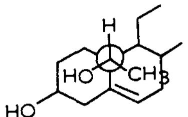
Wicha and Caspi[1b] to predict the formation of the 19*R* configuration by stereochemical considerations. Nevertheless, one could predict a diminished stereoselectivity relative to that for Δ^4-3-one system. We have observed that the stereoselectivity of sodium borohydride reduction decreased from $^9/_1$ to $^2/_1$. Then, Wicha and Caspi's report[1] that the methyllithium reaction on **2a** gave a 90% yield of a single compound seems to be unpredictably stereoselective. In addition, the 19*R* assignment for the major methylated product now clearly contradicts our 19*S* assignment based on the conformational studies described above. For these reasons we undertook a methyllithium reaction of **2a** according to Wicha and Caspi.[1b]

Methyllithium reaction with **2a** gave over 90% yield of 19-methyl-5-androstene-3β,17β,19-triol (**11**) which showed one spot in TLC (ethyl acetate–ethanol, 9:1) exactly as described in the previous reports. This product, however, was separated into two distinct compounds in another TLC system (developed twice in chloroform–acetone, 8:2; **11**-*major*, R_f = 0.50, and **11**-*minor*, R_f = 0.44) and isolated in crystalline form in 63 and 24% yield, respectively. The **11**-*major* was recrystallized from acetone (mp 189–192°; ir, 1066, 1050, 1026 cm^{-1}; ^{1}H NMR (CD$_3$OD), δ 0.80 (18-CH$_3$, 3 H, s), 1.30 (19a-CH$_3$, 3 H, d, J = 7.0 Hz), 5.81 (5-H, 1 H, m); MS, *m/e* 302(M − 18), 276(M − 44), 258(276 − 18), 240(258 − 18), and 225(240 − 15)) and the **11**-*minor* recrystallized from aqueous methanol (mp 188–190°; ir, 1077, 1050, 1029 cm^{-1}; ^{1}H NMR (CD$_3$OD), δ 0.80(18-CH$_3$, 3 H, s), 1.29 (19a-CH$_3$, 3 H, d, J = 7.0 Hz) 5.81 (5-H, 1 H, m); MS, *m/e* 302(M − 18), 276(M − 44), 258, 240, and 225. Each showed no change in characteristics even after heating over its melting point. The **11**-*minor* was grown in single crystals from acetone and the crystal and molecular structure was solved.[17] The absolute configuration at the C-19 of **11**-*minor* was determined to be *R* and the conformation around the C$_{10}$–C$_{19}$ bond showed that the 19-methyl function is at the over-B-ring and the oxygen at the out-of-ring position as shown in Figure 3. Thus the diastereomeric **11**-*major* to which 19*R* was previously assigned should be reassigned as 19*S*. The stereoselectivity of the nucleophilic attacks at the steroidal 19-aldehyde by the two reagents was thus found to be the same. The 19pro*R* hydrogen removal[7] in estrogen biosynthesis was confirmed.

Addendum, The absolute configuration of **11**-major was determined to be 19*S* by X-ray crystallography, after the submission of the manuscript. The conformation around the C$_{10}$–C$_{19}$ bond showed that the methyl function is again at the over-B-ring and the oxygen at the over-A-ring position as shown below. The fact that both diastereomers have the methyl group at the over-B-ring position suggests that this is the least energy conformation. This offers an explanation for the cause of the reversal of the original assignment which relied on Wicha and Caspi's rationalization that the bulkiest of the three substituents on C-19, the methyl group, must be at the out-of-ring position for 3β,19-oxide formation.



11-major 19S

Acknowledgment, This investigation was supported by Research Grants HD04945 and CA10906 from the U.S. Public Health Service, and aided by the American Cancer Society Faculty Award PRA-72 (Y.O.). 3-Ethylenedioxy-5-androstene-17β,19-diol 17-benzoate was prepared by Dr. S. S. H. Gilani and 5-androstene-3β,17β,19-triol 17-*p*-bromobenzoate was synthesized by Dr. K. Yasuda for the crystallography study. Skilled technical assistance was provided by Mrs. Carol Yarborough and Mrs. Mary Erman. All this support is gratefully acknowledged.

References and Notes

(1) (a) E. Caspi and J. Wicha, *Chem. Commun.*, 209 (1966); (b) J. Wicha and E. Caspi, *J. Chem. Soc. C*, 1740 (1968); (c) *ibid.*, 947 (1969), and references cited therein.

(2) (a) S. J. M. Skinner and M. Akhtar, *Biochem. J.*, **114,** 75 (1969); (b) J. Wicha and E. Caspi, *Tetrahedron*, **25,** 3961 (1969); (c) Y. Watanabe, Y. Mizuhara, and M. Shiota, *J. Org. Chem.*, **36,** 2558 (1971); (d) L. L. Engel, *Endocrinology*, **87,** 827 (1970); (e) *Handb. Physiol. Sect. 7: Endocrinol.*, **2** (1), 467 (1973).

