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# ON THE CHEMICAL CONSTITUENTS OF MALT-ROOTLETS WITH SPECIAL REFERENCE TO HORDENINE.

By

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## Introduction.

Up to the present time, only a few chemists have investigated chemical constituents of malt-rootlets, one of the by-products obtained in the course of malting.

In 1865, J. C. Lermer(1) reported on some organic acids, bitter-stuff and asparagine found in malt-rootlets. E. Schulze(2) succeeded in isolating choline and betaine though he failed to find arginine and asparagine. K. Yoshimura(3) investigated the nitrogenous substances contained in the rootlets and ascertained the presence of histidine, choline and betaine, but he failed in detecting arginine, vernine and asparagine. His investigation extended to the possible presence in barley seedlings of cane-sugar, produced by the decomposition of starch at the time of germination, but instead of confirming the presence of cane-sugar, he discovered reducing sugar and maltose.

O. Gaebel(4) and E. Léger(5) found a kind of alkaloid in malt-rootlet, which they named "Hordenine". This substance, they believed, was produced in the course of assimilation of protein in the barley seedling. The authors determined its constitutional formula. L. Camus(6) observed the effect of hordenine sulphate upon pepsine, trypsine, erepsine, and its toxic action on some animals.

The above mentioned researches all dealt with the nitrogenous substance of the rootlets, but little is known of the character of the carbohydrates in them.

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1) J. C. Lermer; Dingler's Polyt., 1865, 71.

2) E. Schulze; Zeitschr. f. physiol. Chem., 1908, **57**, 73.

3) K. Yoshimura; Biochem. Zeitschr., 1911, **31**, 221.

4) O. Gaebel; Archiv. Pharm., 1906, **244**, 435.

5) E. Léger; Compt. rend., 1906, **142**, 108; **143**, 234, 916;
1907, **144**, 208, 482.

6) L. Camus; Compt. rend., 1906, **142**, 110, 350.

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At the time of germination all the reserve materials, such as carbohydrates, fats, protein, etc., are changed first into some other forms, and later transferred to the rootlets, to be used for the growth of the young seedling. We know, that when the purification of malt is imperfect, and when more or less rootlets are mixed with it, there is danger of the quality of beer brewed from it being affected. I observed in the course of this investigation that the extract of the rootlets influenced in many ways the fermentation of the wort and that hordenine also had the same action.

Such being the case, investigations concerning the chemical nature of the constituents of malt-rootlets are necessary and desirable both from scientific and practical points of view. The author has taken much interest in hordenine and will try to describe in this paper the result of his own study of this substance.

This investigation was first undertaken in the laboratory of the Dai-Nippon Brewery Company, Tokyo, at the suggestions of Drs. K. Ueno, K. Yagi and K. Makoshi to whom the author wishes to tender his best thanks.

The author also wishes to acknowledge the valuable help extended to him during his researches by Profs. K. Oshima, K. Miyake, T. Tadokoro and U. Suzuki. The kindly services of Assistant Prof. K. Kondo, Mr. K. Negami, Mr. K. Ukita, and Mr. Y. Yumoto in a similar direction are recognized here.

## Experimental Part.

### General Analysis.

The material used in the present investigation was first washed with water for a little while to remove dust, broken grains and other admixture, and then dried and ground in a mill as usual. It was then analyzed. The results of the analysis are as follows :—

| | Air-dry substance. (%) | Water-free substance. (%) |
|---|---|---|
| Water | 10.21 | — |
| Crude protein | 21.49 | 23.93 |
| Ash | 3.02 | 3.36 |
| (Pure protein) | (13.32) | (14.83) |

| | Air-dry substance.<br>(%) | Water-free substance.<br>(%) |
|---|---|---|
| Crude fat | 3.19 | 3.55 |
| Crude fiber | 18.40 | 20.49 |
| Nitrogen-free extract | 43.69 | 48.67 |
| | 100.00 | 100.00 |
| Reducing sugar (as glucose) | 3.65 | 4.06 |
| Non-reducing sugar (as cane-sugar) | 1.02 | 1.14 |
| Pentosane | 15.41 | 17.16 |
| Cellulose | 12.04 | 13.41 |

The volumetric method of F. Lehmann[1] was applied for determining the sugars.

Pentosane was determined according to the method of K. Oshima and K. Kondo,[2] (Double distillation with 1.06 sp. gr. hydrochloric acid) as in their qualitative determination[3] of methyl pentosane, and it was determined as furfurol phloroglucide. For the calculation Kröber's[4] table was used as usual. These methods are a modification of Tollens and Kröber's.[5]

Cellulose was determined according to T. König's process. Three grams of malt-rootlets were heated for an hour at 133–135°C. in a flask, with reflux condenser, together with 200c.c. glycerine containing 2 per cent of sulphuric acid, and were first purified with hot water and alcohol, then with ether alcohol. They were oxidized with hydrogen peroxide and washed with ammonia liquor, then dried, weighed, and ignited. The loss on ignition represents the amount of pure cellulose. The rest of the analysis was carried out in the usual manner.

From the above analytical results, we may conclude that the malt-rootlets consist chiefly of protein, cellulose and pentosane, and that the amount of sugar is very small.

The percentage of the sugar is variable as it is subject to changes according to the conditions (temperature, stage of germination, dryness, variety of barley, etc.) of malting. This is especially the case with nitrogenous substances.

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[1] F. Lehmann; Ueber Massanalytische Methoden zur Bestimmung von Zuckerarten, Inaug. Dissert., Marburg, 1905.

[2] K. Oshima & K. Kondo; Unpublished, but its analytical results were very satisfactory; it will be published in near future.

[3] K. Oshima & K. Kondo; Jour. Tokyo Chem. Soc., 1918, **39**, 185-198.

[4] E. Abderhalden; Handbuch der Biochemischen Arbeitmethoden, **II**, 154.

[5] E. Abderhalden; Handbuch der Biochemischen Arbeitmethoden **II**, 130.

### Analysis of the Ash.

The above prepared malt-rootlets were burned to a white ash.

The results of the analysis of the ash which was carried out in the usual manner, are as follows :—

| | Case 1. (%) | Case 2. (%) |
|---|---|---|
| $SiO_2$ | 6.01 | 5.87 |
| $SO_3$ | 4.61 | 4.19 |
| $P_2O_5$ | 28.18 | 30.89 |
| $Fe_2O_3$ | 0.58 | 0.23 |
| $Al_2O_3$ | 0.12 | 0.12 |
| $CaO$ | 5.65 | 5.96 |
| $MgO$ | 4.15 | 4.49 |
| $K_2O$ | 43.26 | 44.95 |
| $Na_2O$ | 1.73 | 1.50 |

As seen in the Table, the ash consists chiefly of $P_2O_5$ and $K_2O$.

## On the Carbohydrate.

### I. Microchemical Test and Some Qualitative Tests.

At first, the tissues of the malt-rootlets were examined microchemically under a microscope to get a general idea of their constitution. Then some qualitative tests were also applied for the same purpose.

a) Reaction for congo-red and iodine-zinc-chloride. The section was treated with iodine-zinc-chloride or congo-red and placed under a microscope. The wood tissues were found to consist mainly of lignine as indicated by the yellowish coloration with iodine-zinc-chloride.

The chief constituents of the tissues, especially of the cortical parenchyma and epidermis, are cellulose, as shown by the violet coloration with iodine-zinc-chloride and red coloration by congo-red.

b) Wiesmer's reaction and Maeule's reaction for lignified membrane.

The lignified membrane of the wood tissues, especially the walls of vessels, gave a fine red color when treated with phloroglucine and hydrochloric acid.

After the section of the rootlet was immersed in a one per cent potassium permanganate solution, it was washed with water for a little while. Then some hydrochloric acid was poured on it, and then ammonia liquor: The lignified membrane of the wood tissues and a part of the epidermis changed into a red color.

c) None of the characteristic blue color reactions with ammoniacal gentiana violet solution and hydrochloric acid for cutinified substance, nor with iodine solution for starch, were observed.

d) The distillate with 1.06 sp. gr. hydrochloric acid had the characteristic odor of furfurol and gave also the characteristic furfurol reaction by aniline acetate.

e) To determine the methyl pentosane in malt-rootlets, the double distillation method with 1.06 sp. gr. hydrochloric acid of K. Oshima and K. Kondo[1] was applied, (a modification of the Tollens and Oshima method)[2], and it gave no characteristic absorption spectrum of methyl furfurol.

f) The extract of malt-rootlets reduced Fehling's solution distinctly.

## II. Constituents of the Carbohydrates in the Alcoholic Extract.

### Preparation of the Alcoholic Extract.

After removing impurities as much as possible, the rootlets were washed with water a little while and dried. One kilogram of the sample was extracted with 75 per cent of hot alcohol for two days in a flask with reflux condenser, filtered, and the alcohol recovered by distillation. The organic acid contained in the alcoholic extract was neutralized with calcium carbonate and filtered.

To remove the fat and oil contained in the extract, it was well shaken with ether, the ether layer separated from the fluid, evaporated in a partial vacuum, and the residue extracted with 80 per cent alcohol. The clear solution was concentrated again and then purified twice with 95 per cent alcohol, and then evaporated to syrup. About 40 grams of syrup was thus obtained.

The syrup gave the following reactions :—

1) It reduced Fehling's solution.

2) Fehling's solution was reduced slightly more after the inversion with hydrochloric acid than before.

3) It gave the α-naphtol reaction instantly.

4) It showed + rotation.

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1) K. Oshima & K. Kondo; Jour. Tokyo Chem. Soc., 1918, **39**, 185–198.

2) K. Oshima & B. Tollens; Ber. d. Deutsch. Chem. Ges., 1901, **34**, 1425–1426.

5) Two grams of the syrup were dissolved in 100 c. c. of water and this was fermented by beer yeast with addition of yeast extract free from sugar.

## Detection of Sugars.

1) To determine the nature of the sugars contained in the syrup, seeding experiments with a small crystal of known sugars were first tried. Glucose, fructose, mannose, maltose, galactose, and raffinose were respectively seeded in one drop of syrup which was put on an object glass. It was observed under the microscope that almost all of the crystals disappeared in a few days though glucose and raffinose remained as small crystals.

2) 4 grams of syrup, together with 15 c. c. of a mixture consisting of 0.2 gram phenyl-hydrazine hydrochloride, 4.5 grams sodium acetate and 30 c. c. water, were put in a test tube, and well shaken; then the mixture was warmed for 40 minutes in a water bath. Crude osazones in yellowish needles were obtained. After recrystallization from alcohol, 0.011 gr. of pure osazone was obtained, its melting point being 205–206°C.

3) To determine the presence of ketose, Pinoff's1) and Seliwanoff's2) methods were applied, and both were positive.

4) Mucic acid was not produced by oxidation of 2 grams of syrup with 1.15 sp. gr. nitric acid. Hence it is quite certain that the galactose molecule is absent in the syrup.

5) The syrup was oxidized as for the detection of mucic acid, evaporated nearly to dryness, and acetic acid was then added in the usual manner. By this process 0.0215 gram of saccharic acid was obtained.

6) To one gram of the syrup, 5 c. c. of water and 5 drops of phenyl-hydrazine were added and the mixture was well stirred at room temperature, but the characteristic mannose phenyl-hydrazone was not formed, thereby confirming the absence of mannose.

The results obtained in the above experiments showed the possibility of the presence of fructose, glucose, and a small quantity of some kind of non-reducing sugar.

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1) E. Pinoff; Ber. d. Deutsch. Chem. Ges., 1905, **38**, 3314.

2) T. Seliwanoff; Ber. d. Deutsch. Chem. Ges., 1887, **20**, 181.

### Isolation of Sugars.

The alcoholic extract was repeatedly purified with ether and 95 per cent alcohol and treated first with neutral, then with basic lead acetate to remove impurities. Excess of lead in the filtrate was removed by carbon dioxide. (The precipitate by the basic lead acetate had a slight ketose reaction.) The filtrate was treated with animal charcoal. The lead free liquid was concentrated to a syrup, and small crystals of raffinose, saccharose, glucose and fructose were seeded separately. The syrup was left untouched for several days, but it produced no crystals.

When some strontium hydroxide solution was applied to 50 grams of the syrup, a precipitate was produced, and this was filtered and washed with water. The precipitate thus obtained was suspended in water, treated with carbon dioxide and the filtrate was concentrated to a syrup. The syrup, however, gave neither ketose reaction nor any carbohydrate reaction. It is certain that raffinose and saccharose were absent.

From these experiments we may summarize as follows:—

The sugars present in the alcoholic extract are fructose and glucose but not mannose and galactose. The nature of the non-reducing sugars could not be determined, because of their small quantity but they are probably not saccharose or raffinose.

It was very difficult to confirm the presence of maltose in the syrup, as it contained glucose, for many of the reactions are common for both maltose and glucose. But the assumption that there is no maltose in the syrup is doubtful, because of the condition of the formation of osazone, its melting point, and the amount of saccharic acid produced. Perhaps the process of decomposition caused by malting changed maltose to glucose.

## III. Constituents of the Cell-walls (Hemicellulose)[1]

### Preparation of Sample.

Malt-rootlets were extracted with hot water and then in 95 per cent boiling alcohol in a flask with reflux condenser. The rootlets

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[1] The hemicellulose was first investigated by E. Schulze and his associates; Hoppe-Seyler's Zeitschr. f. Physiol. Chem., 1909, **61**, 279–357. and Ibid. 1910, **68**, 93–108; Ibid. 1910, **69**, 366–382.

thus treated were immersed for 24 hours in 0.25 per cent sodium hydroxide solution, filtered, and then washed well with water until no longer alkaline. 76 grams of the residue thus obtained were heated for about 18 hours in a flask on a boiling water bath, with 3 per cent sulphuric acid. Then the solution was filtered, the filtrate neutralized with pure calcium carbonate, and again concentrated and purified with 95 per cent alcohol. Evaporation and purification by strong alcohol was repeated until no more gummy substance appeared. The decanted solution was concentrated to syrup, which weighed about 18 grams.

