Simple Syntheses of 3-Substituted Indoles and their Application for High Yield $^{14}$C-Labeling

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Methods are described which allow the synthesis of several plant indole alkaloids and their metabolites at different scales. Compounds synthesized include gramine (1) (3-dimethylaminomethylindole) which is directly derived from indole, while its biosynthetic precursors 3-aminomethylindole (3) and 3-methylaminomethylindole (2) are biosynthetic precursors of gramine (1) and constituents of barley shoots [3, 4] while indole-3-carboxylic acid (7) has been described as a degradation product of gramine (1) in barley shoots [5].

These compounds as well as other indole amines have found increasing consideration as components of crop plants which might be toxic for ruminants or at least diminish taste and attractiveness for grazing livestock [6].

Investigations of plant metabolism have often required these and other indole derivatives as substrates or chromatographic references [4-7]. Therefore syntheses of the described compounds are known but they either lack easy reproducibility or lead to low yields, especially when scaled down to amounts necessary for syntheses of compounds with high specific radioactivity.

Indole-3-aldehyde (6), for example, is easily synthesized from indole and a mixture of phosphorous oxychloride and dimethylformamide [8], but this synthesis could not be carried out at the $\mu$molar level. The same is true for the known routes to gramine (1) [9, 10], which need crucial changes before being applicable to synthesis of small quantities. Starting from indole-3-aldehyde (6), indole-3-aldehyde oxime (5) [11] and methyliminomethylindole (4) [8] are almost quantitatively obtained, but the following reduction to the respective amines creates surprising difficulties [4, 11].

We have developed methods which overcome these problems and lead to rather stable products of high purity. High specific radioactivities were obtained in syntheses of specifically labelled substrates used for biochemical investigations of plant metabolism [12].

Results and Discussion

Indole-3-aldehyde (6) has been synthesized with high yields by a simple procedure with indole, dimethylformamide and phosphorous oxychloride as reactants [8]. A 1:1 adduct between phosphorous oxychloride and dimethylformamide was assumed as an intermediate. When dimethylformamide is bound, PO$_2$Cl$_2$ is liberated and subsequently hydrolyzed to orthophosphate and hydrochloric acid. Therefore a strict 1:1 ratio of phosphorous oxychloride and indole seemed to be required [8]. This procedure could not simply be scaled down to the $\mu$molar range because the then necessary amounts could not be handled adequately. Dilution of reaction mixtures with dimethylformamide led to a decreased yield. However, experiments with small amounts of indole but excess of phosphorous oxychloride proved that the reaction was not hindered and even led to higher yields. Fast hydrolysis of the supposedly intermediary 3-dimethylaminomethylindolenine was found to be the crucial step in order to obtain high yields with very little amounts of substrates. When [2-$^{14}$C]indole was used, the

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specific radioactivity of the synthesized [\(^{14}\text{C}\)]indole-3-aldehyde (6) was unchanged.

![Chemical Structure](image)

Table I. Syntheses of labelled indole derivatives.

<table>
<thead>
<tr>
<th>Product</th>
<th>Labelled substrate</th>
<th>Radiochemical yield [%]</th>
<th>Spec. radioact. [MBq/μmol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>[(^{14}\text{C})]Indole-3-aldehyde</td>
<td>[(^{14}\text{C})]Indole</td>
<td>96.0</td>
<td>1.30–1.67</td>
</tr>
<tr>
<td>[(^{14}\text{C})]Gramine</td>
<td>[(^{14}\text{C})]Indole</td>
<td>85.5</td>
<td>1.30–1.67</td>
</tr>
<tr>
<td>[Methylene-(^{14}\text{C})]-gramine</td>
<td>[(^{14}\text{C})]Formaldehyde</td>
<td>96.6</td>
<td>1.30</td>
</tr>
<tr>
<td>[Methyl-(^{14}\text{C})]-gramine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[(^{14}\text{C})]3-Aminomethylindole</td>
<td>[(^{14}\text{C})]Indole-3-aldehyde</td>
<td>68.7</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The known procedure for synthesis of indole-3-aldehyde oxime (5) yields quantitative conversion of the aldehyde (6) [11] and was found applicable to large scale as well as small scale syntheses. Again, an excess of the other reactants over the aldehyde did not negatively influence the reaction. When ethanol was removed by evaporation, the oxime precipitated as fine needles.

