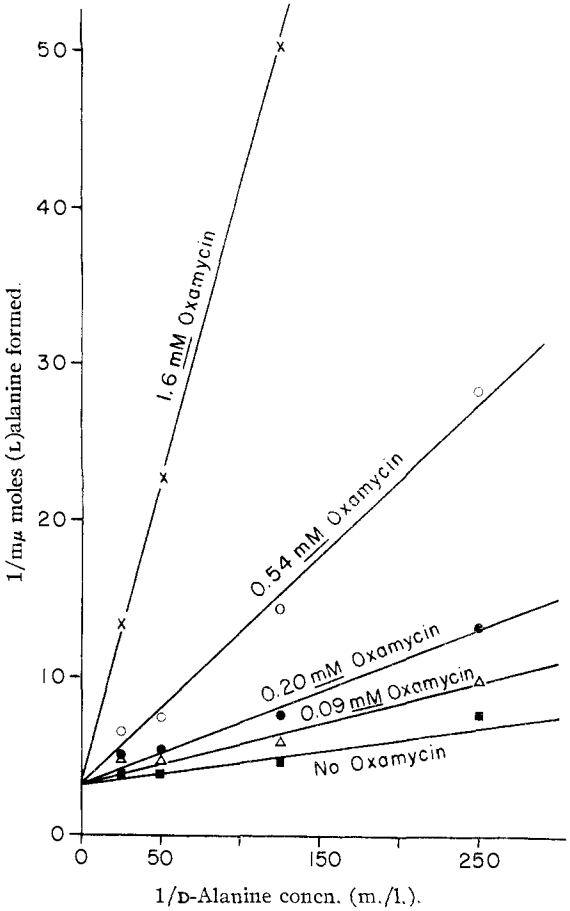

Fig. 1.—Competitive inhibition of alanine racemase by oxamycin. Data are plotted according to Lineweaver and Burk[5] ($1/v$ *vs.* $1/s$). v is expressed as the amount of product formed in the 30 min. assay.

about the same as K_i measured in the direction of (L)alanine formation ($1.0 \times 10^{-4}M$). Pyridoxal phosphate, over a wide range of concentrations, had no effect on the inhibition of the reaction by oxamycin.

However, this information was anomalous in that nucleotide accumulation in whole cells was not reversed by (L)alanine and was *competitively* reversed by (D)alanine.[2] A second point of inhibition was, therefore, sought and it has been found that reaction 2, the dipeptide synthesizing system,[3] is similarly inhibited by the antibiotic (Fig. 2). K_m for (D)alanine is $3\text{–}5 \times 10^{-3}$ M while K_i for oxamycin is $2\text{–}4 \times 10^{-5}$ M. The ratio K_m/K_i = about 100 emphasizes the efficiency of the antibiotic as a competitor for the substrate.

Reaction 3, the addition of the dipeptide to the uridine nucleotide,[3] was not at all inhibited, even by high concentrations of the antibiotic ($4 \times 10^{-3}M$) at $1 \times 10^{-3}M$ (D)ala-(D)ala. The active penetration of C^{14}-(D)alanine into whole cells of *S. aureus* was similarly not affected when the D-alanine concentration in the medium was 10^{-4}, 10^{-5} or $10^{-6}M$ at an oxamycin concentration of $10^{-3}M$.

Thus, the inhibition of these enzymatic reactions by oxamycin provides, for the first time, a definition

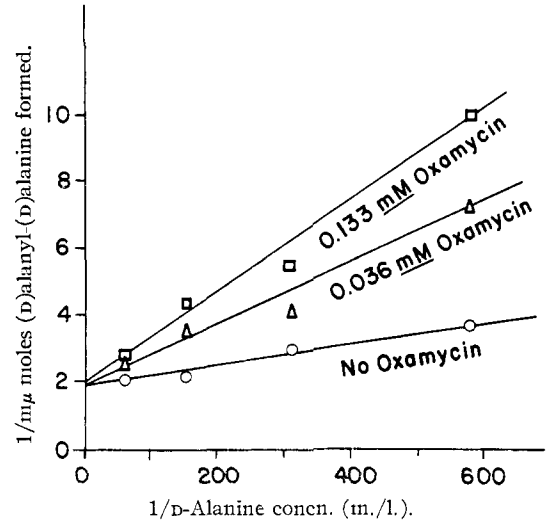

Fig. 2.—Competitive inhibition of the dipeptide synthesizing enzyme by oxamycin. v is expressed as the amount of product formed in the 15 min. assay.

