Pentamethylbenzene Methyl Sulfonate.—The sulfonyl chloride (8 g.) was refluxed for one hour with a solution of sodium methoxide prepared from sodium (3 g.) and methanol (50 cc.). The reaction mixture, poured into water, deposited a solid which was removed and crystallized twice from methanol. The ester (6.0 g.) was white and melted at 91–91.5°. It was readily soluble in ether, benzene, chloroform and warm methanol, and insoluble in water.

Anal. Calcd. for $C_{12}H_{18}O_3S$: C, 59.5; H, 7.49. Found: C, 59.4; H, 7.32.

Summary

1. Subjected to the reagents and conditions which cause the Jacobsen rearrangement to occur, chlorodurene, chloroisodurene, chloroprehnitene, 5-chloropseudocumene, 6-chloropseudocumene, 5-bromopseudocumene, and bromomesitylene rearranged. Chloromesitylene, 4-chlorohemimellithene, hemimellithene, 5-nitropseudocumene, pseudocumidine-5, pentamethylbenzene-methylsulfonate, pentamethylcyclohexane, 2,3-dimethylnaphthalene, and *p*-bromodiphenyl did not rearrange.

2. The ease of migration of groups present in the chloro- and bromotetramethylbenzenes is in the order Br > CH_3 > Cl; in case of the corresponding derivatives of the trimethylbenzenes, the order is Br > Cl > CH_3.

3. Attempts to find mild conditions which would cause Jacobsen rearrangements without producing amorphous by-products were unsuccessful.

4. The limits and mechanism of the Jacobsen rearrangement have been discussed.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

The Chemistry of the Lipids of Tubercle Bacilli. XLI. Part 1. The Composition of the Timothy Bacillus Wax. Part 2. The Isolation of *d*-Eicosanol-2 and *d*-Octadecanol-2 from the Unsaponifiable Matter of the Timothy Bacillus Wax[1]

BY MARY C. PANGBORN[2] AND R. J. ANDERSON

Part 1. The Composition of the Timothy Bacillus Wax

Introduction

All of the acid-fast bacteria contain relatively large quantities of wax-like material; in fact the wax is quantitatively the most important part of the ether-soluble constituents. The bacterial waxes are easily soluble in ether, chloroform or benzene but they are practically insoluble in alcohol or acetone. The method used in this Laboratory of first extracting the bacteria with a mixture of alcohol and ether removes very little of the wax fractions owing to the insolubility of the waxes in alcohol, while the subsequent extraction with chloroform removes the waxes almost completely. The bacterial residues after extraction with alcohol–ether and chloroform are practically free from lipids soluble in neutral solvents.

Since very little information is available concerning either the chemical composition or the biological effects of the bacterial waxes, we have devoted some study to these interesting compounds. Previously the waxes from the human tubercle bacillus have been examined by Anderson[3] and the wax from the BCG has been analyzed by Chargaff.[4] As a further contribution on this subject we wish to report some experiments dealing with the chemical composition of the timothy bacillus wax.

The chief constituents of the wax were found to be optically active fatty acids of high molecular weight, two higher secondary alcohols, a carbohydrate which was identified as trehalose, and glycerol. The wax is therefore a complex compound or mixture containing solid glycerides, esters of fatty acids with trehalose, and esters of fatty acids with higher alcohols. None of the ordinary fatty acids could be found. Only one of the fatty acids could be isolated in a state approaching purity. The composition of this acid corresponded approximately to the formula $C_{70}H_{138}O_6$ and it contained one hydroxyl, one double bond, and apparently two carboxyl groups.

The isolation of the ether-soluble neutral ma-

(1) An abstract of this paper was read before the Division of Organic Chemistry of the American Chemical Society in New York, April, 1935. The present report is a part of a coöperative investigation on tuberculosis and it has been supported partly by funds provided by the Research Committee of the National Tuberculosis Association.

(2) Holder of a National Tuberculosis Association Fellowship at Yale University, 1933–1934.

(3) R. J. Anderson, *J. Biol. Chem.*, **83**, 505; **85**, 327 (1929).

(4) E. Chargaff, *Z. physiol. Chem.*, **217**, 115 (1933).

terial or unsaponifiable matter involved considerable labor; the usual procedure was unworkable in this case owing to the insolubility of the potassium salts of the fatty acids. The unsaponifiable material was found to consist almost entirely of higher alcohols because it could be converted nearly quantitatively into acid phthalates when treated with phthalic anhydride. By laborious fractional crystallizations of the mixed alcohols and of their phenylurethans two new optically active alcohols, *d*-eicosanol-2 and *d*-octadecanol-2, were isolated in pure form. The alcohols were identified by oxidation to the corresponding ketones and comparison of the latter with synthetic 2-eicosanone and *d*-octadecanone. The properties of the new alcohols and several of their derivatives are given in Part 2.

