THE BIOGENESIS OF ALKALOIDS

VI. THE FORMATION OF HORDENINE AND N-METHYLTYRAMINE FROM TYRAMINE IN BARLEY[1]

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ABSTRACT

Tyramine-α-C^{14}, synthesized from C^{14}-barium carbonate, was administered to sprouting barley and radioactive hordenine and N-methyltyramine isolated from the roots. Separation of these alkaloids by chromatography followed by degradation showed that all the activity was located in the α-carbon atom. The specific activity of the N-methyltyramine was about 10 times that of hordenine. From the results it is concluded that tyramine undergoes methylation in the barley root to N-methyltyramine and thence to hordenine.

It has been shown (13) that formate is utilized in barley roots for the production of the N-methyl groups of the alkaloid hordenine (N-dimethyltyramine). Preliminary experiments (13) have also indicated that although formate is also responsible for the formation of the methyl groups of choline, the latter is not involved in the methylation of hordenine. Quite recently Brown and Byerrum (3) have studied the synthesis of nicotine in *Nicotiana rustica* L., and their results seem to indicate that the role of formate consists in the production of the labile methyl group of methionine which subsequently undergoes transmethylation with nornicotine.

The isolation of N-methyltyramine from certain species of barley (12) provided evidence in favor of the assumption that the final step in the synthesis of hordenine by the plant was the N-methylation of tyramine. This assumption could be confirmed by feeding tyramine labelled with C^{14} to the plant and, after a suitable time, isolating the hordenine and determining whether it was labelled on the same carbon atom as in the administered tyramine.

Since tyramine and hordenine are chemically so similar it was essential to devise a method that would separate them completely in order to be sure that any activity found in the isolated hordenine was not due to a trace of the administered active tyramine. Paper chromatography was found to be an excellent method of distinguishing between these amines. Munier and Macheboeuf (19) have described the paper chromatography of various alkaloids, including hordenine. However chromatograms run with the solvents described by these authors failed to afford discrete separation of tyramine, hordenine, and N-methyltyramine. The last base was included in this test since it was found to occur with the hordenine in the roots of Charlottetown No. 80 barley which was the species under investigation. The best separation on untreated filter paper was achieved with *n*-butanol containing ammonia as the developing solvent. The pH of the solvent was found to influence the R_F values of the amines, and since filter paper buffered to a fixed pH had been useful in the

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separation of the solanaceous and ergot alkaloids (4), this technique was investigated. On carrying out a series of chromatograms on paper buffered at different pH values, varying degrees of separation were obtained. As was expected, with acidic paper all the amines had low R_F values while with strongly alkaline paper their R_F values all tended towards 1. The best separation of the amines was obtained with paper buffered at pH 8 and with *n*-butanol as the developing solvent. The alkaloids were detected by spraying the paper with Millon's reagent; as little as 5 μgm. could be detected. On a macro scale it was possible to effect separation by absorption chromatography on alumina, the tyramine being the most strongly absorbed and the hordenine least.

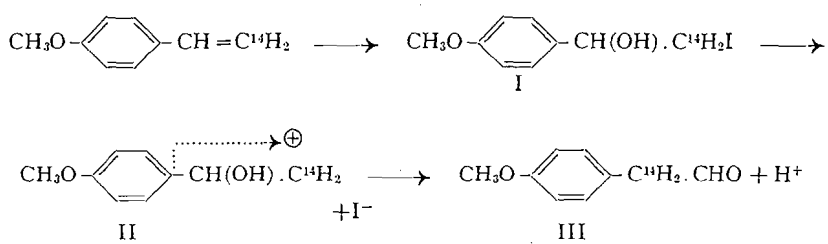
Barley was grown as previously described (13) and the roots harvested when according to Raoul (20) they contained a maximum amount of hordenine (11 days). On subjecting the crude alkaloids from the roots to chromatography it was found that an appreciable quantity of N-methyltyramine was present as well as the hordenine, there being about half as much N-methyltyramine as hordenine. Presumably previous workers have failed to isolate this alkaloid when hordenine was present because of the similarity of its properties to those of hordenine.

