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# *In Vitro* Hepatic and Skin Metabolism of Capsaicin

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Running Title: **CAPSAICIN METABOLISM**

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**ABBREVIATIONS:** ACN, acetonitrile; cGMP, current Good Manufacturing Practice; CYP, cytochrome P450; HBSS, Hank's balanced salt solution; LSC, liquid scintillation counting; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; TRPV1, transient receptor potential vanilloid 1 receptor; VR1, vanilloid receptor 1.

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**ABSTRACT:**

Based on its ability to activate the transient receptor potential vanilloid 1 receptor (TRPV1) expressed in nociceptive sensory neurons, topical and injectable high-concentration formulations of capsaicin are being developed as potential treatments for various pain syndromes. As much of the published literature on capsaicin is based on pepper extracts, which are typically a mixture of capsaicin and other capsaicinoids (including norhydrocapsaicin, dihydrocapsaicin, homocapsaicin and homodihydrocapsaicin), the purpose of this investigation was to study the *in vitro* metabolism of pure capsaicin. The metabolism of capsaicin was similar in human, rat and dog microsomes and S-9 fractions. In these assays, three major metabolites were detected and identified as 16-hydroxy-capsaicin, 17-hydroxy-capsaicin, and 16,17-dehydro-capsaicin. In addition to these three metabolites, rat microsomes and S-9 fractions also produced vanillylamine and vanillin. Biotransformation of capsaicin was slow in human skin *in vitro*, with the majority of the applied capsaicin remaining unchanged and a small fraction being metabolized to vanillylamine and vanillic acid. These data suggest that the metabolism of capsaicin by cytochrome P450 enzymes in skin is minimal, relative to hepatic metabolism.

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Capsaicin is the most abundant pungent molecule produced by pepper plants, and thereby represents an important ingredient in spicy foods consumed throughout the world. The capsaicin content of peppers ranges from 0.1 mg/g to 2.5 mg/g (Parrish, 1996), and the resulting average human capsaicin consumption is in the order of 0.5 to 4 mg/kg/day (EC Scientific Committee on Food, 2002). In addition to its extensive role as a food additive, there is also substantial human exposure to capsaicin in the form of non-prescription (in the US) or prescription (in the European Union) topical analgesics, self-defense products (e.g., pepper spray) and oral herbal supplements.

Capsaicin is a highly selective agonist for the TRPV1 (formerly known as the vanilloid receptor 1 (VR1)). TRPV1 is a ligand-gated, non-selective cation channel preferentially expressed on small-diameter sensory neurons, especially those nociceptors which specialize in the detection of painful or noxious sensations (C-fibres and to a lesser extent Aδ-fibers) (Caterina et al., 1997; Szallasi and Blumberg, 1999). The initial effect of capsaicin is the activation of TRPV1-expressing nociceptors, resulting in a burning sensation, hyperalgesia, allodynia, and erythema (Szallasi and Blumberg, 1999); these events are followed by a reversible defunctionalization of nociceptive sensory axons (Bley, 2004). Defunctionalization of hyperactive nociceptors is thought to underlie the pain relief which follows topical application or intra-articular injections of capsaicin (Bley, 2004).

Much of the published literature of capsaicin relates to extracts of capsaicin derived from peppers; these extracts are typically a mixture of capsaicin, norhydrocapsaicin, dihydrocapsaicin, homocapsaicin and homodihydrocapsaicin. The actual percentage of capsaicin and other capsaicinoids varies depending on the peppers used and method of extraction. This can range from about 65% (USP grade natural capsaicin) to ≥99% (in case of synthetically pure capsaicin). Additionally, extracts may contain chemical entities other than vanilloid compounds. As a

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consequence of these variable impurity profiles, results obtained from metabolism studies with pure capsaicin may differ from results obtained with extracts.

Previous publications suggest that capsaicinoids are metabolized to a great extent by hepatic enzymes (Reilly and Yost, 2006). Early studies demonstrated that capsaicin is converted to metabolites via hydroxylation of the vanillyl ring moiety, leading to the hypothesis that capsaicin is activated by the liver mixed-function oxidase system to an electrophilic intermediate that is capable of covalently binding to hepatic proteins. Additionally, the alkyl side chain of capsaicin was also considered susceptible to enzymatic oxidation, giving rise to a hydroxylated metabolite at the terminal carbon of the side chain when incubated with NADPH and a liver S9 fraction. One-electron oxidation of capsaicin also plays a role in capsaicin metabolism. Liver cytochrome P450 (CYP) 2E1 activity has been shown to be responsible for conversion of capsaicin to the reactive phenoxy radical, which in turn can dimerize or bind to CYP2E1, thereby inactivating the enzyme (Surh and Lee, 1995). This mechanism may be responsible for the inhibition of further activation and the reported chemoprotective activity of capsaicin against some chemical carcinogens and mutagens (Surh and Lee, 1996). Non-oxidative hydrolysis has also been demonstrated in rat tissues at the acid-amide bond. The splitting of the side chain also occurs *in vivo*, which is believed to be the rate-limiting step in overall metabolism of the compound. Oxidative deamination of the resulting vanillylamine produces the aromatic aldehyde, vanillin, which in turn undergoes oxidation to vanillic acid or reduction to vanillyl alcohol for excretion as a free form or as a glucuronic acid conjugate (Surh and Lee, 1995). In addition to CYP2E1, capsaicin can also be metabolized by CYP1A2, 1A1, 3A4, 2B6, 2C8, 2C9, and 2C19 (Reilly et al., 2003). Some of these pathways also represent a detoxification process, as

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opposed to bioactivation pathways discussed earlier, resulting in reduction in cytotoxicity (Reilly et al., 2003; Reilly and Yost, 2006).

The purpose of the presently reported studies was to explore rigorously the *in vitro* metabolism of highly pure capsaicin at physiologically relevant concentrations, using liver microsomes and S9 as well as fresh human skin. Liver microsomes and S9 fractions were used from rat, dog and human for inter species comparison of capsaicin metabolism. S9 fractions were used to detect involvement of any Phase 2 metabolism.