Experimental

Purification of the Wax.—The isolation of the material used in the present investigation was described in paper XXIII of this series.[5] Fractions T-4 and T-5 were combined, total weight 158.4 g., and purified by precipitation with acetone from ethereal solution. After 7 precipitations 112.5 g of a light yellowish solid was obtained. The mother liquors on concentration yielded 41.6 g. of a dark brown semi-solid material which was reserved for future investigation.

The purified wax was almost completely insoluble in hot acetone and in ethyl and methyl alcohols but easily soluble in ether, chloroform and benzene. In the Liebermann–Burchard reaction the wax gave no coloration whatever, thus indicating absence of sterols, but it gave a positive Molisch reaction indicating the presence of carbohydrate.

CONSTANTS OF THE TIMOTHY BACILLUS WAX

Melting point	45°
Ash, 1.7; P, 0.29; N, 0.41%	
Iodine number	20.5
Saponification number	66.9
$[\alpha]_D$ in chloroform	+15.1°

Saponification of the Wax.—Fifty grams of the purified wax was saponified by refluxing for five and one-half hours with 1200 cc. of 5% alcoholic potassium hydroxide in an atmosphere of nitrogen. Most of the substance remained insoluble. The hot alcoholic solution was decanted and the sticky insoluble portion was washed with hot alcohol. As the solution cooled a white amorphous precipitate separated which was filtered off, (the filtrate (a) was saved) washed with alcohol, and digested in 400 cc. of hot alcohol. The solution was decanted from an insoluble sticky residue and cooled whereupon a white precipitate separated. The latter was filtered off, washed with alcohol and dried. The filtrate (b) was saved. The dried precipitate weighed 6 g. and will be designated fraction 1.

The two alcohol-insoluble fractions mentioned above, which were also insoluble in ether, were found to consist principally of potassium salts of higher fatty acids together with a small amount of water-soluble material. The two fractions were combined and separated into ether-soluble fatty acids and water-soluble compounds by treatment with dilute acetic acid and extraction with ether. A carbohydrate was isolated from the aqueous solution as will be described below.

The ethereal solution after being washed with water was concentrated to 300 cc., mixed with an equal volume of alcohol, neutralized with alcoholic potassium hydroxide, and the ether was distilled off. As the solution cooled a heavy white precipitate separated which was filtered off, washed with alcohol and dried. The filtrate (c) was saved. The precipitate, 23 g., had properties similar to those of fraction 1, hence the two products were combined for further purification.

Isolation of a Neutral Fraction Consisting of Higher Alcohols.—The combined filtrates (a, b and c) were concentrated and diluted with water, whereupon a heavy precipitate separated which was filtered off and washed with water. The aqueous filtrate was saved and examined for fatty acids giving water-soluble potassium soaps and for glycerol. The precipitate after being dried *in vacuo* was dissolved in 500 cc. of alcohol and an alcoholic solution of lead acetate was added in excess. The lead salts which separated were filtered off, washed with alcohol, and the fatty acids were liberated as described below. The filtrate was concentrated nearly to dryness, mixed with water, acidified with dilute cold nitric acid and extracted with ether. The ethereal extract was washed with water and with dilute potassium hydroxide and again with water, after which the ether was distilled off. The residue was a neutral substance which weighed 6.5 g. This material, designated fraction 2, was found to consist of two higher optically active alcohols.

The Fatty Acids Precipitated as Lead Salts.—The fatty acids, 10.9 g., obtained from the lead salts on treatment with dilute hydrochloric acid and extraction with ether were found to consist of a complex mixture of higher optically active acids from which no pure substance could be isolated. Several fractions were separated which varied in molecular weight from 546 to 627, iodine number from 37 to 15 and specific rotation from +1.5 to +5.0°.

Examination of the Alkaline Aqueous Solution for Fatty Acids and Glycerol.—The alkaline solution was acidified and extracted with ether. The ethereal solution yielded only 1.7 g. of a brown semi-solid acid, iodine number 35, obviously a mixture from which no pure acid could be isolated.

