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**Biosynthesis of Gramine: The Immediate Precursors of the Alkaloid<sup>1</sup>**BY BOB G. GOWER<sup>2</sup> AND EDWARD LEETE<sup>3</sup>

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3-Aminomethylindole-2-C<sup>14</sup> and 3-methylaminomethylindole-2-C<sup>14</sup> were prepared from indole-2-C<sup>14</sup> and administered to excised shoots of germinating barley resulting in the formation of gramine which was labeled solely at C<sub>2</sub>. The high incorporation of tracer from these amines (14.2 and 24.5%, respectively) compared with the incorporation of DL-tryptophan- $\beta$ -C<sup>14</sup> (0.51%) is a strong indication that these compounds are the immediate precursors of gramine. The chemistry of 3-aminomethylindole was also investigated. Treatment with nucleophilic reagents resulted in the elimination of the primary amino group with the formation of skatyl derivatives. The secondary amine, di-(3-indolylmethyl)-amine, was obtained when 3-aminomethylindole sulfate was dissolved in liquid ammonia. An analogous reaction occurred with 3-methylaminomethylindole.

It is now firmly established that gramine (VII), the main alkaloid found in the leaves of germinating barley, is derived from tryptophan.<sup>4</sup> However, the intermediates between tryptophan and gramine are unknown. Recently Mudd<sup>5</sup> obtained chromatographic evidence for the presence of 3-aminomethylindole (I) and 3-methylaminomethylindole (V) in the shoots of 4-day old barley seedlings. He found that these compounds were N-methylated yielding gramine with S-adenosylmethionine in the presence of a crude enzyme extract isolated from barley.

For several years we have considered the amines I and V to be the immediate precursors of gramine and we have desired to prepare them labeled with carbon-14 for feeding experiments. 3-Aminomethylindole was first described by Putochin<sup>6</sup> who obtained it by the reduction of indole-3-aldoxime with sodium in boiling ethanol. We were unable to duplicate this reaction. More recently Rafelson, Ehrensvar, and co-workers<sup>7</sup> claimed that this oxime, dissolved in diethyl ether at -40°, was reduced with lithium aluminum hydride to 3-aminomethylindole in 90% yield. We have attempted numerous times<sup>8</sup> to repeat this reaction without success. The oxime was recovered unchanged. Reduction with lithium aluminum hydride in tetrahydrofuran or dioxane at various temperatures was also unsuccessful, the oxime either being recovered or polymeric material produced. Reaction of gramine methiodide<sup>9</sup> or 3-hydroxymethylindole<sup>10</sup> with sodamide in liquid ammonia also failed to yield the desired amine. Putochin and Dawydowa<sup>11</sup> also claimed that the O-acetyl derivative of indole-3-aldoxime was reduced with hydrogen in the presence of platinum to 3-aminomethylindole. We were able to repeat this reaction, the amine being isolated as the sulfate. Hydrogenation of 3-cyanoindole in ammoniacal ethanol in the presence of Raney nickel was the most convenient method of obtaining the free amine, which separated from benzene as colorless plates melting at 104-107°. Putochin<sup>6,11</sup> and Rafelson, *et al.*,<sup>7</sup> reported lower melting points for this compound (82-85°). The ultra-

violet spectrum of 3-aminomethylindole is essentially the same as gramine, and its infrared spectrum shows the characteristic absorption of a primary amino group. The free base is unstable in air, and rapidly became pink and finally deep red after a few months. Ammonia was evolved when the amine or its N-acetyl derivative was boiled with aqueous sodium hydroxide. It seems probable that the base removes a proton from the indolic NH and forms 3-methyleneindolenine (II) as illustrated in Fig. 1. This indolenine derivative presumably polymerizes since no crystalline material could be isolated from the reaction mixture. 1-Methyl-3-aminomethylindole, having no proton on the indolic nitrogen, did not eliminate ammonia under these reaction conditions, in support of this hypothesis. When 3-aminomethylindole sulfate was dissolved in liquid ammonia, decomposition occurred and di-(3-indolylmethyl)-amine (IV) was obtained. The structure of this compound was established by conversion to di-(3-indolylmethyl)-dimethylammonium iodide (VIII) previously obtained<sup>9</sup> by the action of methyl iodide on gramine. The secondary amine IV could plausibly be formed by attack of 3-aminomethylindole on 3-methyleneindolenine as illustrated in Fig. 1. Like gramine,<sup>12</sup> 3-aminomethylindole reacts with nucleophilic reagents to yield skatyl derivatives. Lithium aluminum hydride in refluxing dioxane afforded 3-methylindole. Treatment of 3-aminomethylindole with potassium cyanide, thiophenol, and benzylamine yielded 3-cyanomethylindole, (3-indolylmethyl)-phenylsulfide,<sup>13</sup> and 3-benzylaminomethylindole,<sup>14</sup> respectively. On the other hand, 1-methyl-3-aminomethylindole resembled 1-methylgramine in its reactions; no displacement of the amino group occurred in refluxing alcoholic potassium cyanide.

