supplied in Table II. The solubilities of the two diacyl compounds in organic liquids resemble those of the trisubstituted derivatives.

TABLE II

DERIVATIVES OF *o*-AMINOPHENOL

Product	*p*-Anisoyl-*o*-aminophenol	*p*-Anisoyl-*o*-aminophenylbenzenesulfonate	*o*-Benzenesulfonaminophenylbenzoate
Method	Groenvik[3]	Schotten-Baumann	Schotten-Baumann
Yield, %	95	84	70.5
M. p., °C.	171.5–172	113.5–114.5	143.5–144.5
N, % Calcd.	5.76	3.82	3.97
N, % Found	5.56	3.68	4.05
S, % Calcd.		8.73	9.08
S, % Found		8.51	8.75

This occasion is taken to correct the melting point of *o*-hydrocinnamylaminophenyl isovalerate, 92–93°, previously reported from this Laboratory.[4] It has since been found that crystallization of this compound from dilute alcohol, the solvent used at that time, appears never to give a pure product. When crystallized several times from ether, the compound melts at 113.5–114.5°.

(3) Groenvik, *Bull. soc. chim.*, [2], **25**, 177 (1876).

Summary

The N-potassium salt of *o*-benzenesulfonaminophenyl benzenesulfonate has been prepared; on treatment with acyl chlorides and benzyl chloride the corresponding trisubstituted derivatives of *o*-aminophenol are formed. Some other new derivatives of *o*-aminophenol are also described.

(4) Pollard, Sparks and Moore, THIS JOURNAL, **54**, 3283 (1932).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

The Effect of Ammonium Chloride upon the Growth and Production of Acid by *Aspergillus niger*

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In a previous communication by Schopmeyer and Fulmer[1] it was shown that various molds produce yeast growth stimulus when grown on synthetic media containing either glycerol or sucrose as substrate. In order to study this phenomenon more adequately, systematic investigations were made of the effect of the composition of the medium upon the growth of *Aspergillus niger*. In view of the growing importance of the use of molds in the production of chemicals, a subject which has been reviewed by May and Herrick,[2] the production of acid was also followed. The general literature on the production of chemicals by molds has been summarized by Buchanan and Fulmer[3] and Fulmer and Werkman.[4]

The utilization of *Aspergillus niger* for the production of citric acid was stimulated by the well-known work of Currie.[5] Pruess, Peterson, Steenbock and Fred[6] studied the effect of the composition of the medium upon the yield of mycelium by the above organism. Recently, Doelger and Prescott[7] have presented data on the production of citric acid by a species of *Aspergillus niger* selected from eight species on the basis of ability to produce the acid.

There is considerable argument in the literature as to the nitrogen requirement of molds. *Aspergillus niger* is omnivorous in this respect. The standard media reported contain nitrate nitrogen; if ammonium salts alone are used, ammonium nitrate is indicated. Studies in these Laboratories have shown ammonium chloride to be an excellent sole source of nitrogen for the growth of yeast (Fulmer, Nelson and Sherwood,[8] Fulmer, Sherwood and Nelson[9] and Sherwood and Fulmer.[10]) It was found that for each temperature there are two optimal concentrations of the salt. These optimal concentrations, in grams of salt per 100 cc. of medium, are expressed by the relations

$$\text{1st optimum} = 0.096 + 0.00306t^\circ \quad (1)$$

$$\text{2d optimum} = 0.595 + 0.0223t^\circ \quad (2)$$

(1) H. Schopmeyer and E. I. Fulmer, *J. Bact.*, **22**, 23 (1931).

(2) O. E. May and H. T. Herrick, U. S. Dept. Agr. Circ. 216 (1932).

(3) R. E. Buchanan and E. I. Fulmer, "Physiology and Biochemistry and Bacteria," Vol. III, Williams and Wilkins Co., Baltimore, Md.

(4) E. I. Fulmer and C. H. Werkman, "An Index to the Chemical Action of Microörganisms on the Non-Nitrogenous Organic Compounds," C. C. Thomas, Springfield, Ill.

(5) J. N. Currie, *J. Biol. Chem.*, **31**, 15 (1917).

(6) L. M. Pruess, W. H. Peterson, H. Steenbock and E. B. Fred, *ibid.*, **90**, 369 (1931).

(7) W. P. Doelger and S. C. Prescott, *Ind. Eng. Chem.*, **26**, 1142 (1934).

(8) E. I. Fulmer, V. E. Nelson and F. F. Sherwood, THIS JOURNAL, **43**, 191 (1921).