## Materials and Methods

**Materials.** Capsaicin (CAS #404-86-4) was manufactured under current Good Manufacturing Practice (cGMP) conditions and had ≥99% purity. Although there are two geometric isomers of capsaicin, only *trans*-capsaicin occurs naturally (Cordell and Araujo 1993), and thus the term 'capsaicin' is used generically to refer to the *trans*-geometric isomer. The [$^{14}$C]capsaicin (Fig. 1) was synthesized by Chemsyn Science Laboratories (Lenexa, KS) with specific activity of 54.0 mCi/mmol. Radiochemical purity, as well as purity of *trans*-capsaicin, was ≥98%. Vanillin and vanillylamine hydrochloride were purchased from Aldrich Chemical Company (Milwaukee, WI), and vanillic acid and vanillyl alcohol were purchased from Fluka Chemical Co. (Ronkonkoma, NY). Separately pooled male rat, dog, and human hepatic microsomes and S-9 fractions and fresh human skin samples were obtained from In Vitro Technologies, Inc. (Baltimore, MD). Microsomes and S-9 fractions were stored frozen at approximately -70°C.

***Microsomal and S-9 incubations.*** For the hepatic metabolism studies, rat, dog, and human hepatic microsomes and S-9 fractions were diluted with buffer (0.1 M potassium phosphate buffer, pH 7.4, containing 1 mM EDTA) to give final protein concentrations of 1 mg/mL.

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Microsomal and S-9 fractions suspensions were then pre-incubated with a NADPH-solution at 37 °C for 5 min in buffer. Incubations were initiated by the addition of 10 µL of a 100-fold concentrated solution of [$^{14}$C]capsaicin in DMSO to give final concentrations of 1 and 10 µM [$^{14}$C]capsaicin. Control incubations were performed at 1 and 10 µM [$^{14}$C]capsaicin for 0 and 30 min in the absence of the NADPH and medium. The final incubation volume was 1 mL and all incubations were conducted in duplicate. The amount of radiolabeled test article was approximately 120,000 dpm/mL (1 µM [$^{14}$C]capsaicin) and 200,000 dpm/mL (10 µM [$^{14}$C]capsaicin). The final concentration of DMSO in the incubation samples was approximately 1% (v/v). Incubation reactions were terminated at 0, 5, 10, 20, and 30 min by the addition of 1 mL of acetonitrile (ACN), followed by vortex mixing. Precipitated microsomal and S-9 fractions protein was removed by centrifugation (1,400 x g for 15 min, at approximately 4 °C) and the supernatants were analyzed by liquid scintillation counting (LSC) and HPLC analysis.

***Skin Metabolism.*** For the skin metabolism, all incubations were conducted at 37 ± 1 °C, 95% air/5% $CO_2$, and saturating humidity. The medium used in this study was Hank's balanced salt solution (HBSS) supplemented with 1% bovine serum albumin. Fresh skin from the abdominal region of one female human donor was obtained and cut with a dermatome to a thickness of 250 to 350 µm. Skin disks (24 disks, 14 to 18 mm in diameter) were prepared from the skin using a cork-boring tool and stored in supplemented HBSS kept on wet ice until incubation. Skin disks were transferred to 12-well tissue culture plates, each well containing 1 mL solution of 0, 1, 3 or 10 µM [$^{14}$C]capsaicin (up to 200,000 dpm/mL). Skin disks from each concentration were incubated on an orbital shaker for 20 hours. After the 20-h incubation, the skin disks were harvested into individually labeled vials. An equal volume of ACN was added to the medium samples. The samples were harvested into individually labeled cryovials and stored at -70°C.

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The medium samples were transiently removed from -70°C storage to prepare two 100-µL aliquots for analysis by LSC. The skin disks and medium samples were then returned to -70°C storage until analysis. The control and incubation medium samples were analyzed by HPLC directly. Before HPLC analysis, duplicate aliquots of each sample were taken and analyzed by LSC to determine the initial concentration of radioactivity in each sample. The human skin disks were pooled by concentration. The skin disks were cut into small pieces and then ground using a Potter-Elvehjem (P-E) probe. To the ground samples, 5 mL of ACN was added, mixed for 10 min, and then transferred to centrifuge tubes. The samples were then vortexed, sonicated for approximately 15 min, centrifuged for approximately 3300 rpm at room temperature, and the supernatant fractions were decanted to separate tubes. This extraction procedure was repeated, and the corresponding supernatant fractions were combined. The supernatant fractions were dried under nitrogen and reconstituted in 0.1% acetic acid in water:ACN (50:50). Duplicate aliquots were analyzed by LSC to determine the radioactivity concentration in the extracts. Incubated media and human skin samples were analyzed using HPLC with radiochemical detection.

Medium control samples were included to evaluate the chemical degradation of [$^{14}$C]capsaicin in the absence of a metabolically active system. [$^{14}$C]Capsaicin at 1, 3 and 10 µM (1 mL, up to 200,000 dpm/mL) was incubated in 12-well tissue culture plates without skin disks on an orbital shaker for 0 or 20 h. For the 0 h sampling, an equal volume of ACN was added to the medium control samples. Two 100-µL aliquots of the medium control samples were taken for analysis by liquid scintillation counting. The remaining medium control samples were harvested into individually labeled vials. The medium control samples were stored at -70°C until analysis. Following the incubation for 20 h, an equal volume of ACN was added to the medium control

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samples. The samples were harvested into individually labeled cryovials and stored at -70°C. The medium control samples were transiently removed from -70°C storage to prepare two 100-µL aliquots for analysis by LSC. The skin disks and medium control samples were then returned to -70°C storage until analysis. For analysis of the medium control samples, liquid scintillation fluid was added to the aliquot of each medium sample and then placed in the dark at ambient temperature for at least 24 h to allow chemiluminescenece to decay. The samples were analyzed using LSC.