3-Methylaminomethylindole (V) was obtained as an oil by the reduction of the known 3-methyliminomethylindole (III)<sup>15</sup> with sodium borohydride in ethanol. This secondary amine also decomposed in liquid ammonia yielding di-(3-indolylmethyl)-methylamine (VI).

The most convenient way of labeling 3-aminomethylindole and its N-methyl derivative with carbon-14 was by starting with indole-2-C<sup>14</sup>.<sup>16</sup> Treatment with dimethylformamide and phosphorus oxychloride afforded indole-3-aldehyde-2-C<sup>14</sup> which was then used for the syntheses of the amines by the previously described methods.

The radioactive amines and DL-tryptophan- $\beta$ -C<sup>14</sup> were fed to excised shoots of barley. This method of

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(2) General Mills Predoctoral Fellow, 1962-1963.

(3) Alfred P. Sloan Fellow, 1962-1964.

(4) D. O'Donovan and E. Leete, *J. Am. Chem. Soc.*, **85**, 461 (1963), and references cited therein.

(5) S. H. Mudd, *Nature*, **189**, 489 (1961).

(6) N. I. Putochin, *Ber.*, **59**, 1987 (1926).

(7) M. E. Rafelson, G. Ehrensvar, M. Bashford, E. Saluste, and C. Heden, *J. Biol. Chem.*, **211**, 725 (1954).

(8) Besides the present authors the following ex-graduate students of the senior author failed to duplicate Rafelson's procedure: Tzu-Chen Chang, R. I. Fryer, D. W. Henry, J. Moody. A typographical error was suspected since 650 mg. of indole-3-aldoxime will not dissolve in 5 ml. of ether (the reported experimental conditions). Several letters were written to the authors of the paper, but no replies were received.

(9) T. A. Geissman and A. Armen, *J. Am. Chem. Soc.*, **74**, 3916 (1952).

(10) E. Leete and L. Marion, *Can. J. Chem.*, **31**, 775 (1953).

(11) N. I. Putochin and N. P. Dawydowa, *J. Gen. Chem. USSR*, **2**, 290 (1932).

(12) Cf. J. H. Brewster and E. L. Eliel, "Organic Reactions," Vol. 7, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 99.

(13) F. Poppelsdorf and S. J. Holt, *J. Chem. Soc.*, 1124 (1954).

(14) G. N. Walker and M. A. Moore, *J. Org. Chem.*, **26**, 432 (1961).

(15) (a) G. F. Smith, *J. Chem. Soc.*, 3842 (1954); (b) E. Wenkert, J. H. Udelhofen, and N. K. Bhattacharyya, *J. Am. Chem. Soc.*, **81**, 3763 (1959); (c) V. M. Rodionov and T. K. Veselovskaya, *Trudy Moskov. Khim.-Tekhnol. Inst.*, No. 23, 25 (1956) (*Chem. Abstr.*, **53**, 9178 (1959)).

(16) E. Leete and L. Marion, *Can. J. Chem.*, **31**, 1195 (1953).

