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NMR Study of the Solution Structure of Curcumin

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Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is derived from the rhizomes of *Curcuma longa*. Although early studies concluded that curcumin exists predominantly as a keto−enol tautomer, **1b**, in several recent articles the solution structure of curcumin has been represented as a β-diketone tautomer, **1a**. We have investigated the structure of curcumin in solvents ranging in polarity from $CDCl_3$ to mixtures of DMSO-d_6 in water, and in buffered aqueous DMSO-d_6 solutions with pH values varying from 3 to 9. The solution structure of curcumin was determined on the basis of NMR techniques, including DEPT, HMQC, HMBC, and COSY. The results of the NMR studies show definitely that curcumin exists in solution as keto−enol tautomers, **1b**.

Curcumin (diferuloylmethane), the major active component of turmeric, *Curcuma longa* L. (Zingiberaceae), is used as a spice in curry and as coloring agent in yellow mustards, cosmetics, pharmaceuticals, and hair dyes. It has attracted interest because of its antioxidant, anti-inflammatory, and potential cancer chemopreventive activities.[1−8] Recently curcumin has also been found to bind to β-amyloid proteins in models of Alzheimer's disease.[9]

In spite of its range of biological activities, the various mechanisms of action of curcumin have not yet been fully elucidated. In considering potential mechanisms for its range of biological activities, it is important to acknowledge the inherent chemical features of the curcumin molecule. Although the systematic name for curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-hepatadiene-3,5-dione, implies that curcumin is a β-diketone tautomer, **1a**, X-ray crystal structure analyses have established that curcumin and its bisacetoxy derivative exist as keto−enol tautomers, **1b**, in the solid state.[10,11] Relatively few articles, however, explicitly consider the keto−enol tautomers of curcumin. The majority of publications, in a range of journals, illustrate only the β-diketone tautomer, **1a**, of curcumin. (See, for example, refs 8 and 12−16.) In fact, there are three possible structures of curcumin: the β-diketone tautomer, **1a**, and two equivalent asymmetric keto−enol tautomers, **1b**.

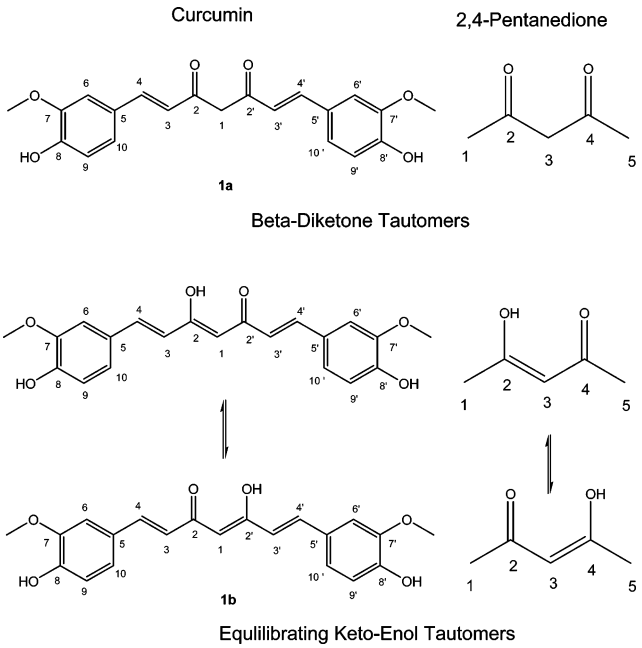

Figure 1. Potential solution structures of curcumin and 2,4-pentanedione.

