Investigation of the Solution Conformation of Coenzyme A and Its Derivatives by Hydrogen-1 and Phosphorus-31 Fast Fourier Transform Nuclear Magnetic Resonance Spectroscopy[1]

Che-Hung Lee and Ramaswamy H. Sarma*

Contribution from the Department of Chemistry, State University of New York at Albany, Albany, New York 12222. *Received August 20, 1974*

Abstract: Coenzyme A and its derivatives—acetyl-CoA, malonyl-CoA, and dephospho-CoASH—have been shown to exist in aqueous solution with a flexible molecular framework. While flexibility is allowed and alternate conformations become accessible, to a considerable extent, these molecules achieve conformational identity by showing preferences—sometimes overwhelming preferences—for certain orientations. The preferred conformation for the 3′,5′-ADP part was found to be anti-^2E-gg-g′g′, the 3′-phosphate being preferentially oriented gauche to H(3′). For the pantetheine part of the coenzymes, an outspoken preference was observed for the gauche″-gauche″ conformation about the C(1′)–O(1′) bond and no preference was observed for any conformation about the C(5′)–C(6′) and C(8′)–C(9′) bonds. It is shown that in aqueous solutions, the coenzymes exist as a fast exchanging system of linear and several-folded species, the population of the folded species being low. It is suggested that during the reorientations of the molecules in solution, the 3′,5′-ADP part and the pantetheine phosphate part can undergo differential segmental motion *via* rotation about the P–O–P linkage and such rotations make possible the existence of linear and folded conformations.

A quarter of a century has elapsed since coenzyme A (Figure 1) was discovered and the basic assay procedures were established by Lipman and Kaplan.[3-7] In the early 50's, Baddiley[8] solved the fundamental structure of coenzyme A and in the late 50's and early 60's, its total synthesis was accomplished by Moffatt and Khorana.[9,10] In this paper we present the first detailed account of its dynamic solution conformation as studied by hydrogen-1 and phosphorus-31 nuclear magnetic resonance spectroscopy.

Experimental Section

Materials and Method. ^{1}H nmr spectra of coenzyme A (CoASH), dephospho-CoASH, acetyl-CoA, malonyl-CoA, pantetheine phosphate, *S*-acetylpantetheine phosphate, and 3′,5′-ADP were obtained in a 100-MHz nmr system in the Fourier mode at 30.5°. The ^{31}P nmr spectra at 40.48 MHz were recorded for coenzyme A and 3′5′-ADP. The details of instrumentation are described elsewhere.[11-13] The concentration and pH employed for the various compounds are given in the different tables which contain the basic data obtained. Usually, three different pH values were chosen: they are (a) pH 8.0, a situation where the 3′-phosphate in the coenzymes, the 1′-phosphate in phosphopantetheines (see Figure 1 for the numbering system employed), and both the 3′- and 5′-phosphate groups in 3′,5′-ADP are doubly ionized; (b) pH 5.0, when all the above phosphate groups are present as monoanions; (c) pH 1.5, when all the above phosphate groups are monoanions and the adenine ring is protonated at N(1) and carries a formal positive charge. The assignments of the absorption peaks of the nonexchangeable protons attached to the adenosine part of the coenzymes were made from the general knowledge that our and other laboratories have accumulated in the analysis of related nucleosides and nucleotides.[11-27] The derived data for the adenosine region are computer fitted and in Figure 2 both the observed and the computer-simulated spectra are given. The agreement between the two in the adenosine region is exceptionally good, especially in view of the strong coupling between the 2′ and 3′ region. In addition, the accuracy of the derived data for 3′,5′-ADP at 100 MHz was checked by recording spectra at 300 MHz[28] and computer simulating the 300-MHz spectrum. The assignments of the peaks from the pantetheine region of coenzyme A and its analogs were made from considerations of (i) electronegativity effects, (ii) effect of substitution on the terminal sulfhydryl group, (iii) comparison with the position of peaks in the monomeric units, *i.e.*, pantetheine and *S*-acetylpantetheine phosphate when the phosphate is a mono- and dianion, and (iv) taking into consideration the fact that protons near the free end of a long chain will experience a considerable degree of freedom of motion and consequently will have sharper absorption peaks. Finally, the spectra were computer fitted and in Figure 2 both the observed and the simulated spectra are given and the agreement between them is good. In Figure 3 the spectra of *S*-acetylpantetheine phosphate and the corresponding simulation are shown. The phosphorus–hydrogen couplings for the ribose region ($J_{5'P(5')}$, $J_{5''P(5')}$, $J_{4'P(5')}$, $J_{3'P(3')}$) and those for the pantetheine region ($J_{1'P(1')}$, $J_{1''P(1')}$) were obtained for CoASH and 3′,5′-ADP from both ^{1}H and ^{31}P spectra. The ^{31}P spectra of 3′,5′-ADP and CoASH, along with their corresponding computer simulations, are illustrated in Figures 4 and 5. The chemical shifts and coupling constants for CoASH, dephospho-CoASH, acetyl-CoA, malonyl-CoA, 3′,5′-ADP, pantetheine phosphate, and *S*-acetylpantetheine phosphate are compiled in Tables I and II.

The various compounds that are investigated in this report are commercial preparations, except for pantetheine phosphate and *S*-acetylpantetheine phosphate. The ^{1}H nmr spectra of the latter two compounds (Figure 3) were examined separately in solutions each of which contained 0.025 *M* of the compound along with 0.025 *M* 3′,5′-ADP. This solution was obtained from 0.025 *M* parent coenzyme by treating it with snake venom phosphodiesterase, as described elsewhere.[29] The employed concentration is low enough[30] as to preclude any intermolecular interaction between the fragments. In addition, a comparison of the chemical shifts in the individual monomers present together with that in the intact coenzyme should give considerable insight into intramolecular interaction between the adenosine and pantetheine part of the coenzyme. Such studies were pioneered by Jardetzky and Wade-Jardetzky.[31] The pH reported for the various solutions in Table I are actual pH meter readings from a Fisher accument Model 320 pH meter and pD may be obtained by adding 0.4 to the reported values.

Results and Discussion

(A) Sugar–Base Torsion Angle. The studies by Schweizer, *et al.*,[32] Chan and Nelson,[33] Evans and Sarma,[34] and Danyluk and Hruska[35] have shown that the adenine moiety in both 5′-AMP and 3′-AMP is oriented preferentially in the anti conformation. In addition, magnetic resonance studies on nucleotide coenzymes, such as pyridine nucleotides,[23,25] and adenosine diphosphoglucose[26] have shown that the purine base in these compounds is also preferentially oriented in the anti conformation. Hence, it is expected that the adenine moiety in coenzyme A most likely has a preferential anti conformation. To confirm this, we have obtained ^{1}H nmr spectra of CoASH and acetyl-CoA (0.01 *M*, pH 8.0)

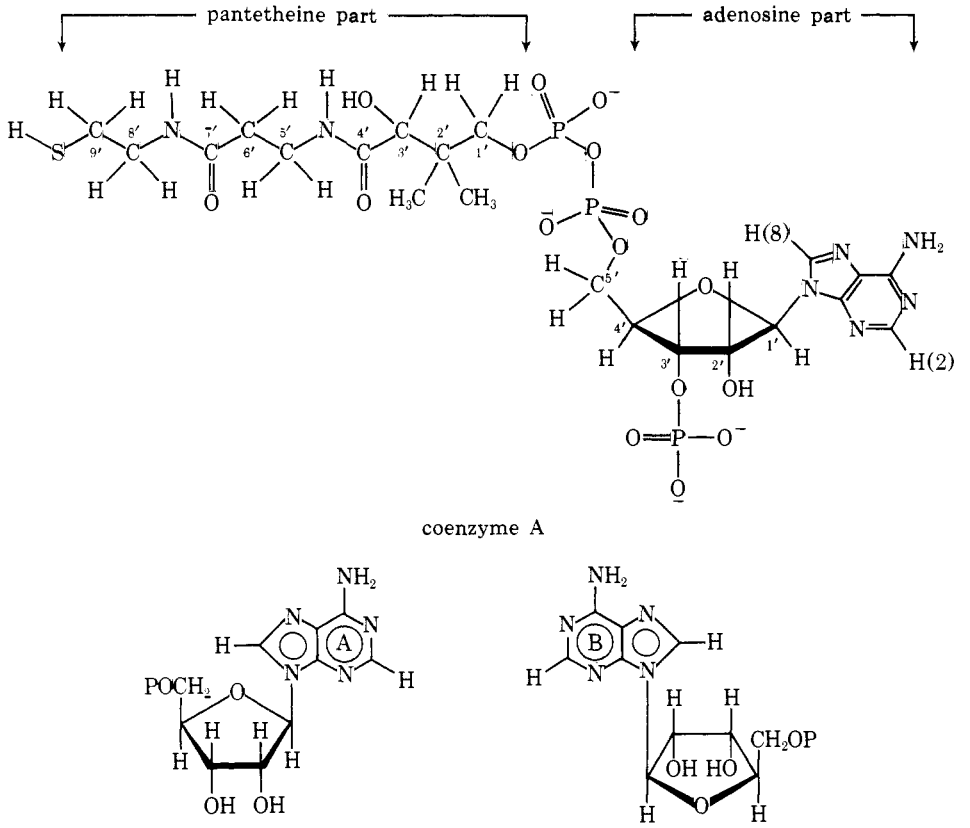


Figure 1. The structure of coenzyme A. Please note the numbering system employed for the adenosine and the pantetheine portions. This system will be used during discussion in the text.

