made to determine the extent of hydrolysis for varying periods of time. Instead, one experiment with each was carried out, using 0.05 *N* hydrochloric acid, 0.05 *N* potassium hydroxide and 9% borax at 100°. With hydrochloric acid, hydrolysis was complete in one hour and with potassium hydroxide, in thirty minutes. With borax, the glucoside was hydrolyzed 62.4% in one hour. The insoluble product of acid hydrolysis was quite pure anthrone (m. p. 154–155.5°), but with potassium hydroxide, some oxidation to dianthrone occurred. The insoluble product of borax hydrolysis was not obtained free from unchanged glucoside.

Summary

Anthranol-β-*d*-glucoside has been prepared and has been found to be hydrolyzed easily in acid and alkaline media.

ST. LOUIS, MISSOURI RECEIVED MARCH 6, 1937

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

The Chemistry of the Lipides of Tubercle Bacilli. XLVII. The Composition of the Avian Tubercle Bacillus Wax[1]

BY R. E. REEVES[2] AND R. J. ANDERSON

In paper X of this series Anderson and Roberts[3] described the extraction and fractionation of the lipides of the avian tubercle bacillus and it was found that the crude chloroform-soluble wax amounted to 70.7% of the total lipides and 10.79% of the dried bacterial mass. The present report deals with the properties and composition of the above mentioned wax. After the wax had been purified and saponified the cleavage products were found to consist of fatty acids, unsaponifiable matter and a water-soluble carbohydrate.

The fatty acids were a complex mixture of optically active hydroxy acids of very high molecular weight whose constitution we could not determine. None of the ordinary fatty acids could be found. The unsaponifiable matter consisted mainly of *d*-eicosanol-2 together with a small amount of *d*-octadecanol-2. The water-soluble carbohydrate was identified as trehalose.

The nature and kind of the cleavage products of the avian wax differentiate this material sharply from the previously examined wax of the human tubercle bacillus.[4] The latter product gave on saponification a mixture of fatty acids from which a hydroxy acid of high molecular weight, designated by the term "unsaponifiable wax," was isolated. The unsaponifiable matter contained the dihydric alcohol, phthiocerol, $C_{35}H_{72}O_3$,[5] while the water-soluble component was a specific polysaccharide which on hydrolysis yielded *d*-arabinose, galactose and other carbohydrates including inosite, mannose and glucosamine.

The avian wax shows considerable resemblance to the timothy bacillus wax which was recently analyzed by Pangborn and Anderson.[6] Both the avian and the timothy wax contain *d*-eicosanol-2, *d*-octadecanol-2 and trehalose together with new and previously unknown fatty acids of undetermined constitutions.

The object of the present investigation was not only to determine the chemical composition of the avian wax, which is quantitatively the most important fraction of the lipides of the avian tubercle bacillus, but also to provide sufficient quantities of the purified wax and of its cleavage products for physiological studies. The physiological experiments are being carried out by Dr. F. R. Sabin and collaborators of the Rockefeller Institute for Medical Research and will be reported independently.

Experimental Part

The crude wax isolated as described by Anderson and Roberts[3] was a non-crystalline powder of light yellow color. It was easily soluble in chloroform, ether, benzene, toluene, ligroin and ethyl acetate but it was insoluble in acetone, alcohol and methyl alcohol. The following constants were determined: m. p. 53–54°, iodine no. 7.8, saponification no. 77, $[\alpha]_D$ in $CHCl_3$ + 25.6°. A trace of phosphorus was present but sulfur, nitrogen and halogen were absent.

(1) An abstract of this paper was read before the Division of Organic Chemistry at the meeting of the American Chemical Society, Pittsburgh, Pa., September, 1936. The data are taken from the dissertation submitted by Richard E. Reeves to the Faculty of the Graduate School, Yale University, 1936, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The present report is a part of a coöperative investigation on tuberculosis; it has been supported partly by funds provided by the Research Committee of the National Tuberculosis Association.

(2) Holder of a National Tuberculosis Association Graduate Student Fellowship at Yale University, 1934–1936.

(3) R. J. Anderson and E. G. Roberts, *J. Biol. Chem.*, **85**, 509 (1930).

(4) R. J. Anderson, *ibid.*, **83**, 505 (1929).

(5) F. H. Stodola and R. J. Anderson, *ibid.*, **114**, 467 (1936).

