

[CONTRIBUTION FROM THE COLLEGE OF PHARMACY OF THE UNIVERSITY OF CALIFORNIA]

The Dipole Moments of Some Bile Acids

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The cyclopentanoperhydrophenanthrene nucleus or one of its modifications occurs in over eight types of physiologically active compounds including the bile acids, sex hormones, antirachitic vitamins, sterols, etc. A knowledge of the structure of this nucleus is of much importance both from a theoretical and practical standpoint.

At present the points of attachment of the rings are known but the evidence is conflicting in regard to whether some of the rings are linked *cis* or *trans* and very little, if anything, is known in regard to whether the individual rings are *cis* or *trans*.

A dipole moment study appeared to offer a new and rather fruitful method of attack on the problem. This nucleus is particularly suited to a dipole moment study for two reasons. First, it is saturated so that complicated resonance effects will not obscure the interpretation of the data. Second, the groups with appreciable dipoles are usually widely separated so steric or inductive effects will not interfere.

The bile acids were used to start the study because a number of these compounds were available and there is considerable evidence that the nuclei in the various bile acids studied have the same configuration. In this paper the dipole moments of ten bile acids have been measured and such factors as association, choleic acids and possible non-rotation of the molecules in the field are considered. In a later paper a detailed analysis of the moments of these compounds will be given in terms of their stereostructure.

Results

The symbols used in the equations and tables are the same as those given previously.² The equations used in calculating the moments are²

$$P_{20} = \frac{3\alpha v_1}{(\epsilon_1 + 2)^2} + (v_1 + \beta) \frac{(\epsilon_1 - 1)}{(\epsilon_1 + 2)}$$

$$P_{20} = p_{20} M_2$$

$$\mu = 0.0127 \sqrt{(P_{20} - P_{E_2})T}$$

The P_{20} values are obtained by a method described previously² which is more accurate than the usual

(1) Abraham Rosenberg Fellow in Pharmaceutical Chemistry 1941-1942.

(2) Halverstadt and Kumler, "A Critical Study of Dielectric Polarisation Concentration Curves," in publication.

method. P_{E_2} values were calculated from the molar refractivities of the atoms present in the molecules.

TABLE I
MEASUREMENTS IN DIOXANE AT 25°

ω_2	ν_{12}	ϵ_{12}	ω_2	ν_{12}	ϵ_{12}
Lithocholic			Cholic		
0.004032	0.97361	2.2157	0.002182	0.97343	2.2177
.007756	.97343	2.2246	.005316	.97315	2.2324
.010121	.97321	2.2290	.007498	.97298	2.2424
.013622	.97300	2.2374	.014975	.97213	2.2779
Desoxycholic			3-Hydroxy-12-keto-choleic		
.004653	.97339	2.2215	.003448	.97326	2.2327
.008239	.97301	2.2342	.005759	.97297	2.2470
.011673	.97264	2.2459	.007714	.97272	2.2576
.015707	.97227	2.2602	.008794	.97266	2.2655
Hyodesoxycholic			Dehydrodesoxycholic		
.004084	.97322	2.2264	.001978	.97342	2.2235
.006502	.97398	2.2343	.004666	.97320	2.2440
.008288	.97276	2.2396	.008451	.97268	2.2729
.010803	.97256	2.2486	.016242	.97182	2.3333
Apocholeic			Reductodehydrocholic		
.002418	.97339	2.2161	.004449	.97303	2.2486
.004160	.97322	2.2214	.006828	.97263	2.2692
.008166	.97272	2.2334	.008716	.97234	2.2852
.015352	.97198	2.2555	.010111	.97217	2.2967
Dehydrolithocholic			Dehydrocholic		
.002806	.97348	2.2232	.003157	.97327	2.2448
.004882	.97334	2.2338	.006077	.97272	2.2744
.006346	.97324	2.2407	.015443	.97122	2.3695
.008862	.97309	2.2523			

Discussion

The bile acids are comparatively large molecules with molecular weights around 400, hence it is possible that they might not orient in the field at the frequency used, which was 680 kilocycles. There is no evidence, however, that such is the case. The moments obtained are of the magnitude expected for compounds having the groups present in these acids.

The bile acids are carboxylic acids and much evidence has accumulated which indicates that carboxylic acids are associated through hydrogen bonds to form dimers both in solvents such as hexane and in the vapor state. If such an association occurs in our solutions, it will make the interpretation extremely difficult. A good criterion for lack of association² is the linearity of the dielectric constant-concentration, $\epsilon_{12}-\omega_2$ curves, plus evidence that the extrapolated ϵ_1 value is approximately equal to ϵ_1 measured. We have plotted these curves for the ten acids in Fig. 1.