Syntheses of 3-aminomethylindole (3) have been described and were indeed found as being little reproducible and giving low yields [4, 8]. A possible route to 3-aminomethylindole (3) is reduction of the 3-cyano compound, but yields of this intermediate had not been satisfactory [6]. In this respect synthesis of the oxime is advantageous and therefore the more commonly used approach. But the simple looking reduction to the amine created a major problem. Putochin [11] tried reduction with metallic sodium in ethanol, but yields were poor or could not be reproduced at all [4]. Equally unsatisfactory results were obtained with lithium aluminum hydride in cold (−40 °C) ether [13] or with lithium aluminum hydride at different temperatures in tetrahydrofuran or dioxane [4]. We found that reduction of indole-3-aldehyde oxime (5) with Devarda’s alloy in alkaline solution was not only a simple and fast method but also led to high yields, even in low scale runs. This can be seen in Table I, where the [\(^{14}\text{C}\)]3-aminomethylindole (3) synthesized is shown to have a specific activity 2500 times higher than described earlier [4]. Rapid extraction of the product from the reaction mixture was very important for good yields, since in warm alkaline solutions 3-aminomethylindole (3) decomposes with concomitant liberation of ammonia. It was possible to perform the reduction with zinc dust in 4 N HCl, which has the advantage to obtain the indole as its more stable hydrochloride. Yields were poor however, since the oxime readily hydrolized, even when it was added in small portions.

3-Aminomethylindole (3) as purified free base was found to be rather unstable, and air and light accelerated decomposition. Conversion to either its sulphate or hydrochloride increased stability to an extent which made storage at room temperature possible without considerable loss even for months.

Synthesis of 3-methyliminomethylindole (4) is the first step towards 3-methylaminomethylindole (2) and created no problem. If desired, crystallization from benzene yielded a pure product, but this was not necessary for subsequent reduction to the amine. The same procedure as for synthesis of 3-aminomethylindole (3) proved to be optimal for good yields and high purity. Crystallization from benzene was possible and melting points as well as other chemical properties could be determined. Hitherto 3-methylaminomethylindole (2) had only been described as an oil which had not been further characterized [4]. Though synthesis with labelled substrates has not yet been performed, the described method proved to be applicable for small scales.

When indole-3-carboxylic acid (7) was synthesized by addition of gramine (1) to melted potassium hydroxide [14], we found heavily fluctuating yields and contamination with unreacted gramine (1) as well as a number of unknown reaction products. Oxidation of indole-3-aldehyde (6) with potassium permanganate in alkaline solution proved to give
good yields and little decomposition of the rather labile acid. Ether extraction first removed unreacted substrate and then, after acidification, yielded the desired acid. Indole-3-carboxylic acid (7) could be stored under nitrogen and in the dark for months.

The reaction of formaldehyde and dimethylamine with indole to yield gramine is well-known (MANICH reaction) and easily done with high yields in the molar range [9, 10]. With weak CH-acidic compounds like phenol or indole the reaction is normally carried out in acetic acid [9]. We found that optimal yields were only obtained when ratios of dimethylamine to acetic acid from 1:1.5 to 1.2 were applied. Higher or lower ratios drastically decreased yields. While it was important to have the reactants cooled, they could be used in high excess without negative influence on the yield of the indole derivative. When the labelled substrate was the limiting compound, products with identical specific radioactivities were obtained. This was done for labelling gramine (1) at different specific positions with high specific radioactivities, as can be seen from Table I. Attempts to synthesize other 3-substituted indoles were not made, but with the exchange of single reactants other indoles should be synthesizable following the presented procedures. Similar approaches have already led to synthesis of 3-diethylaminomethylindole and 3-N-piperidylmethylindole [9]. But not only changes of the side chain are possible, but also different substitutions at the aromatic system [4].

Materials and Methods

Labelled compounds were purchased from C.E.A., Service de Molecules Marquees, Gif-sur-Yvette, (2,14C]indole, spec. act. 1.30-1.67 MBq/μmol and (14C]formaldehyde, spec. act. 1.30 MBq/μmol) or the Radiochemical Centre, Amersham (di-[14C]-methylamine hydrochloride, spec. act. 2.07 GBq/ mmol). All other chemicals were commercially available.

Mass spectra were recorded at 75 eV, 0.8 mA with a Varian MAT 44 S mass spectrometer coupled to a Varian Data 188 computer unit.

1H NMR spectra were recorded with a WH 90- NMR-spectrometer (90 MHZ Bruker).

UV spectra were recorded in methanol using a Pye Unicam SP 8000 photometer.

