TEMPERATURE DEPENDENCE OF AMIDE PROTON CHEMICAL SHIFTS: THE SECONDARY STRUCTURES OF GRAMICIDIN S AND VALINOMYCIN

M. Ohnishi and D. W. Urry
Institute for Biomedical Research
Education and Research Foundation
American Medical Association
Chicago, Illinois 60610

Received May 19, 1969

In cases where hydrogen-deuterium exchange studies are inconclusive in defining hydrogen bonded amide protons, it is useful to have an alternative basis. Kopple et al. (1969) have associated a decrease in temperature sensitivity of chemical shift in amides protons of cyclic hexapeptides with hydrogen bonding. In the present communication we wish to demonstrate how readily temperature dependence of amide proton chemical shifts may be used to arrive at the secondary structures of gramicidin S (Stern et al. 1968) and valinomycin (Ivanov et al. 1969). The former antibiotic is conformationally quite rigid (Quadrifoglio and Urry 1967). Valinomycin, on the other hand, exhibits a dish-like structure with surfactant properties in dimethylsulfoxide (DMSO) whereas in methanol + KBr it exists as a "pore" structure with the proper dimensions and character for selective binding of potassium. The power of the approach is further emphasized by the fact that all three conformations, one for gramicidin S and two for valinomycin, were arrived at, independently and in a short time, without prior knowledge of the work of Stern et al. (1968) and of Ivanov et al. (1969). Thus the present work, in addition to demonstrating the temperature method for elucidating secondary structure of polypeptides, may be taken as a confirmation of the gramicidin S and valinomycin structures.

EXPERIMENTAL: NMR spectra were obtained on a Varian HA-100 spectrometer using an internal lock and frequency sweep and on a Varian HR-220. Variable temperature studies and homonuclear spin decoupling were carried out on both instruments. The ambient temperature for the probe was approximately 30° C for the HA-100 and 24° C for the HR-220.

Gramicidin S was obtained from Mann Research Laboratories lot no. S 3961. Hydrogenation was carried out by Fox Chemical Co. using the method of Ruttenberg et al.(1966). Amino acid analysis showed hydrogenated gramicidin S to have less than 0.01 residue of phenylalanine. Thin layer chromatography with a 2-butanol-3% ammonia solution, 10:4.4, gave a single spot for both gramicidin S and its hydrogenated derivative with Rf.s of 0.41 and 0.38, respectively.

Valinomycin was obtained from Calbiochem, lot No. 860031 with mp = 188-190° C and $[\alpha]_D$ = +30.5. The amino acid analysis indicated a sample of very high purity and the NMR spectrum (Fig. 2) indicates a clean sample. The complex with potassium ion was formed by dissolving equimolar amounts of valinomycin and KBr in a sample tube using a 9:1 methanol:water solvent mixture. The sample was then dried under high vacuum and redissolved in CH_3OH. The same procedure was followed using NaCl instead of KBr.

