that the molecular and empirical formulas were identical, and that the compound contains one double bond, one active hydrogen atom and two acetyl residues. As one of the two acetyl residues is hydrolyzed at a much faster rate than the other, it was concluded that the compound possesses an O-acetyl and an N-acetyl residue. These facts lead to, and are consistent with, the interpretation that the compound $C_{36}H_{69}NO_4$ is a diacetyl-O-tetradecylsphingosine and that the compound originally present in the mother liquor is an O-tetradecylsphingosine.[3]

It therefore appears that aliphatic mono-ethers of sphingosine, analogous to the naturally occurring aliphatic mono-ethers of glycerol, *i. e.*, chimyl, batyl, and selachyl alcohols,[4] are present in nature.

Experimental[5]

The Compound $C_{36}H_{69}NO_4$.—The mother liquor remaining after the recrystallization of fraction C-S-H_1[1] was evaporated to dryness, the residue taken up in ether,[6] and the ethereal phase washed successively with *N* sodium hydroxide and half-saturated salt solution. The dried ethereal extract was evaporated to dryness, the residue taken up in dry pyridine, and acetylated, at 40°, with acetic anhydride. The reaction mixture was worked up, with the aid of acetone, to give a waxy yellow solid, fraction C-S-H_2, and a mother liquor, fraction C-S-H_3. Recrystallization of fraction C-S-H_2 from methyl ethyl ketone gave a colorless solid, m. p. 98.5–99.5°. A second recrystallization from methyl ethyl ketone raised the m. p. of the compound to the maximum value of 102.0–102.5° (cor.), and after a third recrystallization from the same solvent the compound, clusters of thick needles, m. p. 102.0–102.5° (cor.), possessed the following composition.

Anal. Calcd. for $C_{36}H_{69}NO_4$ (579.9): C, 74.6; H, 12.0; N, 2.4. Found: C, 74.7, 74.6; H, 11.8, 11.8; N, 2.3, 2.4.

The mother liquor obtained from the third recrystallization was evaporated to dryness and the residue analyzed.

Anal. Calcd. for $C_{36}H_{69}NO_4$ (579.9): C, 74.6; H, 12.0; N, 2.4. Found: C, 74.6; H, 11.8; N, 2.3.

The microhydrogenation[7] of the thrice-recrystallized compound resulted in the uptake of 1.1 moles of hydrogen per mole of compound. The presence of one atom of active hydrogen (found 0.9, 1.0) was revealed on treating the compound with methylmagnesium iodide[8] and the determination of the molecular weight, by the method of Rast, gave a value of 560 ± 20, in camphor. The estimation of acetyl, via hydrolysis with toluene sulfonic acid, gave a value of 14.2%. The calculated value for two acetyl residues per mole of $C_{36}H_{69}NO_4$ is 14.8%. Application of the method of Kunz and Hudson,[9] as modified by Wolfrom, Konigsberg and Soltzberg,[10] indicated the presence of 1.1 moles of O-acetyl per mole of compound. As the compound failed to evolve nitrogen when treated in the Van Slyke apparatus, it was concluded that a free amino group was absent.

The specific rotation of the thrice-recrystallized compound, dissolved in pyridine, was $[\alpha]^{25}D$ $(-0.22° \times 100)/1.285 = -17.1°$.

Triacetylsphingosine.—Fraction C-S-H_3 was evaporated to dryness, at 25°, and, after standing for some weeks, the residue was taken up in cold isopropyl ether. A fraction of the residue failed to go in solution and this substance was collected, washed with cold isopropyl ether, and dried *in vacuo* over sulfuric acid. After one recrystallization from isopropyl ether and ligroin the substance, m. p. 96.5–97.0° (cor.), possessed the following composition.

Anal.. Calcd. for $C_{24}H_{43}NO_5$ (425.6): C, 67.7; H, 10.2; N, 3.3. Found: C, 67.7; H, 10.2; N, 3.5.

(3) The limited amount of substance at our disposal has made a more complete characterization impossible. However, we are now engaged in obtaining a further quantity of this compound and expect, in the future, to provide additional evidence regarding its structure.

