with acetic anhydride (58 mg, 0.57 mmol) at −60 °C for 10 min. After the reaction was quenched with oxalic acid (26 mg, 0.29 mmol) for 2 h according to method B, it was analyzed by ^{1}H NMR (300 MHz, $CDCl_3$, $\delta(OCH_2CHO)$ (ABX)): (mME) AB 3.73–3.87 (m, 2 H), X 5.82 (m, 1 H), (lME) A 4.10–4.17 (m, 1 H), B 4.22–4.27 (m, 1 H), X 4.92 (m, 1 H), (DE) AB 4.23–4.34 (m, 2 H), X 5.99 (m, 1 H), (1-phenyl-1,2-ethanediol) A 3.56–3.62 (m, 1 H), B 3.67–3.72 (m, 1 H), X 4.76 (m, 1 H).

The Liquid, Solid, and Molecular Force Field Calculated Conformations of Savinin

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Following the unambiguous assignment of the ^{1}H and ^{13}C NMR spectra of the cytotoxic lignan savinin (1), its solution conformation has been studied through ^{1}H NMR spectroscopy. Surprisingly, evidence was found for overlap of the aromatic rings and for the proximate nature of H-2 and H-2′ and H-7 and H-7′. In contrast, the conformation in the solid state was established to be in a "spread-eagle" orientation, the energy of which was calculated to be 45.986 kcal/mol. Molecular force field calculations also gave evidence for two minima at 46.128 and 45.820 kcal/mol whose conformations were in agreement with the NOE data.

Introduction

The conformational analysis of small flexible molecules possessing biological activity is presently an area of great interest. Continuing our studies[2] in this area, we chose to examine the compound savinin (1), an α-arylidene γ-lactone lignan found in several plant species[3-10] and isolated by us as a cytotoxic constituent of *Aristolochia indica* roots.[11] The present study of savinin (1) was originally initiated to establish unambiguous proton and carbon-13 NMR assignments. The interesting results that emanated from the NMR analysis prompted us to pursue a more detailed study of savinin (1) comparing its respective solution, solid, and calculated energy-minimized conformations. In the course of this study, a broad combination of complimentary 1D and 2D NMR techniques, including the homonuclear shift correlation (COSY), two-dimensional nuclear Overhauser experiments (NOESY), and hetero-

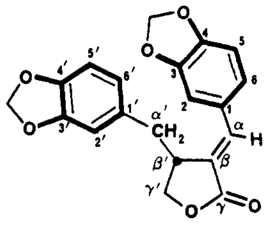

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Table I. ^{1}H NMR Assignments, ^{1}H T_1 Data, and Observed Spatial Assignments of Savinin (1)

proton	δ,[a] ppm	^{1}H T_1,[b] s	cross peaks obsd (δ, ppm)[c]	
α_{ax}-H	2.524	0.6	α'_{eq}H H-6′	(2.930) (6.577)
α'_{ex}-H	2.930	0.5	α'_{ax}-H H-2 H-2′	(2.524) (6.982) (6.609)
β′-H	3.680	0.9	γ'_{cis}-H H-2′	(4.204 or 4.187) (6.609)
$\gamma'_{cis,trans}$-H	4.204 or 4.187	0.7	β′-H H-6′	(3.680) (6.577)
α-H	7.431	2.6	H-6	(7.206)
H-6	7.206	1.4	H-5 α-H	(6.821) (7.431)
H-2	6.982	1.6	H-6′	(6.577)
H-5	6.821	2.8	H-6	(7.206)
OCH_2O[d]	5.985	1.4	H-2 H-5	(6.982) (6.821)
OCH_2O[e]	5.820, 5.881	1.5	H-2′ H-5′	(6.609) (6.675)
H-6′	6.577	1.8	H-5′ H-2 α'_{ax}-H	(6.675) (6.982) (2.574)
H-5′	6.675	2.7	H-6′	(6.577)
H-2′	6.609	2.3	β′-H H-2 α'_{eq}-H	(3.680) (6.982) (2.930)

[a] All chemical shifts are expressed in parts per million relative to tetramethylsilane (TMS) δ = 0.00 ppm. [b] Measured by using an inversion–recovery sequence. Sample concentration = 40 mg/0.5 mL in $CDCl_3$ at 25 °C. The SD for the T_1 data is ±1–5%. [c] Determined from proton 2D NOE (NOESY) results (mixing time = 3.0 s). [d] Conjugated ring. [e] Saturated ring.

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[2] Hamburger, M. O.; Shieh, H. L.; Zhou, B.-N.; Pezzuto, J. M.; Cordell, G. A. *Magn. Reson. Chem.* **1989**, *27*, 1025.

[3] Matsumura, M.; Okumura, F. S. *J. Am. Chem. Soc.* **1955**, *77*, 1906.

[4] Lin, Y. T.; Wang, K. T.; Weinstein, B. *Chem. Commun.* **1965**, 592.

[5] Reisch, J.; Novak, I.; Szendrei, K.; Minker, E. *Pharmazie* **1967**, *22*, 220.

[6] Martinez, E. A.; Funes, J. L. B.; Gonzalez, A. G.; Luis, F. R. *An. Quim.* **1969**, *65*, 809.

[7] Reisch, J.; Szendrei, K.; Novak, I.; Minker, E. *Pharmazie* **1970**, *25*, 435.

[8] Corrie, J. E. T.; Green, G. H.; Ritchie, E.; Taylor, W. C. *Aust. J. Chem.* **1970**, *23*, 133.

[9] Badawi, M. M.; Seida, A. A.; Kinghorn, A. D.; G. A.; Farnsworth, N. R. *J. Nat. Prod.* **1981**, *44*, 331.

[10] Ozaki, N.; Hasegawa, S.; Hirose, Y. *Phytochemistry* **1983**, *22*, 1771.

