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## RESEARCH ARTICLES

# Conformation of Sodium Cromolyn in Aqueous Solution Using Light Scattering and Magnetic Birefringence

J. V. CHAMPION and G. H. MEETEN<sup>▲</sup>

**Abstract** □ The conformation of the sodium cromolyn (disodium cromoglycate) molecule is possibly relevant to its pharmacological activity in the treatment of bronchial asthma. Two optical techniques, depolarized light scattering and magnetic birefringence, were used to study the molecular conformation in dilute solutions (<1 g./100 ml.) of unassociated molecules. Both techniques confirm that the molecule in dilute solution has a planar conformation as found in the crystal.

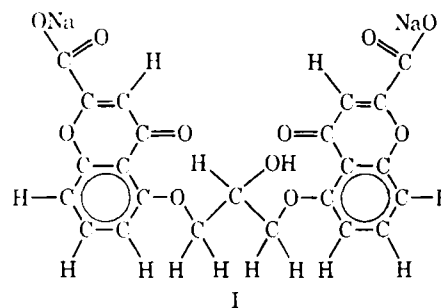
**Keyphrases** □ Sodium cromolyn (disodium cromoglycate)—molecular conformation in aqueous solution, light-scattering and magnetic birefringence techniques □ Molecular conformation, sodium cromolyn—determination in aqueous solution, light-scattering and magnetic birefringence techniques □ Light-scattering techniques—determination of sodium cromolyn conformation in aqueous solution □ Magnetic birefringence techniques—determination of sodium cromolyn conformation in aqueous solution

The conformation of the molecule of sodium cromolyn<sup>1</sup> (disodium cromoglycate) is of particular interest because the compound has been used as a new pharmacological approach to the treatment of bronchial asthma. The solid-state chemistry of sodium cromolyn was recently described (1), and the lattice parameters of the crystalline solid solution at high (90%) relative humidity indicate that the molecule is in a planar con-

formation. In the concentrated solution state, the formation of liquid crystals occurs.

The aim of this study was to determine the conformation of the molecule in aqueous solution. This may be important in assessing the activity and specificity of biological action at a membrane surface.

The chemical structure of sodium cromolyn in the planar conformation is shown here (I), and the molecule may be approximated to a pair of rigid chromones joined by a glycerol bridge [-O-CH<sub>2</sub>-CH(OH)-CH<sub>2</sub>-O-], the hydroxy, carbonyl, and ether groups giving many possibilities of hydrogen bonding, especially in the presence of water molecules. Simple molecular model building shows that numerous different conformations are possible by rotation about the bonds of the glycerol bridge, many of these having a small



<sup>1</sup> Intal, Fisons Ltd., Pharmaceutical Division, Loughborough, England.

energy barrier. Hence, in dilute aqueous solution, the molecule may exist in a form in which the two chromone structures either are randomly oriented with respect to each other or are in a fixed orientation due to intramolecular or intermolecular hydrogen bonding.

Precursory measurements, using viscometric and light-scattering techniques whereby the hydrodynamic volume was determined, showed that aggregation or strong solute-solute interaction occurred at concentrations greater than 0.5 g./100 ml. in aqueous solution. Above 0.8 g./100 ml., the aggregation was time dependent.

The conformation of the molecule can be related to the molecular optical and magnetic anisotropy, which can be measured using depolarized light scattering and magnetic birefringence, respectively. The necessity of using very dilute aqueous (conducting) solutions of relatively low molecular weight material (516) limits the techniques available for observing molecular anisotropy to the two described here.

### OPTICAL AND DIAMAGNETIC ANISOTROPY

It will be shown in the following section that the optical and diamagnetic anisotropies of the sodium cromolyn molecule are sensitive functions of its conformation, hence the use of light-scattering depolarization and magnetic birefringence techniques.

Calculation of the sodium cromolyn molecule optical anisotropy was made using bond polarizability tensor additivity (2). In this calculation, each bond is assigned a polarizability tensor  $\alpha$ . For single bonds,  $\alpha$  is diagonal in coordinates  $x$ ,  $y$ , and  $z$ , where  $x$  is along the bond and  $y$  and  $z$  are perpendicular to it. Thus:

$$\alpha = \begin{pmatrix} \alpha_x & 0 & 0 \\ 0 & \alpha_y & 0 \\ 0 & 0 & \alpha_z \end{pmatrix}$$

For double bonds:

$$\alpha = \begin{pmatrix} \alpha_x & 0 & 0 \\ 0 & \alpha_y & 0 \\ 0 & 0 & \alpha_z \end{pmatrix}$$

where the  $x$  axis lies along the bond and the  $y$  axis is perpendicular to the  $\pi$ -electron plane.

The polarizability of a molecule with many bonds is then calculated by choosing axes 1, 2, and 3 fixed to the molecule and calculating the molecular polarizability tensor:

$$\begin{pmatrix} \alpha_{11} & \alpha_{12} & \alpha_{13} \\ \alpha_{21} & \alpha_{22} & \alpha_{23} \\ \alpha_{31} & \alpha_{32} & \alpha_{33} \end{pmatrix} = \alpha_m$$

The components are:

$$\alpha_{jk} = \alpha_{kj} = \sum_b \sum_u \alpha_u \gamma_{uj} \gamma_{uk} \quad u = x, y, z \quad (\text{Eq. 1})$$

where  $\gamma_{uj}$  is the direction cosine of the angle between the  $u$  ( $u = x, y, \text{ or } z$ ) and the  $j$  axes, and  $j$  and  $k$  take value 1, 2, or 3. The  $\sum_b$  denotes a summation over all bond types in the molecule. In this scheme, benzene rings are considered as bonds having  $x$  axes perpendicular to the ring plane.

Calculation of the molecule diamagnetic susceptibility tensor  $\chi_m$  was made in an exactly similar way as for the optical polarizability tensor, with bond susceptibilities  $\chi_x$ ,  $\chi_y$ , and  $\chi_z$  replacing  $\alpha_x$ ,  $\alpha_y$ , and  $\alpha_z$ .

