dried extract yielded a crystalline residue which was crystallized from petroleum ether, affording fine plates of 8-methylnonanamide (27 mg, 50%), mp 102–103°, not depressed on admixture with an authentic specimen, mp 103.5–104.5°.[24]

Degradation of Veratrylamine. Veratrylamine hydrochloride (100 mg) was heated on a steam bath in water (10 ml) containing sodium hydroxide (0.1 g) and potassium permanganate (0.2 g) for 1 hr. The filtered solution was acidified with hydrochloric acid when veratric acid (62 mg), mp 182–183°, separated out. Veratric acid (20 mg) was refluxed in quinoline (1 ml) in the presence of copper chromite (20 mg) in a current of nitrogen. The liberated carbon dioxide was absorbed in barium hydroxide solution yielding barium carbonate (21 mg). Veratric acid (100 mg) was refluxed with 50% hydriodic acid (5 ml) for 24 hr, and then evaporated to dryness. The residue was refluxed with absolute ethanol (5 ml), benzene (5 ml), and a drop of concentrated sulfuric acid for 24 hr. The solution was diluted with more benzene and washed with dilute sodium bicarbonate solution. The organic layer was dried over sodium sulfate and evaporated and the residue sublimed yielding ethyl 3,4-dihydroxybenzoate (21 mg), mp 133–134°, not depressed on admixture with an authentic specimen.

Degradation of O-Methyl-6,7-dihydroxydihydrocapsaicin. Sodium metaperiodate (200 mg) dissolved in water (10 ml) was added to a solution of the diol **3** (100 mg) in methanol (10 ml). After standing for 4 hr at room temperature, water (20 ml) was added and the mixture steam distilled. Semicarbazide hydrochloride (0.5 g) and sodium acetate (1 g) were added to the distillate. After standing overnight the solution was made basic with sodium carbonate and extracted with ether. The dried (Na_2SO_4) extract was evaporated and the residue crystallized from a mixture of benzene and petroleum ether yielding colorless plates of isobutanal semicarbazone (12 mg), mp 122–123°, not depressed on admixture with an authentic specimen. The aqueous solution which had been subjected to steam distillation was extracted with chloroform. The dried extract was evaporated and the residue crystallized from a mixture of ethyl acetate and petroleum ether affording colorless plates of 5-formyl-(3,4-dimethoxybenzyl)pentanamide (63 mg), mp 72–73°.

Anal. Calcd for $C_{15}H_{21}NO_4$ (279): C, 64.49; H, 7.58; N, 5.01. Found: C, 64.38; H, 7.45; N, 5.28.

The mass spectrum had a molecular ion peak at *m/e* 279, and a prominent peak at 251 due to the loss of CO. The infrared spectrum (KBr pellet) contained the following absorptions: 3300 (NH), 2730 (aldehyde CH), 1714 (aldehyde C=O), and 1640 cm^{-1} (amide C=O).

Kuhn–Roth Oxidation of Isobutanal Semicarbazone. The semicarbazone (90 mg) was added to a solution of chromium trioxide (3 g) in 2 *N* sulfuric acid (20 ml) and the mixture refluxed for 12 hr. The mixture was then distilled, water being added to maintain the volume in the distilling flask at about 20 ml. The distillate (50 ml) was titrated with 0.1 *N* sodium hydroxide (3.1 ml required) and evaporated to dryness. The residue (22 mg) was dissolved in water (2 ml), and α-naphthylamine hydrochloride (30 mg) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (100 mg) were added. The resultant mixture of amides was filtered off, washed with dilute acid, and sublimed (140°, 0.01 mm). Thin layer chromatography indicated the presence of the α-naphthylamides of acetic and isobutyric acid. They were separated on preparative tlc plates of Silica Gel F_{254} (Merck), developing with 5% ethanol in chloroform. The zone with R_f 0.45 afforded acetyl-α-naphthylamide (3.5 mg), mp 159–160° (lit.[25] mp 159–160°). The isobutyryl-α-naphthylamide, R_f 0.65 (4.0 mg), had mp 147–148°, obtained as colorless needles from benzene.