[11] Che, C.-T.; Ahmed, M. S.; Kang, S. S.; Waller, D. P.; Bingel, A. S.; Martin, A.; Rajamehendran, P.; Bunyapraphatsara, N.; Lankin, D. C.; Cordell, G. A.; Soejarto, D. D.; Wijesekera, R. O. B.; Fong, H. H. S. *J. Nat. Prod.* **1984**, *47*, 331.

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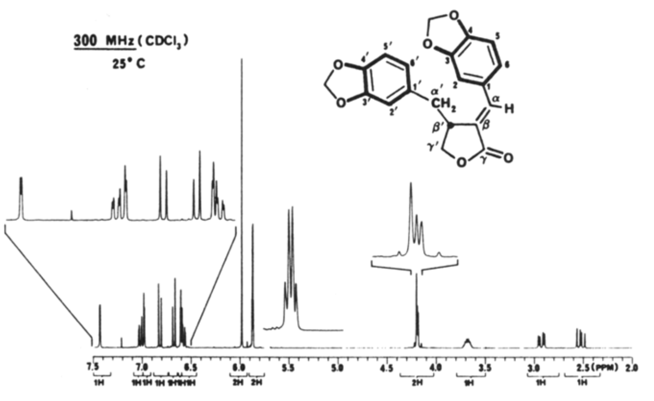

Figure 1. Proton NMR spectrum of savinin (1).

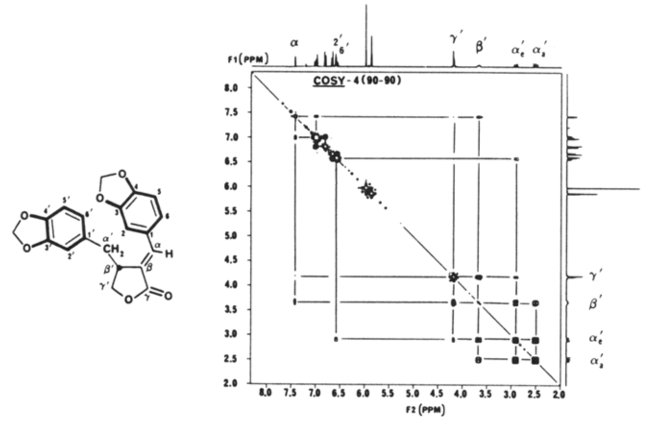

Figure 2. Homonuclear COSY spectrum of savinin (1).

nuclear shift correlation (HETCOR) emphasizing both one-bond and long-range coupling, were employed. As a result, complete and unambiguous assignments of both the carbon and proton chemical shifts of savinin, including several revisions to our previous assignments, have been achieved. In addition, and because of unanticipated results derived from the solution conformation study, an X-ray crystallographic study has been carried out in order to complete the tripartate approach and have available for comparison to solution data the solid-state conformational information.

Results

NMR Studies. The proton NMR spectrum of savinin (1) obtained at 300 MHz is shown in Figure 1. All of the protons are observable and readily assignable in general terms with the exception of the γ'-CH_2O moiety of the lactone ring, whose protons exhibit near chemical shift equivalence. The proton signals of savinin (1) could be accurately assigned (Table I) with the aid of the proton homonuclear two-dimensional shift correlation (COSY) spectrum[12-14] (Figure 2) and the NOESY spectrum. The aromatic proton resonances associated with the individual, but structurally similar, 3,4-methylenedioxy rings could be uniquely assigned in this fashion. Cross-peak intensity was observed between the vinyl proton at C-α and the aromatic protons of the methylenedioxy ring attached to the exocyclic double bond, and the assignments were further substantiated by the NOESY results (Table I). One feature of the proton NMR spectrum of savinin (1) which piqued our initial interest was the observation that one of the two methylenedioxy groups appeared as a singlet (δ 5.985), indicating magnetic equivalence of the geminal protons, whereas the resonance pattern for the other methylenedioxy group appeared as an AB pattern (δ 5.870, 5.881, $J_{AB} = -1.4$ Hz), denoting magnetic nonequivalence of the geminal protons.[15] This suggested that perhaps a preferred conformation may exist for savinin in solution and was further supported by the nature of the vicinal couplings to the proton β' from the chemically nonequivalent methylene protons attached to the α'-carbon. This β'-proton showed two couplings (10.1 and 4.3 Hz) instead of a rotationally averaged (6–7 Hz) vicinal coupling constant with the α'-protons which might be expected for, and characteristic of, a freely rotating system.

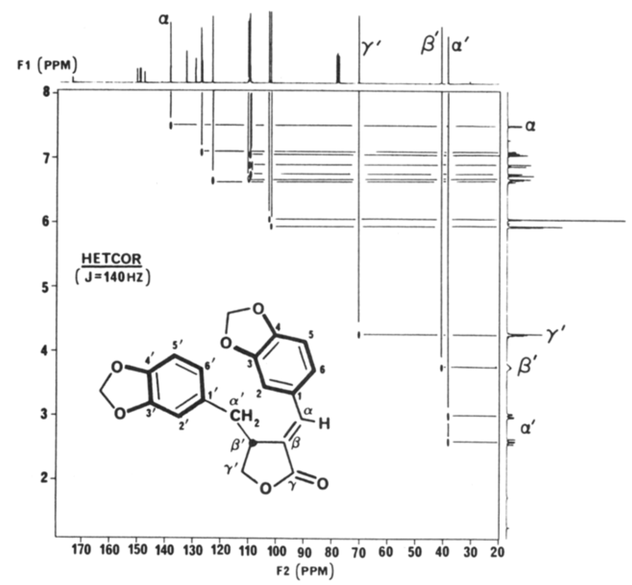

Figure 3. HETCOR spectrum of savinin (1), emphasizing one-bond couplings ($J_{CH} = 140$ Hz).