Two conformations for sodium cromolyn were chosen as representing extreme examples of its conformation: (a) the planar conformation, as suggested by X-ray measurements on the crystal (1); and (b) the perpendicular conformation, obtained when the benzene rings of the molecule lie in perpendicular planes. This perpendicular conformation is possible due to internal rotation around O—C and C—C bonds in the O—C—O bridge connecting the rings. Experiments on a Courtalds space-filling molecular model showed no

Table I—Optical and Diamagnetic Anisotropies

Bond	$\text{\AA}^3$			$10^{-6}$ e.m.u./mole		
	$\alpha_x - \alpha_y$	$\alpha_y - \alpha_z$	$\alpha_z - \alpha_x$	$\chi_x - \chi_y$	$\chi_y - \chi_z$	$\chi_z - \chi_x$
CC	1.43	0	-1.43	1.21	0	-1.21
CH	0.09	0	-0.09	0.9	0	-0.9
CO	1.23	0	-1.23	3.0	0	-3.0
C=O	1.11	0	-1.11	10.3	5.9	-16.2
OH	0	0	0	1.62	0	-1.62
Benzene ring	-5.35	0	-5.35	-58.3	0	58.3
C'H	0.09	0	-0.09	0.49	0	-0.49
C'C	1.43	0	-1.43	2.12	0	-2.12
C'O	1.23	0	-1.23	-0.3	0	0.30

steric hindrance to a perpendicular conformation. Light-scattering and magnetic birefringence theories require principal polarizabilities such that:

$$\alpha_m = \begin{pmatrix} \alpha_1 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & \alpha_3 \end{pmatrix} \quad \text{and} \quad \chi_m = \begin{pmatrix} \chi_1 & 0 & 0 \\ 0 & \chi_2 & 0 \\ 0 & 0 & \chi_3 \end{pmatrix}$$

Since  $\alpha_{jk} = \chi_{jk} = 0$  for molecular axes of symmetry, these are also the principal polarizability or susceptibility axes. For the planar conformation, the 1 axis is coplanar with the benzene rings and parallel to a line joining their centers, the 2 axis is perpendicular to the benzene ring planes, and the 3 axis lies in the plane containing the planes of the benzene rings.

The perpendicular conformation is the result of rotation about the 1 axis of each of the molecular halves such that the 2 axis of one-half of the molecule becomes parallel to the 3 axis of the other half. A large number of —OC<sub>3</sub>O— bridge conformations may attend this rotation, and the average —OC<sub>3</sub>O— bridge conformation is unknown. However, the optical and diamagnetic anisotropies of the —OC<sub>3</sub>O— bridge are small compared with the whole molecule since most anisotropy arises from the ring structures. Thus, to a good approximation:

$$\alpha_2(\text{perpendicular}) = \alpha_1(\text{planar}) \quad (\text{Eq. 2})$$

$$\chi_1(\text{perpendicular}) = \chi_1(\text{planar}) \quad (\text{Eq. 3})$$

$$\alpha_2(\text{perpendicular}) = \alpha_3(\text{perpendicular}) = \frac{1}{2}\alpha_2(\text{planar}) + \frac{1}{2}\alpha_3(\text{planar}) \quad (\text{Eq. 4})$$

$$\chi_2(\text{perpendicular}) = \chi_3(\text{perpendicular}) = \frac{1}{2}\chi_2(\text{planar}) + \frac{1}{2}\chi_3(\text{planar}) \quad (\text{Eq. 5})$$

Vuks' (3) comprehensive and well-tested set of bond polarizabilities was used; relevant values are listed in Table I. The benzene ring value in Table I was derived from light-scattering measurements on gaseous benzene, using Vuks' values for the CH bond polarizabilities. Since further data were lacking, no distinction was made between aliphatic and aromatic carbon atoms, the latter denoted by C'. Thus, C'X and CX are assumed to have the same optical anisotropy.

Bond diamagnetic susceptibility anisotropy data for CC, CH, C'H, and CO in Table I are taken from Bothner-By and Pople (4). The other values in Table I were calculated by the present authors from the measured molecular susceptibility anisotropies of benzene (4), hexamethylbenzene (4), resorcinol (4), and formaldehyde (5).

Models of sodium cromolyn molecules in the planar and perpendicular conformation were constructed using stick models, and the bond angles were measured relative to the symmetry axes. The molecular anisotropies (Table II) were calculated.

Mean values of optical molecular polarizability  $\bar{\alpha}$  were calculated from the sum of mean bond values over the molecule. Tables of bond average polarizabilities were published by several workers for commonly encountered bonds, and there is close agreement on values between tables. No O—Na bond mean polarizability has been reported. Its value was estimated for present purposes by plotting  $\bar{\alpha}^{0.2}$  against the atomic weight of element X, where X was C, H, P, and As as given by LeFevre (6) and Vuks (3), and interpolating for

**Table II—Molecular Anisotropies**

Conformation	$\text{Å}^3$			$10^{-6}$ e.m.u./mole		
	$\alpha_1 - \alpha_2$	$\alpha_2 - \alpha_3$	$\alpha_3 - \alpha_1$	$\chi_1 - \chi_2$	$\chi_2 - \chi_3$	$\chi_3 - \chi_1$
Planar	2.2	21.9	-24.1	5.6	169.6	-175.3
Perpendicular	13.5	0	-13.5	91.7	0	-91.7

X = Na. Table III shows Denbigh's (2) and Vuks' (3) values with  $\alpha^{0-N}$  inserted as estimated previously. The mean molecular polarizabilities according to the table do not differ significantly from 44 Å<sup>3</sup>, and this value was adopted for future calculation. Estimates of mean molecular polarizability may also be made from the crystalline refractive indexes 1.488, 1.66, and 1.705 measured (1) at 40% relative humidity (16.2% water by weight). Assuming additivity of molar refractivities for sodium cromolyn and water in the crystal gives 44.7 Å<sup>3</sup>. The Vuks (7) modification to the molar refractivity scheme gives 44.9 Å<sup>3</sup>. In both cases, a water molecule mean polarizability of 1.59 Å<sup>3</sup> was used (8).

