Observation of Large Solvent Effects on the ^{31}P NMR Chemical Shifts of Nucleotides

Sir:

There is currently much interest in the ^{31}P NMR spectra of polynucleotides due to the sensitivity of the ^{31}P resonance positions of these compounds to changes in the phosphodiester bonds.[1-3] Extensive theoretical studies by Gorenstein indicate that the chemical shift is sensitive to both the O–P–O bond angle[4] and ω and ω' torsional angles[5] which describe rotation of the R–O bond relative to the plane of the O–P–O group. These theoretical studies and earlier experimental studies have prompted many investigators to interpret the ^{31}P resonance positions observed in various polynucleotide systems almost entirely in terms of the conformation of the phosphodiester group.[6,7] Relatively little attention has been given to other factors which might influence ^{31}P chemical shifts. For example, in tRNA molecules a number of resolved resonances are observed in the ^{31}P NMR spectra displaced to both the high- and low-field side of the resonance position characteristic of regular RNA double helices. These shifted resonances have been attributed to phosphodiester groups which have been forced into unusual conformations by the folding of the tRNA molecule. Both Salemink[3] and Gorenstein[8] have attempted to use the Gorenstein theory to correlate the number and position of the shifted resonances with X-ray crystal data. The unusual shifts of the ^{31}P resonances in the left-handed double helix of poly[d(G–C)][1] and drug–DNA[2] complexes are also attributed to unusual backbone conformations.

Although there are suggestions in the literature that solvent effects are negligible (less than 0.1 ppm), we suspected this might not be true, particularly in view of the previously demonstrated solvent effects on the ^{31}P of other phosphorous compounds.[9] We therefore have examined the solvent effects on the ^{31}P chemical shift of a number of nucleotides. Because conformational state has a profound effect on the ^{31}P chemical shift, we have included in our study several cyclic nucleotides in which the conformational state of the phosphodiester group is more or less fixed.[10] In this way we attempted to eliminate the possibility of solvent-induced conformational changes which might complicate the interpretation.

The essential observations are presented in Figure 1 where we show the ^{31}P chemical shift of 3′,5′-cAMP as a function of the mole fraction of organic solvent added to an aqueous solution. Similar shifts were obtained with 3′,5′-cUMP and 2′,3′-cAMP. Contrary to what is claimed in the current literature, the change in solvation caused by the various organic solvents can cause upfield shifts greater than 3 ppm.

The order of upfield shifts correlates with the hydrogen bond donating ability of the solvents (dimethyl sulfoxide is the poorest hydrogen bond donor, 1,1,1,3,3,3-hexafluoro-2-propanol the best), indicating that hydrogen bonding to the phosphate group is the key factor in determining the magnitude of the solvent effect. A second effect of the solvents might be to induce ion pair formation; however, we note there is no correlation between the dielectric constant of the solvent and the magnitude of the shift induced. Furthermore, the possibility of ion-pair formation was tested by examining the conductivity of a mixed aqueous–organic solution of the cyclic nucleotide. At dimethyl sulfoxide levels up to 80% we found no evidence for ion pairing.[11]