Viability control samples were included to assess the mitochondrial function of representative skin disks. Human skin disks were evaluated for viability after incubation for 0 or 20 h. After each incubation time-point, the medium from each viability control well was removed and replaced with supplemented HBSS medium containing tetrazolium dye 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (0.5 mg/mL), and the skin samples were incubated for an additional 3 h. After the 3-h incubation, the MTT dosing solution was removed, and the skin samples were extracted with acidified isopropanol at 4°C for 8 to 12 h to extract the MTT formazan. Samples were analyzed within 24 h of the start of extraction by spectrophotometry at 572 nm (with reference to 690 nm) on a plate reader.

***Radio-HPLC Analysis***. Samples generated from microsomal, S9 and skin incubations were analyzed by radio-HPLC using a Metachem Inertsil 5 µm ODS-2, 4.6 x 250 mm column (25$^{o}$C column temperature) and radioactive detector (Packard 500 series), with Ultima Flo M as scintillation cocktail. The mobile phase was a gradient with A (0.1% acetic acid in RO water) and B (ACN). The flow rate was 1 mL/min. The initial liquid chromatography conditions were held at 2% B for 7 min, followed by a linear gradient from 2% to 90% B over 33 min and back to 2% B over 5 min. The scintillation cocktail flow rate was 3 mL/min.

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***LC/MS Analysis.*** Microsomes and S-9 incubation extracts were first analyzed using a fill-scan LC/MS analysis with a Metachem Intersil 5 μm ODS-2, 250 mm x 4.6 mm reversed phase HPLC column eluted at a flow of rate of 1 mL/min with a mobile phase consisting of 0.02% acetic acid in RO water and acetonitrile, and Micromass Quattro II with an ESP Z-Spray source mass spectrometer. The column temperature was maintained at 25$^{o}$C. During analysis after passing through the column switcher, the HPLC column effluent was split with approximately 30% of the flow diverted to the mass spectrometer and 70% to the radioactivity detector. To minimize contamination of the mass spectrometer source, the first 3.5 min of each run was diverted to waste using the switching valve. The mass spectrometer was equipped with an electrospray ionization source and was set to scan for negative ions in the range of m/z 60-650. The cone voltage was set at 22 volts, source block temperature at 130°C, desolvation temperature at 300 °C, bath gas flow (nitrogen) at 400 L/h, and nebulizer gas flow (nitrogen) at 15 L/h. A number of LC/MS/MS analyses were performed for structural elucidation of metabolites. The LC/MS/MS analyses used the same instrumentation and conditions as for the full-scan LC/MS analyses, with the following exceptions to collision energy (variable), mass range (variable), and collision gas (argon).

***Data Analysis***. For both hepatic and skin studies, statistical analyses of data were limited to descriptive statistics such as mean and standard deviation, as appropriate. Mean and standard deviation values were calculated using Microsoft Excel, Version 8.0e.

## Results

**[$^{14}$C]Capsaicin Metabolism in Rat, Dog and Human Hepatic Microsomes and S-9 Fractions.** The radiopurity of [$^{14}$C]capsaicin ranged from approximately 97% to 98%. Identification of capsaicin metabolites was based on HPLC co-elution with authentic standards



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and on mass spectral analysis of metabolites in rat, dog, and human microsomes and S-9 fractions. Both negative and positive ion electrospray LC/MS were used to analyze metabolites and standards.

**Biotransformation of [$^{14}$C]Capsaicin in Rat Hepatic Microsomes and S-9 Fractions.** In rat microsomes, [$^{14}$C]capsaicin was metabolized rapidly. In control incubations, approximately 95% of the radioactivity was in the form of [$^{14}$C]capsaicin. After 5 minutes of incubation with [$^{14}$C]capsaicin at 1 and 10 μM, only 2.24% and 51.0% of the radioactivity was associated with [$^{14}$C]capsaicin. [$^{14}$C]Capsaicin was completely consumed in 1 and 10 μM incubations at 10 and 20 minutes, respectively. As many as 10 metabolites were detected in incubations at both concentrations. However, some of them accounted for very little of the radioactivity and were not characterized. Major metabolites (Tables 1 and 2) included vanillin, vanillylamine, 16-hydroxy-capsaicin and 16,17-dehydro-capsaicin. 16-Hydroxy-capsaicin was the most abundant metabolite, accounting for 47.0 to 52.5% and 21.3 to 49.4% of the radioactivity in samples at 1 and 10 μM, respectively. 16,17-Dehydro-capsaicin accounted for 18.8% of the radioactivity in the sample at 5 minutes at 1 μM, while at 10 μM 16,17-dehydro-capsaicin accounted for 4.65 to 18.9% of the radioactivity in the sample from 5 to 20 minutes. The transient nature of this metabolite suggests it was further metabolized during the incubations.

The extent of metabolism of [$^{14}$C]capsaicin in rat S-9 fractions was slower compared to microsomes. Unchanged [$^{14}$C]capsaicin detected at 10 minutes accounted for 13.4% of the radioactivity in sample at the 1-μM concentration and by 20 minutes, no unchanged parent drug was detected. At the 10-μM concentration, unchanged parent drug accounted for 16.9% of the radioactivity in sample at 30 minutes. As many as seven metabolites were detected in S-9

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fractions. Vanillylamine, 16-hydroxy-capsaicin and 16,17-dehydro-capsaicin were the major metabolites at both concentrations. 16-Hydroxy-capsaicin accounted for 28.6 to 52.4% and 7.23 to 34.7% of the radioactivity in sample in 1 and 10 μM concentrations, respectively, from 5 to 30 minutes. 16,17-Dehydro-capsaicin accounted for 5.91 to 20.7% and 7.17 to 15.5% of the radioactivity in sample at 1 and 10 μM from 5 to 20 minutes and 5 to 30 minutes, respectively. All remaining unknown metabolites were each ≤ 10% of the radioactivity in the sample.