The acid aqueous solution was neutralized with potassium hydroxide and concentrated to dryness *in vacuo* after which the residue was extracted with absolute alcohol. The alcoholic solution on evaporation to dryness left a sirupy residue weighing 1.5 g. The sirup gave a positive acrolein reaction and after benzoylation according to Einhorn and Hollandt[6] we obtained 1.1 g. of crystalline glyceryl tribenzoate, m. p. 75–76°. A mixed melting point with glyceryl tribenzoate gave no depression.

Isolation of Trehalose.—The dilute aqueous acetic acid solution, after extraction with ether as mentioned above, was neutralized with potassium hydroxide and concen-

(5) E. Chargaff, M. C. Pangborn and R. J. Anderson, *J. Biol. Chem.*, **90**, 45 (1931).

(6) A. Einhorn and F. Hollandt, *Ann.*, **301**, 95 (1898).

trated *in vacuo*. By means of basic lead acetate and ammonia it was possible to isolate a carbohydrate in the usual manner. The aqueous solution after decomposing the lead precipitate with hydrogen sulfide was concentrated *in vacuo* to a thick sirup and the latter was ground in a mortar under absolute alcohol until a white powder was produced. The powder, after filtering, washing and drying weighed 2.3 g. Tests for phosphorus, nitrogen and pentose were negative and the material did not reduce Fehling's solution until it had been boiled for some time with dilute acid. Efforts to crystallize the substance from water or from 80% alcohol were fruitless. Some impurity was apparently present which hindered crystallization.

The carbohydrate was finally purified by means of the acetyl derivative. The latter was prepared by refluxing 0.5 g. of the crude carbohydrate with acetic anhydride and fused sodium acetate. The reaction product weighed 0.84 g. and was purified by crystallization from methyl alcohol yielding 0.4 g. of colorless prismatic needles. After recrystallization from ethyl alcohol the substance melted not sharply at 75–77°, $[\alpha]^{21}D$ in chloroform +162.1°.

Samples of trehalose octaacetate prepared in this Laboratory have all melted not sharply in the region of 80°, but these products after being heated for a few hours *in vacuo* below the melting point have always melted at about 98°. In the present case a sample of the acetyl derivative after being heated at 65° for three hours had lost 1% in weight; further heating for three hours caused no change in weight but the product melted at 97–98°. Mixed with a specimen of trehalose octaacetate which had been heated and which also melted at 97–98° there was no depression. Allowing for the loss in weight the specific optical rotation would be +163.7°. The properties of the substance are identical with those of trehalose octaacetate.

Examination of Fraction 1.—The alcohol-insoluble potassium salts composing fraction 1 represented the principal fraction obtained from the wax. The material was partly soluble in benzene, hence it was separated into benzene-soluble and benzene-insoluble fractions by exhaustive extraction with boiling benzene.

The benzene solutions were combined and concentrated to dryness. The free acids after being liberated in the usual manner by treatment with dilute hydrochloric acid and extraction with ether weighed 8.5 g. The material consisted of an inseparable mixture of higher optically active acids. Various fractions were separated having melting points from 54 to 59°, specific rotations from +4 to +5°, iodine number about 15, molecular weights by titration from 627 to 1168.

The benzene-insoluble portion of the potassium salt was converted into the free acid as described above. The crude acid weighed 12.9 g. It was readily soluble in hot acetone and separated on cooling as an amorphous white powder consisting of fine globular particles. After one precipitation the substance weighed 11 g., m. p. 56–57°, $[\alpha]D$ in chloroform +6.0°, molecular weight by titration 490. The product was reprecipitated fourteen times from 50-cc. portions of acetone which caused no change in melting point but the molecular weight was raised to 517. Five further precipitations from acetone caused no change in properties. The substance weighed 9.5 g., m. p. 56–57°, $[\alpha]D$ in chloroform +6.1°, molecular weight, by titration 518, but by the Rast method a value of 1000 was obtained iodine number 15.2.

Anal. Found: C, 78.27, 78.34; H, 12.98, 12.59.

The simplest formula derived from the analytical data would be that of an hydroxy acid, $C_{35}H_{68}O_3$, but in view of the low iodine number and the high molecular weight in camphor, it is more probable that the acid is dibasic having the formula $C_{70}H_{138}O_6$. An acid of this molecular weight, 1074, with 1 double bond should have an iodine number of 23.6. The values for the acetyl derivative, the methyl ester and the acetyl derivative of the methyl ester also suggest the higher formula. The acetyl derivative prepared in the usual manner separated as a white amorphous powder from alcohol, m. p. 40–41°, $[\alpha]^{25}D$ +6.4° in $CHCl_3$; CH_3CO 4.21. The methyl ester, a white amorphous powder from acetone, melted at 49°, $[\alpha]^{20}D$ +6.0° in $CHCl_3$. Found: C, 78.25, 78.66; H, 12.83, 12.90. The acetyl derivative prepared from the methyl ester, a white amorphous powder from alcohol, melted at 41°, $[\alpha]^{18}D$ +8.1° in $CHCl_3$.