Jovanovic et al. noted that the keto−enol−enolate equilibrium of the heptadienone moiety of curcumin will determine its physi-

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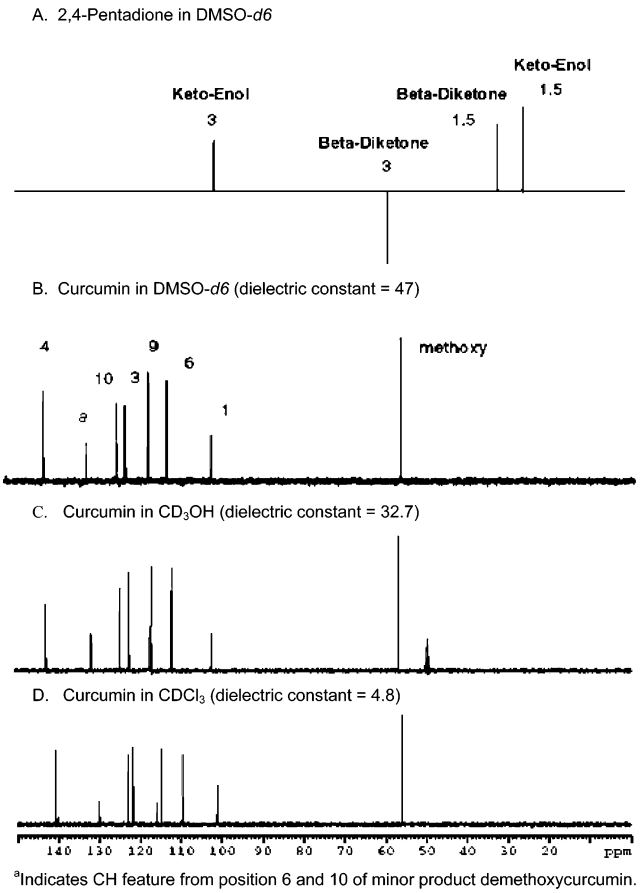

[a]Indicates CH feature from position 6 and 10 of minor product demethoxycurcumin.

Figure 2. DEPT135 spectra. Numbers correspond to ^{13}C positions as presented in Figure 1.

cochemical and antioxidant properties and then stated that the β-diketone tautomer of curcumin is likely to predominate in slightly acidic aqueous solutions and in the interior of cell membranes.[17] No evidence was provided to support the proposal that **1a** predominates under these conditions. Jovanovic et al. also proposed that the central methylene group of **1a** serves as an extraordinarily potent hydrogen atom donor in radical reactions and, on the basis of the observed rates of reaction of curcumin with methyl radicals, concluded that the enolate form of curcumin predominates above pH 8 and that above this pH the phenolic groups of the curcumin enolate become sites of electron donation.[17] Other investigations of curcumin and curcuminoids, however, concluded that in solution curcumin exists predominately as **1b**, at least in solvents like $CDCl_3$ and DMSO-d_6.[18−22] For example, in a detailed study, Roughley and Whiting concluded from the ^1H NMR spectrum of curcumin in $CDCl_3$ that this compound exists entirely as **1b** in this solvent and detected a signal assigned to the enol hydrogen in the ^1H NMR of curcumin in acetone-d_6 at −90 °C.[18] It can be readily seen that the hydrogen-bonding possibilities for the β-diketone, **1a**, and keto−enol tautomers, **1b**, of curcumin differ significantly (see Figure 1). Because this property of curcumin may be of critical importance in determining the selectivity of its binding to proteins, including the β-amyloid protein species in models of Alzheimer's disease,[9] we have reinvestigated the NMR spectrum of curcumin in a series of solvents with modern techniques to determine if the keto−enol forms predominate in protic solvents as well as in $CDCl_3$. We have also extended this study to include a range of pH values in order to address explicitly the suggestion of Jovanovic et al.[17]

The significant difference between **1a** and **1b** lies in the hybridization of the bridging carbon, position 1, as alternatively methylene or methine (Figure 1). NMR spectroscopy is clearly the method of choice to use in making such structural distinctions. The β-diketone core of curcumin is represented by 2,4-pentanedione. The NMR spectra of 2,4-pentanedione in DMSO-d_6 clearly show features from both **1a** and **1b**, with **1b** present at an excess of approximately 12%. Significantly the DEPT135 spectrum of 2,4-pentanedione shows the β-diketone position C-3 methylene, corresponding to position C-1 in curcumin, at 58 ppm, while the keto−