(9) E. I. Fulmer, F. F. Sherwood and V. E. Nelson, *Ind. Eng. Chem.*, **16**, 921 (1924).

(10) F. F. Sherwood and E. I. Fulmer, *J. Phys. Chem.*, **30**, 738 (1926).

It was also shown that both in synthetic medium and in beer wort the concentrations optimal for the growth of yeast were identical with those in which wheat gluten is least swollen.

In order to study the effect of this salt upon the production of mycelium and acid by *Aspergillus niger*, varying concentrations of ammonium chloride were added to a basal medium containing in terms of grams per 100 cc.: 12.5 sucrose, 0.08 $MgSO_4 \cdot 7H_2O$, 0.01 $FeSO_4 \cdot 7H_2O$, 0.01 $ZnSO_4$ and 0.125 KH_2PO_4 with *p*H adjusted to a value of 3.0. The above conditions had been found to be optimum for the production of acid and mycelium. The medium, in 100-cc. quantities, was placed in 300-cc. Erlenmeyer flasks and sterilized at 1 atm. pressure for twenty minutes. Each flask was inoculated with 1 cc. of a suspension of spores from a stock culture kept in the refrigerator. The acidity is expressed in terms of cubic centimeters of 0.100 *N* sodium hydroxide required to neutralize 10.0 cc. of medium. Corrections were made for evaporation. At the end of fourteen days the culture was filtered, washed and the mat dried at 60°. The weight of mycelium is expressed in terms of dry weight per 100 cc. of medium. The incubation temperature was 30°. The data obtained are given in Table I.

TABLE I

THE EFFECT OF AMMONIUM CHLORIDE UPON THE PRODUCTION OF ACID AND MYCELIUM BY *Aspergillus niger*

G. salt per 100 cc.	Acidity, cc. 0.1 *N* base per 10 cc. of medium 3rd day	5th day	7th day	9th day	11th day	14th day	Mycelium per 100 cc., g.
0	1.1	1.6	1.6	1.6	1.6	1.6	0.08
0.005	1.3	1.5	2.5	2.6	2.6	2.6	.18
.050	5.3	5.0	5.5	5.4	5.5	5.5	.66
.100	15.0	16.0	17.5	17.7	18.9	21.3	1.27
.125	18.2	20.5	21.7	22.4	20.6	23.7	1.62
.137	22.5	23.8	25.2	26.5	26.5	27.0	1.71
.163	27.9	36.3	37.9	38.0	38.0	39.5	2.20
.175	28.2	46.9	55.0	55.0	56.3	56.3	2.13
.188	26.3	35.6	74.5	73.5	67.9	64.3	2.66
.200	22.8	61.1	72.3	72.4	66.6	63.2	2.67
.213	20.1	55.6	71.5	70.0	63.2	56.1	2.68
.237	17.7	46.2	60.0	55.0	28.1	9.0	3.06
.250	21.5	54.3	60.1	46.7	14.3	6.6	3.02
.275	16.2	42.7	47.2	30.8	7.3	6.1	3.37
.300	12.8	32.3	36.6	20.8	7.5	6.8	3.36
.325	12.5	30.4	31.2	9.5	7.1	7.1	3.44
.350	13.9	29.3	28.0	8.5	6.6	7.8	3.41
.400	14.1	27.0	21.9	9.2	7.2	7.7	3.36
.500	11.7	16.2	17.3	13.1	11.3	10.5	3.44
.750	10.7	13.2	16.5	17.7	17.2	18.4	2.49
1.00	11.7	14.5	17.3	20.0	18.1	19.4	2.83

It is evident that ammonium chloride is an excellent sole source of nitrogen. It is of especial interest to note that the optimum production of acid is at 0.188 g. of the salt per 100 cc. of medium. This is exactly the optimum as calculated by equation (1) for the optimum growth of yeast. At this concentration the disappearance of acid on prolonged incubation is first apparent. The acid yield of 74.3 on the seventh day is the highest in the series and drops to 64.3 by the fourteenth day. The production of acid shows a minimum of 7.7 at 0.325% of the salt and rises to a value of 19.4 at 1.00%, showing a tendency to approach a second optimum. The second optimum for the growth of yeast at 30°, as calculated by equation (2), is at 1.26%. The concentration of salt optimum for the production of acid is not that for the formation of mycelium. The production of mycelium shows a broad optimum at 0.275 to 0.500% of the salt.

Summary

Ammonium chloride is an excellent sole source of nitrogen for the growth and production of acid by *Aspergillus niger*. The optimum concentration of the salt for the production of acid is identical with that optimum for the growth of yeast and which has been found to produce a minimum hydration of wheat gluten.

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