CIRCULARLY POLARISED LIGHT AS AN ASYMMETRIC PHOTOCHEMICAL AGENT

with additional papers on ASYMMETRIC ENZYME ACTION

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By

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1. FUNDAMENTAL CONCEPTIONS

Plane, Circular, and Elliptical Polarisation

HUYGENS[1] postulated that light is propagated by spherical waves in the ether, and thus accounted in a satisfactory way for reflection and refraction. To extend the theory to double refraction he made a careful study of the optical properties of Iceland spar. In the course of his investigations he found that the ordinary and extraordinary rays travel with the same speed along the axis of the crystal but that at right angles to this direction there is a maximum difference in speed. An experiment with two rhombs of Iceland spar led him to conclude that "the waves of light after having passed through the first crystal acquire a certain form or disposition in virtue of which when meeting the texture of the second crystal, in certain positions, they can move the two different kinds of matter which serve for the two species of refraction and when meeting the second crystal in another position are able to move only one of these kinds of matter."

NEWTON[2] regarded this property of light as an insuperable objection to the Wave Theory. "For pressions or motions propagated from a shining body through an uniform medium, must be on all sides alike; whereas by those experiments it appears that the rays of light have different properties in their different sides." In consequence, Newton devoted his attention to the Emission Theory. He explained reflection by supposing the light particles to be repelled by the reflecting surface and refraction by imagining that they experience a force of attraction when passing into a denser medium. Further[3], he says, "The unusual refraction of Iceland spar looks very much as if it were performed by some kind of Attractive virtue, lodged in certain sides both of the rays, and of the particles of the crystal." This leads to mutual interaction "as the poles of two magnets answer to one another."

The next advance was made by MALUS[4]. One evening he happened to look through a piece of Iceland spar at the image of the setting sun then being reflected from the high windows of the Luxemburg Palace opposite his house in Paris. On rotating the crystal, he noticed that the intensity of the two images varied. He then experimented with a candle and a sheet of glass and found that when the light of the candle was reflected at a particular angle, one of the images disappeared entirely for certain positions of the Iceland spar. Interpreting this result in terms of the Emission Theory, he said that the reflected light was "polarised" in the plane of incidence. The angle which gave the maximum effect was called the "polarising angle". In addition, he found that the ordinary ray from Iceland spar was polarised in the principal section of the crystal, and the extraordinary ray in a plane at right angles.

A further discovery was made in 1811 by ARAGO[5] who polarised light by reflection, passed it through a rhomb of Iceland spar oriented to transmit only one ray and then placed a plate of quartz cut perpendicular to the axis between the reflector and the rhomb. Two coloured images were produced and on rotating the rhomb a series of colours was obtained, the colour of one image being always complementary to that of the other. Arago explained this phenomenon by supposing that the plane of polarisation of the light is rotated on passing through the quartz and that the amount of rotation is different for different colours. He also examined the colours produced with thin pieces of crystalline substances (gypsum, quartz, etc.) cut parallel to the axis.

FRESNEL[6] studied the effect of reflection on polarised light and was led to consider the case of total reflection. Light polarised at 45° to the plane of incidence was used. After total reflection at a glass-air surface it was partly depolarised, and after two total reflections at an angle of 54·5° it was completely depolarised, i.e. it gave two equally bright images with Iceland spar. It was not the same as ordinary light, however, for the following reasons.

(1) When a thin piece of quartz cut parallel to the axis was placed in front of the Iceland spar colours were produced which were intermediate between those obtained from the original polarised light. If the plate was fixed to the rhomb with its axis inclined at 45° to the principal section

of the latter and both were rotated together there was no variation in colour.

(2) When the completely depolarised light was submitted to a further total reflection it was partially depolarised. A fourth total reflection produced complete polarisation in a plane perpendicular to that of the original beam.

In order to elucidate the nature of this completely depolarised light, Fresnel developed his conception of transverse waves. He postulated that ordinary light is propagated by vibrations of the ether at right angles to the path of the light but inclined at all sorts of angles to each other. If the light is polarised, the vibrations are confined to one plane which is perpendicular to the plane of polarisation. When light polarised at 45° to the plane of incidence undergoes total reflection he supposed that it is split up into two components of equal intensity, one polarised in, and the other perpendicular to, the plane of reflection.* He deduced that the former component is retarded relative to the latter, the effect being doubled by a second total reflection. The properties of the light resulting from two total reflections at an angle of 54·5°, he then considered compatible with the idea that the one

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*This may have been suggested by the fact that when polarised light passes from air on to a glass surface at the polarising angle, the reflected and refracted rays are polarised in perpendicular planes. Both rays would be reflected along the same path if the conditions were those required for total reflection.

component had fallen a quarter of a wave length behind the other. The resultant system of waves obtained is one in which the ether particles, instead of vibrating, describe circles round their equilibrium positions with uniform speed. Because of the progressive nature of the waves, the particles are situated at any instant on a helix the pitch of which is equal to the wave length of the light.

Fresnel gave the name CIRCULAR POLARISATION to this new modification of light. He distinguished between right-handed and left-handed circular polarisation according to the direction of rotation of the ether particles. The term ELLIPTICAL POLARISATION was applied to partially depolarised light since, in this case, the ether particles trace out ellipses. Finally, the simplest type of polarisation produced by reflection or double refraction was called PLANE POLARISATION.

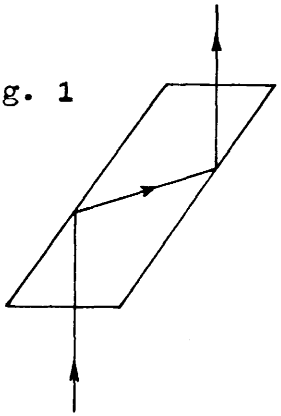
Fresnel pointed out that circular polarisation may also be produced by means of a thin crystalline plate cut parallel to its optic axis and placed normally to a beam of light plane polarised at 45° to its principal section. On entering the plate the light is split up into an ordinary and an extraordinary ray. These have equal intensities but travel with different speeds. When the plate is of such a thickness and the light is of such a wave length that the path difference is equal to an odd number of quarter wave lengths, the emergent light will be circularly polarised.

If the path difference is an even number of quarter wave lengths, the light will remain plane polarised, and for intermediate differences it will be elliptically polarised. When monochromatic circularly polarised light is required, a thin sheet of mica giving a path difference of one quarter wave length is frequently employed and is known as a $\lambda/4$ plate.

Rotation of the Plane of Polarisation

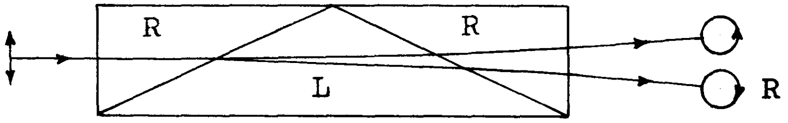
The total reflection method of producing circularly polarised light gives a retardation of one quarter wave length fairly accurately for the whole visible spectrum and can, therefore, be used with white light. Fresnel made glass rhombs for this purpose (Fig. 1). When one of these is placed with its plane of symmetry (i.e. plane of two total reflections) vertical, and the incident light is polarised in a plane turned clockwise through 45° from the vertical, the direction of revolution of the ether particles in the emergent light will be clockwise, or the circular polarisation will be right-handed. If either the plane of polarisation of the incident light or the rhomb is rotated through 90° from this position, left-handed light will be obtained.

Fig. 1



Fresnel carried out a number of experiments with the rhombs and finally tried the following arrangement. He passed plane polarised light through two crossed rhombs and between them placed a thin piece of quartz cut parallel to the axis and with the axis turned at 45° to the planes of double reflection. When he analysed the emergent light with a crystal of Iceland spar, he obtained colour effects very similar to those Arago had observed with quartz plates cut perpendicular to the axis. This suggested that the rotation of the plane of polarisation by quartz might be due to the two kinds of circularly polarised light travelling with different speeds along the axis. By means of the tri-prism of right and left-handed quartz shown in section in Fig. 2, FRESNEL[7] demonstrated that a beam of plane polarised light* can be separated into two circularly polarised beams. When examined through a glass rhomb (Fig. 1) each gave plane polarised light and the polarisation was in perpendicular planes. Fresnel's contention was, therefore, justified.

Fig. 2



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*It was shown subsequently that ordinary light behaves in the same way.

The rotation of the plane of polarisation of light by quartz was further investigated by BIOT[8] who found that "the rotation of the different simple rays is reciprocal to the square of their wave lengths".

Later, Biot discovered that several organic substances, both in the liquid state and in solution, possessed optical rotatory power, and that most of them obeyed the law of inverse squares at least approximately. Solutions of tartaric acid, however, behaved quite differently[9]. The rotations increased with increasing wave length in some parts of the spectrum and under certain conditions the rotation dispersion curves showed maxima.

Biot's apparatus[10] was similar to that employed by Arago. A glass mirror served as polariser, but in place of a simple Iceland spar rhomb he used a double image prism as analyser.

Detection of Elliptical Vibrations

Further study of polarised light was facilitated by the invention by NICOL[11] of the prism which now bears his name, and by the development of the Spectroscope by FRAUNHOFER[12] and SWAN[13].

When a thin piece of quartz cut parallel to the axis is placed between crossed Nicols so that the principal section of the plate is at 45° to the principal section of the polarising Nicol and a beam of parallel white light is passed through the apparatus and then allowed to fall on the slit of a spectroscope, FIZEAU and FOUCAULT[14] found that the resulting spectrum is crossed by one or more dark bands depending on the thickness of the plate. The dark bands occur at wave lengths where the state of polarisation of the light has not been altered in passing through the plate. Fig. 3 shows the kinds of polarisation produced between and in the neighbourhood of the bands.

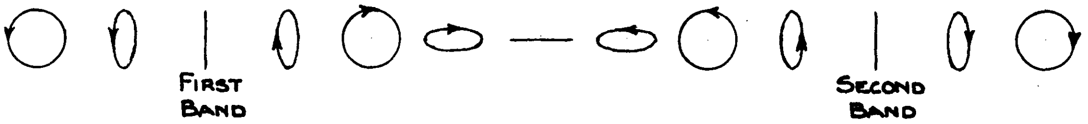


Fig. 3

Dark bands are also obtained with parallel Nicols. In this case the light extinguished by the second Nicol has had its plane of polarisation turned through 90° on traversing the plate. The wave length λ of the centre of a band can be calculated fron the formula.

$$e(n' - n) = p.\lambda/2$$

where n = ordinary index of refraction for wave length λ

n'= extra " " " " " " " "

e = thickness of quartz plate

and p = a number known as the "order" of the band.

When p is even the band is produced with crossed Nicols, when odd with parallel Nicols.

If now, any substance which converts plane polarised light into elliptically polarised light is placed between the Nicols the dark bands are displaced. To render this displacement easily visible it is usual to cut the plate along a direction at 45° to the axis and then put the two pieces together again after turning one over. The two halves of the plate have then their axes at right angles. When placed between the crossed Nicols with the separating line focussed on the spectroscope slit, two spectra are produced, one above the other. Each of these has a black band in the same position. If a substance which converts plane polarised light into elliptically polarised light is now placed between the Nicols the band divides in two, the upper half moving, for example, towards the right and the lower towards the left.

The Cotton Effect

COTTON[15] examined a layer of Fehling's solution in an apparatus of this kind and obtained a displacement of the bands at the red end of the spectrum. He reasoned that the elliptical polarisation resulted from unequal absorption of the two circularly polarised beams which Fresnel had shown to be produced when plane polarised light passes through an optically active medium.

The effect was obviously due to the alkaline copper tartrate in the Fehling's solution, so in his further experiments Cotton used a solution containing simply copper tartrate and potassium hydroxide. He investigated for different wave lengths (1) the shape of the ellipse and (2) the rotation of the plane of polarisation. The ratio of the axes of an ellipse may be expressed as the tangent of an angle which Cotton called the "ellipticity" and which he used as a measure of (1).