(6) M. C. Pangborn and R. J. Anderson, THIS JOURNAL, **58**, 10 (1936).

Purification of the Wax.—For purification 170 g. of the wax was precipitated from solution in chloroform or ether by adding methyl alcohol and cooling. The wax separated as a white, granular powder. The optical rotation increased slowly during purification until the maximum value of +38.6° was attained after forty precipitations. The yield of the purified wax, designated Fraction I, was 69.6 g. The substance had the following properties, m. p. 54–55°, iodine no. 4.5, $[\alpha]_D$ in $CHCl_3$ +38.6°. It contained the merest trace of phosphorus. On combustion it gave C, 75.38, H, 12.14. When titrated in ether–alcohol solution the wax was found to be essentially neutral.

The material contained in the mother liquors was recovered as follows. (a) The mother liquors from the first ten precipitations were concentrated to dryness. The residue which contained most of the coloring matter of the original wax formed a yellowish solid and it weighed 35.1 g. This fraction was set aside and was not examined further. (b) The mother liquors from the subsequent precipitations were concentrated to dryness. The residue was dissolved in chloroform and precipitated by adding methyl alcohol and cooling. The nearly white powder which was obtained weighed 62.2 g. and was designated Fraction II. It had the following properties: m. p. 53–55°; iodine no. 8.7; $[\alpha]_D$ in $CHCl_3$ +17.7°. Except for a lower optical rotation and a somewhat greater solubility Fraction II was very similar to Fraction I.

Since neither fraction could be obtained in crystalline form they were saponified and the cleavage products were separated.

Saponification of the Wax.—The purified wax, Fraction I, was saponified in two lots; in each case 25 g. of the wax was refluxed for ten hours on a water-bath with 500 cc. of 1.6% alcoholic potassium hydroxide. Fraction II was also saponified in two lots using a corresponding quantity of alcoholic potassium hydroxide. The wax melted in the boiling alcohol and as the saponification proceeded the oil was replaced gradually by a brittle cake on the bottom of the flask. The material was never completely in solution.

Separation of the Cleavage Products. The Alcohol-Insoluble Fraction "A."—The hot alcoholic solution was decanted from the insoluble cake and the flask was rinsed several times with hot alcohol. The residue in the flask was extracted with hot benzene and the material obtained on evaporation of the solvent was added to the alcoholic solution. The alcohol and benzene insoluble fraction was soluble in water and was reserved for the isolation of trehalose as will be described later.

Alcohol-Insoluble Potassium Soaps. Fraction "B."—The alcoholic solution containing the potassium salts of fatty acids, unsaponifiable matter, etc., deposited on cooling a large amount of a white precipitate which was found to consist of the potassium salts of high molecular weight fatty acids. The precipitate was filtered off, washed with cold alcohol and dried.

Fatty Acids from the Alcohol-Soluble Potassium Soaps. Fraction "C."—The filtrate from the alcohol-insoluble potassium salts was freed from excess of potassium hydroxide by means of carbon dioxide. The potassium carbonate was filtered off, washed with alcohol and discarded. The filtrate and washings were concentrated to a volume of about 200 cc. and the hot solution was mixed with a hot alcoholic solution of lead acetate. The precipitate which separated on cooling was filtered off, washed with alcohol and decomposed in the usual manner, yielding a small amount of fatty acid.

The alcoholic filtrate from the lead salt was concentrated *in vacuo* to dryness and the residue was mixed with water and extracted with ether. The aqueous solution was reserved and examined for glycerol as will be described below.

The ethereal solution was washed first with dilute acetic acid for the removal of any lead which might be present, and then with water until the washings were neutral, after which it was twice extracted with dilute potassium hydroxide. The ethereal solution was reserved for the isolation of unsaponifiable or neutral material. The alkaline extract was acidified and extracted with ether. The ethereal extract yielded on evaporation a small amount of fatty acids which was combined with the acids obtained from the lead salts.

The Unsaponifiable or Neutral Material. Fraction "D."—The ethereal solution, after the alkaline extraction mentioned above, was washed with water, treated with norite, filtered and the ether was distilled off. The residue, which was a white crystalline solid, was found to consist of two optically active higher alcohols as will be described later.