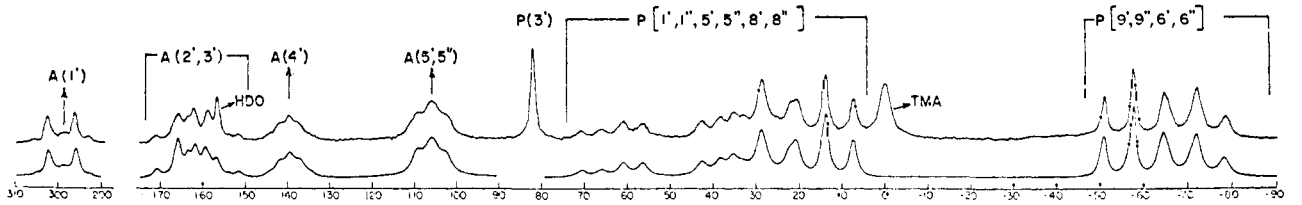


Figure 2. Top: observed ^1H nmr spectrum, recorded in Fourier mode of 0.05 *M*, pH 8.0, coenzyme A. The peaks corresponding to adenine C(8)-H, C(2)-H, as well as pantetheine C(2')-Me_2 are not shown. The internal standard was tetramethylammonium chloride (TMA). In the assignments indicated the letter A refers to adenosine part protons and P refers to protons of the pantetheine part, the numbering system employed being that shown in Figure 1. Bottom: computer-simulated spectrum of CoASH. A comparison of spectra on top and bottom shows excellent agreement between the observed and simulated spectra. Because PH(3') is a singlet, it was not included in the simulation.

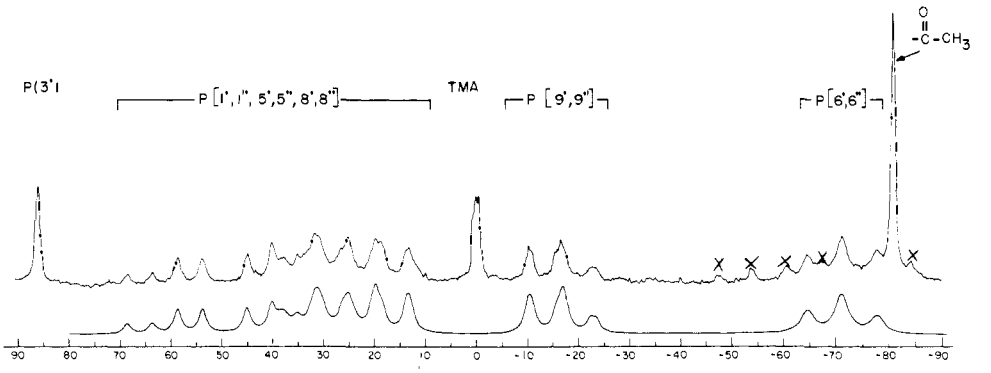


Figure 3. Top: observed ^1H nmr spectrum, recorded in the Fourier mode of 0.025 *M* *S*-acetylpantetheine phosphate at pH 5.0, recorded in presence of 0.025 *M* 3',5'-ADP. The pulse parameters are the same as one used before.[11] The chemical shifts are reported in hertz (100-MHz system) as in Figure 2 from internal TMA. The peaks marked X originate from minor impurities in the preparation. Bottom: the corresponding computer-simulated spectrum.

in the presence of Mn(II) ions at concentrations of 5×10^{-6}, 10×10^{-6}, 15×10^{-6}, 25×10^{-6}, and 35×10^{-6} *M*. The observation that, under the above conditions, the line width of C(8)-H underwent pronounced line broadening with only slight effect on C(2)-H line width suggests a preferential anti orientation for the adenine moiety, according to the criteria of Chan and Nelson.[32] However, a general caution involving all similar Mn(II) ion binding studies should be applied here. For example, since Mn(II) ions neutralize the negative charge on the phosphate and is also re-

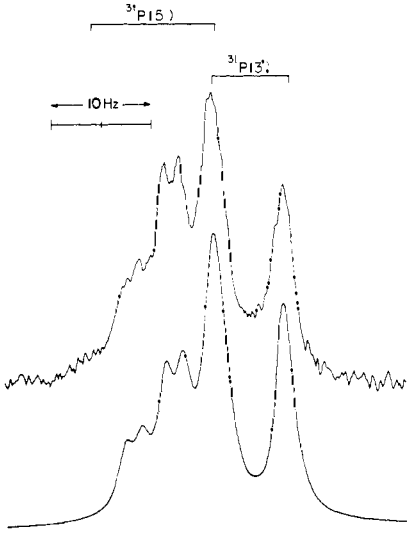


Figure 4. The nondecoupled ^{31}P spectra of 3′,5′-ADP, 0.1 *M*, pH 8.0, recorded at 40.48 MHz in the Fourier mode; P(5′) is 0.212 ppm upfield from P(3′) (top) and the computer-fitted spectrum (bottom).

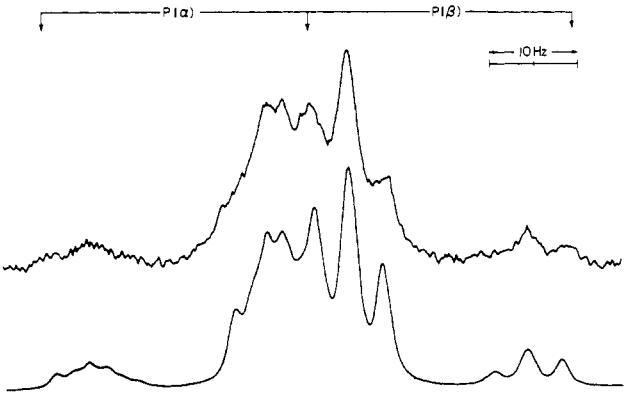


Figure 5. The nondecoupled ^{31}P spectra of CoASH (0.05 *M*, pH 8.0). Only the pyrophosphate region is shown. Also shown at the bottom is the computer-fitted spectrum. P_α is attached to ribose moiety while P_β to pentetheine part. P_α is 0.497 ppm upfield from P_β.

ported to interact with N(7),[36] one may argue that the above conclusion may not be necessarily true in the absence of bound Mn(II) ions. However, in adenosine, Mn(II) ions do not create any significant line broadening,[34] even though in this instance, also, it could be weakly bound to N(7).[36] In addition, the chemical shift data for the C(2)-H and C(8)-H in Table IA at pH 8.0 and pH 5.0 clearly show that the C(8)-H chemical shift in 3′,5′-ADP is highly sensitive to the ionization of the 5′-phosphate. Such an effect is best rationalized based on a preferential anti orientation.[32] The data in Table IA further reveal that ionization of the 3′-phosphate has no significant effect on C(8)-H and C(2)-H chemical shifts in CoASH, acetyl-CoA, and malonyl-CoA. This is exactly what one would expect because of the stringent distance dependence of such effects.

(B) Conformation of the Exocyclic Linkage H(4′)–C(4′)–C(5′)–O(5′)–P(5′) of the Adenosine Part. The preferred conformers constrained to the C(4′)–C(5′) bond may be gauche-gauche (I, gg), gauche-trans (II, gt), and trans-gauche (III, tg). Those constrained to C(5′)–O(5′) are gauche′-gauche′ (g′g′, IV), gauche′-trans′ (g′t′, V), and trans′-gauche′ (t′g′, VI). So, the backbone H(4′)–C(4′)–C(5′)–O(5′)–P(5′) can exist in nine different conformations, *viz.* gg-g′g′, gg-g′t′, gg-t′g′, gt-g′g′, gt-g′t′, gt-t′g′, tg-g′g′, tg-g′t′, and tg-t′g′. The first three conformations in the above sequence are illustrated in Figure 6 by Yathindra-Sundaralingam projections.[37] Because an unambiguous assignment of the two C(5′) protons is not possible, one can-

Table I

Compd	CoASH		De-P-CoASH[c]			Acetyl-CoA			Malonyl-CoA	3′,5′-ADP		
A. Chemical Shifts[a] of Adenosine Protons of Various Compounds												
pH	8.0	5.0	1.5	8.0	1.7	8.0	5.0	1.5	8.0	8.0	5.0	1.5
Concn, *M*	0.05	0.05	0.05	0.02	0.02	0.025	0.025	0.025	0.05	0.1	0.1	0.1
AH(1′)	299.0	299.4	302.7	295.3	301.1	298.7	299.7	303.4	298.0	298.7	294.1	302.0
AH(2′)	164.5	169.1[b]	172.0[b]	158.3	159.7	163.1	168.4[b]	174.9[b]	163.0	170.3	163.1	166.3
AH(3′)	159.7	166.2[b]	167.5[b]	135.5	137.6	158.4	166.1[b]	166.6[b]	158.5	160.0	166.0	170.0
AH(4′)	139.6	142.4	144.4	121.1	124.0	138.6	141.8	144.2	138.6	138.7	139.5	140.2
AH(5′)	108.2	109.4	113.8	106.9	110.7	107.2	108.7	113.6	107.4	88.4	102.5	104.3
AH(5′′)	104.1	105.3	109.7	103.3	107.1	103.0	104.6	109.5	103.2	84.0	98.3	100.1
AH(2)	507.3	506.3	525.1	507.0	527.8	508.8	508.4	527.9	506.5	500.8[d]	497.1	525.8
AH(8)	536.4	535.6	547.0	531.5	547.8	537.4	536.5	549.3	535.1	544.8[d]	527.4	542.3
B. Coupling Constants[e] of Adenosine Protons of Various Compounds												
pH	8.0	5.0	1.5	8.0	1.7	8.0	5.0	1.5	8.0	8.0	5.0	1.5
Concn, *M*	0.05	0.05	0.05	0.02	0.02	0.025	0.025	0.025	0.05	0.1	0.1	0.1
$J_{1'2'}$	6.7	6.3	5.6	5.8	5.4	6.7	6.4	5.5	6.7	6.8	5.6	4.8
$J_{2'3'}$	5.2	5.2[f]	5.2[f]	5.2	5.2	5.2	5.2[f]	5.2[f]	5.2	5.0	5.0	5.2
$J_{3'4'}$	2.4	2.4[f]	2.4[f]	3.7	3.6	2.4	2.4[f]	2.4[f]	2.4	2.4	3.6	3.7
$J_{3'P(3')}$	7.3	7.9[f]	7.9[f]			7.3	7.9[f]	7.9[f]	7.3	7.3	7.9	7.9
$J_{4'5'}$	2.9	2.9	2.9	3.7	3.7	2.9	2.9	2.9	2.9	3.0	3.2	2.8
$J_{4'5''}$	2.7	2.7	2.7	2.3	2.3	2.7	2.7	2.7	2.7	3.0	2.1	2.1
Σ^g	5.6	5.6	5.6	6.0	6.0	5.6	5.6	5.6	5.6	6.0	5.3	4.9
$J_{4'P(5')}$	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	1.8	1.8	1.8
$J_{5'5''}{}^h$	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−12.0	−10.0
$J_{5'P(5')}$	4.1	4.1	4.1	4.5	4.5	4.1	4.1	4.1	4.1	4.2	4.9	4.8
$J_{5''P(5')}$	4.1	4.1	4.1	4.5	4.5	4.1	4.1	4.1	4.1	4.2	4.9	4.8
Σ'^i	8.2	8.2	8.2	9.0	9.0	8.2	8.2	8.2	8.2	8.4	9.8	9.6