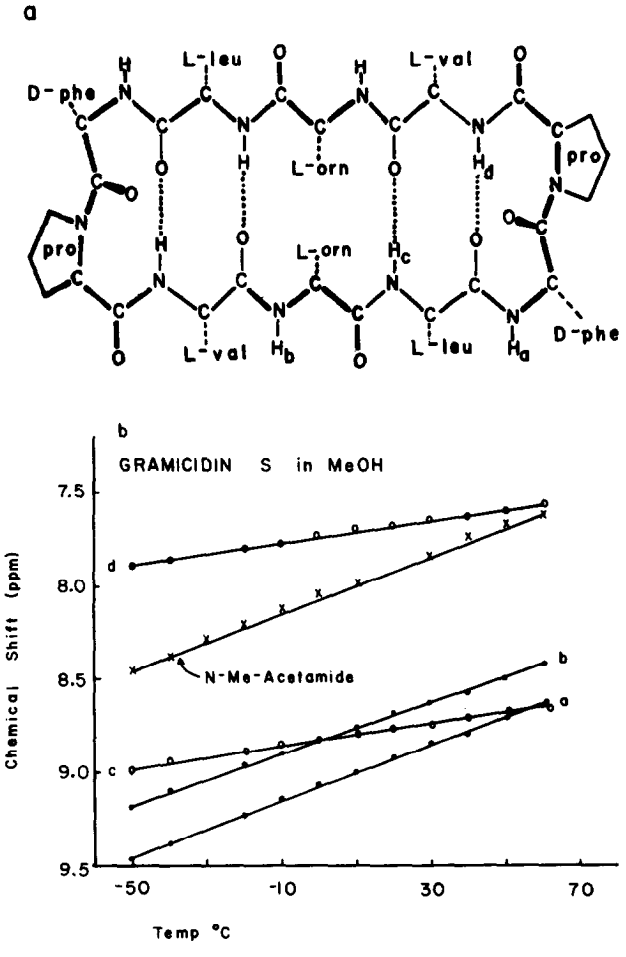

Fig. 1 a) Structure of gramicidin S (Hodgkin and Oughton, 1957; Schwyzer, 1958; Stern et al. 1968). b) Effect of temperature on the chemical shift of amide proton resonances of gramicidin S in methanol. Note the decreased temperature dependence of amide protons c and d as compared to that of amide protons a and b and of the amide proton in N-methyl acetamide. Hydrogenated gramicidin S was also studied to remove the phenyl protons.

RESULTS AND DISCUSSION

Gramicidin S: The β-type structure of gramicidin S which was first suggested on the basis of x-ray studies by Hodgkin and Oughton (1957) is given in Fig. 1a. A similar structure was proposed by Schwyzer (1958). The temperature dependence of amide proton chemical shifts is given in Fig. 1b along with the data on N-methyl acetamide. Note also that proton d is high field shifted due to screening by the D-phe-L-pro amide linkage. Assignments of protons have been made by Conti (1969) and by Stern et al. (1969). These papers may be referred to for the complete spectra. It is seen in Fig. 1b that the hydrogen bonded amide protons, regardless of initial position of the resonance, exhibit a lower slope with temperature than the non-hydrogen bonded amide protons which exhibit the same slope as in N-methyl acetamide. It is perhaps worth noting that the data in Fig. 1b and considerations arising from the studies on cyclic hexapeptides (Kopple et al. 1969) which place bulky side chains as in aromatic residues at the turns are sufficient to suggest the structure in Fig. 1a. In the case of gramicidin S the tritium-hydrogen exchange studies of Laiken et al. (1969), while clearly more laborious, were in excellent correspondence with the interpretation arising from Fig. 1b. Thus in methanol, a decrease in the slope of amide proton chemical shifts with temperature, as compared to that of N-methyl acetamide, may be used to arrive at secondary structure.

Valinomycin: The spectrum of valinomycin taken at 220 MHz in DMSO is given in Fig. 2. The NMR of valinomycin at 60 MHz has recently been reported by Haynes et al. (1969). Two amide proton resonances arising from D and L-Val are observed at 7.86 ppm and 8.34 ppm as measured from a tetramethyl silane (TMS) internal standard. The αCH-NH coupling constant is about 8 Hz. Assignments of the αCH and methyl protons are given in Fig. 2 on the basis of spin-spin coupling with vicinal protons. Double resonance experiments demonstrate that αCH proton a' is coupled with amide proton a and similarly for b' and b (see Fig. 2). The spectrum in CH_3OH (for the amide proton region) and in CD_3OD (for the αCH region) is similar to that in DMSO.