Examination for Glycerol.—The aqueous solution which had been reserved for the examination for glycerol was evaporated to dryness *in vacuo*. The residue was extracted with warm pyridine, filtered and the pyridine was evaporated *in vacuo*. In no case was any weighable residue obtained and we conclude therefore that the wax did not contain any glycerol. Furthermore, no reducing sugar could be detected in the pyridine-insoluble salt residue.

A summary of the cleavage products of the wax fractions is presented in Table I.

TABLE I

CLEAVAGE PRODUCTS OF THE AVIAN WAX

	Purified wax		Wax fraction II
Wax saponified, g.	25.0	25.0	62.2
Carbohydrate, Fraction "A," %	11.3	13.3	5.6
Alcohol-insoluble soaps, Fraction "B," %	80.0	82.0	84.8
Acid from alcohol-soluble soaps, Fraction "C," %	2.2	2.6	6.8
Neutral material, Fraction "D," %	10.8	9.1	9.0
Glycerol	None	None	None

Examination of the Cleavage Products

Isolation and Identification of Trehalose.—The alcohol insoluble Fraction "A" was dissolved in water and the solution was neutralized with acetic acid. The solution was filtered from a slight amount of insoluble material, concentrated *in vacuo* to a small volume, and neutral lead acetate solution added until no further precipitate formed. The slight precipitate was filtered off and discarded. A carbohydrate was isolated from the filtrate by means of basic lead acetate and ammonia in the usual manner. The crude carbohydrate, which was obtained as a white

powder, gave no pentose reactions and no reduction with Fehling's solution until after it had been boiled for some time with dilute acid.

One gram of the substance was acetylated and yielded 1.95 g. of the acetyl derivative. After two crystallizations as described by Pangborn and Anderson[6] the colorless, needle-shaped crystals weighed 1.2 g. and after being heated at 60° *in vacuo* for several hours melted at 97–98°. In chloroform solution $[\alpha]_D$ was +163.7°. On saponification 70.6% of acetic acid was liberated. Trehalose octaacetate requires 70.8% of acetic acid.

The acetyl derivative, 6.5 g., prepared as above, was saponified and the free trehalose was isolated and crystallized from 80% alcohol. Large, colorless, prismatic crystals were obtained which weighed 1.7 g. and melted at 98°, $[\alpha]_D$ in water +178°. On drying at 78° *in vacuo* the crystals lost 9.85% in weight. Calcd. for $C_{12}H_{22}O_{11}\cdot 2H_2O$: H_2O, 9.52.

These data complete the identification of the carbohydrate as trehalose.

Examination of the Acids from the Alcohol-Insoluble Potassium Salts, Fraction "B."—The alcohol-insoluble Fraction "B" was found to consist of potassium salts of fatty acids of high molecular weight. The separation of the free acids by fractional precipitation or by fractionation of the acetyl derivative was difficult and incomplete. A more rapid and satisfactory separation into two distinct fractions was effected by means of the difference in solubility of the potassium salts between ligroin and methyl alcohol. The potassium salts were easily soluble in ligroin and when the ligroin solution was shaken with methyl alcohol two layers separated. The alcoholic solution contained the salt of an acid having a molecular weight of about 500, Fraction BI, while the ligroin solution contained the salt of an acid having a molecular weight of about 1300, Fraction BII. By repeating the procedure several times with each fraction two acids were obtained in about equal amounts whose properties remained essentially unchanged by further attempts at purification. The potassium salt of the acid having the lower molecular weight although insoluble in pure methyl alcohol was easily soluble in methyl alcohol containing ligroin.

An adequate purification of these wax acids was impossible by present available methods. The acids do not crystallize but separate from solvents in the form of colorless, globular particles; hence criteria of purity are entirely lacking. The analytical values for carbon and hydrogen and the molecular weights as determined by titration do not correspond to any definite empirical formula. The reason may depend upon lactone formation.

The analyses of the acids and of their derivatives indicate that the acids contain more than 2 atoms of oxygen but we have been unable to demonstrate conclusively that a third oxygen atom is present as a hydroxyl group although the methyl esters show some active hydrogen[7] and the free acids on acetylation yield products which are partly acetylated. The ratio of C:H is somewhat less than 1:2 but the acids show a low iodine number and on treatment with bromine they yield anomalous bromine derivatives, bromine is absorbed and is also slowly substituted with liberation of hydrobromic acid.

(7) T. Zerewitinoff, *Ber.*, **40**, 2023 (1907).

