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Sub Title	
Author	阿部, 芳郎(Abe, Yoshiro)
Publisher	慶應義塾大学藤原記念工学部
Publication year	1949
Jtitle	慶應義塾大学藤原記念工学部研究報告 (Proceedings of Faculty of Engineering, Keiogijuku University). Vol.2, No.7 (1949. 12) ,p.129(15)- 134(20)
JaLC DOI	
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Notes	
Genre	Departmental Bulletin Paper
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=KO50001004-00020007-0015

Studies on the Chemical Composition of Molds, (II) On the Fatty Material of Penicillium Chrysogenum Q176*

(Recieved Oct 7, 1952)

Yoshiro ABE **

Abstract

Relatively little has yet been reported on the composition of the fatty materials from the mycelium of mycrobes, except special ones, fatty yeasts[2] and acid-fast bacilli.[1] Especially previous investigations of the fatty materials of fungus tissue have been limited mainly to yeasts and to the higher fungi. In 1929, Tanaka[3] described the constituents of the fat extracted from Aspergillus oryzae. Peterson and his coworkers,[4] in 1934, studied the fat extracted from Aspergillus sydowi. Also, in 1934, Ward and Jamieson[5] found the composition of the fat from Penicllium javanicum.

In previous investigations[6] the fatty material contents of some strains of Penicillium notatum, Penicillium chrysogenum, which had produced penicillin, have been determined, and one of fat constituents, ergosterol has been isolated and identified. At the same time, the chemical characteristics of the fat from Penicillum notatum B21 was dealed in the paper.

In the present work, chemical constituents of fatty materials of one of these molds, Penicillium chrysogenum Q176 hove been examined in detail, and the connection with other mycrobes have been studied in the view of fatty constituents.

Experimental Part

Preparation of the Dried Mycelium chrysogenum Q176.

The mold used was grown in 700cc. Roux flasks containing 200cc. of the medium. The depth of the medium was 1.5cm. The penicillin assay is about 300-400u./cc

* This paper was printed in Japanese (Journal of the Oil Chemists' Society, Japan **Vol. 1. No. 3. 1952**)

** Assistant Professor of Keio University

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3) Ryohei Takada, J.Soc. Ind., Chem. Japan. **32.** (1929) 548.

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when mold was cultured at 24°C for a week on the ordinary peptone medium.

The mycelium was separated from the culture medium, washed in cold water, and dried for several days at 65-70°C. When dry, the dark brown mass was finely ground.

The analysis data of the dried mycelium are as follows.

Table 1. The analysis data of Penicillium chrysogenum Q176

Component	Oontent (%)	Content for anhydrous matter (%)
Water	9.0	
Crude fat	2.67	2.95
Crude protein	33.98	37.45
Ash	3.62	3.98

Extraction of the Crude Fats.

The fat was obtained by extracting 2.7kg. of the dry ground mycelium in a Soxhlet apparatus with ether for 24 hours. From ether extract a small amount of light yellow solid, m.p. 133-145°C, was separated out. After the solid was filtered, the filtrate was washed with water, dried with sodium sulfate, and decolourized with active carbon. Then the ether was removed by bubbling carbondioxide through the heated oil. The residual fat was a clear orange yellow coloured which weighed 62.0g., corresponding to 2.55%of the dried mycelium taken for extraction. Table 2 gives the physical and chemical characteristics of this fat.

Table 2. Chemical and physical characteristics of the fat from Penicillium chrysogenum Q176

Melting poing. °C	10-12
Specific gravity, d_4^{20}	0.9175
Acid value	58.8
Saponification value	176.7
Iodine value	89.0

The light yellow solid,which was separated out from ether extracts, was dissolved in hot alcohol, treated with active carbon, and recrystallized two times from alcohol. The white needles, m.p. 158-159°C, were obtained, The colour tests of Liebermann-Burchard, Salkowski, and Rosenheim were positive. The crystals were acetylated by heating with pyridine and acetic acid anhydride. The product, recrystallized from alcohol, melted at 172°C. These results establish the presence of ergosterol in this fat. Wt. 0.1220g., (yield 0.0045%).

Investigation of the Composition of the Fat.

Saponification of the Fat.

