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ARTICLE

Application of Hansch's Model to Capsaicinoids and Capsinoids: A Study Using the Quantitative Structure−Activity Relationship. A Novel Method for the Synthesis of Capsinoids

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We describe a synthetic approach for two families of compounds, the capsaicinoids and capsinoids, as part of a study of the quantitative relationship between structure and activity. A total of 14 capsaicinoids of increasing lateral chain lengths, from 2 to 16 carbon atoms, were synthesized. In addition, 14 capsinoids with identical lateral chains, as well as capsiate and dihydrocapsiate, have been synthesized, and a new method for the synthesis of these compounds has been developed. The yields range from 48.35 to 98.98%. It has been found that the synthetic capsaicinoids and capsinoids present a lipophilia similar to those of the natural compounds and present similar biological activity. The bioactivity of the synthetic capsaicinoids and capsinoids decreases proportionally to the degree of difference in lipophilia (higher or lower) compared to the natural compounds. Biological activity was determined using the etiolated wheat (*Triticum aestivum* L.) coleoptiles bioassay and by comparing results of the synthesis with those presented by their counterpart natural compounds. The bioactivities found correlated directly to the lipophilic properties of the synthesized compounds.

KEYWORDS: Capsinoids; capsaicinoids; synthesis of capsaicinoid and capsinoid; lipophilia; QSAR; Hansch's transport model, Lipinski's rule of five; log *P*; bioassays; IALOGP

INTRODUCTION

Capsaicinoids (*1*) and capsinoids (*2*) are two families of compounds present in pepper (*Capsicum annuum* L.) that are intimately related. Capsaicinoids are responsible for the hot, spicy flavor of peppers (*3*). Chemically, they are a group of acid amides formed from vanillyl amine and fatty acids of 8−13 carbon atoms. Capsaicin is (*E*)-*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methylnon-6-enamide, and dihydrocapsaicin is the 6,7-dihydro derivative of capsaicin. They are notable among the various natural capsaicinoids. These two major capsaicinoids generally account for 90% of the total capsaicinoids in the spicy varieties of peppers (*4*, *5*). In addition to these two major compounds, more than 18 different capsaicinoids have been found as minor compounds (*6*, *7*). Capsaicinoids present antimutagenic and antitumor properties (*8*, *9*), are potent antioxidants (*10*), are employed as topical analgesics for treating pain (*11*), and possess anti-inflammatory (*12*) and antimicrobial (*13*) properties.

Capsinoids are structurally similar to capsaicinoids, except for the way that the carbon chain is bound to the aromatic ring: an amide moiety in capsaicinoids and an ester moiety in capsinoids. Capsinoids have been isolated from the fruit of a sweet pepper cultivar, CH-19 Sweet (*2*, *14*). The most abundant natural capsinoid is capsiate [4-hydroxy-3-methoxybenzyl (*E*)-8-methyl-6-nonenoate]. Other capsinoids, such as dihydrocapsiate [4-hydroxy-3-methoxybenzyl-8-methylnonanoate] and nordihydrocapsiate [4-hydroxy-3-methoxybenzyl-7-methyloctanoate], have also been isolated. The acyl residues of capsiate, dihydrocapsiate, and nordihydrocapsiate are the same as those of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, respectively (*15*).

Capsinoids have biological properties similar to those of capsaicinoids, except that they are not hot or spicy. These compounds have several notable biological properties: they are powerful antioxidants (*16*) and possess chemopreventive and antineoplastic properties (*17*); they promote energy consumption and suppress the accumulation of fats in the organism (*18*); they increase body temperature and the consumption of oxygen in humans (*19*), and present powerful anti-inflammatory activity (*20*). These applications and biological effects of the capsaicinoids, together with their lack of spicy properties, make them extremely important, since their application in both food and pharmacological preparations is not as limited as the capsaicinoids, due to the pungency of the latter.

Many authors have studied the chemical synthesis of capsaicinoids (*21*, *22*), and procedures for the preparation and purification of synthetic capsaicin have been patented (*23*). However, because capsinoids were discovered more recently than their analogues (*2*, *14*), procedures for their synthesis have lagged behind. However, some studies dealing with their synthesis have been published. Kobata et al. reported the chemical synthesis of dihydrocapsiate (*2*), and later synthesized other analogues such as vanillyl nonanoate (*24*). Appendino et al. (*25*) studied the chemoselective esterification of phenolic acids with alcohols, and thereby synthesized vanillyl nonanoate. Recently,

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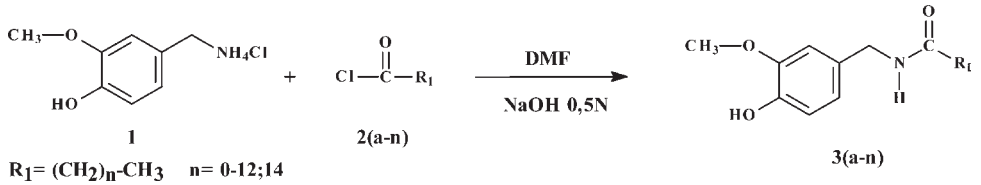


Figure 1. Acylation of 4-hydroxy-3-methoxy benzylamine hydrochloride (**1**) with acyl chlorides (**2a**−**n**).

Torregiani et al. (*26*) developed a new method for the selective esterification of phenolic alcohols, catalyzing the reaction with cerium(III) chloride.

Here we present an easy and selective method for the synthesis of capsinoids. This has been used to synthesize two natural capsinoids (capsiate and dihydrocapsiate) and synthetic analogues of varying lateral chain length, to study the structure−activity relationship.

