Table I. Experimental Free Energies and Calculated Strain Energies[a] of Various Conformations of Cyclododecyne

conformation[b]	symmetry	ΔSE[c]	$\Delta G°$ [d]
$[4_{yne}332]$[e]	C_1	0.0	0.2
$[3_{yne}333]$[e]	C_2	0.8	0
$[3_{yne}243]$	C_1	1.4	
$[3_{yne}234]$	C_1	1.9	
$[4_{yne}233]$	C_1	2.1	
$[4_{yne}323]$	C_1	2.3	
$[3_{yne}324]$	C_1	3.5	

[a] In kilocalories per mole. [b] See note 8 for nomenclature; the acetylene units in these conformations are slightly bent (e.g., internal angles of 177 and 179° for $[4_{yne}332]$), and this allows Boyd's program,[10] which fails for $\theta = 180°$, to be used. [c] Strain energy relative to that of the $[4_{yne}332]$ whose total (force-field) strain energy is 7 kcal/mol (the contributions to angle bending, torsional, and nonbonded strains are 2.0, 1.4, and 3.0 kcal/mol, respectively). [d] Free energy relative to that of the $[3_{yne}333]$ at −133 °C. [e] See Figure 1 for torsional angles.

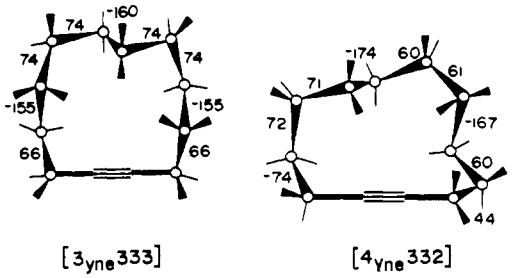

Figure 1. Calculated torsional angles in the $[3_{yne}333]$ and $[4_{yne}332]$ conformations of cyclododecyne.

verify that true (local) energy minima have been obtained.[11]

Of the two lowest energy conformations, one is symmetrical and the other is unsymmetrical (Figure 1), in agreement with the NMR data. The calculated order of these conformations is inverted (Table I), but this is not very significant, given the small energy differences and the expected accuracy (within 1 kcal/mol) of the force-field calculations. The next four conformations (Table I) are not observed at low temperatures, but, because of their fairly low relative strain energies (1.4–2.3 kcal/mol), they may become slightly populated at higher temperatures.[12]

On the assumption that the only mechanism of exchange is the interconversion of the $[3_{yne}333]$ and $[4_{yne}332]$, the ^{13}C NMR data gives[13] a conformational energy barrier of 7.9 ± 0.3 kcal/mol at −95 °C, in agreement with the barrier estimated from the ^1H NMR data. However, the above simple interconversion leads only to a C_2 time-averaged symmetry, whereas the high temperature (>−80 °C) ^1H spectrum of I corresponds to a C_{2v} time-averaged symmetry. Thus, a second conformational process must exist in I, and its barrier must be of the order of 8 kcal/mol in order to accommodate the ^1H NMR data.

It is planned to investigate the conformational properties of other cycloalkynes by the methods described above.

Acknowledgment. This work was supported by the National Science Foundation.

