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Rotatory Dispersions of Some Steroids, Amino Acids and Peptides Using a New Photoelectric Spectropolarimeter

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A new photoelectric spectropolarimeter is described. The precision and accuracy of the instrument were determined from 2400 to 7500 Å. using a standard quartz plate. The standard deviation of each reading of the quartz plate was found to vary from ±0.001 to ±0.004° depending upon the wave length of the incident light. Dispersion data are also presented for several steroids, amino acids, and peptides and three constant equations are developed to fit the results.

Rotatory dispersion studies are valuable for purposes of identification and as criteria for purity.[4] We have obtained rotations from 2500 to 7500 Å. of several biochemically interesting substances using a new photoelectric spectropolarimeter which was developed by one of the authors (B) in cooperation with O. C. Rudolph and Sons of Caldwell, N. J.

The light from a Western Union K-100 zirconium arc lamp in quartz envelope, which is interchangeable with a General Electric Na-1 sodium lamp or a Hanovia 16A13 mercury quartz burner, is passed through a Beckman DU monochromator into a polarimeter. The monochromator has a special base and accessories to facilitate proper alignment of light source, monochromator and polarimeter. The polarimeter has quartz Rochon polarizer and analyzer prisms and an analyzer circle reading to 0.001° arc. No Lippich prism is used. Light intensity is measured by a photoelectric attachment consisting of a photoelectric tube housing allowing rapid interchange of RCA 1P21 and 1P28 photomultiplier tubes and a Photovolt photometer with special scale.[5] Optical rotations are measured by the method of symmetrical angles.[6] The instrument is Rudolph Model 200 S-80.

The precision and accuracy of this new polarimeter were tested between 2300 and 7500 Å. at 25° using a standard quartz plate from the National Bureau of Standards (Table I). The calculated values were obtained from the formula of Lowry, $\alpha^0/\text{mm.} = (9.5639/\lambda^2 - 0.0127493) - (2.3113/\lambda^2 - 0.000974) - 0.1905$[7] where λ is expressed in microns. The thickness of the plate was 0.0377 mm. This was determined from the reading at 5890 Å. as Lowry reports the rotation of quartz at this wave length to be 21.729°/mm.[8] The temperature correction for quartz at 5890 Å. is given by the expression $\alpha^t_D = \alpha^{20}_D + \alpha^{20}_D (0.000143\ [t - 20])$.[9] The corrections at other wave lengths were considered to be of the same relative size. A quartz plate of low rotation was chosen because most of the compounds measured at convenient concentrations had rotations of this same order of magnitude. Twenty-six readings were taken at each wave length and ten readings for each blank. The results were analyzed statistically to obtain the precision of each reading. For the 12 wave lengths between 2500 and 7000 Å. the average difference between the calculated and observed values was only 0.003° and the average standard deviation of each reading between 2400 and 7500 Å. was ± 0.002°.

TABLE I

ROTATORY DISPERSIONS OF QUARTZ AND SUCROSE

Wave length, Å.	Quartz α^0/.0377 mm. Calcd.[a]	Quartz α^0/.0377 mm. Obsd.	Quartz Std. Dev. Reading	Quartz Std. Dev. Blank	Quartz Total Dev.	Sucrose α^0 Calcd.[b]	Sucrose α^0 Obsd.
7500	0.493	0.503	0.003	0.003	0.006	0.480	0.480
7000	.570	.572	.002	.001	.003	.554	.550
6500	.666	.664	.002	.003	.005		
6000	.789	.783	.002	.004	.006		
5890	.820	.819	.003	.003	.006	.798	.797
5500	.949	.946	.002	.001	.003		
5460						.939	.939
5000	1.163	1.166	.002	.001	.003	1.136	1.131
4500	1.461	1.467	.003	.003	.006		
4000	1.894	1.896	.001	.001	.002	1.872	1.868
3500	2.562	2.561	.002	.002	.004	2.566	2.566
3000	3.683	3.685	.002	.002	.004	3.780	3.782
2800	4.360	4.367	.002	.004	.006		
2500	5.825	5.828	.003	.002	.005	6.303	6.288
2400	6.495	6.521	.004	.003	.007		
2300	7.296	7.340	.016	.005	.021		

[a] Calculated values are corrected for 25°. [b] Calculated values are corrected for 25° and a concentration of 1.2 g. per 100 ml.