With the proton NMR spectrum assigned, attention was focused on assignment of the carbon-13 NMR resonances of savinin (1). The multiplicity of each carbon resonance was assigned with the use of the DEPT technique.[16] Correlation of the protons with the carbons to which they are attached was performed by using the HETCOR experiment. The results for the direct bond ($^1J_{CH} = 140$ Hz) coupled carbons are shown in Figure 3. All of the protons in savinin (1) were clearly observable, with the exception of the chemically nonequivalent lactone methylene protons, and were unambiguously assigned from the results of the COSY and NOESY experiments. The corresponding correlation of the protons with the protonated carbons (HETCOR) makes the assignments of the protonated carbons unambiguous.

Finally, it was then necessary to assign the nonprotonated carbons in savinin (1). This was performed by using the long-range HETCOR experiment[17] together with the results of a fully coupled carbon-13 spectrum. The results of the long-range HETCOR experiments are shown in Figures 4 and 5. In savinin (1) there are eight quaternary carbon resonances, three associated with each

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[12] Bax, A.; Freeman, R.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 169.

[13] Bodenhausen, G.; Freeman, R.; Niedermeyer, R.; Turner, D. L. *J. Magn. Reson.* **1977**, *26*, 133.

[14] Bachmann, P.; Aue, W. P.; Muller, L.; Ernst, R. R. *J. Magn. Reson.* **1977**, *28*, 29.

[15] In data reported earlier from this laboratory, this phenomenon was not observable since the data (100 MHz, ^1H) were collected on a lower field (2.3 T) instrument. The data collected at higher field revealed this information very clearly.

[16] Doddrell, D. M.; Pegg, D. T.; Bendal, M. R. *J. Magn. Reson.* **1982**, *48*, 323.

[17] For a recent review, see: Martin, G. E.; Zektzer, A. S. *Magn. Reson. Chem.* **1988**, *26*, 631.

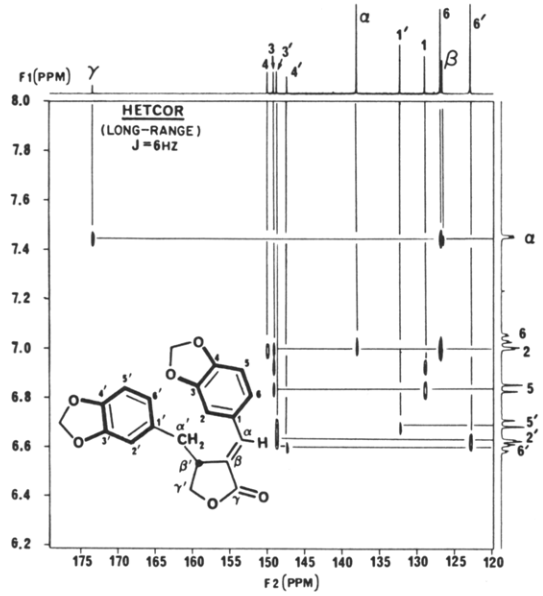

Figure 4. HETCOR spectrum of savinin (1), emphasizing three-bond couplings (J_{CH} = 6 Hz).

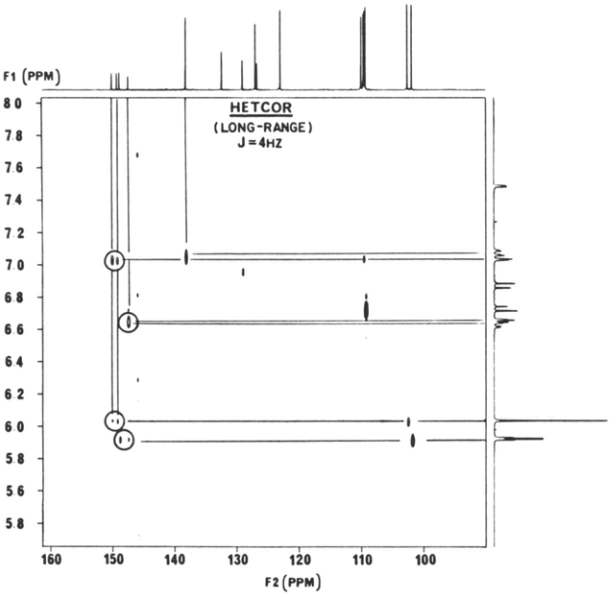

Figure 5. HETCOR spectrum of savinin (1), emphasizing three-bond couplings (J_{CH} = 4 Hz).

aromatic ring, the quaternary carbon of the exocyclic double bond, and the lactone carbonyl resonance. The latter could be assigned initially by inspection and subsequently by the long-range HETCOR results. The quaternary carbon of the olefinic double bond could be assigned since a cross peak was observed correlating with the α-proton of the double bond (two-bond). The ipso carbons of the aromatic ring could be uniquely assigned since correlation peaks from H-5 and H-5′ to C-1 and C-1′, respectively, were observed (three-bond couplings).

The quaternary carbons associated with the methylenedioxy group ring fusion were assignable on the basis of the observation of cross peaks between H-5 and H-6 and the carbons at δ 149.1 and 148.2, as well as the correlation cross peaks between H-5′ and H-6′ and the carbons at δ 147.8 and 146.4, respectively. The intensities of the cross peaks occurring at δ 149.1 and 146.4 appeared to be twice those of the correlation peaks occurring at δ 148.2 and 147.8, respectively. This suggested that the former may be the carbons having *two* three-bond couplings and therefore could be assigned to carbons C-4 and C-4′, respectively, the latter being carbon resonances having only one three-bond coupling and therefore assignable to carbons C-3 and C-3′, respectively. Confirmation was derived by examination of the fully coupled carbon-13 spectrum showing that region of the spectrum for the four carbon resonances in question (Figure 6). The fully coupled resonances for the signals at δ 149.1 and 146.4 were observed to be about twice the width of the signals occurring at δ 148.2 and 147.8. In addition, the methylenedioxy resonances could now be assigned *unambiguously*, since cross-peak correlations were observed between the methylenedioxy protons and the corresponding oxygen-bearing aromatic carbons (J = 4 Hz, Figure 5).[18] Thus,

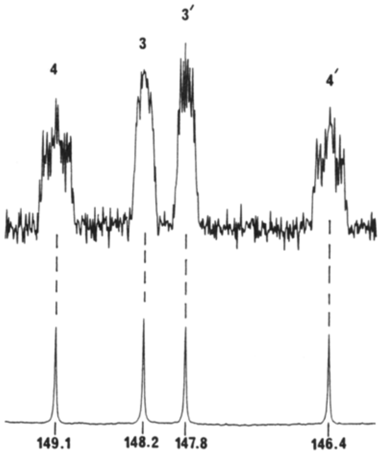

Figure 6. Coupled carbon-13 NMR spectrum of savinin (1) in the region 146–149 ppm.