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(4) All spectra were measured on a superconducting solenoid NMR spectrometer operating at 59 kG; $CHCl_2F$ was used as a solvent and tetramethylsilane as an internal reference. ^1H NMR spectra were obtained in a frequency sweep mode, while ^{13}C spectra were obtained by the Fourier transform technique with protons noise decoupled. In both ^1H and ^{13}C spectra an ^{19}F line of the solvent was employed for lock purposes.
(5) The ^1H NMR spectrum of I at −5 °C has bands having the following chemical shifts: δ 1.44 (8 H), 1.56 (8 H), 2.18 (α-CH_2, 4 H).
(6) If an average chemical-shift difference of 80 Hz is used, $k \simeq 180$ Hz (with $k = \pi\Delta\nu/\sqrt{2}$) and $\Delta G^{\ddagger}$ follows from absolute rate theory with a transmission coefficient of 1. If k is in error by a factor of 2, the error in $\Delta G^{\ddagger}$ is 0.2 kcal/mol.
(7) The ^{13}C NMR spectrum of I at −50 °C shows the following chemical shifts, δ 18.8, 24.4, 24.6, 25.8, 26.1, and 82.1 (acetylenic carbon), in reasonable agreement with a recently published NMR spectrum of I in $CDCl_3$ at room temperature; see P. A. Bartlett, F. R. Green, and E. H. Rose, *J. Am. Chem. Soc.*, **100**, 4852 (1978). At −133 °C, lines of approximately equal intensities at δ 19.3, 23.2, 23.6, 26.5, 27.2, and 82.1 are assigned to the $[3_{yne}333]$ conformation, while weaker lines at 16.5, 20.9, 24.1 (two overlapped lines), 30.7, 78.8, and 84.9 are assigned to the $[4_{yne}332]$ conformation. The remaining lines of the latter conformation are apparently obscured by the stronger lines of the major conformation.
(8) The nomenclature used to describe the various conformations of cyclododecyne is an extension of the shorthand notation proposed by Dale[1] for the conformations of the cycloalkanes. The number of bonds on consecutive sides of a conformation are concentrated and placed in square brackets, starting with the side containing the acetylenic linkage. The direction of numbering around the ring is then dictated by the position of the triple bond and starts at the corner position nearest this bond. In cases where the acetylenic function is symmetrically situated on a side, the direction around the ring is so chosen that the second number is the smallest possible, e.g., $[3_{yne}234]$ not $[3_{yne}432]$. This nomenclature can be applied to a variety of systems including unsaturated and heterocyclic rings where a subscript can be used to designate the functional group present.
(9) The low-energy conformations of cyclododecane in order of increasing energy are calculated to be the [3333], [2334], and [2343]. Experimentally, there is no evidence for population of any conformation except the [3333] (F. A. L. Anet and T. N. Rawdah, *J. Am. Chem. Soc.*, **100**, 7166 (1978); J. Dale, *Acta Chem. Scand.*, **27**, 1115, 1130 (1973)).
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(12) In view of possible errors in the force-field calculations, it is conceivable that the $[3_{yne}243]$ conformation is actually of lower energy than the $[4_{yne}332]$, but this is not considered likely.
(13) Because of the small solubility of I at low temperature, the signal-to-noise ratio of the ^{13}C lines at intermediate rate of exchange is low and this has prevented an analysis of the exchange mechanism to be made. It is planned to prepare a suitably ^{13}C-labeled cyclododecyne for line-shape analysis.

Frank A. L. Anet,* Tarik N. Rawdah
Department of Chemistry
University of California, Los Angeles
Los Angeles, California 90024
Received November 7, 1978

^{13}C NMR Studies of Marine Natural Products. 1. Use of the SESFORD Technique in the Total ^{13}C NMR Assignment of Crassin Acetate

Sir:

The antineoplastic and cytotoxic marine cembranolide, crassin acetate (**1a**),[1] is representative of the burgeoning number of cembrane diterpenes steadily being reported from marine organisms of the coelenerate phylum, as well as from terrestrial plant sources such as tobacco and pines.[2]

Unambiguous ^{13}C NMR spectral assignment has not yet appeared for any member of this interesting group of 14-membered carbocyclic compounds. We now report the complete assignment of the ^{13}C NMR spectra of the naturally occurring crassin acetate (**1a**), and its derivatives crassin (**1b**) and crassin diacetate (**1c**), based largely on the use of the newly developed technique of selective excitation with single fre-

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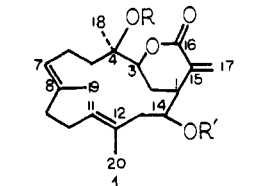
Table I. 25.2-MHz ^{13}C NMR Chemical Shifts, Multiplicities, and T_1 Relaxation Times of Crassin Acetate (**1a**), Crassin (**1b**), and Crassin Diacetate (**1c**)

carbon	m[a]	chemical shift, ppm			T_1 relaxation times, s		
		1a	**1b**	**1c**	**1a**	**1b**	**1c**
1	d	36.7	39.0	37.4	0.62	0.48	0.72
2	t	20.1[b]	19.0	21.0	*d*	0.25	0.44
3	d	81.5	82.4	81.9	0.65	0.45	0.74
4	s	73.2	73.8	84.5	4.91	2.74	6.45
5	t	38.4	38.7	34.9	0.34	0.28	0.38
6	t	21.5[c]	21.9	21.8	0.38	0.29	0.43
7	d	124.9	125.2	125.0	0.68	0.60	0.77
8	s	134.4	135.0	135.8	5.91	4.32	6.42
9	t	39.2	39.8	39.8	0.35	0.28	0.37
10	t	23.3	23.8	24.0	0.38	0.29	0.37
11	d	127.5	127.0	128.1	0.67	0.50	0.66
12	s	136.2	138.1	136.3	7.41	4.78	7.61
13	d	40.9	44.5	41.5	0.34	0.27	0.36
14	d	72.3	71.4	72.5	0.67	0.50	0.66
15	s	129.3	130.7	130.1	7.55	3.98	5.58
16	s	169.5	168.0	169.7	14.08	10.11	9.54
17	t	126.7	127.2	127.7	0.37	0.26	0.40
18	q	23.9	24.4	22.4	0.71	0.67	2.81
19	q	14.1	14.7	14.7	3.02	2.39	*d*
20	q	13.9	14.3	14.7	3.45	2.64	*d*
21	s	166.2		166.8	10.78		7.75
22	q	20.1[b]		20.4[c]	*d*		0.51
23	s			170.0			7.01
24	q			20.8[c]			1.53

[a] Multiplicity. [b] Degenerate chemical shifts. [c] May be permuted. [d] Could not be measured owing to degeneracy.