**Biotransformation of [$^{14}$C]Capsaicin in Dog Hepatic Microsomes and S-9 Fractions.** Metabolism of [$^{14}$C]capsaicin in dog microsomes and S-9 fractions was less extensive than in equivalent rat fractions. Four metabolites (Tables 1 and 2) were detected in microsomal incubations in addition to unchanged [$^{14}$C]capsaicin. By 30 min, unchanged parent drug accounted for 23.8 and 37.7% of the radioactivity in the samples in 1 and 10 μM, respectively. 16-Hydroxy-capsaicin was the major metabolite in microsomal incubations and accounted for 5.68 to 43.7% and 3.10 to 28.3% of the radioactivity in samples. 17-Hydroxy-capsaicin was also a significant metabolite. Additional metabolites detected in dog microsomes and S-9 fractions included hydroxy-capsaicin (determination of the location of hydroxyl group on the alkyl chain was not possible by LC/MS/MS) and 16,17-dehydro-capsaicin.

**Biotransformation of [$^{14}$C]Capsaicin in Human Hepatic Microsomes and S-9 Fractions.** [$^{14}$C]Capsaicin was rapidly metabolized by human microsomal and S-9 fractions. As many as five metabolites were detected. At both the 1 and 10 μM concentrations (Tables 1 and 2), 16-hydroxy-capsaicin and 17-hydroxy-capsaicin were the major metabolites, while vanillin and 16,17-dehydro-capsaicin were detected as minor metabolites. In human S-9 fractions, metabolism of [$^{14}$C]capsaicin was slower than in microsomes. The major metabolites were 16-hydroxy-capsaicin, 17-hydroxy-capsaicin, and 16,17-dehydro-capsaicin.

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**Metabolism of [$^{14}$C]Capsaicin in Human Skin *In Vitro*.** The viability controls indicated that the skin used in the incubations was viable. Conversion of MTT to MTT formazan was observed with skin disks incubated for 0 and 20 h. The optical density of the MTT formazan extracts on average was 0.648 ± 0.237 and 0.740 ± 0.111 for skin disks incubated for 0 and 20 h, respectively. In control incubations for 0 or 20 h with 1, 3, and 10 µM [$^{14}$C]capsaicin, 99 to 100% of the radioactivity consisted of unchanged capsaicin, indicating stability of the test article in control medium under the incubation conditions. During incubation with human skin, [$^{14}$C]capsaicin was metabolized slowly over 20 h. Two metabolites, vanillylamine and vanillic acid, were detected in incubation medium and skin samples at all concentrations, with the exception of vanillic acid, which was not detected in incubation medium at the 1 µM concentration (Figure 2). In the incubation medium, capsaicin, vanillylamine, and vanillic acid accounted for mean values of 91.0 to 95.6%, 4.37 to 8.77% and <0.15% of the radioactivity in the samples, respectively. In skin, capsaicin, vanillylamine, and vanillic acid accounted for 74.0 to 78.7%, 12.9 to 19.8% and 5.25 to 7.97%, respectively, of the radioactivity in the samples.

## Discussion

Although capsaicin is widely consumed orally throughout the world, limited information has appeared regarding its metabolism by liver enzymes. Moreover, there has been no published information on specific metabolism by skin. Therefore, the data presented here represent the first detailed and systematic characterization of capsaicin which would be applicable to the pure form which is utilized in the topical and injectable medical products in clinical development.

In rats, the profile of metabolites of [$^{14}$C]capsaicin was similar in both microsomal and S-9 fractions at both concentrations. However, the extent of metabolism was reduced at the higher concentration, suggesting that the rates of metabolism of [$^{14}$C]capsaicin are saturable. At

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both concentrations in medium control, [$^{14}$C]capsaicin was recovered quantitatively, while in S-9 control samples without NADPH, recovery of [$^{14}$C]capsaicin was approximately 80%. In this system the rest of the radioactivity was found to be vanillylamine. This result indicates that hydrolysis of the amide bond of capsaicin may be in part mediated by amidases in the soluble fraction of liver. As was observed in the rat, metabolism of [$^{14}$C]capsaicin in dog S-9 fractions was qualitatively similar but less extensive than in microsomes. Biotransformation in both human microsomes and S-9 fractions was qualitatively similar at both concentrations, although metabolism of [$^{14}$C]capsaicin was less extensive in S-9 fractions compared to microsomes.

After incubation with rat, dog, and human microsomes and S-9 fractions, capsaicin was metabolized to several products. As proposed in the biotransformation pathway shown in Figure 3, capsaicin was converted in microsomes and S-9 fractions to at least five primary metabolites. A number of side chain-hydroxylated metabolites were detected including two major metabolites, 16-hydroxy-capsaicin and 17-hydroxy-capsaicin. Oxidation of capsaicin or loss of water from the hydroxylated metabolites generated 16,17-dehydro-capsaicin. Hydrolysis of the amide bond of capsaicin produced vanillylamine. Further oxidation of vanillylamine generated a secondary metabolite, vanillin. Proposed generation of vanillylamine from capsaicin and subsequent metabolism to vanillin is similar to the metabolic pathway of dihydrocapsaicin suggested by Kawada and Iwai (1985). Additional metabolites detected in dog microsomes and S-9 fraction included hydroxy-capsaicin.

The extent of metabolism was similar in dog microsomes and S-9 fractions, but was slower than that observed in human and rat. All three major metabolites detected in human were also present in dog microsomal and S-9 fractions samples. Neither vanillylamine nor vanillin was detected in dog. However, in dog a hydroxy-capsaicin was generated which was not present in

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either rat or human. In rat microsomes and S-9 fractions, high levels of vanillylamine and vanillin were detected, indicating high hydrolytic enzyme activities. In rat, two major metabolites, 16-hydroxy-capsaicin and 16,17-dehydro-capsaicin, were detected. A third major metabolite, 17-hydroxy-capsaicin, which was detected in human and dog, was present in rat samples at low levels.

