for the synthesis of isotopically labelled precursors are required. The total synthesis of a carbon-14 labelled version of capsaicin, labelled in the benzylic position, was published by Bodine and Chien.[5] An improved version of this synthesis has subsequently been reported.[6] In this paper we report the synthesis of deuterium labelled versions of vanillylamine and capsaicin, which can be used to probe the biosynthetic pathway through the use of techniques such as ^{1}H, ^{13}C NMR and mass spectrometry.

Results and discussion

Synthesis of the target compounds [^{2}H$_3$]vanillylamine hydrochloride (5) and [^{2}H$_3$]capsaicin (7) is shown in Scheme 1.

Overall yield of [^{2}H$_3$]vanillylamine hydrochloride (5) from (2) was about 9.6%. 3-Bromo-4-hydroxybenzaldehyde (2) was prepared

from 4-hydroxybenzaldehyde (1) using a reported procedure.[7] It was found that the optimum reaction time for this bromination was 30 s to 1 min. Longer reaction times resulted in the formation of 3,5-dibromo-4-hydroxybenzaldehyde or conversion back to starting material.[7] Conveniently, the crude reaction mixture could be used immediately in the next step. The isotopic label was introduced by displacement of bromine from (2) with sodium [^{2}H$_3$]methoxide. This reaction was sensitive to moisture so precautions were taken to ensure that the sodium [^{2}H$_3$]methoxide solution was kept as dry as possible. Sodium [^{2}H$_3$]methoxide was freshly prepared from [^{2}H$_4$]methanol.[8] Vanillyloxime (4) was found to be a 1:1 mixture of *E*- and *Z*-isomers as expected. The benzylic protons of the *E*- and *Z*-isomers had chemical shifts at δ 8.04 and δ 7.91 respectively in the NMR spectrum. The presence of *E*- and *Z*-isomers of (4) had no consequence in subsequent steps. The crude oxime mixture was hydrogenated over palladium on carbon in the presence of hydrochloric acid. Thorough flushing of the hydrogenation vessel with inert gas before introduction of hydrogen was essential to optimize the yield. Vigorous stirring during hydrogenation was also essential. [^{2}H$_3$]Vanillylamine hydrochloride was obtained as its hydrochloride salt in 31.4% yield from (3). (*E*)-8-Methyl-6-nonenoyl chloride (6) was prepared according to a published method.[9] Coupling of [^{2}H$_3$]vanillylamine free base with (6) gave [^{2}H$_3$]capsaicin (7) in 47.5% yield.
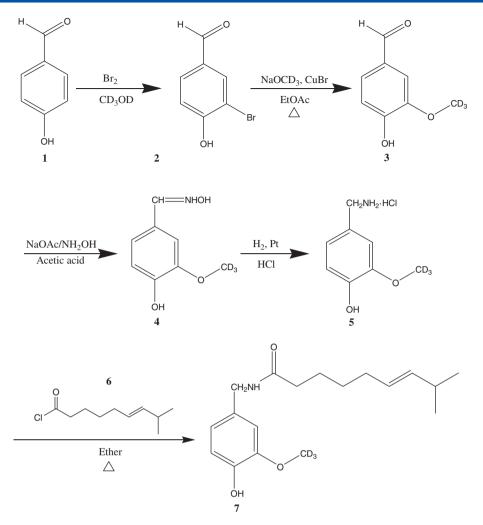
Conclusion

In conclusion, [^{2}H$_3$]vanillylamine hydrochloride (5) and [^{2}H$_3$]capsaicin (7) have been effectively prepared. These compounds can be used to investigate the biosynthesis of capsaicin in the plant genus *Capsicum* and probe the function of CS.

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Scheme 1. Synthetic scheme of deuterated capsaicin.

Experimental

General

All commercial reagents and solvents were purchased from Aldrich and Merck in analytical grade and were used without further purification. Melting point was measured on a Mel-Temp capillary tube apparatus and was uncorrected. ^{1}H, ^{13}C NMR spectra were recorded on a JEOL ECA-500 NMR spectrometer, chemical shift data for the proton resonances were reported on ppm scale with TMS as an internal standard. Mass spectra were recorded on JEOL JMS-AX505WA mass spectrometer, compounds were measured by FAB method using *m*-nitrobenzyl alcohol as a matrix at National Center for Inter-University Research Facilities (NCIRF). Gravity column chromatography was performed on Merck silica gel 60 (70-230 mesh ASTM). Thin layer chromatography was performed on Merck silica gel 60F-254 glass plates and visualized by UV light. The hydrogenation was carried out using our own device at 4 bar.

Synthesis of [^{2}H$_{3}$]vanillin (3)

Flask A: A mixture of 45 mL (180 mmol) of 4.0 M NaOCD$_{3}$/CD$_{3}$OD solution and 2.58 g (18 mmol) of copper(I) bromide was heated at 100°C for 5–7 min under anhydrous conditions.

Flask B: 15 mL (7.5 mmol) of a 0.5 M solution of bromine in CD$_{3}$OD was cooled in an ice-water bath for 5 min. To this solution was

added 1.1 g (9 mmol) of 4-hydroxybenzaldehyde (**1**) and the mixture was stirred at +5°C for 30 s to give 3-bromo-4-hydroxybenzaldehyde (**2**). The contents of this flask were immediately poured into flask A and the resulting mixture was heated at 120°C for 1.5 h.