Only slight modification of the apparatus with which the effect had been discovered was necessary. Fig. 4 shows the arrangement.

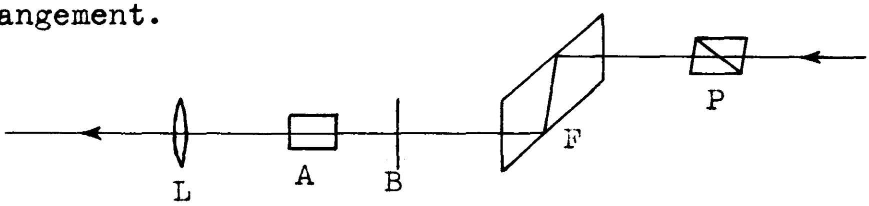


Fig. 4

The polarising Nicol P was supplied with a graduated scale and a Fresnel rhomb F was placed between it and the double quartz plate B. Following B was an analysing Nicol A and then a lens which focussed the dividing line of B on the slit of a spectroscope. The quartz plate used for most of the measurements was $254\mu\mu$ thick. It gave bands with parallel Nicols at $0{\cdot}6574\mu$, $0{\cdot}5222\mu$, $0{\cdot}4371\mu$ and with crossed Nicols at $0{\cdot}5815\mu$ and $0{\cdot}4754\mu$. Some readings were also taken with a thinner plate giving bands at $0{\cdot}5617\mu$ and $0{\cdot}4595\mu$.

To measure the ellipticity for one of these bands, the two halves were first made collinear. A 2·5 cm. cell containing the alkaline copper tartrate solution (1 g. copper tartrate and 1 g. potassium hydroxide per 100 cc.) was then placed between F and B and the band was seen to divide. By turning the polariser an equal elliptical vibration but of opposite sense to that given by the solution could be produced. For this position of P the two halves of the band became collinear again. The angle through which P had been rotated gave the ellipticity and the direction of rotation gave its sign. The same procedure was repeated for the other bands.

To measure the rotations the cell with the solution was placed between P and F and the angle through which P had to be rotated was again found for each of the bands.

Table 1 gives the results obtained. They are closely connected with the absorption band which has its head about 657$\mu\mu$.

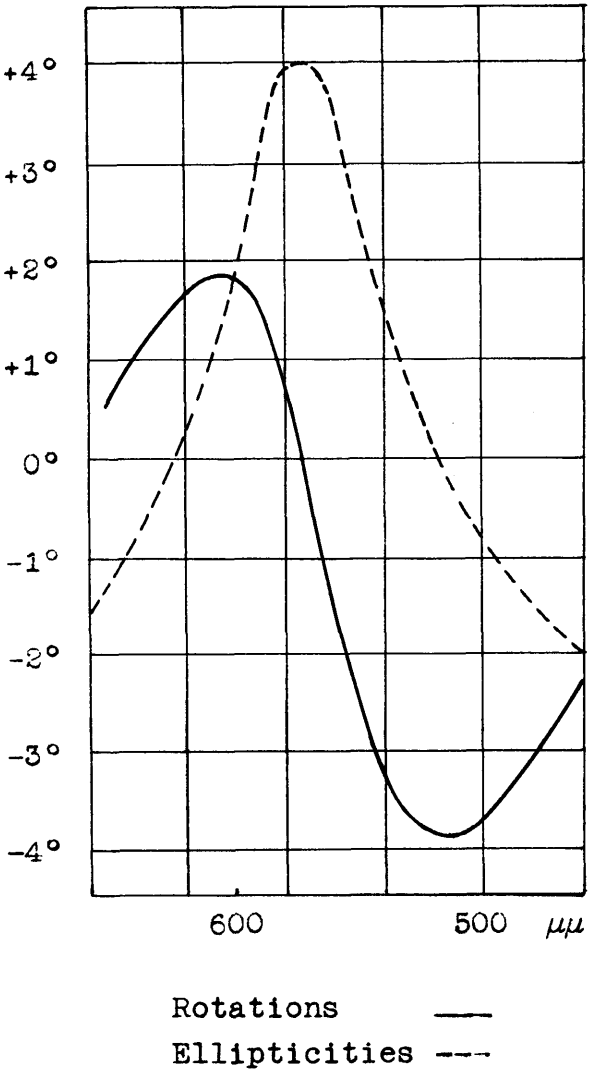
Table 1

Wave length	657	589	581·5	562	475	437
Rotation	0	+2°	+2°16'	+2°29'	+1°56'	+2°
Ellipticity	-2°12'		-1°26'	-51'	0	0

The ellipticity reaches a minimum in this region and the rotation is zero. The rotation shows a maximum some distance from the head of the band.

A further set of results obtained with a 1 cm. layer of a solution containing 10% of the double tartrate of chromium and potassium is shown in Fig. 5. The head of the absorption band in this case is at about 570$\mu\mu$. A maximum ellipticity and zero rotation are found in this region. The rotation

Fig. 5



reaches a maximum value on one side of the band and a minimum on the other. This variation of rotation in the neighbourhood of an absorption band has been called the COTTON EFFECT.

Circular Dichroism

Some direct experiments were also carried out with circularly polarised sodium light. An apparatus was required with two luminous fields, identical and contiguous, one giving right-handed and the other left-handed circularly polarised light. The following arrangement was found to be convenient. Two mica λ/4 plates were placed side by side in a plane polarised beam so that their principal sections were at right angles to each other and at 45° to the plane of polarisation of the incident light. Using a solution containing 2% of copper tartrate and 2% of potassium hydroxide in a 2 cm. cell, Cotton found that the right-handed rays were more strongly absorbed than the left-handed rays. A similar solution prepared from laevo tartaric acid gave the opposite effect. Difference in absorption was also distinctly shown by a 1 cm. layer of the solution containing 10% of the double tartrate of chromium and potassium.

When the sodium light was replaced by white light, and a greater thickness of layer of the chromium solution was employed, the two halves of the field were differently coloured. The part illuminated by left-handed light was rose coloured, whilst the other part, through which right-handed rays passed, was a whitish-green.

This last experiment recalled the phenomenon of Dichroism* which is shown by certain coloured crystals. In these the ordinary and extraordinary rays, which travel at different speeds, are differently absorbed. In Cotton's solutions right and left-handed circularly polarised rays travel at different speeds and are differently absorbed, so he proposed the term CIRCULAR DICHROISM.

Cotton's work on this subject has been extended to other metallic complexes of tartaric acid and to similar compounds of malic and lactic acids by McDOWELL[18], OLMSTEAD[19], and by BRUHAT[20]. The last author has also studied the phenomenon

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*Dichroism was first observed in tourmaline by BIOT[16]. Further examples were discovered by HAIDINGER[17] who compared the colours resulting from the unequal absorption of two rays of white light which had originally the same intensity and were polarised in perpendicular planes.

with the diphenyldithiourethanes of d and l-borneol, and with potassium irido-oxalate[21].

Both ellipticities and rotations were examined in the above cases. A number of examples of the Cotton Effect are also recorded by TSCHUGAEV[22], LIFZSCHITZ[23], and JAEGER[24] where measurements of rotations only have been made.

2. THE ROTATION DISPERSION AND CIRCULAR DICHROISM OF CARYOPHYLLENE NITROSITE

DEUSSEN[25] found that caryophyllene nitrosite in benzene had $[\alpha]_D$ +1626 and later[26] he showed that this remarkably high value varies considerably with concentration and is always greater for sodium light than for mercury green. The latter observation and the blue colour of the substance led TSCHUGAEV[27] to suggest that it might show the Cotton Effect. To settle this point MITCHELL[28] examined the rotation dispersion of a solution of caryophyllene nitrosite in alcohol. The results obtained are shown in Fig. 6. The rotation reaches a maximum positive value about 6250 A.U., becomes zero at 6800 A.U. and is thereafter negative. Subsequent examination of the absorption spectra has revealed a narrow band with its head about 6800 A.U., so that Tschugaev's contention is confirmed.

Preparation of Caryophyllene Nitrosite

The caryophyllene was purchased from Messrs. The British Drug Houses, Ltd., who obtained it by fractional distillation of oil of cloves. The nitrosite was prepared essentially as described by SCHREINER and KREMERS[29], but the following modified procedure was found to give better results.

Caryophyllene	45 cc.
Petroleum ether (b.p. 40-60°)	110 cc.
Sodium nitrite	30 g.
Glacial acetic acid	21 cc.

The entire preparation was carried out in a dark room, only a feeble blue light being employed. The caryophyllene and petroleum ether were put in a 500 cc. bottle, a saturated solution of the nitrite in water was added and the mixture was well cooled in a freezing bath of ice and salt. The acetic acid was next slowly added, 1 cc. every five minutes, the bottle being thoroughly shaken at each addition. After all the acid had been run in, the mixture was kept in the freezing bath for an hour with occasional shaking. The blue crystals of the nitrosite were then filtered off and washed, first with water and finally with a small quantity of alcohol. The product was purified by one crystallisation from hot alcohol when it melted at 113°. The yield was about 4 g. A further crystallisation from cold aqueous acetone raised the melting point to 115°.

Rotation Dispersion

The alcoholic solution for rotation measurements contained 0·3113 g. of caryophyllene nitrosite per 100 g. of solution and was contained in a 6 cm. tube. The readings were taken at 20°, and the density of the solution at this temperature was 0·7932. Eight colours of light were used. A mercury vapour lamp supplied the yellow, green, and violet ones direct, and narrow strips of the spectrum from a Nernst lamp, corresponding to four other fainter mercury lines and to the red hydrogen line, were utilised in the manner described by PATTERSON[30]. Readings were not continued beyond 6908 A.U.; indeed, some difficulty was experienced in taking this last one. The values obtained are shown in Table 2.

Table 2

λ	4358	4916	5461	5790	6324	6563	6717	6908
α	0·78°	1·07°	1·71°	2·31°	3·27°	2·06°	0·66°	-0·97°
$[\alpha]$	526°	722°	1154°	1559°	2207°	1390°	459°	-655°

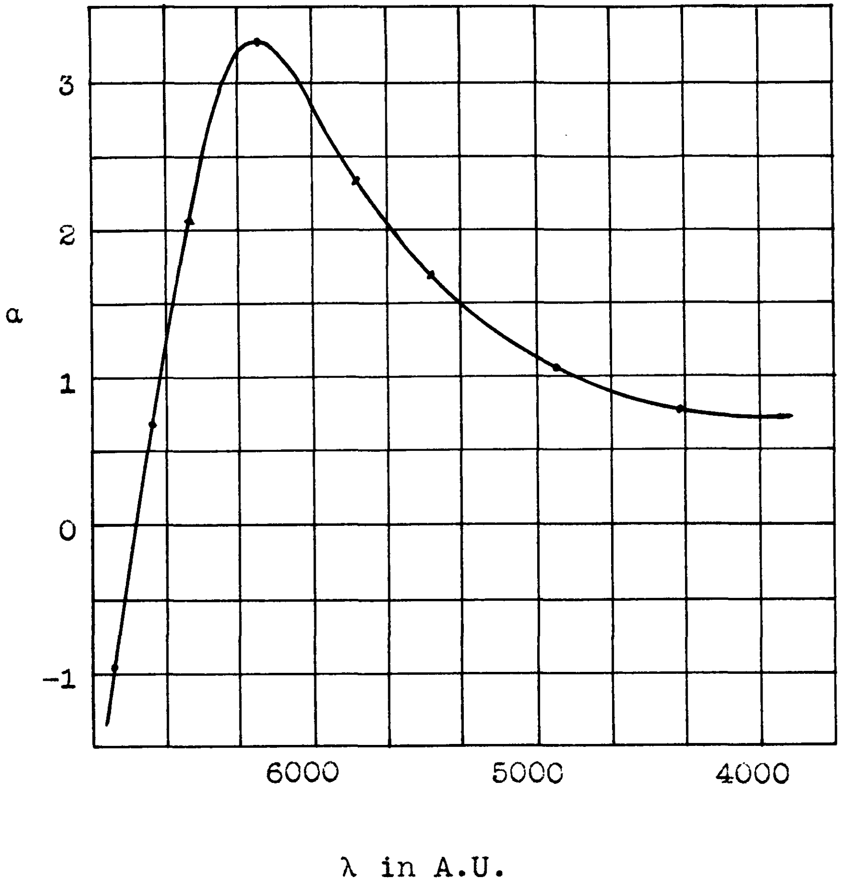


Fig. 6

Circular Dichroism

The rotation dispersion curve for caryophyllene nitrosite is not unlike that obtained by Cotton for alkaline copper tartrate. It is therefore of interest to find if the nitrosite also exhibits circular dichroism.