**LIGHT SCATTERING**

The scattering of light by a transparent medium arises from the inhomogeneities of the medium and may be used to characterize them. In a liquid or solution, the scattering is due to the local statistical fluctuations in the polarizability of the medium, arising from density and concentration fluctuations and, for anisotropic molecules, orientational fluctuations. The total scattering power of such a medium is usually expressed in terms of its Rayleigh ratio,  $R(\theta)$ , defined as the intensity of light,  $I_\theta$ , scattered in a direction  $\theta$  with respect to the incident beam, per unit solid angle per unit scattering volume, divided by the incident light intensity,  $I_0$ .

The total Rayleigh ratio can be separated into an isotropic part and an anisotropic part:

$$R = R_{ia} + R_{an} \tag{Eq. 6a}$$

and:

$$R = KI_\theta \tag{Eq. 6b}$$

for a given scattering system with  $I_0$  constant and with the angular dependence omitted if  $I_\theta$  is in a direction perpendicular to  $I_0$ . The scattered light contributing to  $R_{ia}$  is polarized perpendicularly to the plane determined by the direction of  $I_0$  and the direction of observation of  $I_\theta$  and is termed vertically polarized. The anisotropically scattered light has a horizontally ( $H$ ) as well as a vertically ( $V$ ) polarized component. Hence, Eqs. 6a and b become:

$$R = R_{ia,V} + R_{an,V} + R_{an,H} = R_V + R_H \tag{Eq. 7}$$

and the depolarization of the scattered light for unpolarized incident light is given by:

$$\rho_u = R_H/R_V = I_H/I_V \tag{Eq. 8}$$

where  $I_H + I_V = I_\theta$ .

It has been shown at liquid densities that the isotropic scattering per molecule is strongly reduced (to 5-10%) in comparison with its vapor due to pronounced positional correlations and has a much larger influence on the isotropic than on the anisotropic part of the scattering. The latter, due to orientational correlations, is largely independent of density or concentration fluctuations. Therefore, observations of the anisotropic scattering can lead to information regarding the optical anisotropy of the molecules.

**Experimental**—A light-scattering photometer, developed for depolarization measurements, was used, whereby the sensitivity and signal/noise ratio were maximized by using a 5.08-cm. (2-in.) o.d. low noise photomultiplier tube<sup>2</sup>; the output was fed to a low noise, high gain d.c. amplifier. The amplifier output was averaged with a time constant of 10 sec. and then recorded on a chart. The source was a 250-w. compact arc mercury lamp, giving an output brightness of 20,000 stilb. The smallest signal, the scattered in-

**Table III—Mean Bond and Molecular Polarizabilities**

Bond	Number of Bonds per Sodium Cromolyn Molecular	$\alpha$ per Bond/Å <sup>3</sup>	
		Denbigh (2)	Vuks (3)
C—C	10	0.64	0.536
C <sub>ar</sub> —C <sub>ar</sub>	12	1.07	1.07
C—H	17	0.65	0.65
C—O	11	(0.571) <sup>a</sup>	0.571
C=O	4	1.16	1.32
O—H	1	(0.74)	0.74
O—Na	2	1.1	1.1
Average polarizability per molecule		44.3 Å <sup>3</sup>	43.9 Å <sup>3</sup>

<sup>a</sup> Values in parentheses were not given by Denbigh, and Vuks' (3) values were used.

tensity of the horizontally polarized component of pure water ( $I_H^w$ ), had a signal/noise ratio of 8:1, and the presence of dust could be readily detected by an increase in signal/noise ratio and the occurrence of large spurious peaks in the recorder trace. A high quality polarizing prism<sup>3</sup> was mounted in front of the photomultiplier entrance pupil, enabling  $I_V$  and  $I_H$  to be separately determined.

A rectangular Pyrex glass scattering cell was used. The length of the cell was 25 times the length of the observed scattering volume, removing the scattering volume as far as possible from the exit and entrance windows of the incident beam and thus considerably reducing the intensity of the spurious multiple reflections along the cell walls seen by the detector.

The distilled water was prepared by double distillation over potassium permanganate to remove dust and organic matter. The pure water or solution was then centrifuged at 80,000×g for 1 hr. and transferred to the scattering cell *via* a 50-nm. filter<sup>4</sup> under dust-free atmospheric conditions.

All scattered intensities were measured at the mercury green (5461 Å) and mercury blue (4358 Å) wavelengths using narrow band isolating filters.

All concentrations of sodium cromolyn solutions used were corrected for water content of the solid by weighing at known humidity and using the data of Cox *et al.* (1).

**Theory and Results**—It has been shown (9) that for a system of independent scatters in the liquid state, assuming a Lorentz internal field:

$$R_{ia} = \left( \frac{8\pi^4 k T N^2 \beta_T \bar{\alpha}^2}{\lambda^4} \right) \left( \frac{n^2 + 2}{3} \right)^4 \tag{Eq. 9}$$

and:

$$R_{an} = \left( \frac{8\pi^4 N \gamma^2}{\lambda^4} \right) \left( \frac{13}{90} \right) \left( \frac{n^2 + 2}{3} \right)^2 \tag{Eq. 10}$$

where  $k$  is Boltzmann's constant,  $T$  is the absolute temperature,  $N$  is the number of scatterers per milliliter,  $\beta_T$  is the isothermal compressibility,  $\lambda$  is the wavelength *in vacuo*,  $n$  is the refractive index, and  $\bar{\alpha} = (\alpha_1 + \alpha_2 + \alpha_3)/3$  (the mean polarizability of the particle), and  $\gamma^2 = (\alpha_1 - \alpha_2)^2 + (\alpha_2 - \alpha_3)^2 + (\alpha_3 - \alpha_1)^2$  (the optical anisotropy of the particle).