## General Qualitative Tests of the Syrup.

1) It reduced Fehling's solution.
2) It was fermented by beer yeast.
3) Pentose reaction with aniline acetate was positive.
4) Osazone was produced with phenyl-hydrazine acetate.
5) It produced no characteristic mannose phenyl-hydrazone.

## Detection of Sugars.

a) Xylose.

Bertrand's[1] reaction was used to determine the presence of xylose. About one gram of the syrup was dissolved in little water, then a few drops of bromine water and 0.5 gram of cadmium carbonate were added. The mixture was warmed in a bath for a little while, and then allowed to stand for a day. The product thus obtained was poured into a porcelain basin and dried, and then filtered. After the filtrate was nearly dried up, a small quantity of alcohol was added, when the characteristic crystals of cadmium bromoxylonate formed.

b) Glucose.

Tollens'[2] method of separating potassium saccharate by its solubility was applied to determine the presence of glucose. 0.2275 gram of the characteristic potassium saccharate was produced from 2 grams of the syrup.

c) Galactose.

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1) M. G. Bertrand; Bull. Soc. Chem., 1891, (3), 5, 546.
2) B. Tollens; Handbuch der Kohlenhydrate. 3. Auf. 245.

Two grams of the syrup were oxidized with 1.15 sp. gr. nitric acid to determine the presence of galactose as usual, but the production of mucic acid was doubtful.

d) Fructose

Both Seliwanoff's and Pinoff's reaction for ketose were positive.

From these experiments, it is clear, that the syrup contained xylose, glucose, fructose and galactose (?) but no mannose.

From the Results of these Investigations the Carbohydrates of Malt-rootlets, we may Summarize as Follows:—

(1) The chief constituent of the membrane of the rootlets is cellulose containing some lignine, but no cutine.

(2) Pentosane was confirmed in malt-rootlets, but the reaction for methyl pentosane was negative.

(3) Starch is not present in the rootlets.

(4) Xylose, glucose, fructose, and galactose (?) are produced by hydrolysis of the hemicellulose of the rootlets.

(5) The carbohydrates of the alcohol soluble part of the rootlets consist chiefly of glucose and fructose. Mannose, galactose, saccharose, maltose and raffinose were not detected.

## On the Nitrogenous Substances.

### I. Preparation of Sample.

As the material for the present investigation, malt-rootlets were purified in the usual manner and finely pulverized in a mill. The analysis gave the following composition:—

| | Air-dry substance.<br>(%) | Water-free substance.<br>(%) |
|---|---|---|
| Water | 19.40 | — |
| Ash | 5.19 | 6.44 |
| Crude protein | 23.44 | 29.06 |
| Crude fat | 1.45 | 1.80 |
| Crude fiber | 10.87 | 13.49 |
| Nitrogen-free extract | 39.65 | 49.21 |

## II. Qualitative Reaction of the Water Extract of the Sample.

The sample obtained from the treatment above described, was extracted with water and filtered.

The filtrate gave the following qualitative reactions :—

1) It gave Millon's, Biuret's, Hopkin-Cole's and the Furfuric reactions for proteins.

2) It gave no blue coloration with sulphuric acid and diphenylamine for the nitric group.

3) It gave no Berlin blue coloration with ferric chloride for cyanide compounds.

4) Reaction of Nessler's reagent for the presence of ammonia was negative.

5) It produced blue colored copper salts of amino acids with copper hydroxide, and the formaldehyde method for amino acid was positive.

6) It produced a white precipitate with ammoniacal solution and silver nitrate for purine bases.

7) Jaffe's, Weyl's and Salkowski's1) reactions were made use of in determining the presence of creatinine but none of those reactions were positive. In the case of Salkowski's, however, when the acidified solution was heated with acetic acid and treated with sodium nitroprusside according to Weyl's reaction, it colored slightly blue instead of precipitating prussian blue.

From the above researches (1–7) we may conclude that the nitrogenous substances in the water extract consist of many kinds of proteins, amino acids, and purine bases; cyanides and ammonia are not present, while the presence of creatinine was doubtful.

## III. Quantitative Analysis of Mal - tlets.

Analysis of the nitrogenous substances in malt-rootlets gave results as follows :—

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1) Hoppe-Seyler's Zeitschr. f. physiol. Chem., 1880, **4**, 133. and K. Oshima & M. Ariizumi; On the Occurrence of Creatinine in Leguminous seeds; Jour. of the College of Agric., Tohoku Imp. Univ., Sapporo, 1914, **4**.

| | Water-free substance. (%) | | Ratio of each N. (the total N. was based upon.) | |
|---|---|---|---|---|
| Total nitrogen | 4.65 | | 100.00 | |
| Protein nitrogen | 2.64 | | 56.77 | |
| Water soluble protein N. | 0.31 | | 6.67 | |
| NaCl (10%) soluble protein N. | 0.51 | | 10.97 | |
| Alcohol (60%) soluble protein N. | 0.31 | 2.69 | 6.67 | 57.85 |
| NaOH (0.25%) soluble protein N. | 1.22 | | 26.23 | |
| HCl (2%) soluble protein N. | 0.34 | | 7.31 | |
| Amino acid nitrogen | 0.94 | | 20.22 | |
| Diamino, purine-base and other nitrogen | 1.07 | | 23.01 | |

The following method was applied to determine each of the above mentioned nitrogens, soluble in different solvents: 1–2 grams of the sample were taken and extracted with the solvent; the protein in the filtrate was determined by Stutzer's method.

For the determination of the amino acid nitrogen, Sörensen's method with neutral formaldehyde was used.

The last figure in the above Table is the difference between the total nitrogen and the sum of protein nitrogen and amino acid nitrogen.

## IV. Isolation of Non-protein Nitrogenous Compounds.

### 1) Isolation of Asparagine.

One kilogram of malt-rootlets was immersed in water for 36 hours and then filtered with suction. The residue was washed with water and filtered once more. The filtrates thus obtained were put together and evaporated to about 500 c. c. on a water bath, and 95 per cent alcohol was then added until it contained 30–40 per cent alcohol and filtered. To the filtrate, 95 per cent alcohol was added once more, making it 50–60 per cent, then it was allowed to stand for a few days until the formation of characteristic crystals of asparagine was observed. The crystals were separated from the mother liquor by filtration with suction. By repeating recrystallization from alcohol and decolorization with some animal charcoal, a pure substance was obtained. Yield, 12 grams.

The crystals gave the following composition.

| | | |
|---|---|---|
| 1.4212 g. substance gave | 0.1698 g. | $H_2O$ |
| Found | 11.95% | „ |
| Cal. Asparagine ($C_4H_8O_3N_2.H_2O$) | 12.00 „ | „ |

| | | |
|---|---|---|
| 0.1520 g. water-free substance gave | 0.03227 g. | N |
| Found | 21.23% | ,, |
| Cal. Asparagine ($C_4H_8O_3N_2$) | 21.21 ,, | ,, |

The crystals produced copper salt with copper hydroxide which was of cobalt-color and needle shaped.

Generally the crystals of asparagine could be detected in the extract of malt-rootlets with the naked eye, but in very isolated instances this could not be done, though the crystals were revealed under a microscope.

2) Isolation of Adenine, Histidine, Arginine, Choline and Betaine.

14.8 kilograms of malt-rootlets were extracted for several hours with hot 80 per cent alcohol, this operation being repeated twice.

The extract was concentrated on the water bath at a low temperature, and purified, first with neutral lead acetate, then with basic lead acetate as usual, the excess of lead in the liquor being removed by hydrogen sulphide. The lead-free filtrate was concentrated and sulphuric acid was added till the content of the acid was 5 per cent and then saturated phosphotungstic acid was added until no more precipitate separated out. After 24 hours the precipitate was filtered by suction, and washed with 5 per cent sulphuric acid by stirring it in a mortar.

The precipitate thus prepared was uniformly suspended in water and to it a saturated solution of baryta was added until the solution contained an excess of baryta. It was allowed to stand for 24 hours at room temperature, then filtered, and washed with dilute sulphuric acid. The residue was treated again with baryta. The filtrate and washings were put together and excess of barium was removed by dilute sulphuric acid.

The barium-free filtrate was acidified with dilute nitric acid and 10 per cent of silver nitrate added.

Thus was obtained a brown colored precipitate which was filtered off.

a. Adenine.

The precipitate of silver salt thus obtained, was purified on a porous plate. After the purified precipitate was treated with a little ammonia, it was decomposed by the addition of hydrochloric acid, and the silver chloride formed was filtered off. The filtrate was nearly dried up at a low temperature and purified with alcohol, then some concentrated sodium picrate was added, yellowish needle shaped crystals being obtained. It was recrystallized with hot water, melting at 281–282°C. The melting point coincides with that of adenine picrate. Its double compound of gold chloride was made and found to melt at 215°C., but the gold content was not determined because of its small quantity.

b. Histidine and Arginine.

To the filtrate of the adenine silver compound, a solution of silver nitrate was added and it was neutralized with concentrated baryta until a clear portion of the solution still remained clear on further addition of baryta. It was then filtered. The precipitate here obtained was decomposed by hydrochloric acid along with sulphuric acid and then filtered.

From the silver-free filtrate, a precipitate of phosphotungstate was obtained with sulphuric acid and phosphotungstic acid, and was then decomposed with baryta as usual. Histidine and arginine were sought for by their mercuric salts, and by difference in solubility in water, but neither of them was confirmed.

c. Betaine and Choline.

The mother liquor of the precipitate with silver nitrate and baryta in the portion b. was freed from silver and barium with just the required quantity of hydrochloric and sulphuric acid, then it was concentrated on the water bath and precipitation and decomposition of phosphotungstic salt was carried out as before. After the solution of the free base was dried on the bath under reduced pressure, it was purified with hot alcohol. To the alcoholic solution thus obtained, an alcoholic solution of mercuric chloride was added till no more white precipitate separated out. It was then allowed to stand several days in a cold place. Two weeks afterwards it was filtered and well washed with alcohol. The precipitate here obtained was suspended in water and decomposed by hydrogen sulphide. Then it was filtered. The filtrate was concentrated to a small volume under reduced pressure, and after drying in a desiccator over sulphuric acid, it was divided into two parts, making use of different solubility in absolute alcohol.

c′) Soluble crystals in absolute alcohol (Choline hydrochloride).

The substance consisted of white needle crystals and was very hygroscopic. The crystal obtained out of 14.8 kilograms of malt-rootlets was 8.06 grams, as hydrochloride.

Some of the crystals thus obtained, were dissolved in water, a solution of platinum chloride was added, and then concentrated on a water bath at a low temperature. When it was kept in a desiccator over sulphuric acid, yellowish platelets were observed.

The crystals were separated by filtration and washed with alcohol. The melting point was 234°C. It was insoluble in alcohol.

The crystals showed the following analytical results :—

| | | |
|---|---|---|
| 0.1504 g. substance gave | 0.04788 | Pt |
| Found | 31.78% | „ |
| Cal. $(C_5H_{14}NOCl)_2PtCl_4$<br>(Choline Platinumchloride) | 31.64 „ | „ |

The picrate was produced by treating choline hydrochloride with sodium picrate, its yellowish platelets being soluble in water and alcohol and melting at 130°C.

c'') Insoluble crystals in absolute alcohol (Betaine hydrochloride).

To remove the mineral matter, it was purified with hot alcohol, concentrated at a low temperature, dried in a desiccator as usual and weighed. 4.55 grams of betaine hydrochloride were isolated. Its platinum salt was produced by the same treatment as above.

It formed yellowish platelets, soluble in water but not in alcohol, melting at 250°C. The analytical result was follows :—

| | | |
|---|---|---|
| 0.1538 g. substance gave | 0.0466 g. | Pt |
| Found | 30.30% | „ |
| Cal. $(C_5H_{11}NO_2HCl)_2PtCl_4$<br>(Betaine platinumchloride) | 30.26 „ | „ |

Its picrate formed yellowish platelets, soluble in water but hardly in alcohol, melting at 178–181°C.

3) Isolation of Creatinine.

One kilogram of malt-rootlets was extracted for several hours with hot dilute alcohol, using a reflux condenser. The alcoholic solution was filtered hot, and purified with strong alcohol. The extract thus obtained was concentrated to syrup by evaporating in a partial vacuum, and the syrup was treated with neutral lead acetate to remove impurities. Excess of lead acetate was removed by hydrogen sulphide and filtered off. The filtrate was concentrated to syrup once more under reduced pressure, and to the syrup a little sodium acetate and a saturated solution of zinc chloride, about 1/3 volume of the syrup, were added. The mixture being kept in a desiccator over sulphuric acid for some weeks.

Then, white plate crystals, formed thickly, were observed in the syrup. The crystals were separated with a "Nutsch", washed with strong alcohol, and recrystallized from water. Yield, about two grams.

The crystals thus obtained were found to contain nitrogen and zinc by the usual method. None of the reactions of Jaffe, Weyl and Salkowski for creatinine were positive. Apparently the crystals were not creatinine zinc chloride.

The zinc salts obtained after the above method, were dissolved in water and boiled with some freshly prepared lead hydroxide, and then filtered. The filtrate was concentrated and dried in a desiccator over sulphuric acid. After a few days white needle crystals separated out, and the yield amounted to 0.11 per cent of air dry substance of malt-rootlets.

