s. Even a small increase in the pressure in the cell leads to decreased line intensities, indicating rapid gas-phase dimerization. To the limit of our sensitivity no other lines were observed (apart from weak lines of the small amount of unpyrolyzed precursor).

Work is continuing to improve the sensitivity of the spectrometer to enable other isotopic species to be studied and the full geometry to be determined. The present observations are, however, consistent with the ab initio MO predictions of a short acetylenic C≡C bond rather than the cumulene structure sometimes suggested for benzyne.

Acknowledgment. The work was supported by a grant from the Australian Research Grants Scheme. M.R. acknowledges the award of a Monash Vice Chancellor's Post-Doctoral Fellowship. Burkhard Kleibömer assisted in providing the computer program employed in the trial and error assignment method.

Registry No. Benzyne, 462-80-6.

A New Method for Studying Chain Conformation. Proof of Nonradial Binding to Micelles; Chain-Bending at an Enzyme Surface

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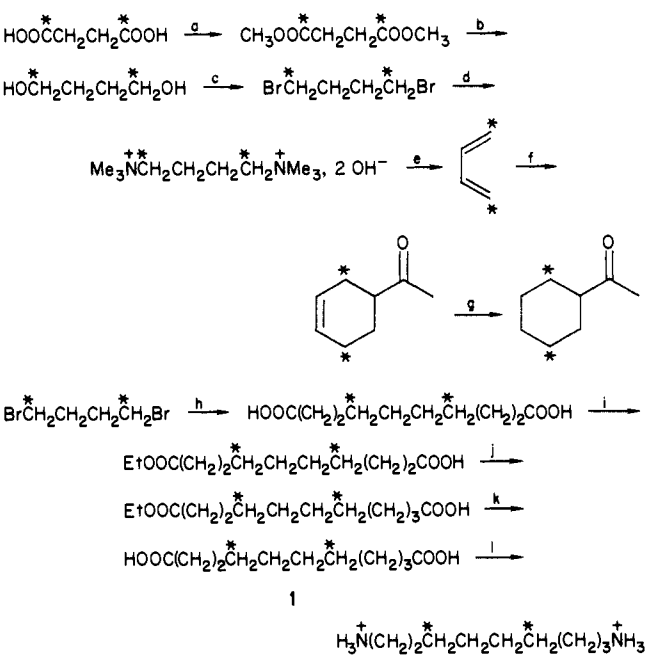
Received July 29, 1985

The ability of hydrocarbon chains to fold influences the structure of micelles,[1] the permeability of membranes,[2] and the binding to enzymes.[3] Given the obvious importance of chain folding, it is surprising how little is known about the subject. Raman scattering reveals the approximate number of gauche linkages per chain,[4] but the exact location of the "kinks" usually cannot be determined. Deuterium NMR provides an "order parameter" which varies from 0 to 1 according to the alignments of a C–D vector in the chain.[5] Since, however, order parameters encompass an extremely complex set of motions, they are primarily useful for qualitative comparisons (e.g., determining whether addition of cholesterol to a membrane increases or decreases the order of the chains). "Mobility parameters" such as T_1 are also difficult to interpret mechanistically.[6] Indeed, even the relationship between "chain order" and "chain mobility" is not well understood. In the present paper, we discuss a new experimental approach for studying chain conformation. The conceptually simple method (a) furnishes the trans–gauche populations at specific sites along a chain, (b) involves no potentially disruptive probe, and (c) works equally well for ordered and disordered systems. As will be shown, the method can be used to examine, for the first time, the conformation of a chain bound to an enzyme in solution.

Our experiments are based upon the long-range coupling, ^3J, between two ^{13}C atoms spaced four carbons apart (C^*_1–C_2–C_3–C^*_4). Past theoretical and experimental work[7,8] indicates that the coupling between C_1 and C_4 depends on the dihedral angle about the C_2–C_3 bond in a typical Karplus-type function. Thus, $^3J_{trans}$ = 3.5–4.0 Hz, whereas $^3J_{gauche}$ = 1.5 Hz. By synthesizing chains bearing two ^{13}C atoms at known locations, we can deduce the time-averaged conformation at a particular linkage.