TABLE II
 MEASUREMENTS IN DIOXANE AT 25°

Acid	Groups on nucleus	ϵ_1 measured	ϵ_1 extra- polated	ϵ_1 extra- polated	α	$-\beta$	P_{20}	P_{E_2}	μ
Lithocholic	1 Hydroxyl (3)	2.2067	2.2068	0.97390	2.240	0.0660	237.3	107.3	2.50
Desoxycholic	2 Hydroxyl (3, 12)	2.2067	2.2053	.97385	3.493	.1015	324.7	108.8	3.22
Hyodesoxycholic	2 Hydroxyl (3, 6)	2.2105	2.2129	.97361	3.283	.0988	310.9	108.8	3.12
Apocholic	2 Hydroxyl (3, 12)	2.2098	2.2086	.97365	3.055	.1099	293.6	108.4	2.98
Dehydrolithocholic	1 Ketone (3)	2.2100	2.2101	.97365	4.794	.0627	393.9	105.8	3.72
Cholic	3 Hydroxyl (3, 7, 12)	2.2067	2.2075	.97369	4.682	.1030	417.7	110.4	3.84
3-Hydroxy-12-keto- cholanolic	1 Hydroxyl (3) 1 Ketone (12)	2.2100	2.2119	.97365	6.032	.1164	484.2	107.3	4.26
Dehydrodesoxycholic	2 Ketone (3, 12)	2.2077	2.2081	.97367	7.707	.1145	589.4	105.8	4.82
Reductodehydrocholic	1 Hydroxyl (3) 2 Ketone (7, 12)	2.2111	2.2109	.97369	8.503	.1528	662.1	107.3	5.16
Dehydrocholic	3 Ketone (3, 7, 12)	2.2093	2.2127	.97377	10.15	.1654	766.2	105.8	5.63

The curves are all linear, showing that the bile acids are not associated in dioxane at concentrations less than weight fraction 0.017.

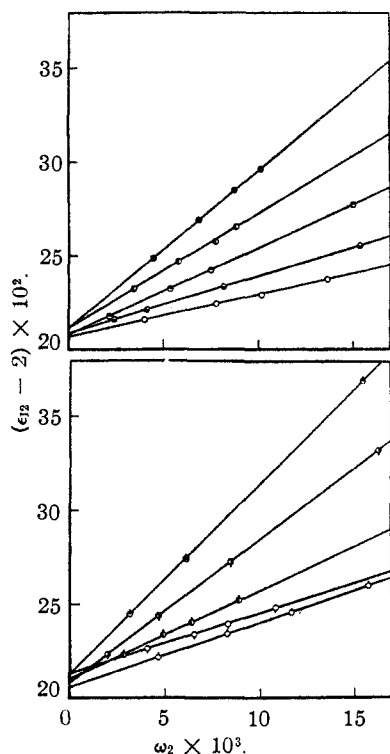


Fig. 1.—○, Lithocholic; ●, apocholic; ●, cholic; ●, 3-hydroxy-12-keto-cholanolic; ●, reductodehydrocholic.

Fig. 1a.—○, Desoxycholic; ○, hyodesoxycholic; ○, dehydrolithocholic; ○, dehydrodesoxycholic; ○, dehydrocholic.

Another point of interest is whether this study gives any evidence of the presence of choleic acids in these solutions. Choleic acids are "molecular compounds" formed between desoxycholic acid and a wide variety of other types of compounds. A logical procedure would be to dissolve a choleic

acid in some non-polar solvent and then determine the resulting dipole moment. Such a procedure does not seem feasible because the only solvent in which choleic acids appear to be soluble is dioxane, and dioxane itself forms a choleic acid. However, this permits some evidence to be obtained from the measurement of desoxycholic acid in dioxane, for if the choleic acid structure persists in the solution then the observed dipole moment of desoxycholic acid should be abnormal with respect to the other bile acids that do not form these molecular complexes. This would be the case if the complex either did or did not orient in the field. If the complex did not orient the apparent moment would be low, if it did orient the apparent moment would be expected to be different from that of a "free" molecule of desoxycholic acid.

An examination of the observed moments in Table II indicates that desoxycholic acid with two hydroxyl groups has a moment of 3.22 about midway between the moment 2.50 of lithocholic acid with one hydroxyl group and the moment of cholic acid 3.84 with three hydroxyl groups; thus the moment of desoxycholic acid is normal with respect to the moments of lithocholic and cholic acids.

The desoxycholic acid moment is also consistent with the moments of hyodesoxycholic and apocholic acids. Hyodesoxycholic acid is an isomer of desoxycholic acid with hydroxyl groups in the 3,6 positions instead of the 3,12 positions. One would thus expect not much difference in their moments and such is the case, hyodesoxycholic having a moment of 3.12 and desoxycholic a moment of 3.22. Apocholic acid differs from desoxycholic acid by having a double bond, which would be expected to alter the moment by only a small amount. The moment of apocholic, 2.98, is 0.24 less than that of desoxycholic acid. Since the moment of desoxy-

cholic acid which forms choleic acids with a large number of substances and the moment of apocholic which also shows this same property to a certain extent are normal with respect to the moment of cholic, lithocholic and hyodesoxycholic acids, which do not form choleic acids, we conclude that the dipole moment data give no evidence for the existence of the choleic acids in our solutions.