Lipophilia, which measures the ability of a compound to cross cell membranes, is a key factor in the absorption of any bioactive compound, as it determines its bioavailability in the cell. This is usually expressed through the logarithm of the octanol−water partition coefficient (log *P*) (*27*, *28*) and is present in many equations of quantitative structure−activity relationship (QSAR), because an optimum equilibrium must exist between water (the usual carrier), solubility, and lipophilia.

The partition coefficient is widely used in pharmacology and pharmacognosy studies, and has recently been highlighted in the so-called "Lipinski's rule of five" (*29*). Using computational methods to study those factors affecting the activity, the rule predicts poor absorption and permeation for those compounds having more than 5 H-bond donors, 10 H-bond acceptors, a molecular mass greater than 500 Da, and a calculated log *P* greater than 5. Those compounds whose characteristics fall outside the limits of this rule are supposed to be either completely inactive or only poorly active.

To detect the bioactivity of these compounds, a bioassay involving elongation of etiolated wheat (*Triticum aestivum* L.) coleoptiles was used. This bioassay was originally devised for evaluating the activity of auxins as plant growth regulators (*30*, *31*), although it has been more recently used to detect pharmacological activities in chemical products, a purpose for which the bioassay has been widely utilized and approved (*32*, *33*).

Therein, the general bioactivity values have been determined, and, finally, an examination to establish a correlation between the degree of lipophilia and bioactivity has been carried out.

MATERIALS AND METHODS

General Experimental Procedures. The purity of each compound was determined by ^{1}H NMR and ^{13}C NMR analyses, and was found to be ≥98%. ^{1}H and ^{13}C spectra were recorded using $CDCl_3$ as the solvent, in a Varian INOVA spectrometer, at 399.952 and 100.577 MHz, respectively. The resonances of residual chloroform for ^{1}H and ^{13}C were set to δ_H 7.25 ppm and δ_C 77.00 ppm, respectively, and used as internal reference. UV−vis spectra were obtained using a Varian Cary 50 BIO spectrophotometer, with chloroform as the solvent.

Starting Materials. 4-Hydroxy-3-methoxy benzylamine hydrochloride (98%) (**1**), propionyl chloride (98%) (**2b**), butyryl chloride (98%) (**2c**), pentanoyl chloride (98%) (**2d**), hexanoyl chloride (99%) (**2e**), heptanoyl chloride (99%) (**2f**), octanoyl chloride (99%) (**2g**), nonanoyl chloride (96%) (**2h**), decanoyl chloride (98%) (**2i**), lauroyl chloride (98%) (**2k**), tridecanoic acid (**4l**), myristoyl chloride (97%) (**2m**), palmitoyl chloride (98%) (**2n**), dihydrocapsaicin (90%) (**3o**), capsaicin (97%) (**3p**), *t*-butyldimethyl silyl chloride (97%), and di-isobutyl aluminum hydride (1 M in toluene) (DIBAL) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Acetic anhydride (98%) (**2a**), hydrochloric acid (37%), nitric acid (65%), sodium chloride (99.0−100.5%), ethanol (99.5%), sodium hydrogen carbonate (99.0−100.5%), sodium hydroxide (98.0−100.5%), methanol (HPLC-grade), *N*,*N*-dimethylmethanamide (DMF) (99%), dehydrated pyridine (99%), and tetrahydrofuran (THF) (99.5%) were purchased from Panreac Química S.A. (Castellar del Vallés, Barcelona, Spain). Undecanoic acid (99%) (**4j**) and 8-methylnonanoic acid (97%) were purchased from Acros Organics (New Jersey, USA). *cis*-8-Methyl-6-nonenoic acid (97%) (**10**) was purchased from Maybridge (Tintagel, Cornwall, UK). Ethyl acetate, chloroform, stabilized with ethanol, and hexane were purchased from Scharlau Chemie S.A. (Sentmenat, Barcelona, Spain). Thionyl chloride (99%) was purchased from Merck (Hohenbrunn, Germany). Sodium nitrite (99%) was purchased from Merck (Darmstadt, Germany). 4-Hydroxy-3-methoxybenzaldehyde (vanillin) (**5**) was purchased from Fluka AG, Buchs SG (Switzerland).

General Procedure for the Synthesis of Capsaicinoids. A modification of the method developed by McIlvain et al. (*23*) was employed for the synthesis of capsaicinoids.

Acylation of 4-Hydroxy-3-methoxy Benzylamine Hydrochloride (***1***) *with Acyl Chlorides* (***2a*****−*****n***). To obtain the various capsaicinoids (**3a**−**n**), acylations of 4-hydroxy-3-methoxy benzylamine hydrochloride (**1**) were performed with the corresponding acyl chlorides (**2a**−**n**), according to the particular capsaicinoid to be synthesized (**Figure 1**).

A small amount of 4-hydroxy-3-methoxy benzylamine hydrochloride (**1**) (0.5−1 g) was placed in a 250 mL round-bottom flask and dissolved in 15 mL of DMF. An atmosphere of argon was introduced, and the mixture was stirred until all of the initial compound was dissolved. Subsequently, 1 mL of 5 N NaOH was slowly added, dropwise, in an ice bath. Once the NaOH has been added, the ice bath was removed and the mixture was left in agitation at ambient temperature for 60 min. Then, the particular acyl chloride (**2a**−**n**) of the required carbonated chain (2.5 equiv), dissolved in 2 mL of dry THF, was slowly added, using a syringe, over an ice bath. Once the appropriate acyl chloride was added, the reaction mixture was magnetically stirred and under argon at ambient temperature for 18 h. The reaction was followed by TLC (eluent: 1% of methanol in chloroform; chromogenic reagent: oleum). Then, the reaction was stopped by addition of water. After purification of the reaction mixture, the various capsaicinoids were obtained. The products formed, and the reaction yields, are shown in **Table 1**.