58g. of the crude fat was saponified by refluxing with 10% alcoholic potassium hydroxide in an atmosphere of hydrogen. After distilling of most alcohol at

diminished pressure and diluting with water, the uusaponifiable material together with unchanged fat was extracted with petroleum ether, resaponified, and the unsaponifiable portion obtained by again shaking out with ether.

The two soap solutions resulting from these operations were combined, acidified with dilute hydrochloric acid, and the fatty acids extracted with ether. Then mixed fatty acids were separated into solid and liquid fatty acids by the alcohol lead salt method of Twitchell.[7] Yields and characteristics of both acids are as follows.

Table 3. Solid-and liquid-fatty acids of the fat from Penicillium chrysogenum Q176

	Yield, g.(%)	Iodine value
Solid fatty acid	11.0 (19.0)	4.0
Liquid fatty acid	39.0 (67.2)	110.32
Total fatty acid	50.0 (86.2)	

Investigation of Unsaponifiables.

Petroleum ether was evaporated in an atmosphere of carbon dioxide from the unsaponifiable portion, dark red thick sirup obtained. Short after ethyl alcohol was added to the sirup white solid matter was separated out. This matter was filtered off, treated with active carbon, and recrystallized from ethyl alcohol, white needles m.p. 158°C, were obtained. Mixed with ergosterol which we obtained previously, there was no depression. Also various colour tests of ergosterol were positive. Yield, unsaphnifiables 2.25g., 3.90% of fatty matter. Isolated ergosterol 0.25g., 1.43% of fatty matter.

Investigation of Liquid Fatty Acids.

Bromination.

5g. of the liguid fatty acid in 50 cc. of anhydrous ethyl ether was brominated according to method of Haller.[8] The absence of an insoluble hexabromide indicated that the fat contained no linolenic acid.

However 5g. of the lquid fatty acids was brominated in 50cc. of cold petroleum ether solution, insoluble bromides were obtained. And the insoluble bromides were filtered, removed the excess of bromine with sodium hydrosulfite, and recrystallized from ligroin (b. p. 60°-80°C). Colourless needles, m. p. 113-114°C. were obtained. When mixed with tetrabromstearic acid there was no depression.

Anal.	Sample		0.1356g	0.1452g
	AgBr		0.1695g	0.1814g
	Br%	Calcd. for	$C_{18}H_{32}O_2Br_4$	53.33%
		Found	53.18%	53.22%

The petroleum ether soluble parts were evaporated in an atmosphere of carbon dioxide with diminished pressure. After the excess of bromine was removed with

7) E.Twitchell. Ind. Eng. Chem,. **13.** (1921) 806.

8) Haller, Compt. rend., **146.** (1908) 250.

sodium hydrosulfite, bromides were carefully debrominated according to the procedure recommended by Kimura.[9] And the debrominated acid was dissolved in 50% alcohol. reacted with 50% alcohol solution of lithium hydroxide. After a night, the separated soap was recrystallized from ethyl alcohol. And the lithium salt was decomposed with dilute hydrochloric acid, the fatty acid separated out. Neutralization value, 195.65; Iodin evalue, 84.93. It seems that these acids are mixed acids of oleic with little higher monoolefinic acid.

Oxidation.

Another sample of the liquid fatty acids was oxidized with alkaline permangnate by the method of Hazura.[10] Namely, 10g. of mixed acids was dissolved in 12cc. of concentrated pottasium hydroxide solution (sp. g. 1.27) and 650cc. of water. Then the solution was cooled at 0 - 2°C, added 650cc. of 1.5% permanganate solution drop by drop.

when the addition of permanganate was over, the solution was more still stirred for 30 minutes, decolourized with sulfite, and reacted with the concentrated hydrochloric acid. The precipitates were filtered, thoroughlly washed with water and petroleum ether, then dried. The dry precipitates were extracted with ether several times. And the ether extracts were combined, concentrated, the crystal was separated. After being recrystallized from alcohol two times, white plates, m.p. 128-130°C, were obtained. Neutralizaation value, 177.58. This corresponds to the dihydroxystearic acid. The insoluble parts were extracted with boiling water.