Determination of radioactivity in solutions was performed by liquid scintillation counting in toluene cocktail (5 g PPO/1 toluene) using labelled toluene as internal standard. Radioactivity on TLC plates was detected on a Berthold TLC scanner equipped with autochroneous recording.

Solvents for TLC on precoated silica gel plates GF254 (Merek) were

A) chloroform/methanol/25% ammonia 93:7:1 and

B) n-butanol/acetic acid/H2O 6:2:2. Compounds were detected on the plates by their extinction of fluorescence at 254 nm.

Melting points were determined on a Kofler heating block (Reichert) equipped with a microscope and are not corrected.

Syntheses of unlabelled compounds

I. 3-Aminomethylindole (3)

1 g (6.25 mmol) Indole-3-aldehyde oxime (5) (synthesized according to [11], m.p. 200-202 °C, lit. [11] 197-198 °C) was dissolved in 40 ml methanol and mixed with 140 ml 1 N sodium hydroxide. After 4 g of Devarda’s alloy (50% Cu, 45% Al, 5% Zn) had been added, stirring was continued without refrigeration for another 15 min. Direct extraction with three 100 ml portions of ether, and evaporation of the Na2SO4 dried ether in vacuo yielded 3-aminomethylindole (3) as a slightly coloured oil (895 mg, 99%)*. Repeated crystallization from hot benzene finally led to small colourless needles.

M.p. 103-105 °C, lit.: [11] 84 °C, [4] 104-107 °C; UV 3max, nm (log ε): 279 (3.76), 287 (3.68); m/e 146 (94%, M+), 130 (100%), 118 (68%), 103 (13%), 91 (25%), 89 (26%), 77 (29%), 63 (23%), 51 (21%).


Salts of 3-aminomethylindole can be obtained by adding to a solution of the indole in ether sulfuric acid dissolved in ether (80 μl/100 ml) to yield amorphous white sulphate (m.p. 175.5-179 °C, no lit.) or leading HCl gas through an ethanolic solution to yield fine colourless leaflets of the hydrochloride (m.p. 168-173 °C, lit. [4] 150 °C under decomposition).

2. 3-Methyliminomethylindole (4)

1 g (6.9 mmol) indole-3-aldehyde (6) was stirred with 20 ml 25% aqueous methylamine (150 mmol) at 40-45 °C for 15 min and then left at room temperature for 4 h. Benzene was used for direct extraction of the mixture, dried with Na2SO4 and evaporated in vacuo. A brownish oil was obtained which crystallized when dried over calcium chloride in vacuo (975 mg, 89.4%)*. From hot benzene, 3-methyliminomethylindole (2) crystallized as thin, slightly yellow leaflets.

* Yields were calculated from UV spectra.
M.p. 122-124 °C, lit.: [12] 122-124 °C; UV \( \lambda_{\text{max}} \) nm (log \( e \)) 214 (4.18), 242 (4.02), 260 (3.95), 296 (4.01); 
m/e 158 (100%), M+, 157 (77%), 142 (43%), 130 (61%), 127 (26%), 102 (12%), 89 (34%), 77 (20%), 63 (31%), 51 (30%).

3. 3-Methylaminomethylindole (2)

0.6 g (3.7 mmol) 3-Methyliminomethylindole (4) was dissolved and reduced as described in I. Evaporation of the ether left a brownish oil which crystallized when dried over calcium chloride in vacuo (562 mg, 92.4%)*. Recrystallization from little hot benzene yielded thick colourless crystals.

M.p. 99-102 °C, lit.: [4] oil; UV \( \lambda_{\text{max}} \) nm (log \( e \)) 279 (3.75), 287 (3.68); 
m/e 160 (37%), M+, 143 (3%), 130 (100%), 118 (18%), 108 (11%), 89 (8%), 77 (22%), 63 (10%), 51 (14%).

\(^1\)H NMR: CD\(_3\)OD [TMS] \( \delta \) (ppm): methyl protons 2.34, methyl en protons doublet 3.84, 3.85, Ü5+6 7.08-7.28, (ppm): 7.96, H\(_7\) 7.34-7.54, H\(_6\) 7.16, H\(_5\) 7.17-7.40, H\(_4\) 7.49-7.64. Sulphate and hydrochloride could be synthesized as described for 3-aminomethylindole.

M.p. (sulphate) 160-162 °C, (hydrochloride) 151.5 to 155.5 °C, no lit..