[a] The chemical shifts in hertz are measured from the peak of internal reference, tetramethylammonium chloride (TMA), with a 100-MHz spectrometer within ±0.1 Hz error. [b] The data are estimated from the experimental spectra. [c] Abbreviation for dephospho coenzyme A. [d] At lower concentrations the chemical shifts move to lower fields. [e] The coupling constants are checked by computer simulation within ±0.1 Hz error. [f] The values are estimated from the experimental spectra. [g] $\Sigma = J_{4'5'} + J_{4'5''}$. [h] The geminal coupling constant $J_{5'5''}$ is set arbitrarily. [i] $\Sigma' = J_{5'P(5')} + J_{5''P(5')}$.

Table II

Compd	CoASH			De-P-CoASH		Acetyl-CoA			Malonyl-CoA	Pantetheine phosphate		*S*-Acetylpantetheine phosphate	
A. Chemical Shifts[a] of Pantetheine Protons of Various Compounds													
pH	8.0	5.0	1.5	8.0	1.7	8.0	5.0	1.5	8.0	8.0	5.0	8.0	5.0
Concn, *M*	0.05	0.05	0.05	0.02	0.02	0.025	0.025	0.025	0.05	0.025	0.025	0.025	0.025
PH(1′)	62.8	64.7	70.3	66.1	70.6	63.5	65.4	71.1	62.3	56.1	60.4	56.2	60.4
PH(1″)	36.8	38.7	46.3	39.5	46.4	36.8	38.8	46.4	35.5	22.6	39.5	22.0	38.9
$PCH_3(2')$	−231.4	−230.0	−223.4	−228.6	−222.6	−231.4	−229.2	−222.8	−233.2	−219.4	−220.9	−219.4	−221.3
$PCH_3(2'')$	−244.5	−242.3	−234.9	−241.1	−234.0	−244.5	−242.0	−235.5	−246.2	−235.0	−228.9	−235.3	−229.5
PH(3′)	82.0	83.2	85.5	82.7	84.8	82.9	84.0	86.7	80.5	94.5	86.4	93.7	86.2
PH(5′)	28.7	29.0	31.2	28.0	31.1	26.9	27.3	29.3	25.9	33.5	33.3	32.4	31.4
PH(5″)	28.7	29.0	31.2	28.0	31.1	26.9	27.3	29.3	25.9	33.5	33.3	32.4	31.4
PH(6′)	−71.2	−71.0	−68.8	−71.9	−68.6	−75.2	−74.9	−72.7	−75.2	−66.6	−66.8	−70.1	−70.9
PH(6″)	−71.2	−71.0	−68.8	−71.9	−68.6	−75.2	−74.9	−72.7	−75.2	−66.6	−66.8	−70.1	−70.9
PH(8′)	13.8	13.8	15.6	12.1	16.5	14.2	14.3	16.9	16.3	17.8	18.4	19.8	19.2
PH(8″)	13.8	13.8	15.6	12.1	16.5	14.2	14.3	16.9	16.3	17.8	18.4	19.8	19.2
PH(9′)	−56.8	−57.0	−55.8	−58.4	−55.1	−21.5	−21.6	−18.6	−15.8	−53.5	−53.3	−14.9	−16.1
PH(9″)	−56.8	−57.0	−55.8	−58.4	−55.1	−21.5	−21.6	−18.6	−15.8	−53.5	−53.3	−14.9	−16.1
$-COCH_3$						−83.1	−84.1	−82.3				−79.8	−80.6
B. Coupling Constants[b] of Pantetheine Protons of Various Compounds													
pH	8.0	5.0	1.5	8.0	1.7	8.0	5.0	1.5	8.0	8.0	5.0	8.0	5.0
Concn, *M*	0.05	0.05	0.05	0.02	0.02	0.025	0.025	0.025	0.05	0.025	0.025	0.025	0.025
$J_{1'1''}$	−9.7	−9.7	−9.9	−9.9	−9.9	−9.7	−9.7	−9.9	−9.9	−10.4	−9.7	−10.3	−9.9
$J_{1'P(1')}$	4.6	4.7	4.4	4.4	4.4	4.6	4.6	4.4	4.6	6.5	4.8	6.6	4.9
$J_{1''P(1')}$	4.2	4.3	4.2	4.2	4.2	4.2	4.4	3.8	4.2	5.0	4.4	5.0	4.9
Σ''[c]	8.8	9.0	8.6	8.6	8.6	8.8	9.0	8.2	8.8	11.5	9.2	11.6	9.8
$J_{5'5''}$[d]	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0
$J_{5'6'}$	6.6	6.6	6.6	6.6	6.7	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
$J_{5'6''}$	6.6	6.6	6.6	6.6	6.7	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
$J_{5''6'}$	6.6	6.6	6.6	6.6	6.7	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
$J_{5''6''}$	6.6	6.6	6.6	6.6	6.7	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
$J_{6'6''}$[d]	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0
$J_{8'8''}$[d]	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0
$J_{8'9'}$	6.6	6.6	6.6	6.7	6.7	5.9	5.7	5.7	5.9	6.6	6.6	5.7	5.7
$J_{8'9''}$	6.6	6.6	6.6	6.7	6.7	6.7	6.9	6.9	6.7	6.6	6.6	6.9	6.9
$J_{8''9'}$	6.6	6.6	6.6	6.7	6.7	6.7	6.9	6.9	6.7	6.6	6.6	6.9	6.9
$J_{8''9''}$	6.6	6.6	6.6	6.7	6.7	5.9	5.7	5.7	5.9	6.6	6.6	5.7	5.7
$J_{9'9''}$[d]	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0

[a] The chemical shifts in hertz are measured from the peak of internal reference, tetramethylammonium chloride (TMA), with a 100-MHz spectrometer within ±0.1 Hz error. [b] The coupling constants are checked by computer simulation within ±0.1 Hz error. [c] $\Sigma'' = J_{1''P(1')} + J_{1''P(1')}$. [d] The geminal coupling constants $J_{5'5''}$, $J_{6'6''}$, $J_{8'8''}$, and $J_{9'9''}$ are set arbitrarily.

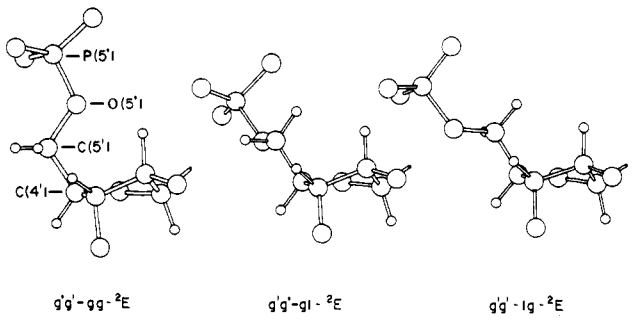
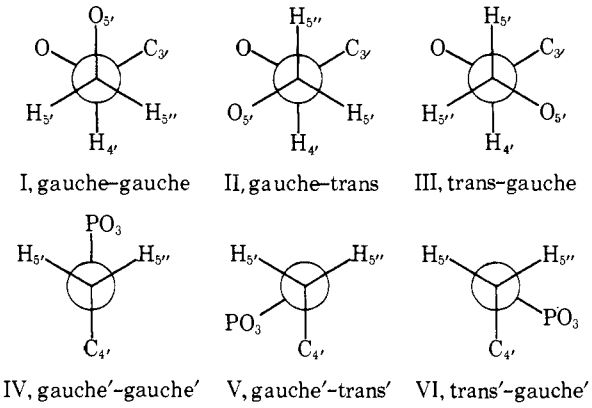


Figure 6. Yathindra-Sundaralingam projections of the gg-g'g', gt'-g'g', and tg-g'g' backbone conformations for a β-5'-nucleotide. The ribose is shown in the ^2E conformation.

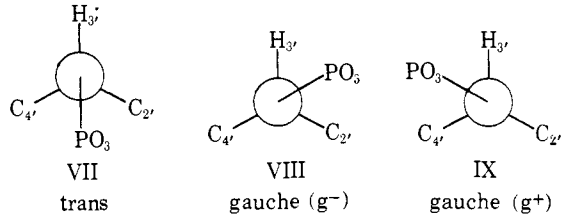


not distinguish between gg-g't' and gg-t'g' or between gt-g'g' and tg-g'g' conformations. Hence, the present nmr methods can enable us to distinguish only between the following four types of conformations: gg-g'g', gg-g'/t', g/t-g'g' and g/t-g'/t'; the abbreviations g/t, g'/t', etc, stand for either gt or tg, g't' or t'g', etc. Similar approaches have been employed to determine the conformational preferences about the *two* C(5')-O(5') bonds present in pyridine nucleotides.[12]

Sarma, *et al.*,[24] have indicated that in the case of 5'-β-nucleotides, when the backbone is gg-g'g', the atoms H(4')-C(4')-C(5')-O(5')-P(5') lie in one plane and the geometric relationship between H(4') and P(4') is a "W." It has been argued[24] by citing the fundamental work of Hall, *et al.*,[38-40] that such a "W" relationship will generate a four-bond coupling between H(4') and P(5'). Sarma, *et al.*,[24] have empirically suggested that an observed value of $^4J_{4'P}$ greater than 1.0 Hz but less than 3.0 Hz suggests that the backbone conformation about C(4')-C(5') and C(5')-O(5') is flexible, but definitely indicative of a preference for the gg-g'g' conformation (Figure 6). The data for CoASH, dephospho-CoA, acetyl-CoA, malonyl-CoA, and 3',5'-ADP in Table 1B show that for these compounds the magnitude of $J_{4'P}$ lies in the range of 1.8-2.2 Hz, clearly suggesting flexibility about C(4')-C(5') and C(5')-O(5') bonds with clear preference for gg-g'g' conformation.