The double quartz plate method was first used. By employing a plate which would give circularly polarised light in the region of selective absorption of caryophyllene nitrosite it was hoped to obtain direct evidence of circular dichroism in addition to a displacement of the Fizeau-Foucault bands. Calculation from the formula on page 10 in conjunction with the refractive index data given by MACE de LEPINAY[31] showed that a plate about 0·132 mm. thick would be suitable. Messrs. R. & J. Beck, Ltd. were able to supply a plate 0·134 mm. thick which has answered the purpose admirably. This plate was mounted between crossed Nicols and with a 500 c.p. Pointolite lamp as light source the dividing line was focussed on the slit of a Hilger quartz spectrograph.

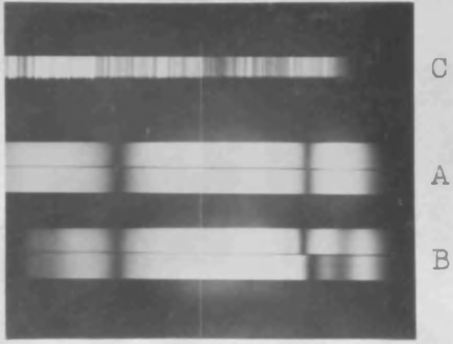


Fig. 7

The resulting spectra were photographed on a panchromatic plate and gave two Fizeau-Foucault bands with their centres at 6120 A.U. and 4520 A.U. respectively (Fig. 7A). A cell 7·5 cms. long containing an N/50 solution of caryophyllene nitrosite in alcohol was then inserted after the polarising Nicol and a second photograph B was taken. It will be seen that the band at 6120 A.U. has divided. The top part is now some 70 A.U. to the left and the bottom part the same amount to the right of the original position. Farther to the right is the head of the caryophyllene nitrosite absorption band. It will be noted that the absorption is less pronounced in the upper half than in the lower one. C is the spectrum of a cobalt arc used for calibration.

The circularly polarised region in the above experiment is very localised and it was thought that a record of the change in absorption with thickness of solution for the two kinds of circularly polarised light over a larger region of the spectrum was desirable. To obtain this an apparatus was fitted up employing a Fresnel quartz tri-prism (described on page 8) to produce accurately both right and left-handed

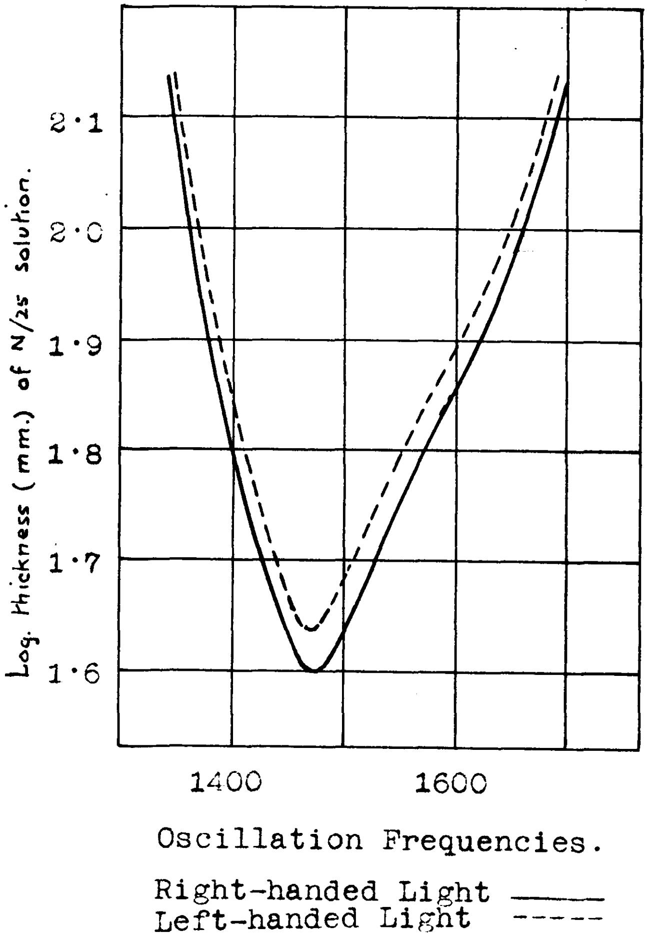


Fig. 8

circularly polarised light of equal intensity and contiguous to each other. The centre prism of left-handed quartz had a refracting angle of 150° and the base was four inches long. The tri-prism was inserted between the collimator and the prism of a Hilger constant-deviation spectroscope, the collimator being displaced the necessary distance from its usual position. A 500 c.p. Pointolite lamp was employed as source of light. The height of the slit was adjusted with a V-shaped slide until the two spectra, produced one above the other by the tri-prism, just ceased to overlap. A Baly tube containing an N/25 solution of the caryophyllene nitrosite in alcohol was placed between the light source and the spectroscope slit. The eye-piece of the telescope was then removed and replaced by a single lens and camera, and a series of photographs was taken on a panchromatic plate for different thicknesses of solution. For calibration purposes the solution was removed and a cobalt arc was photographed at the top and at the bottom of the plate. The results are represented graphically in Fig. 8. A distinct difference between the absorption spectra for right and for left-handed circularly polarised light is apparent. Right-handed circularly polarised light is more absorbed than left-handed.

Modification of Apparatus for Use in the Ultra Violet

In order to use the Fresnel quartz tri-prism for the production of ultra-violet circularly polarised light, it was necessary to replace the Canada balsam employed for joining the prisms by some other liquid which would be more transparent to the ultra-violet rays. Several organic liquids were tried, but none was absolutely satisfactory so Messrs. Adam Hilger, Ltd. were approached regarding the possibility of putting the faces of the prism into optical contact. This consists of making the surfaces so flat and clean that they adhere on being pressed together. The work was undertaken and proved to be highly satisfactory. The tri-prism was then used in conjunction with a Hilger quartz spectrograph. Since it was not convenient to place the tri-prism between the collimator and prism of the spectrograph (as had been done with the constant deviation instrument, page 22), the arrangement shown in elevation in Fig. 9. was used. The various parts were mounted on an optical bench in front of the slit of the quartz spectrograph.

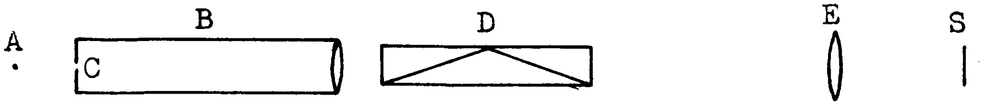


Fig. 9

A Light source (nickel spark).
B Collimator with slit C horizontal and quartz lens.
D Fresnel quartz tri-prism.
E Quartz lens for focussing C on S.
S Vertical slit of quartz spectrograph.

A Baly tube containing the solution to be examined was placed between D and E.

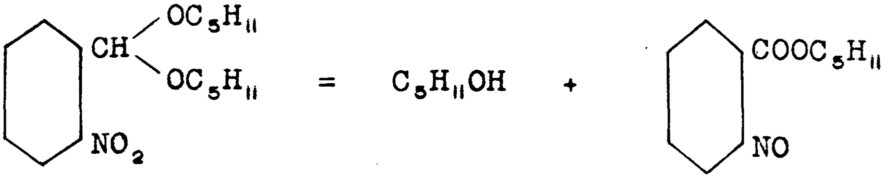
The first substance tried was camphor. In alcoholic solution it shows a band of selective absorption with its head about 2860 A.U.[32] The apparatus was adjusted so that the spectrum was sharply focussed in this region. Layers of different thickness of a N/10 solution were employed, but in no case could any difference be detected between the absorption of right and left-handed circularly polarised light.

A similar experiment was carried out with an N/500 solution of nicotine in alcohol. PURVIS[33] has shown that such a solution gives an absorption band with its head about 2620 A.U. LOWRY and LLOYD[34] examined the rotation dispersion of nicotine and found that a 6 dm. column of the substance gave a rotation of 757° at 6708 A.U. and of 2266° at 4358 A.U. In view of these high values it was thought that near the ultra-violet absorption band it might be possible to detect circular dichroism, even with the dilute solutions which it was necessary to employ. These hopes were not realised, however, and no difference was apparent in the absorption spectra taken with the two kinds of circularly polarised light.

3. SOME UNSUCCESSFUL PHOTOCHEMICAL EXPERIMENTS WITH CIRCULARLY POLARISED LIGHT

VAN'T HOFF[35] appears to have been the first to suggest that circularly polarised light might be employed as an asymmetric photochemical agent. After referring to the production of active substances in Nature with "apparatus consisting essentially of active materials", he says. "It is exceedingly probable that in other asymmetric conditions of experiment the same direct formation of active bodies will result, i.e. in transformations taking place under the action of right or left circularly polarised light ..."

The idea was tried by FREUNDLER[36] who exposed a solution of the racemic diamyl acetal of o-nitrobenzaldehyde in r-amyl alcohol to right-handed circularly polarised light. Under these conditions amyl-o-nitrosobenzoate is formed, but neither this substance nor the amyl alcohol recovered from the experiment showed any rotation after some 400 hours of illumination.



COTTON[37] proposed a slightly different use of circularly polarised light. Instead of allowing it to act during the formation of a potentially active substance, he suggested that it might be employed to bring about asymmetric decomposition. Cotton had previously discovered that alkaline solutions of copper tartrate exhibit unequal absorption for right and left-handed circularly polarised light from the red end of the spectrum and since, in addition, these solutions are decomposed by sunlight, he considered them particularly suitable for asymmetric photochemical experiments. He therefore prepared an alkaline solution of copper racemate and placed it in two glass cells. One was exposed to right-handed and the other to left-handed circularly polarised sunlight in the hope that rotations of opposite sign would be developed. The two circular polarisers used each consisted of a Nicol prism and a Fresnel rhomb. It was intended to examine the solutions for rotation whilst the decomposition was in progress, but the glass ends of the cells became warped and the solutions formed deposits. Cotton had, therefore, to wait till the decomposition was well advanced and then to transfer the solutions to polarimeter tubes. When he did so, no rotation could be detected in either. The cells were exposed from the 10th June till the 23rd July 1897.

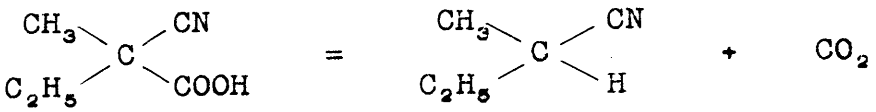
BYK[38] has shown that an alkaline solution of copper tartrate is not acted on by light from the red end of the spectrum as Cotton had thought. The decomposition is caused by ultra-violet rays (4000 to 3500 A.U.) just beyond the visible violet. It is therefore important to ascertain if alkaline solutions of copper tartrate show circular dichroism for these ultra-violet rays.

Cotton did not continue his measurements beyond 4370 A.U. so MITCHELL[39] made observations down to 3500 A.U. in order to settle the question. The apparatus employed was that described on page 23. The solution consisted of 1 g. copper tartrate, 0·5 g. potassium hydroxide, and 0·5 g. sodium hydroxide made up to 100 cc. with distilled water. A 10 cm. layer was first used and it was decreased in stages down to 3 mm., photographs being taken at each stage. The absorption extended at the ultra-violet end to about 4000 A.U. with the thickest layer and gradually receded to about 3500 A.U. with the thinnest layer, but no difference could be detected between the absorption for right and left-handed circularly polarised light in this region. It would appear, therefore, that asymmetric photochemical decomposition of alkaline solutions of copper racemate cannot be expected.