Anal. Calcd. for $C_{74}H_{144}O_7$ (1144): C, 77.62; H, 12.58; CH_3CO, 3.75. Found: C, 77.55; H, 12.35; CH_3CO, 4.05.

The formula suggested for this acid is to be regarded as tentative only since adequate criteria of purity of such an amorphous substance are lacking. Attempts were made to determine the mode of linkage of the supposed sixth oxygen atom but without any results. The acid did not react with semicarbazide and a Zeisel determination gave no volatile iodide.

It is evident from the data presented that the timothy bacillus wax contains practically none of the ordinary fatty acids. The acids that were obtained were dextrorotatory, had low melting points, low iodine numbers and very high molecular weights. The definite identification of these acids will remain a problem for future investigation. The fractions isolated are summarized below.

CLEAVAGE PRODUCTS FROM 50 G. OF TIMOTHY BACILLUS WAX

	G.
Fatty acids giving alcohol-soluble potassium salts	10.9
Fatty acids giving water-soluble potassium salts	1.7
Fraction 1.	
a. Acids giving alcohol-insoluble but benzene-soluble potassium salts	8.5
b. Dibasic hydroxy acid $C_{70}H_{138}O_6$	12.9
Fraction 2. Higher optically active alcohols	6.5
Crude glycerol	1.5
Crude trehalose	2.3

Part 2. Examination of the Unsaponifiable or Neutral Fraction of the Timothy Bacillus Wax

Isolation of *d*-Eicosanol-2.—The neutral fraction, weighing 6.5 g., isolated as already described, was easily soluble

in the ordinary organic solvents. It dissolved easily in warm methyl alcohol; on cooling slowly and scratching a substance was caused to separate in aggregates of fine needles. A crystalline product also separated from a solution in ethyl acetate. After nine recrystallizations from methyl alcohol 1.7 g. of fine lustrous needles was obtained and an additional 0.8 g. of an identical product was recovered from the last four mother liquors. The substance melted at 62–63°, $[\alpha]_D$ +3.5°, and it was saturated as it did not absorb any bromine. Values obtained on analysis indicated a probable formula of $C_{20}H_{42}O$ and the formation of a monoacetate, benozate, phenylurethan and acid phthalate showed that it was a monohydric alcohol. Oxidation with chromic acid to the corresponding ketone showed that it was a secondary alcohol. The ketone obtained by oxidation was shown to be 2-eicosanone, hence the alcohol was *d*-eicosanol-2.

Anal. Calcd. for $C_{20}H_{42}O$(298): C, 80.53; H, 14.09. Found: C, 80.57; H, 14.17.

A molecular weight determination in camphor gave a value of 320.

The alcohol was further recrystallized ten times from methyl alcohol. The melting point was unchanged, 62.5–63°, $[\alpha]_D$ in chloroform +4.2°. The fraction recovered from the mother liquors also melted at 63°. Recrystallization had not caused any change in composition. Found: C, 80.33; H, 14.10.

Acetyl Derivative.—The alcohol, 1.2 g., was acetylated in pyridine solution with acetic anhydride and the reaction product was isolated and crystallized from 95% methyl alcohol from which it separated in small irregular plates, m. p. 35–37°, $[\alpha]_D$ in chloroform +1.5°.

Anal. Calcd. for $C_{22}H_{44}O_2$(340): C, 77.65; H, 12.94; CH_3CO, 12.65. Found: C, 77.58; H, 13.16; CH_3CO, 12.50.

Benzoyl Derivative.—The alcohol was benzoylated in pyridine solution with benzoyl chloride. The reaction product was isolated and crystallized from methyl alcohol yielding small lustrous plates, m. p. 39–40°.

Anal. Calcd. for $C_{27}H_{46}O_2$(402): C, 80.59; H, 11.44. Found: C, 80.80; H, 11.49.

Phenylurethan.—The alcohol, 96 mg., and 0.2 g. of phenyl isocyanate were refluxed in 5 cc. of dry benzene for two hours. The solvent and excess of isocyanate were removed by heating under reduced pressure to 100°. The residue was twice recrystallized from alcohol and was obtained as very thin rectangular plates with brilliant luster. The crystals had a double melting point, first melting sharply at 78–78.5°, solidifying at 76° and remelting at 81°.