Tyramine-α-C^{14} was synthesized by a modification of the method of Koessler and Hanke (14). C^{14}-barium carbonate was converted to C^{14}-potassium cyanide (1, 6, 9) which was reacted with ethyl *p*-chloromethylphenyl carbonate (24) to give *p*-hydroxybenzylcyanide. This product was reduced with hydrogen over Raney nickel, in the presence of ammonia to suppress the formation of secondary amine (23), and the isolated tyramine was purified by sublimation and converted to the water-soluble hydrochloride which was used for feeding to the plant.

Feeding inactive tyramine hydrochloride to the plant had no visible effect on the growth of the barley or on the quantities of hordenine and N-methyltyramine isolated from the roots. On adding active tyramine hydrochloride to the barley on the sixth day of germination followed by isolation of the alkaloids on the 11th day, radioactive hordenine and N-methyltyramine were obtained; no tyramine was detected. Derivatives of the hordenine and N-methyltyramine retained the same specific activities as the parent alkaloids (Table II) showing that their activity was not due to any impurity. In order to determine the localization of the radioactivity, the hordenine was degraded by essentially the same methods used by Leger (16) in the original work on the constitution of hordenine. O-Acetylhordenine when oxidized with potassium permanganate gave rise to inactive *p*-acetoxybenzoic acid, indicating that the radioactivity resided either in the α-carbon atom or in the N-methyl groups or both. Hordenine methiodide was converted to the O-methyl ether and distillation of the corresponding quaternary hydroxide (Hofmann degradation) gave trimethylamine and *p*-vinylanisole. The trimethylamine was isolated as its platinichloride and was found to be inactive. The *p*-vinylanisole was not isolated as such owing to its lack of stability, but was immediately oxidized with yellow mercuric oxide and iodine (17) to homoanisaldehyde which was characterized as the oxime. This had the same specific activity as the original hordenine. All the foregoing

results seem to indicate that the activity resided entirely on the α-carbon atom of hordenine.

The active N-methyltyramine could not be oxidized to *p*-acetoxybenzoic acid since acetylation gave an ON-diacetyl derivative which was stable to oxidation. It was therefore converted to hordenine methiodide by refluxing with methyl iodide in the presence of sodium carbonate. After O-methylation the Hofmann degradation was carried out and it yielded trimethylamine which was converted to the platinichloride, and *p*-vinylanisole which was oxidized with yellow mercuric oxide and iodine. The product of the oxidation, homoanisaldehyde, was converted to the oxime and further oxidized with potassium permanganate. Rather surprisingly the anisic acid thus obtained was active and had the same specific activity as the original N-methyltyramine, apparently indicating that the activity in the N-methyltyramine was in the β-carbon atom adjacent to the benzene ring. However when a similar degradation was carried out on a sample of synthetic tyramine-α-C^{14}, active anisic acid was also obtained. Furthermore oxidation of the *p*-vinylanisole obtained from tyramine-α-C^{14} with potassium permanganate produced inactive anisic acid and active carbon dioxide. Thus, if it be assumed that no rearrangement takes place during the permanganate oxidation, it is obvious that a rearrangement had occurred during the conversion of *p*-vinylanisole to homoanisaldehyde oxime. This type of rearrangement was first observed by Tiffeneau (25) with substituted styrenes. The *p*-vinylanisole reacts with the mercuric oxide and iodine to give the iodohydrin (I) which by ionization of the iodine forms a carbonium ion (II) and this rearranges to homoanisaldehyde (III). The mechanism involving an intermediate π complex is discussed by Dewar (5). Hence, the radioactivity in both N-methyltyramine and



hordenine resides all in the α-carbon atom.

These results indicate that the tyramine has been utilized as such for the synthesis of N-methyltyramine and hordenine; the higher activity of the former is consistent with it being the intermediate in the formation of hordenine from tyramine. The roots from which the hordenine and N-methyltyramine had been extracted had a rather high activity suggesting that the hordenine might be converted to other substances which are utilized by the roots for their development. Its metabolism in the roots does not seem to be a general breakdown to simple carbon compounds containing one or two carbon atoms, for if this were the case one would expect these simple compounds to be utilized throughout the whole of the plant in other synthetic processes, whereas the leaves of the harvested barley were found to be completely inactive. Raoul (20) claimed that no

hordenine was present in the barley seeds. This has been confirmed and no trace of N-methyltyramine or tyramine was detected either. It seems highly likely that the source of tyramine in the roots is tyrosine since it is well established that this amino acid is converted by a variety of bacteria (4, 7, 8, 15, 18, 21) and in the kidney tissue of animals (10, 11, 22, 27) to tyramine. However Werle and Boden (26) were unable to detect an amino acid decarboxylase in yeast extracts. An investigation is at present proceeding of the metabolism of tyrosine in barley roots.