BYK[38] also carried out some experiments with optically active photographic preparations (silver tartrate paper and silver bromide plates sensitised with chlorophyll). Each preparation was exposed simultaneously in a spectroscope to right and left-handed circularly polarised light of the same intensity, but no difference could be observed in the effects produced.

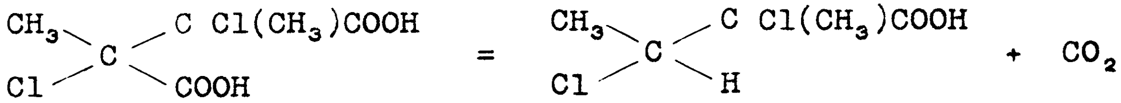
Byk states that the chlorophyll he used was optically active, but this is not confirmed by later observers who consider that chlorophyll is quite inactive.

HENLE and HAAKH[40] studied the photochemical transformation of methylethylcyanoacetic acid into methylethylacetonitrile



$$\begin{matrix}\mathrm{CH_3} & & \mathrm{CN}\\ & \mathrm{C} & \\ \mathrm{C_2H_5} & & \mathrm{COOH}\end{matrix} \quad = \quad \begin{matrix}\mathrm{CH_3} & & \mathrm{CN}\\ & \mathrm{C} & \\ \mathrm{C_2H_5} & & \mathrm{H}\end{matrix} \quad + \quad \mathrm{CO_2}$$

and of dichlorodimethylsuccinic acid into dichlorodimethylpropionic acid.



$$\begin{matrix}\mathrm{CH_3} & & \mathrm{C\ Cl(CH_3)COOH}\\ & \mathrm{C} & \\ \mathrm{Cl} & & \mathrm{COOH}\end{matrix} \quad = \quad \begin{matrix}\mathrm{CH_3} & & \mathrm{C\ Cl(CH_3)COOH}\\ & \mathrm{C} & \\ \mathrm{Cl} & & \mathrm{H}\end{matrix} \quad + \quad \mathrm{CO_2}$$

but although circularly polarised light was used in both cases only inactive products resulted.

BREDIG[41] made a number of attempts during a period of ten years, but they all proved unsuccessful. He used circularly polarised ultra-violet light and acted on inactive diazo-camphor, lactic acid, and asymmetric cobaltammine salts. His experimental procedure was particularly tedious as the diazo-camphor was exposed in benzene solution for 14 weeks and the lactic acid for 6 weeks. It is surprising that Bredig did not examine the active forms of the substances he used for circular dichroism.

JAEGER[42] in the course of work on the complex oxalates discovered that in solution d-potassium-cobaltioxalate shows the Cotton Effect in a remarkable way. The rotation reaches a minimum about 6400 A.U., is zero at 6240 A.U., and shows a maximum about 6000 A.U. On exposure to light the compound is reduced to the corresponding cobaltooxalate, but unfortunately the reaction is complex and the solution becomes very turbid. In consequence, experiments with circularly polarised light, although contemplated[43], do not appear to have been actually carried out.

The author tried some experiments with the cobaltic derivative of hydroxymethylene camphor. LIFZSCHITZ[23] has examined the rotation dispersion of this substance and has found that it shows the Cotton Effect. He records the following values.

Table 3

λ	$[\alpha]$	λ	$[\alpha]$
6840	+ 465	5650	-2670
6650	+ 742	5430	-3168
6450	+ 800	5330	-3178
6250	+ 564	5240	-3138
6100	- 316	5075	-2970
5940	-1310	5000	-2623
5780	-2148	4940	-2227·5

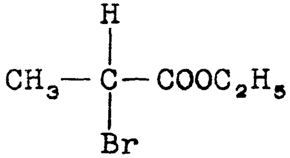
The hydroxymethylene camphor was prepared by the method of BISHOP, CLAISEN, and SINCLAIR[44] and the ter-valent cobalt derivative was obtained from it as described by LIFZSCHITZ[45]. When the green solution of the compound in alcohol was exposed to sunlight the rotation (for mercury green light) gradually diminished and the colour of the solution changed to red. On account of the very strong absorption an N/200 solution had to be employed, however, and with this the initial rotation (about -2° in a 1 dm. tube) was too small to be of any value for the purpose in view. The photochemical change was also extremely slow.

GERNEZ[46] has shown that when ammonium molybdate is added to an aqueous solution of tartaric acid, complexes are formed which have a high rotation. He obtained the maximum effect when 6·866 g. of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ were added to 2·50 g. of tartaric acid and the whole was made up to 100 c.c. with water. For this complex $[\alpha]_D = 781$. Whilst working with this solution the author noticed that it turned blue on exposure to light. The blue solution was tested for circular dichroism but it did not show any. It was also found that the rotation was affected very little by the development of the blue colour. This solution is, therefore, quite unsuitable for asymmetric photochemical work.

4. RECENT RESULTS OF THE HEIDELBERG SCHOOL

Whilst the work described in Section 5 of the present thesis was in progress, two positive results were obtained in Heidelberg.

At the suggestion of Professor Freudenberg, KUHN and BRAUN[47] examined the rotation of the dextro form of



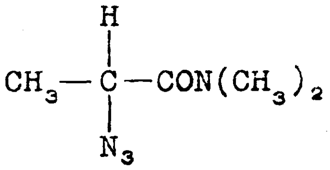
$$CH_3-\overset{\displaystyle H}{\underset{\displaystyle Br}{C}}-COOC_2H_5$$

ethyl-α-bromopropionate

in the ultra-violet region of the spectrum. It showed the Cotton Effect, with a maximum specific rotation of about 4000° at 2450 A.U. followed by a change in sign at shorter wave lengths. In addition, it was decomposed by ultra-violet light, so that the racemic form of the ester was considered to be well suited for asymmetric photochemical experiments. Solutions (4 to 8% in alcohol) were therefore placed in quartz flasks and exposed to monochromatic circularly polarised ultra-violet light ($\lambda = 2800$ A.U.). 50% of the ester was decomposed in each case, a solution of the active ester being used to provide control for the period of illumination required. The alcohol was then distilled off and the ester was transferred to 1 dm. micro polarimeter tubes and was examined for rotation with mercury yellow light. The portion acted on by right-handed circularly polarised light had developed dextro rotation and vice versa. The greatest effect obtained in each case was 0·05°. After standing for a few hours in the polarimeter the activity gradually disappeared again.

These results have been discussed by COTTON[48] who has pointed out that it is for wave lengths in the spectral region where the rotation changes sign that the greatest amount of circular dichroism is to be expected and for which, consequently, the maximum amount of rotation would be developed. Better results might therefore have been obtained with light of shorter wave length than Kuhn and Braun used.

Professor Freudenberg next suggested the dimethylamide of



$$CH_3-\overset{\displaystyle H}{\underset{\displaystyle N_3}{C}}-CON(CH_3)_2$$

α-azidopropionic acid.

KUHN and BRAUN[49] examined the ultra-violet rotation and absorption of a solution of the dextro form of the substance in ether. The results are given in Fig. 10. Curve 1 shows the rotation, and the logarithms of the absorption coefficients are given in curve 2. The weak absorption band situated at 2900 A.U. Kuhn considered due to the N_3 group, as the band is found in other substances containing this group and disappears as soon as it is eliminated. The rotation curve is regarded as the sum of two parts, (1) the contribution of the N_3 band (curve 3), and (2) the rotation

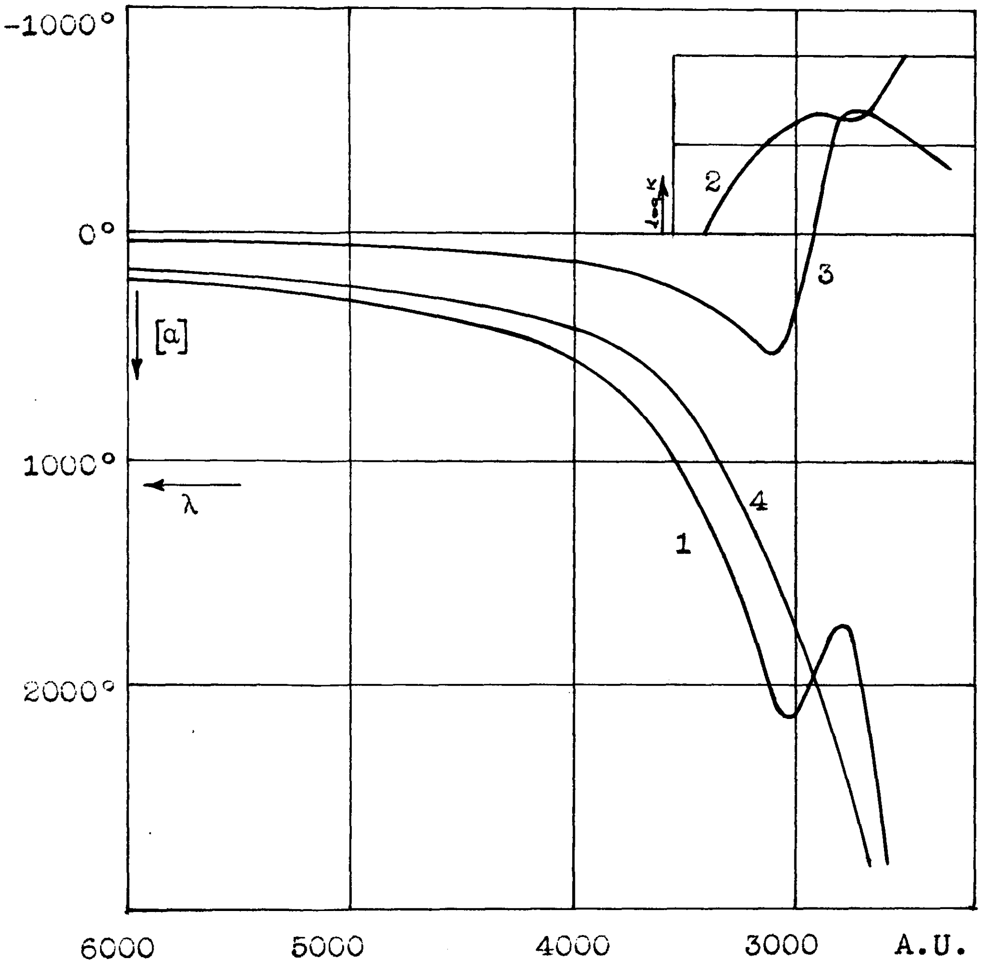


Fig. 10

due to the molecule when this contribution is subtracted (curve 4).

The photochemical work was carried out by KUHN and KNOPF[50]. Hexane solutions of the racemic dimethylamide of α-azido-propionic acid were exposed to right and left-handed circularly polarised light (3200 to 2800 A.U.) obtained from a mercury vapour lamp with a suitable screen. The polarising apparatus was composed of a double image prism and a specially constructed Fresnel rhomb. The latter consisted of a rhomboidal brass case of the required shape. Openings were cut at the ends and at the faces where total reflection had to take place. These openings were closed with thin plates of fused silica and the case was filled with water. Making certain assumptions, it was calculated that the maximum rotation should be developed after the substance had been 40% decomposed. The extent of decomposition was found by measuring the volume of nitrogen liberated. During the experiment some of the reaction product separated on the walls of the tubes and no doubt caused some anxiety. After 37% decomposition by left-handed circularly polarised light (the actual time of irradiation is not stated) the unchanged liquid dimethylamide was separated from the hexane and decomposition product by distillation under reduced pressure. It showed a rotation of -1·04° in a 1 dm. tube. A corresponding experiment with right-handed circularly polarised light with a decomposition of 35% gave a reading of +0·78°. These polarimeter readings were taken with mercury yellow light which is on the long wave side of the absorption band.