The crystals gave the following reactions:—

a) It was very hygroscopic.

b) It gave alkali reaction with litmus paper.

c) It produced needle shaped crystals, orange red inc olor, on treating with potassium iodide and bismuth iodide.

d) It produced a brownish precipitate with iodine-potassium-iodide disappearing after a little while.

e) To the aqueous solution of the crystals some platinum chloride and a little hydrochloric acid were added, whereby the crystals of the platinum salt, insoluble in absolute alcohol, were obtained.

Determination of platinum in the crystals gave the following results:—

| | | |
|---|---|---|
| 0.1032 g. substance gave | 0.03258 g. | Pt |
| Found | 31.57% | „ |
| Cal. $(C_5H_{14}NO.\ Cl)_2Pt.\ Cl_{14}$<br>(Choline platinumchloride) | 31.64 „ | „ |

From the above reactions, it may be concluded that the zinc-salt in hand was no other than choline zinc chloride, and not creatinine salt after all.

4) Isolation of Hordenine.

In a flask with reflux condenser 300 grams of malt-rootlets were boiled in 95 per cent alcohol for several hours and filtered; the residue was washed with alcohol and well pressed. The extract was put together and evaporated to syrup on a water bath under reduced pressure. The syrup was acidified with dilute sulphuric acid and filtered. It was decolorized with animal charcoal and sodium carbonate was added.

Hordenine that was separating in free form was extracted with ether in a separating funnel. On evaporating the ether extract, white needle shaped crystals with a bitter taste were obtained, the yield being 0.39 gram, corresponding to 0.13 per cent of the air dry malt-rootlets.

The crystals showed the following reactions :—

a) It melted at 117–118°C.

b) It gave xanthoproteic and Millon's reactions.

c) It gave a reddish blue coloration with ferric chloride.

d) The analysis gave the following results :—

| | | |
|---|---|---|
| 0.2044 g. substance gave | 0.5433 g. $CO_2$ and | 0.1680 g. $H_2O$ |
| | C | H |
| Found | 72.49% | 9.13% |
| Cal. Hordenine ($C_{10}H_{15}NO$) | 72.67 „ | 9.17 „ |
| 0.2020 g. substance gave | 0.01725 g. | N |
| Found | 8.54% | „ |
| Cal. ($C_{10}H_{15}NO$) | 8.48 „ | „ |

5) Isolation of Tyrosine.

500 grams of malt-rootlets were boiled in a large flask with water, for 24 hours, filtered hot, and the residue was extracted again with some hot water. These two filtrates were put together, concentrated on a water bath, and treated with ether to remove the hordenine, fat, oil, etc., contained in the extract, and purified with lead acetate and filtered. To the filtrate mercuric nitrate was added when a grayish white precipitate separated out. The precipitate thus formed was filtered off and well washed with water to remove impurities. It was suspended in water, decomposed with hydrogen sulphide, and filtered. The filtrate was concentrated to syrup on a water bath and after being purified with ether, Millon's and Piria's reactions for tyrosine were applied. None of them was positive. It was evident that tyrosine was not contained in the malt-rootlets.

From the above experiments (1–5) the following conclusion about non-protein nitrogenous substances may be drawn :—

| | | | |
|---|---|---|---|
| Asparagine | Positive | Betaine | Positive |
| Adenine | „ | Creatinine | Negative |
| Histidine | Negative | Hordenine | Positive |
| Arginine | „ | Tyrosine | Negative |
| Choline | Positive | | |

## On Hordenine.

Since E. Léger[1] in 1906 isolated hordenine from the malt-rootlets, and studied its constitutional formula, many different investigations have been made upon the same substance, each investigator having his own point of view.

G. Denigés[2] carried out researches on the nature of hordenine, and O. Gaebel[3] described its isolation from the malt-rootlets, its characteristic reactions, and its constitutional formula. L. Camus[4] reported on the action of hordenine sulphate upon animals, bacteria and enzymes. Afterwards, the physiological effects and the therapeutic value of hordenine sulphate were examined by J. Sabrazes and G. Guerive.[5]

The synthesis of hordenine was first undertaken by G. Barger,[6] who started with phenylethylalcohol and succeeded in completing the work; his principle being as follows:—

$$<\overline{\underline{\;\;}}>CH_2.CH_2.OH \rightarrow <\overline{\underline{\;\;}}>CH_2.CH_2.Cl \rightarrow <\overline{\underline{\;\;}}>CH_2.CH_2.N(CH_3)_2$$

$$\rightarrow NO_2<\overline{\underline{\;\;}}>CH_2.CH_2.N(CH_3)_2 \rightarrow NH_2<\overline{\underline{\;\;}}>CH_2.CH_2.N(CH_3)_2$$

$$\rightarrow HO<\overline{\underline{\;\;}}>CH_2.CH_2.N(CH_3)_2$$

W. Rosemund[7] obtained hordenine by methylation of *p*-methoxyphenylethylamine with alcoholic potash and methyliodide, the methyl group being converted into hydroxyl with hydriodic acid.

Recently, new synthetic methods were proposed by E. Späth and P. Sobel,[8] the yield proving quite satisfactory. The reactions are indicated by the two methods. One of these was started by the condensation with $CH_3O<\overline{\underline{\;\;}}>CH_2Br$ and $BrCH_2OCH_3$ and the other was started from $CH_3O<\overline{\underline{\;\;}}>CH:CHCOOH$.

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[1] E. Léger; Compt. rend., 1906, **142**, 108; **143**, 234, 916; 1907, **144**, 208, 482.

[2] G. Denigés; Bull. Soc. Chem. France, 1908, (4) **3**, 786.

[3] O. Gaebel; Arch. Pharm., 1906, **244**, 435.

[4] L. Camus; Compt. rend., 1906, **142**, 110, 350.

[5] J. Sabrazes & G. Guerive; Compt. rend., 1908, **147**, 1076.

[6] G. Barger; Jour. Chem. Soc., 1909, **95**, 2193.

[7] W. Rosemund; Ber. d. Deutsch. Chem. Ges., 1910, **43**, 306.

[8] E. Späth u. B. Sobel; Univ. Vienna. Monatsh., 1920, **41**, 77, through A. Chem. Ab., 1920, **14**, 3408.

## 1 Derivatives of Hordenine.

While E. Léger[1] determined the constitutional formula of hordenine by studying its derivatives, the author of the present paper investigated this same subject with some difference, for a different purpose. The results are described as follows:—

1) Hordenine sulphate $(C_{10}H_{15}NO)_2H_2SO_4$.

Some hordenine was dissolved in a little dilute sulphuric acid until the solution showed a weak acidity, and concentrated on a water bath. When cooled, white needle shaped crystals appeared. The crystals were separated by filtration with suction, and washed with strong alcohol. It was again purified by recrystallization from hot alcohol (95%). It was readily soluble in water, but less so in alcohol, its melting point being 209–211°C.

Analysis gave the following results:—

| | | | |
|---|---|---|---|
| 0.2110 g. substance gave | | 0.0134 g. | N |
| Found | | 6.35 % | „ |
| Cal. $(C_{10}H_{15}NO)_2H_2SO_4$ | | 6.54 „ | „ |
| a. 0.3227 g. substance gave | | 0.1732 g. | $BaSO_4$ |
| b. 0.3234 g. substance gave | | 0.1746 g. | „ |
| Found | a. | 22.55 % | $H_2SO_4$ |
| | b. | 22.68 „ | „ |
| Cal. $(C_{10}H_{15}NO)_2H_2SO_4$ | | 22.90 „ | „ |

2) Hordenine hydrochloride $(C_{10}H_{15}NO.HCl)$.

By similar treatment of hordenine as above with hydrochloric acid, white crystals were obtained. Its crystal forms were needles or small plates, soluble both in water and alcohol, the melting point being 176–178°C.

| | | | |
|---|---|---|---|
| 0.1646 g. substance gave | | 0.0116 g. | N |
| Found | | 7.05 % | „ |
| Cal. $(C_{10}H_{15}NO.HCl)$ | | 6.95 „ | „ |
| a. 0.2074 g. substance gave | | 0.1490 g. | AgCl |
| b. 0.2012 g. substance gave | | 0.1436 g. | „ |
| Found | a. | 18.27 % | HCl |
| | b. | 18.15 „ | „ |
| Cal. $(C_{10}H_{15}NO.HCl)$ | | 18.09 „ | „ |

3) Hordenine hydrobromide $(C_{10}H_{15}NO.HBr)$.

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[1] E. Léger; Compt. rend., 1907, **144**, 208–210.

The bromine salt of hordenine was obtained by a treatment similar to that described above with hydrobromic acid. The crystals were long white plates, melting at 173–174°C., easily soluble in water but less so in alcohol and ether. On analysis the following results were obtained:—

| | | | | |
|---|---|---|---|---|
| a. 0.2031 g. substance gave | | 0.01172 g. | | N |
| b. 0.1838 g. substance gave | | 0.01033 g. | | „ |
| Found | a. | 5.77 | % | „ |
| | b. | 5.62 | „ | „ |
| Cal. ($C_{10}H_{15}NO.HBr$) | | 5.69 | „ | „ |
| a. 0.2038 g. substance gave | | 0.1546 g. | | AgBr |
| b. 0.2060 g. substance gave | | 0.1568 g. | | „ |
| Found | a. | 32.69 | % | HBr |
| | b. | 32.80 | „ | „ |
| Cal. ($C_{10}H_{15}NO.HBr$) | | 32.91 | „ | „ |

4) Hordenine acid tartrate ($C_{10}H_{15}NO.C_4H_6O_6$).

When two grams of hordenine together with 1.63 grams of tartaric acid in a little water were dissolved and evaporated on a water bath, white needle crystals were obtained which were purified with water. The crystals were 3.1 grams in weight, melting at 170–171°C., easily soluble in water but not in alcohol. They showed acid reaction with litmus paper.

| | | | |
|---|---|---|---|
| 0.2087 g, substance gave | 0.0097 g. | | N |
| Found | 4.69 | % | „ |
| Cal. ($C_{10}H_{15}NO.C_4H_6O_6$) | 4.45 | „ | „ |

5) Hordenine neutral tartrate ($(C_{10}H_{15}NO)_2C_4H_6O_6$.

This was obtained by treating 2 grams of hordenine with 0.81 grams of tartaric acid. By the above treatment white needle shaped crystals were formed, which were neutral to litmus paper, not easily soluble in water, insoluble in alcohol, and melting at 180–181°C. Analysis gave the following results:—

| | | | |
|---|---|---|---|
| 0.2050 g. substance gave | 0.01151 g. | | N |
| Found | 5.61 | % | „ |
| Cal. ($(C_{10}H_{15}NO)_2C_4H_6O_6$) | 5.83 | „ | „ |

6) Hordenine methyl iodide ($C_{10}H_{15}ON(CH_3)I$).

Some hordenine was dissolved in an excess of methyl iodide, and heated under a reflux condenser. The mixture reacted vigorously, the whole was heated on a water bath until the excess of methyl iodide vanished. The crystalline mass thus obtained was purified by recrystallization from alcohol, and fine white needle crystals were obtained which were soluble in water, and melted at 227–228°C.

| | | | | |
|---|---|---|---|---|
| 0·2356 g. substance gave | | 0.01094 | g. | N |
| Found | | 4.64 | % | „ |
| Cal. ($C_{10}H_{15}ON(CH_3)I$) | | 4.56 | „ | „ |
| 0.2554 g. substance gave | | 0.1968 | g. | AgI |
| Found | | 41.63 | % | I |
| Cal. ($C_{10}H_{15}ON(CH)_3I$) | | 41.34 | „ | „ |

7) Hordenine ethyl iodide ($C_{10}H_{15}ON(C_2H_5)I$).

For this derivative a treatment with ethyl iodide similar to that employed in the case of the above mentioned methyl derivative was adopted, resulting in white needle crystals, soluble in water but hardly soluble in alcohol, and melting at 179–180°C.

| | | | | |
|---|---|---|---|---|
| a. 0.2000 g. substance gave | | 0.00910 | g. | N |
| b. 0.2090 g. substance gave | | 0.00979 | g. | „ |
| Found | a. | 4.55 | % | „ |
| | b. | 4.68 | „ | „ |
| Cal. ($C_{10}H_{15}ON(C_2H_5)I$) | | 4.36 | „ | „ |
| a. 0.2129 g. substance gave | | 0.1544 | g. | AgI |
| b. 0.2096 g. substance gave | | 0.1527 | g. | „ |
| Found | a. | 39.18 | % | I |
| | b. | 39.36 | „ | „ |
| Cal. ($C_{10}H_{15}ON(C_2H_5)I$) | | 39.54 | „ | „ |

8) Acetyl hordenine ($C_{10}H_{14}NO.COCH_3$).

One gram of pure dry hordenine was wetted with a slight excess of acetic anhydride in a flask provided with a calcium chloride tube to exclude atmospheric moisture. After being allowed to stand for a few hours, it was heated on a water bath and cooled. The reaction-product thus obtained was dissolved in water, neutrallized with sodium acid carbonate and extracted with ether in a separating funnel. On evaporating, 1.5 gram of a light yellowish liquid was obtained, insoluble in water, but soluble in alcohol.

| | | | | |
|---|---|---|---|---|
| a. 0.2630 g. substance gave | | 0.0174 | g. | N |
| b. 0.2591 g. substance gave | | 0.0174 | g. | „ |
| Found | a. | 6.62 | % | „ |
| | b. | 6.72 | „ | „ |
| Cal. ($C_{10}H_{14}NO.COCH_3$) | | 6.76 | „ | „ |

| | | | | | |
|---|---|---|---|---|---|
| 0·2256 g. substance gave | 0.5702 g. | $CO_2$ | and | 0.1706 g. | $H_2O$ |
| Found | 68.93 % | C | | 8.40 % | H |
| Cal. ($C_{10}H_{15}NO.COCH_3$) | 69.52 „ | „ | | 8.27 „ | „ |

9) Benzoyl hordenine ($C_{10}H_{14}NO.C_7H_5O$).