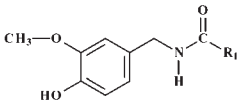
Formation of Acyl Chlorides (***2j,l***) *from their Corresponding Acids* (***4j,l***). Two acids (undecanoic acid (**4j**) and tridecanoic acid (**4l**)) were transformed into their corresponding acyl chlorides (**2j,l**), and the acylations of 4-hydroxy-3-methoxy benzylamine hydrochloride (**1**) were performed later.

The acids were placed in a 50 mL round-bottom flask, and argon was introduced. A quantity of thionyl chloride, sufficient to dissolve the acid, was added very slowly, dropwise, from a syringe, and stirred magnetically, following addition of thionyl chloride, the reaction mixture was heated at 60 °C for 1 h. Subsequently, the excess thionyl chloride was eliminated in vacuo, and a transparent oil was obtained. The acyl chloride formed was dissolved in a known quantity of dry THF to be utilized in the subsequent acylations of 4-hydroxy-3-methoxy benzylamine hydrochloride (**1**).

General Procedure for the Synthesis of Capsinoids. This new method for synthesis comprises four stages. The two first stages are common to all the capsinoids, but the last two stages are different for each capsinoid, since they depend on the chain length required.

Silylation of Vanillin (***5***). The product was taken as the starting point for the synthesis of capsinoids was vanillin (**5**) (4-hydroxy-3-methoxybenzaldehyde). The vanillin (**5**) (5.900 g, 0.038 mol) was placed in a 250 mL round-bottom flask, and dissolved in about 30 mL of dry pyridine. Then 1.2 equiv of *t*-butyldimethyl silyl chloride (TBDMSCl) (7.012 g, 0.046 mol) was added to this solution. The reaction mixture was stirred and under argon at ambient temperature for 24 h. The reaction was

Table 1. Capsaicinoids and Yields Obtained

Structure	R_1	Compound	Yield
	$-CH_3$	**3a**	48.35 %
	$-CH_2CH_3$	**3b**	55.86 %
	$-(CH_2)_2CH_3$	**3c**	60.79 %
	$-(CH_2)_3CH_3$	**3d**	56.42 %
	$-(CH_2)_4CH_3$	**3e**	61.24 %
	$-(CH_2)_5CH_3$	**3f**	58.97 %
	$-(CH_2)_6CH_3$	**3g**	57.60 %
	$-(CH_2)_7CH_3$	**3h**	60.51 %
	$-(CH_2)_8CH_3$	**3i**	62.92 %
	$-(CH_2)_9CH_3$	**3j**	58.54 %
	$-(CH_2)_{10}CH_3$	**3k**	61.25 %
	$-(CH_2)_{11}CH_3$	**3l**	57.10 %
	$-(CH_2)_{12}CH_3$	**3m**	62.48 %
	$-(CH_2)_{14}CH_3$	**3n**	60.36 %
	$-(CH_2)_6CH(CH_3)_2$	**Dihydrocapsaicin (3o)**	available standard
	$-(CH_2)_4CH{=}CHCH(CH_3)_2$	**Capsaicin (3p)**	available standard

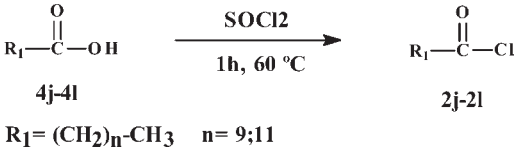


Figure 2. Formation of acyl chlorides (**2j,l**) from their corresponding acids (**4j,l**).

monitored by TLC (eluent: 20% ethyl acetate, 80% hexane; chromogenic reagent: anisaldehyde). Then, the reaction was stopped with ethyl acetate, and, after purification of the reaction mixture, product (**6**) was obtained, which corresponds to 4-*t*-butyldimethylsilyloxy-3-methoxybenzaldehyde, with a 97.56% yield (**Figure 3**).

Reduction of the Carbonyl of 4-t-Butyldimethylsilyloxy-3-methoxybenzaldehyde (**6**). The reaction stage (**Figure 4**) consists of the reduction of the carbonyl group of 4-*t*-butyldimethylsilyloxy-3-methoxybenzaldehyde (**6**) to its corresponding alcohol (**7**). With the alcohol once formed, and the aromatic hydroxyl protected, various esterifications can be performed to produce the capsinoids.

The reduction of the carbonyl group was performed using di-isobutyl aluminum hydride (1 M in toluene) (DIBAL). The 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl (**6**) (3.352 g, 0.0125 mmol) was dissolved in 40 mL of dry THF. Subsequently, 2 equiv of di-isobutyl aluminum hydride (1 M in toluene) was slowly added, from a syringe, in an ice bath, and the reaction mixture was stirred, under argon, at ambient temperature, for 48 h. The reaction was monitored by TLC (eluent: 20% ethyl acetate, 80% hexane; chromogenic reagent: anisaldehyde). Then, the reaction was stopped with water and, after purification of the reaction mixture, the product was obtained, 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl alcohol (**7**), yield 74.42%.

Esterification of 4-t-Butyldimethylsilyloxy-3-methoxybenzyl Alcohol (*7*) *with Acyl Chloride* (***2a*–*p***). The next reaction (**Figure 5**) was the esterification of 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl alcohol (**7**) with acyl chloride (**2a**–**p**) to obtain the various silylated capsinoids (**8a**–**p**) with different lateral chain lengths (i.e., with different degrees of lipophilia).