The mixture was cooled to room temperature and 60 mL (180 mmol) of 3.0 M hydrochloric acid was added. The mixture was transferred to a separating funnel and extracted with 3 × 20 mL of dichloromethane. The combined extracts were dried over sodium sulphate and filtered. The filtrate was concentrated to dryness and the residue was purified by column chromatography eluting with dichloromethane–hexane (9:1 v/v) to give (**3**) as a light yellow solid (410 mg, 2.64 mmol, 26%).

R_{f}: 0.55(DCM:Hexane = 9:1). mp: 81–83°C. ^{1}H NMR (CDCl$_{3}$) δ: 9.83 (s, 1H, –CHO), 7.42 (m, 2H, H-5,6), 7.05 (d, 1H, H-2), 6.24 (s,1H, –OH). ^{13}C NMR (CDCl$_{3}$) δ: 191.05, 151.80, 147.17, 129.99, 127.48, 114.42, 109.07, 55.19 (septet; D$_{3}$). FAB-MS (*m/z*): 156.07 [M+H]$^{+}$.

Synthesis of [^{2}H$_{3}$]vanillylamine hydrochloride (5)

Synthesis of [^{2}H$_{3}$]vanillyloxime

2 g (12.8 mmol) of [^{2}H$_{3}$]vanillin was suspended in 10 mL of glacial acetic acid and combined with 1.1 g (13.4 mmol) of anhydrous sodium acetate. Then, 0.9 g (12.9 mmol) of

564

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hydroxylamine hydrochloride was added, the reaction mixture was heated to 30°C with stirring and stirred for 30 h.

Synthesis of [^{2}H$_3$]vanillylamine hydrochloride

The reaction mixture obtained in the earlier step ('Synthesis of [^{2}H$_3$]vanillyloxime') was combined with 2.6 mL of hydrochloric acid and 0.4 g of Pd/C. Hydrogen was piped into the reaction mixture, with stirring, at 10°C over a period of 4 h under a pressure of 4 bar. After the addition of 5 mL of water the mixture was heated to 60°C and stirred for 1 h. The catalyst was filtered off and the acetic acid was eliminated from the filtrate by evaporation at 60°C. Then the reaction mixture was combined with 10 mL of water in order to dissolve the [^{2}H$_3$]vanillylamine hydrochloride, and the salts were stirred at 60°C for 0.5 h. After the addition of 3 mL of hydrochloric acid was stirred for 1 h, the suspension formed was cooled to 3°C and after 3 h the precipitate was filtered off, washed with acetone and dried at 60°C. The [^{2}H$_3$]vanillylamine hydrochloride was obtained in 31.4% yield (780 mg, 4.02 mmol).

R_f: 0.10 (MeOH:DW = 9:1). mp: 215–216°C. ^{1}H NMR (CDCl$_3$+ DMSO-d$_6$)δ: 9.16 (s, 1H, –OH), 8.24 (s, 2H, –NH$_2$), 7.12 (s, 1H, H-2), 6.81 (d, 1H, H-6), 6.75 (d, 1H, H-5), 3.84 (s, 2H, -CH$_2$NH$_2$). ^{13}C NMR (CDCl$_3$+DMSO-d$_6$)δ: 147.50, 146.82, 124.65, 121.75, 115.26, 113.58, 55.74 (septet; D$_3$), 42.17. FAB-MS (*m/z*): 157.10 [M+H]$^+$.

Synthesis of [^{2}H$_3$]Capsaicin (7)

To a aqueous solution of The [^{2}H$_3$]vanillylamine hydrochloride (780 mg, 4.04 mmol) was added 10 mL of 2 M NaOH. The resulting white solid of free base was filtered by Buechner funnel flask, washed with water, dried over P$_2$O$_5$ in a vacuum desiccator. The (*E*)-8-methyl-6-nonenoic acid (**6**) was prepared according to the reported methods.[9] 334 mg (1.77 mmol) of the **6** and 720 mg (5.67 mmol) of the oxalyl chloride were stirred at room temperature for 2 h under an atmosphere of N$_2$.

The excess oxalyl chloride was evaporated and the residue was added to the suspension of 500 mg (3.20 mmol) of base free vanillylamine in 25 mL of tetrahydrofuran. The mixture was stirred at room temperature for 2 h and then refluxed for an additional 3 h. The reaction mixture was filtered, and the filtrate was evaporated. The residue was purified by column chromatography (hexane:ethyl acetate = 2:1) on silica gel to give a white solid (473 mg, 1.52 mmol, 47.5%).

R_f: 0.68 (DCM:MeOH = 9:1). mp: 64–66°C. ^{1}H NMR (DMSO-d$_6$)δ: 8.77 (s, 1H, –OH), 8.12 (t, 1H, –NH–), 6.76 (s, 1H, H-2), 6.65 (d, 1H, H-6), 6.59 (d, 1H, H-5), 5.30 (m, 2H, HCCH), 4.10 (d, 2H, –CH$_2$NH), 2.17 (m, 1H, CH), 2.07 (t, 2H, CH$_2$CO), 1.90 (q, 2H, CH$_2$CC), 1.47 (p, 2H, –CH$_2$), 1.26 (p, 2H, –CH$_2$), 0.89 (d, 6H, –CH$_3$). ^{13}C NMR (DMSO-d$_6$)δ: 172.40, 147.93, 145.87, 137.89, 131.05, 127.15, 120.16, 115.67, 112.12, 56.02 (septet; D$_3$), 42.31, 35.75, 32.21, 30.90, 29.22, 25.43, 23.08. FAB-MS (*m/z*): 309.22 [M+H]$^+$.

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