Pyridonium dibromocholesteryl sulfate   3,5,6-Cholestantriol I

This reaction is especially interesting because the treatment of cholesterol dibromide with bases or water results in the formation of cholestene-diols, one of which recently has been shown to be Δ^4-cholestenediol-3,6.[3] Thus the presence of the sulfate ester in the 3 position tends to increase substitution in the 5 position instead of loss of hydrogen bromide on treatment with aqueous alkali.

3,5,6-Cholestantriol-I hitherto was obtained either directly from cholesterol by treating it with hydrogen peroxide or by the hydrolysis of α-cholesterol oxide.[4] Its constitution was established by Ellis and Petrow.[5] It has been recently isolated from the ox liver by Haslewood[6] and thus its formation in aqueous medium at room temperature is of some interest in speculating the possible mechanisms of its formation in the animal body from the relatively abundant cholesterol.

Experimental

Preparation of 3,5,6-Cholestantriol I.—Ten grams of pyridonium dibromocholesteryl sulfate[2] was dissolved in 450 ml. of 0.5 molar potassium carbonate. This was placed in a shaking machine for twenty-four hours and three 250-ml. portions of 0.5 molar potassium carbonate added at six-hour intervals during the shaking. The reaction mixture was treated with sulfur dioxide and a precipitate formed which was separated by centrifuging. The precipitate was then transferred with five 40-ml. portions of the hydrolyzing mixture (375 ml. 95% alcohol, 75 ml. water and 50 ml. concentrated sulfuric acid) to a liter Erlenmeyer flask and refluxed for one hour. The reaction mixture was poured into 1500 g. of water and ice and then extracted with two 300-cc. portions of ether. The ether extracts were washed once with dilute potassium carbonate and once more with water and then dried over anhydrous sodium sulfate. The dried ether extract was evaporated to dryness and the dry residue extracted in 50 ml. of hot ethylene dichloride. On cooling, white crystals appeared

(3) J. Lifschütz, Z. physiol. Chem., 106, 271 (1919); O. Rosenheim and W. W. Starling, J. Chem. Soc., 379 (1937); A. Butenandt and E. Hausmann, Ber., 70, 1154 (1937).

(4) R. H. Pickard and J. Yates, J. Chem. Soc., 93, 1678 (1908); T. Westphalen, Ber., 48, 1064 (1915); A. Windaus, ibid., 48, 1064 (1915); V. A. Petrow. J. Chem. Soc., 1077 (1937); F. Pirrone and R. Vanucchi, Gazz. chim. ital., 69, 420 (1934).

(5) B. Ellis and V. A Petrow, J. Chem. Soc., 1078 (1939).

(6) G. A. D. Haslewood, Biochem. J., 35, 709 (1941).

(m. p. 214) which were recrystallized once more; yield 1.0 g., m. p. 234. Calcd. for $C_{27}H_{48}O_3$: C, 77.09, H, 11.52. Found: C, 76.06; H, 11.39.

3,6-Diacetate of Cholestanetriol-3,5,6.—In a 50-cc. glass-stoppered Erlenmeyer flask 100 mg. of the above cholestanetriol was treated with 5 cc. of acetyl chloride reagent (2.36 cc. of acetyl chloride in 100 cc. of toluene) and 0.5 cc. of pyridine at 60° for one hour, cooled and the excess acetyl chloride neutralized by titrating it with 0.1 N sodium hydroxide in the presence of phenolphthalein indicator. The neutralized reaction mixture was extracted with ether. The ether extracts were washed with a little dilute hydrochloric acid and dried over anhydrous sodium sulfate. The dried extracts were filtered, the ether evaporated on a steam-bath and the toluene slowly evaporated in a vacuum desiccator, attached to a water pump. The residue melted at 158–159°; recrystallized twice from alcohol–water, m. p. 166°. Calcd. for $C_{31}H_{52}O_5$: C, 73.78; H, 10.38. Found: C, 73.57; H, 10.33.

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Distribution of Benzoic Acid between Water and Benzene

BY FREDERICK T. WALL

It is well known that the distribution of benzoic acid between water and benzene can be expressed approximately by the equation

$$C_W/\sqrt{C_B} = K_1 \qquad (1)$$

where C_W and C_B are the concentrations of the acid in water and in benzene and K_1 is a constant. Equation (1) can be derived on the assumption that the acid exists as a dimer when dissolved in benzene and as a monomer when dissolved in water. Equation (1) is not exact, however, because some ionization takes place in the aqueous solution and because the association is not quite complete in the benzene. If α equals the degree of ionization in the aqueous solution and β the degree of dissociation of the dimer in the benzene solution, then eqn. (1) should be replaced by the following more exact expression:

$$C_W(1 - \alpha)/\sqrt{C_B(1 - \beta)} = K_2 \qquad (2)$$

As yet eqn. (2) has not been completely verified because sufficient data for the calculation of β have not been available.

