CLXXVIII. THE OPTICAL PROPERTIES OF SOME BILE ACIDS.

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THE rotatory power of the bile acids has acquired a new practical interest in connection with the method of Jenke [1928] for their determination in bile. For most of these acids and their sodium salts the optical rotations have been determined by several investigators, but except for one [Hoppe-Seyler, 1863] all have used only sodium light. In many cases the figures given in the different publications also show quite large differences.

The principle of Jenke's method is, (1) hydrolysis of the conjugated bile acids with strong alkalis, (2) extraction with ether-light petroleum of the free acids precipitated by acidification, and (3) polarimetric determination of the extracted unconjugated acids in alcoholic solution. This alcoholic solution however usually contains a considerable amount of bile pigments, which are optically inactive but make polarimetric reading in sodium light difficult or impossible. As will be shown in a later publication it is even possible in many cases by selection of light of suitable wave length to make the polarimetric determination of the bile acids as salts in the alkaline hydrolysis solution without the time-consuming acid extraction.

The determination of the rotatory dispersion of the common bile acids is also of interest as an addition to other well-known and characteristic physical data for these substances; in particular it seemed desirable to compare the specific rotations and rotatory dispersions of the bile acids and their salts in different solvents.

An investigation of the rotatory dispersions of glycocholic, taurocholic and cholic acids and their salts was made by Hoppe-Seyler [1863]: his substances however were probably impure, as will be seen from the description of their preparation, and his polarimetric apparatus was rather primitive. His values were thus very irregular and do not agree well with those found by later investigators including myself.

My determinations were made in a Schmidt and Haentsch Lippich-polarimeter readable to 0.01° and with a Hoffmann monochromator. Half-shadow angle 5° . The instrument was tested at the various wave lengths with a controlled quartz prism. The temperature was $20 \pm 1^\circ$. All observations were made in 4 dm. tubes.

The following wave lengths were used: 6863 (B), 6563 (C), 5893 (D), 5463 (Hg), 4861 (F) and 4388 (Hg) Å.

The substances examined were the same as in earlier work, where description of the preparations and analyses can be found [Josephson, 1933]. Cholic, deoxycholic, glycocholic and taurocholic acids and their sodium and potassium salts were investigated.

The salts were examined in aqueous solution of 0.5, 1, 2.5, 5 (and 15) % concentration calculated on the bile acids. The cholates and deoxycholates were also tested in KCl solutions and in excess of sodium and potassium hydroxides of various concentrations. The free acids were examined in alcoholic solutions

of various concentrations, depending on their solubilities. Cholic and deoxycholic acids were also examined in solution in acetic acid, acetone, chloroform, benzene and ether. The solubilities in benzene and ether are however so low that no satisfactory values could be obtained, and these observations are therefore not recorded.

The specific rotations and dispersion coefficients found, $\frac{[\alpha]_{\lambda}}{[\alpha]_{6867}}$, are shown in the tables.

For *cholic acid* in alcoholic solution Hoppe-Seyler found $[\alpha]_D$ 33.9°, Pregl [1910] 35.1° and Vahlen [1895] 37.0° independent of the concentration. Pregl's value agrees very well with mine ($[\alpha]_D$ 35.07°, Table I). In acetic acid and

Table I.

Cholic acid.

Solvent ... <i>c</i>	Alcohol 0.432-1.440		Acetic acid 0.5-5.0		Acetone 0.277-1.107		Chloroform 0.197 %	
	$[\alpha]$	<i>d</i>	$[\alpha]$	<i>d</i>	$[\alpha]$	<i>d</i>	$[\alpha]$	<i>d</i>
6867 λ	25.4	—	26.70	—	28.13	—	15.77	—
6563	27.78	1.09	29.21	1.09	30.71	1.09	17.77	1.13
5893	35.07	1.38	37.52	1.40	37.94	1.35	24.11	1.53
5463	41.67	1.64	45.60	1.69	45.88	1.63	30.46	1.93
4861	55.03	2.17	59.19	2.22	60.52	2.15	45.69	2.90
4388	73.44	2.89	77.51	2.89	79.04	2.81	71.07	4.51

In this and following tables $c = g./100$ ml. solution and $d = \frac{[\alpha]_{\lambda}}{[\alpha]_{6867}}$.

acetone the rotation and dispersion were very similar to those in alcohol, but in chloroform the rotation was much less, and the dispersion larger.

