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).

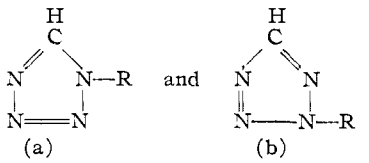
LEDERLE LABORATORIES J. R. D. MCCORMICK
AMERICAN CYANAMID COMPANY URSULA HIRSCH
PEARL RIVER, NEW YORK NEWELL O. SJOLANDER
ALBERT P. DOERSCHUK

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



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).

MICHELSON LABORATORY DONALD W. MOORE
U. S. NAVAL ORDNANCE TEST STATION
CHINA LAKE, CALIFORNIA A. GREENVILLE WHITTAKER

RECEIVED JULY 5, 1960

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.

Search for an activator possessing an easily identified oxidation product led to methionine, the sulfoxide of which can be recognized by its differential chromatographic R_f value. Illumination of a deoxygenated neutral solution of riboflavin and methionine, with simultaneous electrolytic reoxidation of the reduced riboflavin, yielded methionine sulfoxide in an amount twelve times greater than the riboflavin present (Table I). When lumiflavin is used in place of riboflavin, reduced lumiflavin and methionine sulfoxide are obtained. Complete recovery of the lumiflavin is achieved upon subsequent aeration of the solution. This photochemical reaction is neither dependent upon the presence, nor a consequence of the loss of the ribityl side chain, as has been suggested.[6]

The chromatographic demonstration of sulfoxide obviously entails admission of oxygen subsequent to the anaerobic photoreaction. However, methionine is not converted to its sulfoxide by oxygen,[10] by equimolar concentrations of hydrogen peroxide at neutrality, or by electrolytic oxidation. Thus, water must be the source of oxygen for sulfoxide formation and the source of hydrogen for reduction of riboflavin. The mechanism of the photo-reduction of riboflavin (R) in the presence of an activator (A) may therefore be described by (1)

$$R + A + H_2O \longrightarrow [R{-}H_2O{-}A] \xrightarrow{h\nu} RH_2 + AO \quad (1)$$

The water may be regarded as being "suspended" between riboflavin and activator and as being "pulled apart" as the complex dissociates upon absorption of radiant energy. Energy considerations also dictate the water to be bound in a complex between riboflavin and activator: The energy absorbed per mole of light quanta at 440 mμ (a wave length effective for the reaction) is 65 kcal., which is less than the energy requirement calculated from the oxidation-reduction potentials of the reacting substances, considered separately, and assuming the entropy change to be small. Conductance studies indicate that riboflavin forms complexes, in the dark, with methionine and with Na_2EDTA.

TABLE I

PHOTOCHEMICAL CONVERSION OF METHIONINE TO ITS SULFOXIDE[a]

		Concentrations in moles × 10^5/liter			
		[Flavin]	[Dihydro flavin]	[Meth.]	[Sulfoxide]
Expt. I	Initial	5.0	0	50	0
	Final	2.1	2.9	47 ± 1	2.8 ± 0.4
Expt. II[b]	Initial	2.0	0	100	0
	Final	1.8	0.2	72 ± 2	25 ± 2

[a] Methionine and methionine sulfoxide estimated by quantitative chromatographic procedure,[13] riboflavin by spectrophotometry. All solutions deoxygenated by repeated boiling *in vacuo* at room temperature and flushing with purified nitrogen. [b] Riboflavin continuously reoxidized electrolytically.

The photoöxidation of methionine to methionine sulfoxide in the presence of methylene blue has been demonstrated.[11] Supposedly, a "dehydrogenated methionine" was formed by the direct transfer of two hydrogens from methionine to the dye; this "dehydro compound" was assumed to be converted to methionine sulfoxide by its subsequent slow reaction with water. A "dehydromethionine"[12] reputedly is characterized by liberation of iodine from potassium iodide in hydrochloric acid solution. When an air-free solution of riboflavin was photoreduced in the presence of methionine, then treated anaerobically with 1.0 *M* KI and with 0.5 *M* HCl, no iodine could be detected by a spectrophotometric method capable of measuring iodine equivalent to as little as 10% of the riboflavin reduced. Therefore, "dehydromethionine" is not produced in this reaction. Substances that serve as activators for the photochemical reduction of riboflavin (and, presumably, for dyes such as methylene blue) function as acceptors of hydroxyl radicals derived from the photochemical cleavage of water by riboflavin.

INSTITUTE OF MICROBIOLOGY WALTER J. NICKERSON
RUTGERS, THE STATE UNIVERSITY
NEW BRUNSWICK, NEW JERSEY GEORGE STRAUSS

RECEIVED JULY 22, 1960

(10) G. Toennies and J. Kolb, *J. Biol. Chem.*, **128**, 399 (1939).

(11) L. Weil, W. G. Gordon and A. R. Buchert, *Arch. Biochem. Biophys.*, **33**, 90 (1951).

(12) T. F. Lavine, *J. Biol. Chem.*, **169**, 477 (1947).

(13) L. Naftalin, *Nature*, **161**, 763 (1948).

THE COMBINATION OF ENZYMATIC RESOLUTION WITH ASYMMETRIC TRANSFORMATION OF THE OPTICALLY LABILE 3,4-DEHYDROPROLINE AMIDE

Sir:

An optically labile racemic amide of an optically more stable amino acid should be cleaved by an amidase to the L-acid to an extent exceeding the theoretically possible 50% of L-isomer, provided the rate of asymmetric transformation[1] is comparable to the rate of enzymatic hydrolysis. This combination has now been realized with DL-dehydroproline amide (*cf.* II), accessible in 60% yield from pyrrole-2-carboxamide (I) by a modification of E. Fischer's reduction[2] using phosphonium iodide in fuming hydriodic acid (saturated at −20°) for 2 hours and ion exchange resin for separation from 10–20% of the DL-acid (*cf.* III) formed in the process.

The proof of structure for II and III was: (i) the optical lability of the chemically resolved free D-amide II which had a half life time of 48 hours in water, indicating easy abstraction of an allylic proton from C_2 next to the amide carbonyl. This lability is not shown by the L-acid III[3]; (ii) the smooth addition of hydrogen, deuterium or tritium[4] leads to L-proline IV of high optical purity; (iii) the n.m.r. data assigned to II, III and IV[5] expressed in τ-values[5] at 60 Mc./s. (reference internal tetramethylsilane; solvent trifluoroacetic acid) show two vinylic protons, at 3.88, excluding Δ_1-, Δ_2- and Δ_5-structures, and a peak at 4.66 of the proton at C_2 eliminating the Δ_4-structure which would have a peak at 5.2 like proline.[5]

(1) *Cf.* M. M. Harris, "Progress in Stereochemistry," Academic Press, Inc., New York, N. Y., Vol. 2, p. 157 (1958).

(2) E. Fischer and F. Gerlach, *Ber.*, **45**, 2453 (1912).

(3) Solutions of III in 2.0 *N* ammonia lose half of their rotation at 20° after 17 days possibly through base-catalyzed racemization as well as secondary reactions.

(4) L-Proline-3,4-H^3 has been made available for metabolic studies by this procedure through New England Nuclear Corporation, Boston 18, Mass.

(5) F. A. Bovey and G. V. D. Tiers, THIS JOURNAL, **81**, 2870 (1959)