Scheme I[a]



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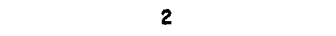

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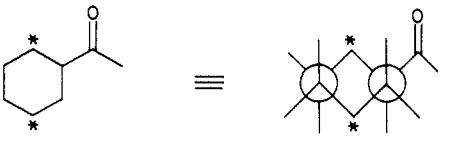
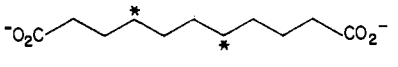
[a] (a) CH_2N_2; (b) DIBAL-H; (c) HBr; (d) Me_3N, Ag_2O; (e) heat; (f) CH_2=$CHCOCH_3$; (g) H_2 + Pd/C; (h) LiC≡CCO_2Li, H_2 + Pd/C; (i) EtOH + H_2SO_4; (j) $SOCl_2$, CH_2N_2, $C_6H_5CO_2Ag$; (k) KOH; (l) NaN_3 + H_2SO_4.

Before embarking on the synthesis of di-^{13}C-labeled chains, we had to assure ourselves that the literature coupling constants, especially $^3J_{gauche}$, are indeed correct. Toward this end, we prepared a di-^{13}C-labeled acetylcyclohexane (Scheme I) in which a



gauche relationship is enforced upon the two ^{13}C atoms. This compound manifests a $^3J_{gauche}$ of 1.8 Hz in three solvent systems ($CDCl_3$, Me_2SO-d_6, and 20% H_2O in Me_2SO-d_6) in agreement with literature data. Coupling constants (±0.1 Hz) were secured from INADEQUATE spectra by using 0.13 M compound (8% dilabeled), a 1602-Hz sweep width, 352 scans, 16.5 s/scan, and a spectrometer programmed for the 32-phase sequence of Bax, Freeman, and Kempsell.[9]

Di-^{13}C-labeled chain experiments focused on the two "bolaform" electrolytes[10] drawn below. Their syntheses are presented in



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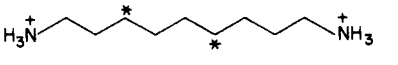

2

Scheme I. Owing to the great expense of starting material (succinic acid-*1,4*-$^{13}C_2$),[11] each reaction was first carried out repeatedly with unlabeled materials in order to optimize the yields. The coupling constants vary neither with the medium nor with the concentration. Thus, ^3J = 3.8, 3.7, 3.6, 3.6, and 3.4 Hz for 50 mM diacid **1** in tetrahydrofuran, ethanol, dioxane, dimethyl sulfoxide, basic deuterium oxide (pD 13), and basic deuterium

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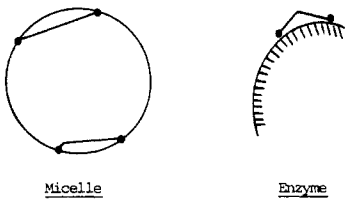

Figure 1.

oxide with 3.0 M KCl. Increasing the diacid concentration to 1.0 M (excess KOH in D_2O) does not change the coupling constant nor does increasing the temperature from 25 to 80 °C. Similarly, 50 mM **2** has a J = 3.8 Hz as the disalt (in acidic D_2O) or as the free amine (in ethanol). Apparently, the chains are fully extended, or nearly so, under all the preceding conditions.

One main reason for selecting **1** and **2** for our initial studies was to determine their behavior when bound to cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) micelles. Note that the fully extended chains of the bolaforms are too short to permit a radial disposition within the micellar host; a radial geometry would force one of the polar groups to reside within the supposedly dry interior. Nonetheless, **1** and **2** can still bind to micelles if they "loop" in order to place both head groups near the surface. Unexpectedly, however, the NMR data show that looping does *not* occur. When 0.03 M **1** in 0.10 M KOH was mixed with 0.09 M CTAB in D_2O, the coupling constant remains at 3.5 Hz. Elevating the concentration of **1** in the micellar CTAB has no effect on ^3J. Likewise, when unprotonated **2** (0.025 M) binds to 0.10 M SDS, the coupling constant also equals 3.5 Hz. Clearly, the two central linkages[12] of the adsorbed guest molecules are substantially *transoid*. Unless the outer C–C linkages possess kinks when the central ones do not (which is just the reverse of what is needed for a major loop), the bolaforms assume extended conformations within the micelles. This can occur only by "tangential" binding (Figure 1),[13] a phenomenon not incorporated into the classical Hartley "asterick" model. Nonradial binding is, however, consistent with "brush-heap" disorder and with the presence of fatty patches near the micelle surface where guests bind hydrophobically.[14]