5. ASYMMETRIC PHOTOCHEMICAL DECOMPOSITION OF THE NITROSITES OF CARYOPHYLLENE AND HUMULENE

Experiments with Caryophyllene Nitrosite

KREMERS and SCHREINER[51] studied the action of sunlight on caryophyllene nitrosite. They found that in benzene solution nitrogen was evolved and crystals of the decomposition product (m.p. 146-148°) separated. From experiments with coloured solutions as light filters they concluded that the reaction was caused by the red and yellow rays. This conclusion was confirmed by placing a row of tubes containing the blue nitrosite solution in the spectrum obtained from an electric arc. The solution in the tubes exposed to red and yellow light turned green, whilst that in the others remained unchanged.

VALENZULA and DANIELS[52] have shown that caryophyllene nitrosite dissolved in various organic liquids is destroyed by heat. Before commencing experiments with circularly polarised light, it was therefore necessary to be sure that rays in the near infra-red did not take part in the reaction. A concentrated benzene solution of the nitrosite was prepared and thickened with some Canada balsam. A film of the resulting viscous greenish-blue liquid was then enclosed between two glass plates and exposed to a spectrum about two inches long produced by a direct-vision spectroscope. The slit was illuminated with light from an electric arc, after it had passed through a filter 3 inches thick through which water circulated. After three hours a narrow yellow band had developed in the region of the film which had been exposed to red light, but no decomposition whatever had been produced by the near infra-red rays.

The rotation dispersion and circular dichroism of caryophyllene nitrosite in alcoholic solution have already been investigated by the author (see Section 2). The rotation reaches a high maximum value about 6250 A.U., becomes zero at 6800 A.U., and is thereafter negative. Absorption measurements revealed a band in the red region with its head about 6800 A.U. When the absorption was examined with circularly polarised light, it was found that the right-handed light was more strongly absorbed than the left-handed. The nitrosite was therefore considered to be very promising for asymmetric photochemical work.

The selection of a suitable solvent presented some difficulty. Ethyl butyrate was used finally because it dissolved both the nitrosite and the decomposition product readily and was not too volatile. A 5% solution showed α_{5461} +36° in a 6 cm. tube. On exposure to sunlight, nitrogen was evolved, the blue solution gradually became colourless, and the rotation decreased to a small negative value. The decomposition could, therefore, be followed conveniently with the polarimeter.

Apparatus

For the experiments with circularly polarised light all parts of the apparatus were rigidly mounted on an optical bench as shown in Fig. 11. A microscope arc lamp with mechanical feed served as source of illumination. Cored carbons, 7 mm. positive and 5 mm. negative, were used with a current of 12 amps. In front of the arc was placed a circulating water filter 3 inches thick, followed by a red gelatin filter transmitting light of 6000-7800 A.U. The light was rendered parallel with a convex lens and was plane polarised by passage through a Nicol prism. Half of the field was then covered with a mica λ/4 plate suitably oriented and of the correct thickness to give right-handed circularly polarised light of about 7000 A.U. The other

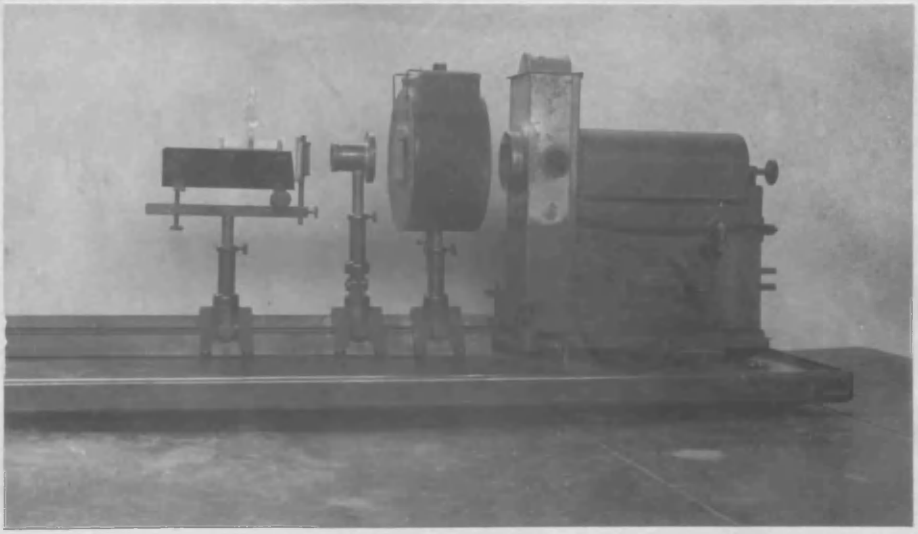


Fig. 11

half was covered with a $\lambda/4$ plate cut from the same piece of mica and oriented to give left-handed circularly polarised light. In order to check the direction of rotation of the circularly polarised light, a solution of the nitrosite is placed after the $\lambda/4$ plates and the double field is viewed through the solution either directly or with a spectroscope. The right-handed circularly polarised light is the type most absorbed. Fig. 12 shows the effects produced when a spectroscope is employed. A is taken with the solution removed, B with the solution in position, and C with the solution again but with the $\lambda/4$ plates reversed.

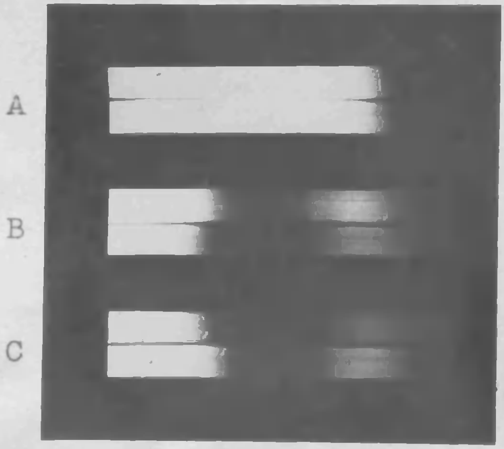


Fig. 12

For the asymmetric photochemical experiments a 4·6% solution of the nitrosite in ethyl butyrate was prepared and placed in a specially constructed double cell. This was held in a V-shaped trough which could be raised, lowered, tilted, or moved laterally so that all necessary adjustments could be made to ensure that the light passed straight through the cell and that the two compartments were illuminated by different kinds of circularly polarised light. An end elevation of the double cell is shown in Fig. 13. Two holes 0·2 inch in diameter were drilled through a 60° glass prism 2·5 inches long, and inlet holes were drilled from the top. The latter were slightly tapered and bubblers of the type shown were ground into them. During the experiment the bubblers were partly filled with solvent. The two horizontal holes were carefully polished until both held exactly the same volume of liquid. The end pieces were made of the best quality optical glass and were cemented in position. A thin brass plate with two holes of equal diameter, just smaller than those through the prism, covered the end of the cell next the light source and so protected the solutions from stray light. This device also facilitated the taking of polarimeter readings. A Hilger polarimeter was used, the adjustable V-shaped trough provided with the instrument being particularly convenient for comparative measurements with the double cell. After each period of illumination, the cell was transferred to the polarimeter where the solutions were allowed to settle for about half an hour before reading the rotation. Throughout the experiment the room was maintained at a temperature of 21°.

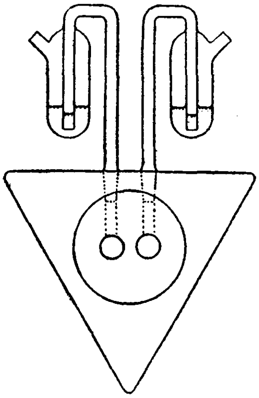


Fig. 13

The results are shown in Table 4. The compartments A and B were illuminated with left-handed and right-handed circularly polarised light respectively.

Table 4

Time of Illumination Hours	Rotations for Compartment A Degrees	Rotations for Compartment B Degrees	Diff.
0	36·20	36·20	0·00
8	24·97	24·90	0·07
12	19·39	19·20	0·19
16	14·77	14·77	0·00

In a second experiment the λ/4 plates were reversed so that the compartments were illuminated with the opposite kinds of circularly polarised light. Table 5 gives the results.

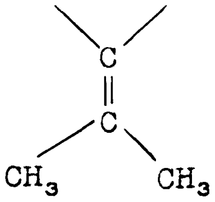
Table 5

Time of Illumination Hours	Rotations for Compartment A Degrees	Rotations for Compartment B Degrees	Diff.
0	35·50	35·50	0·00
4	28·05	27·97	-0·08
12	17·60	17·70	0·10
16	13·38	13·40	0·02

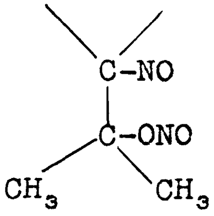
It is seen from Table 4 that up to a point the solution which was absorbing more light was the more quickly decomposed. In addition, Table 5 shows that the effect is reversed by reversing the λ/4 plates. Considering the nature of the experiments, however, the differences are just rather small to be conclusive. Nevertheless, the work served a useful purpose for the technique developed led to success in the experiments which followed.

Experiments with Humulene Nitrosite[39]

Unfortunately, the racemic form of caryophyllene is not known, but the inactive sesquiterpene humulene is very closely related to it and forms a blue nitrosite which is so similar to that of caryophyllene that it was thought advisable to submit the humulene derivative to the action of circularly polarised light. Both hydrocarbons contain the grouping



which adds on N_2O_3 to give



Since the other two groups attached to the top carbon atom are different, this atom will become asymmetric on the addition of nitrous anhydride at the double bond. The existing optical activity in the case of caryophyllene seems to be sufficient to cause the exclusive formation of the dextrorotatory nitrosite. In the case of inactive humulene, however, there is no such directing influence so that a racemic mixture of dextro-and laevo-forms is obtained.

Humulene occurs in hop oil and is separated from it by fractional distillation. It is not possible to obtain more than about 20% of good humulene from the oil, and as the latter costs £4 per ounce the sesquiterpene is rather an expensive substance. The author is much indebted to

Messrs. White, Tomkins and Courage, Ltd. for supplying a fraction of the oil boiling over 160°/10 mm. which was rich in humulene. This was submitted to further distillation, the fraction of b.p. 115-122°/10 mm. being sufficiently pure for the present purpose. The nitrosite was prepared as described by CHAPMAN[53]. It is essential to work with small quantities of humulene, not more than 5 c.c. at a time, and to keep the freezing bath at a temperature of about -12°. As in the case of caryophyllene nitrosite, the preparation was carried out in a dark room, a feeble blue light being used only when necessary. The yield was very small (about 3%). After crystallisation from a cold mixture of alcohol and chloroform the nitrosite melted at 113°.

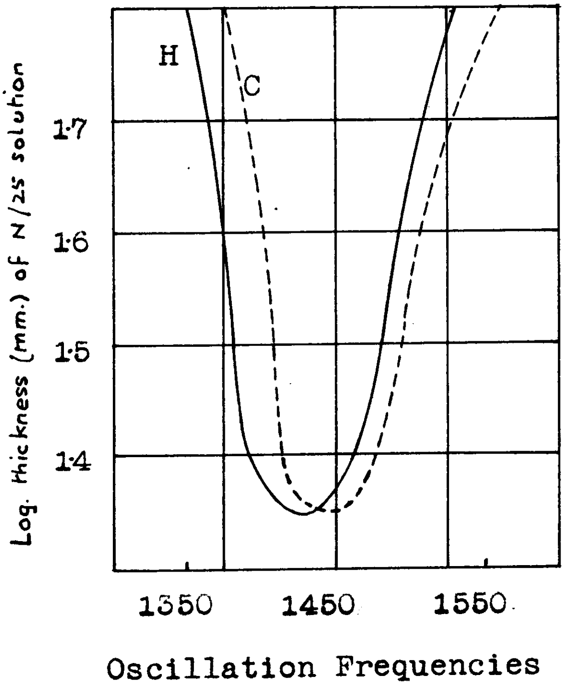


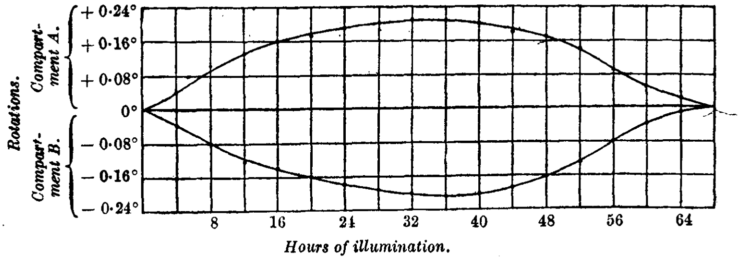
Fig. 14

The absorption spectra of the nitrosites of caryophyllene and humulene were next compared. The results are shown graphically in Fig. 14 from which it is seen that the heads of the bands are so close that no modification of the photochemical apparatus already described (page 37) was necessary for work with the humulene nitrosite.

