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Steryl Sulfates. I. Preparation and Properties

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Because of their natural occurrence and valuable properties, it was the purpose of the present series of investigations to develop methods of preparing steryl sulfates, examine their properties and the possibility of using them in the isolation, thermal decomposition and oxidation of sterols. The present paper deals with the preparation and some of the properties of cholesteryl, dibromocholesteryl, ergosteryl and lanoesteryl sulfates.

As a starting point the preparation and properties of pure pyridonium cholesteryl sulfate were investigated. This compound was at first prepared by condensing pyridine sulfur trioxide in the presence of excess cholesterol, to produce a compound free of unreacted pyridine sulfur trioxide. It was found that even under these conditions the resultant compound retained some unreacted pyridine sulfur trioxide, as shown by the analyses which gave high values for pyridine. After considerable investigation a practical method for the separation of unreacted pyridine sulfur trioxide from pyridonium cholesteryl sulfate was developed by dissolving the sulfate in cold chloroform, in which pyridine sulfur trioxide is insoluble and reprecipitating the pyridonium cholesteryl sulfate by adding petroleum ether to the chloroform extracts. There was practically no loss of the pyridonium cholesteryl sulfate in this process.

With the above method of purification it was possible to convert cholesterol quantitatively to the pyridonium cholesteryl sulfate by using a modification of the process described for the microestimation of cholesterol.[1]

Pyridonium cholesteryl sulfate is a white solid which melts with decomposition at 179°, $\alpha^{26}D$ −23.8° ($CHCl_3$). It is insoluble in petroleum ether, sparingly soluble in benzene, toluene, ether and fairly soluble in chloroform, pyridine, methanol, ethanol and acetone. The pyridonium cholesteryl sulfate is water soluble to the extent of 10 to 15%. It forms a soapy solution which may be colloidal in nature. The ester linkage is stable in strong acids at room temperature and in strong alkalies even at refluxing temperatures. This unusual stability is in contrast to that of most of the esters of cholesterol, which are sometimes stable in acids but are usually saponified with strong alkalies. In reactions such as oxidations where the hydroxyl group needs protection, the pyridonium cholesteryl sulfate can be employed in hot or cold alkali and in cold acid media.

The pyridonium cholesteryl sulfate has certain properties which make it ideal for an attack on the sterol molecule by electrolytic methods. It dissolves in water with ease whereas cholesterol is insoluble and the sulfate group affords protection for the hydroxyl group which is otherwise easily attacked.[2] The development of a method for the electrochemical attack on cholesterol and other sterols would be of special interest in view of the recognized similarity between electrolytic and biochemical reactions (2) and because the electrolytic reactions can be carried out at body temperatures and in the absence of vigorous chemicals. (Experiments along these lines are in progress.)

One of the most interesting of the properties discovered in this investigation was the ease with which the pyridonium radical in the pyridonium cholesteryl sulfate may be replaced by the cation of various salts. A list of the salts prepared is presented in Table I. Most of the salts were stable when kept in a desiccator. However, the barium, cupric and ferric salts were unstable under these conditions. At higher temperatures (100°) the potassium salt was found to be unusually stable, whereas under similar conditions the calcium and magnesium salts were unstable.

The methods employed for the isolation and study of pyridonium cholesteryl sulfate were useful for dibromocholesterol, ergosterol and lanoesterol. With slight variations in technique the pyridonium sulfate derivatives of all the above three compounds were prepared. On treatment with salts the pyridonium lanoesteryl and ergosteryl sulfates exhibited the same versatility in

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(1) A. E. Sobel, I. J. Drekter and S. Natelson, *J. Biol. Chem.*, **115**, 381 (1936).

(2) S. Glasstone and A. Hickling, "Electrolytic Oxidation and Reduction," D. Van Nostrand Co., New York, N. Y., 1936, pp. 327, 351.