EXPERIMENTAL[1]

Paper Chromatography of Tyramine and its N-methyl Derivatives

The ascending method of paper chromatography (28) was used. Whatman No. 1 paper was cut into sheets 18 by 11 in., spots of the alkaloid solution were placed on the long edge about one inch from the bottom, 10 to 20 μgm. in a volatile solvent such as ethanol or methanol were in general used. The paper was stapled together at the shorter edge to give a cylinder 11 in. high, this was placed in a dish of the developing solvent in a closed container and left until the solvent had almost reached the top. The solvent front was marked and, after drying, the paper was sprayed with a solution of Millon's reagent (prepared by dissolving mercury (25 gm.) in concentrated nitric acid (25 cc.) and then diluting to 100 cc. with distilled water). The position of the alkaloids was shown by the formation of a red spot which slowly became brown and faded. The R_F values of these amines determined with a variety of solvents are shown in Table I.

TABLE I

R_F VALUES OF AMINES WITH VARIOUS SOLVENTS

Solvent mixture*	R_F value (at 25°)		
	Tyramine	N-Methyl-tyramine	Hordenine
Methylethyl ketone, water	0.074	0.053	Diffuse spot
Methylethyl ketone, 1% acetic acid	0.10	0.085	Diffuse spot
Ether, water	0.40**	0.48**	0.78**
n-Butanol, water	0.30	0.40	0.50
n-Butanol, 5% acetic	0.35	0.41	0.37
n-Butanol, 20% acetic	0.43	0.49	0.49
n-Butanol, 5*N* ammonia	0.74	0.85	0.95

**The organic solvent being in equilibrium with the second mentioned aqueous solution at 25°.*
***Elongated bands were produced and the maximum distance moved was measured to determine the R_F values.*

Buffered filter paper was obtained by dipping the paper in buffer solutions and then removing the surplus solution by pressing between blotting paper. The pH range 2.2 to 8.0 consisted of mixtures of 0.2 *M* disodium phosphate and 0.1 *M* citric acid; the range from 8.0 to 10.0, of mixtures of 0.1 *M* sodium hydroxide and 0.1 *M* boric acid. The paper was dried at room temperature. The results obtained with this buffered paper are shown graphically in Fig. 1,

[1] *All melting points are corrected.*

n-butanol saturated with water being the developing solvent. The most discrete separation was obtained with paper of pH 8, when the R_F values for tyramine, N-methyltyramine, and hordenine were 0.33, 0.44, and 0.83 respectively.

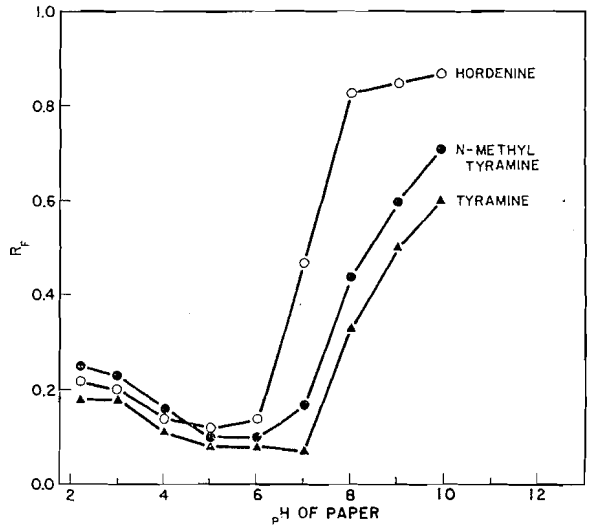


FIG. 1. The R_F values of hordenine, N-methyltyramine, and tyramine at various pH conditions.