The accuracy of our determinations was further checked using 3 ml. of standard sucrose solutions at a concentration of 1.2 g. in 100 ml. This concentration was chosen because it was the lowest at which the instrument error of $\pm 2 \times 0.002°$ would never be more than 1% of the actual reading of the sucrose solution. The calculated values were obtained from the equation $[\alpha] = 21.648/\lambda^2 - 0.0213$[10] where $[\alpha] = 100\alpha/cl$. The temperature correction is independent of wave length and the

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(4) General references in optical activity and rotatory dispersion: (a) T. M. Lowry, "Optical Rotatory Power," Longmans, Green and Co., New York, 1935; (b) W. Heller in A. Weissberger's "Physical Methods of Organic Chemistry," Vol. 2, first edition, Interscience Publishers, New York, N. Y., 1946, pp. 869–987; (c) P. A. Levene and A. Rothen in H. Gilman's "Organic Chemistry," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1938, pp. 1779–1850; (d) "Optical Rotatory Power," A General Discussion of the Faraday Society, April, 1930; (e) J. Kauzmann, J. E. Walter and H. Eyring, *Chem. Revs.*, **26**, 339 (1940).

(5) A more detailed description of this equipment and the methods of measurement will appear in separate papers in forthcoming issues of the *J. Opt. Soc. Am.*

(6) *Cf.* ref. 4b, p. 969.

(7) *Cf.* ref. 4a, p. 258.

(8) *Cf.* ref. 4a, p. 257.

(9) F. J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars," Circular of the National Bureau of Standards, C440, United States Government Printing Office, Washington, 1942, p. 92.

(10) *Cf.* ref. 4a, p. 131.

TABLE II

ROTATORY DISPERSIONS OF SEVERAL STEROIDS

Wave length, Å.	[M] Cholesterol $CHCl_3$ $t = 25°$, c 1 Calcd.	Obsd.[b]	Cholesterol CH_3OH $t = 25°$, c 0.24 Calcd.	Obsd.[b]	Cortisone acetate $CHCl_3$ $t = 28°$, c 0.25 Calcd.	Obsd.[c]	Cortisone acetate CH_3OH $t = 25°$, c 0.25 Calcd.	Obsd.[c]
7500	−89.4	−90.7			515	527	463	466
7000	−104	−106	−71	−74	607	603	546	546
5890	−154	−154[d]	−105	−109	936	936[d]	847	845[d]
5000	−228	−227	−156	−156[d]	1469	1469[d]	1342	1339
4000	−405	−405[d]	−280	−280[d]	2996	3085	2869	2864[d]
3500	−594	−592	−414	−425	5152	5152[d]	5274	5329
3400					6205	5997	3523	3523[d]
3000	−981	−984[d]	−693	−693[d]				
2800	−1266	−1248	−901	−908				
2600			−1238	−1243				
λ_0, Å.	1830		1860		2480		2720	

[a] The chloroform and methanol used were spectro grade Eastman #S-337 and S-467. [b] Average of six determinations. [c] Average of two determinations which did not differ by more than 1%. [d] Indicates values used to calculate equations.

coefficient is $-0.000184 + 0.0000063\ (t - 20)$.[11] The concentration correction at 5890 Å. and 20° is $66.462 + 0.00870\ (c) - 0.000235\ (c^2)$.[12] The correction at other wave lengths was considered to be of the same relative size. From 3000 through 7500 Å. the largest difference between the observed and calculated readings was 0.005°. The average difference was 0.002°. The comparatively large deviation of 0.015° at 2500 Å. is probably due to the effect of stray light which passes through the wider slits necessary in the ultraviolet.

Drude expressed the relationship between optical rotation and wave length as $\alpha = k/\lambda^2 - \lambda_0^2$ where k is known as the rotation constant, λ the wave length of the incident light, and λ_0 the dispersion constant which corresponds to the wave length of the nearest optically active absorption band. The rotation of a compound can be considered as the sum of the contributions of the partial rotations each of which is caused by a different optically active absorbing center, $\alpha = \Sigma k/\lambda^2 - \lambda_n^2$. The dispersion is defined as normal if the rotatory power increases numerically with decreasing wave length and if α, $d\alpha/d\lambda$, and $d^2\alpha/d\lambda^2$ remain constant in sign throughout the range of wave lengths to which the medium is transparent. Anomalous dispersion in a region outside an absorption band is produced by partial rotations of opposite sign. Within an optically active band the unequal indices of extinction for dextro and levo circularly polarized rays cause anomalous dispersion, an effect known as the Cotton effect.

