acidification (V) was obtained as a white precipitate. It was recrystallized from dilute acetic acid; m. p. 178–180°.

Anal. Calcd. for $C_{13}H_{11}NO_4S$: C, 56.31; H, 3.97; N, 5.05; S, 11.55. Found: C, 56.57, 56.41; H, 3.47, 3.11; N, 5.22; S, 11.73, 11.38.

When (IV) was heated for some time in hydrochloric acid solution benzoic acid was obtained. The remaining solution, after removal of sodium chloride, gave a deep purple color with ferric chloride.

Summary

Six-atom heterocyclic compounds formed by several condensation reactions of N-benzoyl-*o*-aminobenzenesulfonamide and its diazotization products are described. A seven-atom ring could not similarly be formed. A new ring system and five new compounds are reported.

FAYETTEVILLE, ARKANSAS RECEIVED JANUARY 9, 1934

[231ST CONTRIBUTION FROM THE COLOR AND FARM WASTE DIVISION, AND OIL, FAT AND WAX LABORATORY, BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

The Chemical Composition of the Fat Produced by Penicillium Javanicum van Beijma[1]

BY GEORGE E. WARD AND GEORGE S. JAMIESON

Introduction

The presence of unusually large quantities of fatty substances in the mycelium of *Penicillium javanicum* van Beijma, as will be reported in a later communication, indicated the desirability of establishing the chemical composition of this material.

Relatively little information is available regarding the composition of fats produced by mold fungi. In 1906 Browne[2] described the chemical characteristics of a fat extracted from a Citromyces which had grown as a scum on the surface of molasses in the hot-room of a sugar factory. The fat, which comprised 27.50% of the air-dried scums, was similar to butterfat in saponification number, iodine number, Reichert–Meissl number, and melting point, and in the melting point and iodine number of the insoluble acids, but differed from fresh butterfat in the acid number and mean molecular weights of soluble and insoluble acids. No absolute identification of the fatty acid constituents was reported. Sullivan,[3] in 1913, found palmitic acid, oleic acid and two unidentified fatty acids in the dried mycelium of *Penicillium glaucum* which was grown on Raulin's solution. In 1927 and 1929 Barber[4] reported the production of fat by a species of Penicillium when it was grown on glycerol, xylose, glucose and sucrose solutions. The fat was composed of palmitic, stearic, oleic and α- and β-linoleic acids, and an x-ray analysis indicated the presence of a fatty acid containing nineteen carbon atoms. The hexabromide test showed the absence of linolenic acid. The fat had chemical characteristics lying within the following ranges, depending on variations in the culture media:

Saponification value	166–201
Iodine value of combined fatty acids	78–101
Melting point of saturated acids, °C.	54–55
Mean molecular weight of saturated acids	264–293
Percentage of saturated acids	16–29

Experimental Part

The fat described in this communication was produced by *Penicillium javanicum* van Beijma when it was cultured on 20% glucose solutions at 30° as will be described in a later paper. The fat was obtained by extracting 6240 g. of the dried mycelium with redistilled petroleum

TABLE I

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE OIL FROM *Penicillium javanicum* VAN BEIJMA

Solidification point, °C.	6–7
Melting point, °C.	about 15
Specific gravity (25°/25°)	0.9145
Refractive index (25°)	1.4680
Acid value	10.6
Saponification value	191
Iodine value (Hanus)	84.0
Reichert-Meissl value	0.3
Acetyl value	10.7
Unsaponifiable matter,[5] %	2.00
Saturated acids (corrected),[6] %	30.8
Unsaturated acids (corrected),[6] %	60.8
Melting points of mixed saturated acids	52.5°
Mean molecular weight of saturated acids	272

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(1) Presented in part before the Section of Biological Chemistry of the American Chemical Society March 27–30, 1933, Washington, D. C.

(2) C. A. Browne, Jr., THIS JOURNAL, **28**, 465 (1906).

(3) M. X. Sullivan, *Science*, **38**, 678 (1913).

(4) H. H. Barber, *J. Soc. Chem. Ind.*, **46**, 200T (1927); *Biochem. J.*, **23**, 1158 (1929).

(5) Modified Kerr-Sorber method—R. Hertwig, G. S. Jamieson, W. F. Baughman and L. W. Bailey, *J. Assoc. Off. Agr. Chem.*, **8**, 439 (1925).

(6) Lead salt-ether method—"Methods of Analysis," Assoc. Official Agr. Chem., 3d ed., 324 (1930).

ether (b. p. 35–65°), removing the solvent by bubbling carbon dioxide through the heated oil, and clarifying with norite and filter-cell. This treatment yielded a clear orange-colored oil which weighed 691 g., corresponding to 11% of the dried mycelium taken for extraction. Table I gives the physical and chemical characteristics of this oil.

Four per cent. solutions of the oil and of the unsaturated fraction in chloroform showed no optical activity.