Table II. Carbon-13 Chemical Shift Assignments and Carbon T_1 Data for Savinin (1)

carbon assignment	chemical shift,[a] δ	carbon NOE	carbon T_1,[b] s
C=O (γ)	172.5	1.3	21.1
C-4	149.1	1.3	19.0
C-3	148.2	1.4	19.9
C-3′	147.8	1.3	19.5
C-4′	146.4	1.2	20.3
C-α	137.2	2.7	0.9
C-1′	131.4	1.5	7.7
C-1	128.1	1.2	9.7
C-6	126.0	2.7	1.0
C-β	125.7	2.4	9.9
C-6′	122.0	2.7	1.2
C-2′	109.1	2.5	1.3
C-5′	108.7	2.6	1.1
C-2	108.6	2.6	1.1
C-5	108.4	2.5	1.2
OCH_2O[c]	101.7	2.8	0.9
OCH_2O[d]	101.0	2.8	1.0
C-γ′	69.4	3.0	0.6
C-β′	39.8	2.9	1.0
C-α′	37.4	2.7	0.6

[a] All chemical shifts are expressed in parts per million relative to TMS (δ = 0.00 ppm). [b] Measured by using an inversion–recovery sequence. Sample concentration = 40 mg/0.5 mL in $CDCl_3$ at 25 °C. The SD for the T_1 data is ±1–5%. [c] Conjugated ring. [d] Saturated ring.

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(18) An alternative approach for assigning the methylenedioxy protons would be to employ a relayed COSY[19] experiment looking for the weak couplings between the methylenedioxy protons and the adjacent aromatic protons on the respective rings to which they are bonded. A series of homonuclear relay coherence transfer experiments unfortunately failed to show any cross peak of that described above, indicating the interproton coupling to be about 0 Hz.

(19) Eick, G.; Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 3731.

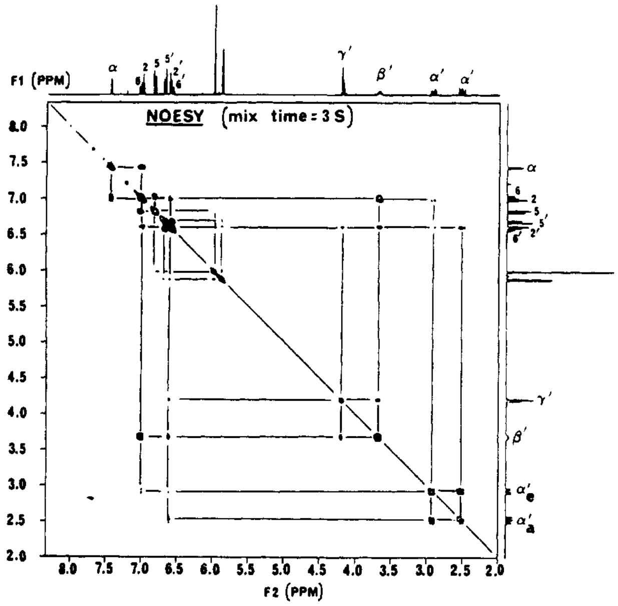


Figure 7. NOESY spectrum of savinin (1).

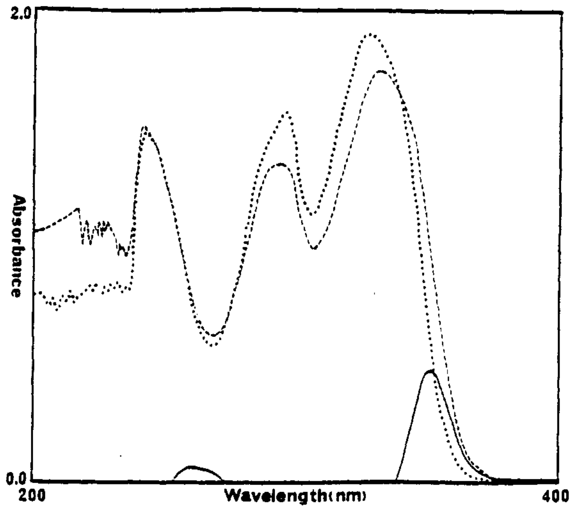
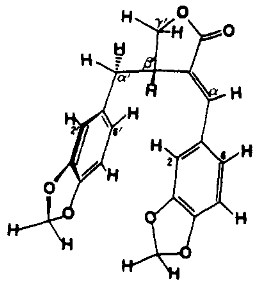


Figure 8. UV absorption spectra of (a) Savinin (1) (···) and (b) a 1:1 mixture of lactones 3 and 4 (···) and (c) difference spectrum (—).

a self-consistent set of assignments for the carbon resonances for savinin (1) emerged. The carbon-13 NMR spectral assignments, together with the carbon T_1 data, are summarized in Table II.