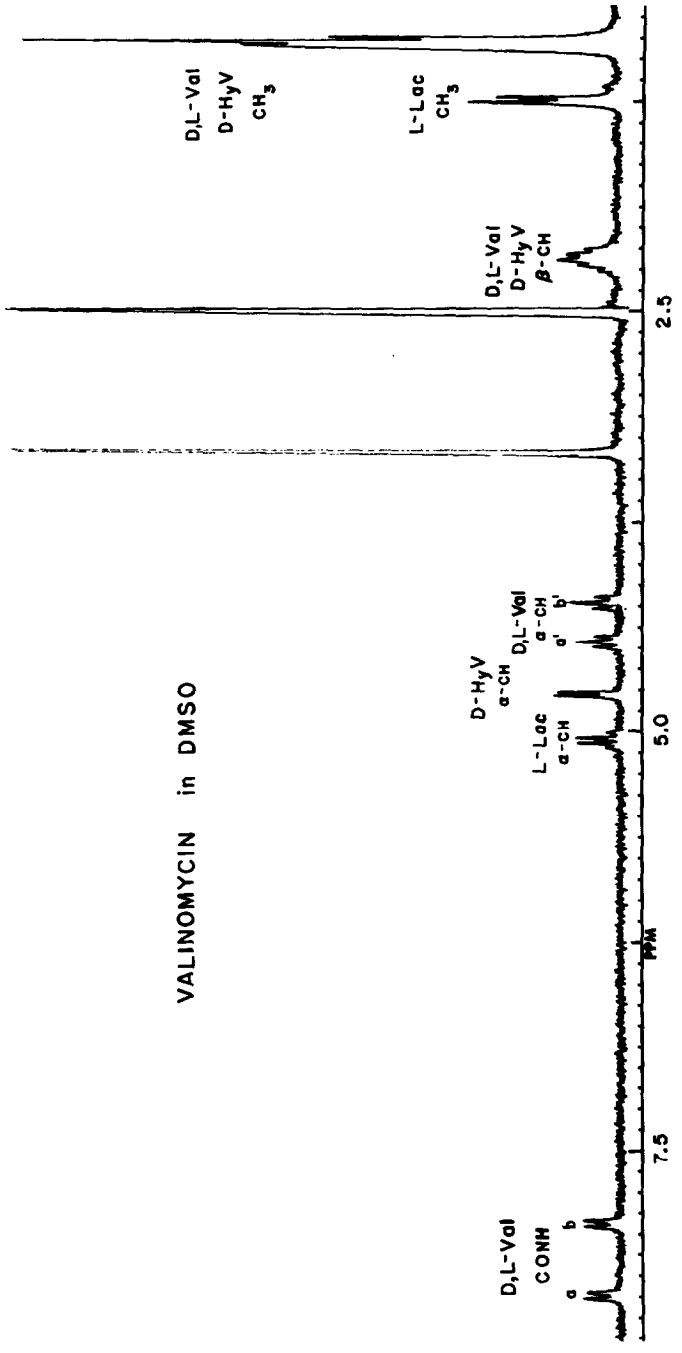

Fig. 2 NMR spectrum of valinomycin obtained at 220 MHz in dimethyl sulfoxide (DMSO). Concentration 14.5 mg/0.5 ml; chemical shifts reported with respect to an internal standard of tetramethyl silane (TMS).