For Na cholate in water from 30 to 9 % concentration Hoppe-Seyler found $[\alpha]_D$ 26.0-28.5° and for K cholate $[\alpha]_D$ 27.2-31.8°. Vahlen found the rotation of a 4 % solution of the Na salt to be $[\alpha]_D$ 27.6° and of a 3 % solution of K salt $[\alpha]_D$ 27.0-27.6°. My values (Table II) coincide fairly well with those of Vahlen

Table II.

Na cholate in water. $[\alpha]$

<i>c</i>	15	5	2.5	1	0.5	<i>d</i>
6867 λ	21.05	21.10	21.50	22.50	23.50	—
6563	22.75	22.80	23.30	24.25	25.00	1.08
5893	28.80	28.85	29.50	31.00	32.50	1.37
5463	34.18	34.25	35.10	37.50	39.50	1.65
4861	45.68	45.80	46.80	49.75	50.50	2.18
4388	57.62	57.75	59.50	62.75	66.00	2.78

but not with those of Hoppe-Seyler. I found the optical activity of the Na and K salts dissolved in water to be very similar, the latter being about 1-2 % lower than the former. For want of space the K values therefore are not recorded. Between 15 and 5 % the activity of the salts seemed to be independent of the concentration, but below 5 % the activity increased with decreasing concentration, and below about 1.5 % this increase became rather large. It therefore seems as if there were some alteration in the molecular state at this concentration limit. The rotatory dispersion of the salts was independent of the concentration and very similar to that of the free acid. As shown in Table III the activity was only very slightly changed by the presence of other electrolytes (KOH, KCl, NaOH).

Table III.

K cholate, $c=2.5$. $[\alpha]$

Solvent	...	Water	KOH 0.5 N	KOH 1.0 N	KCl 1.0 M	KCl 1.7 M
6867 λ		21.50	21.40	21.40	21.40	21.40
6563		23.40	23.30	23.30	23.40	23.30
5893		29.70	29.70	29.40	29.50	29.40
5463		35.20	35.70	34.70	34.90	34.70
4861		46.90	46.40	46.70	46.90	46.70
4388		59.80	59.60	59.20	59.40	59.20

Table IV.

Deoxycholic acid.

Solvent	...	Alcohol 0.5-5.0	Acetic acid 0.212-0.849	Acetone 0.240-0.960	Chloroform 0.213-0.485
c		$[\alpha]$ d	$[\alpha]$ d	$[\alpha]$ d	$[\alpha]$ d
6867 λ		40.91 —	40.47 —	40.63 —	32.11 —
6563		44.30 1.08	44.17 1.09	43.49 1.07	35.29 1.10
5893		55.56 1.36	55.06 1.36	55.99 1.38	44.71 1.39
5463		65.49 1.60	66.25 1.64	66.15 1.63	52.94 1.65
4861		86.60 2.12	87.16 2.15	86.72 2.13	74.12 2.31
4388		109.85 2.69	110.49 2.73	110.68 2.72	117.64 3.66

For *deoxycholic acid* (Table IV) the following figures for $[\alpha]_D$ have been recorded: Langheld [1908] 53.28°; Vahlen [1895] 49.86°; Pregl [1910] 51.86°; Fischer [1911] 55.20°; Wieland and Sorge [1916] 57.02°. No examination of its rotatory dispersion seems to have been published. I found the specific rotation as well as the rotatory dispersion to be almost identical in alcoholic, acetic acid and acetone solutions. As for cholic acid the specific rotation was less and the dispersion higher in chloroform than in alcoholic solution. For this acid also the rotation and the dispersion were rather similar to those of the Na (Table V) and

Table V.

