curred when the Ni(II) atom in III is placed near the oxygens or chelate ring of a neighboring molecule. The tendency for Ni(II) to become octahedrally coördinate apparently overcomes this strain in the solid.

Preliminary studies on a third compound, bis-(3-phenyl-2,4-pentanediono)-Ni(II), IV, (R_1 = R_2 = CH_3, R_3 = C_6H_5) gives additional support to the idea that the paramagnetism in anhydrous β-diketone complexes of Ni(II) can be attributed to intermolecular interactions. Models indicate that the phenyl group in IV cannot rotate into coplanarity with the chelate ring due to the proximity of the methyl groups. Close intermolecular association of the Ni(II) atom with a neighboring molecule would be hindered by the phenyl groups perpendicular to the chelate ring. The anhydrous material, from drying the blue-green hydrate, is red and diamagnetic, a single band being observed in the visible spectrum of the solid at 535 mμ. In toluene the material appears to behave similarly to III, the color of the solution changing from green to red as the temperature is increased from 0 to 50°. It also appears that a green crystalline modification of the material can be formed by melting the red powder.

Studies are also being carried out on the visible spectrum of I in hydrocarbon solutions at fairly high temperatures. It has been observed visually that the color of solutions of I in dibenzyl become reddish-brown near 200° and reversibly return to the green color upon cooling.

Financial support was provided by the U. S. Atomic Energy Commission under Contract No. AT(30-1)-1965 and by the Research Corporation to whom grateful acknowledgment is made. J.P.F. wishes to acknowledge a Fellowship received from the Allied Chemical and Dye Corporation, Semet-Solvay Division.

DEPARTMENT OF CHEMISTRY J. P. FACKLER, JR.
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE 39, MASSACHUSETTS F. A. COTTON

RECEIVED JULY 12, 1960

COSYNTHESIS OF TETRACYCLINES BY PAIRS OF *STREPTOMYCES AUREOFACIENS* MUTANTS.

Sir:

In the course of our studies on the biosynthesis of the tetracyclines we have sought for and found numerous blocked mutant strains of *Streptomyces aureofaciens* Duggar which have lost their ability to elaborate any substantial quantity of tetracycline antibiotics. In order to determine whether such mutants might show biological coöperation in the synthesis of tetracycline antibiotics, they were then grown as pairs in mixed fermentation, and the resulting fermented mashes examined for tetracyclines. Typical data summarized in Table I show that in many cases substantial quantities of one or more tetracyclines were produced in the mixed fermentations even when each mutant grown alone produced no appreciable amount. It should be noted that the methods used resulted in the selection of only those mutants which grew well in a corn steep medium[1] ordinarily used for the elaboration of the tetracyclines. Therefore we have used the term cosynthesis for this phenomenon to distinguish it from the previously reported microbial systems involving cross-feeding of essential nutritional factors (syntrophism).[2,3]

TABLE I

COSYNTHESIS OF TETRACYCLINE ANTIBIOTICS

Mutants[a] grown alone	Principal tetracycline product of parent strain[b]	Tetracyclic product of mutant[b]	Antibacterial assay[c] of fermented mash, g./l.
S-1308	7-Cl-TC	7-Cl-TC plus 7-Cl-5a(11a)-dehydro TC	0.20
W-5	7-Cl-TC	Unknown	Nil
E-504	7-Cl-6-demethyl-TC	Unknown	Nil
T-219	TC	Unknown	Nil
S-2242	7-Cl-TC	Unknown	Nil

Mutants grown as mixtures	Tetracycline products cosynthesized[b]	Antibacterial assay[c] of mixed fermentations,[d] g./l.
S-1308 + W-5	7-Cl-TC	2.7
W-5 + E-504	7-Cl-6-demethyl-TC	0.22
T-219 + S-2242	7-Cl-TC plus TC	0.74
S-1308 + E-504	None	Nil
E-504 + T-219	7-Cl-TC plus 7-Cl-6-demethyl-TC	0.34
W-5 + S-2242	None	Nil

[a] Some of these mutants were obtained through the courtesy of Dr. J. Growich of these laboratories. [b] TC = tetracycline. [c] *S. aureus* turbidimetric assay calculated as 7-chlorotetracycline. [d] Corrected for assay of mutants grown alone.

The mechanisms involved in cosynthesis are assumed to be analogous to those involved in syntrophism, that is, the transfer of biosynthetic intermediates from the mutant of the later block to the mutant of the earlier block or, alternatively, the transfer of the missing facility from either mutant to the other, resulting in the coöperative biosynthesis of the normal product of the parent strain.

We wish to report in greater detail the results of our study of one of these cosynthetic pairs. The two mutants used were coded W-5 and S-1308; both were isolated directly from high 7-chlorotetracycline-producing parent strains. Mutant W-5 was an unpigmented mutant lacking the ability to elaborate significant quantities of 7-chlorotetracycline and presumably blocked early in the biosynthetic pathway. The other mutant of this pair, S-1308, was pigmented; it produced 7-chloro-5a(11a)-dehydrotetracycline[4] to the extent of about 4.0 g./l. together with about 0.2 g./l. of 7-chlorotetracycline. When 48 hour-old shaker flask cultures of W-5 and S-1308 were mixed in equal volume and incubated an additional 72 hours, 2.8 g./l. of 7-chlorotetracycline and correspondingly less of 7-chloro-5a(11a)-dehydrotetracycline were produced.

(1) J. J. Goodman, M. Matrishin, R. W. Young and J. R. D. McCormick, *J. Bact.*, **78**, 492 (1959).
(2) J. Lederberg, *ibid.*, **52**, 503 (1946).
(3) B. D. Davis, *Experientia*, **6**, 41 (1950).
(4) J. R. D. McCormick, P. A. Miller, J. A. Growich, N. O. Sjolander and A. P. Doerschuk, THIS JOURNAL, **80**, 5572 (1958).

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).

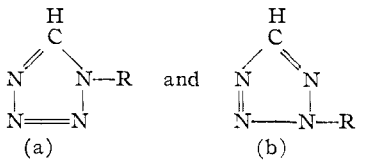
LEDERLE LABORATORIES J. R. D. MCCORMICK
AMERICAN CYANAMID COMPANY URSULA HIRSCH
PEARL RIVER, NEW YORK NEWELL O. SJOLANDER
ALBERT P. DOERSCHUK

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. Scana.*, **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.