With the proton and carbon-13 NMR spectral assignments completed, attention turned to the conformational issues. The nature of the preferred conformation of savinin (1) in solution was evaluated by using phase-sensitive homonuclear 2D nuclear Overhauser enhancement spectroscopy (NOESY).[20] The NOESY spectrum (mixing time = 3.0 s) is shown in Figure 7. Strong cross peaks were observed between the α'-axial and the α'-equatorial protons of the benzyl group. This is generally the case with protons having a geminal relationship. A strong cross peak was observed between the β'-proton of the lactone ring and the γ'-proton positioned cis to it. Strong cross peaks were also observed between the α-proton (vinyl) and the H-6 proton of the methylenedioxy ring attached to the exocyclic double bond, as well as from the β'-proton on the lactone ring to H-2 of the same methylenedioxy ring. A moderately strong cross peak was observed between the β'-proton of the lactone ring and H-2' of the methylenedioxy ring bonded to the pendent CH_2 group attached to the lactone ring. *Notably absent*, however, from the NOESY data were any cross peaks attributed to dipolar interaction between the β'-proton of the lactone ring and the protons α'-axial and α'-equatorial to the benzyl group. Thus the preferred orientation for the benzyl protons is clearly in the opposite direction in space from that of the β'-proton. Several weaker, but significant, cross peaks were also observed, all of which are summarized in Table I. On the basis of a qualitative analysis of the NOESY results, the conformation of savinin in chloroform solution may be formulated as shown in 2.



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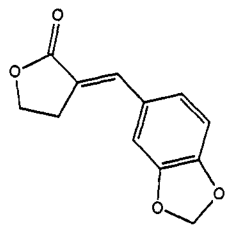
UV Studies. The spatial overlap indicated by the NMR NOE data between the two aromatic ring systems of savinin (the benzyl aromatic ring attached to C-α' being electron rich and the conjugated aromatic ring being weakly electron deficient) in $CHCl_3$ suggested the presence of a charge-transfer (C-T) interaction between these two units which might manifest itself as a band observable in the UV spectrum. The UV spectrum of savinin was obtained in both $CHCl_3$ and MeOH, where maxima were observed at 294 ($\log \epsilon$ 4.39) and 332.5 (4.51), and 292.5 (4.28) and 332.5 nm (4.39), respectively. At concentrations as high as 1 mmol in both $CHCl_3$ and MeOH, no additional bands were observed. Consequently, difference UV spectroscopy (Figure 8) was used at the 0.1 mM level in chloroform. For comparison purposes, the piperonylidene lactone derivative 3 shows $\lambda_{\max}$ 296.5 ($\log \epsilon$ 4.08) and 327 (4.16) in $CHCl_3$, and the benzylidene lactone 4 240 (3.64) and 288 (3.61). The UV spectrum of a 1:1 mixture of these two compounds (spectrum b) was subtracted from the spectrum of savinin (1) (spectrum a) at the same molar concentration. The difference spectrum (c) revealed an absorption at 351 nm which is tentatively assigned to the charge transfer (C-T) between the two phenyl moieties in savinin.



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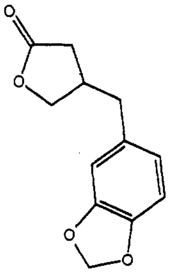

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X-ray Crystallography. Savinin crystallized with two molecules in the asymmetric unit and four molecules in the unit cell, as shown in Figure 9. As may be noted, the compound exists in the crystal state in an unfolded, "spread-eagle" conformation and is in accord with previous data obtained in our laboratories[21,22] for related lignans.

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[20] States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* 1982, *48*, 286.

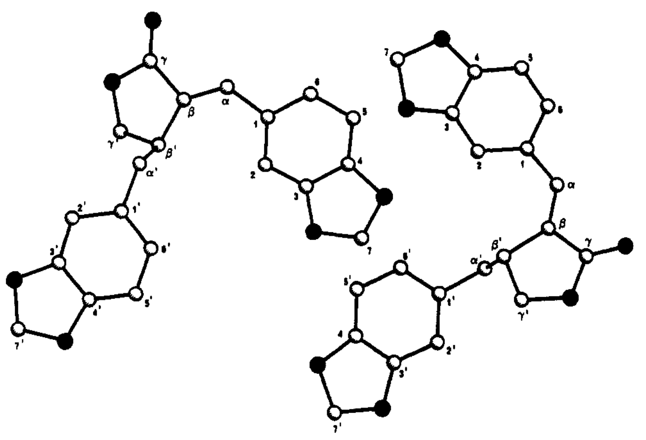

Figure 9. Crystal structure of savinin (1): two molecules in the asymmetric unit with their bond lengths.

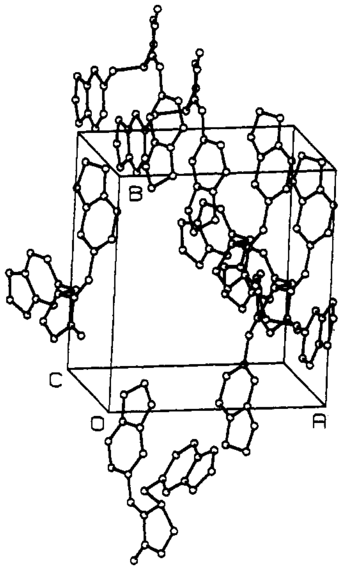

Figure 10. Crystal packing of savinin (1). Some molecules have been omitted for reasons of clarity.

Table III. Angles between Planes in the Crystal Structure of Savinin (1)

plane	angle, deg molecule 1	 molecule 2
lactone/benzodioxolane unprimed	34.9	11.8
lactone/benzodioxolane primed (′)	20.0	34.9
benzodioxolane/benzodioxolane primed (′)	18.5	24.1

The observed conformational difference between the solution (NMR) and solid states (X-ray) could be attributed to crystal packing effects. However, none of the usual indications of such effects were observed. For example, small intermolecular differences are expected for the staggering of the alignment of the aromatic moieties. However, experimentally, all that could be found were weak van der Waals contacts (3.4 Å), as shown in Figure 10. The two molecules in the asymmetric unit differ slightly in their bond lengths, and only the difference in the Cα–Cβ bond (0.09 Å) is larger than three standard deviations. Substantial variations exist between the two molecules with respect to the mutual locations of the planar groups as shown in Table III.