The validity of such a conclusion can easily be checked from three-bond coupling constants $J_{4'5'}$, $J_{4'5''}$, $J_{5'P(5')}$, and $J_{5''P(5')}$. It has been shown elsewhere and discussed extensively[19-21,24,27] how the magnitudes of the sums $J_{4'5'} + J_{4'5''}$ (Σ) and $J_{5'P(5')} + J_{5''P(5')}$ (Σ') can be used to evaluate the populations of the conformers about C(4')-C(5') and C(5')-O(5'). In Table IV, the percentage populations of gg and g'g' conformers computed from expressions in ref 19-21 are given. The data show that for CoASH, dephospho-CoA, acetyl-CoA, malonyl-CoA, and 3',5'-ADP, the most preferred conformation for the backbone is gg and g'g' and no *major* shift in *actual* population is created by ionization of the phosphate groups or protonation of the base at N(1). Thus, both four-bond and three-bond coupling data corroborate the same model.

(C) Conformation of the 3'-Phosphate Group. The Newman projections VII, VIII, and IX show the one trans and two gauche sterochemistry about the C(3')-O(3') bond. In the trans conformer VII, the ^{31}P nucleus (at 3') and H(3') are trans and are expected to yield a $^3J_{H(3')-P(3')}$ of magnitude $\simeq$ 21 Hz; in the gauche conformers VIII and IX, the



expected value for $^3J_{H(3')-P(3')}$ is $\simeq$ 3 Hz.[36-38] The observed value for this coupling for CoASH, acetyl-CoA, malonyl-CoA, and 3',5'-ADP under various conditions of pH ranges from 7.3 to 7.9 Hz (Table 1B) and indicates that the population of the trans (VII) to be about 25% and the combined population of the gauche (VIII, IX) conformers to be $\simeq$75% (Table IV). Hydrogen magnetic resonance spectroscopy cannot distinguish between the two gauche conformers, hereafter labeled as g^-(VIII) and g^+(IX). However, in the favorable case in which ribose is ^2E[C(2') endo] and the 3'-phosphate group is g^+(IX), an almost in plane "W" relation exists between the phosphorus atom and the C(2')-H. Such a "W" relation would be expected to produce a long-range four-bond $^{31}P(3')-^1H(2')$ coupling of maximum magnitude of $\simeq$2.7 Hz,[24,38-40] should all the molecules be populated in the $^2E\cdot g^+$ conformation. It appears that a $^{31}P(3')-^1H(2')$ coupling of magnitude of $\simeq$1 Hz would be observed, should at least 50% of $^2E\cdot g$ conformers contribute toward the time average conformation of the 3'-phosphate group. In the cases of the coenzymes investigated in this report, the observed $^{31}P(3')-^1H(2')$ couplings are less than $\simeq$0.6 Hz and it appears that the percentages of the total gauche populations (VIII, IX) that can be computed from data in Table IV for these compounds mostly reflect the population of the g^- conformer. In the fractional population calculations for the conformer distribution of the 3'-phosphate group (Table IV), it has been assumed that for these compounds, all the three conformers *i.e.*, t(VII), g^-(VIII), and g^+(IX), are accessible in aqueous solution. Because in the crystal structures[41] of several 3'-nucleotides, one has not observed the t conformation and theoretical calculations[42] do not suggest the presence of such orientations, one may process the $J_{3'P}$ coupling data by completely excluding the t conformation. If this were the case the $J_{3'P}$ values in Table IB suggest that for the class of compounds examined, the 3'-phosphate group occupies g^-, g^+ domains in which the time average H(3')-C(3')-O(3')-P(3') dihedral angle is $\simeq\pm50°$.

(D) Conformation of the Ribofuranose Ring. The ribose ring of nucleosides and nucleotides, in aqueous solution, can be qualitatively described to exist as a two-state ^2E[C(2') endo] $\rightleftarrows$ ^3E[C(3') endo] equilibrium,[11,22,25] even though in reality one is dealing with a flexible ring system of continuously exchanging conformations. Recently Altona and Sundaralingam[43] have suggested that the conformation of the ribose ring in aqueous solution can be quantitatively described in terms of pseudorotation parameters and provided equations to compute the percentage populations of N and S conformers.[43] A detailed error analysis of the Altona-

Sundaralingam method was undertaken by Evans and Sarma[27] and it was shown that the pseudorotational methods do not have the claimed reliability. It was concluded that[27] whether one uses the traditional Karplus equations or the Altona–Sundaralingam method, it is necessary to assume at least 10% error in the computed population of conformers. To deter confusion between the two methods, we have proposed to use the terms ^2E and S and ^3E and N synonymously.[2,44] Elsewhere,[2,27,44,45,46] we have provided computed comparison between the percentage populations of ^2E, ^3E puckers with the populations of S, N conformers and have shown that both methods predict the same trend with similar errors. It serves no purpose to continue to present such comparisons and in this paper we will limit ourselves to the study of the $^2E \rightleftarrows {}^3E$ equilibrium as predicted by the traditional Karplus equation. Using basic parameters proposed by Schleich, *et al.*,[18] we have computed the percentage populations of ^2E and ^3E conformers in 3′,5′-ADP, CoASH, dephospho-CoA, acetyl-CoA, and malonyl-CoA under the various states of ionizations investigated (Table III). It is seen that, generally, under all the various pH values, there is a bias for the ^2E pucker, the bias being exceptionally outspoken (over 80%) when the 3′ (and 5′ for 3′,5′-ADP) phosphate is a dianion.

(E) Interrelation between Ribose Pucker and the Conformation of the Main Chain $-CH_2OPO_3$. In all of the common nucleotides investigated by nmr spectroscopy, no one has observed such an overwhelming preference for ^2E pucker; for example, the computed[27] percentage population of ^2E pucker in 5′-AMP is $\simeq$65. However, the ribose ring in β-NMN and β-NMNH is populated $\simeq$80% in ^2E forms. Even though we have no reasonable interpretation for this, we can offer the following rationalization for the observation of the same phenomenon in the case of 3′,5′-ADP as well as in coenzyme A and its analogs.

In Figure 7, we have shown the perspective drawings of 3′,5′-ADP in two different conformations, *viz.* (a) anti-^2E-gg-g′g′ and (b) anti-^3E-gg-g′g′. A remarkably nice feature in Figure 7 is that when the sugar is ^2E, the 3′- and 5′-phosphate groups are far apart and when the sugar is ^3E, the two phosphate groups become juxtaposed. In fact, the calculated[47-49] distance between the two ^{31}P nuclei in Figure 7b (*i.e.*, ^2E) is 7.0 Å and that in Figure 7a (*i.e.*, ^3E) is 5.9 Å, and based on this Sundaralingam has proposed an elegant mechanism for the elongation and contraction of polynucleotide backbones. Obviously, the electrostatic repulsive interactions between the 3′- and 5′-phosphate will be minimum if the sugar assumes the ^2E conformation, and pre-

Table III. Population[a] of Ribofuranose Ring in Various Compounds

Compd	pH	Time-averaged ribose conformation from traditional Karplus equation[b] (populations in parentheses)
3′,5′-ADP	8.0	$^2E(82) \rightleftarrows {}^3E(18)$
	5.0	$^2E(68) \rightleftarrows {}^3E(32)$
	1.5	$^2E(58) \rightleftarrows {}^3E(42)$
	8.0	$^2E(81) \rightleftarrows {}^3E(19)$
CoASH	5.0	$^2E(78) \rightleftarrows {}^3E(24)$
	1.5	$^2E(68) \rightleftarrows {}^3E(32)$
De-P-CoASH	8.0	$^2E(70) \rightleftarrows {}^3E(30)$
	1.7	$^2E(65) \rightleftarrows {}^3E(35)$
	8.0	$^2E(81) \rightleftarrows {}^3E(19)$
Acetyl-CoA	5.0	$^2E(77) \rightleftarrows {}^3E(23)$
	1.5	$^2E(67) \rightleftarrows {}^3E(33)$
Malonyl-CoA	8.0	$^2E(81) \rightleftarrows {}^3E(19)$

[a] The populations are computed from the values of $J_{1'2'}$. [b] The basic Karplus parameters for ribose are taken from ref 18. $J_0 = 9.27$ ($0° < \theta < 90°$); $J_0 = 10.36$ ($90° < \theta < 180°$).

Table IV. Per Cent Conformational Distribution of Various Compounds along Certain σ Bonds

Compds[a]	CoASH		De-P-CoASH		Acetyl-CoA		Malonyl-CoA	Pantethine phosphate		*S*-Acetylpantethine phosphate		3′,5′-ADP		
pH	8.0	5.0	8.0	1.7	8.0	5.0	1.5	8.0	5.0	8.0	5.0	8.0	5.0	1.5
Concn, *M*	0.05	0.05	0.02	0.02	0.025	0.025	0.05	0.025	0.025	0.025	0.025	0.1	0.1	0.1
gg	74.0	74.0	70.0	70.0	74.0	74.0	74.0					70.0	77.0	81.0
g′g′	87.7	87.7	83.3	83.3	87.7	87.7	87.7					86.6	78.8	80.0
Trans	24.0	27.0			24.0	27.0	24.0					24.0	27.0	27.0
g′g′′	84.4	83.3	85.5	85.5	84.4	83.3	87.7	84.4	69.4	82.2	68.8	78.8		
Trans′	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3		
Trans′′	33.3	33.3	33.3	33.3	<33.3	<33.3	<33.3	33.3	33.3	33.3	<33.3	<33.3		

[a] gg = gauche-gauche (I); g′g′ = gauche′-gauche′ (IV); trans = trans (VII); g′′g′′ = gauche′′-gauche′′ (X); trans′ = trans′ (XIII); trans′′ = trans′′ (XVI).