Rotatory dispersion data may be analyzed algebraically and graphically. The Drude equation in its reciprocal form is an equation for a straight line and it is convenient to plot $1/\alpha$ against λ^2. A straight line is usually obtained in the visible region of the spectrum; some deviation from linearity usually occurs in the ultraviolet regions where the rotation is influenced by more than one optically active absorbing center or where the Cotton effect may begin to be seen. The value of λ_0 may be obtained from the x(or λ^2) intercept of the extrapolated curve. The shape of the curve determines the number of terms in the Drude equation necessary for correct expression of the data.[13,14] Two, three and four constant equations of the type

$$\alpha = \frac{k}{\lambda^2 - \lambda_0^2},\ \alpha = \frac{k}{\lambda^2 - \lambda_0^2} \pm \frac{k}{\lambda^2},\ \alpha = \frac{k}{\lambda^2 - \lambda_1^2} \pm \frac{k}{\lambda^2 - \lambda_2^2}$$

can be calculated. Usually no more than two terms are needed to express the results, one corresponding to the partial rotation of the band with the longest wave length and the other to the sum of the other partial rotations. The λ_0 values have no physical significance unless the equation expresses the results close to the region of absorption.

The following compounds were studied from 2500 to 7500 Å.: cholesterol,[15] cortisone acetate,[16] the L-isomers of alanine, glutamic acid, lysine, ornithine and tyrosine[17] and several di- and tripeptides. The results are expressed algebraically. All of the curves obtained, except that of tyrosine, show normal dispersion with a small deviation from linearity in the ultraviolet. The tyrosine curve is anomalous. With decreasing wave length the rotation goes through a minimum, becomes zero, changes sign and approaches $+\infty$. Good agreement is obtained between observed values and those calculated from 3 constant Drude equations. The values of λ_0 calculated from the equations are compared with the wave lengths of maximum absorption reported in the literature.

The results with steroids (Table II) indicate that the presence of many asymmetric centers is not incompatible with normal dispersion. Methanol as well as chloroform was used as a solvent since absorption by chloroform does not permit readings below 2800 Å. The dispersion equations for cholesterol, calculated from results at three wave lengths, are

$$[M]_{CHCl_3} = -\frac{71.6}{\lambda^2 - 0.0335} + \frac{25.8}{\lambda^2};$$

$$[M]_{CH_3OH} = -\frac{50.0}{\lambda^2 - 0.0347} + \frac{19.9}{\lambda^2}$$

(11) *Cf.* ref. 9, p. 93.

(12) *Cf.* ref. 9, p. 82.

(13) H. Hunter, *J. Chem. Soc.*, **125**, 1198 (1924).

(14) *Cf.* ref. 4a, pp. 419–422.

(15) Kindly supplied by Dr. Louis Fieser of Harvard University. He reports [M]D in $CHCl_3$ = −151.

(16) Kindly supplied by Dr. Max Tishler of Merck and Co., Rahway, N. J.

(17) Kindly supplied by Dr. W. H. Stein of Rockefeller Institute, *cf.* THIS JOURNAL, **64**, 724 (1942), described as commercial sample recrystallized from hydrochloric acid and ammonium acetate.

TABLE III

ROTATORY DISPERSIONS OF SEVERAL DIPEPTIDES IN 0.5 N HCl, $[\alpha]^{25}_{c,1.5\text{-}3.6}$

