(3 g.) was boiled for an hour, the color changed to dark red; but after the mixture was filtered and the pyridine and benzaldehyde were distilled at 5 mm. pressure, a mere trace of residue was obtained, which gave a faint reduction with Fehling's solution.

Attempts to Prepare Benzoin with Other Catalysts.—In Table VIII are given the substances which, when boiled with diluted alcohol and benzaldehyde, failed to form benzoin. After the reaction mixture had been heated for the stated time, the solvent was evaporated and the bulk of the remaining benzaldehyde distilled with steam. The residual oil was then tested for benzoin with Fehling's solution, the test being negative in each case.

Action of Hydrochloric Acid on Benzaldehyde.—Benzaldehyde readily absorbs dry hydrogen chloride, with the development of much heat. The liquid assumes a dark red color, and fumes in the air. A mixture of 90 g. of benzaldehyde and 3 g. of hydrochloric acid was allowed to stand for three weeks, but no benzoin was formed. A mixture of 200 g. of benzaldehyde and 12 g. of hydrochloric acid after three weeks was shaken with water, then washed with a very little sodium bicarbonate solution, and distilled; 195 g. of constant-boiling benzaldehyde was recovered. The slight distillation residue gave no test for benzoin.

Summary

1. The condensation of benzaldehyde (2 moles) into benzoin (1 mole) requires the combined action of hydroxyl and of cyano ions.

2. These catalysts also reverse the condensation; but at the same time, they convert benzoin into benzyl benzoate, and then into benzyl alcohol and benzoic acid. This step is not reversible.

3. Heating to 300° converts benzoin partially into benzaldehyde; but heating benzaldehyde yields no benzoin; instead, benzyl benzoate, etc., are formed.

4. A consistent explanation is given for the benzoin and Cannizzaro condensations.

5. A simplified method of preparing benzoin without the use of alcohol is described.

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

THE MULTIPLE NATURE OF BIOS[1]

BY ELLIS I. FULMER, W. W. DUECKER AND V. E. NELSON

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Wildiers came to the conclusion that the yeast dietary must contain, aside from mineral salts and sugar, organic material of biological origin. To this constituent of the medium supposed to be necessary for the growth of yeast he gave the name "bios."[2]

The necessity of bios for the growth of yeast was first seriously questioned by Fulmer and Nelson and co-workers and by MacDonald and McCollum.

[1] Reported before the meeting of the American Chemical Society at Milwaukee, Wisconsin, September, 1923.

[2] Wildiers, *La Cellule*, **18**, 313 (1901).

These investigators came to the conclusion that bios is not a necessary component of media for the growth of yeast but place it in the class of growth stimulants. If this contention be true, bios cannot be considered as analogous to a vitamin, since the vitamins are absolutely necessary for the growth of animals and do not act merely as growth accelerators.[3]

Devloo, Ide, Frankel and Schwarz[4] and others have fractionated various extracts with a view to the isolation of bios. In all of the work which has come to the attention of the authors the procedure was based upon the assumption that bios is a single substance. A similar point of view seems to prevail in attempted isolation of the vitamins.

It seemed to the authors not unreasonable that the extracts used might contain several stimulating materials, these substances having similar or various roles in the nutrition of the yeast. In such a case the name bios is a term for a group of yeast growth stimulants found in various extracts. The studies here described were undertaken with a view to investigate the possible multiple nature of bios.

A water extract of alfalfa was fractionated with ethyl alcohol by a method similar to that used by Osborne and Wakeman.[5] Ground alfalfa was treated for from one to ten hours with water at 70°. The extract was filtered first through filter paper and then through a Chamberland-Pasteur filter and then concentrated in a vacuum.

Alcohol (95%) was added to the extract until a concentration of 40% alcohol by volume was reached, when a finely divided precipitate was formed. After this had settled, the supernatant liquid was siphoned off, the precipitate was washed with 40% alcohol until the washings were colorless, and the material dried at 100°. The fraction was designated as No. 1.

The addition of 95% alcohol to the filtrate produced no further precipitation until the alcohol reached a concentration of 70% by volume, when a copious precipitate was produced. The precipitate was treated as was No. 1 and was called No. 2.

The filtrate from No. 2 was evaporated to a thick sirup and was repeatedly extracted with 95% alcohol in a large separatory funnel, until no more material dissolved. The residue was dried at 100° and designated as No. 3.

The extract from No. 3 was dried and called No. 4. The general properties of the four fractions are given in Table I.

[3] (a) Fulmer, Nelson and Sherwood, THIS JOURNAL, **43**, 186, 191 (1921). (b) Fulmer and Nelson, *J. Biol. Chem.*, **51**, 77 (1922); *J. Infect. Dis.*, **33**, 130 (1923). (c) Fulmer, Nelson and White, *J. Biol. Chem.*, **46**, 77 (1921). (d) MacDonald and McCollum, *ibid.*, **45**, 307 (1920). (e) MacDonald, *ibid.*, **54**, 243 (1923); **46**, 489 (1923).

