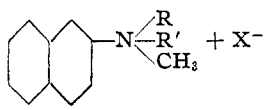
sessing a chain of sixteen carbon atoms show the highest bactericidal activity. These investigators used benzyl or phenyl radicals in their compounds. It was now thought desirable to prepare similar types of compounds possessing a condensed ring system such as naphthalene, and then to study the bactericidal activity of such compounds.

Thus the following invert soaps were prepared: first the N,N-dimethyl-N-cetyl-β-naphthylammonium iodide (II), and then a few short-chain naphthalene invert soaps such as the N-methyl-N,N-di-*n*-butyl-N-β-naphthyl- (III) and N,N,N-trimethyl-N-β-naphthylammonium salts (IV). The structure of these salts is



| | (II) | (III) | (IV) |
|---|---|---|---|
| $X^-$ | = I | I | $CH_3OSO_2O^-$ |
| R | = $CH_3$ | $C_4H_9$ | $CH_3$ |
| R′ | = $C_{16}H_{33}$ | $C_4H_9$ | $CH_3$ |

The salts are slightly soluble in water.

Phenol coefficient determination following the U. S. Bureau of Standards procedure on *Staphylococcus aureus* showed a phenol coefficient of not more than 0.2. This surprising result of complete inactivation indicates that the cetyl group and *per se* the length of the carbon chain alone are not responsible for high bactericidal action.

## Experimental

**N,N-Dimethyl-N-cetyl-N-β-naphthylammonium Iodide (II).**[6,7]—Seven grams of β-naphthylamine was added to a solution of 15 g. of cetyl bromide in 15 cc. of ethyl alcohol. The mixture was refluxed on a steam-bath for fifteen hours. The precipitate, N-cetyl-N-β-naphthylammonium hydrobromide (Ia), was filtered and recrystallized from ethyl alcohol.

Eleven grams of (Ia) was heated to boiling with 50 cc. of 10% sodium hydroxide solution for two minutes. After cooling, the precipitate was filtered and dissolved in ether. The ether solution was filtered and the filtrate evaporated to dryness. The residue, the N-cetyl-β-naphthylamine (I), was recrystallized from ethyl alcohol.

To a solution of 8 g. of (I) in 240 cc. of ethyl alcohol were added 32 g. of methyl iodide and 8 g. of anhydrous sodium carbonate, and the mixture was refluxed for fifteen hours. After this time, the hot alcohol solution was filtered and the filtrate evaporated until a precipitate appeared. The precipitate (sodium iodide) was again filtered and the filtrate evaporated to dryness. The residue, the invert soap (II), was recrystallized from ethyl acetate.

**N-Methyl-N,N-di-*n*-butyl-N-β-naphthylammonium Iodide (III).**—Fourteen grams of β-naphthylamine, 20 g. of butyl bromide, and 10 g. of butyl alcohol were refluxed for twenty hours. After this time, the mixture was cooled and neutralized with 10% sodium hydroxide solution. The oily layer was then separated, dried with magnesium sulfate, and, after addition of 15 g. of butyl bromide, refluxed for thirty hours. The mixture was then cooled, neutralized with sodium hydroxide, and the oily layer dried with magnesium sulfate. The N,N-di-*n*-butyl-β-naphthylamine thus obtained was purified by distillation under reduced pressure.

Three grams of the above tertiary amine and 5 g. of methyl iodide, contained in a stoppered Erlenmeyer flask, were kept at room temperature for three days. The methiodide (III) thus obtained was filtered and washed with ether. The crude product was dissolved in ethyl alcohol and reprecipitated by ether.

**N,N,N-Trimethyl-N-β-naphthylammonium Methosulfate (IV).**—Four grams of β-naphthylamine and 12.5 g. of dimethyl sulfate were heated at 120° in an oil-bath for two hours. After this time, the excess dimethyl sulfate was distilled off under reduced pressure. Upon addition of 100 cc. of methyl alcohol to the oily residue, a precipitate was formed. This precipitate, the methosulfate (IV), was filtered and recrystallized from boiling water.

TABLE I

| | Compounds | Formula | M. p. (uncor.), °C. | Analyses, % N Calcd. | Found |
|---|---|---|---|---|---|
| I | N-Cetyl-β-naphthylamine | $C_{26}H_{41}N$ | 64 | 3.81 | 3.75 |
| | (a) Hydrobromide[6] | $C_{26}H_{42}NBr$ | 161 | 3.12 | 3.07 |
| II | N,N-Dimethyl-N-cetyl-N-β-naphthylammonium iodide | $C_{28}H_{46}NI$ | 106 | 2.67 | 2.52 |
| III | N-Methyl-N,N-di-*n*-butyl-N-β-naphthylammonium iodide | $C_{19}H_{28}NI$ | 157 | 3.52 | 3.19 |
| IV | N,N,N-Trimethyl-N-β-naphthylammonium methosulfate | $C_{14}H_{19}NSO_4$ | 288 | 4.72 | 4.35 |

CONTRIBUTION FROM THE CHEMICAL LABORATORIES
OF NEW YORK UNIVERSITY
WASHINGTON SQUARE COLLEGE
NEW YORK, N. Y. RECEIVED SEPTEMBER 18, 1941

(6) H. Lettre and M. E. Fernholz, *Ber.*, **73**, 436 (1940).

(7) J. R. Stevens and R. H. Beutel, THIS JOURNAL, **63**, 308 (1941).

## A New Hydrolysis Product Derived from Bovine Cerebral and Spinal Tissue

BY CARL NIEMANN

Acetylation of the mother liquor obtained by recrystallizing crude sphingosine sulfate (fraction C–S–$H_1$) from absolute ethanol[1] has led to the isolation of triacetylsphingosine and a hitherto undescribed compound.[2] This latter substance, after repeated recrystallization from methyl ethyl ketone, gives analytical values in good agreement with those demanded by the empirical formula $C_{36}H_{69}NO_4$. Subsequent investigation showed

(1) C. Niemann, THIS JOURNAL, **63**, 1763 (1941).

(2) Parallel experiments were conducted using C–S–$H_1$ fractions obtained from both brain and spinal cord. As the results of these experiments were essentially identical, only those experiments using C–S–$H_1$ fractions prepared from brain will be described.

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).

CONTRIBUTION NO. 856 FROM THE
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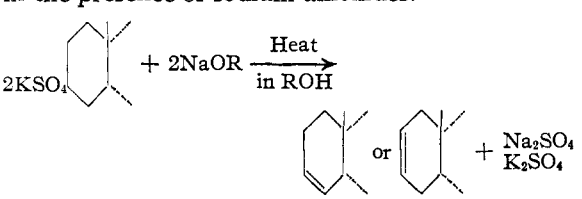
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA RECEIVED SEPTEMBER 22, 1941

## 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).