The quantum yield for the reaction is low (1×10^{-4} at λ 398 nm) and independent of concentration of **1** over the range $1\text{–}6 \times 10^{-5}$ *M*. We have found that **1** exhibits both delayed fluorescence and phosphorescence in degassed solutions at room temperature. The emission yield appears to be fairly concentration insensitive. These observations indicate that dimerization does not involve the luminescent states and suggests that a later-obtained species is the dimer precursor. The most likely path is outlined in eq 1–5,

$$\text{PyPRuCo} \xrightarrow{h\nu} \underbrace{\text{PyPRuCO}^{1*} \rightleftharpoons \text{PyPRuCO}^{3*}}_{= \text{PyPRuCO}^*} \quad (1)$$

$$\text{PyPRuCO}^* \longrightarrow \text{PyPRuCO} + h\nu \quad (2)$$

$$\text{PyPRuCO}^* \longrightarrow \text{PyPRu} + \text{CO} \quad (3)$$

$$\text{PyPRu} + \text{PyPRuCO} \longrightarrow \text{PyPRu=RuPPy} + \text{CO} \quad (4)$$

$$\text{PyPRu} + \text{CO} \longrightarrow \text{PyPRuCO} \quad (5)$$

where Py = pyridine, P = porphyrin, and PyPRu=RuPPy is the dimer. Photoejection of CO followed by formation of the metal–metal bond, although novel for metalloporphyrin complexes, has been demonstrated for other metal complexes.[13,14]

The base complexed to the metalloporphyrin appears to exert a strong influence both in formation of the dimer and in determining its stability. As previously mentioned, **2a** forms readily upon irradiation of **1** in either benzene or pyridine. Irradiation of degassed benzene solutions of the imidazole, triethylamine, piperidine, or tetrahydrofuran complexes of ruthenium(II) carbonyloctaethylporphyrin leads to near-quantitative conversion to products having spectra similar to that of **2a**. The products from pyridine and the other nitrogen bases are stable indefinitely at room temperature.

However, in sealed tubes the tetrahydrofuran product reverts back to the starting material quantitatively within several hours. Irradiation of degassed benzene solutions of ruthenium(II) carbonyletioporphyrin I at 398 nm does not lead to **2** but rather to an unidentified product absorbing at 610 nm. This product is thermally stable at room temperature but readily reverts to starting material upon photolysis at 610 nm. The pyridine dimers **2a** and **2b** are stable indefinitely as solids in the dark; however, they are readily decomposed in solution by weak acids to yield products having characteristic metalloporphyrin spectra.

Further investigation is under way to elucidate full details of the mechanism of this reaction as well as to determine its scope and applicability to other metalloporphyrin systems.

The twisted phenyl rings in the latter complex probably preclude dimer formation on steric grounds.

(13) (a) E. Koerner von Gustorf and F. W. Grevels, *Fortsch. Chem. Forsch.*, **13**, 366 (1969); (b) W. Strohmeier, *Angew. Chem., Int. Ed. Engl.*, **3**, 730 (1964); (c) P. Chini, *Inorg. Chim. Acta*, **2**, 31 (1968); (d) K. L. Waters and W. M. Risen, *ibid.*, **3**, 129 (1969); (e) V. Balzani and V. Carassiti, "Photochemistry of Coordination Compounds," Academic Press, London, 1970, p 324.

(14) The source of the low reaction efficiency and possible details of the mechanism remain to be determined. It is possible that reaction 3 proceeds with very low efficiency or alternatively that cage recapture of CO is important. Possibly a CO-bridged species[13a] is intermediate in dimer formation.

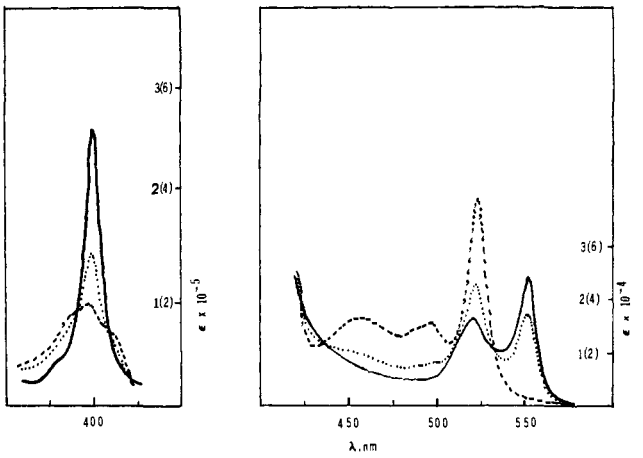


Figure 1. Photodimerization of ruthenium(II) carbonyletioporphyrin I pyridinate: (——) spectrum of **1** in pyridine; (···) intermediate conversion; (- - - -) spectrum of photodimer **2a**. Numbers in parentheses refer to **2a**, others to **1**.