(4) T. P. Hilditch, "The Chemical Constitution of Natural Fats," John Wiley and Sons, New York, N. Y., 1940.

(5) Microanalyses by Dr. G. Oppenheimer and G. A. Swinehart.

(6) Monomethylsphingosine hydrochloride, if present, will precipitate at this point.

(7) A. N. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **12**, 705 (1940).

(8) A. Soltys, *Mikrochemie*, **20**, 107 (1936).

(9) A. Kunz and C. S. Hudson, THIS JOURNAL, **48**, 1982 (1926).

(10) M. L. Wolfrom, M. Konigsberg and S. Soltzberg, *ibid.*, **58**, 490 (1936).

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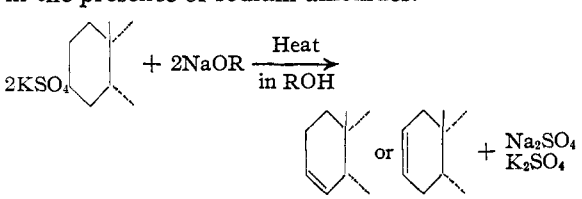
Preparation of Unsaturated Sterids from Steryl Sulfate

BY ALBERT E. SOBEL AND MILTON J. ROSEN

The introduction of double bonds into the sterid nucleus is of interest in a stepwise conversion of rings A and B to the benzenoid type. For this reason the steryl sulfates, which are formed in *quantitative* yields, were further studied as a means of dehydrating sterols.[1,2] A method for dehydrating sterols was found by the thermal decomposition of potassium steryl sulfate in alcohols in the presence of sodium alkoxides.



Potassium cholesteryl sulfate, heated at 177° (in octanol-2 containing sodium octan-2-oxide) decomposed to form 3,5-cholestadiene in practically quantitative yields. On recrystallization the maximum rotation of −123.2° was obtained.

(1) A. E. Sobel and P. E. Spoerri, THIS JOURNAL, **63**, 1259 (1941).

(2) S. Natelson and S. P. Gottfried, *ibid.*, **61**, 971 (1939).

Only the method of Tschugaeff and Gasteff[3] as applied by Eck, *et al.*,[4] has given a product of equal purity but in lower yields. Potassium dihydrocholesteryl sulfate heated at 180° under similar conditions decomposed to form Δ^2-cholestene (neocholestene).[5] At lower temperatures the steryl sulfates do not decompose in the above manner. In the presence of sodium alkoxides, mixed sodium potassium steryl sulfate forms by ionic interchange. In the absence of sodium alkoxides the main reaction is hydrolysis.

Experimental

Thermal Decomposition of Potassium Cholesteryl Sulfate in Capryl Alcohol in the Presence of Sodium Capryloxide.—One gram of sodium was dissolved in 200 ml. of capryl alcohol (octanol-2) contained in a 500-ml. Erlenmeyer flask fitted with a reflux condenser protected by a calcium chloride drying tube, and 5 g. of potassium cholesteryl sulfate was added. After refluxing for one hour at 177°, the reaction mixture was cooled, transferred to a separatory funnel with ether, and washed several times with water. The water washings were ether extracted, the ether extracts added to the washed reaction mixture, and the combined capryl alcohol–ether solution dried over anhydrous sodium sulfate.

The solvents were removed by distillation under reduced pressure, and the residue (3.45 g.), a viscous yellow liquid which solidified upon cooling, recrystallized from ethyl alcohol. The yield of pure white needles was 3.22 g. (88%), m. p. 76.5–79°, $[\alpha]^{23}D$ −120.4° (*c*, 1.38 in $CHCl_3$). After two more recrystallizations, the rotation was $[\alpha]^{26}D$ −123.2° (*c*, 2.517 in CCl_4), m. p. 79.5–80°.

Anal.[6] Calcd. for $C_{27}H_{44}$: C, 87.97; H, 12.03. Found: C, 87.78; H, 12.07.