Ten grams of hordenine were dissolved in a suitable quantity of pyridine, and then ten grams of benzoyl chloride were added drop by drop, the solution being stirred continually. The mixture was allowed to stand for 24 hours at room temperature. The crystalline mass thus obtained was separated by filtration with suction, dissolved in water, and neutralized with ammonia liquor, allowing a brownish oily reaction-product to be separated out on the bottom of the flask. This was separated by extraction with a sufficient quantity of ether.

In order to remove the impurity of pyridine, some powder of pumice was mixed with it and allowed to stand for several days in air until no more smell of pyridine was noticed. It was again extracted with ether. After decolorization by means of animal charcoal it was purified with dilute caustic potash and ammonia. The reaction-product was kept in a desiccator over sulphuric acid. Gradually the oily substance became a crystalline mass. The crystals were white plates insoluble in water but soluble in alcohol and ether, melting at 47–48°C.

The result of analysis was as follows:—

| | | |
|---|---|---|
| a. 0.1952 g. substance gave | 0.01094 g. | N |
| b. 0.2000 g. substance gave | 0.01065 g. | „ |
| Found | a. 5.60 % | „ |
| | b. 5.33 „ | „ |
| Cal. ($C_{10}H_{14}NO.C_7H_5O$) | 5.21 „ | „ |

| | | | | | |
|---|---|---|---|---|---|
| 0.1576 g. substance gave | 0.4326 g. | $CO_2$ | and | 0.1044 g. | $H_2O$ |
| Found | 74.86 % | C | | 7.36 % | H |
| Cal. ($C_{10}H_{14}NO.C_7H_5O$) | 75.79 „ | „ | | 7.12 „ | „ |

10) Benzoyl hordenine hydrobromide ($C_{10}H_{14}(C_7H_5O)NO.HBr$).

Benzoyl hordenine was dissolved in dilute alcohol, and then hydrobromic acid was added. The mixture was concentrated on a water bath. The crystals thus obtained were pressed out on a porous plate and the next day recrystallized from 95 per cent alcohol. They were white plates or needles and soluble in water and alcohol, melting at 171–172°C.

| | | |
|---|---|---|
| 0.2019 g. substance gave | 0.00851 g. | N |
| Found | 4.21 % | „ |
| Cal. ($C_{10}H_{14}(C_7H_5O)NO.HBr$) | 4.00 „ | „ |
| 0.2032 g. substance gave | 0.1073 g. | AgBr |
| Found | 22.47 % | Br |
| Cal. ($C_{10}H_{14}(C_7H_5O)NO.HBr$) | 22.84 „ | „ |

11) Hordenine trimethyl sulphate $C_{10}H_{14}O(CH_3)N(CH_3)(SO_4CH_3)$.

Four grams of hordenine were dissolved in 20 c. c. of 10 per cent caustic potash and were shaken with 4 c. c. of dimethyl sulphate drop by drop. The mixture became warm, and it was then boiled under a reflux condenser for a while, to destroy excess of dimethyl sulphate. After evaporation on a water bath, it was purified with strong alcohol to remove insoluble substances. The alcoholic solution obtained was distilled, the residue again purified on a porous plate, and recrystallized from alcohol. 4 grams of white needle crystals were obtained, which were soluble in water and alcohol but not in ether and benzene, the melting point being 165–166°C. Barium sulphate was not precipitated from the aqueous solution of the crystals, by means of dilute hydrochloric acid and barium chloride. Analysis gave the following results.

| | | | |
|---|---|---|---|
| a. 0.2303 g. substance gave | | 0.01094 g. | N |
| b. 0.2428 g. substance gave | | 0.01122 g. | „ |
| Found | a. | 4.74 % | „ |
| | b. | 4.62 „ | „ |
| Cal. $C_{10}H_{14}O(CH_3)N(CH_3)(SO_4CH_3)$ | | 4.59 „ | „ |
| 0.2448 g. substance gave | | 0.3643 g. | AgI |
| Found | | 19.65 % | (-O.$CH_3$) |
| Cal. $C_{10}H_{14}O(CH_3)N(CH_3)(SO_4CH_3)$ | | 20.33 „ | „ |

The Carius-method (decomposition in a sealed tube) was applied for the determination of sulphur.

The result was as follows :—

| | | |
|---|---|---|
| 0.3052 g. substance gave | 0.2468 g. | $BaSO_4$ |
| Found | 11.10 % | S |
| Cal. $C_{10}H_{14}O(CH_3)N(CH_3)(SO_4CH_3)$ | 10.50 „ | „ |

12) Hydrobromic salt of hordenine bromide $(C_{10}H_{13}NOBr_2)HBr$.

20 grams of hordenine were dissolved in 400 c. c. pure ether, the flask being cooled in a freezing mixture of ice and salt and provided with a calcium chloride tube. To the reaction-mixture some bromine was poured from a dropping funnel, stirring it continually, until the solution showed a slight red color. The mixture was allowed to stand

for a few days. After distilling off the ether, the residue was crystallized from 95 per cent alcohol, and 36.45 grams of needle shaped white crystals were obtained. The crystals gave a weak acid reaction with litmus paper, were barely soluble in water and alcohol, but were not at all soluble in ether. They decomposed at 222.5°C.

| | | | |
|---|---|---|---|
| a. 0.2000 g. substance gave | | 0.0073 g. | N |
| b. 0.2023 g. substance gave | | 0.0075 g. | „ |
| Found | a. | 3.65 % | „ |
| | b. | 3.71 „ | „ |
| Cal. $(C_{10}H_{13}NO.Br_2)HBr$ | | 3.47 „ | „ |

In order to determine the bromine which combined as salt, a suitable quantity of silver nitrate solution was added to an aqueous solution of crystals, and the precipitate here obtained was weighed.

The result was as follows:—

| | | | |
|---|---|---|---|
| a. 0.2034 g. substance gave | | 0.0918 g. | AgBr |
| b. 0.1023 g. substance gave | | 0.0492 g. | „ |
| Found | a. | 19.21 % | Br |
| | b. | 20.37 „ | „ |
| Cal. $(C_{10}H_{13}NOBr_2)HBr$. | | 19.79 „ | „ |

The Carius-method was adopted in determining the total bromine contained, which was as follows:—

| | | | |
|---|---|---|---|
| a. 0.1634 g. substance gave | | 0.2283 g. | AgBr |
| b. 0.1459 g. substance gave | | 0.2042 g. | „ |
| Found | a. | 59.46 % | Br |
| | b. | 59.56 „ | „ |
| Cal. $(C_{10}H_{13}NOBr_2)HBr$ | | 59.38 „ | „ |

Ten grams of the crystals thus obtained were dissolved in water and then neutralized with potassium carbonate, and the white precipitate which separated was purified with hot alcohol. The crystals thus obtained melted at 183–184°C., being hardly soluble in water and alcohol and insoluble in ether. Analysis gave the following results:—

| | | |
|---|---|---|
| 0.1547 g. substance gave | 0.1805 g. | AgBr |
| Found | 49.65 % | Br |
| Cal. $(C_{10}H_{13}NOBr_2)$ | 49.51 „ | „ |
| 0.2032 g. substance gave | 0.0085 g. | N |
| Found | 4.18 % | „ |
| Cal. $(C_{10}H_{13}NOBr_2)$ | 4.34 „ | „ |

The hydrobromide-salt of this substance was prepared by treating it with hydrobromic acid. The analysis of the salt gave the following results:—

| | | |
|---|---|---|
| 0.2340 g. substance gave | 0.0084 g. | N |
| Found | 3.59 % | „ |
| Cal. ($C_{10}H_{13}NOBr_2$)HBr | 3.47 „ | „ |

13) Hordenine sulphonate ($C_{10}H_{14}NO.HSO_3$).

To ten grams of hordenine sulphate placed in a flask provided with an air condenser, were added drop by drop ten grams of concentrated sulphuric acid, and the mixture was heated at 98°C. for 14 hours. After cooling, it was dissolved in a large quantity of water, neutralized with calcium carbonate, filtered hot with suction, and washed with hot water. The filtrate was concentrated on a water bath and once more filtered while hot. The syrup thus obtained was converted into a crystalline mass on cooling. It was purified on a porous plate, and washed with alcohol and cold water. Hordenine sulphonate thus obtained showed white needle shaped crystals, insoluble in cold alcohol, water, and ether, and melting at 270–271°C. It gave a bluish violet color with ferric chloride. The analysis gave the following results:—

| | | |
|---|---|---|
| a. 0.2010 g. substance gave | 0.0113 g. | N |
| b. 0.2012 g. substance gave | 0.0113 g. | „ |
| c. 0.2056 g. substance gave | 0.0118 g. | „ |
| Found | a. 5.62 % | „ |
| | b. 5.62 „ | „ |
| | c. 5.74 „ | „ |
| Cal. ($C_{10}H_{14}NO.HSO_3$) | 5.71 „ | „ |

The Carius-method was adopted to determine sulphur with the following results:—

| | | |
|---|---|---|
| 0.3074 g. substance gave | 0.2978 g | $BaSO_4$ |
| Found | 13.30 % | S |
| Cal. ($C_{10}H_{14}NO.HSO_3$) | 13.08 „ | „ |

## II. Occurrence of Hordenine in Cereals.

Since Léger reported the formation of hordenine in the barley rootlets during germination, many investigators including myself[1], have confirmed it.

It appears to be worth-while to investigate the nature of this substance in relation to beer brewing, especially, its existence in the malt. According to investigations, the derivatives of this substance

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[1] Y. Hashitani; Jour. Tokyo Chem. Soc., 1919, **40**, 647.

generally give an unpleasant taste, so that when they get into the beer, its quality is inevitably affected. E. Ehrlich[1] stated that a certain kind of yeast and fungi utilize hordenine as a nitrogen source, thereby forming the disagreeable bitter tyrosol after decomposition. If, therefore, the malt is attacked by these kinds of fungi or yeast, the tyrosol may appear in the beer.

Moreover, if hordenine exists in plants other than barley, it may possess an interesting physiological meaning. This side of the investigation still remains practically untouched.

As regards hordenine in the germinating barley, T. Torquati[2] stated that dormant barley contains no hordenine, the quantity of this substance reaching its maximum on the fourth day of sprouting, the rootlets containing 0.4–0.45 per cent, the embryo 0.10 per cent (of air dried sub-stanoe), the content diminishing according to the growth of seedlings and ultimately disappearing after 25 days. He tested for hordenine in wheat, pea and lupine with negative results.

So far as the present author is aware, it is only in barley that the formation of hordenine by germination has been found.

In malt making from various kinds of grains, the author was able to obtain a little of their rootlets as by-products and studied these substances as described hereafter.

Method adopted in detecting Hordenine.

The method to be used in determining the presence of hordenine is of first importance in conducting the present investigations, since the hordenine content is so small that its separation by crystallization is almost impossible on account of other impurities.

The following process was made use of for the detection of hordenine :—

The materials were extracted with 95 per cent alcohol and then the same treatment was applied as described in the case of isolating hordenine. The ether soluble syrup obtained here, and containing hordenine, was free from organic acids, protein and tyrosine. Therefore, if Millon's color reaction is obtained with the syrup, this can be attributed to the action of hordenine only.

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[1] E. Ehrlich; Ber. d. Deutsch. Chem. Ges., 1911, **44**, 139; Biochem. Zeitschr., 1916, **75**, 417.

[2] T. Torquati; Arch. Farmakol. Sperim., 1910, **10**, 62, 97. Ref. in Chem. Zentralbl., 1911, **1**, 166.

Even a trace of hordenine in the ether extract could be ascertained by this colorimetric method. Whenever it was possible to obtain pure hordenine, the determination of the melting point and elementary analysis were applied. It is possible to determine the unknown content of hordenine by comparing color reactions of hordenine solution of known concentration with Millon's reagent.

The present investigator proceeded as follows :—

A little 0.2 per cent solution of hordenine sulphate was added to 5 c. c. of water and 7 drops of Millon's reagent, the mixture was boiled for one minute in a test tube and diluted to 15 c. c. with water in a tube, as used for the colorimetric estimation of ammonia. The dilution was compared as to its color intensity with the color of a filtered carmine solution containing 0.5 gram of carmine in 300 c. c. water.

| No. of the tube | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) |
|---|---|---|---|---|---|---|---|---|---|
| Solution of hordenine sulphate (c. c.) | 0.025 | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.35 | 0.40 |
| Quantity of hordenine sulphate (calculated number) (g.) | 0.00005 | 0.0001 | 0.0002 | 0.0003 | 0.0004 | 0.0005 | 0.0006 | 0.0007 | 0.0008 |

The quantity of carmine solution required for coincidence with the color of the above hordenine concentrations was :—

| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) |
|---|---|---|---|---|---|---|---|---|---|
| Carmine solution (c. c.) | 0.1 | 0.3 | 0.5 | 0.7 | 1.1 | 1.5 | 1.7 | 2.1 | 2.5 |

From repeated experiments, the hordenine solutions No. 3 or No. 4, containing 0.0002 or 0.0003 gram of hordenine sulphate respectively, were found to be concentrated enough for recognition of the color reaction with Millon's reagent.

### 1. Barley. (Hordeum sativum, Jess.)

(a) Hordenine was always obtained from malt-rootlets, (0.15–0.23 per cent of the air dried substances), but it was suspected that the hordenine formation in barley seedlings during malting might be an abnormal phenomenon, because of its unnatural growth in the germination apparatus, without light and soil, because of many other phenomena, altogether quite different from natural growth in soil.

Acting on this supposition, I examined barley seedlings cultivated on sand, both in light and darkness, the sand being purified with hydrochloric acid and caustic soda and well washed with water, but in every case hordenine was isolated.