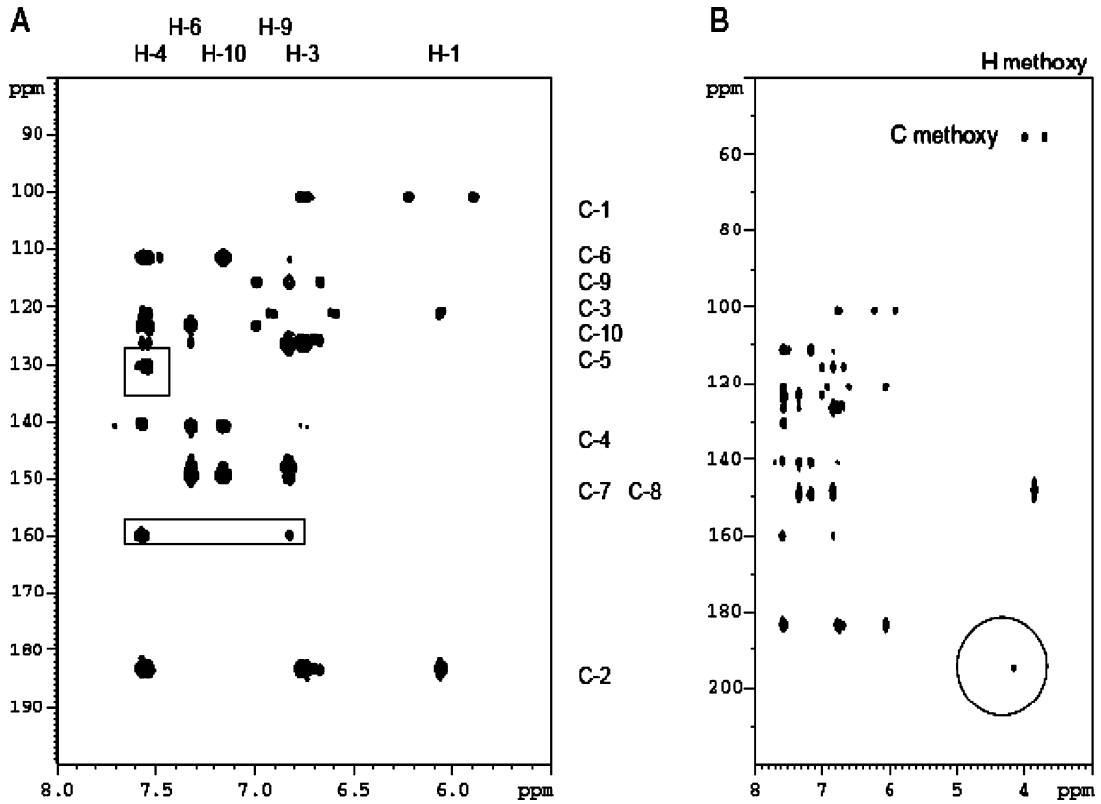

Figure 3. HMBC spectra of 70 mM curcumin in DMSO-d_6. (A) Boxes indicate features from demethoxycurcumin. (B) Circle indicates "spot" that may be the position 1 to position 2 cross-peak of **1a** present at a very low concentration.