(3) A preliminary account was presented at the IVth International Congress on Hormonal Steroids, Mexico City, Sept 1974; K. Shibata, W. L. Duax, and Y. Osawa, *J. Steroid Biochem.*, **5,** 301 (1974).

(4) G. J. Karabatsos, *J. Am. Chem. Soc.*, **89,** 1367 (1967).

(5) D. J. Cram and D. R. Wilson, *J. Am. Chem. Soc.*, **85,** 1245 (1963); D. J. Cram and F. A. Abd Elhafez, *ibid.*, **74,** 5828 (1952); D. J. Cram and K. R. Kopecky, *ibid.*, **81,** 2748 (1959).

(6) M. Cherest, H. Felkin, and N. Prudent, *Tetrahedron Lett.* 2199 (1968); M. Cherest and H. Felkin, *Ibid.*, 2205 (1968).

(7) Y. Osawa, "Endocrinology, Proceedings of the Fourth International Congress of Endocrinology," Washington, D.C. R. O. Scow, Ed., Excerpta Medica, Amsterdam, 1972, p 814.

(8) J. Yates and R. E. Oakey, *Steroids*, **19,** 119 (1972); F. L. Bellino and Y. Osawa, *Biochemistry*, **13,** 1925 (1974).

(9) Measured by the Varian High Resolution NMR Service, Palo Alto, Calif.

(10) Belgium Patent 627,523 (Organon, 1963), *Chem. Abstr.*, **60,** 10764*e* (1964).

(11) M. Kocor and P. Lenkowski, *Bull. Acad. Pol. Sci., Ser. Sci. Chim., 16,* 289 (1968); *Chem. Abstr.*, **69,** 87315 (1968).

(12) P. N. Rao and L. R. Axelrod, *J. Org. Chem.*, **27,** 4694 (1962).

(13) B. Belleau and T. F. Gallagher, *J. Am. Chem. Soc.*, **73,** 4458 (1951), W. G. Dauben and J. F. Easthan, *ibid.*, **73,** 4463 (1951).

(14) W. L. Duax and Y. Osawa, American Crystallographic Association Meeting, Gainesville, Fla., Abstract P13 (1973).

(15) D. C. Rohrer, W. L. Duax, C. M. Weeks, M. G. Erman, Y. Osawa, and M. Soriano, American Crystallographic Association Meeting, Berkeley, Calif., Abstract A7 (1974).

(16) W. L. Duax, C. Eger, S. Pokrywiecki, and Y. Osawa, *J. Med. Chem.*, **14,** 295 (1971).

(17) Detailed crystal and structure data are available from W. L. Duax and are to be published separately.

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Received December 27, 1974

Comment on the Carbon-13 Nuclear Relaxation Measurements in Adenosine Monophosphate

Sir:

Recently Hamill, Pugmire, and Grant[1] reported in this journal the concentration dependence of carbon-13 nuclear relaxation data of 5′-AMP. The observed strong decrease of T_1 with concentration was explained by an increase of the stacking process with concentration. If this explanation is correct, one would expect a significant deviation from the simple Stokes–Einstein relation, namely, a deviation from slope 1 in a double log plot of T_1 vs. η^{-1}. In Figure 2 this plot is presented. The viscosity data for different concentrations were obtained by us (Figure 1) and combined with the ^{13}C relaxation data of Hamill et al.[1] Within the accuracy of the experimental results, the ^{13}C longitudinal relaxation times of the base carbons C-2 and C-8 can be explained by the simple Stokes–Einstein relation without invoking a change of particle size with concentration. This relationship, however, leaves undecided whether the stacking is already complete at the lowest concentration investigated. The following consideration excludes this possibility.

In molecules of the size of a nucleotide the relaxation of a carbon-13 bound directly to a hydrogen atom is completely described by dipole-dipole interaction between the carbon-13 and the proton spin.[2] Assuming isotropic motion of the

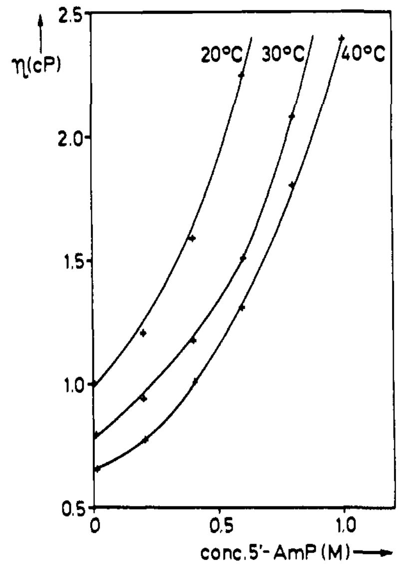

Figure 1. Viscosity of aqueous 5'-AMP solutions as a function of concentration (P_H: 7.0 ± 0.4).