From 44.65 grams of young roots of the seedlings, 2–3 inches in length, cultivated in the dark for two weeks in the above purified sand, 0.033 gram of hordenine was isolated. The leaflets of the same seedlings treated in the manner elsewhere described gave Millon's reaction.

(b) From 11.5 kilograms of malt powder I isolated 0.1978 gram of crystalline hordenine, that is, 0.0017 per cent of the air dried substance. The crystals melted at 117–118°C. and produced a sulphonate compound whose melting point was 270–271°C. A mixture with pure hordenine sulphonate gave the same melting point.

(c) From 278 grams of rootlets of naked barley, obtained by a modification system of floor malting in a wide coop, a quantity of hordenine was isolated corresponding to 0.17 per cent of air dried substance. The melting point and derivatives proved that the substance in hand was hordenine.

### 2. Wheat. (Triticum vulgare, Vill.)

124.7 grams of rootlets, admixed with some leaflets, were obtained by the floor system of malting and out of that quantity 0.0034 gram of oily substance was obtained by means of the hordenine isolation method as already mentioned. The substance we obtained here crystallized in needle form and gave a bitter taste but no Millon's reaction. It is to be regretted that further research was not possible because of its small quantity.

### 3. Oat. (Avena sativa, L.)

From about 50 grams of oat rootlets, prepared by the same system of malting, a little ether soluble substance was obtained which gave Millon's color reaction distinctly, though no crystal formation could be observed.

### 4. Millet. (Panicum miliaceum, L.)

A large quantity of millet was germinated according to the usual system of floor malting and after drying, the rootlets were removed and 1900 grams of rootlets thus obtained were extracted with alcohol and 16,3 grams of ether soluble crystals were isolated, the yield corresponding to 0.86 per cent of air dried substance.

The crystals melted at 117–118°C. and analysis gave the following results :—

| | | |
|---|---|---|
| 0.2002 g. substance gave | 0.0166 g. | N |
| Found | 8.29 % | „ |
| Cal. Hordenine ($C_{10}H_{15}NO$) | 8.48 „ | „ |

A sulphate was produced with sulphuric acid, melting at 210°C. The results of analysis are as follows :—

| | | |
|---|---|---|
| 0.2000 g. substance gave | 0.0132 g. | N |
| Found | 6.60 % | „ |
| Cal. $(C_{10}H_{15}NO)_2H_2SO_4$ | 6.54 „ | „ |
| 0.2000 g. substance gave | 0.1100 g. | $BaSO_4$ |
| Found | 23.11 % | $H_2SO_4$ |
| Cal. $(C_{10}H_{15}NO)_2H_2SO_4$ | 22.90 „ | „ |

The melting point of the sulphonate was found to be 272°C. It was insoluble in cold water, alcohol and ether.

### 5. Crow-foot millet. (Panicum frumentaceum, Hook.)

From 244.5 grams of rootlets with some leaflets admixed, was obtained a little ether soluble matter which had a bitter taste and gave faint Millon's reaction.

### 6. Indian corn. (Zea Mays, L.)

A small quantity of Indian corn was immersed in water and allowed to stand for 72 hours at room temperature. The seeds were allowed to germinate in a packet of rush-mat, water being sprinkled on them from time to time, for 12 days. From 30 grams of rootlets thus prepared, 0.0154 gram of ether soluble substance was obtained which gave a bitter taste and a distinct Millon's reaction.

7. Sorghum. (Andropogon Sorghum, Brot.)

77.3 grams of rootlets, admixed with some leaflets, were taken and 0.0553 gram of rough crystals of hordenine were isolated according to the method previously mentioned. Its sulphonate compounds were melted at 270–271°C. and insoluble in water.

8. Italian millet. (Panicum italicum, L.)

The ether extract of seedlings obtained by the usual operation gave Millon's reaction, and contained a few crystals which could not be isolated because of the small quantity.

9. Rice. (Oryza sativa, L.)

The ether extract obtained by the method previously mentioned gave positive Millon's reaction but the separation of hordenine in a pure state could not be effected.

10. Buckwheat and Soy Bean were both examined and treated as the grains mentioned above, but neither crystalline hordenine nor Millon's reaction could be found.

The results of the foregoing experiments are tabulated below:—

| | |
|---|---|
| Barley | |
| a. Cultivated on sand in light. (Rootlets) | + |
| b. „ „ „ „ darkness. ( „ ) | + |
| c. Cultivated on sand in darkness, growing few inches. (Rootlets) | 0.07 |
| d. Cultivated on sand in darkness, growing few inches. (Leaflets) | + |
| e, Malt | 0.002 |
| f. Naked barley | 0.17 |
| Wheat | – |
| Oat | + |
| Millet | 0.86 |
| Crow-foot millet | + |
| Indian corn | + |
| Sorghum | 0.07 |
| Italian millet | + |
| Rice | + |
| Buckwheat | – |
| Soy bean | – |

The mark (+) represents the presence of hordenine and (−) its absence, the numerals showing the percentage of hordenine as obtained from air dried materials.

Judging from the above experiments, cereals in general form hordenine during germination, it being by no means an abnormal substance. It exists not only in the rootlets, but also in the leaves and stalks. It seems to take some time before it disappears utterly, decreasing gradually with the growth of the plant. Among the several samples treated wheat only showed a negative result.

From the investigation of so few samples it is impossible to conclude whether the presence of hordenine is limited only to the Gramineae or not. Further study is necessary and desirable.

## III. Enzymic Actions of the Extract of Barley, Malt, Malt-rootlets and others on Hordenine.

There is a considerable literature on the enzymes of barley, especially of the sprouting barley. It is devoted mostly to amylase and protease, the former which was discovered by Kirchhoff[1] in 1814, being the most important enzyme in the sprouting barley. Afterwards Payen and Persoz[2] isolated and named it Diastase. The latter, too, is an important enzyme and was investigated by many specialists.

The presence of protease in malt was determined by W. Windisch and B. Schellhorn[3] according to the method of Fermi by means of gelatine.

Oxydases have been described by W. Issajew,[4] V. van Laer[5] and V. Schjerning.[6]

L. Adler[7] made researches on phytase and C. B. Davis[8] isolated a new enzyme in malt, naming it as Hemicellulase, while of late

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[1] Kirchhoff; Schweigg Journ., 1815, **14**, 389.

[2] Payen & Persoz; Annales de Chemie et Physique, 1833, **53**, 73.

[3] W. Windisch u. B. Schellhorn; Wochenschr. f. Brauerei, 1900, **24**, 432.

[4] W. Issajew; Zeitschr. f. d. ges. Brauwesen, 1906, **8**, **116**. durch chem Zentralbl, 1905, **2**, 688.

[5] V. van Laer; Zeitschr. f. d. ges. Brauwesen, **1910**, **9**, 109. durch chem. Zentralbl. 1909, **2**, 836.

[6] V. Schjerning; Zeitschr. f. d. ges. Brauwesen, 1910, **34**, 413.

[7] L. Adler; Zeitschr. f. d. ges. Brauwesen, 1912, **16**, 181; **17**, 193; **18**, 210; **21**, 246; **24**, 277; **25**, 293; 1917, **15**, 113.

[8] C. B. Davis; Zeitschr. f. d. ges. Brauwesen, **1916**, **26**, 207; Wochenschr. f. Brauwerei, 1915, **26**, 226-228.

H. Van Laer[1] determined the presence of lipase in malt extract by the hydrolytic action on esters. M. Horderer[2] also reported on the distribution of enzymes in barley and malt.

Only a few investigators have attempted to study the enzymes of malt-rootlets. V. Schjerning investigated peroxydase, L. Adler proved phytase, and A. Baumann[3] reported on some of the carbohydrate splitting enzymes and protease.

Lately, I[4] examined the presence of enzymes in barley, malt, and malt-rootlets, the results being shown in the following table :—

| | Barley. | Green malt. | Dry malt. | Malt-rootlets. |
|---|---|---|---|---|
| Amylase | + | + | + | + |
| Maltase | + | + | + | + |
| Invertase | + | + | + | + |
| Glucosidase | + | + | + | + |
| Lipase | — | — | ? | + |
| Phytase | + | + | + | + |
| Pepsine | + | + | + | + |
| Trypsine | — | + | + | + |
| Erepsine | — | + | + | + |
| Desamidase | — | ? | — | — |
| Urease | + | + | + | + |
| Chymase | + | + | + | + |
| Catalase | + | + | + | + |
| Oxydase | + | + | + | + |
| Peroxydase | + | + | + | + |
| Tyrosinase | + | + | + | + |

According to L. Camus,[5] hordenine sulphate retards peptic and tryptic action but not the action of maltase, invertase or lipase.

Among the enzymes above mentioned, I examined the actions of urease and oxydase (in a wide sense) on hordenine.

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[1] H. van Laer; Compt. rend. Soc. Biol., 1921, 471, 473, through Jour. Inst. Brew., 1921., 321.

[2] M. Horderer; Wochenschr. f. Brauerei, 1909, **31**, 380.

[3] A. Baumánni; Zeitscher. f. d. ges. Brauwesen, 1916, **50**, 397.

[4] Y. Hashitani; Jour. Soc. of Agr. & Forest., Sapporo, 1921, **13**, 1-36.

[5] L. Camus; Compt. rend., 1906, **142**, 110, 350.

Preparation of the Sample containing Enzymes.

Barley :—

500 grams of finely powdered barley were immersed in 2 liters distilled water and to the mixture 20 c. c. of toluol were added.

After allowing it to stand 24 hours, the mixture was filtered and the filtrate was preserved in a cold place with a little toluol.

Green malt :—

One kilogram of green malt was well pressed and the whole substance was immersed in 2 liters distilled water with 20 c. c. of toluol, and the filtrate containing enzymes was obtained as above.

Malt-rootlets :—

330 grams of finely powdered malt-rootlets were dried at 42–43°C. and ground in a mill as usual, then immersed in 2 liters of distilled water containing 20 c. c. of toluol, and filtered. The filtrate was kept in a cold place with a little toluol.

Soy bean :—

200 grams of the seedlings of soy bean were ground in a mill and extracted with 400 c. c. distilled water. To the filtrate a few drops of toluol were added and it was kept in a cold place.

Potato :—

The filtrate containing enzymes from potato was obtained in similar manner as for soy bean.

### A. The Action of Urease.

A mixture of 20 c. c. of 2 per cent hordenine sulphate solution and a suitable quantity of each sample containing enzymes were prepared and to the mixture was added a little toluol. After allowing the mixture to stand 48 hours at 35–38°C., some freshly prepared magnesia was added to it until the whole became alkaline. It was then distilled below 50°C. under reduced pressure. The ammonia present in the distillate was estimated as usual, and the results obtained were compared with a control. The technique of the operation and the results of the experiments were as follows :—

Case I. Barley

| Mixed with \ No. of experiment | (1) | (2) | (3) |
|---|---|---|---|
| Enzyme Solution (c. c.) | 20 | 20<br>(Boiled) | 20 |
| Solution of hordenine sulphate (2%) (c. c.) | 20 | 20 | 0 |
| Distilled water (c. c.) | 0 | 0 | 20 |
| Toluol (c. c.) | 2 | 2 | 2 |
| Ammonia determined after 48 hours (mg.) | 6.73 | 4.94 | 7.00 |

In order to obtain the boiled solution of enzymes, the sample was immersed in boiling water for about 15 minutes and filtered.

In the following three cases, the operation was the same as in the first case. The results of the experiment are as follows:—

Case 2. Green malt

| No. of experiment | (1) | (2) | (3) |
|---|---|---|---|
| Ammonia determined after 48 hours (mg.) | 2.59 | 1.22 | 2.25 |

Case 3. Malt-rootlets

| No. of experiment | (1) | (2) | (3) |
|---|---|---|---|
| Ammonia determined after 48 hours (mg.) | 0.75 | 0.95 | 0.78 |

Case 4. Soy bean

| No. of experiment | (1) | (2) | (3) |
|---|---|---|---|
| Ammonia determined after 48 hours (mg.) | 1.13 | 0.61 | 0.86 |

It was not possible to confirm from these results, the action of urease on hordenine.

### B. The Action of Tyrosinase.

A colorimetric method was made use of for determining the action of tyrosinase on hordenine. The operation and the results were as follows :—

With the barley extract :—

| Mixed with \ No. of experiment | (1) | (2) | (3) | (4) | (5) |
|---|---|---|---|---|---|
| Enzyme solution (c. c.) | 20 | 20 (Boiled) | 20 | 20 | 20 |
| 3% solution of hordenine sulphate (c. c.) | 30 | 30 | 30 | 0 | 0 |
| 1.5% hydrogen peroxide (c. c.) | 25 | 25 | 0 | 25 | 0 |
| Distilled water (c. c.) | 0 | 0 | 25 | 30 | 55 |
| Toluol (c. c.) | 2 | 2 | 2 | 2 | 2 |

| Observation period \ No. of experiment | (1) | (2) | (3) | (4) | (5) |
|---|---|---|---|---|---|
| Initial | Light yellow | Light yellow | Light yellow | Light yellow | Light yellow |
| After a few minutes | Conc. fine red | „ | „ | Faintly reddish light yellow | „ |
| After 30 minutes | more conc. fine red | „ | „ | „ | „ |

The extract of green malt, malt rootlets, and potatoes gave similar coloration under the same conditions, therefore it was considered not necessary to give any further details.

From the above experiments, it is clear that hordenine was oxydized by the enzyme of barley-extract and the enzyme which acted on hordenine was evidently tyrosinase.[1] For the following reason, the author possesses still greater confidence in this belief:—The extract of potatoes, barley, malt, etc., which contains tyrosinase[2] gave similar coloration with hordenine, while soy bean extract—which contains catalase,[3] oxydase[4] and peroxydase,[5] but no tyrosinase[6]—gave negative reaction, as shown in the next experiment.