It is interesting to see what effect the introduction of an additional hydroxyl group and of an additional ketone group has on the moment. The difference between the moment of the compounds differing by one hydroxyl group and by one ketone group are as follows.

DIFFERENCE—ONE HYDROXYL

Desoxycholic-Lithocholic	3.22 - 2.50 = 0.72
Hyodesoxycholic-Lithocholic	3.12 - 2.50 = 0.62
Cholic-Desoxycholic	3.84 - 3.22 = 0.62
Cholic-Hyodesoxycholic	3.84 - 3.12 = 0.72
3-Hydroxy-12-keto-cholanic-Dehydrolithocholic	4.26 - 3.72 = 0.54
Reductodehydrocholic-Dehydrodesoxycholic	5.16 - 4.82 = 0.34

DIFFERENCE—ONE KETONE

3-Hydroxy-12-ketocholanic-Lithocholic	4.26 - 2.50 = 1.76
Dehydrodesoxycholic-Dehydrolithocholic	4.82 - 3.72 = 1.10
Reductodehydrocholic-3-Hydroxy-12-ketocholanic	5.16 - 4.26 = 0.90
Dehydrocholic-Dehydrodesoxycholic	5.63 - 4.82 = 0.81

The introduction of a hydroxyl group into desoxycholic or hyodesoxycholic acid has about the same effect as its introduction into lithocholic acid. In all the other cases, however, as a polar group (either hydroxyl or ketone) is introduced, its increment to the resultant dipole moment of the molecule decreases as the number of polar groups and moment of the compounds into which it is introduced become larger. This effect can be accounted for on the basis of the vector addition of the dipoles and does not demand the contribution of saturation effects.

Experimental

The measurements were carried out as described in the previous paper.³

(3) Kumlér and Halverstadt, *THIS JOURNAL*, **63**, 2182 (1941).

Materials

The purification and constants of the dioxane were as described previously.³

Lithocholic Acid.—This sample was obtained from Dr. H. L. Mason of the Mayo Foundation; m. p. 187–188° [α]_D²⁵₄₀₁ +40.6°; equivalent weight by titration 378.

Desoxycholic Acid.—The Riedel-de Haen product was converted to the dioxane-choleic acid which was decomposed by boiling with water, then dried; m. p. 174–175°; equivalent weight 391.

Apocholic Acid.—The Riedel-de Haen product was crystallized from dioxane; m. p. 172–173°; equivalent weight 392.

Cholic Acid.—The Riedel-de Haen product was dried in a pistol over phosphorus pentoxide; m. p. 197–198°; equivalent weight 407.

Dehydrodesoxycholic Acid.—The Riedel-de Haen product was used without further purification; m. p. 185–186°; equivalent weight 387.

Dehydrocholic Acid.—The Riedel-de Haen product was used without further purification; m. p. 234–235°; equivalent weight 399.

Hyodesoxycholic Acid, Dehydrolithocholic Acid, Reductodehydrocholic Acid, and 3-Hydroxy-12-keto-cholanic Acid.—These four compounds were obtained from Dr. Willard M. Hoehn of the George A. Breon Co. The constants of the acids are:

Hyodesoxycholic acid; m. p. 192–194°
 Dehydrolithocholic acid; m. p. 138–139°
 Reductodehydrocholic acid, m. p. 186–187°
 3-Hydroxy-12-keto-cholanic acid; m. p. 156–157°

Acknowledgment.—We wish to thank Dr. Willard M. Hoehn for supplying us with the four above mentioned bile acids, Dr. H. L. Mason for the lithocholic acid, and Dr. C. L. A. Schmidt for the samples of the Riedel-de Haen products.

Summary

The dipole moments of ten bile acids have been measured in dioxane. The values are lithocholic 2.50, desoxycholic 3.22, hyodesoxycholic 3.12, apocholic 2.98, dehydrolithocholic 3.72, cholic 3.84, 3-hydroxy-12-keto-cholanic 4.26, dehydrodesoxycholic 4.82, reductodehydrocholic 5.16, dehydrocholic 5.63.

These bile acids are not associated in dioxane in concentrations up to a weight fraction of 0.017. The $\epsilon_{12}-\omega_2$ curves are linear in this range.

The dipole moment evidence points to the non-existence of choleic acids in our solutions. The dipole moment of desoxycholic acid is normal with respect to the moment of other bile acids that do not form choleic acids.

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