Wave length, Å.	H·Ala-Ala·OH $[\alpha] = -\frac{61.0}{\lambda^2-.0085}(\text{LL}) + \frac{50}{\lambda^2}$ c, 2 Calcd.	Obsd.[a]	$[\alpha] = +\frac{37.3}{\lambda^2-.0345}(\text{LD}) - \frac{16}{\lambda^2}$ c, 2 Calcd.	Obsd.[b]	H·Glu-Glu·OH $[\alpha] = +\frac{6.69}{\lambda^2-.0521}(\text{LL}) - \frac{1.8}{\lambda^2}$ c, 2.5 Calcd.	Obsd.[c]	$[\alpha] = +\frac{27.0}{\lambda^2-.0304}(\text{LD}) - \frac{12}{\lambda^2}$ c, 3.6 Calcd.	Obsd.[c]	H·Orn-Orn·OH·2HCl $[\alpha] = \frac{-26.6}{\lambda^2-.0328}(\text{DL}) + \frac{11}{\lambda^2}$ c,1.6 Calcd.	Obsd.[d]
7500					9.50	10.0	30.1	30.9	−30.6	−30.8
7000	−25.0	−25.0	49.5	48.5	11.5	11.6	35.1	35.0	−35.7	−35.4
5890	−36.9	−36.8[e,i]	73.7	73.7[f,i]	17.4	17.4[g,i]	51.8	51.8[h,i]	−52.9	−52.9[i,j]
5000	−53.6	−54.1[j]	110	110[f]	26.4	26.4[j]	76.5	76.5[j]	−78.4	−78.4
4000	−91.7	−92.4	199	198	50.5	50.5	136	135	−140	−140[j]
3500	−130	−131	295	294	80.0	81.0	198	198	−207	−207
3000	−196	−194[j]	507	498	156	156[j]	324	324[j]	−343	−343[j]
2800	−238	−227	651	651[j]	230	227	414	413	−443	−447
2700									−512	−523
λ_0 Å.	920		1860		2280		1740		1810	

[a] Average of four determinations. [b] Average of two determinations. [c] One determination. [d] One determination. Specific rotation calculated on the basis of the free peptide. [e] Rotation previously reported (*cf.* ref. 22a) with $[\alpha]^{24}_{c,2}$D −37.3° in 0.5 N HCl. [f] Rotation previously reported (*cf.* ref. 22a) with $[\alpha]^{23}_{c,2}$D +74.1° in 0.5 N HCl. [g] Rotation previously reported (*cf.* ref. 22b) with $[\alpha]^{24}_{c,1\text{-}2}$D +18.2° in 0.5 N HCl. [h] Rotation previously reported (*cf.* ref. 22b) with $[\alpha]^{24}_{c,1\text{-}2}$D +56.4° in 0.5 N HCl. [i] Rotation previously reported (J. Polatnick, Doctoral Dissertation) with $[\alpha]^{23}_{c,2}$D −52.3° in 0.5 N HCl. [j] Indicates values used to calculate equations.

TABLE IV

ROTATORY DISPERSIONS OF SEVERAL TRIPEPTIDES IN 0.5 N HCl, $[\alpha]^{25}_{c,1.5\text{-}2.5}$

Wave length, Å.	H·Glu-Ala·OH (└Ala·OH) $[\alpha] = +\frac{41.2}{\lambda^2-.0352}(\text{LD}) - \frac{13.7}{\lambda^2}$ c,2.5 Calcd.	Obsd.[a]	$[\alpha] = +\frac{13.3}{\lambda^2-.0401}(\text{LD}) - \frac{2.6}{\lambda^2}$ c,2.4 Calcd.	Obsd.[a]	$[\alpha] = +\frac{10.7}{\lambda^2-.0440}(\text{LL}) - \frac{.53}{\lambda^2}$ c,2.1 Calcd.	Obsd.[a]	H·Ala-Orn-Ala·OH·HCl $[\alpha] = \frac{-30.9}{\lambda^2-.0379}(\text{DLL}) + \frac{11.5}{\lambda^2}$ c,1.7 Calcd.	Obsd.[a,b]	$[\alpha] = \frac{-26.6}{\lambda^2-.0264}(\text{LLL}) + \frac{12.7}{\lambda^2}$ c,1.9 Calcd.	Obsd.[a,b]
7500	53.9	54.8	20.8	20.1	19.8	19.9	−38.4	−38.8	−27.1	−27.4
7000	62.8	62.1	24.2	24.2	23.0	23.3	−44.8	−44.1	−31.6	−31.4
5890	93.0	92.5[c]	35.8	35.4[d]	33.9	33.9[e,h]	−66.6	−66.6[f,h]	−46.5	−46.5[g,h]
5000	137	137[h]	52.9	52.9[h]	50.0	50.2	−99.5	−99.2	−68.4	−68.3
4000	245	245[h]	94.6	94.6[h]	89.3	89.3[h]	−180	−180[h]	−120	−120[h]
3500	361	361	160	157	132	133	−271	−270	−174	−173
3200	480	480[h]								
3000			238	238[h]	228	228[h]	−463	−463[h]	−278	−278
2800			314	311	305	301	−615	−617	−351	−351[h]
2750							−666	−668		
2700					364	361				
λ_0, Å.	1880		2000		2090		1950		1630	

