Nonivamide, a Constituent of *Capsicum oleoresin*

Howard L. Constant and Geoffrey A. Cordell*

Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

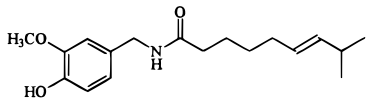
Dennis P. West

Department of Dermatology, Northwestern University Medical School, Chicago, Illinois, 60611, and Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

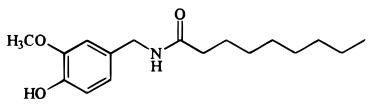
Received November 9, 1995[⊗]

Nonivamide (**2**) has been isolated from capsicum oleoresin for the first time and has been characterized through comparison with an authentic synthetic sample. This work confirms that nonivamide occurs naturally in *Capsicum* species.

Capsaicin (**1**) has recently received increased attention because of its biological properties,[1] and several nonprescription topical products have appeared on the market containing capsaicin or capsicum oleoresin as the active ingredient. *Capsicum* species contain a number of different, closely related capsaicinoid derivatives, whose complete separation by reversed-phase complexation chromatography HPLC has been achieved.[2,3] As a result of this work, it became apparent that the synthetic capsaicinoid nonivamide (**2**) might be identified as a natural product in capsicum oleoresin.



{1}



{2}

For many years, the synthetic alkylvanillylamide nonivamide [nonanamide, *N*-(4-hydroxy-3-methoxyphenyl)methyl] has been regarded as being naturally present in *Capsicum* species. The previous evidence for the existence of nonivamide as a natural product, however, was based on GC or off-line HPLC/MS or by GC retention time comparison with an authentic sample. For example, in 1971, Müller-Stock *et al.* analyzed capsaicinoids from natural sources as TMS derivatives by GC on 1% and 3% JXR and concluded that nonivamide was present in small quantities.[4] Using an off-line HPLC/MS technique, nonivamide, as proposed by mass analysis, was stated as being present in a *Capsicum annuum* var. *annuum* extract in a ratio of 0.05:1 compared to capsaicin.[5] In 1980, using an OV-210 glass capillary column connected to a mass spectrometer, Jurenitsch and Leinmüller achieved baseline separation of the fatty acid methyl esters of the capsaicinoids, and analysis of these capsaicinoid derivatives from natural sources yielded a percentage of nonivamide varying from 0.35 to 1.80, compared to the total capsaicinoid content.[6] In none of these instances was the inference of identity supported by isolation.

Nonivamide has been, and is still, used as an adulterant in the spice and pharmaceutical industries.[7] Nonivamide was first synthesized in 1919 by Nelson[8] and then evaluated for pungency along with several other alkylvanillylamides. Only nonivamide was judged as pungent as capsaicin by a taste-testing panel, when an alcoholic solution of that substance was diluted to achieve the same degree of pungency as capsaicin. It was after this demonstration that nonivamide became known as "synthetic capsaicin", a term that remains current parlance.

The functional requirement of capsicum spice is pungency. Though similar in strength to capsaicin, nonivamide has been added, in varying concentrations, to commercial oleoresins. In one study by Jurenitsch and Leinmüller, samples of "capsicum" extracts had nonivamide percentages from 1.5% to 50% of total capsaicinoids, and in some plasters the ranges seen were from 1% to 29%.[6] Because this range was greater than could be found naturally, it was proposed that a sample containing greater than 3% nonivamide be regarded as adulterated.

Capsaicin is used pharmacologically to decrease pain. Capsaicin and capsicum-containing dosage forms, such as creams and gels, are used as topical analgesics. Capsaicin blocks substance P production and storage in peripheral nerves and blocks C-fiber conduction, thereby reducing neurogenic inflammation and pain response.[9] Nonivamide has been shown to have similar desensitizing activity using animal models, but it has not been proven to be as effective as capsaicin in decreasing the pain response.[10] In our laboratory, we analyzed 20 currently available non-prescription topical products reported to contain capsaicin or capsicum oleoresin and found five (25%) of these to contain only nonivamide, with no detectable capsaicin present.[11] To date, capsaicin is the only individual chemical constituent of capsicum to be recognized and approved by the FDA for human use.

It is known that by using reversed-phase HPLC, capsaicin and nonivamide co-elute.[2] In our studies to separate natural and synthetic capsaicinoids, we used complexation chromatography to separate the co-eluting compounds.[3] Using the same mobile-phase system, nonivamide was purified from capsicum oleoresin. Both ^{1}H- and ^{13}C-NMR experiments were obtained on the purified compound and compared to an authentic sample

[⊗] Abstract published in *Advance ACS Abstracts,* March 1, 1996.

0163-3864/96/3259-0425$12.00/0

of synthetic nonivamide. The ^1H and ^{13}C chemical shifts of the purified natural nonivamide matched those of the authentic sample of synthetic nonivamide. The MS, IR, and UV spectra of nonivamide isolated from capsicum oleoresin correlated with that of the authentic sample. The percentage of nonivamide compared to all capsaicinoids was 0.25% by HPLC analysis. Therefore, it is concluded that nonivamide is a natural product and a component of capsicum oleoresin.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C measurements were recorded in $CDCl_3$ on a Nicolet Model 360 NMR at 360 MHz and 90 MHz, respectively. *J* values are given in Hz. The mass spectrometer used was a Finnigan MAT 90 operating in an electron impact mode.