In addition to the above fact, the similarity of the constitutional formula of tyrosine and hordenine gives weight to this reasoning. Soy bean gave no color reaction, though oxydase and peroxydase were present in it, the result being as follows:—

With the soy bean extract:—

| No. of experiment / Mixed with | (1) | (2) | (3) | (4) | (5) |
|---|---|---|---|---|---|
| Enzyme solution (c. c.) | 20 | 20 (Boiled) | 20 | 20 | 20 |
| 3% solution of hordenine sulphate (c. c.) | 50 | 50 | 50 | 0 | 0 |
| 1.5% hydrogen peroxide (c. c.) | 20 | 20 | 0 | 20 | 0 |
| Distilled water (c. c.) | 0 | 0 | 20 | 50 | 70 |
| Toluol (c. c.) | 2 | 2 | 2 | 2 | 2 |

| No. of experiment / Observation period | (1) | (2) | (3) | (4) | (5) |
|---|---|---|---|---|---|
| Initial | No color | No color | No color | No color | No color |
| After ½ hour | ” | ” | ” | ” | ” |
| After 24 hours | ” | ” | ” | ” | ” |

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1-6) Y. Hashitani; Jour. Soc. of Agric. & Forest., Sapporo, 1921, **13**, 32.

From these experiments it may be concluded that hordenine is easily attacked by tyrosinase but not by catalase, oxydase and peroxidase of barley, malt and malt-rootlets.

## IV. Effect of Hordenine upon the Growth of Micro-organisms and the Products resulting from its Decomposition by their Actions.

### Experiment A.

Hordenine and its derivatives were mixed with wort-gelatine at the rate of 0.2, 0.5 and 1.3 per cent. It was heated while being stirred and well mixed, then the reaction of each medium with litmus paper was found as follows:—

| | | |
|---|---|---|
| Medium without hordenine as control | | weakly acid |
| Medium with free hordenine | 0.2 % | weakly alkaline |
| | 0.5 „ | alkaline |
| | 1.3 „ | strongly alkaline |
| Medium with hordenine ethyliodide | 0.2 % | weakly acid |
| | 0.5 „ | „ |
| | 1.3 „ | „ |
| Medium with hordenine acid tartrate | 0.2 % | acid |
| | 0.5 „ | „ |
| | 1.3 „ | strongly acid |
| Medium with hordenine sulphate | 0.2 % | weakly acid |
| | 0.5 „ | „ |
| | 1.3 „ | „ |

5 c.c. of the solid nutritive substrata thus obtained was put in a test tube and sterilized in Koch's apparatus. Micro-organisms were inoculated, such as Torula, Bacteria, Oidium, Cladosporium, Rhizopus, Penicillium, Chlamydomucor and Alternaria, etc. They were isolated by Dr. K. Yagi from malt, barley, and beer and placed at the author's disposal. The condition of growth of these micro-organisms in the test tube (with the exception of bacterial growth) at room temperature (in summer) was carefully observed for 18 days. The result is shown in the following table:—

| The kind of micro-organisms | Days after inoculation | Control | Free Hordenine | | | Hordenine ethyl iodide | | | Hordenine acid tartrate | | | Hordenine sulphate | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.2% | 0.5% | 1.3% | 0.2% | 0.5% | 1.3% | 0.2% | 0.5% | 1.3% | 0.2% | 0.5% | 1.3% |
| Torula | 2 | − | − | − | − | − | − | − | − | − | + | − | − | ++ |
| | 3 | + | + | + | − | + | + | + | + | + | ++ | + | + | ++ |
| | 4 | + | + | + | − | ++ | + | ++ | + | + | +++ | + | + | +++ |
| | 9 | + | + | + | − | +++ | + | +++ | + | + | +++ | + | + | +++ |
| | 12 | ++ | ++ | + | − | +++ | +++ | +++ | ++ | ++ | +++ | + | + | +++ |
| | 18 | +++ | +++ | + | − | +++ | +++ | +++ | ++ | ++ | +++ | ++ | ++ | +++ |
| Saccharomyces cerevisiae | 2 | + | − | − | − | + | − | − | + | + | + | + | + | + |
| | 3 | + | + | + | − | + | + | + | + | + | + | + | + | + |
| | 4 | + | +++ | + | − | ++ | + | + | + | + | +++ | + | + | +++ |
| | 9 | + | +++ | + | − | +++ | + | + | + | + | +++ | + | ++ | +++ |
| | 12 | + | +++ | + | − | +++ | + | + | + | + | +++ | + | ++ | +++ |
| | 18 | ++ | +++ | + | − | ∞ | ++ | + | + | ++ | +++ | + | ++ | +++ |
| Oidium | 2 | + | + | + | − | + | + | + | + | + | + | + | + | + |
| | 3 | ++ | ++ | ++ | − | ++ | ++ | ++ | ++ | ++ | +++ | ++ | ++ | ++ |
| | 4 | ++ | +++ | ++ | + | ++ | ++ | ++ | ++ | ++ | +++ | ++ | ++ | ++ |
| | 9 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 12 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 18 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Cladosporium | 2 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| | 3 | + | + | + | − | + | + | + | + | + | + | + | + | + |
| | 4 | + | +++ | +++ | − | ++ | + | + | + | + | + | + | + | + |
| | 9 | +++ | +++ | +++ | − | +++ | ++ | + | +++ | +++ | +++ | +++ | +++ | + |
| | 12 | +++ | +++ | +++ | − | +++ | +++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 18 | ∞ | ∞ | ∞ | − | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |

| The kind of micro-organisms | Days after inoculation | Con-trol | Free Horde-nine | | | Hordenine ethyl iodide | | | Hordenine acid tartrate | | | Hordenine sulphate | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.2% | 0.5% | 1.3% | 0.2% | 0.5% | 1.3% | 0.2% | 0.5% | 1.3% | 0.2% | 0.5% | 1.3% |
| Rhizopus (nigricans) | 2 | + | + | + | − | + | + | + | + | + | + | + | + | + |
| | 3 | ++ | ++ | ++ | − | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | 4 | +++ | +++ | +++ | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 9 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 12 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 18 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Penicillium glaucum | 2 | + | + | + | − | + | + | + | + | + | + | + | + | + |
| | 3 | ++ | ++ | ++ | − | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | 4 | +++ | +++ | +++ | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 9 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 12 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 18 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Alternaria | 2 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| | 3 | + | + | − | − | − | − | + | − | + | + | + | + | + |
| | 4 | +++ | +++ | +++ | − | − | − | + | + | + | + | ++ | ++ | ++ |
| | 9 | +++ | +++ | +++ | − | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 12 | +++ | +++ | +++ | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 18 | ∞ | ∞ | ∞ | ++ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Chlamydomucor | 2 | + | + | + | − | + | + | + | + | + | − | + | + | + |
| | 3 | ++ | ++ | ++ | − | ++ | ++ | ++ | ++ | ++ | + | ++ | ++ | ++ |
| | 4 | +++ | +++ | +++ | − | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 9 | +++ | +++ | +++ | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 12 | +++ | +++ | +++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | 18 | ∞ | ∞ | ∞ | +++ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |

| The kind of Micro-organisms | Days after Inoculation | Control | Free hordenine | | | Hordenine ethyl iodide | | | Hordenine acid tartrate | | | Hordenine sulphate | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.2% | 0.5% | 1.3% | 0.2% | 0.5% | 1.3% | 0.2% | 0.5% | 1.3% | 0.2% | 0.5% | 1.3% |
| Bacteria | 2 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| | 3 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| | 4 | + | − | + | − | − | − | − | − | − | − | − | − | + |
| | 9 | ++ | − | + | − | − | − | − | − | − | − | − | − | ++ |
| | 12 | +<br>++ | − | ++ | − | + | − | − | − | − | − | − | − | +<br>++ |
| | 18 | +<br>++ | − | ++ | − | + | − | − | − | − | − | − | − | +<br>++ |

From the above table one sees that the effect of hordenine and its derivatives upon these micro-organisms differed according to kind; that in all cases free hordenine acted as a toxic agent at a concentration of 1.3 per cent, while in some cases it completely checked the growth of micro-organisms even at this concentration. As a rule, the influence of hordenine derivatives upon the growth of micro-organisms was weak, except in the case of bacteria. The bacteria developed at concentrations of 0.5, 0.2 and 1.3 per cent of hordenine, ethyliodide and sulphate respectively. This may be due to their alkalinity or acidity as well as to their concentration.

### Experiment B.

According to E. Ehrlich[1], hordenine is decomposed by some kinds of yeast and fungi by the following reactions:—

$$HO \cdot C_6H_4 \cdot CH_2CH_2N(CH_3)_2 + H_2O = HO \cdot C_6H_4 \cdot CH_2CH_2OH + NH(CH_3)_2$$

$$NH\,(CH_3)_2 + 2H_2O = NH_3 + 2CH_3OH$$

Hence I considered it of practical importance to determine experimentally if it could be utilized as a nitrogen source for micro-organisms. For this purpose, the following nutrient solution containing no nitrogenous compound except hordenine was put in a flask provided with cotton-wool filter and after sterilizing, different micro-organisms as mentioned hereafter were inoculated.

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[1] E. Ehrlich; Ber. d. Deutsch. Chem. Ges., 1911, **44**, 139; Biochm. Zeitsch., 1916, **75**, 417.

The nutrient solution :—

| | |
|---|---|
| Natural Water | 500 c.c. |
| Hordenine sulphate | 5.0 g. |
| Cane sugar | 20.0 g. |
| $KH_2PO_4$ | 1.0 g. |
| $MgSO_4$ | 0.5 g. |
| NaCl | 1.0 g. |
| $Fe_2Cl_6$ | Trace |

The micro-organisms grew well and the fungi formed a compact mass of hyphae on the surface of the liquid. After 3 months all of the micro-organisms except bacteria which grew in the nutritive medium were separated by filtration and then weighed, all the experiments being made at room temperature (18–20°C.).

The mother liquor thus separated was neutralized with acid sodium carbonate and then distilled in a vacuum on a water bath, the distillate being collected in a flask which was cooled with a freezing mixture.

The distillate here obtained possessed an alkaline reaction and a very characteristic odor of amines. It gave Nessler's reaction distinctly.

The residue in the flask was acidified with dilute sulphuric acid and was then extracted with ether. The ethereal solution was evaporated till only a little syrup was left which gradually solidified. After decolorization with animal charcoal it was purified by recrystallization from alcohol, giving white needle crystals.

The crystals showed the following properties :—

a) They were readily soluble in water, alcohol, and ether.
b) They gave a blue coloration with ferric chloride.
c) They gave Millon's reaction.
d) They tasted bitter.
e) They produced white needle crystals of Dibenzoyl-tyrosol by the Schotten-Baumann reaction, insoluble in water but soluble in hot alcohol, and melting at 111°C.

The properties mentioned above indicate that the crystals in hand are surely tyrosol.

When tyrosol was not isolated in a pure state from ether extract on account of its small quantity, only the color reactions for tyrosol were examined.

The results of the above experiments are summarized in the following table :—

| | | Decomposition products of hordenine. | |
|---|---|---|---|
| | Yield of the micro-organisms (g.) | Ammonia or amines | Tyrosol (Yield g.) |
| Rhizopus | 3.3650 | + | 0.04 |
| Cladosporium | 2.9170 | + | 0.10 |
| Penicillium | 0.2088 | + | 0.06 |
| Oidium | 0.0760 | + | ? |
| Mucor | 0.1596 | + | ? |
| Torula | 0.2126 | + | + |
| Beer yeast | 0.1758 | + | ? |
| Bacteria | —— | + | + |

From these experiments it is evident that hordenine can be decomposed by these micro-organisms.

## V. Influence of Hordenine and some of its Derivatives upon Alcoholic Fermentation.

In the present investigation, the author found that the alcoholic extract of malt-rootlets was toxic as well as stimulative upon the fermentation, according to its concentration. This is especially the case with the extract by strong alcohol. The experiments were made in the following manner:—

### Experiment A.

The syrup obtained from malt-rootlets with dilute alcohol was separated into two parts according to its solubility with 95 per cent alcohol. The part soluble in 95 per cent alcohol is designated as syrup (A), the insoluble portion as syrup (B). Both gave positive reactions with Fehling's solution. The syrups were dissolved in distilled water and to each solution some yeast extract, free from sugars, was added. It was then pasteurized in Koch's apparatus for an hour, cooled, and a definite amount of beer yeast was added and made to ferment at 25°C. in an Einhorn's fermentation tube. The result was as follows:—

| Kind of solution | Glucose (2 %) | Syrup B (2 %) | Syrup A (2 %) | Syrup A+ Glucose (2%) (Same volumes) | Syrup A+ Syrup B (Same volumes) |
|---|---|---|---|---|---|
| Volume of $CO_2$ gas produced by fermentation (c.c.) | 1.9 | 4.3 | (−) | 0.7 | (−) |
| Hours required for fermentation | 144 | 144 | (−) | 240 | (−) |

The above experiment shows that some toxic agents for fermentation are contained in the syrup A.

### Experiment B.

To determine the influence of syrup A upon fermentation the following experiment was carried out. To the same amount of syrup B, various quantities of solution of syrup A (2 grams of syrup in 100 c.c. water) were added.

The rest of the treatment was the same as in the first experiment.