Anal. Calcd. for $C_{27}H_{47}O_2N$(417): C, 77.70; H, 11.27. Found: C, 77.75; H, 11.55.

Acid Phthalate.—The acid phthalate was prepared according to the method of Chibnall,[7] 0.3 g. of the alcohol yielding 0.4 g. of the phthalate. The melted substance crystallized on cooling in aggregates of dense needles but owing to its great solubility it could not be obtained in definitely crystalline form from solution. For purification the substance was separated from petroleum ether by cooling and was obtained as a white powder which melted partly at 58°, solidified at 59° and finally melted to a clear fluid at 60–61°. After the substance had been heated at 61° for one hour and cooled a hard crystalline mass was obtained which melted at 60–61°. There was no loss in weight on heating. Evidently there had been a transition from an amorphous to a crystalline state. The molecular weight by titration was found to be 450, $[\alpha]^{18}_D$ in chloroform +27.5°.

Anal. Calcd. for $C_{28}H_{46}O_4$(446): C, 75.34; H, 10.31. Found: C, 75.31, 75.24; H, 10.63, 10.50.

Oxidation with Chromic Acid and Isolation of the Ketone.—The alcohol, 0.85 g., was dissolved in 50 cc. of slightly warm glacial acetic acid and a solution of chromic acid in glacial acetic acid was added in small portions, the temperature being kept between 30–40°, until an excess of chromic acid was present, as shown by the brown color of the solution. The reaction mixture was allowed to stand for one-half hour with a slight excess of chromic acid after which it was diluted largely with cold water. The solid product which separated was filtered off, washed with water, dissolved in ether and the ethereal solution was washed first with dilute sodium hydroxide and then with water. After the solvent had been evaporated the residue was recrystallized from alcohol. Examination of the alkaline extract showed that not more than a negligible trace of acid had been formed during the oxidation. The ketone crystallized in large snow-white lustrous plates. The yield of the purified ketone was 0.8 g., m. p. 58–59°. Four more crystallizations did not change the melting point.

Anal. Calcd. for $C_{20}H_{40}O$(296): C, 81.08; H, 13.51. Found: C, 80.81; H, 13.65.

Semicarbazone.—The ketone, 60 mg., 50 mg. of semicarbazide hydrochloride and 50 mg. of sodium acetate were dissolved in 5 cc. of dilute alcohol and the solution was heated in a water-bath for ten minutes. On cooling, long thin plates separated. The crystals were filtered off and washed with water, alcohol and petroleum ether. The semicarbazone was twice recrystallized from 5-cc. portions of alcohol. The yield was nearly quantitative. The semicarbazone melted at 128°.

Anal. Calcd. for $C_{21}H_{43}N_3O$(353): C, 71.39; H, 12.18. Found: C, 71.06; H, 12.12.

Oxime.—The ketone, 85 mg., was heated for two hours with 50 mg. of hydroxylamine hydrochloride and 0.1 g. of sodium acetate dissolved in 5 cc. of dilute alcohol. On cooling crystals separated and were filtered off and washed with water, alcohol, and petroleum ether. The product on recrystallization from alcohol separated in dense irregular plates, m. p. 73–74°.

Anal. Calcd. for $C_{20}H_{41}ON$(311): C, 77.17; H, 13.18. Found: C, 77.21; H, 13.56.

Examination of the More Soluble Portion of the Unsaponifiable Matter. Isolation of *d*-Octadecanol-2.—The mother liquors from the purification of *d*-eicosanol-2 were concentrated to dryness and an attempt was made to separate the material into pure fractions by means of the acid phthalates, a method successfully employed by Chibnall,[7] but in this case we had no success. It was evident, however, that only alcohols were present as the yield of the

(7) A. C. Chibnall, S. H. Piper, A. Pollard, J. A. B. Smith and E. F. Williams, *Biochem. J.*, **25**, 2095 (1931).

acid phthalates was practically quantitative. The phthalates were saponified; the mixed alcohols were recovered and converted into phenylurethans. The urethans were subjected to exhaustive fractionation from methyl alcohol. The less soluble fraction, 0.76 g., proved to be identical with the phenylurethan of *d*-eicosanol-2.