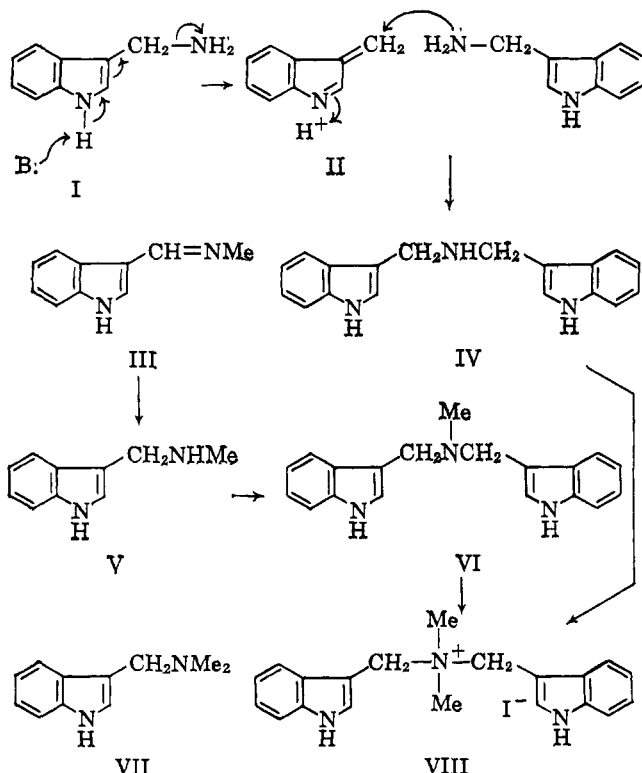


Figure 1.

studying the biosynthesis of gramine was used by Neish and co-workers,<sup>17</sup> who found tryptophan- $\beta$ -C<sup>14</sup> to be one of the most efficient precursors of gramine (0.5–2.7% incorporation). It was found (*cf.* Table I) that 3-aminomethylindole and 3-methylaminomethylindole were much more efficient precursors of

TABLE I

	Compounds fed		
	DL-Tryptophan- $\beta$ -C <sup>14</sup>	3-Aminomethylindole-2-C <sup>14</sup> sulfate	3-Methylaminomethylindole-2-C <sup>14</sup>
Wt., mg.	187	180	125
mmole	0.98	0.92/2	0.78
Total activity, d.p.m.	$2.90 \times 10^7$	$1.63 \times 10^7$	$1.35 \times 10^7$
Spec. act., d.p.m./mmole	$3.16 \times 10^7$	$(1.76 \times 10^7) \times 2$	$1.70 \times 10^7$
Gramine isolated from the above feedings			
Wt., mg.	226	189	190
Spec. activ., d.p.m./mmole	$1.14 \times 10^8$	$2.13 \times 10^8$	$3.03 \times 10^8$
Spec. incorp., %	0.36	12.1	17.8
Incorporation, %	0.51	14.2	24.5
Degradation products of the above gramine, d.p.m./mmole			
Indole-3-carboxylic acid	...	$2.03 \times 10^6$	$2.94 \times 10^6$
Indole	...	$2.21 \times 10^6$	$3.11 \times 10^6$
Barium carbonate	...	0	0
N-Benzoylanthranilic acid	...	0	0
Dimethylethylamine picrate	...	0	0

gramine than tryptophan. The incorporations of tracer from the primary and secondary amines amounted to 14.2 and 24.5%, respectively, compared with 0.51% for the tryptophan- $\beta$ -C<sup>14</sup>. The higher incorporation of 3-methylaminomethylindole as compared with 3-aminomethylindole is consistent with its closer metabolic relationship to gramine. Radioactive assay of paper chromatograms of the gramine isolated from the

(17) F. Wightman, M. D. Chisholm, and A. C. Neish, *Phytochemistry*, **1**, 30 (1961).

shoots established that all the radioactivity was located at a position on the paper corresponding to gramine. The radioactive gramine was degraded by previously described methods.<sup>16</sup> Treatment of gramine with ethyl iodide and sodium ethoxide resulted in the elimination of the dimethylamino group which was detected as ethyldimethylamine picrate. Fusion of gramine with potassium hydroxide yielded indole-3-carboxylic acid which was heated above its melting point affording indole and carbon dioxide. The latter was collected as barium carbonate. The indole was N-benzoylated and oxidized with potassium permanganate yielding N-benzoylanthranilic acid. The activities of the degradation products (Table I) indicate that the gramine derived from both the radioactive amines was labeled solely at C<sub>2</sub> of the indole nucleus. We thus feel confident in claiming that these amines are the immediate precursors of gramine in the plant.