Acknowledgment. We thank the U. S. Public Health Service (Grant No. GM 15,238) for support of this work and Professors M. S. Brookhart and T. J. Meyer for valuable discussion.

(15) A. P. Sloan Foundation Fellow.

G. Wayne Sovocool, Frederick R. Hopf, David G. Whitten*[15]
Department of Chemistry, University of North Carolina
Chapel Hill, North Carolina 27514
Received February 28, 1972

Simultaneous Optical and Electron Spin Resonance Detection of the Primary Photoproduct P700 in Green Plant Photosynthesis[1]

Sir:

It has been known for some time that the initial steps in the photosynthetic mechanism of green plants, algae, and bacteria involve one-electron transfers.[2] These light-induced reactions create free-radical intermediates which may be detected by electron spin resonance (esr). Although these esr signals are thought to arise from components close to the primary photochemical reactions, the precise molecular identity of the free radicals has been a subject of much speculation and study.[3,4]

In certain photosynthetic bacteria (*e.g.*, *Rhodopseudomonas spheroides*), an exact correspondence of the light-induced esr signal with a bleaching at ~870 nm of a uniquely situated bacteriochlorophyll molecule has been demonstrated.[5–7] The situation is not nearly so convincing in green plant or algal systems. The early

(1) Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and to the National Research Council of Canada for support of this research. Contribution No. 35 from the Photochemistry Unit, University of Western Ontario.

(2) E. Rabinowitch and Govindjee, "Photosynthesis," Wiley, New York, N. Y., 1969, pp 190–193.

(3) E. C. Weaver, *Annu. Rev. Plant Phys.*, **19**, 283 (1968).

(4) D. H. Kohl in "Biological Applications of Electron Spin Resonance," H. M. Swartz, J. R. Bolton, and D. C. Borg, Ed., Wiley, New York, N. Y., 1972.

(5) J. R. Bolton, R. K. Clayton, and D. W. Reed, *Photochem. Photobiol.*, **9**, 209 (1969).

(6) P. A. Loach and K. Walsh, *Biochemistry*, **8**, 1908 (1969).

(7) J. D. McElroy, G. Feher, and D. C. Mauzerall, *Biochim. Biophys. Acta*, **172**, 180 (1969).

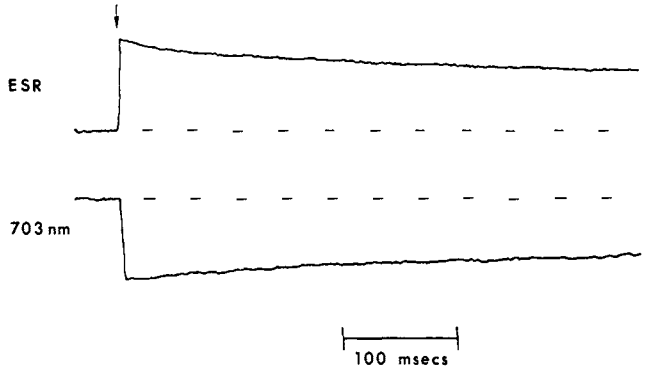


Figure 1. Simultaneous optical and esr decay kinetics in TSF1 particles. The reaction mixture contained 0.5 mM sodium ascorbate and 1 mM benzyl viologen. Chlorophyll a concentration, 0.4 mg/ml. The traces are an average of 64 sequential flashes.