[a] One determination. [b] Calculated on the basis of the free peptide. [c] Rotation prevously reported (*cf.* ref. 22c) with $[\alpha]^{24}_{c,2}$D +92.2° in 0.5 N HCl. [d] Rotation previously reported (*cf.* ref. 22c) with $[\alpha]^{25}_{c,2}$D +34.5° in 0.5 N HCl. [e] Rotation previously reported (*cf.* ref. 22c) with $[\alpha]^{23}_{c,2}$D +32.8° in 0.5 N HCl. [f] Previously reported (J. Polatnick, Doctoral Dissertation) with $[\alpha]^{23}_{c,2}$D −65.4° in 0.5 N HCl. [g] Previously reported (J. Polatnick, Doctoral Dissertation) with $[\alpha]^{23}_{c,2}$D −46.7° in 0.5 N HCl. [h] Indicates values used to calculate equations.

The value of λ_0 is equal to the square root of 0.0335 or 1830 Å for the chloroform results, and to the square root of 0.0347 or 1860 Å. for the methanol results. Heilbron, *et al.*,[18] report that cholesterol has no marked selective absorption down to 2000 Å.

The dispersion of cholesterol was reported in 1910 by Tschugaeff at four wave lengths between 4800 and 6500 Å. The results at 5890 Å. were 15% lower than those found in this investigation.[19]

The two equations calculated from the cortisone acetate results are

$$[M]_{CHCl_3} = +\frac{378}{\lambda^2-0.0616} - \frac{135}{\lambda^2};$$

$$[M]_{CH_3OH} = +\frac{280}{\lambda^2-0.0741} - \frac{62.1}{\lambda^2}$$

The λ_0 values are 2480 and 2720 Å., respectively. The reported absorption maximum is at 2380 Å.[20]

Five dipeptides and five tripeptides, including several sets of optical isomers, were analyzed. The specific rotations at 25° and a concentration of 2 g. in 100 ml. are reported (Tables III, IV).[21,22]

(18) I. Heilbron, R. Morton and W. Sexton, *J. Chem. Soc.*, 47 (1928).

(19) L. Tschugaeff and W. Formin, *Ann.*, **375**, 288 (1910).

(20) L. H. Sarett, *J. Biol. Chem.*, **162**, 630 (1946).

(21) The following abbreviations and symbols are used (*cf.* H. Sachs and E. Brand, THIS JOURNAL, **75**, 4608 (1953)); Ala, NHCH(CH$_3$)CO, C_3H_5ON; Glu, NHCH(CH$_2$CH$_2$COOH)-CO, $C_5H_7O_3N$; Orn, NHCH($C_3H_6NH_2$)CO, $C_5H_{10}ON_2$; peptide linkage indicated by dash, -; configuration follows compound in parentheses, ( ). When the γ-carboxyl group of glutamic acid is substituted, the following symbol is used for the residue: Glu. *e.g.*, L-glutamyl-α-L-alanine-γ-D-alanine: H·Glu(└OH)-Ala·OH (LDL); α-L-glutamyl-D-glutamic acid: H·Glu(└Ala·OH)-Glu·OH (LD); L-glutamic acid: H·Glu·OH (L).

(22) The peptides are reported in the following papers: (a) B. F. Erlanger and E. Brand, *ibid.*, **73**, 3508 (1951); (b) H. Sachs and E. Brand, *ibid.*, **75**, 4608 (1953); (c) H. Sachs and E. Brand, *ibid.*, **76**, 1811 (1954); (d) J. Polatnick and E. Brand, to be published.