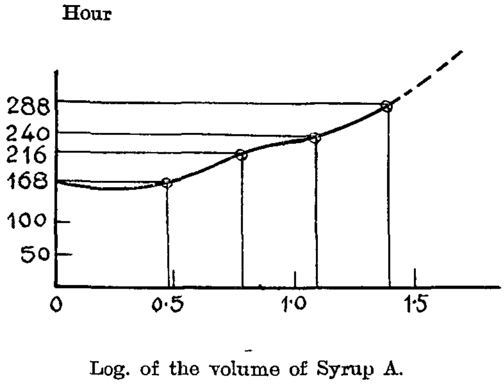
| No. of the tube | (1) | (2) | (3) | (4) | (5) |
|---|---|---|---|---|---|
| Water (c.c.) | 25 | 22 | 19 | 13 | 1 |
| Syrup A (c.c.) | 0 | 3 | 6 | 12 | 24 |
| Yeast extract (c.c.) | 5 | 5 | 5 | 5 | 5 |
| Syrup B (g.) | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |

| | | | | | |
|---|---|---|---|---|---|
| Hours required for fermentation | 168 | 168 | 216 | 240 | 288 |
| Volume of the $CO_2$ produced (c.c.) | 0.2 | 0.4 | 0.5 | 1.0 | 0.6 |

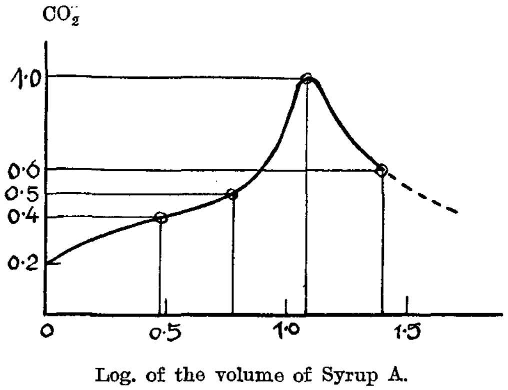
The results may be shown in the following graph, where in Fig. I. 1 the number of hours was put on the ordinate and the Log. of the volume of syrup A on the abscissa, while in Fig. I. 2 the volume of carbon dioxide was put on the ordinate and the same Log. was put on the abscissa.

Fig. I.

1



2



It is clear from the above curves, that Fig. I. 1 represents the growth of yeast decreasing in inverse ratio to the quantity of the syrup A, while Fig. I. 2 shows the action of the syrup A, as a stimulating as well as a toxic substance according to its concentration.

Judging from the good solubility in strong alcohol and the bitter taste which are common to both hordenine and syrup A, the toxic action of the syrup A must be due to the hordenine in it.

The isolation of hordenine by Léger's method from the syrup A, was then tried and crystals of hordenine amounting to 1.94 per cent of syrup were obtained. Calculating on this basis, the tubes in the above experiment contained the following amounts of hordenine:—

| | | | | | |
|---|---|---|---|---|---|
| No. of Einhorn's tube | (1) | (2) | (3) | (4) | (5) |
| Per cent of hordenine | 0 | 0.00388 | 0.00776 | 0.01552 | 0.03104 |

### Experiment C.

The influence of hordenine and some of its derivatives upon the fermentation was then examined in the same way as in the two previous experiments. The solution for fermentation was mixed with various amounts of hordenine or its derivatives and allowed to ferment in Einhorn's fermentation tube at 27°C. The concentration of the hydrogen ions in the culture media was determined by the colorimetric method of Clark and Lubs.1)

### CASE I.

For Hordenine

| No. of the tube | (1) | (2) | (3) | (4) | (5) | (6) |
|---|---|---|---|---|---|---|
| Wort (Ca. 2% extract contained) (c.c.) | 30 | 30 | 30 | 30 | 30 | 30 |
| Hordenine % | 0 | 0.004 | 0.02 | 0.10 | 0.20 | 0.50 |
| Emulsion of beer yeast (c.c.) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| PH value | 5.6 | 5.8 | 6.7 | 7.6 | 8.4 | 9.2 |

Carbon dioxide which was produced by fermentation.

| No. of the tube / Hours after yeast inoculation | (1) | (2) | (3) | (4) | (5) | (6) |
|---|---|---|---|---|---|---|
| 20 hours | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 „ | Trace | 0.5 | 0.2 | 0 | 0 | 0 |
| 44 „ | 3.4 | 8.8 | 1.7 | 0 | 0 | 0 |
| 51 „ | 6.9 | 13.2 | 3.1 | 0 | 0 | 0 |
| 68 „ | 12.9 | 16.8 | 6.7 | Trace | 0 | 0 |
| 75 „ | 15.3 | 17.3 | 8.7 | „ | 0 | 0 |
| $CO_2$ ratio at 75 hrs. | 1.00 | 1.13 | 0.57 | „ | 0 | 0 |

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1) Clark and Lubs; H. A. J. Bact., 1917, 1-34, 109-136.

## CASE II.

For Hordenine sulphate

| No. of the tube | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
|---|---|---|---|---|---|---|---|---|
| Wort (Ca. 2% extract contained) (c.c.) | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| Hordenine sulphate % | 0 | 0.004 | 0.02 | 0.10 | 0.20 | 0.50 | 1.25 | 2.50 |
| Emulsion of beer yeast (c.c.) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| PH value | 5.7 | 5.7 | 5.8 | 5.7 | 5.7 | 5.6 | 5.6 | 5.6 |

Carbon dioxide which was produced by fermentation.

| No. of the tube<br>Hours after yeast inoculation | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
|---|---|---|---|---|---|---|---|---|
| 20 hours | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 " | 0.2 | Trace | 0 | Trace | Trace | Trace | 0 | 0 |
| 44 " | 8.2 | 4.2 | 6.5 | 8.8 | 1.4 | 0.6 | 0 | 0 |
| 51 " | 11.0 | 8.0 | 9.5 | 13.0 | 2.8 | 1.0 | 0 | 0 |
| 68 " | 13.9 | 14.4 | 13.0 | 18.0 | 14.4 | 12.3 | 0 | 0 |
| 75 " | 14.2 | 14.7 | 15.5 | 18.0 | 15.8 | 14.0 | 0 | 0 |
| $CO_2$ ratio at 75 hours | 1.00 | 1.04 | 1.09 | 1.27 | 1.11 | 0.99 | 0 | 0 |

## CASE III.

For Hordenine methyl iodide

| No. of the tube | (1) | (2) | (3) | (4) | (5) | (6) | (7) |
|---|---|---|---|---|---|---|---|
| Wort (Ca. 2% extract contained) (c.c.) | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| Hordenine methyl iodide % | 0 | 0.004 | 0.02 | 0.10 | 0.20 | 0.50 | 1.25 |
| Emulsion of beer yeast (c.c.) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| PH value | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 |

Carbon dioxide which was produced by fermentation.

| No. of the tube<br>Hours after yeast inoculation | (1) | (2) | (3) | (4) | (5) | (6) | (7) |
|---|---|---|---|---|---|---|---|
| 20 hours | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 „ | 0.2 | 0.14 | 0.1 | 0.2 | Trace | 0 | 0 |
| 44 „ | 7.5 | 11.6 | 8.1 | 10.1 | 6.8 | 7.4 | 0 |
| 51 „ | 9.5 | 12.7 | 10.1 | 11.7 | 9.8 | 10.0 | 0 |
| 68 „ | 11.6 | 13.5 | 12.9 | 12.5 | 11.2 | 12.0 | 0 |
| 75 „ | 12.6 | 13.7 | 13.6 | 12.9 | 11.9 | 12.0 | 0 |
| $CO_2$ ratio at 75 hours | 1.00 | 1.09 | 1.08 | 1.02 | 0.94 | 0.95 | 0 |

## CASE IV.

For Hordenine trimethyl sulphate

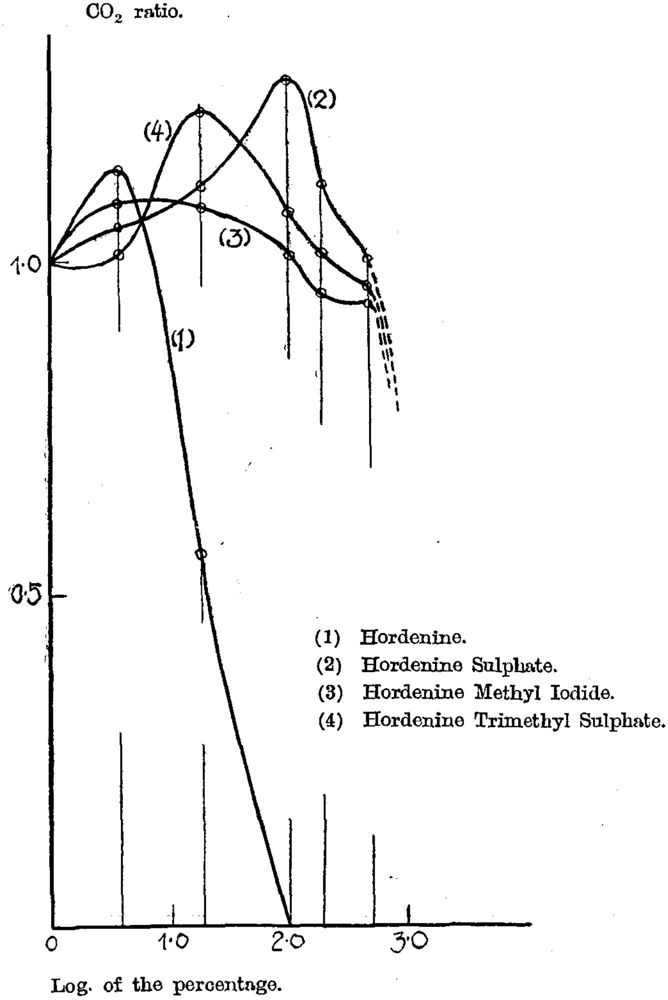
| No. of the tube | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
|---|---|---|---|---|---|---|---|---|
| Wort (Ca. 2% extract contained) (c.c.) | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| Hordenine trimethyl % sulphate | 0 | 0.004 | 0.02 | 0.10 | 0.50 | 1.25 | 2.50 | 5.0 |
| Emulsion of beer yeast (c. c.) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |

Carbon dioxide which was produced by fermentation.

| No. of the tube<br>Hours after yeast inoculation | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
|---|---|---|---|---|---|---|---|---|
| 20 hours | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 „ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44 „ | 7.0 | 5.3 | 9.3 | 7.5 | 5.1 | 0 | 0 | 0 |
| 51 „ | 9.2 | 9.0 | 12.3 | 9.8 | 7.1 | 0 | 0 | 0 |
| 68 „ | 9.7 | 9.3 | 12.6 | 10.4 | 7.7 | 0 | 0 | 0 |
| 75 „ | 11.0 | 10.9 | 13.4 | 11.8 | 9.4 | 0 | 0 | 0 |
| $CO_2$ ratio at 75 hours | 1.00 | 0.99 | 1.22 | 1.07 | 0.85 | 0 | 0 | 0 |

From the above tables, the following relations can be observed :—In cases II and III, the concentration of hordenine derivatives in the culture media was gradually increased but $P_H$ value was almost constant. Hence it may be said that in these cases alcoholic fermentation was influenced only by the concentration of hordenine derivatives, while in case I, $P_H$ value increased (becoming alkaline reaction) with the increase of hordenine concentration, so the influence upon fermentation is due to both the action of the $P_H$ value and the concentration of hordenine.

Fig. II.



In case I, when the $P_H$ value was 6.7, the percentage of hordenine was 0.02, in which concentration the fermentation was depressed rapidly, while in cases II, III and IV, with the same concentration of hordenine derivatives, fermentation was still accelerated.

From the above experimental results, a graph is traced in which the ratio of the volume of carbon dioxide is put on the ordinate and the Log. of the percentage of hordenine as well as its derivatives, on the abscissa.

From these three experiments the following conclusion may be derived:—

1. The action of hordenine and its derivatives on alcoholic fermentation resembles that of the alcoholic extract of malt-rootlets.
2. The substances used for the said experiments act as a stimulating as well as a toxic agent upon the alcoholic fermentation according to their concentration.
3. Free hordenine is the most poisonous of all four substances studied. It checked completely the alcoholic fermentation in 0.2 per cent concentration, while the other three substances were not toxic even over 0.5 per cent.
4. It was shown that the toxic action of free hordenine was intensified by its alkalinity.

Remarks.

Einhorn's fermentation tube was made use of in the present study, but strictly considered, it was probably not perfect, because it was impossible to determine the total amount of carbon dioxide produced in the tube, and the result, therefore, varied according to the size, length and angle of curvature of the tube. The volume of the carbon dioxide evolved by fermentation was changeable according to the physiological state of the yeast, its previous treatment, the condition of fermention medium, etc.

It is satisfactory to note, however, that even with the possible imperfection of the tube, this study enabled me to observe the comparative relation of those influences upon fermentation.

## VI. Physiological Effects of Hordenine and some of its Derivatives on Animals.

According to L. Camus[1] and J. Sabrazés and G. Guérive[2] hordenine

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1) L. Camus; Compt. rend., 1906, **142**, 110.

2) J. Sabrazés & G. Guérive; Compt. rend., 1908, **147**, 1076.

sulphate has the characteristic action (similar to those generally possessed by derivatives of *p*-hydroxy phenylethylamine, as, for example, adrenaline.) of producing contraction of blood vessels and a rise in blood pressure, although the action of hordenine sulphate is not so intense, being less active than digitalis and of weak toxicity. The fatal dose by intravenous injection for a dog is 0.3 gram and for a rabbit 0.25 gram, per Kg., of body weight. By subcutaneous injection, it is 2 grams for a dog and 1 gram for a rat per Kg. of the body weight. The poisonous effect appears first on the nervous system, followed by erethism and a fit of madness, the subject of the experiment finally making a dash. After a little while the respiratory center begins to be affected, causing difficulty of breathing, the subject is next overcome by suffocation lapsing to apparent death, though the heart continues to beat for a while. It then dies.