Biotransformation of [$^{14}$C]capsaicin was rapid in rat microsomal and S-9 fraction incubations compared to dog and human incubations. In human, the rate of formation of the metabolites was faster than in dog. Generally, the rate of metabolism in microsomes was faster than in S-9 fractions. For all species the metabolism of [$^{14}$C]capsaicin was less extensive at the 10 µM concentration as compared to the 1 µM concentration, suggesting saturability of metabolism. The metabolism of capsaicin was similar in microsomes and S-9 fractions from rat, dog, and human. In rat, dog, and human, three major metabolites were detected and identified as 16-hydroxy-capsaicin, 17-hydroxy-capsaicin, and 16,17-dehydro-capsaicin. These metabolites are previously reported by Reilly, et al. (2003). However, other metabolites reported by Reilly et al. were not detected. These undetected metabolites include one of the two aliphatic dehydrogenation products (current study detected only 16,17-dehydro-capsaicin), two aromatic hydroxylation products, an O-demethylation product, an N-dehydrogenation product and an N-dehydrogenation and ring oxygenation product. However, in the current study, rat microsomes and S-9 fractions also produced vanillylamine and vanillin metabolites which were not reported by Reilly, et al. These differences could possibly be attributed to using a higher concentration of capsaicin (100 µM, as opposed to 1 and 10 µM in the current study) and a longer incubation time (60 min, as opposed to 30 min in the current study). It is possible that higher capsaicin concentration, combined with longer incubation time, may have changed some of the metabolic

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pathways, which resulted in formation of more metabolites as reported by Reilly, et al. that were not observed in the current study. Vanillylamine and vanillin metabolites have been reported previously in rats after oral administration of dihydrocapsaicin by Donnerer, et al. (1990) and Kawada and Iwai, (1985). However, no vanillyl alcohol or vanillic acid was detected in our studies.

There are no published data on the oral bioavailability of capsaicin. However, evidence from a rat intestinal *ex vivo* study predicts that the molecule will be readily absorbed from the intestinal tract (Monsereenusorn, 1980). Therefore, capsaicin ingested from food is probably well absorbed and subjected to significant first-pass hepatic metabolism. Although capsaicin consumption has been speculated to be linked with increased rates of stomach carcinogenesis by one group (Lopez-Carrillo et al., 1994), the vast majority of literature suggests that capsaicin actually has anti-cancer properties (Surh and Lee, 1995; Surh and Lee, 1996). A recent assessment of capsaicin in a transgenic mouse model showed no evidence of carcinogenic potential (Chanda et al., 2007). The absence of an epidemiological link between capsaicin ingestion and toxicities suggests that the three major hepatic metabolites display no unexpected toxicities. This inference is reinforced by an *in silico* toxicological analysis of these metabolites (unpublished data). Additionally, similarity of metabolites observed between rat, dog and human provides evidence that extrapolation of results from studies carried out in rats and dogs are likely to be valid in humans.

In contrast to the rapid metabolism of capsaicin by hepatic enzymes, *in vitro* biotransformation was very slow in human skin. After incubation with human skin, capsaicin was slowly metabolized to two metabolites. Although the majority of the sample radioactivity was associated with unchanged capsaicin, as shown in the proposed biotransformation pathway

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(Fig. 4), hydrolysis of the amide bond of capsaicin produced vanillylamine as a primary metabolite. Further oxidation of vanillylamine generated vanillic acid as a secondary metabolite. In control medium samples, unchanged capsaicin was recovered quantitatively, indicating that the formation of vanillylamine and vanillic acid was due to skin metabolism. In this tissue, capsaicin was metabolized to vanillylamine and vanillic acid, although the majority of the sample radioactivity was associated with unchanged capsaicin. Both of these metabolites, vanillylamine and vanillic acid, are also metabolites generated from the common flavor vanilla (Suresh et al., 2003; Odink et al., 1988). The implication for topical capsaicin-containing analgesic products is that capsaicin can reach its peripheral target site intact and can reside at the site of action unchanged for a longer period of time. Additionally, a lack of oxidative metabolism in skin would indicate that there is lack of potential for formation of covalently bound toxic metabolites.

In summary, capsaicin is rapidly metabolized *in vitro* by hepatic enzymes from three species, but not by human skin. These studies have not identified any unexpected metabolites, metabolic pathways or safety concerns for oral or topical capsaicin exposure.

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## Figure Legends

FIG. 1. Structure of capsaicin. * Denotes position of the radiolabel.

FIG. 2. Graphical representation of mean percent of radioactivity in human skin and the incubation medium samples. Human skin samples were incubated with [$^{14}$C]capsaicin for 20 h at concentrations of 1, 3, and 10 μM.

FIG. 3. Proposed biotransformation pathway for capsaicin in liver

FIG. 4. Proposed biotransformation pathway for capsaicin in skin

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TABLE 1

*Percent of radioactivity in incubation samples of rat, dog and human hepatic microsomes and S-9 fractions at 1 mg protein/mL with [$^{14}$C]capsaicin at 1 μM for 0, 5, 10, 20, and 30 min*

| | Timepoint | Vanillylamine | | Vanillin | | 16-HC | | 17-HC | | 16, 17-DHC | | Capsaicin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Matrix | Min | M | S-9 | M | S-9 | M | S-9 | M | S-9 | M | S-9 | M | S-9 |
| Rat | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 97.8 | 92.3 |
| | 5 | 3.52 | 3.25 | 11.7 | ND | 49 | 28.6 | ND | ND | 18.8 | 13.9 | 2.24 | 37.3 |
| | 10 | 5.48 | 9.73 | 11.7 | ND | 51.1 | 43.8 | ND | ND | ND | 20.7 | ND | 13.4 |
| | 20 | 3.47 | 13.6 | ND | 2.04 | 52.5 | 52.4 | ND | 2.77 | ND | 5.91 | ND | ND |
| | 30 | 2.48 | 9.83 | 23.6 | 11.7 | 47 | 51.4 | ND | ND | ND | ND | ND | ND |
| Dog | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 98.2 | 98.2 |
| | 5 | ND | ND | ND | ND | 5.68 | ND | ND | ND | ND | ND | 87.6 | 91.8 |
| | 10 | ND | ND | ND | ND | 14.8 | 2.75 | ND | ND | ND | 2.23 | 74 | 81.2 |
| | 20 | ND | ND | ND | ND | 18.8 | 13.2 | 10.2 | ND | 3.51 | ND | 46.7 | 62.6 |
| | 30 | ND | ND | ND | ND | 43.7 | 16.7 | 9.21 | 5.59 | ND | 7.88 | 23.8 | 42.5 |
| Human | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 95.9 | 96 |
| | 5 | ND | ND | ND | ND | 16.8 | 8.34 | 42.8 | 16.5 | 6.72 | 9.92 | 26.7 | 61.7 |
| | 10 | ND | ND | ND | ND | 22.7 | 12.5 | 52.4 | 29.1 | 4.84 | 13 | 5.54 | 37.6 |
| | 20 | ND | ND | ND | 1.51 | 23.5 | 25.9 | 56.1 | 40.6 | ND | 11.4 | ND | 10.5 |
| | 30 | ND | ND | 2.27 | ND | 24.8 | 27.7 | 63.5 | 49 | ND | 4.47 | ND | 5.1 |