Anal. Calcd for $C_{14}H_{15}NO$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.71; H, 7.10; N, 6.80.

(24) L. F. Fieser, *et al.*, *J. Am. Chem. Soc.*, 70, 3174 (1948).

(25) E. Leete, H. Gregory, and E. G. Gros, *ibid.*, 87, 3475 (1965).

Chlorophyll Diastereoisomers. The Nature of Chlorophylls a′ and b′ and Evidence for Bacteriochlorophyll Epimers from Proton Magnetic Resonance Studies[1]

Joseph J. Katz, Gail D. Norman, Walter A. Svec, and Harold H. Strain

Contribution from the Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439. *Received June 17, 1968*

Abstract: Chlorophylls a′ and b′ are shown by pmr to be 10-epichlorophylls a and b. By the use of deuteriochlorophyll a containing ordinary hydrogen at the C-10 position, two kinds of dimer are shown to exist in CCl_4 solution. Bacteriochlorophyll also occurs in diastereoisomeric forms, and pmr shows small amounts of 10-epibacteriochlorophyll to be present in solution.

In 1942, Strain and Manning[2] observed that both chlorophylls a and b were often accompanied by small quantities of a minor green pigment which appeared as a well-resolved, less-sorbed, companion or satellite chromatographic zone. The spectroscopic properties (in the visible region) of the chlorophyll from the a satellite zone were remarkably similar to those of a, and the properties of the other were similar to those of b. The satellite and the principal chlorophyll in each case could be reversibly interconverted by heating in alcohol or pyridine solution, as judged by chromatographic criteria. Consequently, the satellite and the corresponding chlorophyll were considered to be isomers, hence the satellite pigments were designated chlorophyll a′ and b′, respectively. The methyl and ethyl chlorophyllides, which lack the phytyl group, were found to undergo a similar isomerization; hence the reversible isomerization of the chlorophylls must involve the ring system rather than a *cis–trans* isomerization about the vinyl bond in the phytyl moiety. Allomerization products formed from chlorophylls a and b[3] failed to form isomers when heated in propanol solution. Because allomerization is essentially oxida-

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

(2) H. H. Strain and W. M. Manning, *J. Biol. Chem.*, **146**, 275 (1942).

(3) G. R. Seely in "The Chlorophylls," L. P. Vernon and G. R. Seely, Ed., Academic Press, New York, N. Y., 1966, p 91.

Table I

Compound	Mg present[a]	R	R′	R″	R‴
Chlorophyll a	+	CH_3	H	CO_2CH_3	Phytyl
10-Epichlorophyll a[c]	+	CH_3	CO_2CH_3	H	Phytyl
Chlorophyll b	+	CHO	H	CO_2CH_3	Phytyl
10-Epichlorophyll b[c]	+	CHO	CO_2CH_3	H	Phytyl
Bacteriochlorophyll[b]	+	CH_3	H	CO_2CH_3	Phytyl
10-Epibacteriochlorophyll[c]	+	CH_3	CO_2CH_3	H	Phytyl
Methylpheophorbide a	−	CH_3	H	CO_2CH_3	CH_3
Pyrochlorophyll a	+	CH_3	H	H	Phytyl
Methyl(pyro)pheophorbide a	−	CH_3	H	H	CH_3
10-Methoxymethyl(pyro)pheophorbide a	−	CH_3	H	CH_3O	CH_3
10-Epimethoxymethyl(pyro)pheophorbide a[c]	−	CH_3	CH_3O	H	CH_3

[a] In the Mg-free compounds, the Mg is replaced by 2H. [b] In bacteriochlorophyll, the vinyl group at position 2 is replaced by an acetyl group, and two additional hydrogens are present at positions 3 and 4. [c] We have followed the usage of Inhoffen, *et al.*,[5] in naming these compounds. This nomenclature is also sanctioned by E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 40. We use the designation chlorophyll a′ interchangeably with 10-epichlorophyll a.

tion at C-10, and results in removal of the C-10 proton,[3] Strain[4] suggested that "spatial changes of the hydrogen and the carboxyl group normally attached to this carbon atom might account for the reversible isomerization of the chlorophylls and chlorophyllides." As no experimental data was brought to bear on the isomerization problem, the details of the isomerism and the relationship between chlorophylls a and a′ and b and b′ continued a puzzle.