[4] (a) Devloo, *La Cellule*, **23**, 361 (1906). (b) Ide, *Centr. Bakt. Parasitenk.*, **18**, 193 (1907). (c) Frankel and Schwarz, *Biochem. Z.*, **112**, 207 (1920).

[5] Osborne and Wakeman, *J. Biol. Chem.*, **49**, 63 (1921).

TABLE I

THE PROPERTIES OF THE FOUR FRACTIONS

No.	Color	Nitrogen	Ash	Solubility in water
1	dark brown	1.56	34.1	fairly soluble
2	buff	1.27	16.9	slightly soluble
3	dark brown	1.20	0.0	very soluble
4	similar to 3	1.20	4.0	very soluble

It is of interest to note that while the nitrogen content of the fractions does not vary greatly, the content of ash is at a minimum in the third fraction. Fractions 3 and 4 formed the largest part of the total amount of the material. Fractions prepared from various samples of alfalfa gave similar results in all respects.

Each fraction was tested for bios using a medium containing the following per 100 cc.: 0.280 g. of ammonium chloride, 0.100 g. of dipotassium phosphate, 0.100 g. of calcium chloride, 0.040 g. of calcium carbonate, and 10 g. of cane sugar. All data were obtained at 30°. It will be noted that the concentration of ammonium chloride in this medium is greater than the optimum of that salt that was determined by Fulmer, Nelson and Sherwood.[2a] The higher concentration of the salt was used in order to detect stimulation which might not be apparent in the better medium.

The yeast used was obtained originally from a cake of Fleischmann's yeast, designated as *Saccharomyces cerevisiae* Race F, and had been growing for more than three years on a synthetic medium. The stock medium was made sufficiently strong to compensate for the dilution due to the addition of the inoculation and of the extracts. All of the flasks were inoculated in such a way as to make an initial "count" of one (250,000 per cc.). In Table II are given the results of typical experiments with the four fractions. The numbers represent the yeast count after 48–72 hours of growth.

TABLE II

ESTIMATION OF BIOS IN THE FRACTIONS OF ALFALFA

1 cc. of solution = 0.0050 g. of dry material

Cc. of solution	1		2		3		4	
0	225	155	215	250	230	265	220	240
0.5	222	...	120	370	217	...	...	...
1	232	...	125	310	...	...	...	...
2	230	...	262	282	...	...	...	...
3	215	...	...	312	245	497	...	...
4	230	270	220	362	...	...	...	...
5	260	372	170	337	250	565	340	...
6	210	295	100	360	360	612	442	...
7	...	...	122	405	345	505	475	...
8	140	...	125	332	900	782	490	422
9	...	...	190	355	352	732	495	437
10	...	...	...	...	...	770	512	522
11	...	...	...	...	...	767	512	512
12	...	...	...	...	...	...	487	480
13	...	...	...	...	...	...	435	442

It will be noted in Table II that the results obtained from No. 2 are very irregular. This irregularity is due to the fact that the material is practically insoluble in water, making uniform suspensions difficult. A wide range of concentrations of No. 2 was added to the optimum concentration of each of the other fractions, but in no case did No. 2 improve the yields.

The optimum concentrations of Fractions 1, 3 and 4 were tested in various combinations, typical results being given in Table III.

TABLE III

OPTIMUM CONCENTRATION

Opt. concn. of	1	2	3	4	1–3	1–4	3–4
Series 1	372	192	345	440	737	452	350
Series 2	355	180	320	422	640	497	377

It is apparent that the combination of Nos. 1–3 gives much greater stimulation than the optimum of either fraction alone. Nos. 1 and 3 cannot be identical materials since the increase in concentration of either alone above the optimum would decrease the yeast crop.

Conclusion

These results indicate that extracts containing bios contain at least two different yeast growth stimulants.

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

BAUER OIL, THE HIGH-BOILING RESIDUE FROM MOLASSES FUSEL OIL.[1] A SOURCE OF CAPRIC ACID

BY C. S. MARVEL AND F. D. HAGER

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Many investigations have been carried out on the composition of fusel oil from various sources but most of the work has been done on the lower-boiling fractions. A few articles have appeared in which the composition of the higher-boiling portions is discussed.

Rowney[2] has reported *iso*amyl caprate in corn fusel oil. Fischer[3] and later Grimm[4] have reported this ester in wine fusel oil and Johnson, in potato fusel oil.[5] More recently, Hilger[6] has found capric, lauric and palmitic acids in corn fusel oil. Luce[7] has reported pelargonic, capric and lauric acids in fusel oil. The fusel oil from sweet

[1] The material used in this investigation was furnished by the U. S. Industrial Alcohol Company, New Orleans division.

[2] Rowney, *Ann.*, **79**, 236 (1851).

[3] Fischer, *Ann.*, **118**, 312 (1861).

[4] Grimm, *Ann.*, **157**, 267 (1871).

[5] Johnson, *J. prakt. Chem.*, **62**, 262 (1854).

[6] Hilger, *Chem. Zentr.*, **1894**, I. 981.

[7] Luce, *J. pharm. chim.*, **22**, 136 (1920).