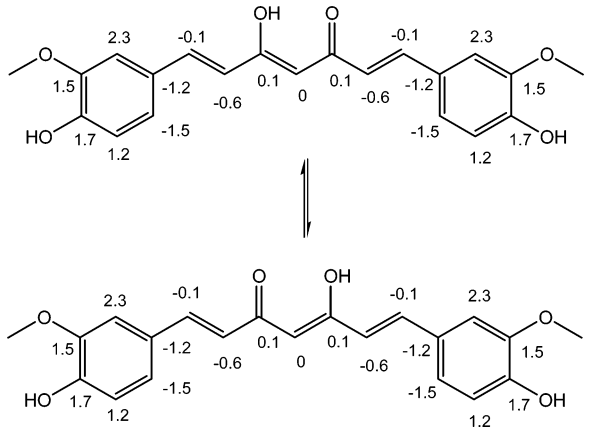

Figure 4. Solvent-induced changes in ^{13}C NMR chemical shifts (DMSO-d_6 to $CDCl_3$).

enol position C-3 methine is observed at 100 ppm (see Figure 2A). The ^{13}C NMR spectrum of 2,4-pentanedione shows separate carbonyl peaks for the β-diketone and keto−enol forms, occurring at 203 and 191 ppm, respectively. Positions C-1 and C-2 in the 2,4-pentanedione keto−enol tautomers are equivalent, respectively, to positions C-5 and C-4. Thus, the two asymmetric keto−enol tautomers rapidly interconvert in solution (see Figure 1). NMR spectra of DMSO-d_6 solutions of curcumin show only features from the keto−enol spectrum. No position C-1 methylene, characteristic of **1a**, is evident in the DEPT135 spectrum (Figure 2B). Complete assignments of the ^{13}C and ^{1}H NMR spectra of curcumin in DMSO-d_6 were made using DEPT, COSY, HMQC, and HMBC spectra and calculated ^{13}C chemical shifts (see Figure 3). The ^{13}C assignments are in complete agreement with those given in refs 18 and 20. Demethoxycurcumin, a natural product of *Curcuma longa*, which is co-isolated with curcumin,[23] is also present at approximately 30%, and this species also occurs entirely as keto−enol tautomers (assignments not shown). As with 2,4-pentanedione, the two asymmetric keto−enol tautomers of curcumin and demethoxycurcumin rapidly interconvert in solution.

While no β-diketone tautomer, **1a**, is evident in any of the 1D NMR spectra, a spot occurring in one HMBC spectrum of curcumin in DMSO-d_6 may represent the β-diketone position C-1 methylene ^{1}H to position C-2 ^{13}C cross-peak (see Figure 3B). No corresponding methylene peak is observed in the DEPT135, nor are any other spectroscopic features assignable to **1a** observed in the HMBC spectrum or any other spectra. This "spot" did not reproduce in other curcumin solutions; however, it may represent **1a** present at a concentration of less than 1%.

The NMR spectroscopic analysis performed on the DMSO-d_6 solution of curcumin was repeated in other solvent systems, including a D_2O/DMSO-d_6 mixture (3:7), CD_3OD, CD_3COOD, and $CDCl_3$. This is a set of solvents that spans both polar and nonpolar environments, encompasses a range of dielectric constants from 4.8 to 47 or greater, and includes both protic and nonprotic solvents. In all these solutions, the spectra of curcumin and demethoxycurcumin indicate only the presence of rapidly interconverting keto−enol tautomers. Small changes in the ^{13}C chemical shifts were observed when going from the more polar solvents to $CDCl_3$; however these changes occurred mainly in the aromatic rings rather than the keto−enol core (see Figure 4).

As noted above, it has been suggested that **1a** predominates in acidic aqueous DMSO solutions below pH 7.[17] However, characterization of curcumin under these solvents and pH conditions using ^{1}H, ^{13}C, DEPT45, DEPT90, and DEPT135 spectra clearly indicates that only the keto−enol tautomers, **1b**, are present at any observable concentration (see Figure 5). The absence of a negative methylene feature in the DEPT135 spectra clearly indicates that no observable quantity of **1a** is present either at pH values between 7 and 3, where

