Table I. Results of Incorporation of Nine-Membered Ring Intermediates into *V. rosea* L. and *V. minor* L. by Various Techniques

Expt	Compd[a] fed	Plant	Feeding method	Alkaloid isolated	—% incorporation— Plant	Blank[b]
1	[*ar*-^{3}H]Carbomethoxycleavamine (IV, HCl salt)	*V. rosea* L.	Cotton wick into stems, 8 days	Catharanthine (VIII)	<0.015	0.04
2	[*ar*-^{3}H]Carbomethoxycleavamine (IV, HCl salt)	*V. rosea* L.	Vacuum infiltration of leaf discs, 42 hr	Catharanthine	<0.008	
3	[*ar*-^{3}H]Carbomethoxycleavamine (IV, acetate salt)	*V. rosea* L.	Leaf vein injection,[c] 6 days	Catharanthine	<0.05	
4	[*ar*-^{3}H]Carbomethoxycleavamine (IV, HCl salt)	*V. rosea* L.	Hydroponic, cut stems, 46 hr	Catharanthine	<0.011	
5	[*ar*-^{3}H]Quebrachamine (I)	*V. minor* L.	Hydroponic, cut stems, Tween 20 emulsion, 4 days	Aspidospermidine (V)	0.48	3.4
6	[*ar*-^{3}H]Vincaminoreine (II, acetate salt)	*V. minor* L.	Absorption through leaf sections, 4 days[d]	Vincamine (IX) Aspidospermidine Minovine (VI)	<0.001 <0.008 0.7	 0.3

[a] The preparation of the various precursors employed in these studies will be discussed in our detailed paper. [b] In all instances, blank experiments were run under similar conditions to those involved in the plant feedings. [c] In these experiments, incorporation of precursors was performed by capillary injection into leaves of growing plants. [d] In these experiments, fine incisions were made on the leaves of the growing plant and the precursor was absorbed from plastic bags carefully fitted over these sections.

Table II. Results of Incorporation of Various Intermediates into *V. rosea* L. under Identical Conditions

Expt	Compd[a,b] fed	—% incorporation— Catharanthine (VIII)	Vindoline (X)	Ajmalicine (XI)
7	[3-^{14}C]DL-Tryptophan	0.05	0.15	0.8
8	[*ar*-^{3}H]Tryptamine	0.01	0.003	0.4
9	[*ar*-^{3}H]III	<0.001	<0.001	<0.001
10	[*ar*-^{3}H]Tabersonine (VII)	0.05	0.03	<0.001
11	[*ar*-^{3}H]Carbomethoxycleavamine (IV)	0.03	<0.001	Inactive
12	[*ar*-^{3}H]Carbomethoxycleavamine (IV) blank experiment	0.04	Inactive	Inactive
13	[*ar*-^{3}H]-XII	<0.001	<0.001	0.004
14	[*ar*-^{3}H]-XIII	<0.001	<0.001	<0.001

[a] The preparation of the various precursors employed in these studies will be discussed in our detailed paper. [b] In all instances, the acetate salts were utilized.

salts confirmed the level of radioactivity. By this sequence even very low levels of incorporation could be easily detected. The pertinent results for catharanthine (VIII), vindoline (X), and ajmalicine (XI) are summarized in Table II, and a brief analysis of these is appropriate.

Experiments 7 and 8 illustrate that (a) the age of the plants selected for this study was suitable for biosynthesis and (b) the experimental method chosen at least provides positive incorporation of established precursors. Experiments 9 and 10 provide an important comparison between two closely related compounds in their role as potential precursors in the biosynthetic pathway. While the alkaloid tabersonine (VII) is converted into catharanthine and vindoline, the 6,7-dehydrovincadine derivative (III) is not incorporated. The latter compound is the immediate precursor of this alkaloid (VII) in the laboratory conversion which utilizes the transannular cyclization reaction.[12] The incorporation of tabersonine into these alkaloids furthermore establishes that the experimental method employed allows the incorporation of higher molecular weight "precursors" into the plant system. The level of incorporation of the cleavamine derivative IV into the Iboga system is also negligible, as shown in expt 11 and 12. All of these experiments strongly suggest that the transannular cyclization reaction is probably not significant in either Aspidosperma or Iboga biosynthesis, although it is clear that negative results must be interpreted with caution.

One of the most interesting results obtained in these investigations concerns the conversion of tabersonine to catharanthine in the plant.[13] In order to accommodate this transformation, it is necessary to rearrange the carbon skeleton of the tabersonine molecule. The manner in which this latter process occurs is of distinct relevance, and experiments in this direction are now in progress.