M: Liver microsomes; ND: not detected; 16-HC: 16-hydroxy-capsaicin; 17-HC: 17-hydroxy-capsaicin; 16,17-DHC: 16, 17-dehydro-capsaicin



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TABLE 2

*Percent of radioactivity in incubation samples of rat, dog and human hepatic microsomes and S-9 fractions at 1 mg protein/mL with [$^{14}$C]capsaicin at 10 $\mu$M for 0, 5, 10, 20, and 30 min*

| | Timepoint | Vanillylamine | | Vanillin | | 16-HC | | 17-HC | | 16, 17-DHC | | Capsaicin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Matrix | Min | M | S-9 | M | S-9 | M | S-9 | M | S-9 | M | S-9 | M | S-9 |
| Rat | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 98.4 | 98.8 |
| | 5 | 4.52 | 4.44 | ND | ND | 21.3 | 7.23 | ND | ND | 15.4 | 7.17 | 51 | 79 |
| | 10 | 8.83 | 7.37 | 6.69 | ND | 36.6 | 13.8 | 1.56 | ND | 18.9 | 11.1 | 21.2 | 63.1 |
| | 20 | 10.2 | 15.3 | 8.78 | 1.22 | 49.4 | 25.4 | 1.53 | ND | 4.65 | 15.5 | ND | 35.1 |
| | 30 | 9.69 | 17.8 | 27.4 | 5.05 | 42.8 | 34.7 | 3.58 | 3.26 | ND | 13.2 | ND | 16.9 |
| Dog | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 99.3 | 98.9 |
| | 5 | ND | ND | ND | ND | 3.1 | ND | 1.24 | ND | ND | ND | 90.2 | 96 |
| | 10 | ND | ND | ND | ND | 10.1 | 1.14 | 4.97 | ND | 2.91 | ND | 75.4 | 92.1 |
| | 20 | ND | ND | ND | ND | 19.7 | 6.48 | 7.92 | 3.87 | 3.93 | ND | 56.4 | 77.5 |
| | 30 | ND | ND | ND | ND | 28.3 | 8.21 | 14.2 | 2.21 | 6.05 | 1.41 | 37.7 | 70.2 |
| Human | 0 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 93.2 | 98.5 |
| | 5 | ND | ND | ND | ND | 14.2 | 3.21 | 27.4 | 6.42 | 7.95 | 4.04 | 46.9 | 83.6 |
| | 10 | ND | ND | 1.36 | ND | 19.9 | 6.33 | 42.6 | 12 | 8.81 | 6.84 | 21.7 | 71.7 |
| | 20 | ND | ND | 7.9 | ND | 23.8 | 13.2 | 54.2 | 25.4 | 4.33 | 12.3 | 4.03 | 44.5 |
| | 30 | ND | ND | 10.3 | ND | 27 | 20.6 | 55.6 | 31.6 | ND | 13.8 | ND | 28.1 |

M: Liver microsomes; ND: not detected; 16-HC: 16-hydroxy-capsaicin; 17-HC: 17-hydroxy-capsaicin; 16,17-DHC: 16, 17-dehydro-capsaicin



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FIG. 1.