The apparatus is calibrated for the distilled water-sodium cromolyn scattering system from Eq. 7, whereby  $R^w = k$ ;  $I_s = k(I_H^w + I_V^w) = 0.87 \times 10^{-6}$  cm.<sup>-1</sup> at  $\lambda = 5461$  Å and  $R^w = 2.32 \times 10^{-6}$  cm.<sup>-1</sup> at  $\lambda = 4360$  Å, as given by Cohen and Eisenberg (10). Thus,  $k$  can be determined in terms of the instrumental values of ( $I_H^w + I_V^w$ ). The superscripts  $w$ ,  $s$ , and  $d$  represent pure water, solution of sodium cromolyn-water, sodium cromolyn, respectively.

For dilute solutions, the excess horizontally polarized anisotropic scattering of sodium cromolyn molecules is:

$$R_{an,H}^d = k(I_H^s - I_H^w) \tag{Eq. 11}$$

<sup>2</sup> E.M.I. 9502S.

<sup>3</sup> Glan-Thompson.  
<sup>4</sup> Millipore VMWP.

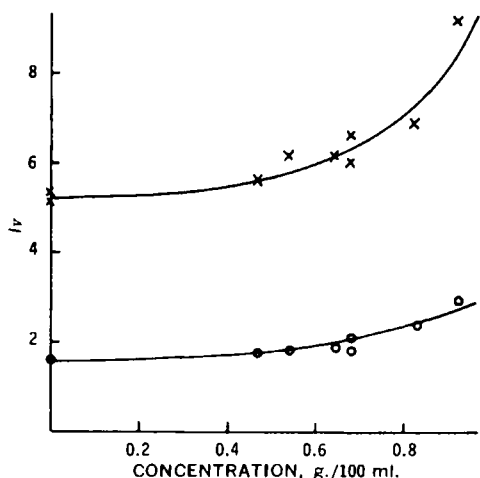


Figure 1—Vertically polarized scattered intensities as a function of sodium cromolyn concentration and wavelength. Key: ○, 5461 Å; and ×, 4358 Å.

giving  $R_{\text{an},V}^d = R_{\text{an},V}^d + R_{\text{an},H}^d = K13/6 (I_H^s - I_H^w)$  making use of the result  $R_{\text{an},V}/R_{\text{an},H} = 7/6$  obtainable from the Cabbannes (11) depolarization factors. By using Eqs. 10 and 11 then:

$$I_H^s - I_H^w = \left( \frac{8\pi^4 \gamma^2 (I_H^w + I_V^w)}{15\lambda^4 R^w} \right) \left( \frac{n^2 + 2}{3} \right)^2 \left( \frac{N_0}{M} \right) c \quad (\text{Eq. 12})$$

where  $N_0$  is Avogadro's number,  $M$  is the molecular weight of sodium cromolyn,  $c$  is the concentration in grams per milliliter, and  $n$  is the refractive index of the medium surrounding the sodium cromolyn molecules (i.e., water).

It was found from the experiment that  $I_H^s$  and  $I_V^s$  were time dependent. For concentrations below 1.0 g./100 ml., this time dependence was relatively slow. No change was observable within the first few hours of making up a solution, while 50% changes were found after several days. Also  $I_H^s$  was markedly less sensitive to time than  $I_V^s$ . Hence, all the observations reported here were made within 3 hr. of making up the solutions, and low (<1 g./100 ml.) concentrations were used.

The scattered intensities  $I_H$  and  $I_V$  and the depolarization ratio  $\rho_u^d$  as a function of concentration are shown in Figs. 1-4. The scattered intensities ( $I$ 's) are in arbitrary instrument units, calibration being obtained from the known scattering power of water (10) as described previously. A graph of  $I_H^s - I_H^w$  as a function of concentration is shown in Fig. 5. From the slopes of these curves and Eq. 12, values of  $\gamma^2$  at the two wavelengths are  $\gamma_{5461}^2 = 1390 \text{ \AA}^6$  and  $\gamma_{4358}^2 = 1430 \text{ \AA}^6$ , using a molecular weight of 516 for sodium cromolyn. The refractive index correction  $[(n^2 + 2)/3]^2$  in Eq. 12 converts the independent particle (gas) value of  $\gamma^2$  to the value appropriate to the molecule being surrounded by water. These values of  $\gamma^2$  are directly comparable to those derived from the polarizability anisotropy tensor additivity scheme.

An alternative approach is to obtain the depolarization ratio

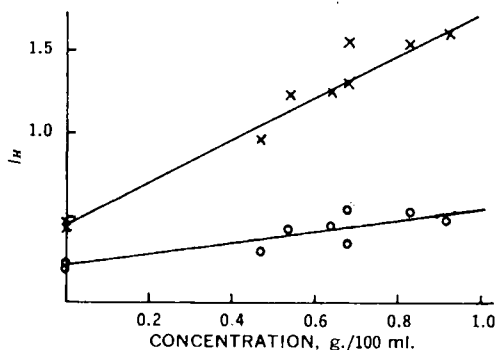


Figure 2—Horizontally polarized scattered intensities as a function of sodium cromolyn concentration and wavelength. Key: ○, 5461 Å; and ×, 4358 Å.

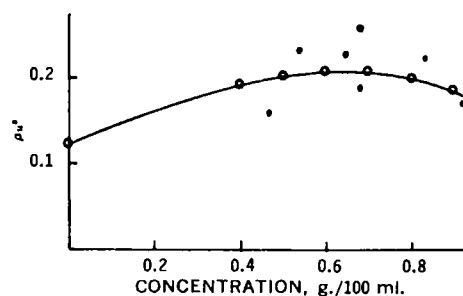


Figure 3—Depolarization ratio as a function of sodium cromolyn concentration at 5461 Å. Key: ○, interpolated points from Figs. 1 and 2.

