STUDIES ON PUTREFACTIVE PRODUCTS OF BEER YEAST.

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Formerly, as putrefactive products of yeast, ethylamine, trimethylamine, isoamylamine, caprylamine and sepsin were isolated by Hesse,(1) Müller(2) and Faust(3); and recently the writer(4) reported with Dr. Yoshimura on the occurrence of hypoxanthine, histamine, putrescine, cadaverine, tyramine and leucine in a putrefied yeast. In this experiment, the author isolated the phenylethylamine, adenine, hypoxanthine, histamine, putrescine, cadaverine, tyramine and leucine from putrefied beer yeast.

Experimental Part.

The beer yeast experimented was obtained by pressing and drying the settled yeast in the bottom of fermenting tunk after main-fermentation of beer; and the various forms of nitrogen in the dried sample were as follows:

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(1) Hesse, *Jahresber. Fortschr. Chem.*, (1857) 403.

(2) Müller, *J. prakt. Chem.*, **70** (1859), 65.

(3) Faust, *Arschiv. exper. Path. Pharm.*, **51** (1904), 248.

(4) K. Yoshimura and K. Nishida, *J. Agr. Chem. Soc. Japan*, **8** (1932), 309.

	Moisture 9.64%	Dry matter 90.36%
	In 100 parts of dry matte	Ratio (Total N as 100)
Total N	10.153	100.0
Protein N	6.883	67.8
Non-protein N	3.270	32.2
In which:		
Ammonia N	0.097	1.0
Organic base N	1.481	14.6
Other N	1.692	16.6

In earthen pot, 9 kg. of the sample were allowed to stand in 36 liters of water for 28 days, stirring the mixture one time in every day. In this period, average of maximum temperature daily was 28.3°C., average of minimum temperature 10.0°C., and average of temperature at 10 a.m. daily 22.5°C. In the above conditions the putrefaction took place, and still the liquid was kept an acidic reaction.

The various forms of nitrogen in the filtrate of the putrefied substance were determined:

	In 100 parts of original sample	Ratio (Total N as 100)
Total N	8.277	100.0
Protein N	0.625	7.6
Non-protein N	7.652	92.4
In which:		
Ammonia N	1.192	14.4
Organic base N	2.370	28.6
Other N	4.090	49.4

Isolation and Identification of Organic Bases. To remove protein and other impurities, neutral- and basic-lead acetate were added to the above putrefied yeast; when the excess of lead was removed by sulphuric acid and the mother liquor from the lead sulphate was evaporated to a small volume, the considerable amounts of inorganic substances (ammonium- and potassium-sulphate) crystallised out. The filtrate from the preceding inorganic substances was diluted with 5% sulphuric acid, and then researches were carried out on the organic bases, fractionally precipitated by adding phosphotungstic acid to the above solution.

First Precipitate by Phosphotungstic Acid.

This precipitate was decomposed by baryta, and excess of baryta was removed by CO_2 and the solution was fractionated into five fractions.

I. Volatile Base (Phenylethylamine). The diluted solution of free bases obtained by the above treatment was distilled under reduced pressure, and the distillate was received into dilute hydrochloric acid, and then the alcohol soluble base was isolated as hydrochloride. The yield was 2.90 gr. The hydrochloride formed colourless thin plates of silky lustre, and melted at 215–216°C. (uncorr.). Its analytical results agreed with the hydrochloride of the compound having the formula $C_8H_{11}N$.

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C%	H%	N%	Cl%
(1)	3.261	7.270	2.350	60.80	8.06	—	—
(2)	3.854	8.620	2.807	61.00	8.15	—	—
(3)	4.545	0.335 c.c. N(22.5°C., 761. 8 mm.)				8.53	—
(4)	6.245	0.461 c.c. N(22.5°C., 761. 2 mm.)				8.54	—
(5)	4.780	4.245 mg. AgCl		—	—	—	21.98
(6)	6.720	5.970 mg. AgCl		—	—	—	21.98
Calc. for $C_8H_{11}N{\cdot}HCl$				60.93	7.68	8.89	22.50

The chloroaurate, readily soluble in water and alcohol, crystallised in yellow thin plates of pearly lustre.

Anal. : Subst. = 0.1088, 0.2470, 0.4952; Au = 0.0468, 0.1059, 0.2115 gr.
Found : Au = 43.01, 42.87, 42.71%.
Calc. for $C_8H_{11}N{\cdot}HCl{\cdot}AuCl_3$ (Phenylethylamine chloroaurate): Au = 42.76%.

The chloroplatinate of the base, hardly soluble in water and alcohol, crystallised in small bright golden yellow prisms and melted at 247–248°C. (uncorr.) with decomposition.