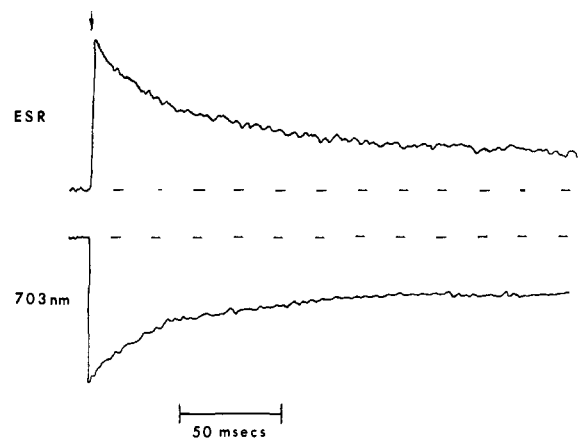


Figure 2. Simultaneous decay kinetics of spinach D144 particles incubated with 1 μM PMS, 0.5 mM sodium ascorbate, and 1 mM benzyl viologen. Chlorophyll a concentration, 0.8 mg/ml. The traces are an average of 64 sequential flashes.

tentative assignment of the green plant esr analog (signal 1) to a bleaching of a chlorophyll component at 700 nm[8] (P700) has not been disproven. However, attempts to establish a correlation between the number of light-induced free radicals and the number of bleached moieties have not been entirely successful. Although recent experiments with subchloroplast particles have indicated a P700 to signal 1 ratio near unity,[9,10] earlier investigations on intact cells by Beinert and Kok yielded ratios of 0.5 or less.[11] It should be noted that these measurements require not only accurate esr quantitation, but also the value for $\Delta\epsilon$ (the change in the molar absorptivity between the light and dark states) must be precisely known at the wavelength of interest.[12]

The qualitative correlation between optical and esr signal behavior is better when green plant or algal species, which have been subjected to various chemical and physical treatments, are studied. For example, addition of the herbicide DCMU (dichlorophenyldimethylurea) inhibits oxygen evolution by blocking electron flow from system 2 (the oxygen evolving photosystem) to system 1 (the NADP reducing photosystem) leading to an increase in both the P700 and signal 1 intensities. Likewise, addition of the photophosphorylation stimulant PMS (phenazine methosulfate) leads to an accelerated decay of both signal 1 and P700 upon cessation of illumination. Although an imprecise kinetic correlation between signal 1 and P700 in Triton subchloroplast particles has been reported,[13] the uncertainty of using different samples, consecutive measurements, and a limited instrumental response time renders the correlation as dubious.

Recently, we have initiated a study of transient electron transfer reactions in system 1 of chloroplasts using the new technique of flash photolysis electron spin resonance.[14,15] Although our findings are in basic agreement with analogous optical experiments, the basic problem of biological variability from sample to sample makes a 1:1 correlation impossible. Realizing these limitations, we chose to construct an apparatus which would allow the simultaneous monitoring of optical-density changes and esr signals following a flash of light.

The esr spectrometer (Varian E-12) has been fitted with an optical transmission cavity, modified to allow transmission of the monitoring and photolytic light beams through the cavity. The photolysis source is a Synergetics Chromabeam 1070 dye laser emitting a light pulse of $\sim$0.5-μsec duration and up to 200-mJ energy at 620 $\pm$ 5 nm using rhodamine B as the active medium. The photomultiplier and esr outputs are simultaneously acquired by a Nicolett Corp. Fabritek 1070 computer of averaged transients. Details of the apparatus will be published later.[15]

Spinach subchloroplast particles were prepared from greenhouse grown spinach (USDA no. 7) by either digitonin fractionation, according to the method of Anderson and Boardman (D144)[16] or the Triton method as developed by Vernon and Shaw (TSF1).[17]

When the monitoring light monochromator was set at 703 nm, a clear correspondence between the optical and esr kinetic response was obtained in a TSF1 preparation as shown in Figure 1. The arrow indicates the time of the laser flash. Both P700 and signal 1 decay by first-order kinetics with a half-life of 0.90 $\pm$ 0.02 sec. The same kinetic correlation is also observed for the D144 particles to which PMS and sodium ascorbate had been added to accelerate the decay (see Figure 2). The same identical kinetic response has been observed in many samples under a variety of conditions. These results strongly support the proposition that signal 1 and the bleaching at 700 nm are physical attributes of the same chemical species. The fact that both signals appear within $\sim$100 μsec (the time resolution of the esr spectrometer) of the laser flash suggests that the species is the primary photoproduct. Other work has implicated that this species is most probably the cation radical of a specific chlorophyll a molecule.[18]

Experiments in progress are designed to allow simultaneous quantitation of both optical and esr tran-