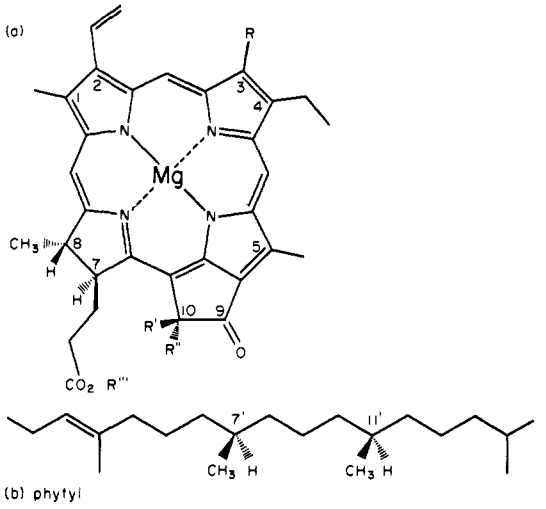


Figure 1. Structural formulas and nomenclature of compounds: (a) ring numbering and stereochemistry, (b) phytyl moiety (see Table I for compound formulas).

We have observed that in pyrochlorophyll a the two protons at position C-10 have different chemical shifts,[5] indicative of different magnetic environments for the two positions occupied by the protons at the tetrahedral C-10 carbon. Inhoffen, *et al.*[6] have likewise observed differences in the chemical shifts of the C-10 proton in the two diastereomeric 10-methoxymethyl(pyro)pheophorbides a. We now provide proton magnetic resonance (pmr) data that show chlorophylls a′ and b′ to be 10-epichlorophylls a and b, which are diastereoisomeric compounds related to the parent pigments by inversion of configuration at position C-10. Further, because Fleming,[7] and, more recently, Brockmann[8] have established the absolute configurations of chlorophyll a and bacteriochlorophyll, we can assign the absolute configurations of the epichlorophylls. We can also show that bacteriochlorophyll similarly occurs in epimeric forms that have not as yet been isolated by chromatography.

Nomenclature, numbering, and structural formulas for the compounds treated here are shown in Figure 1 and Table I.

C-10 Chemical Shifts in Pyrochlorophyll a and 10-Methoxymethyl(pyro)pheophorbide a. The two C-10 resonances in pyrochlorophyll a (0.08 *M* in tetrahydrofuran-d_8 solution) form a strongly perturbed AB pattern (Figure 2), with two large peaks at 5.07 and 5.02 ppm, and two small peaks at 5.26 and 4.82 ppm, measured from internal tetramethylsilane (TMS). The chemical shift differences between the two C-10 protons is 15.07 Hz, with a *gem*-coupling constant $|J| = 20.2$ Hz. The chemical shift values we observe for these protons in methyl pyropheophorbide a (0.16 *M* in $CDCl_3$) are very similar and agree well with those given by Inhoffen, *et al.*[6]

Inhoffen, *et al.*,[6] find a chemical shift difference of 26 Hz in the C-10 proton of the two epimeric 10-methoxymethyl(pyro)pheophorbides a; they did not detect epimeric forms of methyl pheophorbide a.