Anal. : Subst. = 0.2156, 0.1523, 0.0498; Pt = 0.0645, 0.0452, 0.0148 gr.
Found : Pt = 29.92, 29.68, 29.72%.
Calc. for $(C_8H_{11}N{\cdot}HCl)_2\,PtCl_4$ (Phenylethylamine chloroplatinate): Pt. = 29.94%.

The hydrochloride was converted into picrate by adding Na-picrate to its aqueous solution. It formed yellow prisms and melted at 170°C. (uncorr.). From these results, the volatile organic base obtained from the putrefied yeast is no doubt phenylethylamine.

II. The Base Crystallised out as Nitrate (Adenine). The residual solution after the distillation of volatile base was slightly acidified with nitric acid and by evaporation of the solution 11.50 gr. of adenine nitrate were crystallised out. Analytical results of the nitrate are as follows.

Anal.: Subst. = 0.2348; H_2O = 0.0111 gr.
Subst. = 0.2491; Nitron nitrate = 0.4477 gr.
Found: H_2O = 4.73%; HNO_3 = 30.19%.
Calc. for $C_5H_5N_5 \cdot HNO_3 \cdot \frac{1}{2}H_2O$ (Adenine nitrate): H_2O = 4.35%; HNO_3 = 30.43%.

The picrate of characteristic hairly needles is yellow and sparingly soluble in water and it decomposes at 282°C. (uncorr.).

The chloroaurate, which was converted from the picrate, crystallised in yellow prisms and melted at 263°C. (uncorr.).

Anal.: Subst. = 0.1067, 0.0723; Au = 0.0505, 0·0341 gr.
Found: Au = 47.33, 47.16%.
Calc. for $C_5H_5N_5 \cdot 2HCl \cdot 2AuCl_3 \cdot H_2O$ (Adenine chloroaurate): Au = 47.35%.

III. Precipitate by Silver Nitrate (Hypoxanthine). The precipitate by silver nitrate was decomposed by hydrochloric acid. When the filtrate from AgCl was evaporated under reduced pressure, crystals of the hydrochloride of the base (13.70 gr.) separated out. Its chloroaurate forms yellow prisms and decomposes at 246–247°C. (uncorr.).

Anal.: Subst. = 0.3015; Au = 0.1246 gr. Found: Au = 41.33%.
Calc. for $C_5H_4N_4O \cdot HCl \cdot AuCl_3$ (Hypoxanthine chloroaurate): Au = 41.42%.

The hydrochloride was also analysed as its chloroplatinate.

Anal.: Subst. = 0.2309; Pt = 0.0658 gr. Found: Pt = 28.50%.
Calc. for $(C_5H_4N_4O \cdot HCl)_2\ PtCl_4$ (Hypoxanthine chloroplatinate): Pt = 28.62%.

The above results agree fairly well with the data of hypoxanthine derivatives.

IV. Precipitate by Silver Nitrate and Baryta. The precipitate, prepared by silver nitrate and baryta, was treated with hydrochloric acid and sulphuric acid to remove the silver and baryta. Then phosphotungstic acid was added to the above filtrate, and the precipitate was decomposed by baryta. The filtrate from barium phosphotungstate after removing the excess of baryta by carbon dioxide was concentrated to a small volume under reduced pressure; and then hydrochloric acid was added to the solution of free base obtained by the above treatment.

The hydrochloride freed from water was treated with cold absolute alcohol and was separated as follows:

(A) *Insoluble Portion in Absolute Alcohol* (*Histamine*). From this portion 7.80 gr. of colourless hygroscopic substance were obtained, which gives intensive diazo-reaction. Its picrate formed rhombic plates of bright yellow and decomposed at 232∼233°C. (uncorr.).

The chloroaurate of the base prepared from the hydrochloride crystallised in yellow prisms and decomposed at 218°C. (uncorr.).

Anal.: Subst. = 0.4131; Au = 0.2061 gr. Found: Au = 49.89%.
Calc. for $C_5H_9N_3 \cdot 2HCl \cdot 2AuCl_3$ (Histamine chloroaurate): Au = 49.85%.

(B) *Soluble Portion in Absolute Alcohol* (*Histamine*). The yield of the base from the precipitate, which was formed by adding saturated alcoholic solution of $HgCl_2$ to this portion, was 1.30 gr. as hydrochloride. The hydrochloride was hygroscopic, colourless prisms and gave intensive diazo-reaction. The natures of its picrate and chloroaurate were as follows: The picrate formed yellow rhombic crystals and decomposed at 231°C. (uncorr.). The chloroaurate of the base crystallised in golden yellow prisms and melted at 218°C. (uncorr.) with decomposition.