( $\rho_u^d$ ) of the sodium cromolyn molecule. According to Sicotte and Rinfret (12) and using Eq. 8:

$$\frac{I_s^d \rho_u^d}{I_s^w (1 + \rho_u^d)} = \rho_u^d \left( \frac{I_s^s}{I_s^w (1 + \rho_u^d)} \right) - \left( \frac{\rho_u^d - \rho_u^w}{1 + \rho_u^w} \right) \phi_w \quad (\text{Eq. 13})$$

where  $\phi_w$  is the volume fraction of water in the solution. For dilute solutions in the concentration range  $0 \rightarrow 1.0$  g./100 ml.,  $\phi_w$  varies from 1.00 to 0.99 but may be assumed constant, the variables being  $\rho_u^d$  and  $I_s^s (= I_H^s + I_V^s)$ . Figure 6 shows a plot of  $\{ (I_s^s/I_s^w) [\rho_u^d / (1 + \rho_u^d)] \}$  versus  $[I_s^s/I_s^w (1 + \rho_u^d)]$ . The slope of the linear part of the curve, below concentrations of 0.6 g./100 ml., gives a value of  $\rho_u^d = 0.31$ . It is expected that values for both wavelengths should fall on the same curve due to the negligibly small variation of polarizability with wavelength.

It was shown (10) that the depolarization ratio of a particle in a surrounding medium (sodium cromolyn in water) is given by:

$$\rho_u^d = \frac{6 \left( \frac{n^2 + 2}{3} \right)^2 \gamma^2}{90 N_0 k T \beta_T d \left( \frac{n^2 + 2}{3} \right)^4 \bar{\alpha}^2 + 7 \left( \frac{n^2 + 2}{3} \right)^2 \gamma^2} \quad (\text{Eq. 14})$$

where the density  $d$ ,  $\beta_T$ ,  $M_w$ , and  $n$  relate to the medium surrounding the sodium cromolyn molecule. The terms containing these quantities convert the independent particle value of optical anisotropy  $\gamma^2$  and the average polarizability  $\bar{\alpha}$  to a value appropriate to the molecule in a surrounding medium.

Using the values  $\beta_T = 4.5 \times 10^{-11} \text{ cm.}^2/\text{dyne}$  (10),  $d = 1.0$  g./ml.,  $M_w = 18$ ,  $n = 1.33$ , and  $\bar{\alpha} = 44 \text{ \AA}^3$  with  $\rho_u^d = 0.31$ , one gets  $\gamma^2 = 1100 \text{ \AA}^6$ .

From the bond polarizability data in the preceding section,  $\gamma_{\text{planar}}^2 = 1060/\text{\AA}^6$  and  $\gamma_{\text{perpendicular}}^2 = 365 \text{ \AA}^6$ . The average experimental value is 16% higher than that calculated for the planar conformation and 300% higher than the calculated perpendicular conformation of the sodium cromolyn molecule. With the large (20%) error in individual experimental observations, this must give very strong evidence for the existence of the planar conformation in very dilute solutions.

## MAGNETIC BIREFRINGENCE

The Cotton-Mouton constant  $C$  is defined as  $\Delta n/\lambda B^2$ , where  $\Delta n$  is the birefringence induced in a specimen by a magnetic field  $B$ , measured using light of wavelength  $\lambda$ . The effect is due to the small

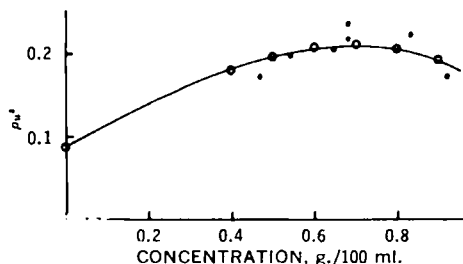


Figure 4—Depolarization ratio as a function of sodium cromolyn concentration at 4358 Å. Key: ○, interpolated points from Figs. 1 and 2.

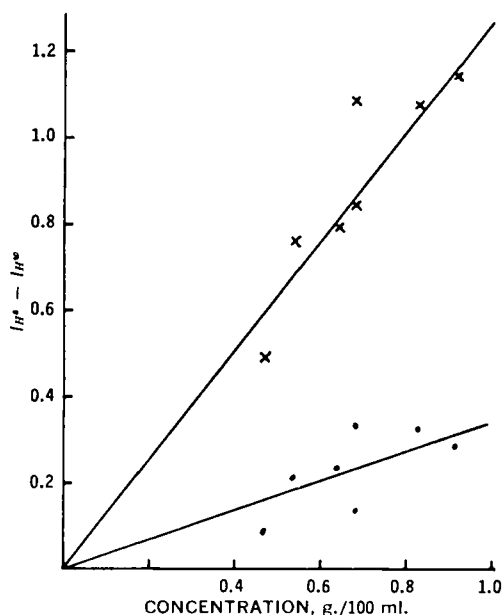


Figure 5—Horizontally polarized excess scattering of sodium cromolyn solution. Key: ×, 4358 Å; and ○, 5461 Å.

degree of nonrandom alignment of molecular optic axes caused by interaction between the magnetic field and the anisotropy of molecular diamagnetism.

In the measurement of  $C$ , it is necessary to pass a beam of monochromatic linearly polarized light through length  $L$  of the specimen and traverse to a known field  $B$ . The birefringence  $\Delta n = C\lambda B^2$  produces ellipticity in the emergent beam. This ellipticity is a maximum when the incident beam polarization azimuth is  $\pm 45^\circ$  to  $B$ , the phase difference  $\delta$  between the principal vibration components being  $(2\pi/\lambda)L\Delta n$ .

