Further experiments showed that mutant W-5 was not able to hydrogenate added 7-chloro-5a-(11a)-dehydrotetracycline[5] and did not respond to S-1308 fermented mash filtrate, but that S-1308 growing in the presence of previously prepared W-5 filtrate did show the full cosynthetic response. This active substance elaborated by W-5 and by most other *S. aureofaciens* strains, has been designated Cosynthetic Factor I. Isolation and preliminary characterization of this substance are presented in an accompanying communication.[6]

[5] In contrast, *S. aureofaciens* mutants BC-41 and V-138 have been shown to hydrogenate 7-chloro-5a(11a)-dehydrotetracycline to 7-chlorotetracycline: J. R. D. McCormick, N. O. Sjolander, P. A. Miller, U. Hirsch, N. H. Arnold and A. P. Doerschuk, THIS JOURNAL **80**, 6460 (1958).

[6] P. A. Miller, N. O. Sjolander, S. Nalesnyk, N. Arnold, S. Johnson, A. P. Doerschuk and J. R. D. McCormick, *ibid.*, **82**, 5002 (1960).

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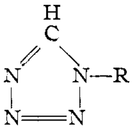
RECEIVED AUGUST 12, 1960

SUBSTITUTION EFFECT IN NUCLEAR MAGNETIC RESONANCE SPECTRA OF TETRAZOLE AND ITS DERIVATIVES

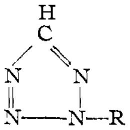
Sir:

Proton magnetic resonance spectra of N-substituted tetrazole compounds reveal a systematic shift of the C-substituted proton line depending on whether the substituent group is in the 1- or 2-position. Chemical shifts of four pairs of compounds of the forms



(a)

and



(b)

have been measured at 40 mc. using the concentric-tube external reference method. All eight compounds were run as pure liquids at room temperature. In addition, tetrazole (R = H) was run in dimethylformamide solvent, since this material is solid at room temperature and decomposes upon heating to its melting point. Results are summarized

R	—CH shift, relative to H_2O, c.p.s. 1-Substituted (a)	 2-Substituted (b)
Ethyl	−163.6	−137.0
Isopropyl	−166.6	−138.5
Vinyl	−166.5	−134.9
Allyl	−161.6	−136.1

The corresponding shift of proton lines in the substituent groups is small, on the order of 3–4 c.p.s. for protons nearest the ring, so it seems unlikely that the observed shift of the –CH line arises from any difference in the screening contribution of the substituents. Rather, the shift must arise from the variations in bonding systems shown in the structural formulas.

The measured position of the –CH line in solutions of tetrazole in dimethylformamide was found to be nearly independent of concentration. At 9.1% the shift relative to H_2O was −171 c.p.s., and at 53.2% it was −169 c.p.s. These values suggest that tetrazole, which can exist as either 1- or 2-protonated tautomers, is predominantly in the 1-substituted form.

The same conclusion has been reported from dipole moment studies.[1] The dipole moments of 1- and 2-ethyltetrazole were found to be 5.46 D. and 2.65 D., respectively, while tetrazole itself has 5.11 D.; indicating 1-position substitution of the proton.

[1] M. H. Kaufman, F. M. Ernsberger and W. S. McEwan, THIS JOURNAL, **78**, 4197 (1956).

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THE PHOTOCHEMICAL CLEAVAGE OF WATER BY RIBOFLAVIN[1]

Sir:

In deoxygenated aqueous solution, riboflavin is reduced to dihydroriboflavin on exposure to visible light.[2] In this system, riboflavin can function catalytically if suitable oxidants are incorporated. Inclusion of an "activator" increases the reaction rate and permits photoreduction of riboflavin in the presence of oxygen. These findings have been confirmed and extended,[3–5] and the mechanism of this reaction has been investigated in several laboratories.[6–8] As we have reported,[9] illumination of an air-free aqueous solution of riboflavin results both in reduction of the flavin and in production of an equimolar amount of hydrogen peroxide. This is evidence that water serves as hydrogen donor in the photoreduction of riboflavin. The hydrogen peroxide formed anaerobically must be distinguished from that resulting from the oxidation of leuco riboflavin on the subsequent admission of oxygen.

The list of "activators" includes tertiary amines, thioethers, and other substances, possessing in common an electronegative nitrogen or sulfur atom, but excludes compounds, such as aryl amines, wherein the nitrogen can contribute electrons to the ring. Such activators have been described as special reducing agents effective only with photo-excited dye molecules.[7,8] Inasmuch as some of the known activators are quite resistant to molecular oxygen and generally are oxidizable only with difficulty, a more detailed explanation of their mode of action was sought.

[1] This investigation was supported in part by Grants from the National Science Foundation and from the National Institutes of Health, U. S. Public Health Service.

[2] W. J. Nickerson and J. R. Merkel, *Proc. Nat. Acad. Sci. U. S.*, **39**, 901 (1953); J. R. Merkel and W. J. Nickerson, *Biochim. et Biophys. Acta*, **14**, 303 (1954).

[3] W. J. Rutter, *Acta Chem. Scand.*, **12**, 438 (1958).

[4] B. Commoner and B. B. Lippincott, *Proc. Nat. Acad. Sci. U.S.*, **44**, 1110 (1958).

[5] W. R. Frisell, C. W. Chung and C. G. Mackenzie, *J. Biol. Chem.*, **234**, 1297 (1959).

[6] G. K. Oster, G. Oster and G. Prati, THIS JOURNAL, **79**, 595 (1957).

[7] G. Oster and N. Wotherspoon, *ibid.*, **79**, 4836 (1957).

[8] D. Mauzerall, *ibid.*, **82**, 1832 (1960).

[9] G. Strauss and W. J. Nickerson, Am. Chem. Soc. Meeting, Atlantic City, N. J., Sept., 1959, p. 50-C.