4. Indole-3-carboxylic acid (7)

0.7 g (4.8 mmol) Indole-3-aldehyde (6) was dissolved in 40 ml ethanol and alkaliwized with 5 ml 0.25 N potassium hydroxide. 0.8 g (50 mmol) potassium permanganate was dissolved in 60 ml H\(_2\)O heated to 50-60 °C, and added to the aldehyde with stirring. After 5 min. MnO\(_2\) is filtrated from the hot solution and the ethanol evaporated in vacuo. The remaining solution was extracted twice with 50 ml ether and again three times after acidification with HCl. The Na\(_2\)SO\(_4\) dried acidic ether extract was concentrated to dryness in vacuo and yielded indole-3-carboxylic acid (7) as a yellow powder (728 mg, 94.2%)*. This was dissolved in acetone, and the acid precipitated as light, thin needles by the addition of water.

M.p. 204 °C (decomposition), lit.: [14] 209 °C (decomposition);

UV, \( \lambda_{\text{max}} \) nm (log \( e \)) 280 (4.01), 286 (3.98); 
m/e 161 (100%), M+, 144 (86%), 132 (4%), 116 (32%), 104 (5%), 89 (46%), 77 (18%), 72 (15%), 63 (49%), 51 (26%).

\(^1\)H NMR: CD\(_3\)OD [TMS] \( \delta \) (ppm): H\(_{5,6}\) 7.08-7.28, H\(_4\) 7.34-7.54, H\(_2\) 7.96, H\(_1\) 8.00-8.15.

Syntheses of labelled compounds

I. [2-\(^{14}\)C]Indole-3-aldehyde (6)

85 mg (0.55 mmol) Phosphorous oxychloride and 95 mg (1.3 mmol) dimethylformamide were added to 0.5 \( \mu \)mol [2-\(^{14}\)C] indole (0.74 MBq) and kept at room temperature for 45 min. Then the mixture was added to 1 ml hot 5 N NaOH (boiling water bath) and left at that temperature for 5 min. The ether from 3 extractions with 3 ml was concentrated and streaked onto a silica gel plate which was developed in solvent system A. The radioactive band with an identical \( R_f \) value as authentic non-labelled indole-3-aldehyde (6) was scraped off the plate and eluted with methanol. For storage at -18 °C, methanol was evaporated and the product dissolved in 0.01 N HCl.

2. [2-\(^{14}\)C]Indole-3-aldehyde oxime (5)

0.25 \( \mu \)mol [2-\(^{14}\)C] Indole-3-aldehyde (6) (0.37 MBq) in 5 ml ethanol were mixed with 0.35 g (5 mmol) hydroxyl ammonium chloride and 0.25 g sodium carbonate in 1 ml H\(_2\)O and stirred at 50-60 °C for 30 min. Chromatographic control in solvent system A confirmed synthesis of labelled oxime, but no further purification was attempted, since the complete reaction mixture was used for further synthesis of 3-aminomethylindole (3).

3. [2-\(^{14}\)C] 3-Aminomethylindole (3)

5 ml H\(_2\)O were added to the reaction mixture containing labelled oxime, and the ethanol removed by evaporation. Without refrigeration the mixture was stirred with 1 ml 5 N NaOH and 1 g Devarda’s alloy for 10 min. Extraction with three 10 ml portions of ether was followed by an analogous procedure as described for indole-3-aldehyde (6).

4. [2-\(^{14}\)C] Gramine (1)

Solutions of formaldehyde (5 \( \mu \)l 35%, 63 \( \mu \)mol) and dimethyamine (6 \( \mu \)l 40%, 47 \( \mu \)mol) were premixed in an ice bath and added to cold acetic acid (5 \( \mu \)l 99%, 88 \( \mu \)mol). This mixture was added to 1.25 \( \mu \)mol [2-\(^{14}\)C] indole (1.85 MBq) and subsequently kept at room temperature for 6 h. The complete mixture was then chromatographed in solvent system B and further handled as described above.

5. [methylene-\(^{14}\)C] Gramine (1)

With [\(^{14}\)C] formaldehyde (40 \( \mu \)l, 1.48 MBq, 1.14 \( \mu \)mol) and non-labelled indole (1 mg, 8.5 \( \mu \)mol) synthesis was performed exactly as described for [2-\(^{14}\)C] gramine (1).

6. [methyl-\(^{14}\)C] Gramine (1)

This compound was synthesized from di-[\(^{14}\)C] methyamine hydrochloride (3.7 MBq, 1.8 \( \mu \)mol) as described for other labelled gramine syntheses.

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