C-10 Chemical Shifts in Chlorophylls. We have now observed, in chlorophylls a and b and bacteriochlorophyll, that the C-10 proton resonance is accompanied by a small resonance peak at slightly higher field. We assign this satellite resonance peak to an epi-C-10 proton on the basis of chemical shift observations on pyrochlorophyll and the diastereomeric 10-methoxymethyl(pyro)pheophorbides, and on exchange and kinetic data to be described below. In addition, because the epi-C-10 resonance occurs in the AM portion of the AMX pattern arising from the vinyl group in position 2 of the chlorophylls a and b, we have made observations on deuteriochlorophyll a, where the C-10 proton resonances can be seen without interference from the vinyl group. With all these chlorophylls, dissolved in an electron-donor solvent so that they are

(4) H. H. Strain, *Agr. Food Chem.*, 2, 1222 (1954).
(5) F. C. Pennington, H. H. Strain, W. A. Svec, and J. J. Katz, *J. Am. Chem. Soc.*, 86, 1418 (1964).
(6) H. Wolf, H. Brockmann, Jr., H. Biere, and H. H. Inhoffen, *Ann. Chem.*, 704, 208 (1967).
(7) I. Fleming, *Nature*, 216, 151 (1967).
(8) H. Brockmann, Jr., *Angew. Chem.*, 80, 233, 234 (1968).

monomeric, the epi-C-10 resonance absorption is observed at about 12 Hz to higher field than is the principal C-10 resonance peak (Table II). In pyridine-d_5 and tetrahydrofuran-d_8 solution, the epi-C-10 peak is clearly visible, but in acetone-d_6, both C-10 resonances are overlapped by the vinyl resonances (Figure 2). The magnitude of the upfield shift of the epi-C-10 proton resonance in the chlorophylls is consistent with that observed in pyrochlorophyll a and the diastereomeric 10-methoxymethyl(pyro)pheophorbides a.

Table II. Chemical Shifts of C-10 Protons in Epimeric Chlorophylls

Compound	Solvent	Concn, *M*	Chem shift,[a] δ C-10	δ epi-C-10	Δδ, Hz
Chlorophyll a	THF-d_8	0.11	619.1	606.4	12.7
Chlorophyll a	THF-d_8	0.07	620.8	608.1	12.7
Chlorophyll a	C_5D_5N	0.09	661.1	647.0	14.0
Chlorophyll a	CF_3COOH	0.06	669.0	658.9	10.1
Chlorophyll b	C_5D_5N	0.08	656.7	643.3	12.4
Chlorophyll b	THF-d_8	0.07	613.2	600.9	12.3
Methylbacteriopheophorbide	$CDCl_3$	0.04	607.7	594.8	12.9
Bacteriochlorophyll	C_3D_6O	0.12	570.2	558.9	11.3

[a] Chemical shifts measured in parts per million from internal tetramethylsilane.

In the preparation of chlorophylls a and b,[9] the a′ and b′ are separated. Consequently, freshly prepared solutions of pure chlorophylls a or b do not show the epi-C-10 resonance. This resonance can be observed to grow in. The half-time for the generation of the epi-C-10 resonance is somewhat, but not greatly, different for different polar solvents, being about 2 hr in pyridine or tetrahydrofuran. Basic solvents, it is reasonable to suppose, will facilitate the enolization of the C-10 proton by stabilizing the enol relative to the keto form of the chlorophylls, and so facilitate isomerization at the C-10 position. In benzene, equilibration of the epimeric chlorophylls is slower and requires at least 24 hr to be complete. By integration of the resonance peaks, we estimate the relative amounts of a and a′ to be between 80:20 and 85:15, close to the value estimated by Strain in pyridine solution by chromatography. The relative amounts of a and a′ in pyridine appear to be nearly independent of temperature in the range 30–70°. As the epi-C-10 resonance absorption grows in, small satellite peaks appear very near the low-field methine resonances.[10] These shoulders on the methine resonances are generally about 1.5 Hz on the high-field side of the peaks, and have often been observed previously in chlorophyll pmr spectra. It is now clear that these "shoulders" arise from the presence of 10-epichlorophylls in the equilibrium mixture. Either the ring currents, or the geometries, or both, are slightly but detectably different in the epimeric chlorophylls.