Recently Wall and Rouse[1] studied the association of benzoic acid in benzene and showed that the dissociation constant K for the dimer is subject to the following numerical equation

$$\log_{10} K = 3.790 - (1977/T) \qquad (3)$$

Using K calculated from (3), it is possible to compute β and thus provide a check for (2). This

TABLE I

$t = 6°C.$

C_W	C_B	β	$C_W/\sqrt{C_B}$	$\dfrac{C_W(1 - \alpha)}{\sqrt{C_B}}$	$\dfrac{C_W(1 - \alpha)}{\sqrt{C_B(1 - \beta)}}$
0.00329	0.0156	0.120	0.0263	0.0230	0.0245
.00435	.0275	.092	.0264	.0234	.0246
.00493	.0355	.081	.0262	.0235	.0245
.00579	.0495	.069	.0261	.0235	.0244
.00644	.0616	.063	.0260	.0236	.0244
.00749	.0835	.054	.0259	.0237	.0244
.00874	.1144	.046	.0258	.0237	.0243
.00993	.148	.041	.0258	.0238	.0243
.0114	.195	.036	.0258	.0240	.0244

(1) F. T. Wall and P. E. Rouse, Jr., THIS JOURNAL, **63**, 3002 (1941).

procedure has been applied to the results of Creighton,[2] the data being summarized in Table I.[3] In this table the concentrations C_W and C_B are expressed in *normal* moles per liter. It will be observed that whereas $C_W/\sqrt{C_B}$ and $C_W(1 - \alpha)/\sqrt{C_B}$ show definite trends with changing concentration, the values of $C_W(1 - \alpha)/\sqrt{C_B(1 - \beta)}$ leave little to be desired in the way of consistency. We thus have a check on the theory from which eqn. (2) was derived.

It should not be supposed that the above described agreement provides a rigorous check on eqn. (3). It so happens that the numbers in the last column of the table are not very sensitive to the value of K used. Ordinarily eqn. (3) could not be expected to be very reliable at 6° because that temperature corresponds to a fairly long extrapolation from the temperature range for which the equation was fitted.

(2) Creighton, *J. Franklin Inst.*, **180**, 63 (1915).

(3) With the exception of the columns involving β, the data of Table I were taken from Millard, "Physical Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1941. p. 361.

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COMMUNICATION TO THE EDITOR

THE LOCATION OF THE DOUBLE BOND IN CLIONASTEROL

Sir:

In a previous communication (F. R. Valentine and W. Bergmann, *J. Org. Chem.*, **6**, 452 (1941)) one of us has pointed out that the striking similarity existing between clionasterol and the sterol of the fresh-water sponge, *Spongilla lacustris*, strongly suggests the identity of the two sterols. Recently Mazur [THIS JOURNAL, **63**, 2442 (1941)] has presented evidence intended to prove that the spongilla sterol, and therefore in all probability also clionasterol, are 5,6-dihydrostigmasterol.[1] It can now be shown that clionasterol is different from this dihydrostigmasterol, and that it possesses a double bond in the 5,6-

(1) 5,6-Dihydrostigmasterol has already been prepared by Marker and Wittle [THIS JOURNAL, **59**, 2707 (1937)]. Its melting point is 50° higher than either of the two sponge sterols.

position. The presence of this linkage has been convincingly demonstrated by a number of oxidation reactions in which clionasterol shows the same behavior as cholesterol and steroids of similar constitution. Thus oxidation of clionasterol with aluminum isopropoxide yields clionastenone of m. p. 79° and αD +80.0° (Calcd. for $C_{29}H_{48}O$: C, 84.4; H, 11.8. Found: C, 84.2; H, 11.9); 3,5-dinitrophenylhydrazone, m. p. 230° (Calcd. for $C_{35}H_{52}O_4N_4$: C, 70.8; H, 8.9; N, 9.4. Found: C, 70.6; H, 8.9; N, 9.6). Clionastenone shows the typical absorption spectrum of an α,β-unsaturated ketone, and its strongly positive rotation indicates that the usual shift of the double bond from the 5,6- to the 4,5-position has taken place during the oxidation. The presence of a 5,6-double bond was further demonstrated by the oxidation of clionasterol with hydrogen peroxide to give clionastantriol-3,5,6; m. p. 238°