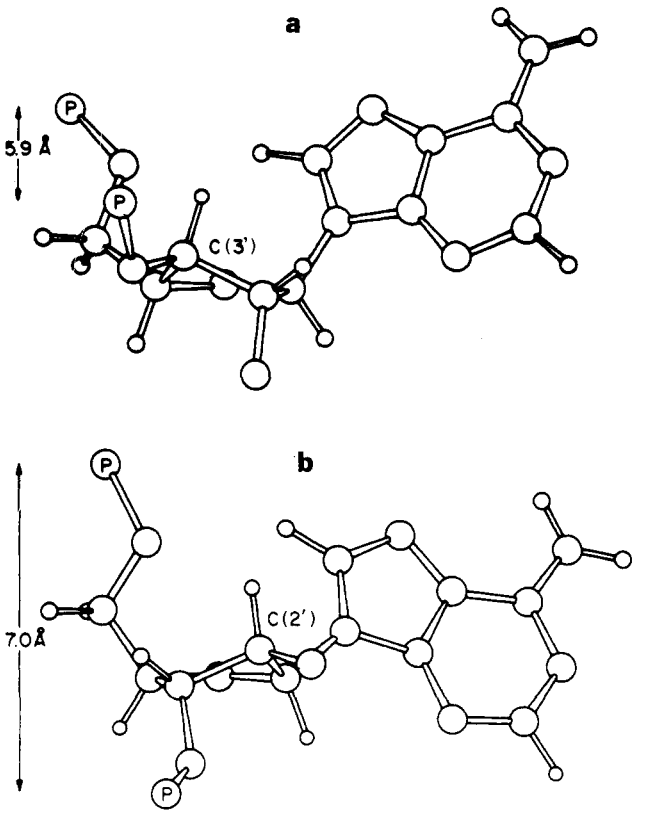


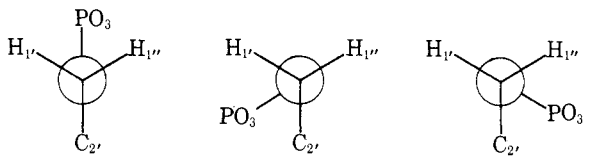
Figure 7. The Yathindra-Sundaralingam perspectives of (a) anti-^3E-gg-g'g' and (b) anti-^2E-gg-g'g' conformations for 3',5'-ADP (from ref 47 and 48).

sumably this accounts for the outspoken preference for the ^2E pucker in 3',5'-ADP, CoASH, acetyl-CoA, and malonyl-CoA at pH 8.0. At pH 8.0, the 5'- and 3'-phosphates in 3',5'-ADP are dianions and the electrostatic repulsion between the two will be very strong in ^3E pucker causing its molecular framework to assume the ^2E pucker. At pH 8.0, in coenzyme A, acetyl-CoA, and malonyl-CoA, the 3'-phosphate is a dianion and the backbone pyrophosphate is a dianion. In these cases as well, the electrostatic repulsions between the backbone and the 3'-phosphate that will be present in a ^3E sugar pucker will force the molecules to adopt the ^2E conformation. The observation that in dephospho-CoA the ^2E population is only 70%, a 11% reduction compared to the rest of the compounds at pH 8.0, is in general agreement with our rationalizations based on electrostatic repulsions between the 3'- and 5'-phosphates. In this connection, it should be pointed out that the percentage populations presented in Tables III and IV have an error of 10% and one may *prima facie* conclude that an *observed* difference of 10% in population has no significance or meaning. If one is discussing *actual* conformer populations in Tables III and IV, most certainly one may not attach significance to a difference in 10% in populations. This is because, in the basic Karplus equations used for computing conformer populations, one does not know with certainty and precision the magnitudes of the J values in the "pure" conformers, and this uncertainty introduces the large error. However, the error involved in measuring coupling constants and computer fitting the spectra is very small (±0.1 Hz) and this contributes only very little error to the *actual* conformer populations. So when we discussed *actual* conformer populations in previous sections, we did not take into consideration whether the population is 80% ^2E or 70% ^2E but, in general, whether there is a "bias" or "predominance" for particular type of pucker; but in a *relative sense*, the differences are *very real;* and the error in the differences is considerably smaller than in the actual populations.[45,46] For example, the dephospho-CoA, at pH 8.0, has a ^2E population which is 11 ± 2% less than that of CoASH at pH 8.0. There is no other reasonable way one can rationalize the magnitudes of $J_{1'2'}$ and $J_{3'4'}$, in CoASH and dephospho-CoA (Table IB), but for a small electronegativity effect on $J_{3'4'}$ from the 3'-phosphate.

The theory[47] that electrostatic repulsions between the 3'- and 5'-phosphates as the prime factor in shaping sugar conformation derives elegant support from the observation that the value of $J_{1'2'}$ decreases by 1.2 Hz when 3',5'-ADP goes from the dianion state (pH 8.0) to the monoanion state (pH 5.0). A reduction of 1.2 Hz in $J_{1'2'}$ indicates a ^2E depopulation of 14 ± 2%. Obviously, a ^3E conformation will tolerate better, from electrostatic considerations, a situation in which the 3'- and 5'-phosphates are monoanions than a situation in which they are dianions. Similar considerations can rationalize all the data in Table III. In the case of the coenzymes, it should be noted that at pH 5.0, 3'-phosphate is a monoanion and the backbone is still a pyrophosphate dianion.

The coupling constant data in Table IB show that in the case of 3',5'-ADP, the magnitude of Σ decreased by 0.7 ± 0.2 Hz under conditions in which ^3E sugar population (Table III) increases by 14 ± 2%; *i.e.*, the C(4')-C(5') bond becomes increasingly gg as the sugar pucker becomes increasingly ^3E. Hruska[22] has reported similar observations in the case of nucleosides. It is difficult to say whether the observed concomitant change in Σ' (Table IB) with pH is conformationally related to the effect of ionization on coupling constants.

(F) Conformation about the O(1')-C(1') Bond of the Pantetheine Moiety. The Newman projections X, XI, and XII show the three preferred conformers constrained to the



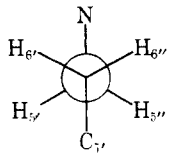
X, gauche''-gauche'' XI, gauche''-trans'' XII, trans''-gauche''

O(1')-C(1') bond of the pantetheine moiety. Using the equations in ref 19-21 one could compute the percentage populations of the gauche''-gauche'' (X, g''g'') and the combined populations of the g''t'' and t''g'' (XI and XII) conformers from the magnitude of Σ'' ($J_{1'P} + J_{1''P}$, Table IIb). The results are compiled in Table IV. It is seen that the C(1')-O(1') bond of the pantetheine moiety of CoASH, dephospho-CoA, acetyl-CoA, and malonyl-CoA shows an overwhelming preference for the g''g'' (X) conformer; and the various cofactors continue to maintain the same preference whether the 3'-phosphate of the adenosine moiety or the base itself is protonated or not.

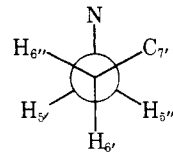
A contrasting picture is presented by the monomeric units, pantetheine phosphate and *S*-acetylpantetheine phosphate. These monomers, when their phosphate group is a monoanion (*i.e.*, same state of ionization as the parent coenzymes), display the same high degree of preference for the g''g'' conformer. However, when the phosphate group is doubly ionized, g''g'' population decreases by 10-13%, suggesting that under these conditions, g''t'' and t''g'' populations become increasingly accessible (Table IV). More dramatic is the effect of phosphate ionization on the C(1')-H, C(1'')-H, C(2')-CH_3, C(2'')-CH_3, and C(3')-H chemical shifts[50] (Table IIa). For example, in pantetheine phosphate it is seen that one of the C(1')-H is shift-

ed to lower field by 4.3 Hz and the other by 16.9 Hz in the same direction. Also, the chemical shift of one of the C(2′)-CH_3 group seems to be unaffected by phosphate ionization, whereas the other one shows a shift of 6.1 Hz to the lower field. On the other hand, protonation of the phosphate causes the C(3′)-H to shift to *higher* field by 8.1 Hz. Equally important to note is that the difference in chemical shifts between the geminal 1′,1″ protons decreases from 33.5 to 20.9 Hz, as the system goes from pH 8.0 to 5.0. The corresponding data for the C(2′) geminal methyl groups are 15.6 and 8.0 Hz. These strong perturbations in the chemical shifts and differences in chemical shifts of the 1′,1″ protons as well as those of the C(2′) methyl groups and C(3′)-H brought about by phosphate ionization, along with the information that ionization of the phosphate increases 10–13% the population of g′t″ and t″g″ rotamers, suggest some intramolecular interaction between the doubly ionized phosphate and the 3′-OH group in the free phosphopantetheine and *S*-acetylphosphopantetheine. It is conceivable that the 3′-OH is involved in hydrogen bonding formation with one of the phosphate oxygens to form an eight-membered ring; and protonation of the phosphate annihilates such interaction. This finding has little biological relevance because in coenzyme A, and in its analogs as well as in acyl carrier proteins involved in fatty acid synthesis, the phosphate group in the pantetheine phosphate moiety is a monoanion.