Anal.: Subst. = 0.2936, 0.1876; Au = 0.1464, 0.0930 gr.
Found: Au = 49.86, 49.57%.
Calc. for $C_5H_9N_3 \cdot 2HCl \cdot 2AuCl_3$ (Histamine chloroaurate): Au = 49.85%.

These results agree precisely with histamine derivative in all respects.

V. Filtrate from Silver Nitrate- and Baryta-Precipitate. The filtrate was treated with hydrochloric acid and sulphuric acid to remove an excess of silver and baryta. Then phosphotungstic acid was added to the above solution, and the precipitate obtained thus was decomposed by baryta. The filtrate from barium phosphotungstate after removing the excess of baryta by carbon dioxide was concentrated to a small volume under reduced pressure. The solution of free base obtained from the above treatment was converted into the hydrochloride by adding hydrochloric acid. And the hydrochloride freed from water was treated with cold absolute alcohol and was separated as follows:

(A) *Insoluble Portion in Absolute Alcohol* (*Tyramine*). The yield was 2.30 gr. It was colourless plates of silky lustre and gave strong Millon's reaction. The picrate formed deep yellow prisms and melted at 203°C. (uncorr.). The chloroplatinate crystallised in orange yellow, long prisms, hardly soluble in water, decomposed at 224–225°C. (uncorr.) and remarkably gave phenol odour by ignition.

Anal.: Subst. = 0.1131, 0.0762; Pt = 0.0323, 0.0216 gr.
Found: Pt = 28.56, 28.35%.
Calc. for $(C_8H_{11}NO \cdot HCl)_2PtCl_4$ (Tyramine chloroplatinate): Pt = 28.48%.

These results agree well with tyramine derivatives.

(B) *Soluble Portion in Absolute Alcohol.* The alcohol soluble portion gave a white precipitate by the addition of saturated alcoholic solution of mercuric chloride.

(a) Precipitate by Mercuric Chloride (Putrescine). When the mercury was removed by sulphuretted hydrogen and the liquid freed from mercuric sulphide was evaporated to a small volume, the hydrochloride of the base was crystallised out (1.10 gr.). The picrate formed greenish yellow prisms, hardly soluble in water, and decomposed at 252–253°C. (uncorr.). The hydrochloride was also analysed as its chloroaurate (Decomp. point = 231°C.).

Anal.: Subst. = 0.3055; Au = 0.1564 gr. Found: Au = 51.19%.
Calc. for $C_4H_{12}N_2 \cdot 2HCl \cdot 2AuCl_3$ (Putrescine chloroaurate): Au = 51.35%.

The chloroplatinate of the base formed glistening orange yellow prisms, and decomposed at 222–223°C. (uncorr.). The result of the analysis was as follows:

Anal.: Subst. = 0.1994; Pt = 0.0781 gr. Found: Pt = 39.17%.
Calc. for $C_4H_{12}N_2 \cdot 2HCl \cdot PtCl_4$ (Putrescine chloroplatinate): Pt = 39.13%.

(b) Filtrate from $HgCl_2$-precipitate (Tyramine). The alcoholic solution filtered from the $HgCl_2$-precipitate was evaporated and the residue was suspended in water, treated with sulphuretted hydrogen gas and the mercuric sulphide was filtered off. Then the hydrochloride of the base (2.40 gr.) was obtained from the above filtrate. It was colourless thin plates of silky lustre and gave strong Millon's reaction. The picrate prepared from the hydrochloride formed yellow prisms and decomposed at 203°C. (uncorr.). The chloroplatinate of the base crystallised in orange yellow prisms and decomposed at 223–224°C. (uncorr.). It gave remarkably odour of phenol by ignition, and the amount of platinum in it was as following.

Anal : Subst. = 0.1557; Pt = 0.0438 gr. Found: Pt = 28.13%.
Calc. for $(C_8H_{11}NO \cdot HCl)_2PtCl_4$ (Tyramine chloroplatinate): Pt = 28.48%.

Second Precipitate by Phosphotungstic Acid.

I. Volatile Base (Phenylethylamine). Dilute solution of free bases prepared from the precipitate which was deposited by phosphotungstic acid was distilled under reduced pressure and the distillate was received into dilute hydrochloric acid, and then alcohol soluble base was separated as hydrochloride. The yield was 4.30 gr. It was colourless thin plates having silky lustre and its M.P. being 214°C. (uncorr.). The chloroaurate of the base crystallised in yellow thin plates of pearly lustre.

Anal.: Subst. = 0.1329, 0.3435, 0.4889; Au = 0.0564, 0.1470, 0.2083 gr.
Found: Au = 42.44, 42.79, 42.61%.
Calc. for $C_8H_{11}N \cdot HCl \cdot AuCl_3$ (Phenylethylamine chloroaurate): Au = 42.76%.