Thus, the 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl alcohol (**7**) (approximately 1 mmol) was dissolved in 10–15 mL of dry pyridine in a 50 mL round-bottom flask. Argon was introduced. Next the corresponding acyl chloride (2 equiv) was slowly added to this solution and was agitated for 18 h. The reaction was monitored by TLC (eluent: 20% ethyl acetate, 80% hexane; chromogenic reagent: anisaldehyde). The reaction was stopped with ethyl acetate, and, after purification of the reaction mixture, the different silylated capsinoids were obtained, as a function of the lateral chain introduced. The products formed (silylated capsinoids (**8a**–**p**)) and the yields are shown in **Table 2**.

Desilylation of Silylated Capsinoids (***8a*–*p***). The last stage (**Figure 6**) was the desilylation of the various silylated capsinoids obtained previously. The *t*-butyldimethyl silyl group was eliminated from these silylated capsinoids (**8a**–**p**) by the addition of a mixture of 0.25 M HCl/ethanol (1:5). The silylated capsinoids (**8a**–**p**) were placed in a 250 mL round-bottom flask, and approximately 80 mL of the 0.25 M HCl/ethanol (1:5) mixture was added. Argon was introduced and the reaction mixture was agitated for 18 h, at ambient temperature. The reaction was monitored by TLC (eluent/20% ethyl acetate, 80% hexane; chromogenic reagent/anisaldehyde).

Then, the reaction was stopped with, brine and, after purification of the reaction mixture, the different capsinoids (**9a**–**p**) were obtained, as a function of the lateral chain introduced. The capsinoids formed (**9a**–**p**) and the reaction yields are given in **Table 3**.

Formation of Acyl Chlorides (***2***) *from Their Corresponding Acids* (***4***). The synthesis of capsaicinoids paralleled that of the capsinoids. Several

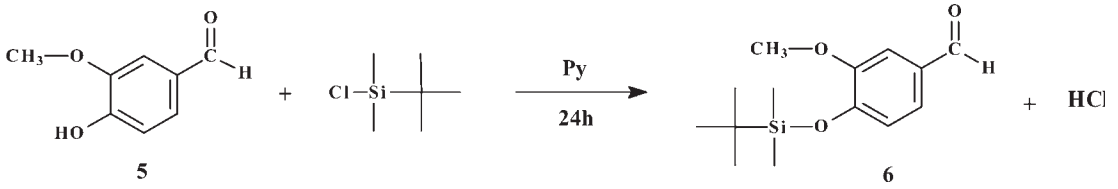


Figure 3. Silylation of vanillin (**5**) (4-hydroxy-3-methoxy benzaldehyde).

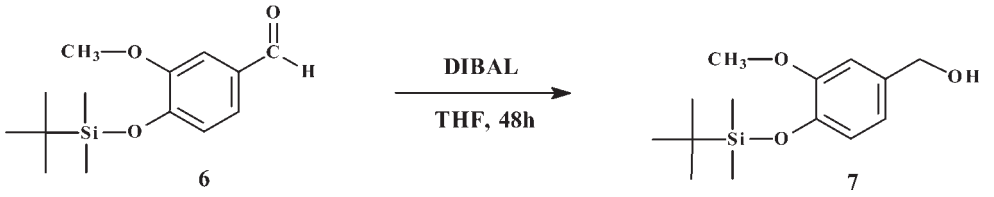
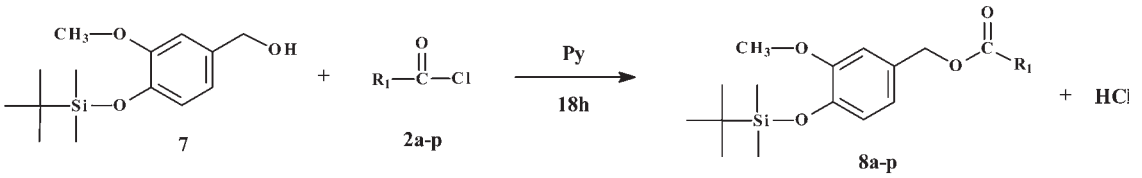


Figure 4. Reduction of carbonyl of 4-*t*-butyldimethyl silyloxy-3-methoxy benzaldehyde (**6**).



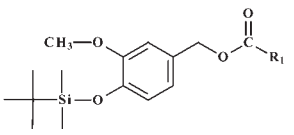
$R_1 = (CH_2)_n\text{-}CH_3$ n= 0-12;14 (8a-n)

$R_1 = (CH_2)_6CH(CH_3)_2$ (8o)

$R_1 = (CH_2)_4CH{=}CHCH(CH_3)_2$ (8p)

Figure 5. Esterification of 4-t-butyldimethyl silyloxy-3-methoxybenzyl alcohol (**7**) with acyl chloride (**2a**−**p**).