There is no doubt that the dianionic bolaform **1** does indeed bind to the CTAB micelles. An Armstrong experiment[15] was carried out in which the bolaform was thin-layer chromatographed on a Brinkmann Polyamide-6 plate with 0.025 M aqueous CTAB as the mobile phase. The bolaform, moving with the CTAB front, displayed an R_f of 0.60 compared to 0.08 when pure water was the mobile phase. Partition coefficients, estimated from the R_f values,[15] indicate that >98% of **1** is micelle bound under NMR conditions. This is hardly surprising: amphiphiles of opposite charge are known to form tight complexes.[16,17] In the case of neutral **2** in SDS micelles, there is also no doubt of complete association because the diamine will not dissolve in water when SDS is absent.

Binding of bolaform **2** to trypsin (known to have a specificity pocket for lysine[18]) produced quite different results. Solutions containing 7.5×10^{-4} M bolaform and excess trypsin (20 mg/mL) at pD 6.0 were examined by ^{13}C NMR (9056 scans, 1612 sweep width, 8% dilabeled compound). The presence of the enzyme reduced ^3J from its solution value of 3.8 Hz to only 0.8 Hz, a value corresponding to a dihedral angle of about 70°. Competitive inhibition studies (carried out by measuring the enzyme-catalyzed hydrolysis rate of arginine methyl ester at pH 5.5 with the aid of a pH-stat) gave a K_i = 0.05 M. Under our NMR conditions, therefore, less than 10% of the bolaform is bound to the active site. Thus, the striking reduction in ^3J must be attributed to noncompetitive binding which induces a chain "kink" as depicted in Figure 1.

The di-^{13}C-labeled NMR method can be used to study chain folding in molecules attached to receptor sites, antibodies, membranes, and a host of other biologically important systems. We ourselves are currently synthesizing lipids that are di-^{13}C-labeled at several locations, and the results will be reported when the experiments are completed.

Acknowledgment. This work was supported by the National Institutes of Health. We thank Professor C. G. Trowbridge for his assistance with the enzyme kinetics.

(12) The NMR method examines only one of two identical C–C bonds in the center of the chain.
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Stereochemical Course of the Phosphoryl Transfer from Adenosine 5′-Diphosphate to Alcohols in Acetonitrile and the Possible Role of Monomeric Metaphosphate

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Received October 2, 1985

Following the initial proposal of Westheimer[1] and of Bunton[2] much evidence has been cited in support of the intermediacy of a monomeric metaphosphate in nucleophilic displacement reactions of monosubstituted phosphate esters.[3] However, recent stereochemical studies on the solvolysis of the monoanion of phenyl [^{16}O,^{17}O,^{18}O]phosphate and the dianion of 2,4-dinitrophenyl [^{16}O,^{17}O,^{18}O]phosphate in hydroxylic solvents have been shown to proceed with complete (within experimental error) inversion of configuration.[4] The previous evidence in favor of a metaphosphate intermediate can be reconciled with the stereochemical course of the reaction in terms of a preassociative mechanism, in which the intermediate is never "free" and indeed is only formed productively when the nucleophile is already preassociated in the encounter complex. Much of the direct evidence in favor of a free metaphosphate has come from reactions in aprotic solvents.[3,5] Pertinent to this study, Ramirez and co-workers[5] have demonstrated that aryl phosphate esters and the tri and tetra anions of ATP in acetonitrile will phosphorylate the hindered alcohol *tert*-butyl alcohol and they argue that this may be a good criterion for the participation of a metaphosphate. Despite the compelling evidence in favor of a metaphosphate-like intermediate the extent to which this can be freely solvated, particularly in aprotic solvent, is unclear. Utilizing isotopically chiral [β-^{16}O,^{17}O,^{18}O]adenosine 5′-diphosphate we have sought stereochemical information pertinent to this point.

We have shown that adenosine 5′-diphosphate tris(tetra-*n*-butylammonium) salt in dry acetonitrile phosphorylates alcohols under conditions analogous to those previously reported by Ramirez et al. for ATP.[5] *tert*-Butyl alcohol was phosphorylated at

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