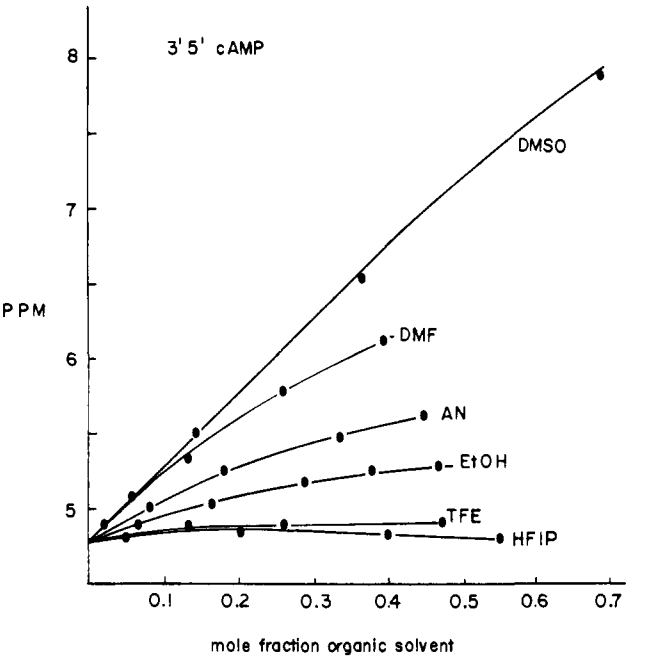

Figure 1. Variation in the ^{31}P NMR chemical shift of 3′,5′-cAMP as a function of the mole fraction of organic solvent in the various mixed aqueous-organic solvents. An aqueous solution of 3′,5′-cAMP containing 10 mM NaCl and 10mM cacodylate buffer, pH 7, was diluted with the appropriate organic solvent to yield the mole fractions indicated in the figure. Spectra were recorded at 26 °C by using a 40.3-MHz spectrophotometer. Chemical shifts of the sample were referenced to an external trimethyl phosphate (TMP) standard contained in a capillary tube in the center of the sample and positive shifts correspond to upfield shifts. The shift of the TMP resonance due to bulk susceptibility effects was measured by reference to an external D_2O sample permanently mounted in the probe and found to be negligible (less than 0.05 ppm for the pure solvents and less for the mixed solvents). The solvent abbreviations are DMSO, dimethyl sulfoxide; DMF, *N,N*-dimethylformamide; AN, acetonitrile; EtOH, ethanol; TFE, 2,2,2-trifluoroethanol; and HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol.

It is interesting to note that none of the solvents tested cause a downfield shift relative to H_2O. Considering the range of solvents studied, it is unlikely that any uncharged organic solvent molecule would be capable of producing downfield shifts of the ^{31}P resonances relative to those observed in H_2O. In view of this, one might speculate that in those cases where significantly downfield shifted resonances are observed (as in drug–DNA complexes, z-DNA, tRNA) conformational effects are dominant. The overall shifts would, therefore, represent some combination of solvent and conformational effects.

These observations of large solvent effects on the ^{31}P chemical shifts in simple cyclic nucleotides render questionable any interpretation of ^{31}P chemical shifts observed in polynucleotides which are based solely on considerations of the phosphodiester bond conformations. For example, no simple correlation is expected between the variety of ^{31}P resonances observed in the tRNA spectra and the crystal structure. Rather, there may be significant effects due to hydrogen bonding between the phosphate groups and other proton-donating groups in the tRNA molecule. Steric constraints which prevent full hydration of the phosphate groups by solvent molecules might also induce large shifts. Conceivably, mixed solvent studies on the tRNA could provide evidence of such effects.

It is clear from these studies that a careful examination of the role of solvation must now accompany any attempt to relate ^{31}P chemical shifts to conformational state of the polynucleotide. We hope in future studies to more precisely delineate those properties of the solvent which are responsible for the large solvent effects on the ^{31}P NMR of nucleotides and polynucleotides.

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0002-7863/80/1502-7611$01.00/0

Acknowledgment. The support of Research Grant GM 22969 from the National Institutes of Health is most gratefully acknowledged. We also thank Dr. John M. Wright for his help with the ^{31}P instrumentation and Wayne J. Becktel for helpful discussions.

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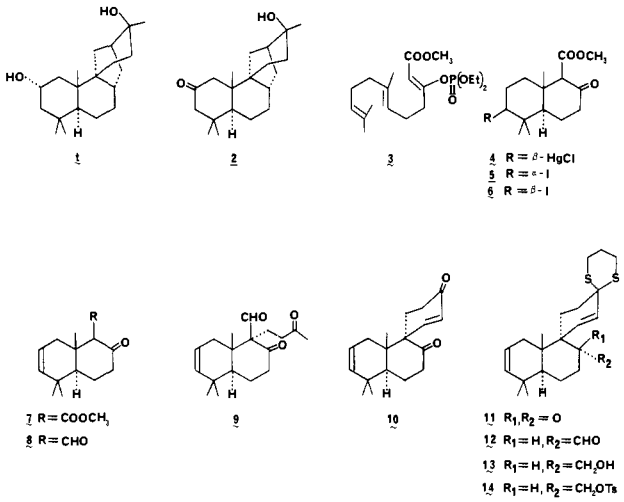
Received June 30, 1980

Total Synthesis of (±)-Stemodin and (±)-Stemodinone

Sir:

The tetracyclic diterpenes stemodin (**1**) and stemodinone (**2**),



obtained from the leaves of *Stemodia maritima* L. (Jamaican "sea mint"),[1] are structurally related to the antiviral and antimitotic fungal metabolite aphidicolin,[2] differing mainly in the stereorelationship of the C and D rings. Subsequent to the completion of the total synthesis of (±)-aphidicolin,[3] we have undertaken cognate studies in the stemodin series. Successful stereoselective syntheses of racemic **1** and **2** have now been realized by the approach which is outlined herein.