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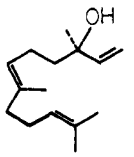
a R = H R′ = $COCH_3$

b R = H R′ = H

c R = $COCH_3$ R′ = $COCH_3$



2

quency off resonance decoupling (SESFORD).

Multiplicity information for all resonances in a molecule is essential for accurate assignment of the ^{13}C NMR spectra of complex natural products. For simple molecules, unambiguous multiplicities normally can be obtained from the residually coupled spin multiplets routinely acquired by the single frequency off resonance decoupling (SFORD) technique.[3,4] However, in more complex systems, e.g., **1a–c** in which numerous resonances have closely similar chemical shifts, overlap of SFORD spin multiplets presents a complex problem of interpretation.[5,6] This difficulty may be further aggravated by the appearance of second-order effects in SFORD spectra.[7-10]

In principle, a resolution of these difficulties in the interpretation of complicated coupled and SFORD spectra is provided by the selective excitation procedure of Bodenhausen, Freeman, and Morris.[11] When used in conjunction with gated decoupling,[12] this technique has been successfully employed

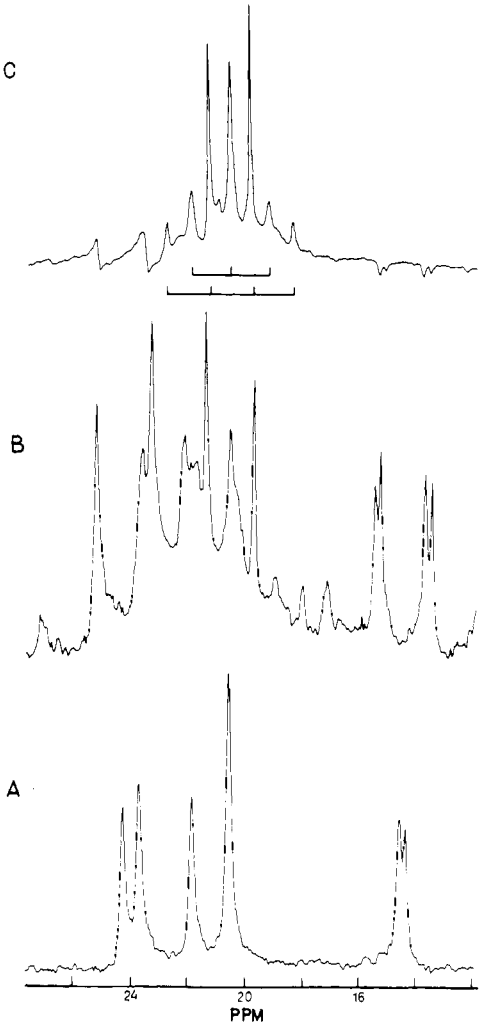


Figure 1. 25.2-MHz ^{13}C spectral segments of crassin acetate (**1a**) in deuteriochloroform: A, decoupled spectrum of a portion of the methylene region; B, SFORD trace; SESFORD trace of the resonance at δ 20.1.

in the total assignment of aromatic[13,14] and certain other types of simpler systems.[11,15] However, in highly saturated compounds such as **1a–c**, extensive ^1H–^{13}C long-range couplings have the effect of substantially complicating spin-multiplet splittings, thereby significantly reducing signal intensities. For such compounds, selective excitation with conventional gated decoupling is thus a relatively inefficient or even impractical method of obtaining multiplicity information.

We have completely overcome these limitations by combining the inherent advantages of both the SFORD and selective excitation techniques in a single experiment: SESFORD.[16] In this experimental sequence, the individual ^{13}C resonance under study is selectively excited by a train of precisely spaced "soft" pulses.[12,13,17] In this manner, the vector associated with the resonance being selectively excited is tipped 90° into the XY plane, which subsequently produces a maximal response in the receiver. During the excitation sequence, all other resonances remain as essentially unperturbed Z magnetization and thus produce no signal. The relative degree of selectivity in this experiment is a function of both the number of pulses applied in the train and the proximity of the resonance to the carrier frequency.[13,15] Following excitation and immediately prior to accumulation, the new technique electronically gates the decoupler from broad-band irradiation to a preselected coherent single-frequency irradiation of the proton spectral window. The weaker long-range couplings are suppressed (as in the simple SFORD experiment) and only the stronger residual geminal couplings of the selectively excited

resonance are observed. Because all other resonances are excluded from the subspectrum, clear and unambiguous multiplets are observed for each resonance in turn.