Table 2. Silylated Capsinoids and Yields Obtained in the Esterification Stage

Structure	R_1	Compound	Yield
	$-CH_3$	**8a**	84.93 %
	$-CH_2CH_3$	**8b**	88.02 %
	$-(CH_2)_2CH_3$	**8c**	92.79 %
	$-(CH_2)_3CH_3$	**8d**	88.11 %
	$-(CH_2)_4CH_3$	**8e**	94.85 %
	$-(CH_2)_5CH_3$	**8f**	98.98 %
	$-(CH_2)_6CH_3$	**8g**	95.25 %
	$-(CH_2)_7CH_3$	**8h**	93.42 %
	$-(CH_2)_8CH_3$	**8i**	96.45 %
	$-(CH_2)_9CH_3$	**8j**	92.60 %
	$-(CH_2)_{10}CH_3$	**8k**	97.07 %
	$-(CH_2)_{11}CH_3$	**8l**	90.32 %
	$-(CH_2)_{12}CH_3$	**8m**	94.93 %
	$-(CH_2)_{14}CH_3$	**8n**	93.78 %
	$-(CH_2)_6CH(CH_3)_2$	**8o**	89.69 %
	$-(CH_2)_4CH{=}CHCH(CH_3)_2$	**8p**	84.15 %

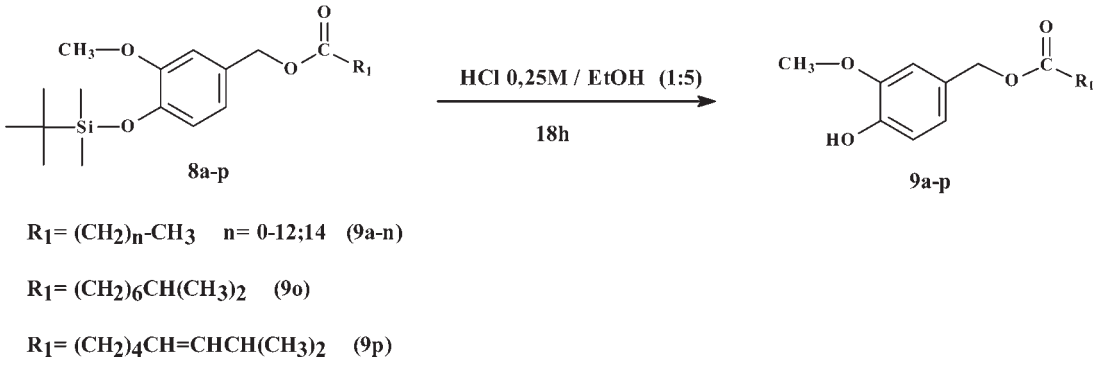


Figure 6. Desilylation of silylated capsinoids (**8a**−**p**).

Table 3. Synthesized Capsinoids and Yields Obtained after Desilylation

starting compound	R_1	resulting compound	yield (%)
8a	$-CH_3$	**9a**	78.59
8b	$-CH_2CH_3$	**9b**	81.78
8c	$-(CH_2)_2CH_3$	**9c**	85.30
8d	$-(CH_2)_3CH_3$	**9d**	82.46
8e	$-(CH_2)_4CH_3$	**9e**	86.97
8f	$-(CH_2)_5CH_3$	**9f**	79.24
8g	$-(CH_2)_6CH_3$	**9g**	85.02
8h	$-(CH_2)_7CH_3$	**9h**	80.69
8i	$-(CH_2)_8CH_3$	**9i**	85.55
8j	$-(CH_2)_9CH_3$	**9j**	78.83
8k	$-(CH_2)_{10}CH_3$	**9k**	84.28
8l	$-(CH_2)_{11}CH_3$	**9l**	79.11
8m	$-(CH_2)_{12}CH_3$	**9m**	84.09
8n	$-(CH_2)_{14}CH_3$	**9n**	82.54
8o	$-(CH_2)_6CH(CH_3)_2$	**9o**	77.25
8p	$-(CH_2)_4CH{=}CHCH(CH_3)_2$	**9p**	79.42

acids (undecanoic acid, tridecanoic acid, and 8-methyl nonanoic acid) were transformed into their corresponding acyl chloride in order subsequently to perform the acylations of 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl alcohol (**Figure 2**).

Synthesis of Capsiate. Capsiate (**9p**) is the major capsinoid found in the "CH-19 Sweet" pepper. It has the same lateral chain as capsaicin (**2**), and a double trans bond is similarly present. Hence, it is more complex to synthesize than its preceding analogues. The synthesis has been accomplished using *cis*-8-methyl-6-nonenoic acid, which is commercial available, as the initial reagent. Therefore, the first stage in the synthesis of capsiate was the isomerization of the double bond to trans.

Isomerization of cis-8-Methylnon-6-enoic Acid (***4q***). For the isomerization of cis-8-methylnon-6-enoic acid (**4q**) a quantity of 0.310 g (1.823 mmol) of this acid was reacted with 310 μL of 2 M $NaNO_2$ and 215 μL of 6 M HNO_3 (**Figure 7**). The mixture reaction was submitted to vigorous stirring, under argon, and was heated to 70 °C for 1.5 h. Then, the reaction mixture was left to cool. Once cooled, the reaction was stopped with about 100 mL of ethyl ether, and, after purification of the mixture, a whitish oil was obtained that corresponded to *trans*-8-methyl-6-nonenoic acid (**4p**), 98% yield.

Formation of Acyl Chloride (***2p***) *from trans-8-Methylnon-6-enoic Acid* (***4p***). The procedure for the formation of the acyl chloride from *trans*-8-methyl-6-nonenoic acid (**4p**) was the same as that described for the capsinoids and capsaicinoids (**Figure 2**).

Esterification of 4-t-Butyldimethylsilyloxy-3-methoxybenzyl Alcohol (*7*) *with trans-8-Methylnon-6-enoyl Chloride* (***2p***). The next reaction was the esterification of 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl alcohol (**7**) with *trans*-8-methylnon-6-enoyl chloride (**2p**), to obtain 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl (*E*)-8-methylnon-6-enoate (**8p**). The process is similar to that described for capsinoids (**Figure 5**).