Investigation of the Alkaloids from the Roots of Germinating Barley

Charlottetown No. 80 barley (720 gm.) was grown as previously described (13) and harvested on the 11th day of sprouting. The crude alkaloid fraction was obtained by an established method (12), consisting in extracting the dried roots with methanol, evaporating to dryness, dissolving the residue in 2*N* sulphuric acid, extracting this with ether to remove nonbasic products, alkalizing the sulphuric acid solution with ammonia, and extracting with ether. The alkaloids were extracted from this ether extract with sulphuric acid, the acid solution alkalized again with ammonia, and extracted with ether. The residue (0.554 gm.) left after the evaporation of this final ether extract was a brown semisolid mass which was dissolved in 10 cc. of methanol and an aliquot part containing about 50 μgm. was subjected to paper chromatography along with control samples of hordenine, N-methyltyramine, and tyramine. It was found that with a variety of developing solvents and with paper of different pH spots were obtained from this crude extract corresponding in position to hordenine and N-methyltyramine. From the density of the spots it was estimated that there was about twice as much hordenine as N-methyltyramine. No trace of tyramine was detected.

The methanol solution of the crude alkaloids was diluted with 200 cc. of benzene and chromatographed on alumina (activity 0-1). The composition of the eluates was determined by taking a few drops of the eluate and chromatographing it on paper along with controls. Hordenine was eluted with a 10% solution of methanol in benzene, the N-methyltyramine remaining on the

column, this was eluted by washing the column with pure methanol. Combination of the eluates yielded 0.216 gm. of hordenine and 0.080 gm. of N-methyltyramine (in a repeat experiment 0.241 gm. of hordenine and 0.097 gm. of N-methyltyramine were obtained from 720 gm. of barley). The identity of these fractions was confirmed by the preparation of hydrochlorides and picrates which were not depressed by admixture with the corresponding authentic derivatives of hordenine and N-methyltyramine.

Tyramine-α-C^{14}

Barium C^{14}-carbonate (12.087 mgm. with a total activity of 3.95×10^8 disintegrations per minute) was converted to hydrogen cyanide by fusing with sodium azide and then to potassium cyanide by absorption in potassium hydroxide as previously described (1, 6). The product was diluted with inactive potassium cyanide to give a total amount of 1.068 gm. ethyl *p*-chloromethylphenyl carbonate (24) (b.p. 112-5° at 0.8 mm., 3.522 gm.), was dissolved in ethanol (5 cc.), and added to the potassium cyanide dissolved in a minimum of water. The mixture was refluxed for two hours, the potassium chloride filtered off, and the filtrate evaporated to dryness *in vacuo*. The viscous residue was dissolved in ethanol (10 cc.) and refluxed with 2 cc. of a settled suspension of Raney nickel in ethanol for one hour. The filtered solution was concentrated *in vacuo* to give crude *p*-hydroxybenzylcyanide as a semisolid mass (in preliminary experiments with inactive cyanide it was obtained crystalline). The nitrile was dissolved in ethanol (3 cc.) and 3 cc. of a settled suspension of Raney nickel added, followed by liquid ammonia (3 cc.). The mixture was hydrogenated at 110° and 140 atmospheres in a high pressure autoclave for 15 hr. The product which was light brown in color was dissolved in ethanol, filtered, and then evaporated to dryness. The residue was dissolved in 2*N* hydrochloric acid (100 cc.) and extracted with ether, the aqueous layer was alkalized with ammonia and extracted with ether in a continuous extractor. The dried ether extract was evaporated to yield a viscous oil which was distilled at 120-130° at 10^{-3} mm. to yield tyramine-α-C^{14} as a white crystalline solid (0.677 gm., 30% yield from the potassium cyanide). A portion when crystallized from ethanol separated as colorless plates, m.p. 161.5-162.5° (Barger (2) reported m.p. 161°). The rest of the tyramine (0.521 gm.) was dissolved in ethanol and hydrochloric acid added to the solution when tyramine hydrochloride immediately separated out. It was crystallized from ethanol to yield colorless plates m.p. 273.5-275° (0.226 gm.). Found: C, 55.44; H, 6.63; N, 7.95; Cl, 20.85. Calcd. for $C_8H_{12}ONCl$: C, 55.34; H, 6.97; N, 8.07; Cl, 20.43%. The tyramine hydrochloride had a specific activity of $1.27 \pm .01 \times 10^5$ disintegrations per minute per mgm.[5] or $2.20 \pm 0.02 \times 10^7$ disintegrations per minute per millimole. By adding inactive tyramine hydrochloride to the mother liquor and concentrating the solution, further tyramine hydrochloride was obtained with a lower specific activity.