In a preliminary experiment, the double cell was filled with a 3·5% solution of humulene nitrosite in ethyl butyrate. On polarimetric examination no rotation was observed in either compartment. As in the earlier experiments with caryophyllene nitrosite, one side was then illuminated with right-handed and the other with left-handed circularly polarised red light. After four hours the solutions were again examined in the polarimeter, and it was found that the side which had been exposed to left-handed light had developed a slight dextrorotation, whilst the other side had developed a small laevorotation. On further illumination the difference between these values increased until after 12 hours one side showed a rotation of +0·11° and the other side −0·09° (λ = 5461). On still further illumination the difference between these values decreased.

The results of a further experiment with a 7% solution of the nitrosite are given in Table 6. Remarkable symmetry is revealed when these results are represented graphically (Fig. 15).

Fig. 15.



A maximum rotation of 0·21° (λ = 5461) on each side of the zero was developed after 36 hours of illumination.

Additional readings taken at this stage with mercury yellow light gave rotations of +0·30° and −0·30°. Assuming that the solution was then 50% decomposed (which is probably an underestimate) this would give $[\alpha]_{5790}$ ± 13·5°. During the earlier part of the experiment observations with yellow light were impossible because it was too strongly absorbed by the solution, but this difficulty diminished as the decomposition proceeded.

Table 6

Time of Illumination Hours	Rotations for Compartment A Degrees	Rotations for Compartment B Degrees	Diff.
4	+ 0·04	− 0·04	0·08
8	+ 0·10	− 0·08	0·18
12	+ 0·13	− 0·12	0·25
16	+ 0·16	− 0·14	0·30
20	+ 0·17	− 0·16	0·33
24	+ 0·19	− 0·18	0·37
28	+ 0·20	− 0·19	0·39
32	+ 0·21	− 0·20	0·41
36	+ 0·21	− 0·21	0·42
40	+ 0·20	− 0·20	0·40
44	+ 0·18	− 0·19	0·37
48	+ 0·17	− 0·16	0·33
52	+ 0·14	− 0·13	0·27
56	+ 0·09	− 0·08	0·17
60	+ 0·05	− 0·04	0·09
64	+ 0·02	− 0·01	0·03

Compartment A was illuminated with left-handed circularly polarised light.

Compartment B was illuminated with right-handed circularly polarised light.

It is interesting that these effects have been produced by visible light and that it has been possible to take polarimeter readings during the course of the reaction. It should be noted that the readings were taken with light on the short wave side of the absorption band. Since the rotation dispersion curve for the component which predominates when left-handed light is used would be of the form shown in Fig. 16, the readings would have had the opposite sign if they had been taken with light on the long wave side of the band. Kuhn and his collaborators used mercury yellow light which lies on the long wave side of the ultra-violet absorption band of the dimethylamide of α-azidopropionic acid, and they found that left-handed light produced laevorotation and vice versa. These results and those of the author are therefore in agreement as regards sign.

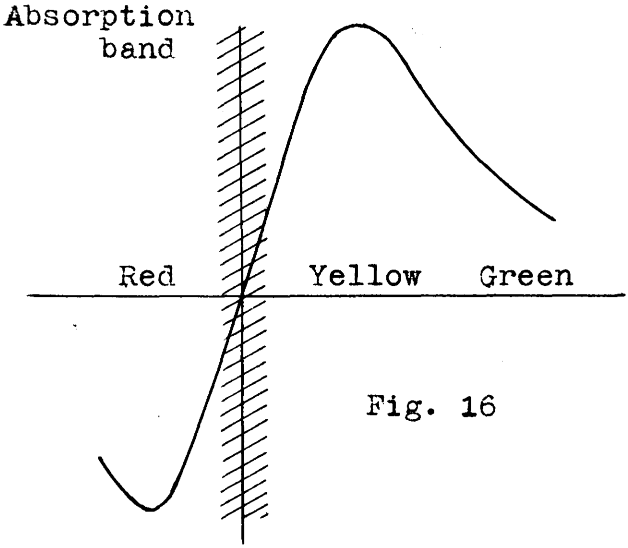


Fig. 16

Humulene nitrosite has proved to be an excellent substance for demonstrating the asymmetric photochemical action of circularly polarised light. The use of visible light has permitted a simpler experimental procedure than Kuhn could employ and the choice of a suitable solvent has enabled a series of readings to be taken.

Suggested Further Work

Some years ago BORN[54] put forward the idea that optical rotation is due to a coupling between the various resonators inside a molecule and his mathematical treatment has been developed by other authors. KUHN[55] pointed out the importance of the term $\frac{n_l - n_r}{n}$ which occurs in the equations, n_l, n_r, and n being the refractive indices for left-handed light, right-handed light, and ordinary light respectively. He showed that it is equal to $\frac{\varepsilon_l - \varepsilon_r}{\varepsilon}$ the relative difference of the absorption coefficients for left-handed and right-handed light inside an absorption band. He called this the "anisotropy factor" and deduced that a weak absorption band coupled with a strong band would give a high value for this factor. He then considered the case of the dimethylamide of α-azidopropionic acid. A weak band (at λ = 2900 A.U.) due to the N_3 group is present, but he found that it could not be coupled with the $CON(CH_3)_2$ band and concluded that there must be a strong band in the farthest ultra-violet (Schuman region) with which it is coupled. Data for verifying this deduction are, however, not available.

If Kuhn's theory is correct, then it is a matter for future research to decide whether the NO group in nitrosites is coupled with the ONO group or with some other unsaturated centre which displays absorption at smaller wave lengths. Even in the latter case there is a good chance of locating the band in the accessible part of the ultra-violet, since the weak absorption band due to the NO group lies in the red region.

But it is perhaps rather early for theorising, and probably a number of further examples of circular dichroism will require to be investigated before we are able to control the "anistropy factor". Nevertheless, the author is hopeful that sufficient progress will shortly be made to enable circularly polarised light to be used for resolving certain types of compounds. The complete resolution of humulene nitrosite could be accomplished by a series of experiments with circularly polarised light, but this would be a costly process. The next step is to look for another nitrosite which can be prepared more cheaply than the humulene compound and, if possible, which will yield components more strongly circularly dichroic.

5. SIGNIFICANCE OF THE RESULTS

Pasteur pointed out in his "Lectures on the Molecular Asymmetry of Natural Organic Products" (1860) that optically active substances had never been obtained without the intervention of life. He thus revived in a modified form the belief in a "vital force". At the same time, he conceived the possibility of asymmetric synthesis by means of non-living asymmetric forces, and he asks, "Do these asymmetric actions, possibly placed under cosmic influences, reside in light, in electricity, in magnetism, or in heat? Can they be related to the motion of the earth, or to the electric currents by which physicists explain the terrestrial magnetic poles? It is not even possible at the present time to express the slightest conjecture in this direction."

As recently as 1898 Japp, in an address to the British Association on "Stereochemistry and Vitalism", strongly upheld Pasteur's vitalistic views. He discussed in detail the various methods employed in producing optically active compounds and showed that in all cases the intervention of life was essential. His remarks on the possible use of asymmetric physical agents are of interest:- "All attempts to form optically active compounds under the influence of magnetism or circularly polarised light have hitherto signally failed. These forces do not distinguish between the two equally exposed points of attack which present themselves in the final stage of the transformation of a symmetric into an asymmetric carbon atom. But even if such an asymmetric force could be discovered - a force which would enable us to synthesise a single enantiomorph - the process would not be free from the intervention of life. Such a force would necessarily be capable of acting in two opposite asymmetric senses; left to itself it would act impartially in either sense, producing, in the end, both enantiomorphs in equal amount. Only the free choice of the living operator could direct it consistently into one of its two possible channels." He concludes, therefore, that "the absolute origin of the compounds of one-sided asymmetry to be found in the living world is a mystery as profound as the absolute origin of life itself."

A few years later BYK[38] showed that there has always been an excess of one type of circularly polarised light at the surface of the earth due to the reflection of plane polarised sky light. He emphasised the importance of Cotton's discovery of Circular Dichroism, and considered that if this phenomenon was exhibited by a suitable substance asymmetric photochemical action should be possible. "As by the artificial making of urea by Wöhler an argument against vitalism has been created, so by the artificial making of a one-sided optically active substance in excess a new halting-place would be reached on the way of organic and inorganic nature."

Reviewing the position of Vitalism in his "Lectures on the Principle of Symmetry" (1919) Jaeger says: "Continual research leads us to believe that the hope need not be given up of overcoming within a not too distant future all experimental difficulties, and that the dynamics of the asymmetrical synthesis will then be as accessible to us as those of our common laboratory processes. If the experiments should some day have a real positive result, we shall then have completely solved one of the most fundamental riddles in the chemical and biological sciences, and we shall once more effectually have contributed to the final understanding of one of the most important phenomena, which have puzzled scientists ever since they have been able to think of problems on this level."

The recent results of Kuhn together with those of the author definately show that in principle no vital force is necessary for the production of optically active compounds. Japp's objection that an operator must control the production of circularly polarised light has been met since there is an excess of one type of circularly polarised light at the surface of the earth.

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Physical Chemistry Department,
University of Glasgow,
15th October, 1930

ADDITIONAL PAPERS ON ASYMMETRIC ENZYME ACTION

From the
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HYDROLYSIS OF THE d-GLUCOSIDES OF d- AND 1-METHYL-n-HEXYLCARBINOL WITH EMULSIN.

By STOTHERD MITCHELL

(From the Physical Chemistry Department, University of Glasgow, Glasgow, Scotland.)

(Received for publication, February 20, 1929)

The classical investigations of Dakin on the hydrolysis of esters with lipase and of Fischer on the action of trypsin on peptides have established the asymmetric nature of enzyme action. FISCHER[1] has also recorded the following results with emulsin. d-Mandelamide-d-glucoside was unhydrolyzed after 48 hours, while under the same conditions 1-mandelamide-d-glucoside was completely hydrolyzed after 20 hours. Further, both d-mandelonitrile-d-glucoside and 1-mandelonitrile-d-glucoside were attacked at about the same rate and practically to the same extent. MITCHELL[2] showed that emulsin hydrolyzes the d-glucoside of 1-borneol 3·4 times as rapidly as the corresponding compound of d-borneol.

The action of emulsin on the d,β-glucosides of d- and 1-methyl-n-hexylcarbinol has now been examined. These glucosides have not been prepared previously, so a quantity of the alcohol as obtained commercially was used to test the method before resolving the alcohol. The method employed was essentially that described by KOENIGS and KNORR[3]. Commercial methyl-n-hexylcarbinol is not absolutely inactive. It usually has a small laevorotation. The sample used in these experiments had a rotation $\alpha^{20}_{5461} = -0{\cdot}21^\circ$ in a 1 dm. tube.