The oleoresin used was purchased from MacFarlan Smith Ltd. (Edinburgh). Synthetic nonivamide was purchased from Fluka (Ronkonkoma, NY). MeOH (HPLC Grade), $CHCl_3$, and anhydrous Na_2SO_4 were purchased from Fisher Scientific (Pittsburgh, PA), and water was drawn from a Barnstead NANOpure system (Dubuque, IA). The $AgNO_3$ was purchased from Baker (Phillipsburg, NJ). All solvents were filtered with a 0.45-μm filter and degassed before use.

The HPLC system used was a Waters LCModule I system (600 model pump, autoinjector, and 486 model detector set at 280 nm) with a 996 photodiode array detector add-on. The column was purchased from YMC (Wilmington, NC), and the column specifications were 10 × 250 mm with 5-μm C_{18} packing material. The mobile-phase was composed of a MeOH/H_2O (60%/40%) mixture with a $AgNO_3$ concentration of 2.0×10^{-2} M at a flow rate of 4 mL/min. Capsicum oleoresin was repeatedly injected, and the peak of interest was collected. The collected eluent was partitioned with $CHCl_3$ three times, and the $CHCl_3$ fractions were collected and dried using anhydrous Na_2SO_4 and then filtered. The filtrate was then evaporated *in vacuo* to dryness.

Nonivamide (**2**): UV ($CHCl_3$) λ max (log ϵ) 239 (3.16), 275 (3.17); IR (dry film) ν max 3400 br, 2922, 2846, 1638, 1462, 1273, 1033, 746; ^1H-NMR ($CDCl_3$, 360 MHz) δ 6.83 (1 H, d, J = 8, H-5′), 6.78 (1 H, d, J = 2, H-2′), 6.74 (1 H, dd, J = 8, 2, H-6′), 5.60 (1 H, s, OH), 4.32 (2 H, d, J = 5, H-1″), 3.85 (3 H, s, OMe), 2.18 (2 H, t, J = 8, H-2), 1.62 (2 H, m, H-3), 1.24 (10 H, br m, H-4, H-5, H-6, H-7, H-8), 0.82 (3 H, t, J = 7 H-9); ^{13}C-NMR, ($CDCl_3$, 90 MHz) δ 173.48 (C-1), 147.19 (C-3′), 145.60 (C-4′), 130.90 (C-1′), 121.35 (C-6′), 114.88 (C-5′), 111.20 (C-2′), 56.30 (OMe), 44.11 (C-1″), 37.46 (C-2), 32.39 (C-7), 29.90 (C-5, C-6), 29.74 (C-4), 26.38 (C-3), 23.23 (C-8), 14.69 (C-9); EIMS (70 eV) *m*/*z* 294 (9) [M + 1], 293 (45) [M^+), 246 (3), 231 (3), 195 (21), 194 (3), 152 (17), 137 (100), 122 (10).

Acknowledgment. The authors thank the Research Resources Center at UIC for the provision and maintenance of the high-field NMR facilities and R. Dvorak for the provision and maintenance of the UIC MAT 90 Mass Spectrometry Facility. This work was supported in part by GenDerm Corporation, Lincolnshire, Illinois.

References and Notes

(1) Holzer, P. *Pharm. Rev.* **1991**, *43*, 143−201.
(2) Jurenitsch, J.; Kampelmühler, I. *J. Chromatogr.* **1980**, *193*, 101−110.
(3) Constant, H.; Cordell, G. A.; West, D. P.; Johnson, J. H. *J. Nat. Prod.* **1995**, *58*, 1925−1928.
(4) Müller-Stock, A.; Joshi, R. K.; Büchi, J. *J. Chromatogr.* **1971**, *63*, 281−287.
(5) Heresch, F.; Jurenitsch, J. *Chromatographia* **1979**, *12*, 647−650.
(6) Jurenitsch, J.; Leinmüller, R. *J. Chromatogr.* **1980**, *189*, 389−397.
(7) Cordell, G. A.; Araujo, O. E. *Ann. Pharmacother.* **1993**, *27*, 330−336.
(8) Nelson, E. K. *J. Am. Chem. Soc.* **1919**, *41*, 2121−2130.
(9) Mapp, P.; Kidd, B. *Semin. Arthritis Rheum.* **1994**, *23* (Suppl. 3), 3−9.
(10) Szolcsányi, J.; Jancsó-Gábor, A. *Arzneim.-Forsch.* **1975**, *26*, 1877−1881.
(11) Constant, H.; Cordell, G. A. *Proceedings and Abstracts*, International Congress on Natural Products Research, Halifax, Nova Scotia, Canada, 31 July−4 August, 1994; Abstract P:116.

NP9600816