[5] *This activity and all subsequent ones were determined on thin samples with a Radiation Counters Laboratory "Nucleometer" making the usual corrections for self-absorption etc.*

Administration of Tyramine-α-C^{14} Hydrochloride to the Barley and Isolation of the Alkaloids

Barley (720 gm.) was grown as previously described and on the sixth day of sprouting radioactive tyramine hydrochloride (186.1 mgm. having a total activity of 2.36×10^7 disintegrations per minute and a specific activity of 2.20×10^7 disintegrations per minute per millimole) was fed to the barley in 600 cc. of distilled water. The addition of the tyramine did not affect the normal growth of the barley. On the 11th day of sprouting the roots were harvested and extraction as previously described (13) yielded the crude alkaloid fraction (0.491 gm.) with an activity of 2090 disintegrations per minute per mgm. The dried shoots were found to be completely inactive. The roots (74.5 gm.) which had been extracted with boiling methanol for 48 hr. had a residual activity of 106 disintegrations per minute per mgm. The crude alkaloid extract was dissolved in methanol (10 cc.) and several aliquot parts were chromatographed on paper of pH 8. Part of the paper was sprayed with Millon's reagent to detect the alkaloids. A photograph of the sprayed paper is shown in Fig. 2. The crosses along the line *A B* are the spots where the alkaloid solutions were initially placed, *C D* is the final solvent front. The developed chromatogram from another spot, not sprayed with Millon's reagent, was cut into strips 1 cm. wide. The strips were extracted with methanol and evaporated on to separate aluminum disks. The activity of these extracts (the actual counts observed above the background with the Nucleometer without any corrections) plotted against the distance of the strip from the initial spot of alkaloid solution is shown in Fig. 3.

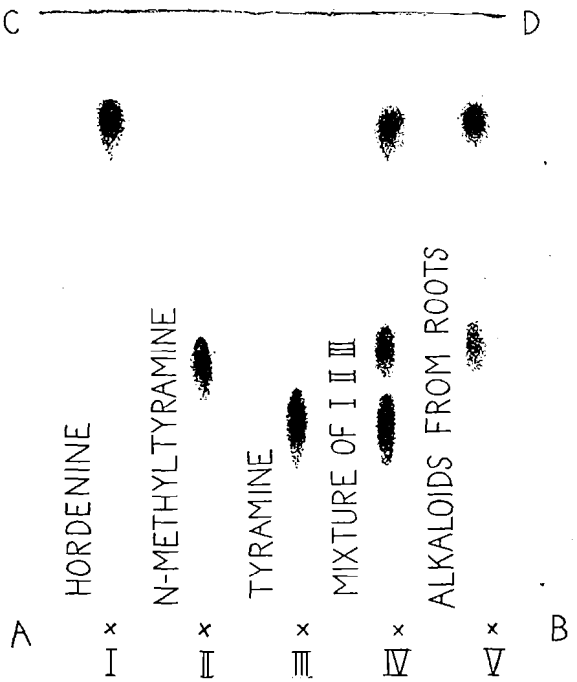


FIG. 2. The developed paper chromatogram.