### Experimental<sup>18</sup>

**3-Aminomethylindole (I).** (a) **From 3-Cyanoindole.**—3-Cyanoindole (3 g.) dissolved in a mixture of ethanol (150 ml.) and concentrated ammonia solution (3 ml.) was hydrogenated in the presence of Raney nickel-W-2<sup>19</sup> (1 g.) at a pressure of 38 p.s.i. for 18 hr. until the theoretical amount of hydrogen had been absorbed. The mixture was filtered and the solvent removed *in vacuo*. The residual pale yellow oil was dissolved in ether, dried over magnesium sulfate, and then evaporated yielding 3-aminomethylindole (2.95 g., 96%). After several crystallizations from benzene the amine was obtained as odorless, colorless plates, m.p. 104–107°;  $\lambda_{\max}$ ,  $m\mu$  (log  $\epsilon$ ) in 95% ethanol: 221 (4.55), 274(3.86), 281(3.87), and 288(3.74); the infrared spectrum had absorptions at 3325 and 3300  $\text{cm}^{-1}$  (NH<sub>2</sub>). Gramine and related compounds do not show a well defined NH absorption in this region of the spectrum.

*Anal.* Calcd. for C<sub>9</sub>H<sub>10</sub>N<sub>2</sub>: C, 73.94; H, 6.90; N, 19.16. Found: C, 74.18; H, 6.89; N, 18.81.

The picrate of 3-aminomethylindole was obtained as yellow plates from ethanol, m.p. 160° dec.

*Anal.* Calcd. for C<sub>9</sub>H<sub>10</sub>N<sub>2</sub>·C<sub>6</sub>H<sub>3</sub>N<sub>3</sub>O<sub>7</sub>: C, 48.00; H, 3.49; N, 18.66. Found: C, 48.13; H, 3.63; N, 18.95.

The sulfate of 3-aminomethylindole separated when an ether solution of concentrated sulfuric acid was added to an ether solution of the amine. Recrystallization from aqueous ethanol afforded colorless plates, m.p. 150° dec.

*Anal.* Calcd. for (C<sub>9</sub>H<sub>10</sub>N<sub>2</sub>)<sub>2</sub>·H<sub>2</sub>SO<sub>4</sub>: C, 55.37; H, 5.68; N, 14.35. Found: C, 54.61; H, 5.69; N, 14.09.

(b) **From O-Acetylindole-3-aldoxime.**—O-Acetylindole-3-aldoxime<sup>21</sup> (10.0 g.) was dissolved in absolute ethanol (100 ml.) and hydrogenated in the presence of platinum oxide (0.3 g.) at a pressure of 40 p.s.i. for 18 hr. The mixture was filtered and evaporated to small volume (30 ml.). This solution was diluted with ether (200 ml.) and treated with a solution of concentrated sulfuric acid in ether until 3-aminomethylindole sulfate (7.3 g., 75%) separated.

**3-Acetylaminomethylindole.**—3-Aminomethylindole (1.0 g.) was added to a solution of sodium acetate trihydrate (1.0 g.) in acetic anhydride (5 ml.) cooled to 0°. After stirring for 5 min. the acetyl derivative (0.98 g., 80%) separated from the clear solution. Crystallization from benzene afforded colorless plates of 3-acetylaminomethylindole,<sup>20</sup> m.p. 133–134°.

*Anal.* Calcd. for C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O: C, 70.18; H, 6.43; N, 14.88. Found: C, 70.47; H, 6.61; N, 14.58. When this acetyl derivative was refluxed with 10% sodium hydroxide ammonia was liberated (identified by its odor and reaction with litmus paper). The amorphous material in the aqueous solution could not be crystallized and had a low melting point (60–75°).

**1-Methylindole-3-aldoxime.**—1-Methylindole-3-aldehyde<sup>21</sup> (20 g.) was refluxed in a mixture of ethanol (75 ml.) and water (50 ml.) in which had been dissolved hydroxylamine hydrochloride (20 g.) and sodium hydroxide (15 g.). After 30 min. the mix-

(18) Melting points are corrected. We thank Mrs. Olga Hamerston and her assistants at the University of Minnesota for the analyses. Radioactive compounds were assayed in a Nuclear Chicago model D-47 flow counter or a Nuclear Chicago model 724 liquid scintillation spectrometer.