We have prepared pure chlorophyll a′ by equilibrating chlorophyll a in pyridine, and separating the epimers by column chromatography. A solution of chlorophyll a′ in tetrahydrofuran isomerizes rapidly, and equilibrium between the epimers is quickly re-

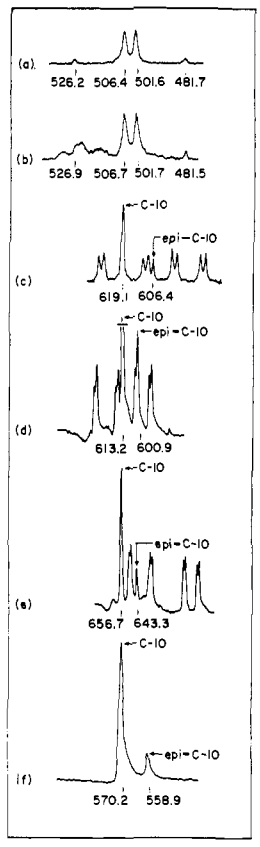


Figure 2. C-10 and epi-C-10 proton chemical shifts (δ values in parts per million downfield from TMS): (a) methyl(pyro)pheophorbide a (0.16 *M* in $CDCl_3$), (b) pyrochlorophyll a (0.08 *M* in THF-*d*), (c) chlorophyll a (0.11 *M* in THF-*d*), (d) chlorophyll b (0.07 *M* in THF-*d*), (e) chlorophyll b (0.08 *M* in C_5D_5N), (f) bacteriochlorophyll (0.12 *M* in C_3D_6O). Spectra c, d, e, and f were collected by time averaging for improved signal-to-noise ratio.

established at 10°. By observing the pmr spectrum of chlorophyll a′ solution in tetrahydrofuran at low temperature as quickly as possible after forming the solution, it can be seen that the epi-C-10 resonance is distinctly larger than in the equilibrium mixture, and that isomerization is rapid even at low temperatures. A less basic solvent, we now infer, might be desirable to stabilize the a′ form.

To make the assignment of the high-field C-10 proton resonance more certain, we have observed the exchange behavior of these resonances with added CD_3OD. The C-10 proton is known to undergo exchange,[11] and the epi-C-10 resonance is observed to be exchanged at sensibly the same rate as the C-10 resonance in chlorophyll a. This conclusively establishes the epi-C-10 resonance as indeed a C-10 proton. Because the exchange is slow, the possibility that the epi-C-10 resonance is due to enolic OH is excluded.

Chemical Shifts of C-10 Carbomethoxy Methyl Protons. If chlorophylls a and a′ are epimeric, then we should expect to find chemical shift differences in the proton resonances of the carbomethoxy group at position 10. Inspection of our large collection of (unpublished) pmr chlorophyll spectra clearly reveals the expected epimeric C-10-$COOCH_3$ resonances. The carbomethoxy methyl proton resonance is found at 3.76

(9) H. H. Strain and W. A. Svec, ref 3, pp 22–66.
(10) J. J. Katz, R. C. Dougherty, and L. J. Boucher, ref 3, pp 215–234.
(11) R. C. Dougherty, H. H. Strain, and J. J. Katz, *J. Am. Chem. Soc.*, 87, 104 (1965).

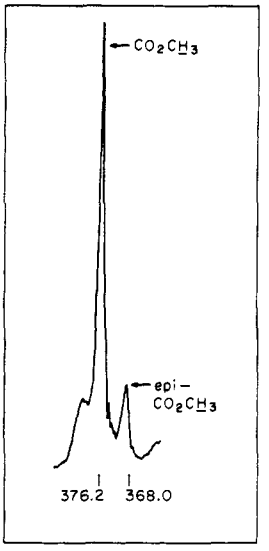


Figure 3. Chemical shifts of 10-carbomethoxymethyl group (δ values in parts per million from TMS); chlorophyll a, 0.11 *M*, in THF-*d*.