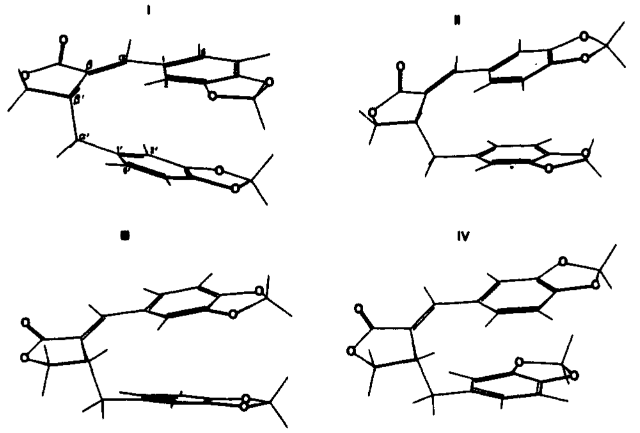

Figure 11. Energy-minimized conformations I–IV of savinin (1).

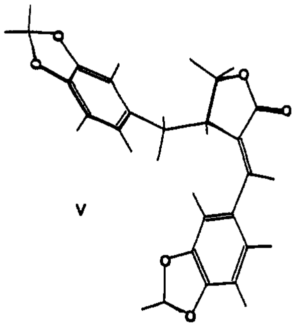

Figure 12. Energy-minimized conformation V of savinin (1).

Table IV. Distances[a] between Two Protons of the Conformations I–IV of Savinin (1)

	I	II	III	IV
2-H-β′-H	2.046	5.368	2.021	5.350
2-H-2′-H	3.868	6.296	2.960	3.838
6-H-β′-H	5.374	1.991	5.567	1.990
OCH_2O	3.176	3.393	2.467	2.612

[a] In angstroms.

Molecular Force Field Calculations. The CHEMLAB-II program[23] was used to perform the molecular force field calculations of the energies associated with some potential conformations of savinin; four of these are shown in Figure 11. In these conformers, the β′-piperonyl and β-piperonylidene units of savinin overlap, and the main difference between conformations I, II, III, and IV is that the 2-H, 2′-H, 6-H, and 6′-H lay on either the same or opposite edges of the molecule. The calculated steric energy of each conformation is 45.315, 46.032, 45.824, and 46.189 kcal/mol, respectively. As shown in Table IV, the distances between β′-H and 2-H are 2.046 Å in conformation I, 5.368 Å in conformation II, 2.021 Å in conformation III, and 5.350 Å in conformation IV. The distances between β′-H and 6-H are 5.374, 1.991, 5.567, and 1.990 Å in the conformations I, II, III, and IV. The other important geometrical characters from the NMR studies are the distances between the pairs of methylenedioxy protons on the two aromatic rings; these are calculated to be 2.467 and 2.612 Å in conformations III and IV and 3.176 Å and 3.393 Å in conformations I and II, respectively.

Conformation V (Figure 12) was generated from X-ray crystallographic data[24] and has a minimum steric energy

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(21) Zhuang, L.-G.; Seligmann, O.; Lotter, H.; Wagner, H. *Phytochemistry* **1983**, *22*, 263.

(22) Woo, W. S.; Shin, K. H.; Wagner, H.; Lotter, H, *Phytochemistry* **1987**, *26*, 1542.

(23) CHEMLAB-II Revision 10.0 (1988), Chemlab Inc. Distributed by Molecular Design Limited, 2131 Farallon Dr., San Leandro, CA 94577.

(24) Wang, Y.; Cheng, M. C.; Jan, S. T.; Cheng, S. C. *Acta Crystallog.* **1987**, *C43*, 1005.

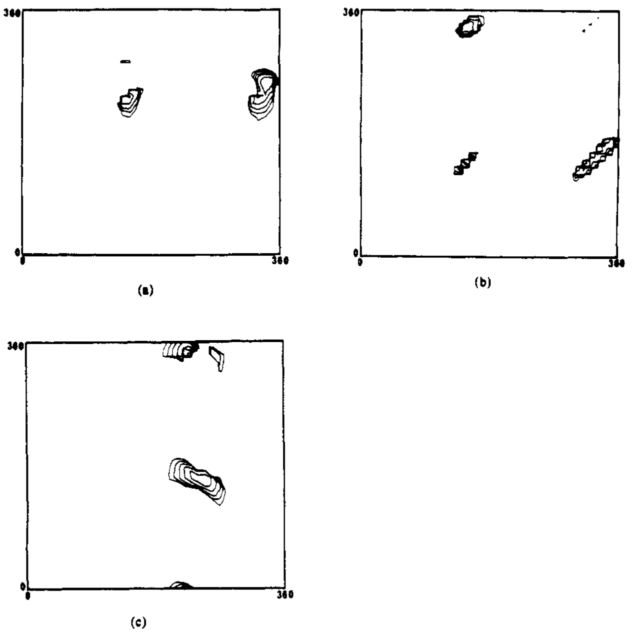

Figure 13. Energy contour plot for conformation V of savinin (1). Contours have been drawn at intervals of 2 kcal/mol. Torsional angles (a) Cα′–Cβ′, (b) C1′–Cα′, and (c) C1′–Cα′ have been varied along the vertical axis, and (a) C1–Cα, (b) C1–Cα, and (c) Cα′–Cβ′, along the horizontal axis.