The 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl alcohol (**7**) (0.958 g, 3.57 mmol reagent in excess) was dissolved in 15 mL of dry pyridine in a 50 mL round-bottom flask and argon was introduced. Next, *trans*-8-methylnon-6-enoyl chloride (0.337 g, 1.786 mmol) was slowly added to this solution, and it was agitated for 18 h. Then, the reaction was stopped with ethyl acetate and, after purification of the reaction mixture, 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl-(*E*)-8-methylnon-6-enoate (**8p**) was obtained, 84.15% yield.

Desilylation of 4-t-Butyldimethylsilyloxy-3-methoxybenzyl-(E)-8-methyl-6-nonenoate (***8p***). The final reaction was the desilylation of 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl (*E*)-8-methylnon-6-enoate (**8p**) obtained in the preceding stage, to obtain 4-hydroxy-3-methoxybenzyl (*E*)-8-methylnon-6-enoate (**9p-capsiate**). The *t*-butyldimethyl silyl group was separated from the 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl (*E*)-8-methyl-6-nonenoate (**8p**), and also from the rest of the synthesized capsinoids by the addition of a mixture of 0.25 M HCl/ethanol (1:5) (**Figure 6**). The 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl (*E*)-8-methylnon-6-enoate (**8p**) (0.630 g, 1.50 mmol) was placed in a 250 mL round-bottom flask, and 80 mL of 0.25 M HCl/ethanol (1:5) was added. Argon was introduced and the reaction mixture was agitated for 18 h, at ambient temperature. The reaction was monitored by TLC (eluent: 20% ethyl acetate, 80% hexane; chromogenic reagent/anisaldehyde).

Then, the reaction was stopped with brine and, after purification, 4-hydroxy-3-methoxybenzyl-(*E*)-8-methylnon-6-enoate (**9p-capsiate**) was obtained, 79.42% yield.

Calculation of IC_{50} and log *P*. The phytotoxicity data were fitted to a sigmoidal dose−response model (constant slope) by employing the GraphPad Prism v. 4.00 software package (*34*). *c* log *P* data were acquired using the OSIRIS property explorer (*35*, *36*). This software uses the Chou and Jurs algorithm, based on computed atom contributions (*37*).

Bioassays. Coleoptiles were obtained from 3-day-old wheat seedlings sown on 15 cm diameter Petri dishes fitted with Whatman No. 1 filter paper and grown at 24 °C in the dark. The etiolated seedlings were removed from the dishes and selected for uniformity of size under a green safelight. Then seedlings were placed in a Van der Wij guillotine, and the apical 2 mm was cut off and discarded. The following 4 mm lengths were selected for bioassay and kept in an aqueous nutritive buffer for 1 h before being used, to synchronize growth.

Products were purified (+99%) by HPLC prior the bioassay and were tested at 1000 μM to 10 μM in a buffered nutritive aqueous solution (citric acid-sodium hydrogen phosphate buffer, pH 5.6; 2% sucrose). Mother solutions of pure compounds were prepared in dimethyl sulfoxide (DMSO) and diluted to the proper concentration with the buffer to a 0.5% v/v DMSO final maximum concentration. The following dilutions were prepared maintaining the same buffer and DMSO concentrations. Bioassays were performed in 10 mL test tubes as follows: five coleoptiles were placed per tube each containing 2 mL of test solution; three replicates were prepared for each test solution, and experiments were duplicated. Test tubes were placed in a roller tube apparatus and rotated at 6 rpm for 24 h at 22 °C in the dark. Increments of coleoptile elongation were measured by digitalization of their photographic images, and data were statistically analyzed.

RESULTS

Preparation of Capsinoids. In this study, a procedure for the synthesis of capsinoids was developed. The starting point was vanillin (**5**) and it comprises four stages: protection of the hydroxyl group of vanillin (**5**) with *t*-butyldimethyl silyl chloride;

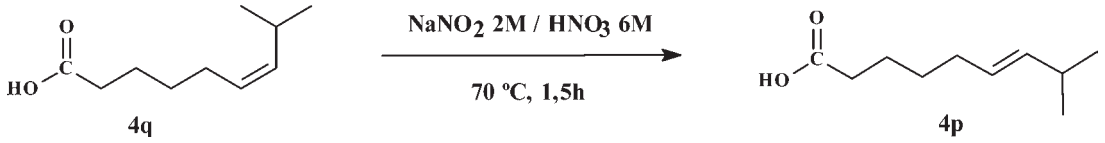


Figure 7. Isomerization of cis-8-methyl-6-nonenoic acid (**4q**).

reduction of the carbonyl group of 4-*t*-butyldimethylsilyloxy-3-methoxybenzaldehyde (**6**) with di-isobutyl aluminum hydride (1 M in toluene); esterification of 4-*t*-butyldimethylsilyloxy-3-methoxybenzyl alcohol (**7**) with acyl chlorides (**2a**−**p**); and deprotection of the silylated capsinoids (**8a**−**p**) in order to obtain the various capsinoids (**9a**−**p**).

The advantages of the procedure are (1) the reactions give good yields; (2) the products obtained can be separated easily and purified; secondary reactions are not produced; (3) the reactions take place in regioselective way; (4) the reactions are simple to put into practice; and (5) the reagents employed are inexpensive. This makes the procedure useful in synthesizing this family of compounds.

Characterization of Compounds. The structure of the all the compounds synthesized was confirmed by spectroscopic methods (^{1}H and ^{13}C NMR spectra; see Supporting Information). A total of 14 capsaicinoids and 16 capsinoids were synthesized (**Tables 1** and **2**, respectively).