of the mechanism of action of an antibiotic at an enzymatic level. Although competitive inhibition of enzymatic utilization of natural substrates has previously been demonstrated for substances which are not selectively toxic for bacterial cells (*e.g.*, ref.[5,6]), special interest in inhibition of alanine racemase and of the dipeptide synthesizing enzyme lies in the fact that the inhibited reactions are found uniquely in bacterial cells. Hence, the substance is a usefui chemotherapeutic agent.[7]

(5) H. Lineweaver and D. Burk, THIS JOURNAL, **56**, 658 (1934).

(6) B. Levenberg, I. Melnick and J. M. Buchanan, *J. Biol. Chem.*, **225**, 163 (1957).

(7) Blood levels in human beings undergoing therapy with oxamycin are between 10^{-4} and 10^{-3} M. Thus, the levels obtained are sufficiently high to inhibit these enzymatic reactions *in vivo*.

(8) Supported by NSF Grant G-7619 and NIAID Grant E-1902.

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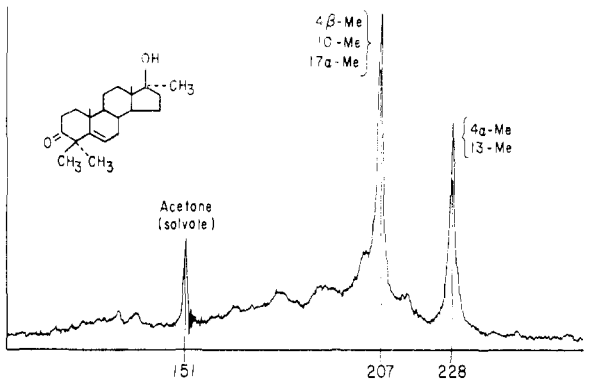
NUCLEAR MAGNETIC RESONANCE STUDIES USING PYRIDINE SOLUTIONS

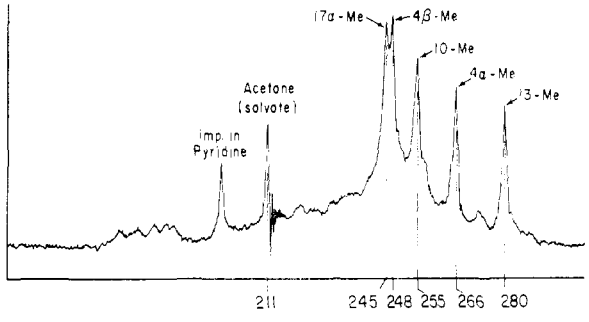
Sir:

Nuclear Magnetic Resonance spectroscopy has become very useful as a method for identification of unknown steroids.[1] The problem of interpretation of spectra and assignment of structures of complex molecules often is acute because of superposition of resonance lines arising from similar constituents. An observation is recorded herein which helps resolve such absorptions and facilitates correct assignments.

(1) J. N. Shoolery and M. T. Rogers, THIS JOURNAL, **80**, 5121 (1958); W. E. Rosen, J. B. Ziegler, A. C. Shabica and J. N. Shoolery, *ibid.*, **81**, 1687 (1959); G. Slomp and B. R. McGarvey, *ibid.*, **81**, 2200 (1959); R. L. Hirschmann, G. A. Bailey, R. Walker and J. M. Chemerda, *ibid.*, **81**, 2822 (1959); H. L. Slates and N. L. Wendler, *ibid.*, **81**, 5474 (1959); A. L. Nussbaum, F. E. Carlon, D. Gould, E. P. Oliveto, E. B. Hershberg, M. L. Gilmore and W. Charney, *ibid.*, **81**, 5230 (1959).



Cps. at 60 mc., relative to water.



Cps. at 60 mc., relative to water.

Fig. 1.—Portions of the spectra of 4,4,17α-trimethyl-17β-hydroxy-5-androsten-3-one in deuteriochloroform (upper) and in pyridine (lower).