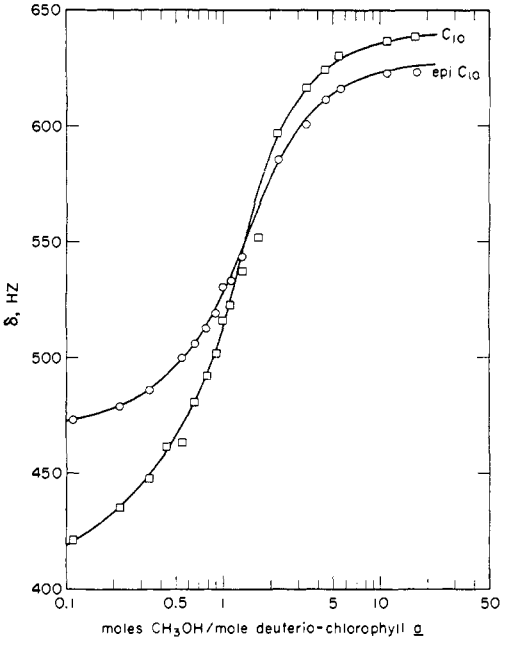


Figure 4. Chemical shifts (δ values in parts per million downfield from hexamethylsiloxane) of dimers from a methanol titration of deuteriochlorophyll a (0.089 *M* in CCl_4) by methanol. The curve marked C-10 arises from dimers containing deuteriochlorophyll a in the normal configuration. The curve marked epi-C-10 is given by dimers in which one unit of the aggregate is 10-epideuteriochlorophyll a.

ppm (0.11 *M* in tetrahydrofuran). In the equilibrated solution, a small peak is observed at 3.68 ppm, which can be assigned to the epimeric $COOCH_3$ resonance (Figure 3). The epi-C-10-$COOCH_3$ resonance in the chlorophylls generally is found at about 8 Hz to higher field, and can be observed to grow into a solution of pure chlorophyll a at the same rate as the epi-C-10 and epimethine resonances.

Epi-C-10 Resonances Observed in Deuteriochlorophyll Solutions. As mentioned above, the epi-C-10 resonance occurs in the same region of the spectrum as do the vinyl protons in chlorophylls a and b. To minimize ambiguities in interpretation, we have observed the epi-C-10 proton resonances in solutions of deuteriochlorophyll. Because the C-10 hydrogens are exchangeable, deuteriochlorophyll can easily be obtained with H at position C-10. The vinyl protons, being ^2H in deuteriochlorophyll, are invisible, and the pmr spectrum is thus greatly simplified. We have carried out a methanol titration of[12] (equilibrated) deuteriochlorophyll (with H at C-10) in CCl_4 solution (Figure 4). In CCl_4 solution, chlorophyll a occurs as dimers.[12] In the dimer, the C-10 proton is subject to a large diamagnetic ring current shift. As the dimers are disaggregated by the addition of the base (CH_3OH), the C-10 proton resonance moves downfield, being the weighted average of the low-field C-10 proton in the chlorophyll monomer with the high-field C-10 in the dimer. In the present experiment, *two* C-10 resonances are observed. Thus, two dimeric species are present in the partially disaggregated deuteriochlorophyll a solution. As increasing amounts of alcohol are added, both resonances move downfield, coalesce, and finally, in monomeric form, the C-10 resonance, which was originally at higher field, ends up at lower field (12 Hz) relative to the epimeric C-10 resonance. On a statistical basis, if the ratio of the epimeric chlorophylls is 80:20, we expect 64% a–a dimer, 32% a–a′ dimer, and 4% a′–a′ dimer. We conclude from the shape of the titration curves[13] that the a–a′ dimer is tighter than is the a–a, and we can likewise conclude that stereochemical factors in dimer formation must be quite important. Thus, the experiments with deuteriochlorophyll not only made it possible to observe the epimeric C-10 resonances without the confusion resulting from other proton resonances, but new and otherwise unobservable details about the dimerization process are also revealed.