TABLE I

STERYL SULFATES

Substance	Formula	M. p. (dec.), °C.	Cation (C_5H_5NH, K, Na etc.), % Found	Calcd.	Sulfur, % Found	Calcd.
		Cholesteryl sulfate				
Pyridonium	$C_{27}H_{45}SO_4C_5H_5NH$	179	14.67 ± 0.46	14.67	5.74 ± 0.06	5.89
Potassium[a]	$C_{27}H_{45}SO_4K \cdot H_2O$	210	7.46 ± .00	7.47		
Sodium	$C_{27}H_{45}SO_4Na \cdot 6H_2O$	177–178.5	3.80 ± .02	3.86		
Calcium[b]	$(C_{27}H_{45}SO_4)_2Ca$	136	4.11 ± .01	4.12		
Barium	$(C_{27}H_{45}SO_4)_2Ba \cdot 3H_2O$	124	12.33 ± .02	12.24		
Magnesium	$(C_{27}H_{45}SO_4)_2Mg \cdot 6H_2O$	152–154	2.20 ± .02	2.28	6.15	6.03
Silver[c]	$C_{27}H_{45}SO_4Ag$	124	17.69 ± .07	18.85		
Lead	$(C_{27}H_{45}SO_4)_2Pb$	132–134	22.03 ± .72	18.21		
Mercuric	$(C_{27}H_{45}SO_4)HgOAc$	152–171	27.35 ± .30	27.6		
Cupric[d]		150				
		Ergosteryl sulfate				
Pyridonium	$C_{28}H_{43}SO_4C_5H_5NH$	194–196	15.76	14.42	5.30	5.77
Potassium	$C_{28}H_{43}SO_4K \cdot H_2O$	211	7.27	7.31	5.95	6.01
Sodium	$C_{28}H_{43}SO_4Na \cdot 3H_2O$	164–166	4.15 ± .02	4.16		
Calcium	$(C_{28}H_{43}SO_4)_2Ca \cdot 5H_2O$	135	3.65 ± .08	3.69	5.88 ± .03	5.92
Magnesium	$(C_{28}H_{43}SO_4)_2Mg \cdot 8H_2O$	145–148	2.15	2.17	5.66 ± .05	5.72
Barium[d]		145				
		Lanoesteryl sulfate				
Pyridonium	$C_{30}H_{50}SO_4C_5H_5NH$	160–168	17.54 ± .09	13.67	6.28	5.47
Potassium	$C_{30}H_{50}SO_4K$	199–200	8.57	7.18	6.43 ± .07	5.88
		Dibromocholesteryl sulfate				
Pyridonium-[e]	$C_{27}H_{45}Br_2SO_4C_5H_5NH$	135	11.07 ± .17	11.30	4.85	4.54
Sodium[f]	$C_{27}H_{45}Br_2SO_4Na$		3.51	3.55		
Potassium[f]	$C_{27}H_{45}Br_2SO_4K$		4.67	5.87		
Calcium[f]	$Ca(C_{27}H_{45}Br_2SO_4)_2 \cdot 1.5CaSO_4$ (tentative)		6.76	6.69		
Mercuric[g]	$1.5HgO \cdot Hg(C_{27}H_{45}Br_2SO_4)_2$ (tentative)	123	27.15 ± .05	28.1		

[a] Decomposition point 239 when precipitated from hot water. [b] Various batches decomposed at 140, 137, 124, 122°, and analyzed for Ca gave 3.64, 3.82, 3.94%. This variation is probably due to various degrees of hydration. [c] Turns red at 117°. [d] Decomposed before it could be analyzed. [e] Br calculated 22.7, found 22.5 ± 0.8. [f] The dry compounds were unstable and decomposed on standing. [g] Begins to decompose at 123°; further observation was difficult because of black mass found. The original precipitate was white on drying; it turned red, suggesting the presence of HgO.

forming derivatives as cholesterol. The dibromocholesteryl sulfate, however, formed water-soluble derivatives with most of the salts. Thus a method for the separation of dibromocholesterol from the other sterols is suggested.

Pyridonium dibromocholesteryl sulfate was stable for at least four months when kept in a stoppered bottle. Under similar conditions the cholesterol dibromide always decomposed in ten days, with the evolution of hydrogen bromide even when kept in a dark bottle.

It must be pointed out that "lanoesterol" is not a true sterol but a polyterpene. The close resemblance of this compound to the sterols is confirmed in these studies.

The steryl sulfates prepared and some of their properties are presented in Table I. This by no means presents all of the compounds prepared but only those examined.

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Experimental

Pyridonium Cholesteryl Sulfate.—Method A: An excess of cholesterol was used in the earlier experiments. Four grams of cholesterol was dissolved in 50 cc. of dry benzene in a three-neck flask equipped with a reflux condenser, a calcium chloride tube, and a mercury-sealed stirrer. One gram of solid pyridine sulfur trioxide (prepared as previously described[1]) was added and the mixture heated between 56° and 60° for twenty minutes with stirring. The reaction mixture was then cooled to room temperature and 200 cc. of petroleum ether (30–60°) was added. A white flaky looking precipitate appeared, replacing the gelatinous mass. The flask was stoppered, placed in an ice-box for several hours and the precipitate

finally filtered on a Büchner funnel. The white precipitate was washed with a 1:5 benzene petroleum ether mixture and then dried in a vacuum desiccator; yield 2.8 g. (The mother liquors were concentrated and treated as in method B.)