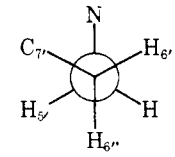
(G) Conformation about the C(5′)–C(6′) Bond of the Pantetheine Moiety. The preferred conformers about the C(5′)–C(6′) bond of the pantetheine moiety are trans′ (XIII), gauche′ (XIV), and gauche′ (XV). Pople, *et al.*,[51]



XIII, trans′



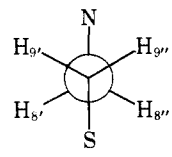
XIV, gauche′



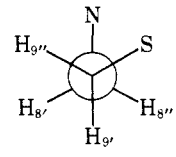
XV, gauche′

and Bovey[52] have discussed in detail the manifestation of differential conformer population such as XIII, XIV, and XV on the relevant coupling constants. Experimentally it was observed (Table IIb) that $J_{5'6'} = J_{5'6''} = J_{5''6'} = J_{5''6''} =$ 6.6 Hz in all the parent coenzymes as well as in the component monomers of pantetheine phosphates. Hence, it appears that in CoASH, dephospho-CoA, acetyl-CoA, malonyl-CoA, and the pantetheine phosphates, all the three rotamers (XIII, XIV, and XV) are equally populated, and this population is independent of the state of ionization of the 3′-phosphate as well as the protonation of the base (Table IV). It may be noted that the present nmr methods cannot distinguish between H(5′) and H(5″) as well as between H(6′) and H(6″) geminal protons; hence, rotamers XIV and XV are indistinguishable.

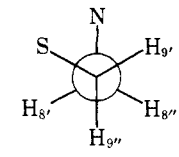
(H) Conformation about the C(8′)–C(9′) Bond of the Pantetheine Moiety. The Newman projections XVI, XVII, and XVIII show the preferred conformations about the C(8′)–



XVI, trans″



XVII, gauche″



XVIII, gauche″

C(9′) bond of the pantetheine moiety. In CoASH, dephospho-CoA, and pantetheine phosphate, it was found that $J_{8'9'} = J_{8'9''} = J_{8''9'} = J_{8''9''} =$ 6.6–6.7 Hz, indicating that in these compounds the rotamers XVI, XVII, and XVIII are equally populated. Even though we cannot distinguish between 8′ and 8″ as well as between 9′ and 9″, it was found that in acetyl-CoA, malonyl-CoA, and *S*-acetylpantetheine phosphate the relevant *J* values are no longer equal; *i.e.*, $J_{8'9'} \neq J_{8'9''}$, etc. Because the sum $J_{8'9'} + J_{8'9''}$ was found to be detectably smaller in acetyl-CoA, malonyl-CoA, and *S*-acetylpantetheine phosphate (12.6 Hz) compared to the same in CoASH, dephospho-CoA, and pantetheine phosphate (13.2–13.4 Hz), one can conclude that substitution at the -SH group tends to cause a slight depopulation of the trans″ (XVI) conformer (Tables I, II, IV).

(I) Overall Intramolecular Conformation of CoASH and Its Analogs. From the discussion in the preceeding sections, it follows that coenzyme A and its analogs have a molecular framework which is flexible in aqueous solution. However, the molecule is not totally and uncontrollably flexible; rather the framework is such that, while flexibility is allowed and alternate conformations become accessible to a considerable extent, the molecule achieves conformational identity by showing preferences—sometimes overwhelming preferences—for certain orientations. The experimentally determined preferred conformation is essentially what one would project from energy minimization considerations based on semiempirical potential energy calculations[37,47-49,53] and those based on molecular orbital computations.[42,54-57] Equally encouraging to note is the fact that the presently observed conformation is that which is observed in solid state and in solution of a host of other similar molecules.[11-27,32-34,44-46,41] It should be emphasized that whatever the conformational preferences coenzyme A and its analogs show are also exhibited by their components, *viz.* 3′,5′-ADP and phosphopantetheine and its analogs. This is in agreement with the concept of conformational "rigidity" advocated by Sundaralingam.[41,58]

In order to complete our story on the intramolecular conformation of coenzyme A and its analogs, it is necessary to examine whether any intramolecular interactions exist between the 3′,5′-ADP and pantetheine phosphate part of the coenzymes. We provide below three lines of arguments which suggest that such interactions indeed exist and a detectable amount of the coenzymes exists in folded conformations.

(i) The data in Table IIa and Table V, columns A and D, show that when coenzyme A and acetyl-CoA are taken from pH 5.0 to 8.0, the 1′1″ and 3′ hydrogens as well as the two methyl groups at 2′ of pantetheine residue move to higher fields; *i.e.*, the ionization of the 3′-phosphate of the 3′,5′-ADP portion causes an upfield shift in the chemical shifts of pantetheine hydrogens at 1′1″,3′ and the two C(2′) methyl groups; and no detectable effect is experienced by the remaining protons. Since, the affected protons are located 12–14 chemical bonds away from the 3′-phosphate oxygens, it is reasonable to conclude some time-average spatial juxtaposition between the 3′-phosphate of the adenosine part and the $POCH_2C(CH_3)_2CH$- region of the pantetheine part in the coenzymes. Data in Table V, column H, to be discussed later support such an interpretation.

(ii) The data in Table IIa as well as those in Table V, columns B and E, further show that, when the coenzymes are taken from pH 1.5 to 5.0, all the protons of the pantetheine part are shifted to higher field to different magnitudes, the effect being pronounced for the protons of the $POCH_2C(CH_3)_2CH$- regions of the pantetheine side chain; *i.e.*, protonation of the adenine and consequent reduction in its ring current field perturb the chemical shifts of the pantetheine protons. The data indicate that on a time-average basis, some interaction exists between the adenine moiety and the phosphopantetheine side chain and that in such an

Table V. Chemical Shift Differences of Pantetheine Protons on Various Compounds in Different Conditions

Nuclei	CoASH		De-P-CoASH,	Acetyl-CoA		Pant.-P,[a]	SAPP,[b]	H, (De-P-CoASH, pH 8) − (CoASH, pH 8)	I, (CoASH, pH 8) − (Pant.-P, pH 5)	J, (De-P-CoASH, pH 8) − (Pant.-P, pH 5)	K, (Acetyl-CoA pH 8) − (SAPP, pH 5)
	A, (pH 5.0) − (pH 8.0)	B, (pH 1.5) − (pH 5.0)	C, (pH 1.7) − (pH 8.0)	D, (pH 5.0) − (pH 8.0)	E, (pH 1.5) − (pH 5.0)	F, (pH 5.0) − (pH 8.0)	G, (pH 5.0) − (pH 8.0)				
PH(1′)	1.9	5.6	4.5	1.9	5.7	4.3	4.2	3.3	2.4	5.7	3.1
PH(1″)	1.9	7.6	6.9	2.0	7.6	16.9	16.9	2.7	−2.7	0.0	−2.1
$PCH_3(2')$	1.4	6.6	6.0	2.2	7.4	−1.5	−1.9	2.8	−10.5	−7.7	−10.1
$PCH_3(2'')$	2.2	7.4	7.1	2.5	6.5	6.1	5.8	3.4	−15.6	−12.2	−15.0
PH(3′)	1.2	2.3	2.1	1.1	2.7	−8.1	−7.5	0.7	−4.4	−3.7	−3.3
PH(5′)	0.3	2.2	3.1	0.4	2.0	−0.2	−1.0	−0.7	−4.6	−5.3	−4.5
PH(5″)	0.3	2.2	3.1	0.4	2.0	−0.2	−1.0	−0.7	−4.6	−5.3	−4.5
PH(6′)	0.2	2.2	3.3	0.3	2.2	−0.2	−0.8	−0.7	−4.4	−5.1	−4.3
PH(6″)	0.2	2.2	3.3	0.3	2.2	−0.2	−0.8	−0.7	−4.4	−5.1	−4.3
PH(8′)	0.0	1.8	4.4	0.1	2.6	0.6	−0.6	−1.7	−4.6	−6.3	−5.0
PH(8″)	0.0	1.8	4.4	0.1	2.6	0.6	−0.6	−1.7	−4.6	−6.3	−5.0
PH(9′)	−0.2	1.2	3.3	−0.1	3.0	0.2	−1.2	−1.6	−3.5	−5.1	−4.6
PH(9″)	−0.2	1.2	3.3	−0.1	3.0	0.2	−1.2	−1.6	−3.5	−5.1	−4.6
$-COCH_3$				−1.0	1.8		−0.8				−2.5

[a] Pant.-P = pantetheine phosphate. [b] SAPP = *S*-acetylpantetheine phosphate.

interaction, the time-averaged juxtaposition between the adenine moiety and phosphopantetheine region is such that the $POCH_2C(CH_3)_2CH-$ fragment of the side chain is effected the most.

(iii) The data in Table IIa and those in columns I and K of Table V provide comparison of the chemical shifts of coenzyme A and acetyl-CoA (pH 8.0) with those of phosphopantetheine and *S*-acetylphosphopantetheine at pH 5.0. The comparison is made for the pantetheines at pH 5.0 when the phosphate group is a monoanion because under these conditions they resemble the same fragments in the parent coenzymes at pH 8.0. The data show the total effect of the 3′,5′-ADP part on the chemical shifts of the pantetheine side chain. It is seen that in going from phosphopantetheine monoanion to coenzyme A, the C(1′)-H is deshielded,[50] and the remaining protons have undergone shielding, the effect being maximum on the two CH_3 groups at C(2′).

The data discussed clearly show that some detectable interactions happen between the adenosine and the pantetheine part and this finding is best rationalized on the ground that some folded conformations are accessible for these coenzymes. Folded conformations can be achieved from linear conformations by simple rotations about the P–O–P bond and this can be done while maintaining conformational integrity about the various other bonds. Sundaralingam[41,58] as well as us[23] has discussed this point *in extenso* elsewhere. The magnitude of the chemical shift change, induced by the adenosine portion (Table V, columns I and K) on the pantetheine moiety, is too small to be rationalized on the ground that the molecular framework, on a time-average basis, exists predominantly in folded conformations. In fact, qualitative estimation, based on ring current consideration[59] (*vide infra*), alone suggests that they are not present any more than 30%.