The presence of glycerol in the oil was established by applying the acrolein test to the ether–alcohol extracts of the neutralized and concentrated aqueous solution remaining after the separation of the fatty acids from a saponified sample of the oil.

Examination of the Unsaturated Fraction

Hydrogenation.—Seven grams of unsaturated acids was dissolved in 80 cc. of 95% alcohol and hydrogenated with the aid of 0.2 g. of Voorhees and Adams platinum oxide catalyst.[7] The resulting solution yielded, upon fractional crystallization, only material melting at 69.5 to 71.6°, which is the melting point range reported for pure stearic acid. This indicated that only fatty acids containing eighteen carbon atoms were present in the unsaturated fraction.

Bromination.—3.85 grams of unsaturated acids in 50 cc. of anhydrous ethyl ether was brominated according to the method of Eibner and Muggenthaler.[8] The absence of an insoluble hexabromide indicated that the fat contained no linolenic acid. However, 1.44 g. of linoleic tetrabromide (m. p. 114°) was obtained; equivalent to 0.67 g. of α-linoleic acid, corresponding to 17.4% of the unsaturated acids taken for bromination. As subsequent calculations show the presence of 47.9% linoleic acid in the unsaturated fraction, it is apparent that a considerable portion of this is the so-called β-linoleic acid, whose tetrabromide is soluble in petroleum ether.

As there are only two unsaturated acids present, it is possible to calculate the composition of the unsaturated portion of the oil, using the iodine value (84.0) and the percentage of unsaturated acids (60.8).

	In unsaturated fraction, %	In oil % Acid	In oil % Glyceride
Oleic acid	52.1	31.7	33.2
Linoleic acid	47.9	29.1	30.5
Total	100.0	60.8	63.7

Examination of the Saturated Acids.—Six fractions and a small residue were obtained by distillation at 6–12 mm. pressure of 82.5 g. of saturated ethyl esters, as previously described by Jamieson and Baughman.[9] The composition of these fractions was calculated from their saponification and iodine values, as already described.[10]

The composition of the saturated portion of the oil was as follows:

	In satd. fraction, %	In oil % Acid	In oil % Glyceride
Palmitic acid	69.5	21.4	22.4
Stearic acid	28.0	8.6	9.0
Tetracosanic acid	2.5	0.8	0.8
Total	100.0	30.8	32.2

That the saturated portion consisted of only these three acids was ascertained by identifying the fatty acids liberated by saponification of the various ester fractions. These acids were recrystallized from 95% alcohol, and their melting points were determined.

Palmitic acid, melting at 62.5°, was obtained from the first four fractions. Stearic acid, melting at 69–71°, was isolated from fractions 2, 3, 4, 5 and 6.

The first crops of crystals from fractions 5, 6 and the residue from the distillation were snow-white and were far less soluble in alcohol than palmitic or stearic acid. After several recrystallizations from alcohol the substance melted at 83.0–83.5°.

Anal. Calcd. for $C_{24}H_{48}O_2$: C, 78.18; H, 13.13; neut. equiv., 368. Found: C, 78.16, 78.39; H, 13.32, 13.37; neut. equiv., 371, 374.

That the substance was a tetracosanic acid was verified by an x-ray examination of the acid and of its ethyl ester by Dr. S. B. Hendricks of the Bureau of Chemistry and Soils. The spacing of the C-form of the acid was 52.4 ± 0.1 Å. and the spacing of the ethyl ester was 32.5 ± 0.3 Å. Corresponding values of 52.8, 52.7 Å., and 32.1, 32.9 Å., have been reported by Francis, Piper and Malkin[11] for the acids and esters of the synthetic normal lignoceric acid and of the lignoceric acid isolated from beechwood tar. These authors give the melting point of normal tetracosanic acid as 83.5–84.0°, and that of its ethyl ester as 54.4°. The ethyl ester of the acid isolated from mold fat also melted at 54.4°.

In the x-ray study of the acid, eleven orders of reflection were obtained, and this fact, coupled with the very close agreement of the spacing measurements with those of normal lignoceric acid, would seem to indicate that the acid from the mold fat was of a high degree of purity and was not accompanied by neighboring homologs, as is often the case in fats produced by higher plants.

Discussion

The oil produced by *Penicillium javanicum* van Beijma is entirely different from the fat isolated by Browne from a Citromyces; however, it is somewhat similar to the fat obtained from a Penicillium by Barber, as both of these fats contain palmitic, stearic, oleic and α- and β-linoleic acids, but no linolenic acid.

The present investigation is the first to report normal tetracosanic acid as a product of mold metabolism.

The authors are indebted to Mr. Lewis B. Lockwood and Dr. O. E. May for general assistance in

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(7) V. Voorhees and R. Adams, THIS JOURNAL, **44**, 1397 (1922).

(8) J. Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," 6th ed., Vol. I, p. 585, 1921.

(9) G. S. Jamieson and W. F. Baughman, THIS JOURNAL, **42**, 1200 (1920).