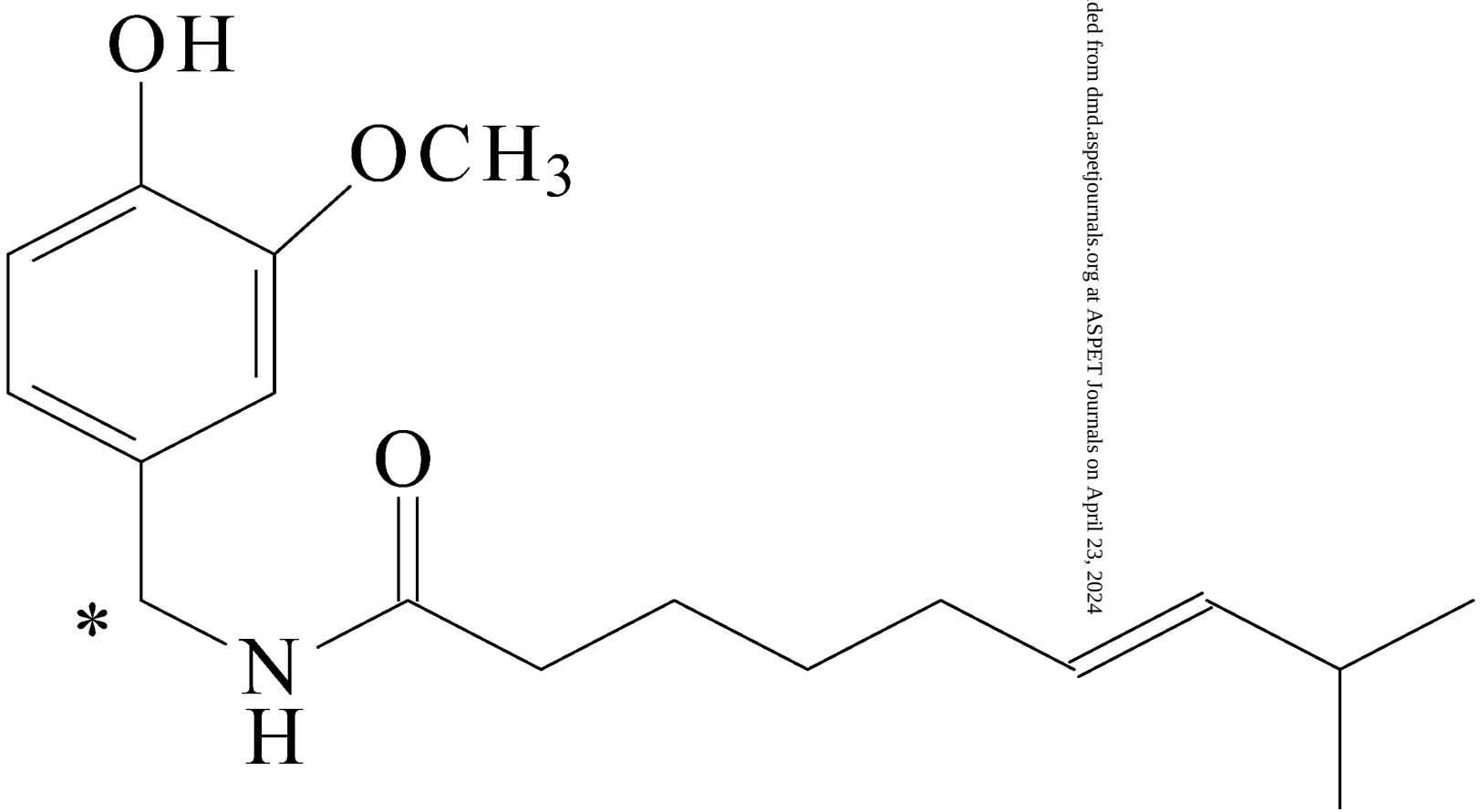


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FIG. 2.

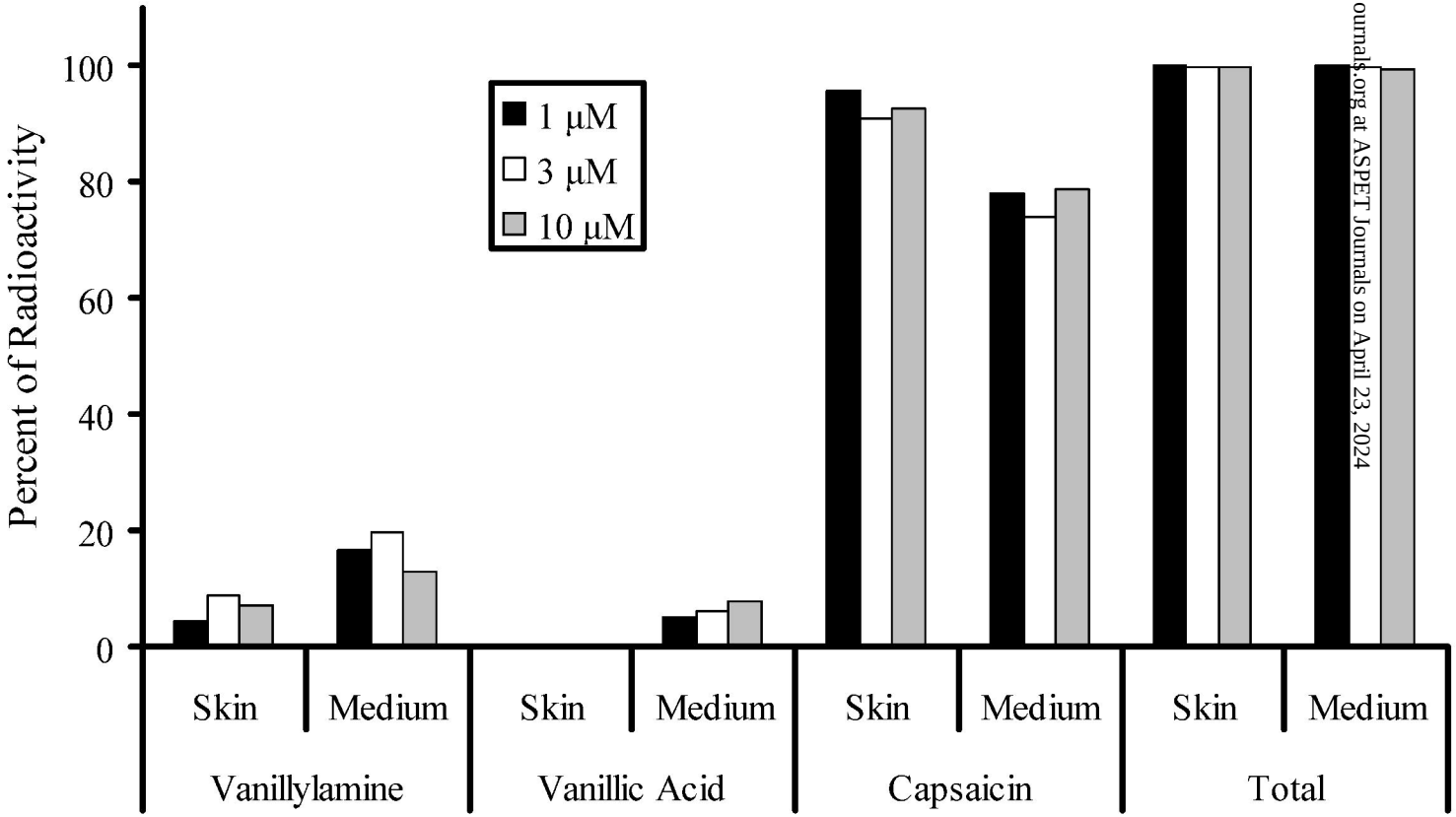


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FIG. 3.