Finally, expt 13 and 14 reveal briefly the results of some of our initial investigations which utilize totally synthetic substances as potential precursors. It is noted that the cyanoacetic ester analog XII is incorporated at a low level into ajmalicine, while the malonic ester intermediate XIII is not utilized. Clearly the former substance cannot be seriously considered as a precursor, although the nitrile function is probably converted into an aldehyde or similar grouping in the living plant. Further experiments in this area are now being conducted.

Acknowledgment. Financial aid from the National Cancer Institute of Canada, National Research Council of Canada, and Medical Research Council of Canada is gratefully acknowledged.

(12) The facile cyclization of vincadine and its derivatives to the pentacyclic vincadifformine family is now well documented in our laboratory.

(13) At a recent Natural Products Symposium at the University of West Indies, Professor A. I. Scott, Sussex University, reported the conversion of tabersonine to vindoline and catharanthine in germinated seeds of *V. rosea* L.

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On the Structure of the Liquid Crystalline State of Cholesterol Derivatives

Sir:

It is well established that liquid crystals in the cholesteric phase have a helical structure.[1] The long axes

(1) G. W. Gray, "Molecular Structure and The Properties of Liquid Crystals," Academic Press Inc., New York, N. Y., 1962.

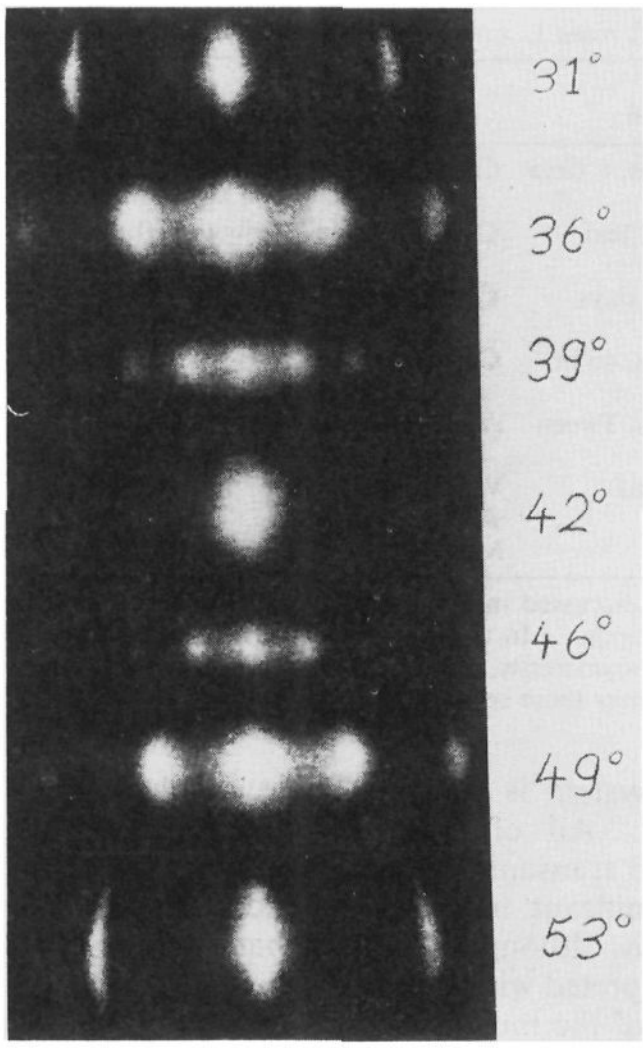

Figure 1. Diffraction pattern of a 1.75:1 by weight mixture of cholesteryl chloride and cholesteryl myristate. The mixture is nematic at about 43°. The pitch calculated from eq 1 is, from top to bottom, 4.0, 6.6, 11.0, ∞, 11.4, 6.5, 3.8 μ. Polarizers oriented to transmit light with the electric vector perpendicular to the magnetic field were placed on both sides of the sample.

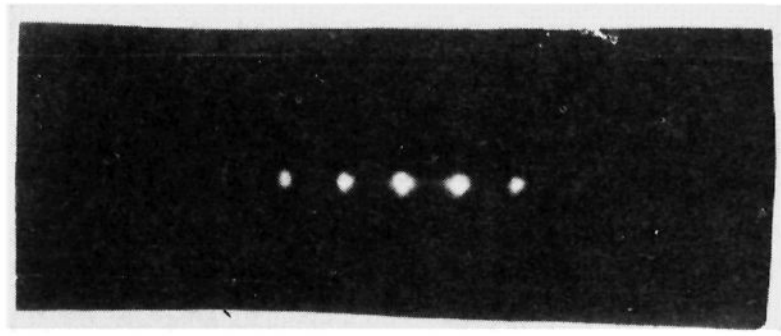

Figure 2. Diffraction pattern of an ordered glass at −50°. The sample was held for 5 hr at +40° in a magnetic field of 25 kOe and then cooled to −50° and removed from the magnetic field.