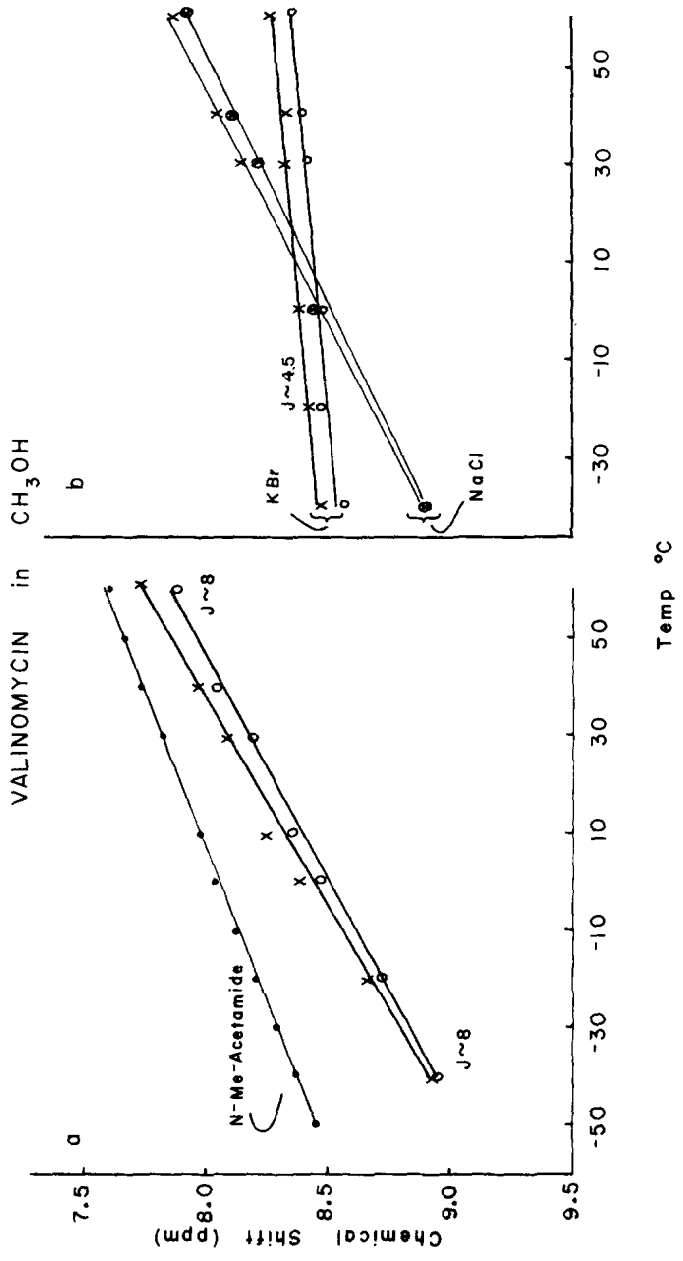

Fig. 3 a) Effect of temperature on the chemical shift of amide proton resonances of free valinomycin in CH_3OH. b) Effect of KBr and NaCl on the chemical shift temperature dependence. Note that NaCl has little effect and that KBr greatly reduces the temperature dependence

Temperature studies on the amide proton resonances in methanol are given in Fig. 3a. Both resonances a and b give similar slopes and similar αCH-NH coupling constants. In Fig. 3b it is seen that addition of NaCl has little effect whereas addition of KBr greatly decreases the change in chemical shift with temperature. In the latter, the αCH-NH coupling constants are 4.5 Hz. These temperature studies indicate that all amide protons are hydrogen bonded in the valinomycin-potassium complex. H-D exchange studies using small amounts of CD_3OD in CH_3OH were inconclusive. Ivanov et al. (1969) used infra red studies to arrive at the essential statement that all amide protons are hydrogen bonded. The temperature dependence technique, herein reported, provides a more decisive argument for the secondary structure. The resulting "pore" structure of valinomycin is given in Fig. 4.

Temperature studies on the amide proton resonances of valinomycin in

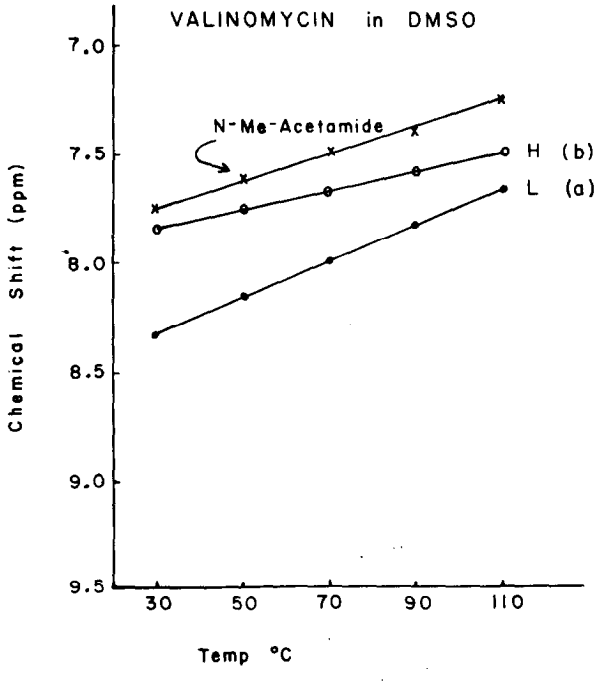

Fig. 4 Proposed structure for valinomycin when binding K+. $R_1 = CH_3$, $R_2 = R_3 = R_4 = CH(CH_3)_2$ The drawing is an unrolled perspective showing the stacking of turns of an antiparallel β type structure.