Bioassays. Bioassays were conducted on etiolated wheat coleoptiles for 14 capsaicinoids and 16 capsinoids, and for capsaicin and dihydrocapsaicin. The latter two are the major capsaicinoids present in the spicy pepper varieties and that are commercially available.

Both the synthesized capsaicinoids and capsinoids that are generally the most active are those that have a chain length of 8−11 carbon atoms, and a degree of lipophilia similar to that of the natural capsaicinoids and capsinoids.

log *P*. Values of log *P* were calculated using the algorithms IALOGP and CLOGP. IALOGP was chosen for this study, because the commonly used algorithm CLOGP failed to give coherent results several times. Data generated by both algorithms are presented in **Table 4**.

DISCUSSION

A series of 14 analogues of capsaicinoids with different linear chain lengths (**2a**−**p**) were synthesized (**Table 1**), by amidation of 4-hydroxy-3-methoxy benzylamine hydrochloride with the acyl chlorides of these linear acids. Similarly, 16 analogues of capsinoids (**9a**−**p**) have been synthesized by esterification of vanillyl alcohol.

The degree of lipophilia of the synthesized capsaicinoids increased linearly with the increased chain length. Therefore, a series of capsaicinoids and capsinoids have been obtained that present a wide range of values of log *P*. The log *P* values of the natural capsaicinoids (capsaicin and dihydrocapsaicin) and those of the natural capsinoids (capsiate and dihydrocapsiate) lie in intermediate positions among the values of all the compounds synthesized.

The IC_{50} values for the capsaicinoids assayed were calculated, using a sigmoidal dose−response model of constant slope, from the activities obtained in the wheat coleoptile assay. These IC_{50} values are expressed in mol/L. The chemical characteristics of the synthetic capsaicinoids and their effects are presented in **Table 5**. **Table 6** shows the results for the caspsinoids.

In this study, the IA log *P* values and the IC_{50} values were correlated and expressed in the form [log(1/IC_{50})] for all the compounds prepared. A linear fit, logarithmic, and second-order polynomial, was performed. The values obtained show a very good fit to a second-order polynomial, fitted using a second-order equation (eqs **1** and **2**). In the case of the capsaicinoids, it was noted that the products **3o** and **3k** were not well fitted to eq **1**; consequently, they were discarded for the calculation of this equation. To obtain eq **2**, the compounds **9p** and **9m** were discarded, for the same reason. In both cases, the correlation coefficients are >0.95, at 0.9696 and 0.9521 for eqs **1** and **2**, respectively, for 14 samples (n = 14).

$$\log(1/IC_{50}) = -0.1108(IAlogP)^2 + 0.9109(IAlogP) + 1.8213 \quad (1)$$

$$\log(1/IC_{50}) = -0.0881(IAlog\,P)^2 + 0.8919(IAlog\,P) + 1.3394 \quad (2)$$

In light of **Figure 8**, it is noted that the capsaicinoid presenting the most activity is capsaicin (**3p**); this is the major natural capsaicinoid. Capsaicinoids **3g** and **3h** also present excellent

Table 4. Values of IALOGP and CLOGP for the Synthesized Capsaicinoids and Capsinoids

capsaicinoid	IALOGP	CLOGP	capsinoid	IALOGP	CLOGP
3a	0.94	−1.79	**9a**	1.26	−1.14
3b	1.3	−1.16	**9b**	1.71	−0.51
3c	1.73	−0.77	**9c**	2.18	−0.12
3d	2.17	−0.37	**9d**	2.66	0.28
3e	2.64	0.03	**9e**	3.17	0.68
3f	3.12	0.42	**9f**	3.7	1.07
3g	3.62	0.82	**9g**	4.24	1.47
3h	4.12	1.21	**9h**	4.79	1.86
3i	4.62	1.61	**9i**	5.34	2.26
3j	5.12	2.01	**9j**	5.9	2.66
3k	5.61	2.4	**9k**	6.44	3.05
3l	6.09	2.8	**9l**	6.97	3.45
3m	6.56	3.2	**9m**	7.47	3.85
3n	7.45	3.99	**9n**	8.4	4.64
3p	3.87	1.29	**9p**	4.6	1.94
3o	4.33	1.55	**9o**	5.16	2.2

Table 5. Chemical Characteristics of the Synthesized Capsaicinoids and Results Obtained[a]

compound	IA log *P*	$IC_{50} \times 10^3$(mol/L)	log (1/IC_{50})	HAB	HDG	MW
3a	0.94	2.774	2.5569	4	2	195
3b	1.3	1.408	2.8514	4	2	209
3c	1.73	0.645	3.1902	4	2	223
3d	2.17	0.728	3.1379	4	2	237
3e	2.64	0.385	3.4146	4	2	251
3f	3.12	0.265	3.5770	4	2	265
3g	3.62	0.197	3.7051	4	2	279
3p	3.87	0.188	3.7251	4	2	305
3h	4.12	0.223	3.6525	4	2	293
3o	4.33	1.080	2.9666	4	2	307
3i	4.62	0.252	3.5979	4	2	307
3j	5.12	0.222	3.6527	4	2	321
3k	5.61	0.161	3.7937	4	2	335
3l	6.09	0.625	3.2041	4	2	349
3m	6.56	0.714	3.1460	4	2	363
3n	7.45	4.062	2.3912	4	2	391

[a] HAB (H acceptor bonds); HDG (H donors groups); MW (molecular weight).