Na deoxycholate in water. $[\alpha]$

c	5	2.5	1	0.5	d
6867 λ	32.70	32.90	34.25	38.00	—
6563	35.90	36.10	37.50	41.50	1.10
5893	45.40	45.70	47.50	52.50	1.39
5463	53.70	54.00	56.00	62.00	1.64
4861	69.25	69.60	72.50	80.50	2.12
4388	88.30	89.00	93.75	104.00	2.72

Table VI.

Deoxycholates, $c=1$. $[\alpha]$

Salt	...	Na	Na	Na	K	K	K	K
Solvent	...	Water	NaOH 0.2 N	NaOH 0.4 N	Water	KOH 0.5 N	KCl 0.25 M	KCl 0.75 M
6867 λ		34.25	32.50	30.75	35.50	30.00	32.50	28.00
6563		37.50	35.75	34.00	39.00	32.75	35.50	30.75
5893		47.50	45.00	42.50	49.25	41.50	44.50	40.25
5463		56.00	53.25	50.50	58.00	46.75	53.00	48.50
4861		72.50	68.75	65.25	74.00	63.75	68.50	60.00
4388		93.75	89.00	84.50	96.25	—	—	—

the K (Table VI) salts in water, the K salt being about 2-4 % more active than the Na salt. As for the cholates the specific rotation increased with decreasing

concentration, and this increase was much more rapid below a concentration of about 2 %, indicating some change in the molecule. The dispersions were very similar for the free acid and the alkali salts, and somewhat less than that of cholic acid. The deoxycholates are much more sensitive to other electrolytes in the solution than the cholates; it seems however as if the decrease in optical activity caused by electrolytes depends more on their concentration than on their chemical nature (Table VI), as it is approximately proportional to the molecular concentration of the added electrolyte.

Glycocholic acid and its sodium salt have been investigated by Hoppe-Seyler and by Letsche [1909]. The former found $[\alpha]_D$ for the free acid in alcoholic solution to be 29.0° and for the Na salt in water 21.8° , both independent of the concentration. Letsche found for the free acid $[\alpha]_D$ 32.3° and for the salt in 8.5 % aqueous solution 24.5° . The figures of Letsche agree well with mine. I did not, as for cholic and deoxycholic acids, find the rotation of the free acid in alcohol to be independent of the concentration; there was a slight increase in activity with decreasing concentration. Even the rotatory dispersion increased slightly with decreasing concentration (Table VII). The Na and K salts in

Table VII.

Glycocholic acid in alcohol.

c	10.0		5.0		2.5		1.0		0.5	
	$[\alpha]$	d	$[\alpha]$	d	$[\alpha]$	d	$[\alpha]$	d	$[\alpha]$	d
6867 λ	22.55	—	22.60	—	22.60	—	22.75	—	22.75	—
6563	25.40	1.13	25.50	1.13	25.50	1.13	25.50	1.13	25.50	1.12
5893	31.85	1.41	32.30	1.43	32.50	1.44	33.50	1.48	33.50	1.48
5463	37.75	1.67	38.25	1.69	38.80	1.72	39.50	1.74	40.00	1.77
4861	49.05	2.18	49.75	2.20	50.40	2.23	51.50	2.27	52.00	2.30
4388	63.10	2.80	64.30	2.85	65.80	2.91	67.75	2.99	69.00	3.03

water also showed an increase at decreasing concentration, which was obviously larger below about 1.75 %. The activities of the Na and K salts were almost identical, and the dispersions seemed to be nearly independent of the concentration (Table VIII). The dispersion was also somewhat larger for the free acid than for the salts.

Table VIII.