**Experimental**—The apparatus was constructed around an electromagnet<sup>5</sup> having a maximum field of about 23 kOe. The solution was contained in standard 20-cm., fused quartz polarimeter tubes with the glass windows replaced by spectroil fused quartz. Practically monochromatic light was produced by a 250-w. mercury arc lamp, and a filter<sup>6</sup> was used to isolate the 5461 Å green line. Light from the lamp, after being collimated by means of a pinhole and two lenses, passed through a polarizer<sup>7</sup>. After traversing the specimen, the emergent beam's ellipticity was analyzed using a quarter-wave plate specially constructed to be free from optical strain and another polarizer<sup>8</sup> as an analyzer. Intensity minima were observed using a telescope focused for infinity on the pinhole and a filter<sup>9</sup> fitted to the eyepiece to remove a small amount of red light not removed by the other filter<sup>6</sup>. The whole apparatus was used in a dark room at ambient temperatures between 21 and 25°.

Since the field was not constant over the optical path length in the solution, the average value of  $B^2$  (denoted by  $\bar{B}^2$ ) is necessary to calculate  $C$ . This was measured for various magnet currents using nitrobenzene, which has  $C = 262 \times 10^{-13} \text{ Oe.}^{-2} \text{ cm.}^{-1}$  (13) at 5461 Å and 23°. Point-to-point measurement of  $B$  along the optical path using a gaussmeter and calculation of:

$$\bar{B}^2 = \frac{1}{L} \int_0^L \beta^2(dL) \quad (\text{Eq. 15})$$

agreed to within  $\pm 1\%$  with the calibration using nitrobenzene.

The experimental procedure was thus to rotate the quarter-wave plate and analyzer for an intensity minimum with the specimen between the magnet pole-pieces, the polarizer azimuth having been previously set at  $\pm 45^\circ$  to the direction of  $B$ . Energizing the magnet then produced a weak illumination in the field of view. The inten-

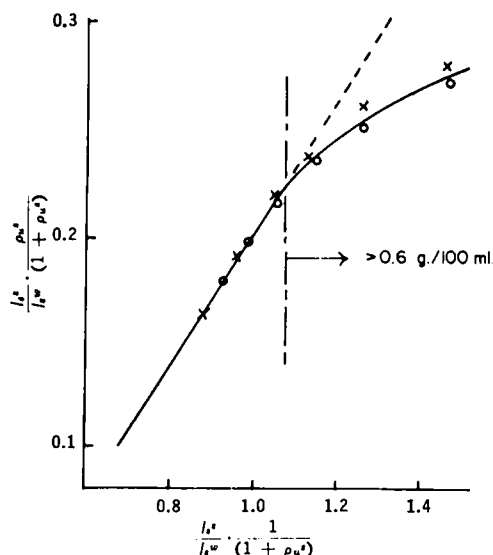


Figure 6—Interpolated data from Figs. 1–4. Key: ○, 5461 Å; and ×, 4358 Å.

sity minimum could be restored by adjusting the analyzer azimuth by a rotation  $\Delta\theta$ , whereupon  $\delta = \pm 2(\Delta\theta)$ .

Sources of error were Faraday rotation in the specimen and residual birefringence in the polarimeter tube windows. These were reduced to negligible proportions by accurate alignment and selection of low strain windows, respectively.

The solutions were prepared by shaking the sodium cromolyn powder with distilled water and using 50- or 100-nm. filters<sup>10</sup> before making the measurements. Weighing tests had shown that these filters did not remove any of the dissolved sodium cromolyn. No glass was allowed to contact the solutions at any stage. Aged or unfiltered solutions often showed a nonlinearity of  $\Delta n$  with  $\bar{B}^2$ . This dependence of  $C$  upon  $B$  is typical of solutions of very large particles or liquid crystals. Solutions exceeding about 2% concentration invariably showed this behavior, which varied with time in a nonreproducible manner. Accordingly, all measurements were made immediately after filtering at less than 1.5% concentration. Any result showing a field dependence of  $C$  was rejected. Figure 7 shows a typical graph of  $\Delta n$  versus  $\bar{B}^2$ . Each point is the average of between 3 and 10 analyzer readings for each value of  $\bar{B}^2$ . The concentration dependence of  $C$  is shown in Fig. 8, where the intercept on the  $C$  axis (pure water) was taken from the Landolt-Bornstein

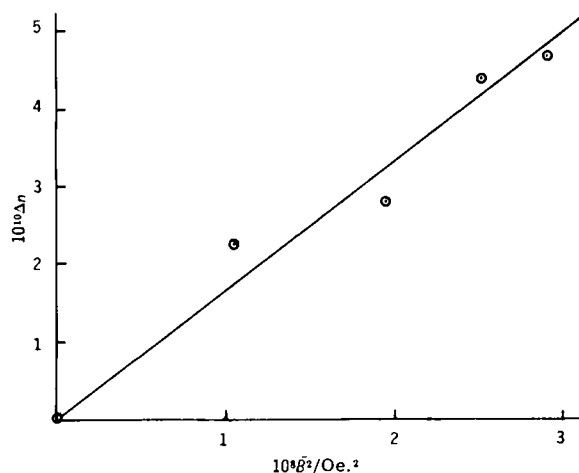


Figure 7—Birefringence  $\Delta n$  as a function of mean square field  $\bar{B}^2$  for 0.82 g./100 ml. sodium cromolyn solution. Filter pore size was 100 nm.; immediate use after filtration.

<sup>5</sup> Newport type E.

<sup>6</sup> Wratten 77A.

<sup>7</sup> Polarex, type Ko-MIK, Kasemann Ltd.

<sup>8</sup> Polarex.

<sup>9</sup> Ilford Tricolour, type 404.

<sup>10</sup> Millipore, type VMWP and VCWP.