Enol phosphate **3**[4] was treated at −20 °C in anhydrous nitromethane with 1.2 equiv of mercuric trifluoroacetate[3] in the same solvent. Warming to 0 °C followed by treatment with aqueous sodium chloride gave bicyclic ketoester **4** [mp 179–181 °C; IR_{max} ($CHCl_3$) 1760, 1725 cm^{-1}; ^1H NMR δ 2.90 (dd, 1 H, ClHgCH, J_1 = 10 Hz, J_2 = 5 Hz)] in 60% yield along with 10–15% of monocyclic material. Replacement of chloromercury by iodide was accomplished by slow addition of 1.07 equiv of potassium triiodide to an oxygen-free solution of **4** in 90% aqueous dioxane.[5] Reductive workup with aqueous sodium bisulfite solution and ether extraction furnished in quantitative yield a mixture of 3α- and 3β-iodo keto esters **5** and **6** (5:1), R_f 0.56 and 0.49, respectively, on silica gel plates by using a single development with 25% ethyl acetate in hexane. The reaction of **4** with iodine or iodine monochloride in chloroform ($CHCl_3$) gave equal quantities of **5** and **6**. The crude mixture of iodides was treated at 23 °C for 48 h with powdered anhydrous lithium chloride in dimethylformamide (DMF) to afford after extractive workup and chromatography on silica gel the unsaturated keto ester **7** [mp 76–80 °C; IR_{max} ($CHCl_3$) 1750, 1718, 1630 cm^{-1}; ^1H NMR δ 5.4 (m, 2 H, CH=CH), 3.26 (s, 1 H, O=CCH)] in 70% overall yield from **4**.

The unsaturation in **7** allowed the introduction of oxygen at C-2 at the end of the synthesis. The conversion of **7** to keto aldehyde **8** [mp 113–116 °C; IR_{max} ($CHCl_3$) 1718, 1620–1585 cm^{-1}; ^1H NMR δ 9.01 (d, 1 H, CHO, J = 3.2 Hz)] was accomplished by the following sequence: (1) protection of the ketone as the ethylene ketal (ethylene glycol in benzene, *p*-toluenesulfonic acid, reflux, 2 h; 90%, mp 115–117 °C), (2) reduction of the carbomethoxy appendage to hydroxymethyl (lithium aluminum hydride in ether at 23 °C; 90%), (3) oxidation (pyridinium chlorochromate[6] in CH_2Cl_2 at 23 °C; 92%), (4) ketal hydrolysis (10:1:1 acetone–water–70% aqueous perchloric acid at 23 °C, 3 h; 96%). A direct method for the reduction of carbomethoxy to formyl was not found. An excess of diisobutylaluminum hydride in refluxing benzene gave no detectable reduction, perhaps as a consequence of the shielding provided by the two quaternary centers flanking the ester function.

This short and efficient preparation of rings A and B set the stage for the introduction of the spiro ring. The Michael reaction of keto aldehyde **8** at 23 °C with methyl vinyl ketone in 1:1 tetrahydrofuran (THF)–*tert*-butyl alcohol and 0.2 equiv each of 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) and potassium carbonate gave adduct **9** [mp 90–95 °C; IR_{max} ($CHCl_3$) 1730–1700 cm^{-1}; ^1H NMR δ 10.1 (d, 1 H, CHO, J = 1.5 Hz), 2.07 (s, 3 H, $COCH_3$)] in 70% yield, along with 20% of unreacted **8**. Aldol closure to spiro diketone **10** [mp 139–141 °C] was effected in 93% yield by using pyrrolidinium acetate[7] in THF–methanol at 23 °C. Selective reaction at the less hindered conjugated carbonyl group with bis(*S*-trimethylsilyl)propane-1,3-dithiol[8] in $CHCl_3$ at 23 °C in the presence of zinc iodide gave thioketal **11** [mp 153–154 °C] in quantitative yield.

The conversion of **11** to aldehyde **12** was accomplished by using the methodology described previously in connection with the total synthesis of aphidicolin:[3] (1) treatment of **11** with 10 equiv of trimethylsilyl cyanide[9] [87%; mp 165–167.5 °C], (2) reduction to the α-trimethylsilyloxy aldehyde [80%; mp 135–137 °C] with 4 equiv of diisobutylaluminum hydride in toluene at −10–5 °C, (3) addition of 0.95 equiv of trimethylsilyllithium[10] in ether–hexamethylphosphoramide (HMPA) at −35 °C to the formyl function (80% yield), and (4) treatment with 3 equiv of lithium diisopropylamide in THF containing 5% HMPA at 23 °C under positive argon pressure to give aldehyde **12** [IR_{max} ($CHCl_3$) 1710 cm^{-1}; ^1H NMR δ 9.77 (s, 1 H, CHO)] in 80% yield after workup with aqueous acid.

The reduction of aldehyde **12** in THF–ethanol at 0 °C with sodium borohydride gave primary alcohol **13**, which was converted to tosylate **14** (*p*-toluenesulfonyl chloride, 4-(dimethylamino)-pyridine,[11] and pyridine in $CHCl_3$ at 23 °C). Thioketal cleavage by using 2.2 equiv of 1,3-diiodo-5,5-dimethylhydantoin[12] at −20 °C for 30 min in 5:5:1 acetone–THF–water produced enone tosylate **15** which was transformed to the tetracyclic ketone **16** in two steps: (1) treatment with 1.1 equiv of potassium *tert*-butoxide in THF at 23 °C, followed by (2) enone reduction by use of lithium

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