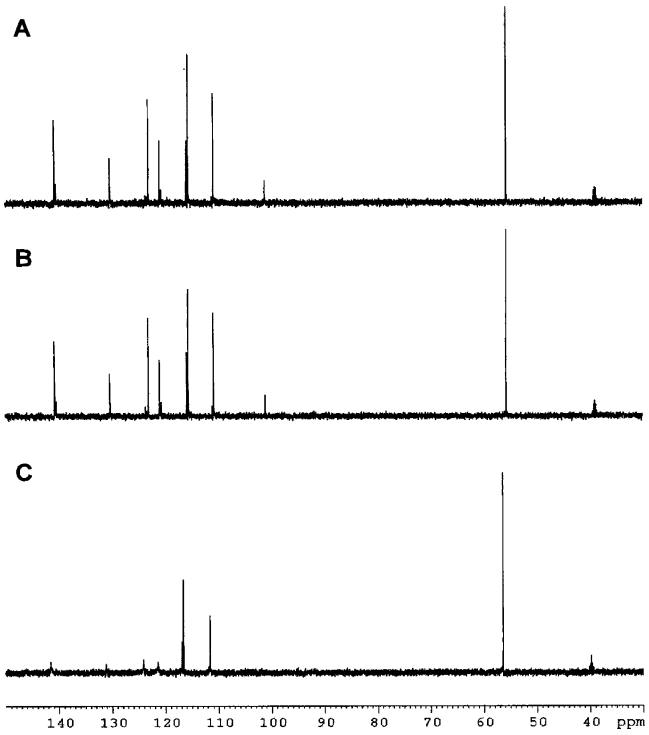

Figure 5. DEPT135 NMR spectrum of 23 mM curcumin in 30% D_2O/70% DMSO-d_6 over a range of pH's: (A) pH 3, (B) pH 6, (C) pH 9.

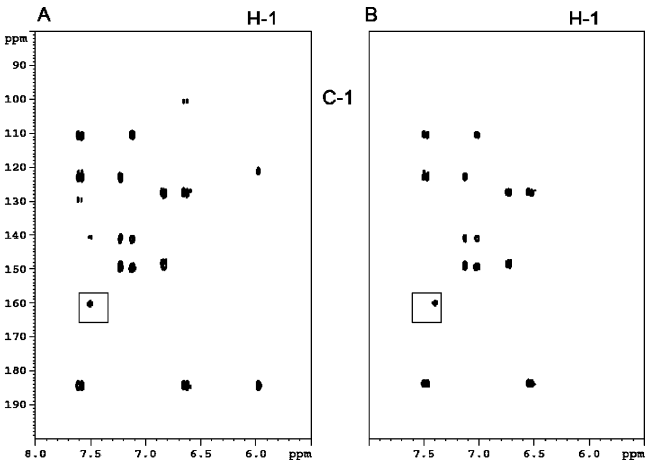

Figure 6. HMBC of 7 mM solutions of curcumin (A) in CD_3OH, (B) in CD_3OD. Note absence of any position 1 features in B. Boxes indicate features from demethoxycurcumin.

curcumin is chemically stable, or at pH values between 7 and 9, where curcumin rapidly degrades to other chemical species.[24]

Although no dramatic solvent-induced change was observed in the spectra of curcumin, in CD_3OD and CD_3COOD complete substitution of the C-1 methine ^{1}H for ^{2}H occurs, completely eliminating the C-1 features from the 1D ^{1}H, DEPT, HMQC, and HMBC spectra. Repeating the NMR experiments in CD_3OH restores the C-1 methine ^{1}H features (see Figure 6). The observed exchange of the position C-1 methine hydrogen of curcumin in protic solvents implies the presence of an unobservable amount of **1a** in equilibrium with **1b**.