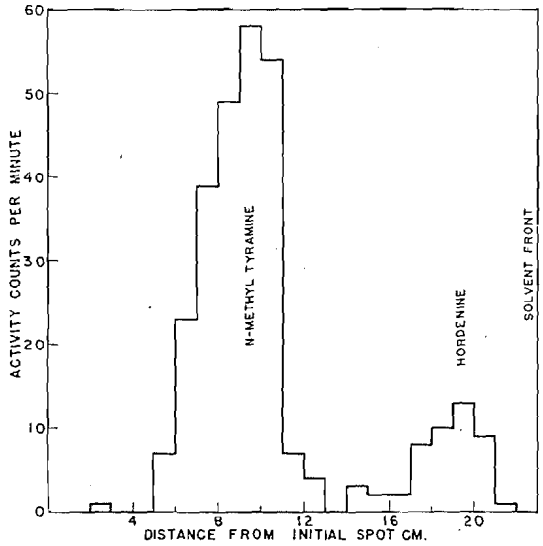


FIG. 3. Radioactivity of strips of the paper chromatogram.

It is seen that the positions of activity correspond to the positions of N-methyltyramine and hordenine as detected by Millon's reagent. After dilution of the methanol solution of the alkaloids with benzene it was chromatographed on alumina to yield 0.137 gm. of hordenine and 0.087 gm. of N-methyltyramine. The hordenine was diluted with 2.000 gm. of inactive hordenine and distilled at 130-140° at 10^{-3} mm. The crystalline distillate was crystallized from benzene–petroleum ether to give 1.8655 gm. By determining the activity of this diluted hordenine it was calculated that the hordenine isolated from the plant had an activity of 1.08×10^5 disintegrations per minute per millimole. In a similar way the N-methyltyramine was diluted with 1.865 gm. of inactive N-methyltyramine, distilled *in vacuo* 140-150° at 10^{-3} mm., and the distillate crystallized from anisole to yield 1.7147 gm. The activity of this diluted sample indicated that the N-methyltyramine from the plant had an activity of 1.04×10^6 disintegrations per minute per millimole. All subsequent derivatives and degradation products of these alkaloids were obtained from these diluted samples and their specific activities multiplied by the dilution factor.

Derivatives of the Active Hordenine

Picrate: small yellow prisms from aqueous ethanol, m.p. 140-140.5°.

Hydrochloride: colorless needles from ethanol, m.p. 179-180°.

Platinichloride: This was obtained by dissolving the hordenine hydrochloride in 5*N* hydrochloric acid and then adding an excess of chloroplatinic acid. The orange precipitate was crystallized from hydrochloric acid containing chloroplatinic acid; hordenine platinichloride separated as orange plates, m.p. 115-116°. Found: C, 31.92; H, 4.47; Pt, 26.9%. Calcd. for $(C_{10}H_{15}ON)_2.H_2PtCl_6$: C, 32.44; H, 4.35; Pt, 26.37%.

Methiodide: Active hordenine (1.300 gm.) was refluxed with methyl iodide (6 cc.) in methanol (25 cc.) for 15 min., and the solution evaporated to a small

bulk. The crystalline methiodide which separated was filtered and washed with ether–methanol, yield, 2.247 gm., m.p. 232-233°.

O-Methylhordenine methiodide: Hordenine methiodide (2.000 gm.) was dissolved in 10 cc. of a 10% aqueous solution of sodium hydroxide and dimethyl sulphate (1.6 cc.) was added with stirring. After stirring at room temperature for six hours, acetic acid (6 cc.) and sodium acetate (3.0 gm.) were added to the solution which was then concentrated to 5 cc. On cooling the O-methyl ether separated as colorless microscopic prisms (2.008 gm.) having no definite melting point.

Hofmann Degradation

The active O-methylhordenine methiodide (1.50 gm.) was dissolved in water (30 cc.) and silver hydroxide (obtained from 1.0 gm. of silver nitrate and sodium hydroxide) was added. After stirring for five hours at room temperature in the dark, the mixture was filtered to remove the silver iodide and the filtrate was introduced into a sublimation bulb. The solution was evaporated almost to dryness under reduced pressure and the sublimation bulb was then connected to a high vacuum system through two traps, the first cooled to 0° in ice and the second to −70° in a mixture of dry ice and acetone. The bulb was heated in an air bath to 120-130° when the *p*-vinylanisole distilled; most of it condensed in the first trap, while the second trap collected the trimethylamine. The contents of the traps were treated with dilute hydrochloric acid and the spherical droplets of *p*-vinylanisole extracted with ether (20-30 cc.).