Tetraacetyl-β-Methyl-n-Hexylcarbinyl-d-Glucoside

30 gm. of methyl-n-hexylcarbinol were dissolved in 100 cc. of anhydrous ether, and 5 gm. of acetobromoglucose[4] together with 5 gm. of freshly prepared and thoroughly dried silver carbonate were added. The mixture was shaken for 20 hours. A second 5 gm. of acetobromoglucose and 5 gm. of silver carbonate were then put in, and the shaking was continued as before. Two further quantities of acetobromoglucose and silver carbonate were added later with further shaking, so that in all 20 gm. of acetobromoglucose and 20 gm. of silver carbonate were used. The ether was then evaporated off, and the uncombined alcohol was removed by steam distillation. The tetraacetyl compound was crystallized first from aqueous alcohol and finally from alcohol. From the latter solvent it separated in fine needles which melted at 95°.

Found. C 57·2, H 7·7.

$C_{22}H_{36}O_{10}$ requires C 57·4, H 7·8.

β-Methyl-n-Hexylcarbinyl-d-Glucoside

4 gm. of tetraacetyl-β-methyl-n-hexylcarbinyl-d-glucoside were put into a stoppered bottle containing 240 cc. of water, 75 cc. of alcohol, and 16 gm. of barium hydroxide. The mixture was kept at 55-60° for 5 hours, and was frequently shaken until the whole passed into solution. The remaining barium hydroxide was precipitated by bubbling in carbon dioxide, the barium carbonate was filtered off, and the solution was evaporated to dryness. The residue was then extracted with alcohol, and the solution was again taken to dryness. The glucoside thus obtained was crystallized from anhydrous ether.

Found, C 53·9, H 9·4

$C_{14}H_{28}O_6 \cdot H_2O$ requires C 54·2, H 9·2.

A quantity of methyl-n-hexylcarbinol was resolved by the method described by PICKARD and KENYON[5]. The d-glucosides of the d and l forms of the alcohol were then prepared as described above for the racemic alcohol. For determining the rotations of the compounds 5 per cent solutions were employed.

d-Methyl-n-hexylcarbinyl-d-glucoside gave $[\alpha]^{17}_{5461} = -37{\cdot}8°$.

l-Methyl-n-hexylcarbinyl-d-glucoside " $[\alpha]^{17}_{5461} = -46{\cdot}6°$.

Hydrolysis Experiment

Two 25 cc. graduated flasks were taken; in one was placed 0·1 gm. of the first glucoside and in the other an equal weight of the second glucoside. The flasks were then put in a thermostat at 37°. The enzyme solution was prepared by mixing 0·05 gm. of emulsin with 60 cc. of water, filtering, and allowing to come to 37° in the thermostat.

The hydrolysis of the d-methyl-n-hexylcarbinyl-d-glucoside was commenced by filling the graduated flask to the mark with the emulsin solution. 1 cc. was withdrawn at intervals and

Table 1

Rate of Hydrolysis of d and l-Methyl-n-Hexylcarbinyl-d-Glucosides

Time	A	c	K
d-Methyl-n-hexylcarbinyl-d-glucoside			
min.			
18 (= t_x)	4·12	3·73	
33	6·00	1·85	0·0468
48	6·93	0·92	0·0467
63	7·40	0·45	0·0470
78	7·55	0·30	0·0464
91·5	7·72	0·13	0·0457
∞	7·85	Average	0·0465
l-Methyl-n-hexylcarbinyl-d-glucoside			
105 (= t_x)	3·20	4·73	
150	4·24	3·69	0·00552
180	4·76	3·17	0·00559
278	6·10	1·83	0·00549
311	6·43	1·50	0·00558
405	7·02	0·91	0·00550
∞	7·93	Average	0·00554

the volume of 0·01 N sodium thiosulfate solution equivalent to the amount of glucose set free was determined by MacLEAN'S[6] method. After a few readings had been taken, the hydrolysis of the l-methyl-n-hexylcarbinyl-d-glucoside was started, and thereafter the two experiments were carried on side by side. The results given in Table 1 were obtained. In each case A is the number of cc. of 0·01 N sodium thiosulfate solution equivalent to the glucose set free in 1 cc. of solution, and c is proportional to the concentration of unchanged glucoside present at times stated. K is calculated from the usual formula for unimolecular reactions.

$$K = \frac{2 \cdot 303}{t_y - t_x} \log \frac{c_x}{c_y}$$

Hence d-methyl-n-hexylcarbinyl-d-glucoside is hydrolyzed by emulsin 8·4 times as rapidly as l-methyl-n-hexylcarbinyl-d-glucoside.

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EXPONENTIAL ANALYSIS APPLIED TO THE HYDROLYSIS OF THE d-GLUCOSIDES OF SOME RACEMIC ALCOHOLS WITH EMULSIN.

By STOTHERD MITCHELL and JOHN MacARTHUR

The rates of hydrolysis of the d-glucosides of d- and l-methyl-n-hexylcarbinol with emulsin have already been examined by MITCHELL[1] and it was thought that an extension of the work to the glucosides of other optically active aliphatic alcohols would be of interest. In order to avoid, as far as possible, the tedious and costly process of resolving the alcohols the possibility of employing exponential analysis was considered, the previously prepared β-glucoside of racemic methyl-n-hexylcarbinol being used to test the validity of the method.

Methyl-n-hexylcarbinyl d-Glucoside

A small quantity (0·0818 gm.) of the glucoside was weighed into a 50 cc. graduated flask which was then placed in a thermostat at 37°. The enzyme solution was prepared by mixing 0·100 gm. of emulsin with 100 cc. of water, filtering, and allowing it to come to 37° in the thermostat. The hydrolysis experiment was started by filling the graduated flask to the mark with the emulsin solution. Two cc. were withdrawn at intervals during about 10 hours and the volume

(T_n) of 0·01 sodium thiosulphate solution equivalent to the amount of glucose set free was determined by the method of MacLEAN[2]. The final reading (T_∞) was obtained some 24 hours later. Table 1(A) gives the values of T_n and $T_\infty - T_n$ at the times shown. The differences $T_\infty - T_n$, which are proportional to the concentrations of unchanged glucoside, were graphed against time as in Fig. 1 and the method of exponential analysis outlined by WALSH[3] was applied assuming two components present, i.e. that the curve is of the form

$$y = a_1 e^{-k_1 t} + a_2 e^{-k_2 t} \quad \ldots\ldots\ldots\ldots \quad (1)$$

where a_1, a_2, k_1, k_2 are constants, t is time, and y is $T_\infty - T_n$.

Let y_0, y_1, y_2, y_3 be the values of y corresponding to the values 0, d, 2d, 3d of t, zero time and d being arbitrarily chosen. Then where

$$\left.\begin{aligned} e^{-k_1 d} &= \alpha_1 \\ e^{-k_2 d} &= \alpha_2 \end{aligned}\right. \quad \ldots\ldots\ldots\ldots \quad (2)$$

we have

$$\begin{aligned} y_0 &= a_1 + a_2 \\ y_1 &= a_1\alpha_1 + a_2\alpha_2 \\ y_2 &= a_1\alpha_1^2 + a_2\alpha_2^2 \\ y_3 &= a_1\alpha_1^3 + a_2\alpha_2^3 \end{aligned}$$

Let p, q be such that α_1, α_2 are the roots of the equation

$$f(x) = x^2 + px + q = 0$$

Then $y_2 + py_1 + qy_0 = a_1 f(\alpha_1) + a_2 f(\alpha_2) = 0$

and $y_3 + py_2 + qy_1 = a_1\alpha_1 f(\alpha_1) + a_2\alpha_2 f(\alpha_2) = 0$

Elim. p, q, $\begin{vmatrix} x^2 & x & 1 \\ y_2 & y_1 & y_0 \\ y_3 & y_2 & y_1 \end{vmatrix} = 0$

The roots of this quadratic α_1, α_2 are thus easily obtained and the values so found give k_1 and k_2 from equations (2) and a_1 and a_2 from any two of equations (3).

Probability Correction

This exponential analysis being based only on four points from the mean curve, and being rather sensitive even to slight error in the chosen points, it is desirable to use the set of four parameters so obtained as a first approximation to the set which gives an equation in closest agreement with all the available reliable data.

The following method was adopted.

Let $\phi(a_1, a_2, k_1, k_2) \equiv a_1 e^{-k_1 t} + a_2 e^{-k_2 t}$ where (1) is the equation best representing the data of hydrolysis.

Let $\bar{a}_1, \bar{a}_2, \bar{k}_1, \bar{k}_2$ be the parameter set obtained by preliminary exponential analysis.

Let
$$\begin{aligned} a_1 &= \bar{a}_1(1 + \beta_1) \qquad & k_1 &= \bar{k}_1(1 + \lambda_1) \\ a_2 &= \bar{a}_2(1 + \beta_2) \qquad & k_2 &= \bar{k}_2(1 + \lambda_2) \end{aligned} \qquad \ldots\ldots (4)$$

then where $\beta_1, \beta_2, \lambda_1, \lambda_2$ are small

$$\delta_n \equiv \phi(a_1, a_2, k_1, k_2)_{t=t_n} - \phi(\bar{a}_1, \bar{a}_2, \bar{k}_1, \bar{k}_2)_{t=t_n}$$

$$= \left[\frac{\partial\phi}{\partial\bar{a}_1}\cdot\bar{a}_1\beta_1 + \frac{\partial\phi}{\partial\bar{a}_2}\cdot\bar{a}_2\beta_2 + \frac{\partial\phi}{\partial\bar{k}_1}\cdot\bar{k}_1\lambda_1 + \frac{\partial\phi}{\partial\bar{k}_2}\cdot\bar{k}_2\lambda_2\right]_{t=t_n}$$

to first order

$$\text{i.e. } \delta_n = \beta_1(\bar{a}_1 e^{-\bar{k}_1 t_n}) + \beta_2(\bar{a}_2 e^{-\bar{k}_2 t_n}) + \lambda_1(-a_2 k_2 t_n e^{-\bar{k}_1 t_n}) + \lambda_2(-\bar{a}_2 \bar{k}_2 t_n e^{-k_2 t_n}) \quad \ldots\ldots\ldots\ldots (5)$$

$$\equiv \gamma_{n1}\beta_1 + \gamma_{n2}\beta_2 + \gamma_{n3}\lambda_1 + \gamma_{n4}\lambda_2 \qquad (n = 1 \text{ to } 16.)$$

For each point $P_n(t_n, y_n)$ of some 16 readings an 'equation of condition' of type (5) in β_1, β_2, λ_1, λ_2 was thus formed, t_n being calculated from P (the first of the four selected points for the preliminary exponential analysis) as zero, and δ_n being

$$y_n - (\bar{a}_1 e^{-k_1 t_n} + \bar{a}_2 e^{-k_2 t_n})$$

From the 16 equations of condition were formed as usual in the method of Least Squares[4] the four 'normal equations'

$$p_{r1}\beta_1 + p_{r2}\beta_2 + p_{r3}\lambda_1 + p_{r4}\lambda_2 = q_r \quad \ldots(6)$$
$$(r = 1,2,3,4)$$

where $$p_{rs} \equiv \sum_{n=1}^{16} \gamma_{nr} \cdot \gamma_{ns}$$

and $$q_r \equiv \sum_{n=1}^{16} \gamma_{nr} \cdot \delta_n$$

Equations (6) are then solved for β_1, β_2, λ_1, λ_2 either as ratios of determinants or by successive approximation, and the results inserted in (4) give the required best set of parameters a_1, a_2, k_1, k_2 whence equation (1).

For the approximate exponential analysis of the octyl glucoside graph the following set of four points was selected, the time for the first point P being taken as zero. P is chosen some distance from the beginning so as to avoid initial irregularities.

t (hours)	0	2·5	5	7·5
y	4·795	1·845	1·078	0·640

The values obtained were

$\bar{a}_1 = 3{\cdot}054$ $\bar{k}_1 = 0{\cdot}208$

$\bar{a}_2 = 1{\cdot}741$ $\bar{k}_2 = 1{\cdot}610$

After applying the probability correction, the equation for the curve became

$$y = 3{\cdot}13e^{-0{\cdot}213t} + 1{\cdot}65e^{-1{\cdot}84t}$$

from which $k_2/k_1 = 8{\cdot}6$. Table 2(A) shows the extent of agreement between the mean curve for the octyl glucoside on Fig. 1 and the values calculated from the final equation.