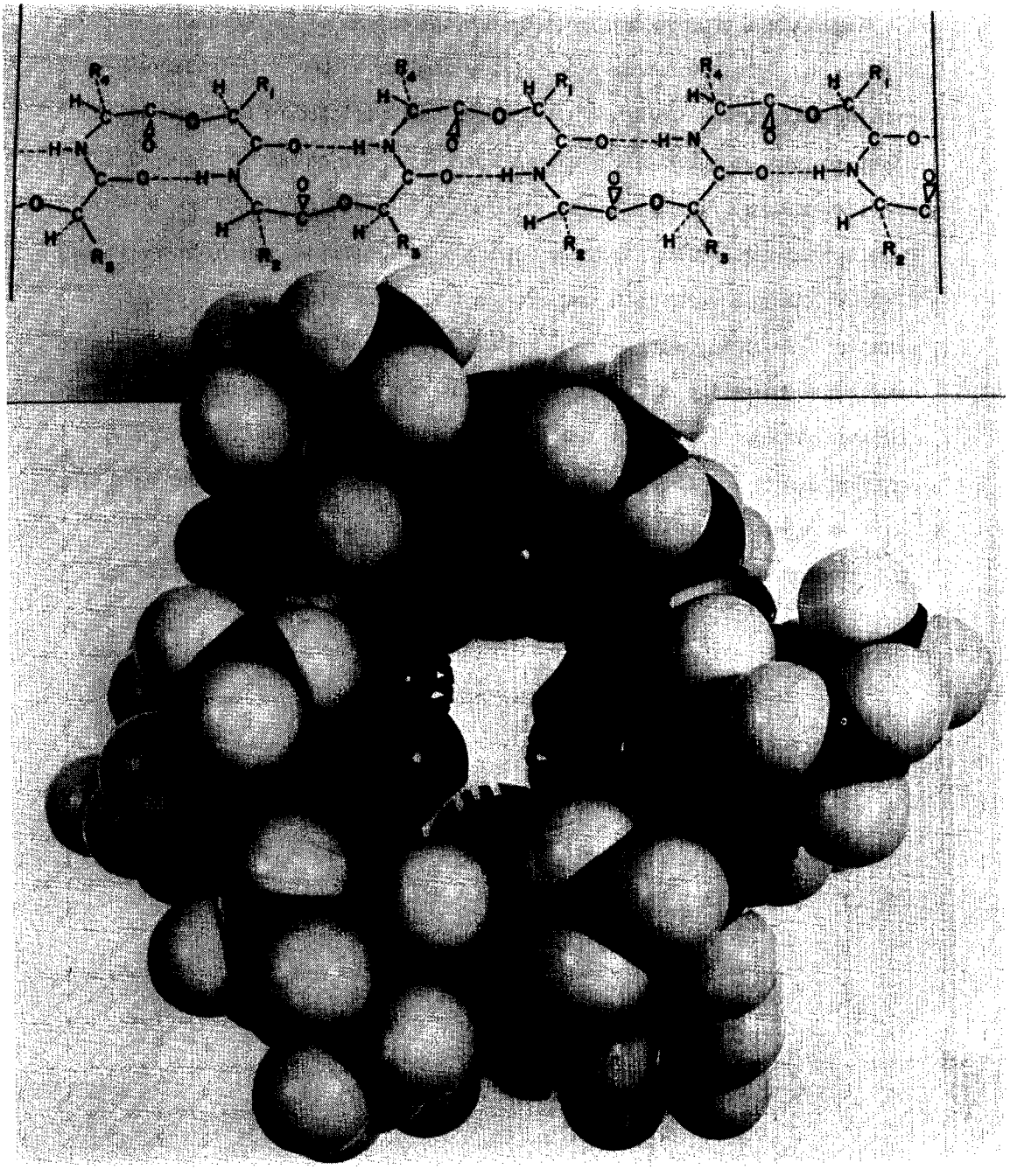

Fig. 5 Effect of temperature on the chemical shift of amide proton resonances of valinomycin in DMSO.