The hydrochloric acid solution was evaporated to dryness and the white crystalline residue dissolved in ethanol, filtered, and added to an excess of chloroplatinic acid in ethanol. The pale orange precipitate was filtered (0.198 gm.) and crystallized from aqueous ethanol. Trimethylamine platinichloride separated as orange prisms, m.p. 231° (dec.).

The ether solution of *p*-vinylanisole was stirred with yellow mercuric oxide (1.5 gm.) and iodine (1.5 gm.) added. After two hours the solution was filtered and the brown filtrate was shaken with aqueous sodium thiosulphate to remove excess iodine. The pale yellow ether solution containing homoanisaldehyde was shaken with a solution of sodium bisulphite (3.0 gm.) in water (10 cc.). The bisulphite derivative, which separated as an amorphous yellow precipitate (0.142 gm.), was suspended in water (1 cc.) and to the suspension was added a solution of hydroxylamine hydrochloride (0.1 gm.) and sodium carbonate (0.1 gm.) in water (1 cc.). The mixture was stirred at room temperature; it was semisolid at first, but gradually became crystalline. The oxime was filtered and crystallized from aqueous methanol from which it separated as glistening plates, m.p. 116-119°, not depressed in admixture with homoanisaldehyde oxime, m.p. 119-120°.

Oxidation of Hordenine

Hordenine (0.102 gm.) was warmed with acetic anhydride (1 cc.) for three hours at 100°. The solution was diluted with water (10 cc.) and almost neutralized with potassium carbonate. Potassium permanganate (25 cc. of a 3% aqueous

solution) was added to the solution at 60-70°. After keeping for 15 min. at this temperature the excess permanganate was destroyed by addition of a few drops of ethanol and the manganese dioxide filtered. The alkaline filtrate was acidified with concentrated hydrochloric acid and the evolved carbon dioxide passed into a barium hydroxide solution. The precipitated barium carbonate was filtered, dried, and its activity determined. The total activity in the isolated barium carbonate was found to be 517 disintegrations per minute. If all the activity in the original amount of hordenine had been transferred to the barium carbonate an activity of 4400 disintegrations per minute would have been expected.

The acidified solution was extracted with ether, the extract dried over sodium sulphate and evaporated to dryness. There was left a colorless residue which crystallized from water as glistening colorless plates (0.033 gm.), m.p. 188-189°, not depressed in admixture with *p*-acetoxybenzoic acid, m.p. 193.5-194°. Found: C, 59.74; H, 4.64%. Calcd. for $C_9H_8O_4$: C, 60.00; H, 4.48%. The activities of the derivatives and degradation products of hordenine obtained from the plant are summarized in Table II.

TABLE II

ACTIVITIES OF THE DERIVATIVES AND DEGRADATION PRODUCTS OF HORDENINE

Compound	Disintegrations per minute per millimole
Hordenine	1.08×10^5
Hordenine picrate	1.07×10^5
Hordenine hydrochloride	1.04×10^5
Hordenine platinichloride	0.99×10^5
Hordenine methiodide	1.09×10^5
O-Methylhordenine methiodide	1.03×10^5
Trimethylamine platinichloride	0
Homoanisaldehyde oxime	0.96×10^5
p-Acetoxybenzoic acid	0

Derivatives and Degradation of Active N-methyltyramine

Picrate: yellow prismatic plates from aqueous ethanol, m.p. 149°.

Hydrochloride: colorless plates from ethanol, m.p. 148°.

Platinichloride: microscopic orange prisms, m.p. 208-209° (dec.).

Conversion to hordenine methiodide: A mixture of N-methyltyramine (1.300 gm.), sodium bicarbonate (0.74 gm.), methyl iodide (6 cc.), and methanol (20 cc.) was refluxed, with stirring, for two hours. It was filtered hot and the insoluble material washed well with methanol. The combined filtrate and washings were evaporated to a small bulk and allowed to cool when hordenine methiodide crystallized (2.018 gm.), m.p. 232°. Hordenine methiodide was methylated and degraded by the method already described, to homoanisaldehyde oxime and trimethylamine.