Epimeric Bacteriochlorophylls. In speculating about the nature of the relationship between chlorophylls a and a′ and b and b′, it has been an embarrassment that no corresponding bacteriochlorophyll isomer had ever been detected. Chlorophylls a and b and bacteriochlorophyll have identical chemical structures in the vicinity of the alicyclic ring V. Consequently, it was difficult to see why there existed no isomeric bacteriochlorophyll, if in fact the stereochemistry about C-10 were all that was involved. The pmr spectra of bacteriochlorophyll solutions clearly show the presence of an epi-C-10 resonance (Table II and Figure 1). Because bacteriochlorophyll lacks a vinyl group, the epi-C-10 resonance is clearly seen. The equilibrium appears to be more displaced in the direction of the normal, or major, configuration, and we estimated that 10-epibacteriochlorophyll occurs in an equilibrium concentration of less than 10% in tetrahydrofuran solution. The reason 10-epibacteriochlorophyll is not observed during chromatography must be that the solvent system that resolves chlorophylls a and a′ and b and b′ is not optimum for the separation of the relatively small amounts of 10-epibacteriochlorophyll present at equilibrium. Another possible reason for the failure to resolve 10-epibacteriochlorophyll may be a more rapid interconversion of the isomers.

(12) G. L. Closs, J. J. Katz, F. C. Pennington, M. R. Thomas, and H. H. Strain, *J. Am. Chem. Soc.*, 85, 3809 (1963).

(13) J. J. Katz, H. H. Strain, D. L. Leussing, and R. C. Dougherty, *ibid.*, 90, 784 (1968).

10-Epichlorophylls in Nature. We have every reason to suppose that the 10-epichlorophylls are artifacts because they are not found in carefully prepared plant extracts. The epimeric chlorophylls must be taken into consideration, however, in studying the properties of solid chlorophyll or chlorophyll solutions, and the extent to which spectroscopic properties of chlorophyll will require emendation when a mixture of dimers of various structures are present is a task for the future. Configuration may not significantly affect electronic spectra, but the fact that a mixture of epimers or dimers may exist in solution could affect the interpretation of ir spectra, and could be important in the formation of the ordered aggregates of chlorophyll and water that we have described elsewhere.[14] It would appear that still another variable must be added to the already complicated behavior of chlorophyll in defined solution.

Absolute Configurations of the 10-Epichlorophylls. On the basis of the absolute configurations of chlorophyll a and bacteriochlorophyll established by Fleming[7] and Brockmann,[8] the absolute configuration of 10-epichlorophyll a is 7*S*,8*S*,10*S*,7′*R*,11′*R*, with similar configurations for the other 10-epichlorophylls (see Figure 1).

Experimental Section

The chlorophylls were prepared and purified by standard procedures.[9,15] Pmr spectra were recorded on a Varian HA-100 spectrometer.

For the formation of the epichlorophylls a and b, the parent chlorophyls were dissolved in pyridine and heated to 100° for 20–30 min. Under these conditions, there was no allomerization of the pigments, as sometimes occurs in *n*-propyl alcohol, which was first employed,[2] and there was scarcely any trace of the pyrochlorophylls[6] as evidenced by chromatography.

For preparation of the 10-epichlorophyll a, the mixture formed in the pyridine solution was freed of most of the solvent at reduced pressure. The resultant green residue was dissolved in a little diethyl ether, which was then diluted with petroleum ether. This solution was washed with water and adsorbed in columns of powdered sugar, which were washed with petroleum ether plus 0.25–0.50% *n*-propyl alcohol. To retard the reconversion of the a′ to the a, the columns were cooled with a jacket of ice water. The a′ separated as a green zone below the a. The pyro a formed a very faint zone above the a. The washing was continued until the a′ was carried into the percolate, which was collected in a cooled flask. This percolate was washed quickly with ice water, then evaporated, at reduced pressure, in an ice bath and with a condenser cooled with solid CO_2. The green residue was the 10-epichlorophyll a, which kept unchanged for several days, at least, in an evacuated, refrigerated flask.