(8) H. Beinert, B. Kok, and G. Hoch, *Biochem. Biophys. Res. Commun.*, 7, 209 (1962).
(9) L. P. Vernon, B. Ke, and E. R. Shaw, *Proc. Int. Congr. Biochem., 7th*, Abstract 14–31 (1967).
(10) E. C. Weaver and H. E. Weaver, *Science*, **165**, 906 (1969).
(11) H. Beinert and B. Kok, *Biochim. Biophys. Acta*, **88**, 278 (1964).
(12) T. Hiyama and B. Ke, manuscript in preparation.
(13) L. P. Vernon, B. Ke, and E. R. Shaw, *Biochemistry*, **6**, 2210 (1967).
(14) B. J. Hales and J. R. Bolton, *Photochem. Photobiol.*, **12**, 239 (1970); *J. Amer. Chem. Soc.*, **94**, 3314 (1972).
(15) J. R. Bolton and J. T. Warden, manuscript in preparation.
(16) N. K. Boardman and J. M. Anderson, *Biochim. Biophys. Acta*, **112**, 403 (1966).
(17) L. P. Vernon and E. R. Shaw, *Methods Enzymol.*, **23**, 277 (1971).
(18) D. C. Borg, J. Fajer, R. H. Felton, and D. Dolphin, *Proc. Nat. Acad. Sci. U. S.*, **67**, 813 (1970).

sients. In addition, the role of donor or acceptor molecules is being surveyed.[19]

Acknowledgment. The authors wish to acknowledge Messrs. B. Lapier and R. Kovach for their skilled technical assistance.

(19) NOTE ADDED IN PROOF. We have now extended these measurements to whole chloroplasts and can report that the same kinetic correspondence of esr response and bleaching at 703 nm is found for the intact system. In addition, preliminary results on a quantitative comparison show that the ratio of P700 molecules bleached to signal I radicals formed is very close to unity for several types of preparations. Details will be reported later.

Joseph T. Warden
Department of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455

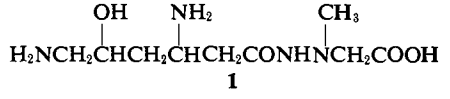
James R. Bolton*
Photochemistry Unit, Department of Chemistry
University of Western Ontario
London 72, Ontario, Canada

Received April 18, 1972

The Total Syntheses of Negamycin and the Antipode

Sir:

The structure of negamycin (**1**) and its partial synthesis have bteen reported.[1] The mechanism of action of negamycin is similar to that of most aminoglycosidic antibiotics including streptomycin and kanamycin; that is, negamycin causes inhibition of protein synthesis and misreading of the genetic code.[2]

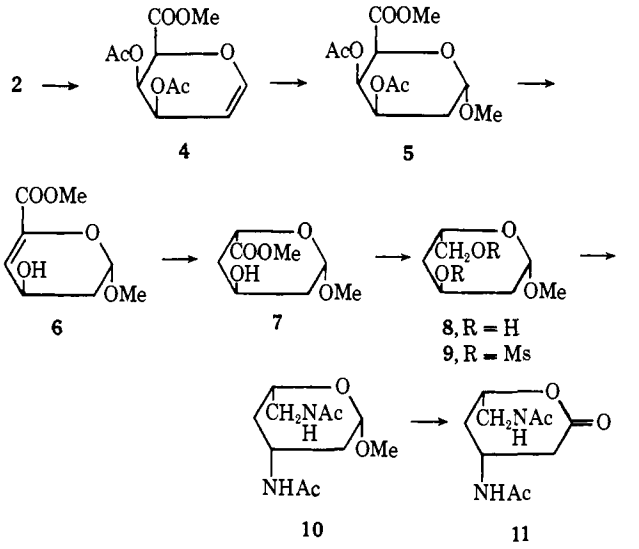


1

Therefore, it is important to investigate the possibility of a close relationship between hydroxy amino acid and aminoglycosidic antibiotics. We report herein the stereoselective conversions of D-galacturonic acid[3] (**2**) and 3-amino-3-deoxy-D-glucose[4] (**3**) to δ-hydroxy-β-lysine, the amino acid moiety of **1**, which have led to the total syntheses of **1** and the antipode, respectively. In addition, it has been shown that a difference in the absolute configuration of δ-hydroxy-β-lysine causes a marked difference in biological activity.