To separate any unreacted pyridine sulfur trioxide from the pyridonium cholesteryl sulfate the reaction product was dissolved in (12–20 cc. per g.) chloroform and placed in an ice-box. The insoluble pyridine sulfur trioxide was filtered off and washed with cold chloroform. Three to four volumes of petroleum ether were added to the filtrate for each volume of chloroform, with shaking. The purified pyridonium cholesteryl sulfate is reprecipitated under these conditions, $\alpha^{32}D$ −24.0 (in chloroform).

Method B: This procedure was employed whenever quantitative conversion of the cholesterol to the pyridonium cholesteryl sulfate was desired. The product obtained by this method did not melt as sharply as that obtained by method A (173–181°) but was satisfactory for most purposes.

A solution of 4 g. of cholesterol in 50 cc. of benzene was treated as above with 5 cc. of pyridine, 5 cc. of acetic anhydride and 4 g. of pyridine sulfur trioxide. The rest of the procedure including the method of purification was similar to that in method A. The yield of the crude product was 5.0 g., only traces of cholesterol being present in the washings as tested by the Liebermann–Burchard reaction.

Preparation of Cholesteryl Sulfates.—The general procedure consisted of adding to a 5% suspension of the pyridonium cholesteryl sulfate an equal volume of 10% salt solution and mixing thoroughly. A precipitate formed, which in most cases rose to the surface. In the case of the cations of higher molecular weight the precipitate settled to the bottom. The precipitate was filtered off on a Büchner funnel, washed with water and dried by continued suction. The dried precipitate was washed with chloroform to remove any unreacted pyridonium cholesteryl sulfate, and placed in a vacuum desiccator for several hours.

The following salts gave derivatives, K_2CO_3, KOH, KCl, KOAc, NaCl, NaOH, $CaCl_2$, $BaCl_2$, $MgCl_2$, $CuSO_4$, $FeCl_3$, $AgNO_3$, $Pb(OAc)_2$, $Hg(OAc)_2$. These salts by no means exhaust all the possibilities, but they are a cross section of the type of salts that were of interest. These derivatives were white, flaky in appearance.

Ergosteryl Sulfates.—The method of preparation was similar to that of the corresponding cholesteryl sulfates. The ergosteryl sulfates are less stable than the corresponding cholesteryl salts.

Lanoesterol Sulfates.—The method of preparation was similar to that of the corresponding cholesteryl sulfates. The pyridonium lanoesteryl sulfate is water soluble to the extent of 5% at room temperature. On heating this solubility is increased to about 15%. This solution of pyridonium lanoesteryl sulfate is water-clear and not soapy, like the corresponding salts of cholesterol and ergosterol. The addition of potassium, sodium, calcium and barium chlorides causes the formation of the corresponding insoluble salts.

The lanoesterol used in these experiments was prepared by saponifying 100 g. of crude wool grease in 500 cc. of boiling 95% ethanol with 50 g. of finely divided barium hydroxide for one hour. Carbon dioxide was passed to remove the excess barium hydroxide as the insoluble barium carbonate. The reaction mixture was filtered while hot and washed with hot 95% alcohol. The alcoholic extracts were concentrated to dryness under vacuum and the lanoesterol was isolated as described by Marker, *et al.*[3] The yield of "isocholesterol" was 4.7 g. It melted at 120 to 122°.

Dibromocholesteryl Sulfates.—The dibromocholesterol was prepared according to the method outlined by Bills, *et al.*[4] The pyridonium dibromocholesteryl sulfate was prepared similarly to the procedure described for pyridonium cholesteryl sulfate, except that the temperature of the reaction mixture was kept below 37°. The yields of the purified product by method A were 58% and by method B 90 to 95%.

The pyridonium cholesteryl sulfate forms a clear solution when dissolved in hot water. On cooling a gel forms in concentrations above 6%. The derivatives of pyridonium dibromocholesteryl sulfate were made as described for the corresponding cholesteryl sulfate. These derivatives, however, were soluble in water to an appreciable degree. In concentrated solutions a slimy precipitate appeared which was difficult to characterize. None of the derived compounds were stable whereas the pyridonium salt was stable for at least four months in the dark under anhydrous conditions.

Summary

The preparation and properties of pyridonium cholesteryl, ergosteryl, lanoesteryl and dibromocholesteryl sulfates were investigated.

The pyridonium radical in these sulfates may be replaced by the cations of various salts. This was the basis for the preparation of a series of steryl sulfates.

The properties observed may be useful in the isolation, separation, thermal decomposition and oxidation of sterols.

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