It is tempting to propose some reasonable model for the low populated folded conformations. One could take advantage of the ring current effects of the adenine moiety felt by the pantetheine protons to propose such a model. Such approaches to solve structural and conformational problems have been used successfully by Shulman[60] and Kearns[61] to assign the chemical shifts of the hydrogen-bonded base pairs in the helical region of tRNA. Sarma and Mynott,[23,25] Evans and Sarma,[30] and Kondo, *et al.*,[62] have also used theoretical ring current calculations[59] to arrive at the preferred stacking geometry in certain 5′-β-nucleotide derivatives. To extend such studies to coenzyme A, it is necessary to know the effect of the adenine moiety alone on the chemical shifts of the pantetheine protons. The data in Table V, columns I and K, show the combined effect of adenosine as well as 3′-phosphate on pantetheine proton chemical shifts. So the first order of business is to separate these two effects.

In order to accomplish this we have done detailed conformational analysis of dephospho-CoA and the discussion presented in earlier sections clearly shows that this coenzyme has identical conformational preferences as CoASH and acetyl-CoA. The only difference is in the degree of ^2E pucker populations; the reasons for this were given earlier. We do not think this difference will have any marked influence on the overall time-average conformation. The identity of the chirality shift summarized in Table VI, for CoASH, dephospho-CoA, acetyl-CoA, and malonyl-CoA, further suggests that all these compounds have the same time-average conformations. So it is reasonable to use the difference in chemical shifts for the pantetheine protons between CoASH and dephospho-CoA as the effect of a 3′-phosphate group on the chemical shifts of pantetheine protons in CoASH. And the data in Table V, column H, show that the

Table VI. Chemical Shift Differences of Some Geminal Groups on Various Compounds

Compd	CoASH			De-P-CoASH			Acetyl-CoA			Malonyl-CoA	Pantetheine phosphate		S-Acetylpantetheine phosphate	
pH	8.0	5.0	1.5	8.0		1.7	8.0		1.5	8.0	8.0	5.0	8.0	5.0
Concn, M	0.05	0.05	0.05	0.02		0.02	0.025		0.025	0.05	0.025	0.025	0.025	0.025
PH(1′)-PH(2′)	26.0	26.0	24.0	26.6		24.2	26.7		24.7	26.8	33.5	20.9	34.2	21.5
$PCH_3(2'')$-$PCH_3(2'')$	13.1	12.3	11.5	12.5		11.4	13.1		12.7	13.0	15.6	8.0	15.9	8.2

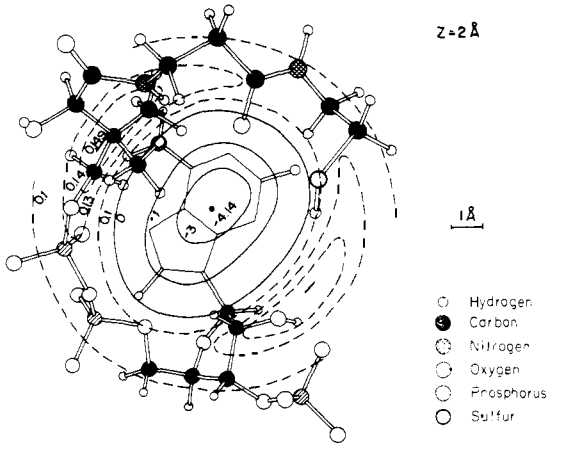


Figure 8. A perspective drawing of a plausible folded conformation of coenzyme A with isoshielding lines (z = 2 Å) as the background. It should be emphasized that the isoshielding surface in Figure 8 lies at a z value of 2 Å whereas the z value for the pantetheine protons ranges from 2 to 7 Å. The theoretically predicted values for shielding in Table VII were derived according to ref 63.

3′-phosphate group in CoASH principally causes the shielding of the $POCH_2C(CH_3)_2$- fragment of the pantetheine part of CoASH with minor deshielding effect on the 8′,4″,9′,9″ protons. This finding, along with the observation that in coenzyme A and its analogs the ionization of the phosphate perturbs the chemical shifts of the $POCH_2C(CH_3)_2CH$- fragment of pantetheine, indicates that detectable folding interactions take place on the side of the adenine moiety which contains the 3′-phosphate group. According to Sarma-Mynott nomenclature,[23,25] this is side A of adenine (Figure 1). Obviously, we cannot rule out, because of the inability of the methods to detect, the interaction with the B side.[23,25]

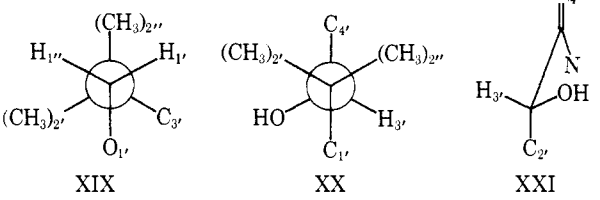
Now, the difference in chemical shifts between dephospho-CoA at pH 8.0 and phosphopantetheine at pH 5.0 (Table V, column J) can be, in general, taken as equal to the observed ring current effect of the adenine moiety on the pantetheine proton chemical shifts in these coenzymes. A very important observation, such separation of effects, uncovered is that one of the 1′ hydrogens of the pantetheine fragment undergoes deshielding by adenine (by 5.7 Hz) while the other is unaffected (Table V, column J). All the other protons of the pantetheine side chain have moved to higher fields to varying extent by the ring current effect of the adenine moiety (Table V, column J).

The difficult part is to translate the observed effects of adenine on the chemical shifts of pantetheine protons (Table V, column J) to a reasonable folded conformation. The attempt is hampered by the fact that the data in Table V, column J, are not contributed by 100% of a uniquely folded conformation. However, the data can be used to determine whether one is dealing with a simple fast, two-state equilibrium between a linear and a folded conformation or a situation in which fast exchange exists among linear and a variety of folded species.

In Figure 8 is shown a perspective of the molecular framework of a plausible folded conformation along with the isoshielding lines (z = 2 Å) as the background. In constructing the folded conformation we have attempted to generate a model which will, as much as possible, account for the direction and magnitudes of the observed ring current effects[59,63] (Table V, column J), while introducing no unusual distortions about the various covalent bonds. It may be noted that in the folded form shown (Figure 8), the pantetheine tail is not folded over the adenine surface, but it is coiled around the edges. This arrangement becomes nec-

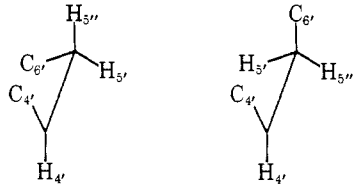
essary to rationalize the direction and relative magnitudes of the ring current effects on pantetheine 1′,1″ hydrogens and the methyl groups at C(2′). The model is such that the pantetheine tail coils around the adenine moiety which is burried in the cavity.

In both the folded and linear conformations[64] the adenine is anti, the ribose is ^2E, the C(3′)-O(3′) torsion is trans; *i.e.*, C(4′)-C(3′) is trans to O(3′)-P(3′), the C(4′)-C(5′) and C(5′)-O(5′) bonds are gg-g′g′; in the pantetheine part P(1′)-O(1′)-C(1′)-C(2′) bonds are trans. These are the observed preferred conformations (*vide supra*) except that conformation VIII was adopted for the 3′-phosphate group even though we cannot distinguish between VIII and IX. This is in accordance with the X-ray and ^{13}C nmr data on 3′-AMP.[14,65] No effort was made to keep C(5′)-C(6′) and C(8′)-C(9′) bonds in any specific orientation. Conformations about C(1′)-C(2′), C(2′)-C(3′), and C(3′)-C(4′), which cannot be determined by the present methods, were kept in the maximum staggered conformation in both the folded and linear species. These arrangements are illustrated in XIX, XX, and XXI.



XIX XX XXI

The two major differences between the folded and linear forms lie[64] in the P-O-P bond rotation and that about N-C(5′). In the folded form the pyrophosphate oxygens are staggered[41] and they are eclipsed in the open form. The geometric difference in the N-C(5′) is shown in projections XXII (folded) and XXIII (linear).



folded linear

The cylindrical coordinates[66,67] p and z for the folded conformation in Figure 8 are given in Table VII. Also given are the theoretically computed and experimentally observed shifts for the pantetheine protons, induced by the adenine ring.

No theoretical data are shown for most of the protons in the pantetheine tail because for these protons the magnitudes of p and z are such that no data are available. However, the theoretically predicted shieldings for these protons are expected to be very small and negative in direction. Inspection of the data in Table VII indicates that there is agreement in the direction of the observed and theoretically projected shifts. There is no agreement between the two with respect to the magnitudes. This is because the theoretical projections were derived on the assumption of 100% folded *static* species. One would expect the observed shifts to be proportional to the theoretical shifts, if one is dealing with a simple linear $\rightleftarrows$ folded equilibrium. This is not observed especially for the pantetheine tail protons suggesting the total freedom in segmental motion this part of the coenzyme will have in several possible folded conformers. On the other hand, there is a general trend for constant proportionality between the observed and theoretical shifts for

Table VII. Cylindrical Coordinates p and z for the Pantethine Protons in Figure 8[a]

Nuclei	z, Å	p, Å	Theor predicted shielding[b]	Obsd shielding
PH(1′)	2	5	13	5.7
PH(1″)	2	3	0	0.0
PCH_3(2′)	5	4.0	−15	−7.7
PCH_3(2″)	3	2.5	−40	−12.2
PH(3′)	4	6.5	Very small (−)	−3.7
PH(5′)	7	5.0	Very small (−)	−5.3
PH(5″)	7	5.0	Very small (−)	−5.3
PH(6′)	7	6.0	Very small (−)	−5.1
PH(6″)	7	6.0	Very small (−)	−5.1
PH(8′)	5	5.0	−8	−6.3
PH(8″)	5	5.0	−8	−6.3
PH(9′)	6	6.0	Very small (−)	−5.1
PH(9″)	6	6.0	Very small (−)	−5.1

[a] Measurement error = 0.5 A. [b] See ref 63.