Table 6. Chemical Characteristics of the Synthesized Capsinoids and Results Obtained[a]

compound	IA log *P*	$IC_{50} \times 10^3$(mol/L)	log(1/IC_{50})	HAB	HDG	MW
9a	1.26	3.638	2.4391	4	1	196
9b	1.71	2.835	2.5474	4	1	210
9c	2.18	1.170	2.9318	4	1	224
9d	2.66	1.210	2.9172	4	1	238
9e	3.17	0.601	3.2210	4	1	252
9f	3.7	0.386	3.4132	4	1	266
9g	4.24	0.356	3.4487	4	1	280
9p	4.6	0.517	3.2863	4	1	306
9h	4.79	0.186	3.7305	4	1	294
9o	5.16	0.220	3.6570	4	1	308
9i	5.34	0.181	3.7423	4	1	308
9j	5.9	0.309	3.5100	4	1	322
9k	6.44	0.467	3.3305	4	1	336
9l	6.97	0.559	3.2527	4	1	350
9m	7.47	2.433	2.6138	4	1	364
9n	8.4	2.451	2.61066	4	1	392

[a] HAB (H acceptor bonds); HDG (H donors groups); MW (molecular weight).

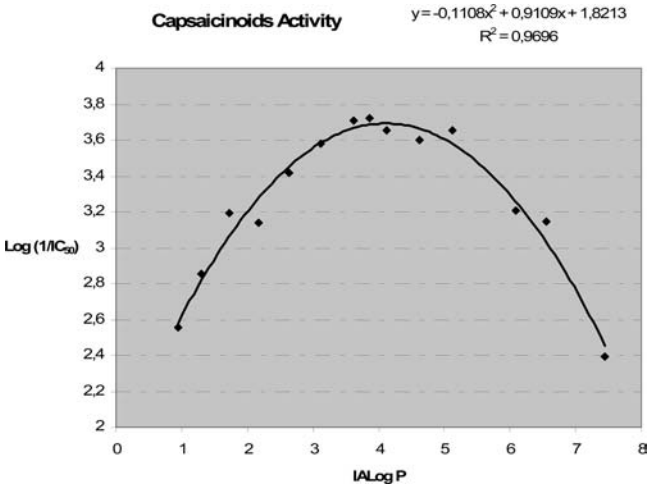


Figure 8. Representation of IAlog *P* vs log(1/IC_{50}) for synthesized capsaicinoids.

activity; these products have lipophilia values very similar to that of capsaicin. A similar effect was observed in **Figure 9**, where the most active products are **9h**, **9i**, and the natural capsinoid, dihydrocapsiate (**9o**). In general, the further the lipophilia values (log *P*) were from those found naturally, whether higher or lower, the more reduced were the biological properties of the synthesized compounds.

The results show that, out of all the capsaicinoids and capsinoids assayed, the natural capsaicinoids and capsinoids, and those synthetic compounds with lipophilia values similar to those of the natural products, are the most active biologically. This is more important in the case of capsinoids; although these natural compounds are found in peppers, very few varieties of pepper possess congeners. To obtain sufficient volume of these capsinoids to take advantage of their beneficial properties, they will need to be synthesized.

Because of these results, a logical question is why the plant employs modified fatty acids for the production of these compounds, instead of linear, more accessible fatty acids. A similar case exists in sorgoleone, whose analogues with simpler chains give activities similar to those of the natural products (*37*). It has been suggested that the plant possibly utilizes these transformations to mark, in some way, compounds that are necessary, to thus regulate the biosynthesis of these metabolites (*38*).

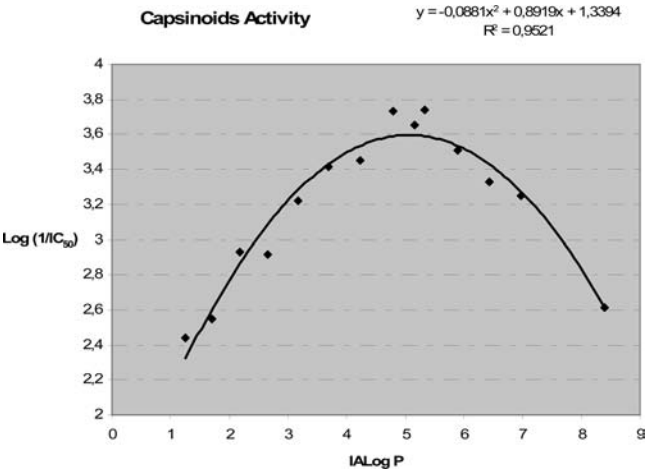


Figure 9. Representation of IAlog *P* vs log(1/IC_{50}) for the synthesized capsinoids.

With respect to the biological properties of the capsaicinoids and capsinoids, it was observed (**Figures 8** and **9**) that the two families of compounds exhibit similar biological properties. Both similar inhibit coleoptile growth, in those products with the most activity. The only observable difference between capsaicinoids and capsinoids is the following: in the capsaicinoids, the maximum activity is with products having 8 and 9 carbon atoms in the lateral chain ($3.62 < \text{IAlog } P < 4.12$), including capsaicin, but, in the capsinoids, the maximum activity is with those having 9 and 10 carbon atoms in the lateral chain ($4.79 < \text{IAlog } P < 5.34$).

The synthesis of natural capsaicinoids and capsinoids can also be performed chemically, but the nature of their lateral chains (methyl terminals and double links) makes their synthesis complicated and very costly. The possibility of obtaining these congeners much more easily, and economically, means that they may be of commercial value in the food and pharmaceutical industries.

Supporting Information Available: Details of capsaicinoids and capsinoids. This material is available free of charge via the Internet at http://pubs.acs.org.

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