Multiplicity information for **1a**, shown in Table I, was obtained exclusively by the application of the SESFORD technique. Particularly illustrative of its capability is the SESFORD trace (Figure 1) for the degenerate resonance at δ 20.1 for the C-2 methylene and the C-22 acetate methyl, which showed a symmetrically overlapped triplet and quartet pattern. Chemical shift assignments for **1a** were made largely from nerolidol (**2**),[18] which serves as an excellent model for the C-4 through C-12 portion of the carbocyclic nucleus. Signal assignments for the remainder of the molecule were based on empirical chemical shift relationships[19,20] in conjunction with T_1 inversion-recovery relaxation measurements[21-23] for discrimination among methyl[24,25] and carbonyl[26] resonances.

Complete correspondence between the observed SESFORD multiplicities and the multiplicities inferred from T_1 inversion recovery spin-lattice relaxation studies further served to establish that **1a** is subject to isotropic tumbling (see Table I).[5] The multiplicities shown in Table I for **1b** and **1c** were consequently established from T_1 studies alone. Chemical shift assignment for the latter two compounds, also shown in Table I, are based on those established for **1a**, with adjustments by standard empirical correlations as appropriate for their functional modification.

The successful application of SESFORD to the problem of crassin acetate multiplicities amply demonstrates the utility of the technique with complex molecules. It is evident that SESFORD should be useful for the unequivocal resolution and determination of the spin multiplicity of any resonance in even the most complex natural product.[27]

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(27) Selective excitation of resonances separated by 1 Hz (at 25 MHz) can comfortably be achieved by this technique. With some effort, resonances separated by 0.2 Hz have been resolved.

Gary E. Martin,* James A. Matson, James C. Turley, Alfred J. Weinheimer

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Houston, Houston, Texas 77004

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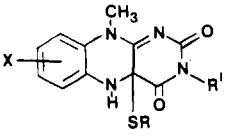
Reduction of 1,3-Dimethyl-5-(*p*-nitrophenylimino)barbituric Acid by Thiols. A High-Velocity Flavin Model Reaction with an Isolable Intermediate[1]

Sir:

Strong kinetic evidence exists for a thiol addition intermediate, **1**, involving the C(4a)—N(5) bond, in nonenzymatic reductions of flavins and analogues by thiols.[2-5] We have re-



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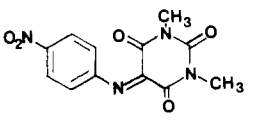
cently proposed the C=N bond of 5-aryliminobarbituric acids as a simple model for the C(4a)—N(5) bond of flavins.[6] We report here our observation of an isolable covalent intermediate, **3b**, in the reduction of the highly activated imine **2** by a thiol. This provides the first *direct* nonkinetic demonstration of such an intermediate in a flavin model reaction and confirms the structural assignment of flavin–thiol intermediates **1** previously proposed on the basis of kinetic evidence.



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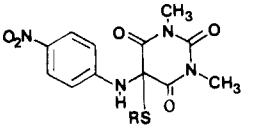
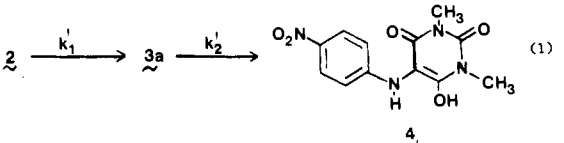


3a R=$CH_3OC(O)CH_2$–
3b R= HOC_2H_4–

The reaction of 1,3-dimethyl-5-(*p*-nitrophenylimino)barbituric acid (**2**, prepared by a modification of the published procedure[6] for the 5-*p*-tolylimino derivative) with excess methyl thioglycolate at 25 °C exhibits biphasic kinetics at 360 nm (Figure 1A) consistent with accumulation and decay of an intermediate (eq 1). Kinetics of the two phases could be studied



(1)

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independently, by stopped-flow spectrophotometry, at 379 nm, the isosbestic point for intermediate and product (Figure 1B), and at 415 nm (Figure 1C). The reaction followed at 379 nm was pseudo first order as indicated by agreement of two successive half-times for the reaction, and k_1' was determined from $t_{1/2}$ or from linear plots of $(A_\infty - A_0)$ vs. time; similar plots at 415 nm were linear after an initial lag phase and were used for determination of k_2'. No evidence[7] was obtained for significant reversal of the initial step. Both processes, k_1' and k_2', are dependent on the first power of the thiol anion concentration, as shown from the dependence of the rate on total thiol concentration and on pH at pH 3.8–5.2. The corresponding rate laws are given by

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