1′,1″ protons and the protons of the 2′- and 2″-methyl groups. This suggests that in the several possible folded conformations, the initial turn for folding is likely to be similar and indicates that the total population of the folded species to be not more than 30%. The assignments are such that if 1′ hydrogen (as assigned above) is replaced by deuterium, the configuration of C(1′) will be *S*. Similarly, if the hydrogens of the 2′-methyl group, as assigned here, are replaced by deuterium, the configuration of C(2′) will be *S*. It appears likely that during the reorientation of the molecules in solution, the 3′,5′-ADP part and the pantetheine phosphate part can undergo differential segmental motion *via* rotation about the P-O-P linkage and such rotations make possible the existence of open and folded conformers. It is gratifying to note that Sundaralingam[58] proposed that the monomeric units of polynucleotides and related molecules maintain their conformational integrity in their parent compounds and that the parent compound achieves its three-dimensional geometry by rotation about the P-O bonds.

Acknowledgments. This research was supported by Grant CA 12462 from the National Cancer Institute and Grant B-28015-001 from the National Science Foundation. We thank M. Sundaralingam for providing us with the perspective drawings of 3′,5′-ADP before publication. We thank professors M. Sundaralingam and F. E. Hrusk for several stimulating discussions in the last 2 years which contributed toward a greater understanding of the stereochemical behavior of nucleotides and their coenzyme derivatives. The authors thank Professor B. Pullman and Dr. C. Giessner-Prettre for providing us with the isoshielding surface for adenine for various z values.

Supplementary Material Available. Drawings of folded and linear conformations will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-75-1225.

References and Notes

(1) (a) Preliminary communication on this has appeared elsewhere.[2] (b) Presented in part at the Fourth Harry Steenback Symposium, University of Wisconsin, Madison, Wis., June 16–19, 1974.
(2) C. H. Lee and R. H. Sarma, *FEBS Lett.*, **43**, 271 (1974).
(3) F. Lipmann, *J. Biol. Chem.*, **160**, 173 (1945).
(4) F. Lipmann and N. O. Kaplan, *J. Biol. Chem.*, **162**, 743 (1946).
(5) F. Lipmann, N. O. Kaplan, G. D. Novelli, L. C. Tuttle, and B. M. Guirard, *J. Biol. Chem.*, **167**, 869 (1947).

(6) N. O. Kaplan and F. Lipmann, *J. Biol. Chem.*, **174**, 37 (1948).
(7) F. Lipmann, N. O. Kaplan, G. D. Novelli, L. C. Tuttle, and B. M. Gulrard, *J. Biol. Chem.*, **186**, 235 (1950).
(8) J. Baddiley, E. M. Thain, G. D. Novelli, and F. Lipmann, *Nature (London)*, **171**, 76 (1953).
(9) J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, **81**, 1265 (1959).
(10) J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 663 (1961).
(11) R. H. Sarma and R. J. Mynott, *J. Amer. Chem. Soc.*, **95**, 1641 (1973).
(12) R. H. Sarma, R. J. Mynott, F. E. Hruska, and D. J. Wood, *Can. J. Chem.*, **51**, 1843 (1973).
(13) R. H. Sarma and R. J. Mynott, *Org. Magn. Resonance*, **4**, 577 (1972).
(14) I. C. P. Smith, H. H. Mantsch, R. D. Lapper, R. Deslauriers, and T. Schleich, *Conform. Biol. Mol. Polym., Proc. Jerusalem Symp. Quantum Chem. Biochem.*, **5**, 381 (1973).
(15) B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **95**, 2873 (1973).
(16) R. D. Lapper, H. H. Mantsch, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **96**, 2878 (1973).
(17) R. D. Lapper and I. C. P. Smith, *J. Amer. Chem. Soc.*, **95**, 2880 (1973).
(18) T. Schleich, B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *Biochemistry*, **11**, 137 (1972).
(19) F. E. Hruska, D. J. Wood, R. J. Mynott, and R. H. Sarma, *FEBS Lett.*, **31**, 153 (1973).
(20) D. J. Wood, F. E. Hruska, R. J. Mynott, and R. H. Sarma, *FEBS Lett.*, **34**, 323 (1973).
(21) D. J. Wood, F. E. Hruska, R. J. Mynott, and R. H. Sarma, *Can. J. Chem.*, **51**, 2571 (1973).
(22) F. E. Hruska, *Conform. Biol. Mol. Polym., Proc. Jerusalem Symp. Quantum Chem. Biochem.*, **5**, 345 (1973).
(23) R. H. Sarma and R. J. Mynott, *J. Amer. Chem. Soc.*, **95**, 7470 (1973).
(24) R. H. Sarma, R. J. Mynott, D. J. Wood, and F. E. Hruska, *J. Amer. Chem. Soc.*, **95**, 6457 (1973).
(25) R. H. Sarma and R. J. Mynott, *Conform. Biol. Mol. Polym., Proc. Jerusalem Symp. Quantum Chem. Biochem.*, **5**, 591 (1973).
(26) R. H. Sarma, C. H. Lee, F. E. Hruska, and D. J. Wood, *FEBS Lett.*, **36**, 157 (1973).
(27) F. E. Evans and R. H. Sarma, *J. Biol. Chem.*, **249**, 4754 (1974).
(28) 300-MHz spectra were obtained at the NMR center, Institute of Polymer Science, University of Akron, Akron, Ohio. We thank Everett R. Santee, Jr., and Toshimitsu Suzuki for providing us with the spectra in an extremely short time.
(29) R. H. Sarma and N. O. Kaplan, *Biochemistry*, **9**, 539 (1970).
(30) F. E. Evans and R. H. Sarma, *Biopolymers*, **13**, 2117 (1974).
(31) O. Jardetzky and N. G. Wade-Jardetzky, *J. Biol. Chem.*, **241**, 85 (1966).
(32) M. P. Schweizer, A. D. Broom, P. O. P. T'so, and D. P. Hollis, *J. Amer. Chem. Soc.*, **90**, 1042 (1968).
(33) S. I. Chan and J. H. Nelson, *J. Amer. Chem. Soc.*, **91**, 168 (1969).
(34) F. E. Evans and R. H. Sarma, *FEBS Lett.*, **41**, 253 (1974).
(35) S. S. Danyluk and F. E. Hruska, *Biochemistry*, **7**, 1038 (1968).
(36) G. Kotowycz and K. Hayamizu, *Biochemistry*, **12**, 517 (1973).
(37) N. Yathindra and M. Sundaralingam, *Biopolymers*, **12**, 297 (1973).
(38) L. D. Hall and R. B. Malcolm, *Can. J. Chem.*, **50**, 2092 (1972).
(39) L. D. Hall and R. B. Malcolm, *Can. J. Chem.*, **50**, 2102 (1972).
(40) B. Donaldson and L. D. Hall, *Can. J. Chem.*, **50**, 2111 (1972).
(41) M. Sundaralingam, *Biopolymers*, **6**, 189 (1969).
(42) B. Pullman, D. Perahia, and A. Saran, *Biochim. Biophys. Acta*, **269**, 1 (1972).
(43) C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.*, **95**, 2333 (1973).
(44) C. H. Lee and R. H. Sarma, "Proceedings of the Fourth Harry Steenback Symposium," S. T. Rao and M. Sundaralingam, Ed., University Park Press, Baltimore, Md., June 1974, in press.
(45) C. H. Lee, F. E. Evans, and R. H. Sarma, *J. Biol. Chem.*, in press.
(46) F. E. Evans and R. H. Sarma, *J. Amer. Chem. Soc.*, submitted for publication.
(47) M. Sundaralingam in "Proceedings of the Sanibel Quantum Biology Symposium," Jan 1974, in press.
(48) See M. Sundaralingam in ref 44.
(49) N. Yathindra and M. Sundaralingam, *Biopolymers*, **12**, 2261 (1973).
(50) We cannot at this point in discussion distinguish between C(1′)-H and C(1″)-H or between C(2′)-CH_3 and C(2″)-CH_3. Later we have presented a method to distinguish between the above set of protons. What is important to note is that the chemical shifts of the two C(1′) protons and the two C(2′) methyl groups are affected differentially by phosphate ionization.
(51) J. A. Pople, W. G. Schneider, and H. J. Bumstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N.Y., 1959, pp 380–381.
(52) F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy," Academic Press, New York, N.Y., 1969, pp 136–137.
(53) N. Yathindra and M. Sundaralingam, *Biopolymers*, **12**, 2075 (1973).
(54) D. Perahia, B. Pullman, and A. Saran, *Biochim. Biophys. Acta*, **340**, 299 (1974).
(55) A. Saran, B. Pullman, and D. Perahia *Biochim. Biophys. Acta*, **287**, 211 (1972).
(56) C. Giessner-Prettre and B. Pullman, *J. Theor. Biol.*, **40**, 441 (1973).
(57) C. Giessner-Prettre and B. Pullman, *J. Theor. Biol.*, in press.
(58) M. Sundaralingam, *Proc. Int. Symp. Conform. Biol. Mol. Polym., Symp. Quantum Chem. Biochem.*, **5**, 417 (1973), and references cited therein.
(59) C. Giessner-Prettre and B. Pullman, *J. Theor. Biol.*, **27**, 87 (1970).
(60) See R. Shulman in ref 44.
(61) See D. Kearns in ref 44.
(62) N. S. Kondo, K. N. Fang, P. S. Miller, and P. O. P. Ts'o, *Biochemistry*, **11**, 1991 (1972).
(63) The theoretical shielding data in Giessner-Prettre and Pullman[59] are for a z value of 3.4 Å. The same authors have supplied us the theoretical shielding data for z values in the range of 2.0–5.2 Å for increments of 0.2 Å. It is these data which were used in all the calculations in the present paper.
(64) Two perspective drawings of the folded conformation and one of the linear conformation, all drawn to scale, are deposited for the microfilm edition. See paragraph at end of paper regarding supplementary material.
(65) M. Sundaralingam, *Acta Crystallogr.*, **21**, 495 (1966).
(66) C. E. Johnson, Jr., and F. E. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).
(67) R. B. Mallion and C. W. Haigh, *Org. Magn. Resonance*, **4**, 203 (1972).