DMSO (see Fig. 5) show resonance b (see Fig. 2 for assignment) to have a lesser slope and a to have a greater slope than the amide proton resonances

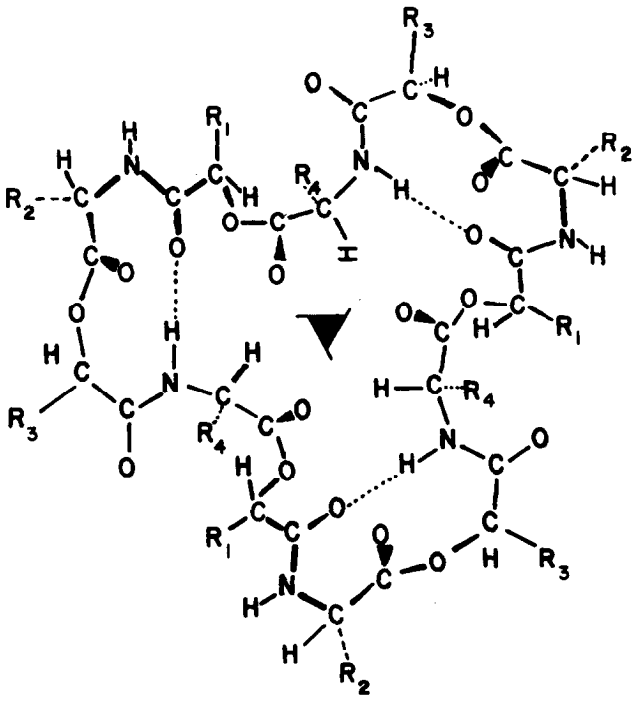

Fig. 6 Proposed structure for valinomycin in DMSO. Cleavage of alternate H-bonds and opening of "pore" structure in Fig. 6 gives rise to two possible dish-shaped structures which interconvert via the "pore" structure. From steric considerations it is expected that $R_1 = CH_3$ would be the favored conformation.

---

of N-methyl acetamide. Deuterium exchange experiments show both resonances a and b to exchange within about 30 minutes with resonance b exchanging only slightly slower than resonance a. The low temperature dependence of the chemical shift of the high field amide proton resonance (see Figs. 2 and 5) and the large $J_{\alpha CH-NH}$ are interpreted in terms of the structure in Fig. 6. This is one of two nearly equivalent and interconverting conformations with the "pore" structure in Fig. 5 functioning as the intermediate. We believe the spectrum in Fig. 2 to represent a weighted average of the two structures. Another feature of the spectrum in Fig. 2 is the association by decoupling of the high field amide proton with the high field αCH proton which is explained in the structure by ester shielding of both protons.

It has been demonstrated with three secondary structures that the temperature dependence of amide protons chemical shifts in DMSO and in methanol is a useful means of arriving at the secondary structure of cyclic peptides. On the basis of shielding considerations it may be anticipated that the slope of solvent accessible and non-hydrogen bonded amide proton resonances would be reversed in an aromatic solvent such as benzene and that a solvent system such as pyridine would exhibit a steeper temperature dependence than observed in DMSO.

ACKNOWLEDGEMENTS: The authors wish to thank Prof. G. L. Closs of the Dept. of Chemistry at the University of Chicago for use of the HA-100 NMR spectrometer, Dr. H. Klinger for running the HR-220 spectra, Varian Associates for use of their HR-220, and M. Schutte for preparing the manuscript.

References

Conti, F., Nature, 221, 777 (1969).
Haynes, D. H., Kowalsky, A., Pressman, B. C., J. Biol. Chem., 244, 502 (1969).
Hodgkin, D. C., and Oughton, B. M., Biochem. J., 65, 752 (1957).
Ivanov, V. T., Laine, I. A., Abdulaev, N. D., Sanyavini, L. B., Popov, E. M., Ovchinnikov, Yu. A., and Shenyakin, M. M., Biochem. Biophys. Res. Commun., 34, 803 (1969).
Kopple, K. D., Ohnishi, M., and Go, A., J. Am. Chem. Soc. in press (1969).
Laiken, S. L., Printz, M. P., and Craig, L. C., Biochemistry, 8, 519 (1969).
Liguori, A. M., DeSantis, P., Kovacs, A. L., and Mazzarella, L., Nature, 211, 1039 (1966).
Quadrifoglio, F., and Urry, D. W., Biochem. Biophys. Res. Commun., 29, 785 (1967).
Ruttenberg, M. A., King. T. P., and Craig, L. C., Biochem., 5, 2857 (1966).
Schwyzer, R., Chimia, 12, 53 (1958).
Stern, A., Gibbons, W., Craig, L. C., Proc. Natl. Acad. Sci., 61, 734 (1968).