And the product from cold water after being recrystallized once from alcohol melted at 170-171°C. Neutralization value, 161.19. This indicates that the presence of α-satibic acid in the oxidation products of liquid acids.

As the consequence of the oxidation investigation, the main constituents of liquid fatty acids were linoleic and oleic acid.

Investigation of Solid Fatty Acids.

10g. of solid fatty acids was esterified with 8cc.of concentrated sulfuric acid and 200cc. of anhydrous ethyl alcohol. The ethyl esters of fatty acids were distilled at diminished pressure, and six distillates were obtained. The results of distillation were shown in Table 4.

Table 4. Distillation of the saturated esters

Fraction	B. P., °C	Weight, g	Sap. equiq, of corresponcing esters
I	145-150	1.68	217.55
II	150-155	2.82	197.17
III	155-158	3.59	193.79
IV	158-165	0.09	183.22
V	165-175	0.14	163.18
VI	175-200	0.27	144.20
Residue	—	1.50	—

9) Wasaburo Kimura, J. Soc. Ind., Chem. Japan. **34.** (1932) 958.

10) Hazura, Monatsh., 8. (1837) 463. 472.

From the fraction 1, the free fatty acid was obtained in the usual manner. The acid after several recrystallizations from alcohol melted at 53.0°C. Neutralization value, 246.65. Mixed with pure myristic acid there was no depression.

The acid from fraction 2 after several recrystallized from alcohol melted at 61.0°C. Neutralization value, 218.78. Mixed with pure palmitic acid there was no depression.

The combined fatty acids obtained from fractions 3 and 4 were crystallized fractionally from alcohol. Then two crystals. m.p. 63-65°C and 68-70°C, were separated. The acid from the latter after once recrystallized from alcohol melted at 69-70°C. Neutralization value, 197.52. Mixed with pure stearic acid, there was no depressin,

The detailed investigation of the former and the fraction 5 was not succeded for the lack of quantity.

The acid from fraction 6 and the distillation residue after three recrystallizations from alcohol melted at 81-82°C. Neutralization value, 153.44. This acid seems to be n-tetracosanoic acid.

Investigation of glycerin.

The aqueous solution after the separation of unsaponifiable matter and fatty acids was evaporated to dryness at diminished pressuse. Then alcohol-ether (1:1) mixed solvent was added to this concentrates and the insoluble part filtered off. The filtrate was conenetrated to a thick sirup, taken up in absolute alcohol, filtered and evaporated. To remove more water this treatment was repeated several times, and 3.15g. of a thick red brown sirup was obtained. The acrolein test of this sirup is positive. The data of glycerin analysis by the use of triacetin method are as follows.

Sample	1.3850g	1.4101g
Consumed 1N-NaOHaq.	33.95cc	34.25cc
Glycerin%	75.24%	74.00%

As the consequence of above data, the yield of glycerin was 2.35g.

The content of glpcerin of the fat from penicillium chrysogendm Q176 was 4.05%.

Composition of the Fat.

The approximate quantitative composition of the fatty material of Penicillium chrysogenum Q176 as calculated from neutralization value and iodine value is given in Table 5.

Table 5. Composition of the Fat from Penicillium Chrysogenum Q176

Fatty acids	86.2%
Saturated acids	19.0
myristic	3.2
palmitic	10.3
stearic	4.9
n-tetracosanic	0.6
Unsaturated acids	67.2

oleic	46.2
linoleic	17.5
C22-22	3.5
Unsaponifiable	3.9
ergosterol	0.62
Glycerol	4.05

Summary

1. The fatty material of Penicillium chrysogenum Q176 was extracted and the physical and chemical characterestics were determined.
2. The following fatty acids were isolated and identified: myristic, palmitic stearic, n-tetracosanoic, oleic and linoleic.
3. It was shown that fatty material of Penicillium chrysogenum Q176 was mainly consitiuted from glyceride.
4. Ergosterol was present in the unsaponifiadle of this fatty material.
5. The approximate quantitative composition of the fatty material was given.

Acknowledgement

The authour wishes to express his thaks to Prof. Dr, S.Umezawa to whom he is greatly indebted. The authour is also grateful to members of Umezawa's laboratory staff, who supplied him the mycelium of mold. This work was supported in part by a subsidy from the Educational Department.