(19) E. C. Horning, Ed., "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 181.

(20) This compound was also obtained in small yield by Miss Tzu-Chen Chang by reducing a solution of indole-3-aldoxime in a mixture of acetic acid and acetic anhydride with hydrogen in the presence of Raney nickel (M.S. Thesis, University of Minnesota, 1960).

(21) H. Wieland, W. Konz, and H. Mittasch, *Ann.*, **513**, 1 (1934).

ture was cooled and the oxime separated (20 g., 92%). Recrystallization from aqueous ethanol afforded colorless plates, m.p. 137–138°.

*Anal.* Calcd. for  $C_{10}H_{10}N_2O$ : C, 68.95; H, 5.79. Found: C, 68.71; H, 5.67.

**1-Methyl-3-aminomethylindole.**—1-Methylindole-3-aldoxime (2.7 g.) was dissolved in a mixture of ethanol (100 ml.) and concentrated ammonia solution (2 ml.), and hydrogenated in the presence of Raney nickel (1 g.) at a pressure of 38 p.s.i. at 50–60° for 18 hr. Evaporation of the filtered reaction mixture yielded an oil which was dissolved in ether and dried over magnesium sulfate. Addition of an ether solution of concentrated sulfuric acid yielded the sulfate (3.22 g., 88%). Crystallization from ethanol afforded colorless plates of 1-methyl-3-aminomethylindole sulfate, m.p. 170° dec.

*Anal.* Calcd. for  $(C_{10}H_{12}N_2)_2 \cdot H_2SO_4$ : C, 57.39; H, 6.26; N, 13.39. Found: C, 57.25; H, 6.01; N, 13.33.

The picrate of 1-methyl-3-aminomethylindole was obtained from ethanol as bright yellow plates, m.p. 160° dec.

*Anal.* Calcd. for  $C_{10}H_{12}N_2 \cdot C_6H_3N_3O_7$ : C, 49.36; H, 3.88; N, 17.99. Found: C, 49.29; H, 3.88; N, 18.26.

**3-Methylaminomethylindole (V).**—3-Methyliminomethylindole<sup>18</sup> (1.3 g., m.p. 125–126°, having  $\lambda_{max} m\mu$  (log  $\epsilon$ ) in 95% ethanol at 212(4.38), 243(4.13), 260(4.07), and 297(4.12)), dissolved in absolute ethanol (50 ml.) at room temperature was treated with sodium borohydride (1 g.) and the mixture stirred for 3 hr. Water was then added and the mixture extracted with benzene. Evaporation of the dried benzene extract afforded 3-methylaminomethylindole as a colorless oil (1.3 g.). The picrate was obtained from ethanol as yellow plates, m.p. 176–176.5° dec.

*Anal.* Calcd. for  $C_{10}H_{12}N_2 \cdot C_6H_3N_3O_7$ : C, 49.36; H, 3.88; N, 17.99. Found: C, 49.30; H, 4.04; N, 18.03.

**Di-(3-indolylmethyl)-amine (IV).**—3-Aminomethylindole sulfate (0.75 g.) was added to liquid ammonia (100 ml.) and stirred for 10 min. Ether (100 ml.) was then added followed by water after most of the ammonia had evaporated. The separated ether layer was dried over magnesium sulfate and evaporated, yielding the amine IV (0.38 g., 73%). Crystallization from benzene afforded the amine as colorless plates, m.p. 128–129°;  $\lambda_{max} m\mu$  (log  $\epsilon$ ) in 95% ethanol: 244(4.57), 274(4.07), 281(4.09), and 288(4.01);  $\nu_{NH}$  at 3370  $cm^{-1}$  in Nujol.

*Anal.* Calcd. for  $C_{18}H_{17}N_3$ : C, 78.51; H, 6.22; N, 15.26. Found: C, 78.54; H, 6.35; N, 15.69.

**Di-(3-indolylmethyl)-methylamine (VI)** was obtained in 70% yield from 3-methylaminomethylindole by stirring its sulfate with liquid ammonia using essentially the same procedure as described for the amine IV. The tertiary amine crystallized from benzene as colorless plates, m.p. 164–165°;  $\lambda_{max} m\mu$  (log  $\epsilon$ ): 221(4.76), 275(4.08), 281(4.11), and 289(4.01).