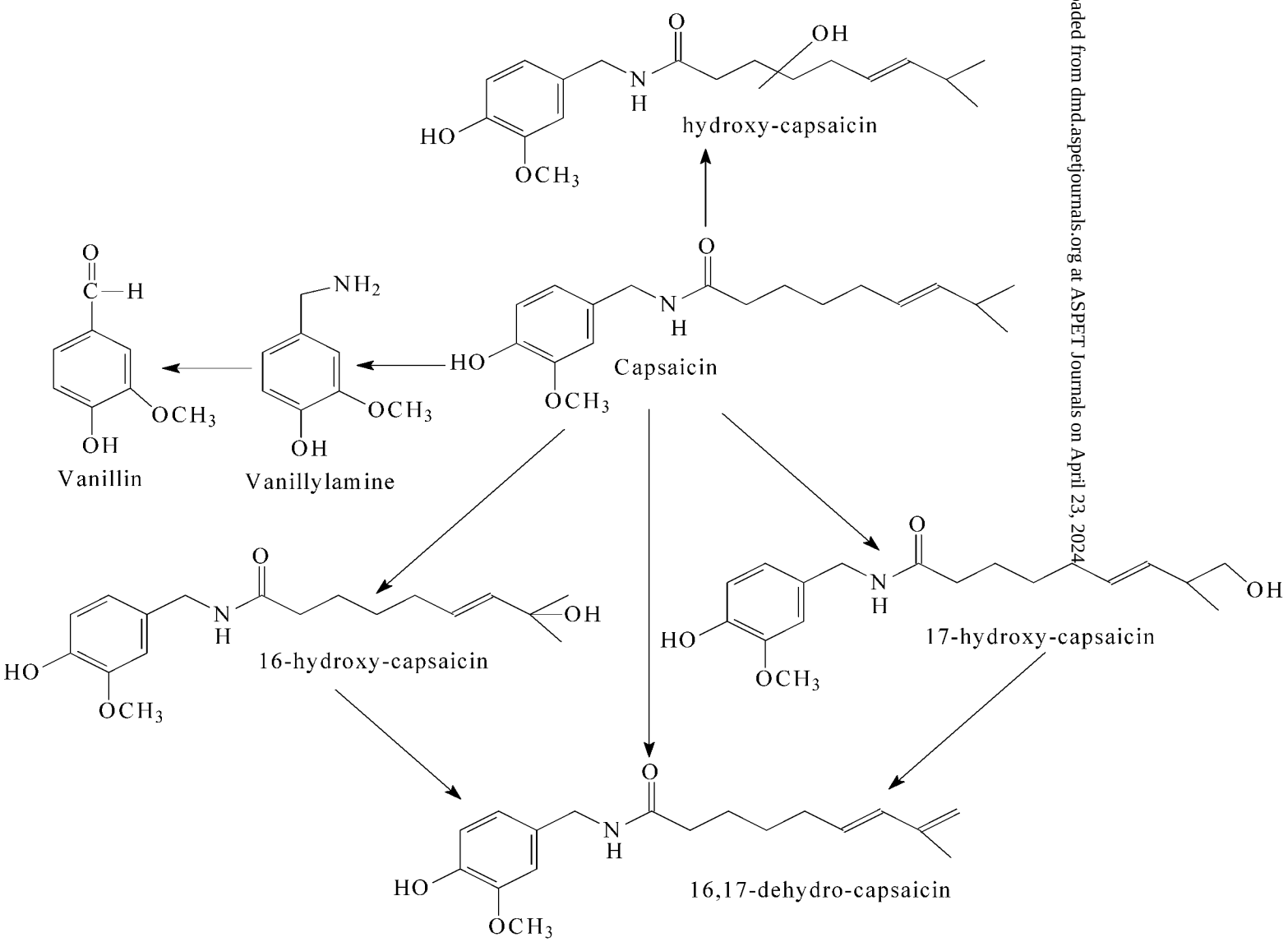


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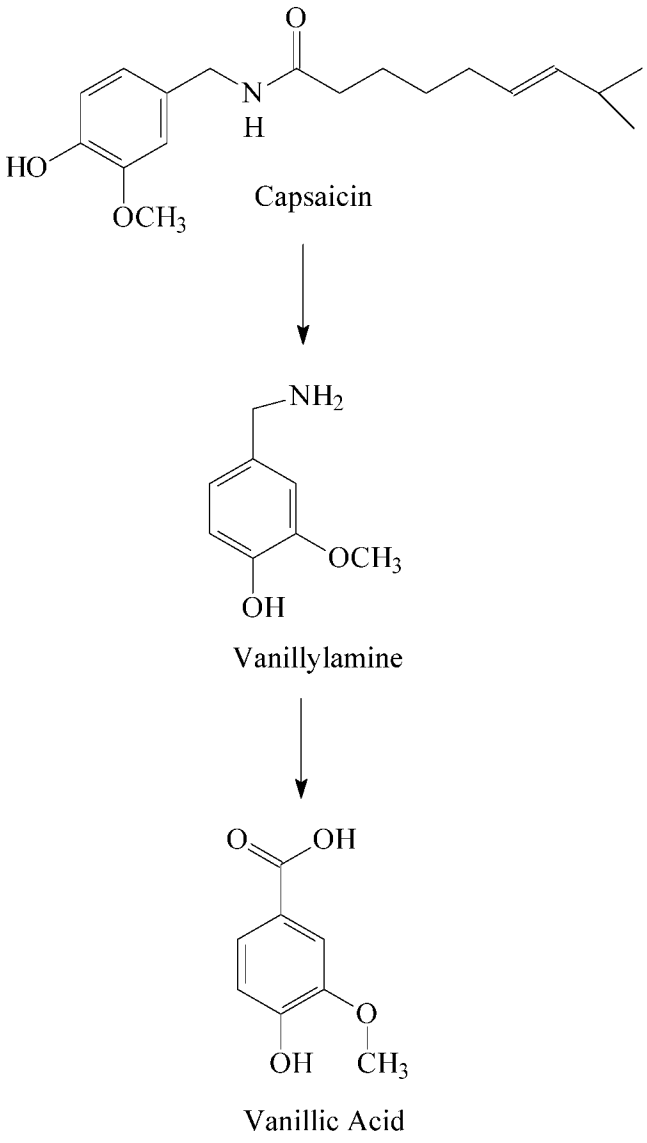
FIG. 4.

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