The NMR experiments presented above establish that the keto−enol tautomer, **1b**, form of curcumin is essentially the only form of this molecule present in a variety of solvents ranging from chloroform to mixtures of dimethylsulfoxide and water, and buffered aqueous DMSO-d_6 solutions varying in pH from 3 to 9. These results are consistent with previous NMR investigations of curcumin

and curcumin derivatives.[18−22] Since it is possible to write hydrogen-bonded structures for the keto−enol tautomers, **1b**, of curcumin with peptide bonds, these tautomers may be involved with the binding to β-amyloid protein species in the models of Alzheimer's disease, as observed by Yang et al.[9] The chelating properties of **1b** may also be important in binding to the Zn ion during inhibition of aminopeptidase N.[13] Recently Balasubramanian used high-level ab initio computations to calculate the structure of curcumin.[25] Balasubramanian's calculations predict that, as described here, the keto−enol forms of curcumin are favored. It was also concluded that the keto−enol tautomers of curcumin will possess the properties of an ideal antioxidant and the features required to penetrate the blood−brain barrier and to bind to amyloid β-protein. Due to the impressive range of biological activities observed for curcumin, it is important to acknowledge its inherent chemical structure in order to understand its properties. Future articles describing the various biological activities of curcumin should consider the possible physicochemical properties of the keto−enol tautomers, **1b**, and explicitly include these forms whenever illustrating the structure of curcumin.

Experimental Section

General Experimental Procedures. Curcumin and 2,4-pentanedione were purchased from Sigma Aldrich (St. Louis, MO) and used without further purification. Curcumin has a ~70% purity. Deuterated NMR solvents, DMSO-d_6, D_2O, $CDCl_3$, CD_3OD, CD_3OH, and acetic acid-d_6, were purchased from Cambridge Isotope Laboratories (Cambridge, MA). NMR samples of 2,4-pentanedione in DMSO-d_6 were prepared at a concentration of 20% by volume. NMR samples of curcumin in $CDCl_3$, CD_3OD, CD_3OH, and acetic acid-d_4 were prepared at 7 mM, just below the saturation point of curcumin in these solvents. NMR samples of curcumin in unbuffered DMSO-d_6 were prepared at 70 mM. In preparing pH-buffered NMR samples a stock solution of 35 mM curcumin was made up in DMSO-d_6, and 700 μL of this solution was mixed with 300 μL of 200 mM H_2O−phosphate buffer at the appropriate pH, giving a final curcumin concentration of 23 mM.

NMR Experiments and Analysis. NMR spectra were run on a 500 MHz Bruker AVANCE DRX instrument using a broadband probe equipped with a z-gradient coil (Bruker-Biospin, Freemont, CA). All NMR samples were 600 μL and run in standard 5 mm NMR tubes at 25 °C. Pulse programs used were standard sequences taken from the Bruker XWINNMR pulse sequence library. NMR experiments were set up and processed generally using the parameters suggested in *200 and More NMR Experiments: A Practical Course*.[26] The pulse programs selected for the 2D COSY, HMQC, and HMBC experiments employed z gradients for coherence pathway selection. ^{1}H and ^{13}C chemical shifts were calibrated relative to the solvents and TMS. ^{1}H and ^{13}C chemical shift calculations were performed using the tables found in ref 27.