The free acids were prepared from the potassium salts in the usual manner and purified by precipitation from ethereal solution by the addition of alcohol. The acids were easily soluble in ether, chloroform, ligroin and benzene but insoluble in alcohol or acetone. Dr. F. R. Sabin examined these acids and found that they were acid-fast

In general the properties of the avian wax acids resemble those of the analogous acids encountered in the wax from human tubercle bacillus[4] and in the timothy bacillus wax[6] but they are not identical.

The complete elucidation of the composition and structure of these interesting but very complex wax acids must be left for future investigations. For the present we record the observed properties of the free acids and of some of their derivatives in Table II.

TABLE II

PROPERTIES OF THE AVIAN WAX ACIDS

	Fraction BI	Fraction BII
Free acid, m. p., °C.	69–70	60–61
$[\alpha]_D$ in $CHCl_3$	+5.6°	+5.5°
Analysis	C, 78.99; H, 12.78	C, 82.46; H, 13.49
Mol. wt. by titration	501-520	1280–1300
Approximate formula	$C_{28}H_{74}O_2$	$C_{88}H_{178}O_2$
Acetyl derivative, m. p., °C.	54–55	48–57
Methyl ester, m. p., °C.	54–55	49–50
Active hydrogen of ester,[7] %	0.92	0.82
Bromo derivative	m. p. 47–49° Br. 22.4%	m. p. 43–49° Br. 22.9%
Iodine number (Hanus)	6.5	5.5

Fraction C.—The acids obtained from Fraction C represented only a small percentage of the total acids. They were semi-solid, had low iodine numbers and were obviously a mixture from which no pure substance could be isolated, nor could any of the usual fatty acids be found.

The Neutral Material. Fraction D. Isolation of *d*-Eicosanol-2.—The neutral material was found to consist largely of *d*-eicosanol-2 but a small amount of *d*-octadecanol-2 was also present. The isolation of *d*-eicosanol-2 was accomplished readily by crystallization. After the crude neutral material had been crystallized three times from ethyl acetate and twice from methyl alcohol, colorless prismatic crystals were obtained which melted at 62–63° and gave no depression when mixed with an authentic specimen of *d*-eicosanol-2; $[\alpha]_D$ in ether +6.79°.

Anal. Calcd. for $C_{20}H_{42}O$(298): C, 80.55; H, 14.18. Found: C, 80.54; H, 14.25; mol. wt. (Rast), 294.

The 3,5-dinitrobenzoate was prepared and recrystallized from alcohol. Colorless, needle-shaped crystals were obtained which melted at 77.5–78°; $[\alpha]_D$ in $CHCl_3$ +23.4°.

Anal. Calcd. for $C_{27}H_{44}O_6N_2$(492): C, 65.82; H, 9.01. Found: C, 65.62; H, 8.91.

Oxidation of the alcohol by the procedure employed by Pangborn and Anderson[6] yielded eicosanone-2, colorless thin plates, m. p. 60–61°. The melting point was not depressed when the ketone was mixed with a sample of synthetic eicosanone-2.

Anal. Calcd. for $C_{20}H_{40}O$(296): C, 80.99; H, 13.61. Found: C, 80.73; H, 13.44.

The semicarbazone was prepared and crystallized from alcohol. Colorless, prismatic crystals were obtained which

melted at 130–131°. The melting point was not depressed when the semicarbazone was mixed with the semicarbazone of eicosanone-2.

Isolation of *d*-Octadecanol-2.—The material recovered from the mother liquor from *d*-eicosanol-2 yielded very small quantities of *d*-octadecanol-2. A portion of this material, 0.9 g., was converted into the phenylurethan. The latter after more than 100 recrystallizations yielded 60 mg. of needle-shaped crystals which had the correct melting point, 76–76.5°, of the phenylurethan of *d*-octadecanol-2. A mixed melting point with an authentic specimen gave no depression. However, this method of purification was too laborious and the yield too small,

Another portion of the crude material, 2.53 g., was first fractionated by distillation through a modified Widmer column into 3 fractions. The first fraction, 0.73 g., which distilled at 170–190° and 3 mm. pressure, was converted into the 3,5-dinitrobenzoate. The product, after treatment with norite, was recrystallized from alcohol until the melting point was constant at 71–72°. The colorless needle-shaped crystals weighed 0.45 g.; $[\alpha]_D$ in $CHCl_3$ +25.3°.