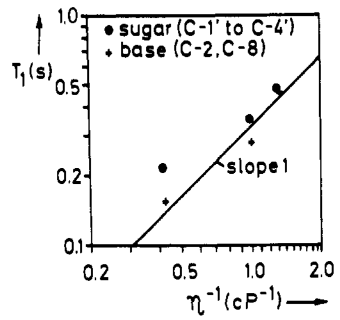

Figure 2. Double log plot of the average longitudinal ^{13}C relaxation rates of 5'-AMP[1,2] vs. the reciprocal viscosity of the solutions: points, average of C-1' to C-4'; crosses, average of C-2, C-8.

nucleotide one calculates the rotational correlation time τ_c in the extreme narrowing case from[3]

$$1/T_1 = N\hbar^2\gamma_C^2\gamma_H^2 r_{CH}^{-6}\tau_c \quad (1)$$

with N the number of protons bound to the carbon and γ_C and γ_H the magnetogyric ratios of the two nuclear spins involved. Taking the isotropic rotating sphere as a valid approximation for the motion of 5'-AMP in water, τ_c is described by

$$\tau_c = 4\pi\eta a^3/3kT \quad (2)$$

Inserting 0.105 ± 0.005 nm for r_{CH} one obtains at all concentrations a value of a, commonly taken as the diameter of the diffusing particle, of 0.45 ± 0.05 nm, which certainly is less than the actual dimensions of the single nucleotide. The relaxation times of the base carbons at the different concentrations are therefore determined by the macroscopic viscosity, and it is unnecessary to invoke any specific microscopic model to explain the experimental results. On the other hand, the relaxation rates of the sugar carbon, which possess several possibilities of internal motion, depend on two correlation times: the correlation time for overall molecular reorientation which increases with increasing viscosity and a correlation time for the internal motions which to a first approximation should not depend on the viscosity.[4,5] It is then clear that the effect of the internal motions can only be observed in the more concentrated solutions, that is when the overall reorientation is slowed down.

Rhodes and Schimmel[6] measured the energy of activation for the rotation around the glycosidic bond (syn ⇄ anti equilibrium) in some purine (β)ribosides to be 6.2 kcal mol^{-1}. Therefore the segmental motion may not come from rotation around the glycosidic bond. Röder et al.[7] have determined the activation energy for the conformational mobility of the furanoside ring of the ribose moiety, as for example described by the N ⇄ S model of Altona and Sundaralingam[8] by variable temperature ^{13}C relaxation measurements and comparison with the 2',3'-isopropylidene nucleosides to be 4.7 ± 0.5 kcal mol^{-1}. Assuming that these results are applicable to 5'-AMP one must conclude that the deviations observed in the more concentrated solutions for the relaxation rates of the sugar carbons from those of the base should not be explained by a greater diffusive mobility of the ribosephosphate moiety around the glycosidic bond but rather by transitions between the different possible conformations of the ribose ring.

References and Notes

(1) W. D. Hamill, Jr., R. J. Pugmire, and D. M. Grant, *J. Am. Chem. Soc.*, **96**, 2885 (1974).
(2) A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.*, **55**, 189 (1971).
(3) A. Abragam, "The Principles of Nuclear Magnetism", Oxford University Press, London, 1961, p 300.
(4) D. Doddrell and A. Allerhand, *J. Am. Chem. Soc.*, **93**, 1558 (1971).
(5) G. C. Levy, R. A. Komoroski, and J. A. Halstead, *J. Am. Chem. Soc.*, **96**, 5456 (1974).
(6) L. M. Rhodes and P. R. Schimmel, *Biochemistry*, **10**, 4426 (1971).
(7) O. Röder, H.-D. Lüdemann, and E. v. Goldammer, *Eur. J. Biochem.*, **53**, 517 (1975).
(8) C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **94**, 8205 (1972).

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Received October 15, 1974

Simple Criteria for Distinguishing between Inner- and Outer-Sphere Electrode Reaction Mechanisms

Sir:

The well-known and widely applied distinction[1] between inner-sphere and outer-sphere mechanistic pathways in electron-transfer reactions involving homogeneous reactants has been extended to a few heterogeneous reactions proceeding at electrode surfaces.[2-7] The clearest examples of inner-sphere electrode reactions involve certain transition metal isothiocyanate complexes[6,7] which are strongly adsorbed on the surface of mercury electrodes by means of sulfur-mercury bonds. The kinetics of the electroreduction of these complexes in the adsorbed state have been investigated, and further testimony to their proceeding by inner-sphere pathways appeared in the form of unusual apparent reaction orders and potential dependences.[6,7] Strong, although less direct evidence for inner-sphere electrochemical pathways has also been obtained for a second class of reactions involving the oxidation of $Cr(OH_2)_6^{2+}$ at mercury electrodes in the presence of anions which are adsorbed on the electrode and appear in the inner coordination sphere of the Cr(III) complexes resulting from the oxidation.[2-5] This result is analogous to the classical and unequivocal demonstration of a ligand-bridging mechanism by means of ligand transfer between homogeneous reactants.[8] However, anation of both $Cr(OH_2)_6^{2+}$ and mercury electrode surfaces proceeds rapidly and reversibly so that it is not always possible to distinguish between prior complexation and ligand bridging by adsorbed anions as the mechanism leading to anated chromium(III) complexes during the electrooxidation of $Cr(OH_2)_6^{2+}$.[5]