(10) W. F. Baughman and G. S. Jamieson, *Ibid.*, **42**, 156 (1920).

(11) F. Francis, S. H. Piper and T. Malkin, *Proc. Roy. Soc.* (London), **A128**, 214 (1930).

carrying out this work, and to Mr. J. R. Spies for the micro-combustions.

Summary

The physical and chemical characteristics of the fat extracted from the dried mycelium of *Penicillium javanicum* van Beijma have been determined. The fat is composed of palmitic, stearic, tetracosanic, oleic and α- and β-linoleic acids, in addition to glycerol and a small quantity of unsaponifiable matter.

Normal tetracosanic acid has for the first time been identified as a constituent of mold fat.

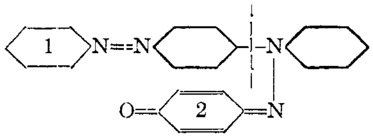
WASHINGTON, D. C. RECEIVED JANUARY 10, 1934

[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF THE WASHINGTON SQUARE COLLEGE OF NEW YORK UNIVERSITY]

Studies in the Di-aryl Acyl Hydrazine Series. III. The Oxidation of Acetylhydrazobenzene

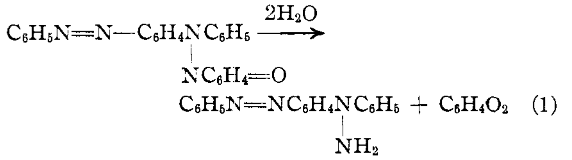
BY FRANK O. RITTER

In a previous communication in this series[1] the formation of a highly colored oxidation product on treatment of acetylhydrazobenzene with dichromate in glacial acetic acid solution was described. Further study of this product has proved it to have the structure shown.

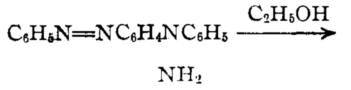


The correctness of this structure is established from the following considerations: (1) the molecular formula is $C_{24}H_{18}N_4O$, (2) the products of reduction are *p*-aminodiphenylamine, aniline and *p*-aminophenol, (3) titration with stannous chloride requires eight equivalents of hydrogen, (4) treatment with alcoholic potassium hydroxide forms *p*-phenylaminoazobenzene.

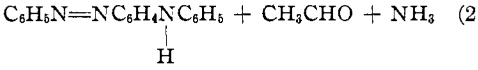
The latter substance was prepared by Witt[2] by coupling of the benzenediazonium salt with diphenylamine. The amount of this substance theoretically obtainable from our product is 72.2, while that actually isolated is 65 to 70%. Its formation from our product appears to involve hydrolysis and then reduction of the resulting secondary hydrazine as follows



$$\underset{\displaystyle \overset{|}{\mathrm{NC_6H_4{=}O}}}{\mathrm{C_6H_5N{=}N{-}C_6H_4NC_6H_5}} \xrightarrow{\mathrm{2H_2O}} \underset{\displaystyle \overset{|}{\mathrm{NH_2}}}{\mathrm{C_6H_5N{=}NC_6H_4NC_6H_5}} + \mathrm{C_6H_4O_2} \quad (1)$$



$$\underset{\displaystyle \mathrm{NH_2}}{\mathrm{C_6H_5N{=}NC_6H_4NC_6H_5}} \xrightarrow{\mathrm{C_2H_5OH}}$$



$$\underset{\displaystyle \overset{|}{\mathrm{H}}}{\mathrm{C_6H_5N{=}NC_6H_4NC_6H_5}} + \mathrm{CH_3CHO} + \mathrm{NH_3} \quad (2)$$

The latter point was corroborated by investigation of the action of alcoholic potassium hydroxide upon diphenylhydrazine, which was found to yield diphenylamine and ammonia This reaction probably involves oxidation of the alcohol to acetaldehyde. The isolation of benzoquinone in this reaction is not possible because it undergoes further reaction with alkali.

The structure of the oxidation products shows that its formation involves two molecules of the original acetylhydrazobenzene. If the nitrogen linkage of the original acetylhydrazobenzene is unbroken during the transformation to the oxidation product (the simplest assumption), one can readily trace the two original molecules in the structure of the final product. These have been indicated in the above structure by a dotted line.

It is also evident why only acetyl hydrazo compounds with an open para position[1] can yield these oxidation products. The open para position in one molecule is necessary to form a new bond, while the open para position in the second molecule is necessary to develop the quinonoid structure.

Furthermore, since the oxidation product contains a semidine nucleus, one is led to speculate whether the semidine transformation of hydrazo compounds does not also involve an analogous bimolecular compound, which, subsequently, by rejection of two phenyl nuclei (marked (1) and (2)) yields the semidine and the "bases of splitting." If we assume that this transitory union of the two molecules of the hydrazo compound can take place in only one sense because of the differ-

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(1) Ritter, THIS JOURNAL, **53**, 670 (1931).

(2) Witt, *Ber.*, **12**, 261 (1879).