*Anal.* Calcd. for  $C_{19}H_{19}N_3$ : C, 78.86; H, 6.62; N, 14.52. Found: C, 78.68; H, 6.79; N, 14.72.

On addition of methyl iodide to an ether solution of this amine and allowing to stand overnight, di-(3-indolylmethyl)-dimethylammonium iodide (VIII), m.p. 169–170° dec., separated. This material was identical (infrared spectrum, mixture m.p.) with an authentic specimen prepared from gramine.<sup>9</sup> This methiodide was also obtained from the secondary amine IV by treatment with methyl iodide in ethanol.

**Reactions of 3-Aminomethylindole.** (a) **Hydrogenolysis.**—3-Aminomethylindole (1.0 g.) was added to a suspension of lithium aluminum hydride (1.0 g.) in dry dioxane (40 ml.) and the mixture refluxed in a nitrogen atmosphere for 16 hr. Water was then added and the mixture filtered. The filtrate was evaporated and the residue chromatographed on alumina (Activity I). Elution with petroleum ether (b.p. 60–70°) yielded 3-methylindole (0.22 g., 24%), m.p. 93–95°, not depressed on admixture with an authentic specimen.

(b) **With Thiophenol.**—3-Aminomethylindole (0.5 g.) and thiophenol (2 ml.) were heated at 150–160° for 45 min. The mixture was then cooled, dilute sodium hydroxide solution added, and then extracted with ether. The solid residue obtained after evaporation of the dried ether extract was crystallized from petroleum ether affording colorless plates of (3-indolylmethyl)-phenyl sulfide (0.72 g., 89%), m.p. 83–84°, not depressed on admixture with an authentic specimen prepared from gramine and thiophenol.<sup>18</sup> 3-Aminomethylindole reacts at a slower rate with thiophenol in ethanol than gramine. When the reaction was carried out in boiling ethanol for 1 hr. the yield of sulfide was 37%, 52% of the 3-aminomethylindole being recovered. Under identical conditions an 84% yield of sulfide was obtained from gramine.

(c) **With Benzylamine.**—3-Aminomethylindole (0.5 g.) was heated with freshly distilled benzylamine (1 ml.) at 150–160° for 30 min. After cooling, water was added and the mixture extracted with ether. Evaporation of the dried ether extract afforded 3-benzylaminomethylindole (0.44 g., 59%) which was crystallized from a mixture of benzene and petroleum ether,

affording colorless plates, m.p. 92–95°, identical with an authentic specimen prepared by the sodium borohydride reduction of the Schiff base obtained from indole-3-aldehyde and benzylamine.<sup>14</sup>

(d) **With Potassium Cyanide.**—3-Aminomethylindole (0.5 g.) was refluxed with a solution of potassium cyanide (0.23 g.) in 50% aqueous ethanol (50 ml.) for 18 hr. After cooling and dilution with water the mixture was extracted with ether. Evaporation of the dried ether extract afforded a pale yellow oil which was dissolved in methanol and treated with a solution of 1,3,5-trinitrobenzene in methanol, when the trinitrobenzene derivative of 3-cyanomethylindole (0.82 g., 66%) separated, m.p. 136–138°, not depressed on admixture with an authentic specimen.<sup>22</sup> Under identical reaction conditions 1-methyl-3-aminomethylindole was recovered unchanged.