**Table IV—Summary of Experimental Results**

Technique	Parameter	Value of Parameter		
		Experiment	Planar (Calculated)	Perpendicular (Calculated)
Depolarized light scattering	$\gamma^2/\text{Å}^6$	1100	1060	365
Magnetic birefringence	$10^{12}(dC/dc)/\text{Oe.}^{-2}\text{cm.}^{-1}\text{g.}^{-1}\text{ml.}$	3.6	{ 2.26 4.2 (relative method)	{ 0.71 1.3 (relative method)

tables. Errors in  $C$ , judged from the scatter of experimental  $\Delta n$  values, are shown as vertical bars in Fig. 8. The error in concentration  $C$  is negligible. The line through the points in Fig. 8 is a line of best fit, assuming the value of  $C$  for water to have zero error. This was done since the value of  $C$  for water is of higher precision than the values of  $C$  for the measured solutions.

Values of incremental refractive index  $dn/dc$  and density  $d\rho/dc$  were measured using a high precision Abbé refractometer and weighing bottle, respectively. The values at 23°, 5461 Å, were found to be  $dn/dc = 0.233 \pm 0.005 \text{ ml. g.}^{-1}$  and  $d\rho/dc = 0.49 \pm 0.02$ .

A relative method, not requiring absolute values of  $\alpha$  and  $\chi$ , is to measure the Cotton-Mouton constant for a compound similar to sodium cromolyn but having a known conformation. Such a compound is 9C in which the  $-\text{O}-\text{C}_3-\text{O}-$  bridge connecting the benzene rings in sodium cromolyn is replaced by a  $-\text{O}-\text{C}_9-\text{O}-$  bridge. Due to the increased bridge length (therefore more possibilities for internal rotation about C-C bonds), the bridge is thought to be flexible. Additional evidence for flexibility comes from Raman spectra of linear carbon chains in  $n$ -alkanes. The spectra show (16) that there are no rigid all-*trans*-isomers present when the chain exceeds nine carbon atoms in length. Thus, the orientation of one end of the 9C molecule by the applied magnetic field may take place without affecting the other end. When neglecting the flexible bridge, which has a low optical and diamagnetic anisotropy, this lack of correlation between the molecule ends means that each 9C molecule contributes to the Cotton-Mouton constant as though it were two free halves of a sodium cromolyn molecule.

**Theory and Results**—Bottcher (14) gave an expression for the refractive index of a mixture in terms of the number densities  $N$  and effective polarizabilities  $\alpha^*$  of each component:

$$\frac{(n^2 - 1)(2n^2 + 1)}{12\pi n^2} = N_1\alpha_1^* + N_2\alpha_2^* \quad (\text{Eq. 16})$$

where  $\alpha_i^* = \bar{\alpha}_i/(1 - f_i\bar{\alpha}_i)$ , and  $f_i = (1/a_i^3)[(2n^2 - 2)/(2n^2 + 1)]$ ;  $a_i$  is the effective radius of the particles of the  $i$ th sort and  $\bar{\alpha}_i$  is their mean polarizability. According to the Langevin theory (15) of the Cotton-Mouton effect of diamagnetic molecules, an applied field  $B$  in the  $x$  direction produces an average polarizability difference:

$$\bar{\alpha}_x - \bar{\alpha}_y = \frac{3}{2} \theta_1 B^2 \quad (\text{Eq. 17})$$

where  $\theta_1 = (1/45kT)[(\alpha_1 - \alpha_2)/(\chi_1 - \chi_2) + (\alpha_2 - \alpha_3)(\chi_2 - \chi_3) + (\alpha_3 - \alpha_1)(\chi_3 - \chi_1)]$ . Differentiating Bottcher's equation for small changes in  $\alpha^*$  of both components and in the limit of low concentrations of Component 1 gives:

$$\Delta n = \frac{(18\pi^2 N_1)(\Delta\bar{\alpha}_1)}{(2n^2 + 1)^2(1 - f_1\bar{\alpha}_1)^2} + \Delta n_2 \quad (\text{Eq. 18})$$

where  $\Delta\alpha_1 = 3/2\theta_1 B^2$  using Langevin's theory. Bottcher's equation may also be used to calculate  $dn/dc$ , if low concentrations are again assumed:

$$\frac{dn}{dc} = \frac{18\pi^2 N_0}{(2n^2 + 1)^2} \left[ \frac{\alpha_1^*}{M_1} - \frac{\alpha_2^*}{M_2} \left( 1 - \frac{d\rho}{dc} \right) \right] \quad (\text{Eq. 19})$$

where  $N_0$  is Avogadro's number. By assuming, as  $c \rightarrow 0$ , that  $\alpha_2^* = \alpha_{\text{water}}^*$ , then:

$$\alpha_1^* = \frac{M_1(2n^2 + 1)}{36\pi^2 N_0} \left[ 2(2n^2 + 1) \frac{dn}{dc} + 3n(2n^2 - 1) \left( 1 - \frac{d\rho}{dc} \right) \right] \quad (\text{Eq. 20})$$

Thus  $(1 - f_1\bar{\alpha}_1) = \bar{\alpha}_1/\alpha_1^* = 0.815$ , using the bond polarizability value of 44 Å<sup>3</sup> for  $\bar{\alpha}_1$ , and measured values of  $dn/dc$  and  $d\rho/dc$ .