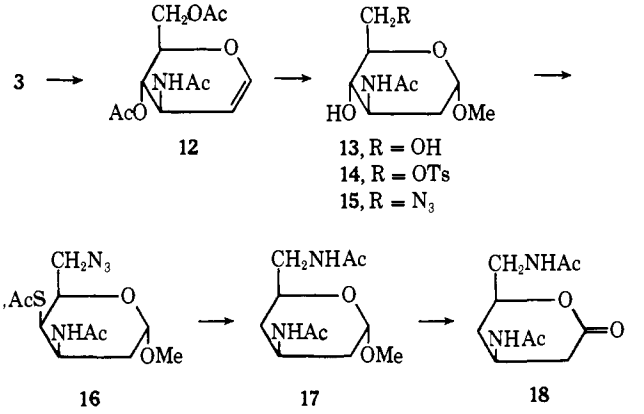
D-Galacturonic acid commercially available has the 3*S* and 5*S* configurations. A transformation to the amino acid moiety of **1** has been realized in the following way (Scheme I). A glycal compound **4** was obtained in a good yield from **2**[5] by the usual methods. 2-Deoxy derivative **5** was obtained by treatment of **4** with iodine in methanol in the presence of silver acetate followed by catalytic hydrogenation over Pd/C. The method of Schmidt and Neukom[6] was applied to **5** to afford **6**, $[\alpha]^{21}D$ +234° (*c* 0.85, MeOH). Catalytic hydrogenation of **6** gave methyl (methyl 2,4-dideoxy-β-L-*erythro*-hexosid)uronate (**7**) as a major product[7] (60% yield): mp 60–62°; $[\alpha]^{21}D$ +113° (*c* 0.8, $CHCl_3$); M^+ = 190; δ 4.55 (H-5, quartet at −30° with $J_{4,5}$ = 7.5 and $J_{4',5}$ = 2.5 Hz). The reduction of the carboxyl group with $LiAlH_4$ afforded methyl 2,4-dideoxy-β-L-*erythro*-hexopyranoside (**8**), and then two hydroxyl groups were mesylated to afford **9**. The treatment of **9** with sodium azide followed by hydrogenation with Pd/C afforded a diamino compound which was acetylated with Ac_2O to afford methyl 3,6-diacetamido-2,3,4,6-tetradeoxy-β-L-*threo*-hexopyranoside (**10**). After hydrolysis and oxidation with aqueous bromine, a crystalline lactone of (3*R*,5*R*)-3,6-diacetamido-5-hydroxyhexanoic acid (**11**), mp 183–185°, $[\alpha]^{21}D$ −5.8° (*c* 1.0, H_2O), was obtained. It was identical in all respects with the compound derived from natural negamycin.

Scheme I



3-Amino-3-deoxy-D-glucose (**3**) has the 3*S*,5*R* configuration. Therefore, the antipode of δ-hydroxy-β-lysine of **1** can be synthesized by replacing two hydroxyl groups at C-2 and C-4 with hydrogen and converting the primary alcohol at C-6 to the primary amino group without disturbing the stereochemistry at C-3 and C-5. Such a transformation has been realized in the following way (Scheme II). 3-Acetamido-4,6-di-*O*-acetyl-1,2,3-

Scheme II



(1) S. Kondo, S. Shibahara, S. Takahashi, K. Maeda, H. Umezawa, and M. Ohno, *J. Amer. Chem. Soc.*, **93**, 6305 (1971).

(2) S. Mizuno, K. Nitta, and H. Umezawa, *J. Antibiot.*, **23**, 581, 589 (1970).

(3) J. Staněk, M. Cerny, J. Kocourek, and J. Pacák, "The Monosaccharides," Academic Press, New York and London, 1963, p 703.

(4) This amino sugar is now easily available by fermentation of *Bacillus aminoglycosidicus*, and the absolute structure is already known: S. Umezawa, K. Umino, S. Shibahara, M. Hamada, and S. Omoto, *J. Antibiot., Ser. A*, **20**, 355 (1967).

(5) T. Kondo and T. Goto, *Agr. Biol. Chem.*, **35**, 912 (1971).

(6) H. W. H. Schmidt and H. Neukom, *Carbohyd. Res.*, **10**, 361 (1969).

(7) The diastereomer at C-5 was obtained as a minor product and the purification of **7** was easily carried out on silica gel chromatography.