Repeated attempts to isolate 10-epichlorophyll a as a definitely oriented or aggregated solid were unsuccessful. On standing in vacuum the substance did not form a brittle mass as is characteristic of chlorophyll a. 10-Epichlorophyll a was very soluble in petroleum ether and did not separate from concentrated solutions. None of the a′ was precipitable from a solution in petroleum ether (bp 30–60°) by shaking with water, whereas the a is completely removed by this procedure.[9] This observation strongly implies that the configuration at C-10 is decisive in the procedure of Jacobs, Vatter, and Holt[16] for the "crystallization" of chlorophyll; unless the C-10 carbomethoxy group and the C-9 ketone oxygen function can both be hydrogen bonded by one water molecule, aggregates of sufficient size cannot be formed by shaking a chlorophyll solution in petroleum ether with water, and this can occur only when the chlorophyll is in the normal, not epi, configuration.[14]

10-Epichlorophyll b was prepared from the b under the same conditions employed for formation of the epichlorophyll a. For the chromatography, the sugar columns were washed with petroleum ether plus 0.5–0.75% *n*-propyl alcohol.

In the course of the preparation of bacteriochlorophyll by chromatography, a small zone of a similar pigment was often observed immediately below the principal chlorophyll. When the colorless cellular contaminants, that tend to sharpen the separations, were removed, as by chromatography and by crystallization of the pigment, the separation of this less-sorbed substance became so indistinct and uncertain that it could not be isolated.

Because of their rapid reconversion into the more stable isomers, the labile epimers could be preserved in solution only at low temperatures. As the rate of this reversion to the equilibrium mixture is time, temperature, and solvent dependent, it was always necessary to determine the composition of the preparations by chromatography.

Acknowledgment. We wish to thank Dr. F. C. Pennington for helpful conversations. We are grateful to Dr. R. C. Dougherty for a careful reading of the manuscript and for guidance in the interpretation of the data.

(14) J. J. Katz, K. Ballschmiter, M. Garcia-Morin, H. H. Strain, and R. A. Uphaus, *Proc. Natl. Acad. Sci. U. S.*, 60, 100 (1968).
(15) H. H. Strain and W. A. Svec, *Advan. Chromatog.*, in press.
(16) E. E. Jacobs, A. E. Vatter, and A. S. Holt, *Arch. Biochem. Biophys.*, 53, 228 (1954).

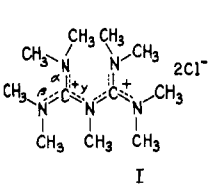
Communications to the Editor

Restricted Rotation in Biguanide Dications

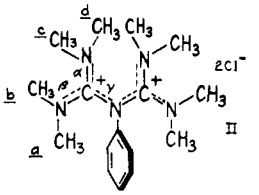
Sir:

The preparation of 1,1,2,2,4,4,5,5-octamethylbiguanide perchlorate,[1] the most highly alkylated biguanide reported to date, initiated our study of polysubstituted biguanides. We now describe the syntheses and properties of several even more highly substituted compounds, the dicationic nonasubstituted biguanides.

Thus, the exothermic reaction of tetramethylchloroformamidine chloride[1,2] and 1,1,2,3,3-pentamethylguanidine[3,4] yielded colorless hygroscopic crystals, mp 244° dec, of 1,1,2,2,3,4,4,5,5-nonamethylbiguanide dichloride (I). A similar reaction with 1,1,3,3-tetramethyl-2-phenylguanidine[5] in boiling acetonitrile gave



I



II

(1) V. J. Bauer and S. R. Safir, *J. Med. Chem.*, 9, 980 (1966).
(2) H. Eilingsfeld, G. Nebauer, M. Seefelder, and H. Weidinger, *Ber.*, 97, 1232 (1964).
(3) H. Lecher and F. Graf, *ibid.*, 56, 1326 (1923).
(4) V. J. Bauer, W. Fulmor, G. O. Morton, and S. R. Safir, *J. Am. Chem. Soc.*, 90, 6846 (1968).
(5) H. Bredereck and K. Bredereck, *Ber.*, 94, 2278 (1961).