TABLE V

ROTATORY DISPERSIONS OF L-α-AMINO ACIDS IN 0.5 *N* HCl,[a] $[\alpha]_{c,2}^{25}$

Wave length, Å.	H·Glu·OH $[\alpha] = \frac{9.8}{\lambda^2 - .0482} - \frac{.32}{\lambda^2}$ Calcd.	Obsd.	H·Ala·OH $[\alpha] = \frac{6.0}{\lambda^2 - .0541} - \frac{2.1}{\lambda^2}$ Calcd.	Obsd.	H·Lys·OH $[\alpha] = \frac{8.8}{\lambda^2 - .0493} - \frac{1.3}{\lambda^2}$ Calcd.	Obsd.	H·Orn·OH $[\alpha] = \frac{9.3}{\lambda^2 - .0489} - \frac{1.1}{\lambda^2}$ Calcd.	Obsd.	H·Tyr·OH $[\alpha] = \frac{2.3}{\lambda^2 - .0826} - \frac{6.8}{\lambda^2}$ Calcd.	Obsd.
7500	18.4	18.6	8.00	8.70	14.8	15.9	16.1	16.9		
7000	21.4	21.1	9.40	9.43	17.3	17.6	18.8	18.8	−8.26	−7.95
5890	31.8	31.7[b]	14.3	14.3[b]	25.8	25.8[b]	28.0	28.0[b]	−11.0	−11.0[b]
5000	47.1	47.2	22.1	21.9	38.6	38.5	41.8	41.7	−13.6	−13.8
4700									−14.3	−14.3
4500									−14.6	−14.6[b]
4300									−14.5	−14.4
4000	85.3	85.3[b]	43.3	43.3[b]	71.3	71.3[b]	76.7	76.7[b]	−13.1	−12.9
3520									−0.06	+1.16
3500	129.0	130.0	70.0	70.9	109	110	117	117	+1.22	+2.76
3300									+23.2	+23.2[b]
3000	230	231	144	146	202	202	214	213		
2800	319	319[b]	220	220[b]	285	285[b]	300	300[b]		
2700	391	393								
2600			414	389	461	458				
2500							664	650		
λ_0, Å.	2200		2330		2220		2210		2870	

[a] All results are the average of two determinations. [b] Indicates values used to calculate equations.

The calculated values of λ_0 lie between 900 and 2300 Å. Saidel and Goldfarb report a peptide bond absorption band near 1850 Å.[23]

The rotatory dispersions of the α-amino acids have been studied by Patterson and Brode between 4400 and 6600 Å.[24] We have extended this study to sixteen wave lengths between 2500 and 7500 Å. (Table V). The first term of the calculated equations is positive for each of the five L-acids and the λ_0 values are between 2200 and 2900 Å. The absorption of amino acids, *i.e.*, alanine, valine, leucine, glycine, cystine, in the ultraviolet starts at approximately 2500 Å. and continues to rise at 1800 Å.[25]

The tyrosine curve is an excellent example of anomalous dispersion outside an absorption band and is due to two rotatory contributions of opposite sign. The presence of an aromatic nucleus also causes anomalous dispersion in a series of alcohols, reported by Pickard and Kenyon.[26] The dispersion of these aromatic alcohols was very susceptible to temperature changes. Tyrosine was run at 25 and 20°. The results at 25° are about 14% higher than those at 20°. The dispersion ratio is the same. The three-constant equation calculated from the results at 25° does not express the data very well. A four-constant equation was attempted with three different sets of four values but each time an imaginary result was obtained. The reported absorption peak of tyrosine is at 2740 Å.[27]

(23) L. J. Saidel and R. Goldfarb, *Science*, **114**, 156 (1951).

(24) J. W. Patterson and W. R. Brode, *Arch. Biochem.*, **2**, 247 (1943).

(25) H. Ley and B. Arends, *Z. physik. Chem.*, **B17**, 177 (1932).

(26) R. H. Pickard and J. Kenyon, *J. Chem. Soc.*, **105**, 1115 (1914).

(27) N. Kretchmer and R. Taylor, THIS JOURNAL, **72**, 3291 (1950).

Experimental

All dilutions were made in 3-ml. volumetric flasks. One decimeter glass polarimeter tubes with quartz end plates were used. The strain effect on the quartz was minimized by adjusting the end plates so that the empty tube never read more than ±0.02° from the zero reading of the instrument at any wave length. The blank was read in the same tube without changing the strain on the end plates or the position of the tube in the trough. To eliminate the determination of a blank for each set of data several tubes with cemented quartz end plates were tried, but none was satisfactory. A water jacketed trough was developed and used in conjunction with an Aminco constant temperature bath. All solvents were kept in the bath and the filled polarimeter tube was allowed to stand in the trough for 20 minutes before readings were taken. About one hour is needed to obtain data at 10 wave lengths.

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