Oxidation of Homoanisaldehyde Oxime Derived from N-methyltyramine

The active oxime (9.17 mgm.) together with inactive oxime (98.5 mgm.) was dissolved in water (20 cc.) containing sodium hydroxide (0.1 gm.). Potassi-

um permanganate (15 cc. to a 3% aqueous solution) was added and the resulting solution refluxed for 30 min. Alcohol was then added to destroy the excess permanganate and the mixture filtered. The filtrate was evaporated to a small bulk and acidified with hydrochloric acid when a white crystalline precipitate separated (34.3 mgm.), m.p. 183-184°, undepressed in admixture with authentic anisic acid (m.p. 184°).

To a solution of the anisic acid (28.2 mgm.) obtained from the oxidation in 10% aqueous sodium hydroxide (1 cc.), *p*-bromophenacyl bromide (52 mgm.) in ethanol (10 cc.) was added and the mixture refluxed for one hour. Most of the alcohol was then evaporated and on cooling the *p*-bromophenacyl derivative of anisic acid crystallized (32.6 mgm.), m.p. 150-151°, not depressed in admixture with an authentic specimen of the derivative, m.p. 151-152°.

Degradation of Tyramine-α-C^{14}.

The degradation experiments were carried out on a sample of the product synthesized for administration to the plant and diluted with inactive tyramine.

Tyramine (1.226 gm.), sodium bicarbonate (1.70 gm.), and methyl iodide (6 cc.) were added to methanol (20 cc.) and refluxed for three hours with stirring. Hordenine methiodide was isolated from the reaction mixture as described in its preparation from N-methyltyramine, yield 2.413 gm. This was methylated and degraded as before to trimethylamine and *p*-vinylanisole which was in part converted to homoanisaldehyde oxime.

The remainder of the *p*-vinylanisole (0.120 gm.), freshly distilled from the Hofmann degradation, was suspended in water (10 cc.) containing sodium hydroxide (0.1 gm.) and to the suspension potassium permanganate (30 cc. of a 3% aqueous solution) was added. After allowing to stand two hours at room temperature the mixture was refluxed for 30 min. The excess permanganate was destroyed by the addition of a little ethanol and the reaction mixture filtered. The filtrate was acidified with hydrochloric acid and the evolved carbon dioxide

TABLE III

ACTIVITIES OF DERIVATIVES AND DEGRADATION PRODUCTS OF N-METHYLTYRAMINE AND OF TYRAMINE-α-C^{14}

Compound	Disintegrations per minute per millimole	
	From N-methyltyramine	From tyramine-α-C^{14}
N-Methyltyramine	1.04×10^6	
N-Methyltyramine picrate	1.14×10^6	
N-Methyltyramine hydrochloride	1.13×10^6	
N-Methyltyramine platinichloride	1.05×10^6	
Hordenine methiodide	1.11×10^6	2.00×10^5
O-Methylhordenine methiodide	1.15×10^6	1.92×10^5
Trimethylamine platinichloride	0	0
Homoanisaldehyde oxime	1.01×10^6	2.01×10^5
Anisic acid (from oxidation of homoanisaldehyde oxime)	1.01×10^6	1.97×10^5
p-bromophenacyl deriv.	0.98×10^6	1.91×10^5
Anisic acid (from oxidation of *p*-vinylanisole)		0
p-bromophenacyl deriv.		0

absorbed in barium hydroxide. The activity of the isolated barium carbonate accounted for 45% of the activity originally present in the *p*-vinylanisole. The acidified solution on evaporation yielded anisic acid (0.053 gm.) which was converted to the *p*-bromophenacyl derivative.

The activity of the derivatives and degradation products of the N-methyltyramine and of tyramine-α-C^{14} are summarized in Table III.

Extraction of Barley Seeds

Barley seeds were ground in a Wiley mill and the ground product (300 gm.) was extracted with methanol for 48 hr. in a Soxhlet extractor. The extract, on distillation *in vacuo* to remove the methanol, left a pale yellow viscous residue (23.2 gm.). This when subjected to the same treatment as described for the crude extract of the roots gave a final ether extract which on evaporation yielded a pale yellow viscous liquid (0.204 gm.). An aliquot part of this liquid was chromatographed on paper of pH 8 along with controls of hordenine, N-methyltyramine, and tyramine, but no trace of any of these alkaloids was detected.

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