**3-Aminomethylindole-2-C<sup>14</sup>.**—Indole-2-C<sup>14</sup><sup>16</sup> (0.88 g.) dissolved in dimethylformamide (5 ml.) was added dropwise to phosphorus oxychloride (0.75 ml.) stirred in an ice bath. After 30 min. the reaction mixture was allowed to warm to room temperature and then stirred for 4 hr. The mixture was then poured onto ice and 40% sodium hydroxide added until a basic solution was obtained. The mixture was boiled for 1–2 min. and then cooled until indole-3-aldehyde-2-C<sup>14</sup> separated (0.90 g., 83%,  $1.80 \times 10^7$  d.p.m./mM.). This aldehyde (0.60 g.) was dissolved in boiling ethanol (5 ml.) and a solution of hydroxylamine hydrochloride (0.38 g.) in water (1 ml.) added. A solution of sodium hydroxide (0.38 g.) in water (1 ml.) was added dropwise with stirring. After cooling for 1 hr. the mixture was poured onto ice and carbon dioxide passed into the solution when indole-3-aldoxime-2-C<sup>14</sup> separated (0.47 g., 72%,  $1.71 \times 10^7$  d.p.m./mM.). The oxime (0.45 g.) was dissolved in absolute ethanol (50 ml.) containing acetic anhydride (0.3 g.) and hydrogenated overnight at room temperature in the presence of platinum oxide (0.1 g.) at 40 p.s.i. The catalyst was filtered off and the filtrate evaporated to 10 ml., diluted with ether (100 ml.), and a solution of concentrated sulfuric acid (0.08 ml.) in ether (100 ml.) added slowly with stirring. The sulfate of 3-aminomethylindole-2-C<sup>14</sup> separated (0.365 g., 64%,  $3.53 \times 10^7$  d.p.m./mM.).

**3-Methylaminomethylindole-2-C<sup>14</sup>.**—Indole-3-aldehyde-2-C<sup>14</sup> (0.150 g.) was stirred with 40% aqueous methylamine solution (10 ml.) for 2 hr. Sodium borohydride (1 g.) was added and the mixture stirred for an additional 2 hr. Ether extraction of the reaction mixture with subsequent drying over magnesium sulfate and evaporation yielded 3-methylaminomethylindole-2-C<sup>14</sup> as a colorless oil (129 mg., 78%,  $1.70 \times 10^7$  d.p.m./mM.).

**Administration of the Tracers to Barley and Isolation of the Gramine.**—Charlottetown No. 80 barley (*Hordeum distichum*)<sup>23</sup> (500 g.) was dusted with a fungicide (Semasan, manufactured by Bayer Co., New York, having as active principle 2-chloro-4-(hydroxymercuri)-phenol) and placed on cotton cloths in Pyrex dishes. Distilled water was added and the barley allowed to germinate in a closed cabinet in the absence of light. When the shoots were 5–6 cm. in length (about 5 days after germination) they were cut off close to the roots with scissors and placed in 250-ml. beakers containing a solution of the tracer dissolved in dilute acetic acid. Water was added as required to maintain a depth of about 1 cm. The tracers were also fed in the dark germination cabinet. Five days after the administration of tracers, the shoots were removed from the beakers and dried at 50°. They were then macerated in a Waring blender with a mixture of chloroform (1 l.) and concentrated ammonia solution (50 ml.). After standing 2 days the mixture was filtered through cloth and the yellow chloroform layer evaporated to dryness *in vacuo*. The residue was dissolved in 2 *N* sulfuric acid (200 ml.) and extracted several times with ether which was discarded. The aqueous solution was cooled, made basic with sodium hydroxide, and extracted with ether. Evaporation of the dried ether extract afforded crude gramine which was purified by sublimation (90–100°,  $10^{-4}$  mm.).

The activities of the tracers fed and the isolated gramine are recorded in Table I. The radioactive gramine which was isolated from the barley which had been fed the aminomethylindoles was subjected to paper chromatography. Whatman No. 1 paper was impregnated with a buffer solution composed of 97% 0.2 *M* dipotassium hydrogen phosphate and 3% 0.1 *M* citric acid and then dried in air. The developing solvent was a mixture of 70% 2-propanol and 30% of the buffer solution. With this system the following  $R_f$  values were obtained: 3-aminomethylindole, 0.60; 3-methylaminomethylindole, 0.69; gramine, 0.85; di-(3-indolylmethyl)-amine, 0.90; di-(3-indolylmethyl)-methylamine, 0.98. These amines were detected by spraying with Millons reagent. All the radioactivity of the gramine was located in a position coincident with gramine.

**Degradation of the gramine** was carried out as previously described.<sup>16</sup> The activities of the degradation products are recorded in Table I.

(22) D. W. Henry and E. Leete, *J. Am. Chem. Soc.*, **79**, 5254 (1957).

(23) We thank Mr. R. B. MacLaren of the Experiment Station, Charlottetown, Prince Edward Island, Canada, for a generous supply of this barley.