Anal. Calcd. for $C_{25}H_{40}O_6N_2$(464): C, 64.61; H, 8.68. Found: C, 64.78; H, 8.56.

The dinitrobenzoate, 392 mg., was saponified by refluxing with 4% alcoholic potassium hydroxide. After the alcohol had been isolated and recrystallized four times from methyl alcohol, 197 mg. of colorless needles was obtained. The crystals melted at 53–54° and there was no depression when mixed with *d*-octadecanol-2; $[\alpha]_D$ in $CHCl_3$ +4.84°.

Anal. Calcd. for $C_{18}H_{38}O$(270): C, 79.91; H, 14.17. Found: C, 80.15; H, 13.98; mol. wt. (Rast), 271.

The octadecanol-2, 88 mg., was oxidized to the ketone and the latter was recrystallized three times as described by Pangborn and Anderson.[6] The yield was 32 mg. of colorless, plate-shaped crystals. The ketone melted at 50–51° and there was no depression when mixed with a sample of synthetic octadecanone-2.

Summary

1. The chloroform-soluble wax of the avian tubercle bacillus has been purified, saponified and the cleavage products investigated.

2. The principal constituents of the wax are hydroxy fatty acids of very high molecular weight. The acids were acid-fast and were optically active. None of the usual fatty acids were found.

3. The unsaponifiable matter of the wax consisted of *d*-eicosanol-2, $C_{20}H_{42}O$, together with a small amount of *d*-octadecanol-2, $C_{18}H_{38}O$.

4. The water-soluble component of the wax liberated on saponification was identified as the disaccharide trehalose, $C_{12}H_{22}O_{11}$.

5. Glycerol could not be detected among the cleavage products.

NEW HAVEN, CONN. RECEIVED MARCH 8, 1937

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MISSISSIPPI]

The Oxidation of Substituted Phenols. The Effect of Iodine in the Ortho and Para Positions

BY G. H. WOOLLETT, F. M. DAVIS, C. N. JONES AND MARY NEILL

Upon oxidation 2,6-dimethoxyphenol is converted almost quantitatively into cedriret (3,5,-3′,5′-tetramethoxydiphenoquinone-4,4′)[1] while similar phenols such as 2,6-dimethylphenol[2] and 2,6-diacetamidophenol[3] yield 70% or more of dinuclear quinones. These are highly colored conspicuously crystalline sparingly soluble compounds whose structure has been verified amply. The reaction is commonly cited as an example of steric hindrance. Carboxyl, chlorine, bromine,[4] hydroxyl, methoxyl[2] and methyl para to the hydroxyl prevent the reaction.

Another group of substances often confused with the true dinuclear quinones is the highly colored resinoids obtained by the action of iodine and alkali on phenols having ortho and para positions unsubstituted. One of these (Lautemann's Red), obtained from common phenol, was said to be 3,5,3′,5′-tetraiododiphenoquinone-4,4′, by Kammerer and Benzinger[5] shortly after the report on the structure of cedriret by Hoffman, the similarity in color no doubt being the cause of the mistake. The analogous product from thymol[5,6] (aristol) was also thought to be a dinuclear quinone. Later investigators[7–10] agree that Lautemann's Red and aristol are amorphous and have high molecular weights, although it has been sug-

(1) A. W. Hoffman, *Ber.*, **11**, 329 (1878).
(2) Auwers and others, *ibid.*, **38**, 226 (1905); **57**, 1270 (1924).
(3) Fromm and Ebert, *J. prakt. Chem.*, **108**, 75 (1924).
(4) Hunter and Levine, THIS JOURNAL, **48**, 1608 (1926).
(5) Kammerer and Benzinger, *Ber.*, **11**, 557 (1878).
(6) Messinger and Vortmann, *ibid.*, **22**, 2314 (1889); Vortmann, *ibid.*, **56B**, 234 (1923).
(7) Bougault, *Compt. rend.*, **146**, 1404 (1908).
(8) Carswell, *Chem. News*, **68**, 87, 99, 131, 153, 166, 181 (1893).
(9) Hunter and Woollett, THIS JOURNAL, **43**, 131, 135 (1921).
(10) Woollett and others, *ibid.*, (a) **38**, 2474 (1916); (b) **43**, 553 (1921); (c) **52**, 4018 (1930); (d) **55**, 2909 (1933).