of the molecules are on the average aligned parallel to each other within planes; while the direction of this alignment rotates smoothly on moving in a path perpendicular to these planes. In an earlier note[2] we reported nmr observations on benzene dissolved in mixtures of cholesteryl chloride and cholesteryl myristate, which are known to have helical structures of opposite screw sense. It was concluded that the pitch of the helical structure can be adjusted by varying the composition of the mixture, and that in fact a composition exists at which complete compensation occurs; that is, the liquid crystal is nematic. The nmr results also indicated that these nearly compensated liquid crystals align macroscopically in a magnetic field, and that the dissolved benzene preferentially orients with its plane perpendicular to the applied magnetic field. This suggests that the cholesteryl molecules also align perpendicular to the magnetic field, and thus the screw axis of the cholesterol helix would align parallel to the field. If this is true, then a magnetically aligned cholesteric liquid crystal would exhibit a periodically varying refractive index, and thus act as an optical grating. The present note reports observations confirming this conclusion.

The following experiment was done to test our picture of these ordered states. A flat glass cell 1 cm × 3 cm and 0.025 cm thick was filled with a 1.75:1 by weight mixture of cholesteryl chloride and cholesteryl myristate and placed in a magnetic field of 20 kOe with the plane of the cell parallel to the field. The sample was located in a simple glass dewar with flat windows and heated with an N_2 stream of controlled temperature. The sample was allowed to align in the magnetic field for 4–5 hr at a temperature at which the sample is cholesteric. Then the beam of a He–Ne laser (λ 6328 Å) was directed on the sample so that the laser beam was perpendicular to the field. On a screen behind the sample the typical diffraction pattern of an optical grating was observed as shown in Figure 1. For the initial alignment, several hours were necessary to produce the diffraction pattern. Once the alignment was reached at one temperature, a change in temperature resulted in change of the distances of the diffraction spots without loss of the initial alignment. At 42° the mixture is nematic and only one diffuse central spot is visible.

This experiment proves that the ordered cholesteric liquid crystal phase has a periodic refractive index in the direction of the magnetic field. With varying temperature the pitch of the helical stacking and thus the "grating constant" changes. Only light polarized in a direction perpendicular to the magnetic field sees a periodic refractive index. It is indeed found that the diffraction spots are mainly polarized perpendicular to the magnetic field, whereas a sharp central spot is mainly polarized parallel to it. Perfect polarization is not observed because the light is depolarized to some degree by scattering in the sample.

The theory used by Debye and Sears[3] for their explanation of light scattering by ultrasonic waves in liquids applies here. Therefore, as in ordinary gratings, the angles, ϑ_n, of the different orders n with respect to the central image are given by

$$\sin \vartheta_n = \frac{n\lambda}{{}^1/_2 P} \qquad (1)$$

where P is the pitch of the helix. Our experiment provides us with a direct measure of the pitch. For the diffraction patterns reproduced in Figure 1, the calculated pitch is given in the figure caption. We find that the pitch is inversely proportional to the temperature departure from the nematic temperature.

We have found that it is possible to freeze in the ordered state of the liquid crystal by rapid cooling to about −50°. A clear oriented glass results, which shows a diffraction pattern (Figure 2) similar to that observed before freezing. The glass does not lose its alignment when removed from the magnetic field. This colorless glass can be used as an anisotropic matrix for studies of the polarization of optical transitions in ori-

(2) E. Sackmann, S. Meiboom, and L. C. Snyder, *J. Am. Chem. Soc.*, **89**, 5981 (1967).

(3) P. Debye and F. W. Sears, *Proc. Natl. Acad. Sci. U. S.*, **18**, 409 (1932).

ented solute molecules, as will be shown in a subsequent paper.

One final remark should be made about the conclusions reported in ref 2. It is now clear that, in discussing the behavior of cholesteric liquid crystals in a magnetic field, two cases must be differentiated. In the first, exemplified by the optically active amyloxyazoxybenzene, the long axis of the molecules tends to align parallel to the magnetic field. In this case no macroscopic alignment takes place unless the magnetic field is strong enough to unwind the helical structure. Only then will a high-resolution nmr spectrum of solute molecules be observed. In the second case, exemplified by the cholesterol derivatives, the long axis of the molecules tends to align perpendicular to the magnetic field, the axis of the helical structure aligns parallel to the magnetic field, and no unwinding takes place. In this case a high-resolution nmr spectrum of solute molecules should be observable in the cholesteric structure. It has indeed been found that in these compounds an accurate compensation to obtain a nematic phase is not required in order to obtain high-resolution nmr spectra.

(4) On leave of absence from the Max-Planck Institut für Spektroskopie, Göttingen, Germany.