Putting  $M_1 = 516$  for sodium cromolyn and  $n = 1.335$  gives absolute  $dC/dc$  values as follows:

$$\left( \frac{dC}{dc} \right)_{\text{abs}} = 2.26 \times 10^{-12} \text{ Oe.}^{-2} \text{ cm.}^{-1} \text{ g.}^{-1} \text{ ml.} \quad (\text{planar conformation}) \quad (\text{Eq. 21})$$

$$\left( \frac{dC}{dc} \right)_{\text{abs}} = 0.71 \times 10^{-12} \text{ Oe.}^{-2} \text{ cm.}^{-1} \text{ g.}^{-1} \text{ ml.} \quad (\text{perpendicular conformation}) \quad (\text{Eq. 22})$$

Neglecting the optical and diamagnetic anisotropy of the 9C flexible bridge and assuming complete flexibility give  $\theta_1$  (planar sodium cromolyn) =  $2\theta_1(9C)$ . Bond polarizability calculations predict a ratio of 3.3 between the values of  $\theta_1$  sodium cromolyn in the planar and perpendicular conformation; thus, it may be assumed that:

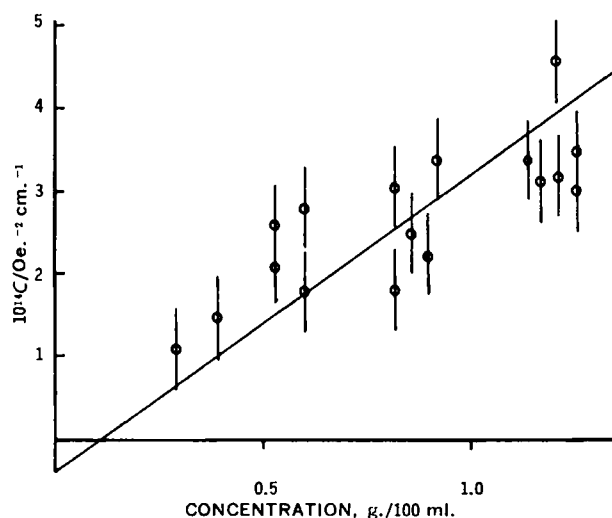
$$\theta_1(\text{perpendicular sodium cromolyn}) = \frac{2}{3.3} \theta_1(9C) = 0.61\theta_1(9C) \quad (\text{Eq. 23})$$

Only a small quantity of 9C was available, and two experiments were made on one solution of concentration 0.625%. These gave  $0.66 \times 10^{-14}$  and  $0.85 \times 10^{-14} \text{ Oe.}^{-2} \text{ cm.}^{-1}$  for the Cotton-Mouton constant (Fig. 9). Adopting  $(0.75 \pm 0.1) \times 10^{-14} \text{ Oe.}^{-2} \text{ cm.}^{-1}$  as a mean value and using the Landolt-Bornstein value of  $C$  for water allow an estimate of  $dC/dc$  for the two sodium cromolyn conformations after allowing for the molecular weight difference between sodium cromolyn (516) and 9C (600). This gives, for sodium cromolyn:

$$\left( \frac{dC}{dc} \right)_{\text{rel}} = 4.2 \times 10^{-12} \text{ Oe.}^{-2} \text{ cm.}^{-1} \text{ g.}^{-1} \text{ ml.} \quad (\text{planar}) \quad (\text{Eq. 24})$$

$$\left( \frac{dC}{dc} \right)_{\text{rel}} = 1.3 \times 10^{-12} \text{ Oe.}^{-2} \text{ cm.}^{-1} \text{ g.}^{-1} \text{ ml.} \quad (\text{perpendicular}) \quad (\text{Eq. 25})$$

Comparing the  $dC/dc$  values, both absolute and relative, with the



**Figure 8**—Cotton-Mouton constant  $C$  as function of sodium cromolyn concentration. Vertical lines through points represent domain of measurement error.

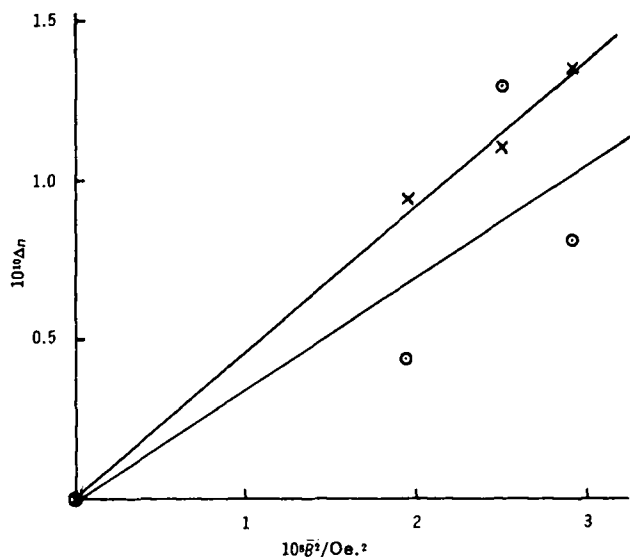


Figure 9—Birefringence  $\Delta n$  of 0.625 g./100 ml. 9C solution. Key: O, lower line, first experiment; and X, upper line, second experiment. Both lines are least-square fits to the experimental points.

experimental value of  $3.6 \times 10^{-12} \text{ Oe.}^{-2} \text{ cm.}^{-1} \text{ g.}^{-1} \text{ ml.}$  from Fig. 8 shows that the planar conformation is favored.

#### CONCLUSION

The summary of experimental and calculated data relating to the conformation is shown in Table IV. Both techniques show conclusively that the planar rather than the perpendicular or random conformation exists in very dilute solution within the estimates of error discussed in the text. The larger errors apparent in the magnetic birefringence comparisons are thought to arise from uncertainties in some of the bond magnetic anisotropy values.

Due to flexibility in the  $-\text{O}-\text{C}_3-\text{O}-$  bridge between the two identical halves of the sodium cromolyn molecule, a planar conformation of the isolated molecule is unexpected. The molecule's affinity for water (1) provides an explanation in terms of water molecules bridging the chromone groups by hydrogen bonding and thus forming a rigid structure. Experiments using scale space-filling

molecular models show that several water molecules can be accommodated to form this bridge. Due to their low optical and diamagnetic anisotropy, the water molecules would not greatly affect the values of  $\gamma^2$  and  $dC/dc$  and thus not show up in the experimental measurements.

Both techniques are sensitive indicators of molecular association or liquid crystal formation, and the time-dependent and nonlinear behavior found at about  $> 1 \text{ g./100 ml.}$  shows that these phenomena are occurring at much lower concentrations than previously thought (1).

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