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).

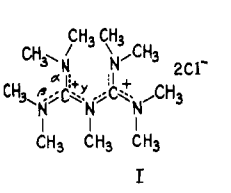
Communications to the Editor

Restricted Rotation in Biguanide Dications

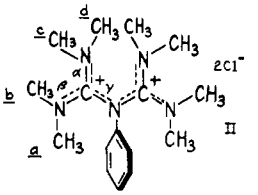
Sir:

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).

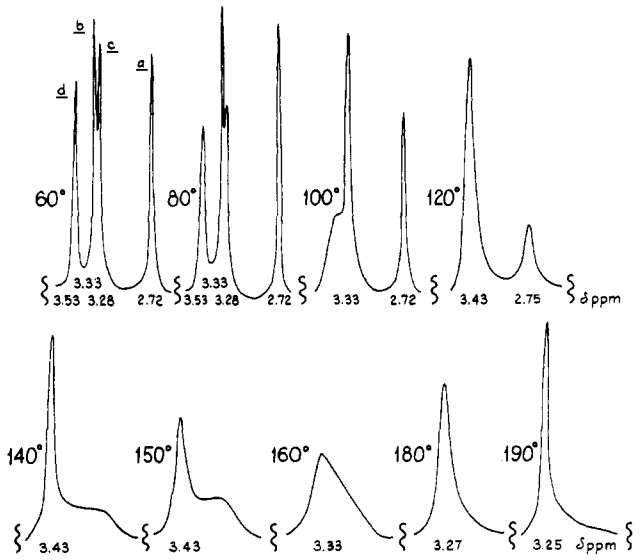


Figure 1. Nmr spectrum of 1,1,2,2,4,4,5,5-octamethyl-3-phenylbiguanide dichloride.

1,1,2,2,4,4,5,5-octamethyl-3-phenylbiguanide dichloride (II), colorless hygroscopic crystals, mp 281° dec.

The nmr spectrum[6] of II ($DOCD_2CD_2OD$), which at 60° exhibits four equally intense methyl singlets at δ 3.53, 3.33, 3.28, and 2.72 (Figure 1), provides considerable insight into the spatial configuration of the molecule. This spectrum is compatible only with a molecular geometry in which the biguanide system is planar, or nearly so,[7] and in which rotation about each skeletal bond (α, β, γ) is slow with respect to the nmr time scale. The highest field peak at δ 2.72 can be assigned to methyl groups a because of their proximity to the shielding π system of the benzene ring.[8] Because of the deshielding effect resulting from van der Waals compression,[9] the peak at δ 3.53 can be attributed to crowded methyl groups d. As the temperature of the nmr solution is raised, the signals at δ 3.53 and 3.28 broaden, then collapse, and by 120° reemerge as a single composite peak at δ 3.43. The coalescence of these peaks requires that they be associated with methyls on the same nitrogen atoms, and, since the δ 3.53 signal corresponds to methyls d, the δ 3.28 peak must be assigned to methyls c. By elimination, the δ 3.33 resonance represents methyls b. The appearance of the δ 3.43 composite peak indicates that the energy barrier to rotation about bond α has been overcome. At 100°, the δ 3.33 and 2.72 signals begin to broaden, and by 150° have virtually flattened; rotation about bond β has become rapid. Finally, as the temperature is raised to 190°, a single peak forms at δ 3.25; rotation about bond γ has also been freed, and all eight methyl groups have become magnetically equivalent.

The nmr spectrum (D_2O, 33°) of I consists of three peaks at δ 3.38 (1 CH_3, sharp), 3.28 (6 CH_3, broad), and 3.02 (2 CH_3, broad). The similarity of this spectrum to the 120° spectrum of II suggests that one bond (α) may be free to rotate, while the restricted rotation about the others (β, γ) is essentially unchanged.

The syntheses of other biguanide dications appropriate to a study of the influence of steric and electronic effects on the rotation process is in progress.

(6) Nmr spectra were determined with a Varian Associates A-60 spectrometer with DSS as an internal standard.

(7) The severe steric interaction of the N-methyl groups d can be partially alleviated by either a modest deviation from planarity or deformation of the biguanide skeleton bond angles.

(8) K. Tori, K. Kitahonoki, H. Tanida, and T. Tsuji, *Tetrahedron Letters*, 559 (1964).

(9) W. Nagata, T. Terasawa, and K. Tori, *J. Am. Chem. Soc.*, **86**, 3764 (1964); R. W. Frank and K. Yanagi, *J. Org. Chem.*, **33**, 811 (1968).

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Restricted Rotation in Guanidines

Sir:

The nmr spectrum of 1,1,2,2,4,4,5,5-octamethylbiguanide perchlorate (**1**) consists of a lone sharp singlet at δ 2.90.[1] This result was unexpected, since the presence of double bonds and severe steric crowding of methyl groups in the molecule should hinder rotation and, therefore, introduce methyl nonequivalence. A ready explanation for this phenomenon, as well as a new aspect of guanidine structure, was revealed by an examination of the spectra of several guanidine bases and cations.

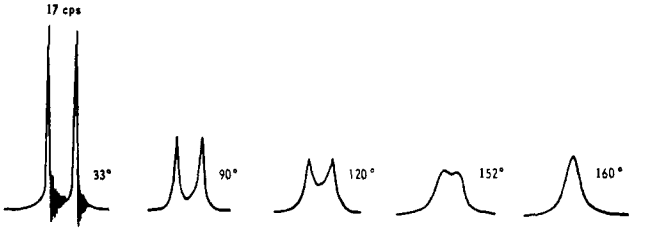

Figure 1. Nmr spectrum of 2-methoxy-1,1,3,3-tetramethylguanidine (**2b**): $N(CH_3)_2$ signals.

The nmr spectrum[2] ($CDCl_3$, 33°) of 1,1,2,3,3-pentamethylguanidine (**2a**)[3] consists of three singlets at δ 2.93, 2.77, and 2.67 of relative intensity 1:2:2. In this compound, rotation about the C=N bond must be restricted, while rotation about each C—N bond is rapid with respect to the nmr time scale.[4] The $N(CH_3)_2$ methyls appear at different chemical shifts because of their "*syn-anti*" relationships to the imine methyl. In contrast, the nmr spectrum (D_2O, 33°) of 1,1,2,2,3,3-hexamethylguanidine perchlorate (**3a**), mp not below 320°, the product of reaction[5] between tetramethylchloroformamidine chloride[1,6] and dimethylamine, is a sharp singlet at δ 2.92. This simplicity is a necessary result of the complete molecular symmetry of **3a**.

(1) V. J. Bauer and S. R. Safir, *J. Med. Chem.*, **9**, 980 (1966).

(2) Nmr spectra at 33° were determined with a Varian Associates A-60 spectrometer with TMS or DSS as an internal standard. High-temperature nmr spectra were determined by Dr. J. E. Lancaster, American Cyanamid Co., Stamford, Conn., with a Varian Associates HA-100 spectrometer.

(3) H. Lecher and F. Graf, *Ber.*, **56**, 1326 (1923).

(4) (a) H. Kessler [*Tetrahedron Lett.*, 2041 (1968)] has described restricted rotation in aryltetramethylguanidines. (b) An alternative explanation, free rotation about the C=N bond and restricted rotation about each C—N bond, would also accommodate the observed spectrum, but is unlikely. This possibility is under investigation.

(5) *Cf.* C. Jutz and E. Müller, *Angew. Chem.*, **5**, 724 (1966).

(6) H. Eilingsfeld, G. Nebauer, M. Seefelder, and H. Weidinger, *Ber.*, **97**, 1232 (1964).