The chloroplatinate crystallised in golden yellow small prisms, hardly soluble in water and alcohol. It turned to black at about 230°C. and decomposed at 246–247°C. (uncorr.).

Anal.: Subst. = 0.1530, 0.2444; Pt = 0.0459, 0.0735 gr.
Found: Pt = 30.00, 30.07%.
Calc. for $(C_8H_{11}N \cdot HCl)_2PtCl_4$ (Phenylethylamine chloroplatinate): Pt = 29.94%.

II. Non-Volatile Bases. After the volatile base was removed, the concentrated residual solution was acidified with hydrochloric acid and evaporated to dryness on a water-bath. The residual mass was separated into soluble and insoluble portions by using absolute alcohol.

(A) *Insoluble Portion in Absolute Alcohol* (KCl). The amounts of this portion obtained were 19.80 gr. It was colourless short prisms and gave violet flame reaction. The analysis of its chloroaurate is as follows.

Anal.: Subst. = 0.1957; Au = 0.1019 gr. Found: Au = 52.07%.
Calc. for $KCl \cdot AuCl_3$ (Potassium chloroaurate): Au = 52.15%.

(B) *Soluble Portion in Absolute Alcohol.* The alcoholic solution filtered from the above insoluble portion was treated with the saturated alcoholic solution of mercuric chloride.

(a) Precipitate by Mercuric Chloride (Cadaverine). From the precipitate by mercuric chloride, 4.10 gr. of colourless hygroscopic crystals were obtained as hydrochloride. The picrate formed bright yellow prisms, hardly soluble in water, and melted at 223°C. (uncorr.) with decomposition. The chloroaurate crystallised in deep yellow irregular prisms and decomposed at 225°C. (uncorr.).

Anal.: Subst. = 0.4743; Au = 0.2376 gr. Found: Au = 50.09%.
Calc. for $C_5H_{14}N_2 \cdot 2HCl \cdot 2AuCl_3$ (Cadaverine chloroaurate): Au = 50.38%.

The chloroplatinate of the base formed short prisms with glistening golden yellow colour.

Anal.: Subst. = 0.1417; Pt = 0.0542 gr. Found: Pt = 38.25%.
Calc. for $C_5H_{14}N_2 \cdot 2HCl \cdot PtCl_4$ (Cadaverine chloroplatinate): Pt = 38.06%.

(b) Filtrate from Mercuric Chloride-Precipitate (Tyramine). 7.70 Gr. of hydrochloride of the base were obtained from this portion. It was colourless thin plates of silky lustre and gave intensive Millon's reaction. The picrate formed yellow prisms and decomposed at 203°C. (uncorr.). The chloroplatinate of the base was orange yellow thin plates of pearly lustre, and it decomposed at 222°C. (uncorr.) and gave a strong odour of phenol by ignition.

Anal.: Subst. = 0.1470, 0.1164; Pt = 0.0415, 0.0334 gr.
Found: Pt = 28.23, 28.69%.
Calc. for $(C_8H_{11}NO \cdot HCl)_2 \cdot PtCl_4$ (Tyramine chloroplatinate): Pt = 28.48%.

Filtrate from Phosphotungstic Acid-Precipitate. (*Leucine*).

The filtrate from the second precipitate by phosphotungstic acid was alkalified by adding the saturated solution of baryta, and the precipitates of Ba-sulphate and Ba-phosphotungstate were filtered off. Then the filtrate which was freed exactly from the excess of baryta with sulphuric acid was concentrated to a small volume and 324.90 gr. of crystals were obtained.

Anal.: Subst. = 0.1018; N = 0.01107 gr. Found: N = 10.87%.
Calc. for $C_6H_{13}NO_2$ (Leucine): N = 10.69%.

It was converted into copper salt and the content of copper was determined.

Anal.: Subst. = 0.0585, 0.0917; CuO = 0.0142, 0.0228; Cu = 0.01822, 0.01135 gr.
Found: Cu = 19.40, 19,87%.
Calc. for $(C_6H_{12}NO_2)_2Cu$ (Leucine copper): Cu = 19.64%.

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Summary.

The quantities of substances isolated from 9 kg. of the dried beer yeast by putrefaction were as follows:

Phenylethylamine (as hydrochloride)	7.20 gr.
Adenine (as nitrate)	11.50 „
Hypoxanthine (as hydrochloride)	13.70 „
Histamine (as hydrochloride)	9.10 „
Tyramine (as hydrochloride)	12.40 „
Putrescine (as hydrochloride)	1.10 „
Cadaverine (as hydrochloride)	4.10 „
Leucine	324.90 „
Ammonia	128.74 „

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