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On the Polarization of Optical Transitions of Dye Molecules Oriented in an Ordered Glass Matrix

Sir:

In a previous[1] paper we reported the preparation of highly oriented organic glasses, obtained by aligning a mixture of liquid crystalline cholesterol derivatives in a magnetic field, followed by rapid cooling. The glasses are transparent and colorless, and they will align solute molecules to a very appreciable extent too. Thus these glasses provide a suitable solvent for polarization studies of the absorption spectrum of dissolved dye molecules. Such measurements give direct information on the direction of the transition moment characterizing the absorption. In this note we report preliminary observations on a number of dyes.

The solvent used consisted of a mixture of cholesteryl chloride and cholesteryl myristate. The composition was adjusted so that the mixture was "compensated," *i.e.*, nematic, at some convenient temperature T_{nem}, and cholesteric above and below this temperature. Slight changes in composition will vary T_{nem}. The compositions actually used are given in the figure captions.

Solutions of dyes in the liquid crystal solvent were placed in flat cells with dimensions 1 cm × 3 cm and 0.01–0.025 cm thick. The cells were put in a magnetic field of 20 kOe with the flat cell surface parallel to the field. After alignment for several hours, the cells were rapidly cooled to −50° and a clear glass resulted. The glass keeps its alignment after removal from the magnetic field. The absorption spectra were taken

(1) E. Sackmann, S. Meiboom, L. C. Snyder A. E. Meixner, and R. E. Dietz, **90**, 3567 (1968).

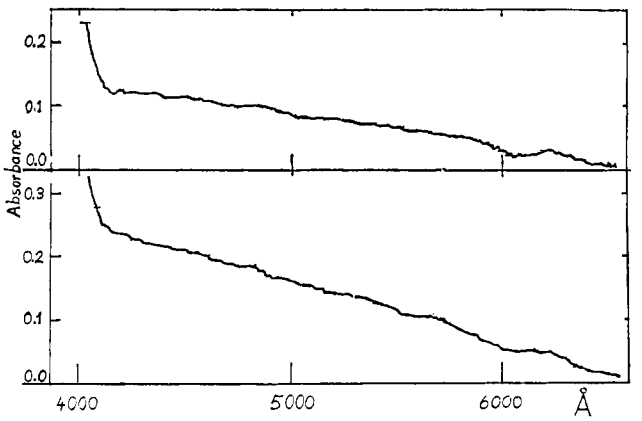

Figure 1. Polarized absorption spectra of the ordered glass at −50° of a 1.75:1 by weight mixture of cholesteryl chloride and cholesteryl myristate (T_{nem} = 54°). The sample was aligned for 12 hr in a 20-kOe magnetic field at 54°. In the upper spectrum the electric vector of the light is perpendicular, and in the lower spectrum parallel, to the magnetic field.

with a Cary 15 double-beam spectrometer with the light beam directed perpendicular to the magnetic field and traversing the cell along its thin dimension of 0.01–0.025 cm. Polarizing films, with their direction of polarization parallel, were placed on each side of the cell. These films absorb below about 4000 Å and cause the short-wavelength cutoff in the spectra shown in the figures. There is appreciable light scattering and depolarization by the solvent; the use of two polarizers minimizes this effect.

For reference purposes, Figure 1 gives the observed spectrum of the solvent without dye. The apparent absorbance is mainly due to light scattering. It will be noted that the variation of the absorbance with frequency is approximately linear. This observation has been used in correcting for solvent absorption in the dye spectra.

In Figure 2 the spectra of $Me_2NC_6H_4N{=}NC_6H_4NO_2$ are given. In interpreting the spectra, a correction was applied for the absorption and scattering of the solvent in accordance with Figure 1. It is found that the absorption coefficient for light polarized parallel to the magnetic field is smaller by a factor of 2.2 than that for perpendicularly polarized light. We make the reasonable assumption that the rather long dye molecules are aligned with their long axes parallel to those of the solvent molecules, *i.e.*, perpendicular to the magnetic field. We also assume that the only order in the cholesterol solvent is the helical arrangement of the long molecular axes. Specifically, this means that all orientations of a solvent molecule about its long axis are equally probable. If this is the case, then the dissolved dye molecules must also be randomly oriented about their long axes. On the basis of these assumptions we conclude that the long-wavelength absorption of the dye is polarized along the long molecular axis. This is in accordance with other experiments.[2]

In Figure 3 the polarized spectra of β-carotene are given. The absorption coefficient is smaller by a factor of 2.5 when the light is polarized parallel to the magnetic field. The carotene molecule is again aligned with its long molecular axis parallel to the solvent molecules.

(2) M. B. Robin and W. T. Simpson, *J. Chem. Phys.*, **36**, 580 (1962).