Na glycocholate in water. $[\alpha]$

c	15.0	5.0	2.5	1.0	0.5	d
6867 λ	16.93	18.90	20.10	23.00	25.00	—
6563	18.88	20.90	22.20	25.00	27.00	1.09
5893	24.29	26.35	27.80	32.00	34.00	1.38
5463	28.31	31.35	32.90	37.50	40.50	1.64
4861	36.82	40.80	43.40	49.50	52.50	2.14
4388	48.82	53.85	56.70	66.00	73.00	2.86

Taurocholic acid has been examined by Kazumo and Yamazaki [1934], who found $[\alpha]_D$ 38.8° in diluted alcohol, but this value was calculated from one reading ($\alpha = +0.2^\circ$), and they do not mention the concentration of the alcohol. The specific rotation of the Na salt was measured by Hoppe-Seyler, who found $[\alpha]_D$ in water 22.4° independent of the concentration, and by Hammarsten [1909], who found $[\alpha]_D$ 23.27 – 24.14° between 2.32 and 5.42 %. Tanaka [1933] found $[\alpha]_D$ 23.87° ($c = 1.28$), Makimo [1934] 24.44° ($c = 0.9$) and Kazumo and Yamazaki 23.9° in diluted alcohol (from one value $\alpha = 0.1^\circ$). For the free acid, dissolved in alcohol, I found, as for glycocholic acid, slightly increasing specific rotation and rotatory dispersion with decreasing concentration (Table IX). The Na salt in

Table IX.

Taurocholic acid in alcohol.

<i>c</i>	1.64			0.82			0.41		
	[α]	<i>d</i>		[α]	<i>d</i>		[α]	<i>d</i>	
6867 λ	21.43	—		21.62	—		21.77	—	
6563	23.08	1.08		23.05	1.07		23.17	1.06	
5893	28.74	1.34		29.21	1.35		29.49	1.36	
5463	33.20	1.55		33.97	1.57		34.46	1.58	
4861	43.80	2.04		45.19	2.09		46.34	2.13	
4388	65.15	3.04		66.68	3.08		68.29	3.14	

Table X.

Na taurocholate in water. [α]

<i>c</i>	5.0	2.5	1.25	0.625	0.33	<i>d</i>
6867 λ	16.05	16.40	16.80	18.20	20.33	—
6563	17.40	17.70	18.00	19.60	21.96	1.08
5893	21.65	22.10	22.60	24.80	28.03	1.36
5463	25.70	26.60	27.30	29.60	33.12	1.62
4861	35.05	35.60	36.40	39.30	43.85	2.17
4388	70.65	72.50	75.60	84.10	90.73	4.44

water also showed increasing activity with decreasing concentration, and this increase was larger below about 0.75 % (Table X). The rotatory dispersion of the sodium salt seemed to be independent of the concentration; it was much larger than that of the free acid.

None of the substances examined satisfied the Lommel or the Drude formula, so their rotatory dispersions may be characterised as complex. As the deviation in most cases was rather large, the calculated values are not given.

SUMMARY.

The specific rotation and rotatory dispersion were determined for the following bile acids and their alkali salts: cholic, deoxycholic, glycocholic and taurocholic acids.

For cholic and deoxycholic acids the specific rotations were very similar in alcohol, acetone and acetic acid, and the rotatory dispersions were nearly identical in all these solvents and for the alkali salts in water. In chloroform the rotation was lower and the dispersion higher. The rotatory dispersions of these two acids were very similar.

The specific rotations of the sodium and potassium salts showed only minimum differences. The optical activity of the salts of cholic acid was scarcely influenced by other electrolytes in the solution, but that of the deoxycholic salts was obviously decreased.

In alcoholic solution glycocholic and taurocholic acids have higher specific rotations at low concentrations than at higher. Their dispersions were somewhat similar to those of the unconjugated acids.

It was found that in aqueous solution the alkali salts of all the acids examined showed increasing optical activity at decreasing concentration. This increase was much larger below a certain critical concentration, which was different for the different acids; for taurocholic acid it was about 0.75 % and for the others between 1.5 and 2.0 %. This change in the rate of increase may possibly be due to some alteration in the state of the bile acid ion.

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