Pyridine was suggested[2] as a useful solvent for n.m.r. studies on polar steroids. Its n.m.r. spectrum has been studied in detail[3] and it consists of only two groups of lines at rather low frequencies. It was expected that resonance frequencies from samples dissolved in pyridine would be uniformly shifted from those ordinarily observed in chloroform due to the different magnetic susceptibilities of the two solvents.[4]

When steroid spectra obtained in deuteriochloroform and pyridine solvents were compared, marked differences appeared. Some of the lines were shifted much more than others. It appeared that the pyridine preferred certain sites for coördination and that the large shifts were due to the anisotropy associated with its ring current effects.[5] Absorptions from similar types of hydrogens were shifted by different amounts because of small differences in environment. We have consistently found that lines which were superimposed in deuteriochloroform solution spectra were rearranged in pyridine solution spectra.

This information is valuable in characterizing unknowns. It permits confirmation of uncertain assignments for superimposed lines and spin multiplets. An interesting example of the former case was 4,4,17α-trimethyl-17β-hydroxy-5-androsten-3-one. The n.m.r. spectrum (see Fig. 1) in deuteriochloroform solvent showed only two lines ascribable to methyl hydrogens but in pyridine solvent all five methyl absorptions were resolved.

Spin multiplets usually retained the same spacing in both solvents and therefore were easily distinguished from chemically shifted groupings of lines. This method of differentiation usually was more convenient than obtaining a spectrum at a different frequency.

Solvent shifts have been compiled for the unique protons of about thirty steroids. They ranged from +20 cps. for the 21-CH_2 of the dihydroxyacetone side chain to +60 for the 19-methyl of 3-keto-Δ^4-steroids with no other substituents in the A, B or C rings. Nearby F, C=O and OH increased the solvent shift in that order. Axial methyls were shifted more than those which were equatorial.

N.m.r. spectra were observed with a Varian 4300-2 spectrometer operating at 60 mc. on solutions (*ca.* 0.3 ml., *ca.* 0.15 molar) of the steroids in deuteriochloroform and in pyridine. The spectra were calibrated against internal tetramethylsilane[6] using the audiofrequency side-band technique.[7] Frequencies are reported relative to water.

(2) S. M. Kupchan, W. S. Johnson and S. Rajagopalan, *Tetrahedron*, **7**, 47 (1959), and W. S. Johnson, J. J. Korst, R. A. Clement and J. Dutta, THIS JOURNAL, **82**, 614 (1960).

(3) H. J. Bernstein and W. G. Schneider, *J. Chem. Phys.*, **24**, 469 (1956); W. G. Schneider, J. A. Pople and H. J. Bernstein, *Can. J. Chem.*, **35**, 1487 (1957).

(4) A. A. Bothner-By and R. E. Glick, *J. Chem. Phys.*, **26**, 1651 (1957).

(5) J. A. Pople, *ibid.*, **24**, 1111 (1956).

(6) G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

(7) J. T. Arnold and M. E. Packard, *J. Chem. Phys.*, **19**, 1608 (1951).

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STRUCTURE AND EXCHANGE IN METHYL ALUMINUM CHLORIDES

Sir:

Trimethylaluminum and the methylaluminum halides are dimeric in the liquid state. In the partially halogenated methyl compounds the methyl groups are bridging between the two aluminum atoms in the dimer.[1] Therefore in trimethylaluminum dimer and in dimethylaluminum chloride dimer there should be two different types of methyl groups. The two kinds of methyl groups recently have been observed by proton resonance spectroscopy at −75°.[2] At higher temperatures the two peaks coalesce to a single one due to rapid chemical exchange of the methyl groups, and allow an activation energy for the process to be calculated.[3]

We had previously obtained the proton resonance spectra of trimethylaluminum, dimethylaluminum chloride and methylaluminum dichloride in cyclohexane solution. In each case a single line was observed for the aluminum alkyl. The chemical

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(2) N. Muller and D. E. Pritchard, THIS JOURNAL, **82**, 248 (1960).

(3) H. S. Gutowsky and C. H. Holm, *J. Chem. Phys.*, **25**, 1228 (1956).