The author examined some of the physiological effects of hordenine and its derivatives upon frogs, guinea-pigs and rabbits, the results being described in the following:—

## 1. EFFECTS ON FROGS.

### a. When hordenine sulphate was used.

Repeated experiments by subcutaneous injection of 5 per cent aqueous solution of hordenine sulphate on frogs (body weight generally 20 grams) showed that with 0.1 c.c. no effects appeared, while with 0.2 c.c. the subjects lapsed into a state of apparent death after 40 minutes (some being restored to normal conditions after 4 hours), but with 0.3 c.c., 0.5 c.c., 1.0 c.c. and 2.0 c.c. they lapsed into apparent death after 40 minutes, 25 minutes, 20–25 minutes, and 12 minutes, respectively, and were never restored to normal again.

A case is given below:—

An injection of 0.2 c.c. was made on a frog (♂) weighing 23.8 grams. After 10 minutes its eyelids quivered and after 20 minutes it closed its eyes in a state of paralysis. After 25 minutes it had no power to reverse itself when put on its back. After 30 minutes it made convulsive movements, and after 35 minutes no reflex movement responded to stimulus, though heart beating was continued, for 1 hour when it ceased altogether. In short, the frog was killed by hordenine sulphate of 0.3 c.c., or over 0.075 grams per 100 grams of its body weight.

### b. When hydrobromic salt of hordenine bromide was used.

The subcutaneous injection of this substance made a frog extremely sensitive, even a slight shock subjecting it to alarm. It then croaked and struggled to jump. When more than 0.040 gram per 100 grams of body weight was injected, it stretched its limbs, became stiff and died in a few minutes. A few cases are given below:—

Case I. A frog (♂), 13.45 grams in body weight, received a subcutaneous injection of 0.1 c.c. of 1.7 per cent aqueous solution of the substance, but no reaction was observed.

Case II. A frog (♂) weighing 13.5 grams was injected with 0.15 c.c. of 1.7 per cent aqueous solution of the substance. It became extremely sensitive and showed signs of distress. After 6 minutes it was reduced to a state of apparent death, and became stiff with its limbs stretched. It was restored to normal conditions the next morning.

Case III. When injected with 0.5 c.c. of the same solution, a frog (♀) weighing 22.25 grams became sensitive. After 6 minutes it stretched its limbs in a state of apparent death and at last died, becoming stark.

### c. When acetyl hordenine was used.

Acetyl hordenine is an oily substance neutral to litmus paper. The effect of subcutaneous injection of this substance is tabulated below. The injection caused local paralysis and loss of reflex movement to stimulus which gradually extended all over the body. No frogs subjected to this injection regained their vitality.

| Body weight (g.) | 23.55 (♀) | 18.15(♀) | 22.05(♀) | 23.35 (♀) | 17.45(♀) |
|---|---|---|---|---|---|
| Acetyl hordenine (g.) | 0.0089 | 0.0178 | 0.0178 | 0.0267 | 0.0267 |
| Time till it lost reflex movement (m.) | 65 | 48 | 75 | 55 | 30 |

| Body weight (g.) | 19.55 (♀) | 23.95 (♀) | 24.05 (♂) | 20.15 (♂) |
|---|---|---|---|---|
| Acetyl hordenine (g.) | 0.0356 | 0.0356 | 0.0445 | 0.0445 |
| Time till it lost reflex movement (m.) | 35 | 35 | 30 | 30 |

## 2. Effects on Guinea-pigs.

### a. When hordenine sulphate was used.

A few moments after the subcutaneous injection of 10 per cent aqueous solution at the groin, a guinea-pig slightly moved its head and body spasmodically, showed signs of distress, and then became active in movements. At length it grew mad and made a dash, in the meantime breathing hard. The mucous membrane of the nostrils, mouth and inside of the eyes became markedly anæmic and turned pale. Then it lay down suddenly lapsed, and entered slowly into a state of apparent death. When the injected substance did not amount to a fatal quantity, the subject was restored to normal conditions after a few hours. The fatal dose was 0.8 gram per Kg. of body weight, but a larger quantity was necessary for those guinea-pigs repeatedly treated with a non-fatal dose. After injection the temperature of the body rose for a time and then fell to below normal, but was soon restored. Below are given a few cases from among the many experiments carried out.

Case I. Fifteen minutes after subcutaneous injection of 3 c.c. of 10 per cent solution on a guinea-pig (♂), weighing 589.3 grams it became active, moved its head in slight spasms, with faster and deeper breathing, all the time dashing about, tumbling over and showing signs of distress. Then its movements gradually relaxed and it was restored to normal conditions after 2 hours, regaining its appetite.

Changes in the body temperature were as follows :—

| | | |
|---|---|---|
| Before injection | | 38°.3 C. |
| After injection | 15 m. | 38°.5 „ |
| „ „ | 30 „ | 38°.9 „ |
| „ „ | 1 h. | 38°.0 „ |
| „ „ | 1.5 „ | 37°.5 „ |
| „ „ | 2.0 „ | 36°.5 „ |
| „ „ | 2.5 „ | 36°.7 „ |
| „ „ | 3.0 „ | 37°.3 „ |
| „ „ | 3.5 „ | 38°.3 „ |
| „ „ | 4.0 „ | 38°.6 „ |

Case II. A guinea-pig (♂) weighing 520 grams. The subcutaneous injection of 4 c.c. of 10 per cent solution made the subject wild, followed by hard breathing. After 20 minutes it was suddenly suffocated, then overcome by syncope. For more than ten minutes its heart continued to beat; finally it died. Part of the hordenine sulphate that entered its body was found in its urine after about an hour. Its urine in health was negative in both Millon's reaction and in color reaction to ferric chloride, but after the injection its Millon's reaction became marked.

Case III. From 2 to 3 c.c. of 10 per cent hordenine sulphate was injected into a guinea-pig (♂) 560 grams in weight once every 24 hours, till a total amount of 10 c.c. was used. (the hordenine sulphate contained being 1.0 gram.)

Then the urine was collected in a vessel sterilized by toluol till no trace of the hordenine was found in it. 300 c.c. of the urine thus obtained was acidified by sulphuric acid, evaporated on a water bath, and decolorized by animal charcoal. The separation of hordenine was next effected by the process already mentioned and as a result 0.123 gram was obtained in the form of hordenine sulphate, this being approximately 1/10 of the quantity injected. In other cases where 1.4 grams were injected, the amount obtained by separation was 0.35 gram or 2.5/10 of the quantity injected.

A similar experiment was carried out with the droppings with no trace of hordenine found.

### *b.* When acetyl hordenine was injected.

Case I. To a guinea-pig (♂) weighing 543 grams, acetyl hordenine of 0.109 or 0.20 gram per Kg. of body weight was injected subcutaneously. It made the animal somewhat drowsy, but evidently free from pain, for it took food from time to time.

Case II. A guinea-pig (♂) weighing 510 grams was subjected to subcutaneous injection of 0.303 or 0.59 gram per Kg. of body weight. It was reduced to a drowsy state but evidently felt no pain. The next day when 0.605, or 1.19 grams per Kg. of body weight, were injected, it lost activity but no other considerable changes were observed except at the injected locals where the skin was more or less rotten.

Case III. A subcutaneous injection of 1.009, or 2.13 grams per Kg. of body weight, was made on a guinea-pig (♂) weighing 473 grams. The leg that received the injection lost sensitivity. After 20 minutes the animal's movements became active and its breathing more rapid.

The next day it regained its normal conditions. An injection was again effected of 1.15, or 3.19 grams per Kg. of body weight. After 25 minutes the animal became active and then grew out of spirits, the activity ceasing after 4 hours. The animal died after 5 hours. From the above it may be said that the poisonous effect of acetyl hordenine is weak as compared with hordenine sulphate.

### 3. EFFECTS ON RABBITS.

Case I. Into the vein of a rabbit (♀) weighing 1,710 grams was injected 4.25 c.c. of 10 per cent aqueous solution of hordenine sulphate. After 5 minutes it lay down, the pupils of its eyes contracting markedly while the mucous membranes became perceptibly anæmic. The auricles of the ears did not bleed when they were cut. Its distress appeared to lessen gradually and it recovered the normal conditions in 40 minutes. When it was injected again with 3.0 c.c. it was at once suffocated to a state of syncope, and later died.

Case II. A rabbit (♀) weighing 1,640 grams was subjected to intravenous injection of 4.1 c.c. of the above solution. After 2 minutes syncope supervened. The conditions were the same as in Case I.

The subjects in a state of apparent death as in Case I. and II. were dissected and upon examination it was found that the lungs were anæmic. Further, the auricles of the heart continued their activities, but the ventricles only intermittently. Between 1.5 and 2 hours afterwards the heart ceased to beat.

Case III. 4.3 c.c. of the same solution of hordenine sulphate was injected intravenously into a rabbit (♂) weighing 1,700 grams. As soon as it became syncopic (in 2 minutes) it was subjected to dissection. First, the blood circulation was stopped and next the internal organs were taken out and their water extracts were made. To the extracts was added some excess of Millon's reagent and the mixture was then filtered. The filtrates were heated and examined as to Millon's reaction. A rabbit in health was slaughtered and its organs were subjected to similar experiment as a control for the above.

The results of these examinations, as given below, show that the organs from the healthy rabbit are negative in reactions while those from the injected one present a markedly positive reaction, this being specially strong in those organs abounding in veins :—

| | Injected | Control |
|---|---|---|
| Heart | + + + | – |
| Lungs | + + | – |
| Kidneys | + + + | – |
| Spleen | + + + | Trace |
| Liver | + + + | – |
| Brain | + | – |

Case IV. A rabbit (♂) weighing 1,500 grams was subjected to subcutaneous injection of 10 per cent hordenine sulphate solution to the amount of 15 c.c. After 3 minutes the pupils of its eyes were contracted a little and the auricles of the ears did not bleed when cut. The mucous membranes became anæmic. After 5 minutes its breathing became hard and deep, it developed greater activity and moved its head and body slightly and spasmodically, all the time showing signs of pain. After 10 minutes the pupils were dilated somewhat, and the auricles of the ears began to bleed. After 15 minutes it fell down and was in apparent death. The body was then dissected, with the same result as in the case of intravenous injection.

Case V. At the groin a rabbit (♂), weighing 2,200 grams, was injected with 3 grams acetyl hordenine. The paralysis that started in the limb gradually extended to upper parts of the body and in 2 hours the whole body was affected. The animal lay still, but its breathing kept on for a few minutes further. Some difference was noticed in its breathing, etc., as compared with the results of injection of hordenine sulphate. The anæmia of mucous membranes and erethism was also less.

Judging from the above experiments, hordenine, one of the derivatives of tyramine, has a property common to all these derivatives, that of affecting the circulatory system, as stated by Camus and others. The action of hordenine differs more or less according to the kind of the derivatives used and the animals acted upon. This may be seen in the results obtained by injecting frogs and guinea-pigs with hordenine sulphate and bromine salt, and also in the case of acetyl hordenine. The poisonous effect of hordenine sulphate is weak, the fatal dose by subcutaneous injection for a frog is 0.075 gram per 100 grams of body weight, for a guinea-pig 0.8 gram per Kg. of body weight, and for a rabbit over 1.0 gram, while by intravenous injection it is over 0.25 gram. The effect appears on the nervous system, causing difficulty of breathing, suffocation, and death. The poisonous action of acetyl hordenine is weaker than that of hordenine sulphate and bromine salt.

Part of the hordenine that enters the body of animals is found unchanged in their urine.

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## SUMMARY.

1) The cell-wall of malt-rootlets consists chiefly of cellulose, and hemicellulose with some lignine. Glucose, fructose, xylose, and galactose (?) were detected in hydrolytic products of the cell-wall.

2) The sugars in malt-rootlets were glucose and fructose.

3) The ash of the rootlets consists chiefly of $P_2O_5$ and $K_2O$.

4) The nitrogenous substances consist of proteins which are largely soluble in NaOH (0.25%) and NaCl (10%), amino acids, purine bases, etc.

5) Among non-protein nitrogenous substances, asparagine, adenine, choline, betaine and hordenine, were isolated.

6) The derivatives of hordenine which have been prepared are as follows :—

Hordenine sulphate, Hordenine hydrochloride, Hordenine hydrobromide, Hordenine acid tartrate, Hordenine neutral tartrate, Hordenine methyl iodide, Hordenine ethyl iodide, Acetyl hordenine, Benzoyl hordenine, Benzoyl hordenine hydrobromide, Hordenine trimethyl sulphate, Hordenine bromide, Hydrobromic salt of hordenine bromide, Hordenine sulphonate.

7) It was confirmed that hordenine is widely distributed in the seedlings of Gramineæ.

8) Hordenine is easily attacked by tyrosinase of barley, malt and malt-rootlets, but not by urease, catalase, oxydase and peroxydase.

9) Hordenine in a free state, according to its concentration, acts as a toxic agent upon the growth of micro-organisms, with the sole exception of bacteria, which are slightly affected by its derivatives. If the concentrations of hordenine and its derivatives are not strong, then they can be utilized as a nitrogen source for micro-organisms.

10) Hordenine and its derivatives act as an agent, stimulating and toxic, upon alcoholic fermentation according to their concentration ;

free hordenine checked fermentation completely at 0.2 per cent and its derivatives at 1.25 per cent.

11) The physiological influence of the derivatives of hordenine varies according to their composition and the kind of animals used. Hordenine sulphate acts on the circulatory and nervous systems of animals but its toxicity is very weak, though it sometimes causes suffocation to death. A part of hordenine injected into the body of animals appeared unchanged in their urine. Acetyl hordenine is less poisonous than hordenine sulphate and bromine salt, but increases the paralysing power possessed by hordenine.

June, 1922.