From these results it would appear that the original assumption that two exponential components are present is justified. The two β-glucosides, d-methyl-n-hexylcarbinyl d-glucoside and l-methyl-n-hexylcarbinyl d-glucoside, in the mixture are hydrolysed at different rates, and the ratio of the reaction constants (8·6) obtained by exponential analysis is in good agreement with the value (8·4) previously obtained (loc. cit.) when the two glucosides were hydrolysed separately.

Methyl-n-butylcarbinyl d-Glucoside

The alcohol was obtained by reduction of methyl-n-butyl ketone[5]. Tetraacetyl-β-methyl-n-butylcarbinyl d-glucoside was then prepared exactly as described by MITCHELL[1] for the methyl-n-hexylcarbinyl compound. M.P. 99°

Found C 55·8, H 7·3

$C_{20}H_{32}O_{10}$ requires C 55·6, H 7·4

Subsequent removal of the acetyl groups gave β-methyl-n-butylcarbinyl d-glucoside. M.P. 115°

Found C 54·6, H 9·0

$C_{12}H_{24}O_6$ requires C 54·6, H 9·1

For the hydrolysis experiment 0·0701 gm. of this glucoside was weighed into a 50 cc. graduated flask as described for the octyl glucoside, and a solution of emulsin (0·0504 gm. in 100 cc.) was added. Two cc. were withdrawn at intervals and the final reading was taken next day. The readings are given in Table 1(B). The four points selected from the graph on Fig. 1 were as follows

t (hours)	0	2	4	6
y	5·465	2·150	0·895	0·388

and the preliminary exponential analysis gave the values

$\bar{a}_1 = 3{\cdot}517$ $\bar{k}_1 = 0{\cdot}384$

$\bar{a}_2 = 1{\cdot}948$ $\bar{k}_2 = 0{\cdot}662$

After applying the probability correction the following equation was obtained.

$$y = 3{\cdot}49e^{-0{\cdot}398t} + 1{\cdot}97e^{-0{\cdot}602t}$$

from which $k_2/k_1 = 1{\cdot}5$. The agreement between the mean curve and the calculated values is shown in Table 2(B).

Methyl-ethylcarbinyl d-Glucoside

In this case the alcohol was obtained by the reduction of methyl-ethyl ketone[5]. Tetraacetyl-methyl-ethylcarbinyl d-glucoside was then prepared. M.P. 125°

Found C 53·7, H 7·0

$C_{18}H_{28}O_{10}$ requires C 53·5, H 6·9

Subsequent removal of the acetyl groups gave β-methyl-ethylcarbinyl d-glucoside. M.P. 95°

Found C 50·6, H 8·5

$C_{10}H_{20}O_6$ requires C 50·8, H 8·5

For the hydrolysis experiment 0·1311 gm. of this glucoside was used in 50 cc. of an emulsin solution containing 0·2026 gm. per 100 cc. The readings are given in Table 1(C). For these 1 cc. of solution was withdrawn at the times stated. The four points selected from the graph on Fig. 1 were as follows.

t (hours)	0	2	4	6
y	5·255	2·045	1·175	0·790

and the preliminary exponential analysis gave the values.

$\bar{a}_1 = 2{\cdot}152$ $\bar{k}_1 = 0{\cdot}170$

$\bar{a}_2 = 3{\cdot}103$ $\bar{k}_2 = 0{\cdot}899$

After applying the probability correction, the following equation was obtained.

$$y = 2{\cdot}16e^{-0{\cdot}166t} + 3{\cdot}08e^{-0{\cdot}909t}$$

from which $k_2/k_1 = 5{\cdot}5$. The agreement between the mean curve and the calculated values is shown in Table 2(C).

Table 1

A Octyl glucoside			B Hexyl glucoside			C Butyl glucoside		
Time mins.	T_n	$T_\infty - T_n$	Time mins.	T_n	$T_\infty - T_n$	Time mins.	T_n	$T_\infty - T_n$
0	0·00	6·98	0	0·00	7·08	0	0·00	7·015
5·0	0·24	6·74	6·0	0·18	6·90	6·5	0·25	6·765
26	2·185	4·795	28·5	1·31	5·77	27	1·58	5·435
48·5	3·29	3·69	49·5	2·15	4·93	48	2·675	4·340
68·5	3·875	3·105	71	2·895	4·185	69·5	3·41	3·605
90·5	4·22	2·76	92	3·545	3·535	111·5	4·34	2·675
111	4·53	2·45	113	4·09	2·99	132·5	4·76	2·255
132	4·77	2·21	134	4·48	2·60	152·5	4·95	2·065
153	4·98	2·00	155	4·915	2·165	172·5	5·225	1·790
176	5·115	1·865	179·5	5·305	1·775	194·5	5·38	1·635
227·5	5·465	1·515	204	5·60	1·48	215	5·54	1·475
278	5·715	1·265	226	5·705	1·275	236·5	5·635	1·380
311	5·84	1·14	305	6·34	0·74	258	5·79	1·225
362	6·02	0·96	336	6·525	0·555	321	6·04	0·975
401	6·17	0·81	376	6·65	0·43	366	6·16	0·855
479	6·355	0·625	419	6·735	0·345	409	6·258	0·757
525	6·42	0·56	460·5	6·845	0·235	440·5	6·337	0·678
577	6·51	0·47	565·5	6·975	0·105	501	6·405	0·610
∞	6·98	0·00	∞	7·08	0·00	560	6·485	0·530
						584	6·533	0·482
						∞	7·015	0·00

The first two values of each set were not used in making the probability correction in order to avoid possible initial irregularities, but such would seem to be small since agreement is still good, as indicated in Table 2 row 1, for the values of t, -0·1, -0·5, and -0·4 respectively taken from P as zero time.

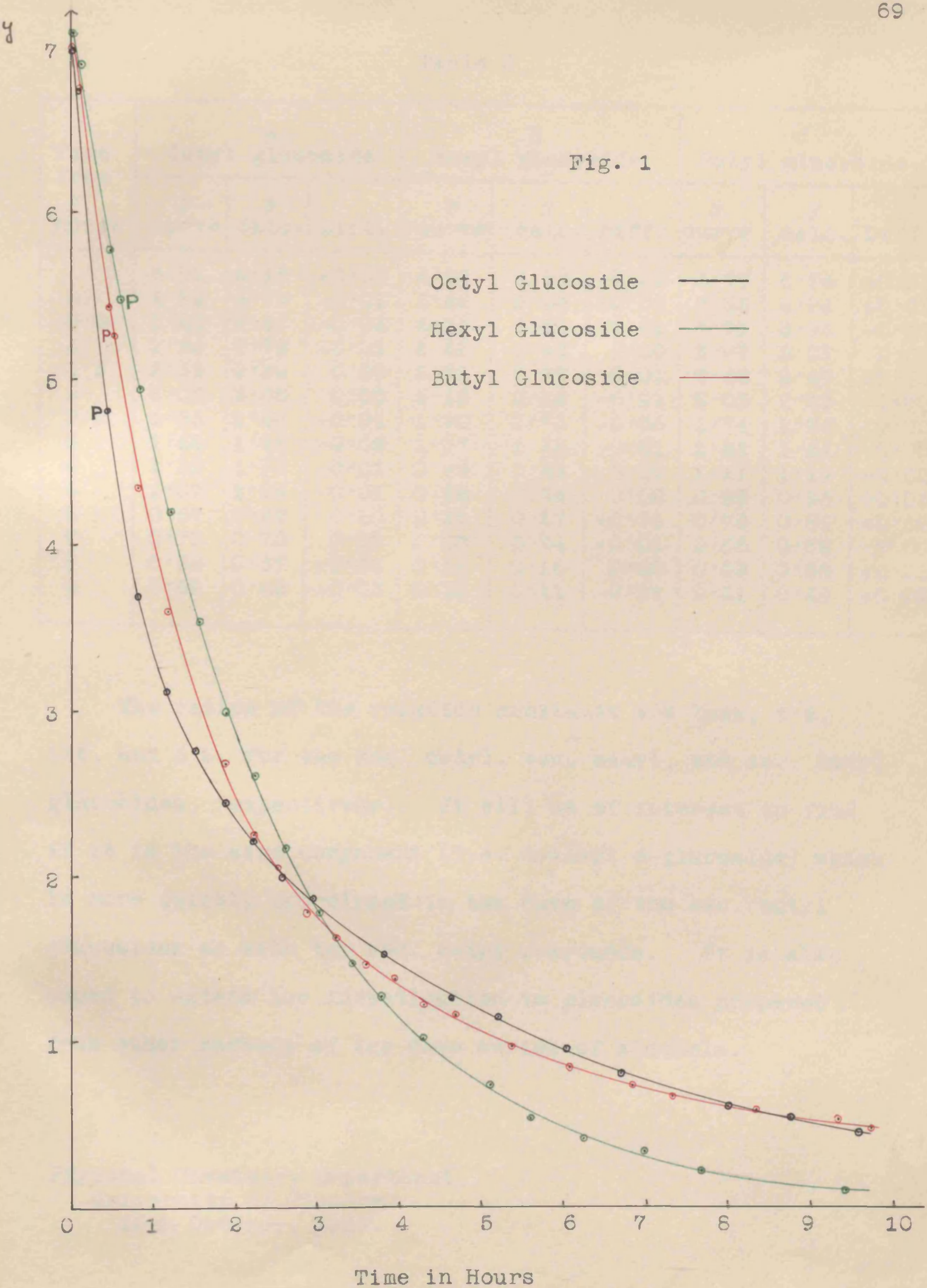

Fig. 1

y
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10
Time in Hours
Octyl Glucoside
Hexyl Glucoside
Butyl Glucoside
P
P
P

Table 2

Time from P hours	A Octyl glucoside			B Hexyl glucoside			C Butyl glucoside		
	y curve	y calc.	Diff.	y curve	y calc.	Diff.	y curve	y calc.	Diff.
	5·21	5·18	+0·03	6·90	6·92	−0·02	6·77	6·74	+0·03
0	4·79	4·78	+0·01	5·43	5·46	−0·03	5·25	5·24	+0·01
0·5	3·43	3·47	−0·04	4·33	4·32	+0·01	3·91	3·94	−0·03
1	2·80	2·79	+0·01	3·42	3·42	0·00	3·07	3·07	0·00
1·5	2·39	2·39	0·00	2·71	2·72	−0·01	2·46	2·47	−0·01
2	2·08	2·08	0·00	2·15	2·16	−0·01	2·05	2·05	0·00
2·5	1·85	1·86	−0·01	1·70	1·73	−0·03	1·74	1·74	0·00
3	1·64	1·66	−0·02	1·37	1·38	−0·01	1·51	1·51	0·00
4	1·32	1·33	−0·01	0·89	0·89	0·00	1·17	1·19	−0·02
5	1·07	1·08	−0·01	0·58	0·58	0·00	0·95	0·98	−0·03
6	0·87	0·87	0·00	0·38	0·37	+0·01	0·79	0·81	−0·02
7	0·70	0·70	0·00	0·25	0·24	+0·01	0·68	0·68	0·00
8	0·58	0·57	+0·01	0·16	0·16	0·00	0·59	0·58	+0·01
9	0·49	0·46	+0·03	0·10	0·11	−0·01	0·51	0·49	+0·02

The ratios of the reaction constants are thus, 8·6, 1·5, and 5·5, for the sec. octyl, sec. hexyl, and sec. butyl glucosides, respectively. It will be of interest to find if it is the same component (i.e. d-alkyl d-glucoside) which is more quickly hydrolysed in the case of the sec. butyl glucosides as with the sec. octyl compounds. It is also hoped to extend the investigation to glucosides prepared from other members of the same series of alcohols.

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15th October, 1930.

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