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References and Notes

(1) Ammon, H. P. T; Wahl, M. A. *Planta Med.* **1991**, *57*, 1−7.
(2) Tønnesen, H. H. In *Phenolic Compounds in Foods and Their Effects on Health*; Ho, C.-T., Lee, C. Y., Huang, M.-T., Eds.; ACS Symposium Series 506; American Chemical Society: Washington, DC, 1992; Chapter 11, pp 143−153.
(3) Sharma, O. P. *Biochem. Pharmacol.* **1976**, *25*, 1811−1812.
(4) Toda, S.; Miyase, T.; Arichi, H.; Tanizawa, H.; Takino, Y. *Chem. Pharm. Bull.* **1985**, *33*, 1725−1728.
(5) Srimal, R C.; Dhawan, B. N. *J. Pharm. Pharmacol.* **1973**, *25*, 447−452.
(6) Rao, T. S.; Basu, N.; Siddiqui, H. H. *Ind. J. Med. Res.* **1982**, *75*, 574−578.
(7) Conney, A. H.; Lou, Y.-R.; Xie, J.-G.; Osawa, T.; Newmark, H. L.; Liu, Y.; Chang, R. L.; Huang, M.-T. *Proc. Soc. Exp. Biol. Med.* **1997**, *216*, 234−245.
(8) Surh, Y-J. *Nat. Rev. Cancer* **2003**, *3*, 768−780.
(9) Yang, F.; Lim, G. P.; Begum, A. N.; Ubeda, O. J.; Simmons, M. R.; Ambegaokar, S. S.; Chen, P.; Kayed, R.; Glabe, C. G.; Frautschy, S. A.; Cole, G. M. *J. Biol. Chem.* **2005**, *280*, 5892−5901.
(10) Tønnesen, H. H.; Karlsen, J.; Mostad, A. *Acta Chem. Scand. Ser. B* **1982**, *36*, 475−479.
(11) Mague, J. T.; Alworth, W. L.; Payton, F. L. *Acta Crystallogr.* **2004**, *C60*, 608−610.
(12) Huang, M.-T.; Ma, W.; Yen, P.; Xie, J.-G.; Han, J.; Frenkel, K.; Grunberger, D.; Conney, A. H. *Carcinogenesis* **1997**, *18*, 83−88.
(13) Shim, J. S.; Kim, J. H.; Cho, H. Y.; Yum, Y. N.; Kim, S. H.; Park, H-J.; Shim, B. S.; Choi, S. H.; Kwon, H. J. *Chem. Biol.* **2003**, *10*, 695−704.
(14) Wortelboer, H. M.; Usta, M.; van der Velde, A. E.; Boersma, M. G.; Spenkelink, B.; van Zanden, J. J.; Rietjens, I. M. C. M.; van Bladeren, P. J.; Cnubben, N. H. P. *Chem. Res. Toxicol.* **2003**, *16*, 1642−1651.
(15) Dalton, L. *Chem. Eng. News* **2003**, *81* (35), 8.
(16) Halford, B. *Chem. Eng. News* **2004**, *82* (17), 9.
(17) Jovanovic, S. V.; Steenken, S.; Boone, C. W.; Simic, M. G. *J. Am. Chem. Soc.* **1999**, *121*, 9677−9681.
(18) Roughley, P. J.; Whiting, D. A. *J. Chem. Soc., Perkin Trans. 1* **1973**, *20*, 2379−2388.
(19) Matthes, H. W. D.; Luu, B.; Ourisson, G. *Phytochemistry* **1980**, *19*, 2643−2650.
(20) Unterhalt, B.; Z. *Lebens. Unters. Forsch.* **1980**, *170*, 425−428.
(21) Pedersen, U.; Rasmussen, P. B.; Lawesson, S.-O. *Liebigs Ann. Chem.* **1985**, *8*, 1557−1569.
(22) Bong, P.-H. *Bull. Korean Chem. Soc.* **2000**, *21*, 81−86.
(23) Aggarwal, B. B.; Kumar, A.; Aggarwal, M. S.; Shishodia S. *Phytopharmaceuticals in Cancer Chemoprevention*; Bagchi, D., Preuss, H., Eds.; CRC Press: Boca Raton, FL, 2005; Chapter 23, pp 349−387.
(24) Wang, Y. J.; Pan, M. H.; Cheng, A. L.; Lin, L. I.; Ho, Y. S.; Hsieh, C. Y.; Lin, J. K. *J. Pharm. Biomed. Anal.* **1997**, *15*, 1867−1876.
(25) Balasubramanian, K. *J. Agric. Food Chem.* **2006**, *54*, 3512−3520.
(26) Berger, S.; Braun, S. *200 and More NMR Experiments: A Practical Course*; J. Wiley & Sons: New York, 2004; pp 185−190, pp 526−529, pp 538−545.
(27) Crews, P.; Rodríguez, J.; Jaspars, M. *Organic Structure Analysis*; Oxford University: New York, 1998; pp 53−95.

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