Table V. Distances[a] between Two Protons and Steric Energy of Four Low-Energy Conformations of Savinin (1)[b]

	I	II	III	IV
2-H-β′-H	2.011	5.343	1.979	5.347
2-H-2′-H	4.115	6.083	2.832	3.982
6-H-β′-H	5.354	1.985	5.331	1.980
OCH_2O	4.076	3.066	2.659	2.888
steric energy[c]	45.328	46.138	45.938	46.333

[a] In angstroms. [b] These conformations were generated by rotating the bonds C1′–Cα′, Cα′–Cβ′, and C1–Cα, of the conformation V. The rotations were developed according to the energy map which was created by the SCAN option of the CHEMLAB-II program. [c] In kilocalories/mole.

of 45.986 kcal/mol. In this conformation, the β′-piperonyl moiety is turned away from the β-piperonylidene moiety of the molecule. The energy maps were generated as shown in Figure 13 by scanning of C1′–Cα′, Cα′–Cβ′, and C1–Cα of conformation V. There are four minimal centers which are (155° ± 5°, 240° ± 10°, 340° ± 10°), (340° ± 10°, 245° ± 5°, 150° ± 10°), (345° ± 5°, 230° ± 10°, 340° ± 10°), and (155° ± 5°, 230° ± 10°, 150° ± 10°) corresponding to the rotation of (C1′–Cα′, Cα′–Cβ′, C1–Cα). The populations of these conformations are 49.2%, 1.1%, 20.0%, and 6.3%. After energy minimization, as shown in Table V, their geometrical characters are similar to the conformations I–IV as well as to the minimal steric energies.

In the course of studying the stable conformations of savinin, we were able to compare the conformations of savinin in the solid state (from X-ray crystallographic data),[24] in the unsolvated "free state" (from computer modelling),[20] and in solution (from the NMR study).[25] According to the molecular force field calculations, the most energetically favorable conformation of savinin is conformation I. The differences in steric energy between conformation I and the others are not great, but these four conformations have different populations. In the NMR study, β′-H shows a NOE with 2-H and 6-H, and besides β′-H, 2-H has a NOE with 2′-H. The distances between 2-H and β′-H in conformation I and III and 6-H and β′-H in conformation II and IV obtained from computer modelling suggested that they are close enough for NOE effects to be observed. In conformation III, the distance between 2-H and 2′-H is calculated to be 2.960 Å, which also corresponds to the observation of a cross peak in the NOESY spectrum.[25] As a result of rotating the bonds C1′–Cα′, Cα′–Cβ′, and C1–Cα as shown in Figure 13, the conformation of savinin is rigid at four minima. No matter how these bonds are rotated within the four minima, the conformers obtained after molecular mechanics calculation are closely related to the conformations I–IV in Figure 11. Some examples are shown in Table V in which the distances between two protons are displayed. As a result of the NMR and computer modelling study, it appears that all four conformers of savinin can exist at the same time in the solution and free state with different populations.[26]

Conclusions. In order to study the conformation of savinin (1) in solution, it was necessary to categorically assign the proton spectrum, which was achieved using the results of the COSY and NOESY experiments. From these results an unambiguous assignment of the carbon-13 NMR spectrum followed. Based on 2D NOE spectroscopy, evidence was obtained that the two phenyl moieties in savinin were overlapped. Further support for this was apparent from the difference UV spectroscopic data on comparison with the corresponding monomer units.

In contrast to the solution results, the conformer present in the crystalline form of savinin (conformer V) is structurally quite different from the other calculated and, energetically speaking, potentially accessible structures. As the energy maps (Figure 13) illustrate, this is not the favorable conformation in the unsolvated "free" state, even though the minimum steric energy is close to the other conformations. However, as the NOE study also indicates, conformation V, which is not capable of eliciting *intramolecular* NOE effects between the aromatic rings, is not a favorable conformer in solution either.

On the basis of the results described here, we urge that great caution be exercised in drawing conclusions, based on the results of X-ray crystallographic analysis, about the solution conformation of molecules that posses varying degrees of flexibility. This is especially important when attempting to correlate structure with biological activity and, in particular, in experimental assessments of the structural changes attendant with the binding of a small molecule to a receptor site.[27]

Experimental Section

General. All spectra were collected on a Varian VXR-300 instrument (7.05-T field) for proton observation at 300 MHz and carbon-13 observation at 75 MHz using a 5-mm broad-band switchable probe in $CDCl_3$ solution (40 mg/0.5 mL) at 25 °C. Both 1D and 2D spectra were collected in the double-precision mode.

COSY-4(90–90): sequence D1–(90)–t_1–(90)–acquire; relaxation delay (D1), 1.0 s; PW90 (^{1}H), 17.5 μs; acquisition time (AT), 270 ms; spectral window in F1 and F2 domains, 1899.0 Hz; size, 1024 data points; number of increments (NI), 512 zero-filled to 2048 data points in both t_1 and t_2; number of transients/increment (NT), 16; quadrature detection with pseudoecho weighting in both dimensions (absolute value, AV display).

Phase-sensitive NOESY: sequence D1–(90)–t_1–(90)–MIX)–(90)–acquire); relaxation delay (D1), 4.0 s; PW90, 17.5 μs;

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(25) Cordell, G. A. *Saengyak Hakhoechi* 1988, *19*, 153.

(26) The calculations described here do not take into consideration the observed C–T interaction. This influence would certainly contribute to a further lowering of the calculated energy of conformation I.

(27) See, for example: Behling, R. W.; Yamane, T.; Navon, G.; Jelinski, L. W. *Proc. Natl. Acad. Sci. U.S.A.* 1988, *85*, 6721.

mixing time (MIX), 3.0 s; acquisition time (AT), 269 ms; spectral window (SW) in F1 and F2 domains, 1902.2 Hz; size, 1024 data points; number of increments, 2 × 256 employing the States–Haberkorn–Rueben hypercomplex data collection;[20] quadrature detection with line broadening (LB and LB2), 0.1 Hz, and apodization (AF and AF2), 0.135 s in both dimensions; zero-filled to 2048 data points in both t_1 and t_2.