The more soluble fraction finally yielded 0.5 g. of the urethan of a new alcohol, *d*-octadecanol-2. This urethan also showed a double melting point; melting first at 72–73°, solidifying at 66° and remelting at 76–77°; $[\alpha]^{22}D$ in chloroform +7.9°. The substance separated from solutions in two different crystal forms depending upon concentration and rate of cooling, either in lustrous plates or in long fine needles. The needle-shaped crystals had the higher melting point.

Anal. Calcd. for $C_{25}H_{43}O_2N$(389): C, 77.12; H, 11.05. Found: C, 76.95, 76.93; H, 11.04, 11.26.

Isolation of the Free Alcohol.—The urethan was saponified by refluxing for eight hours with 20 cc. of 10% alcoholic potassium hydroxide. The solution was diluted with water, extracted with ether, and the ethereal solution was washed, first with dilute hydrochloric acid and then with water. The ethereal solution on evaporation to dryness yielded 0.4 g. of a faintly yellowish residue. The substance was easily soluble in warm methyl alcohol and separated in colorless needles when the solution was cooled. After three recrystallizations the substance weighed 0.18 g., m. p. 56°, $[\alpha]^{25}D$ in chloroform +5.7°; in benzene +7.3°.

Anal. Calcd. for $C_{18}H_{38}O$(270): C, 80.00; H, 14.07. Found: C, 80.13; H, 14.17.

Oxidation of the Alcohol to the Ketone.—The alcohol was oxidized in glacial acetic acid with chromic acid and the product was isolated and purified as described above; 90 mg. of the alcohol gave 60 mg. of the recrystallized ketone as thin, snow white plates from alcohol, m. p. 52°.

The semicarbazone was prepared from 25 mg. of the ketone. It separated in thin long plate-shaped crystals from alcohol, m. p. 127.5°.

The properties of the ketones obtained by oxidation of the two alcohols from the timothy bacillus wax agreed with those of 2-eicosanone and 2-octadecanone which were synthesized and described by Morgan and Holmes.[8] In order to identify our two ketones definitely we synthesized 2-eicosanone and 2-octadecanone as mentioned below and found that these ketones and their derivatives were identical in every respect with the ketones obtained by oxidation.

(8) G. T. Morgan and E. Holmes, *J. Soc. Chem. Ind.*, **44**, 108T (1925).

Preparation of 2-Eicosanone.—The ketone was synthesized by heating 4.5 g. of *n*-heptadecyl bromide, 2.5 cc. of ethyl acetoacetate, 0.4 g. of sodium and 8 cc. of absolute alcohol in a sealed tube for eight hours. The condensation product was saponified with potassium hydroxide in 50% alcohol and the ketone was recrystallized 5 times from alcohol yielding 1.2 g. of thin colorless plates. The pure ketone melted at 58° and gave no depression when mixed with the ketone prepared from the C_{20} alcohol.

Anal. Found: C, 81.06; H, 13.90.

The oxime and the semicarbazone were prepared. The oxime melted at 73–74° and the semicarbazone melted at 128°; mixed melting points with the corresponding derivatives of the ketone obtained by oxidation gave no depression.

Preparation of 2-Octadecanone.—The 2-octadecanone was synthesized from *n*-heptadecylic acid chloride and methyl zinc iodide and purified by crystallization from alcohol. Starting with 2 g. of heptadecylic acid, prepared from cetyl alcohol by the method of Levene and Taylor,[9] we obtained 0.7 g. of the purified ketone, m. p. 52°.

Anal. Calcd. for $C_{18}H_{36}O$(268): C, 80.60; H, 13.44. Found: C, 80.67; H, 13.56.

The semicarbazone was prepared and purified by crystallization from alcohol; long thin prismatic crystals, m. p. 127.5°.

Anal. Calcd. for $C_{19}H_{39}N_3O$(325): C, 70.15; H, 12.00. Found: C, 70.00; H, 12.13.

Mixed melting points with the ketone and semicarbazone prepared from the C_{18} ketone gave no depression.

Summary

The principal constituents of the timothy bacillus wax are:

1. Higher optically active fatty acids. None of the ordinary fatty acids could be found. An unsaturated hydroxy dibasic acid of the probable formula $C_{70}H_{138}O_6$ was isolated.

2. The unsaponifiable matter contains two new optically active alcohols, *d*-eicosanol-2 and *d*-octadecanol-2.

3. The chief water-soluble constituents of the wax are trehalose and glycerol.

NEW HAVEN, CONN. RECEIVED OCTOBER 21, 1935

(9) P. A. Levene and F. A. Taylor, *J. Biol. Chem.*, **59**, 905 (1924).