Treatment of Potassium Cholesteryl Sulfate with *n*-Butyl Alcohol in the Presence of Sodium Butyloxide.—One gram of sodium was dissolved in 125 ml. of *n*-butyl alcohol, 5 g. of potassium cholesteryl sulfate added, and the mixture refluxed for six hours at 120°. Upon cooling, a gelatinous precipitate appeared, which was filtered off on a Büchner funnel. The precipitate was extracted several times with ether and then with water, and dried *in vacuo*. Ash tests and analyses for sodium and potassium on samples of this precipitate showed it to be a mixture of potassium and sodium cholesteryl sulfates, corresponding to the formula $NaSO_4C_{27}H_{45}\cdot 2KSO_4C_{27}H_{45}$, m. p. (dec.) 174–178°; yield, 4.5 g. (90%).

The ether extracts of this precipitate were added to the filtrate, and the combined solution washed several times with water. Removal of the solvents left 281 mg. of material, which was mainly cholesterol.

Thermal Decomposition of Potassium Cholesteryl Sulfate in a Sealed Tube.—3.1 g. of dry potassium cholesteryl sulfate was sealed into an evacuated tube, and heated at various temperatures to determine the minimum decomposition point.

At 100° and at 120° there was no change at the end of one hour. After one hour at 140°, in refluxing xylene vapor, a slight yellowish tinge had appeared, together with partial liquefaction evidenced by the adhesion of some of the material to the sides of the tube. After one hour at 180°, in refluxing capryl alcohol vapor, the contents of the tube had melted completely to a yellow-brown oil, which was crude cholesterilene.[2]

This experiment was repeated three more times at 160°, with results similar to that obtained at 180°, and the cholesterilene purified by recrystallization from alcohol and benzene–alcohol. The specific rotations of the three preparations at 160°, observed after three to four recrystallizations, were $[\alpha]^{21}D$ −78.3°, $[\alpha]^{27}D$ −65.2°, and $[\alpha]^{28}D$ −80.2°, respectively ($CHCl_3$).

Thermal Decomposition of Potassium Cholestanyl Sulfate in Capryl Alcohol in the Presence of Sodium Caprylloxide, at 180°.—The potassium cholestanyl sulfate used in these investigations was prepared similarly to the method employed for the corresponding cholesteryl salt[1] m. p. (dec.) 236°.

Two grams of sodium was dissolved in 185 ml. of capryl alcohol contained in a 500-ml. Erlenmeyer flask fitted with a reflux condenser protected by a calcium chloride drying tube, and 5 g. of potassium cholestanyl sulfate added. After refluxing for six hours at 180°, the reaction product was isolated as described above for the cholesteryl sulfate. The yield of white crystals, m. p. 62–66°, recrystallized from methyl alcohol was 2.58 g. (70%). After one more recrystallization from acetone–methanol, pure white needles melting at 65.5–66.5°, $[\alpha]^{23}D$ +65.5°, were obtained. After two further recrystallizations from acetone–methanol, the product melted at 68–69°, $[\alpha]^{25}D$ +64.4°.

Anal.[6] Calcd. for $C_{27}H_{44}$: C, 87.50; H, 12.50; I_2 no., 68.8. Found: C, 88.00; H, 12.40; I_2 no., 66.3.

Treatment with antimony trichloride in $CHCl_3$ and 90% trichloroacetic acid gave negative reactions.

Treatment of Potassium Cholestanyl Sulfate with Capryl Alcohol in the Presence of Sodium Capryloxide, at 169°.—One gram of sodium was dissolved in 200 ml. of capryl alcohol and 5 g. of potassium cholestanyl sulfate added. The mixture was refluxed at 169° for one hour, cooled, shaken with ether and water simultaneously, and filtered. The white precipitate was washed with a mixture of ether and water, and dried *in vacuo*. Analyses for sodium and potassium on samples of this precipitate showed it to be a mixture of sodium and potassium cholestanyl sulfates corresponding to the formula $NaSO_4C_{27}H_{47}\cdot KSO_4C_{27}H_{47}$, m. p. (dec.) 234°; yield, 2.7 g. (54%).

The filtrate and washings were combined, washed with water, and the water washings extracted with ether. The washed filtrate and ether extract were combined, and dried over sodium sulfate. Removal of the ether and capryl alcohol left a few milliliters of high boiling material which could not be crystallized.

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