HETCOR (^{1}H,^{13}C-heteronuclear shift correlation):[17] direct ($^1J_{CH}$) correlation; sequence D1–90(H)–$t_{1/2}$–180(H,C)$t_{1/2}$–90-(H)–90(C)–acquire; relaxation delay (D1), 1.0 s; J1XH, 140 Hz; PW90$_H$ (PP), 17.5 μs; PW90$_C$, 12.5 μs; acquisition time (AT), 85 ms; spectral window (SW2)$_H$ in F1, 2089.4 Hz; spectral window (SW)$_C$ in F2, 12 019.2 Hz; size, 1024 data points in F2; number of increments (NI), 256 zero-filled to 1024 data points in t_1; quadrature detection with pseudoecho (absolute value, AV display) weighting in both dimensions. **Long-range HETCOR** data collection was performed as above except that J1XH = 6.0 Hz. Data processing was performed as above except that the weighting functions (RE, RE2, AF, AF2) were optimized by using the interactive weighting feature of the Varian software.

DEPT spectral editing:[16] sequence D1–90(H)–D3–(90)-(180)(90)(H), 90(C)–D3–T(H), (90)(180)(90)(C)–D3–acquire, where D3 = $^1/_{2}J$ and T = 45°, 90°, 90°, 135°; relaxation delay (D1), 3.0 s; D3 delay; 2.57 ms; pulse widths, decoupler pulse width (PP), 17.5 μs, and PW90 (C), 12.5 μs; acquisition time (AT), 1.0 s; spectral window (SW), 26 000 Hz; size, 16 000 data points zero-filled to 32K data points with 1.0-Hz line broadening; number of transients (NT), 1024.

T_1 Measurements. Both proton (^{1}H) (Table I) and carbon (^{13}C) (Table II) T_1 data were collected by using the inversion–recovery sequence: D1–(180)–D2–(90)–acquire. The standard Varian software calculated the T_1 data by using a three-parameter exponential-least-squares fit of the peak amplitudes as a function of the D2 delay, which was an arrayed parameter of 11 values. The acquisition time (AT) was 3.7 s, zero-filled to 65K data points for the T_1 calculation.

X-ray Structure Analysis of Savinin. The compound crystallized with difficulty from a very low concentration in hexane (0.05 mg in 1 mL) with slow evaporation at room temperature to afford disordered bunches of transparent crystals. From one of these crystals was cut a suitable piece of crystal (0.4 × 0.2 × 0.1 mm) for X-ray structure analysis. Preliminary unit cell and space group information was obtained from oscillation and Weissenberg photographs. The final unit cell constants were determined by centering 25 reflections on a Nicolet R3m diffractometer and by least-squares refinement. Crystal data: a = 11.605 (2) Å, b = 11.902 (1) Å, c = 12.354 (2) Å, β = 77.04 (1)°, density measured by flotation in KI/H$_2$O = 1.36 g cm^{-3}, d_{calcd} = 1.40 g cm^{-3}, Z = 4, space group monoclinic $P2_1$ with two molecules in the asymmetric unit, four in the unit cell.

The intensity data of 2374 unique reflections (2182 observed with $I < 3\sigma(I)$ were measued by using Ni-filtered Cu K$_\alpha$ radiation, ω-scan with scan speed 2°/min up to $(2\vartheta)_{max}$ = 114°. The data were scaled by Wilson plot and corrected for Lorentz and polarization effects in the usual way. Absorption correction, empirical method, was applied (μ = 8.3 cm^{-1}).

The structure was solved by direct methods using SHELXTL.[28] An E map with the best phase set from the N-Quest criterion revealed 60% of the atoms of the two molecules in the asymmetric unit. Partial structure expansion and subsequent difference Fourier synthesis led to the complex structure. The positions of the hydrogens were calculated from the C atoms to which they are bound. Final cycles of anisotropic refinement (with the non-hydrogen atoms only) converged at R = 4.3% (unit weights). The atomic parameters and bond lengths and angles are deposited at the Cambridge Crystallographic Data Centre.

Molecular Force Field Calculations. The molecular model of savinin was generated from the Build and Edit options of the CHEMLAB-II program.[21] In the study of the different conformations of the rotation along the bonds C1′–Cα′, Cα′–Cβ′, and C1–Cα, the energy maps were generated by scanning these three bonds between 0° and 360° in 10° increments. The minimal steric energy of each conformation was calculated by the MMFF option of the program. X-ray crystallographic data[24] of savinin were initially used to create the conformation which corresponded to savinin in the solid state.

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(28) Sheldrick, G. M. SHELXTL (Release 4.1), A progam for crystal Strucure Determination, Cambridge, England, 1983.

Kinetic vs Thermodynamic Factors in α-Hydrogen Atom Abstractions from Alkyl Aromatics

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The relative reactivities of several 9-alkylanthracenes toward bromine atom have been determined. Whereas for alkylbenzenes it is observed that isopropyl > ethyl > methyl, an inverted reactivity order was observed in the 9-anthryl system: methyl (1.00) > cyclopropyl (0.33) > ethyl (0.063) ≫ isopropyl (<0.001), per α-hydrogen at 80 °C. Stereoelectronic factors, specifically the alignment of the α-C–H bond with the anthryl π-system, are implicated as bearing primary responsibility for this unusual reactivity order. In a general context, the relative importance of stereoelectronic effects vs the stability of the incipient radical in hydrogen atom abstractions from alkyl aromatics is discussed.

Introduction

The selectivity of a free radical in hydrogen atom abstractions from carbon can generally be correlated to the "nature" of the attacking radical and the strength of the C–H bond. Thus, the familiar reactivity order, 3° > 2° > 1°, observed for a variety of abstracting radicals can be directly related to the thermodynamic stability of the resulting free radical. This correlation between carbon radical stability and transition-state energetics was recognized over a half-century ago by Evans and Polanyi[1] and

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(1) Evans, M. G.; Polanyi, M. *Trans. Faraday Soc.* **1938**, *34*, 11.

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