Synthesis of 1-(β-D-Ribofuranosyl)indol-3-acetic Acid

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By condensation of ethyl indolin-3-acetate (**4**) and 2,3,5-tri-*O*-benzoylribofuranosyl-1-acetate (**5**), ethyl 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indolin-3-acetate (**6**) was obtained in good yield. The indoline nucleoside **6** was aromatized to ethyl 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indol-3-acetate (**7**) with DDQ. The treatment of the indole nucleoside with barium hydroxide and methanol gave the methyl ester **8**, which was further treated in water to give the desired 1-(β-D-ribofuranosyl)indol-3-acetic acid (**9**).

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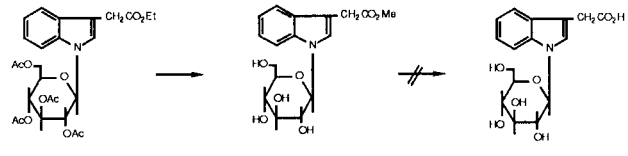
Indol-3-acetic acid is a well known plant hormone [1]. It was first inferred by Darwin in 1880 that grass coleoptiles tended to curve towards light, and found that this response could be negated by shading the coleoptile tip. This suggested the formation of a diffusible substance in the tip which promoted the growth of the coleoptile tissue. These observations eventually led to the demonstration that decapitated coleoptiles could be induced to curve in the dark by the application of a diffusible substance obtained from coleoptile tips in agar blocks [2]. Indeed, this material could replace the tip of the plant and the substance was found to have identical diffusion and physiological properties to the compound, indol-3-acetic acid (IAA) [3]. Later, this growth promoting hormone was isolated from higher plant tissues [4]. It has been shown that tryptophan is a precursor of IAA although the details of the pathways may differ between species [5]. IAA undergoes oxidation by oxidase *in vivo*. When exogenous IAA is supplied to plants, it is usually converted either to 1-(indol-3-ylacetyl)-β-D-glucose [6] or indol-3-ylacetyl-L-aspartic acid [7]. One of the most striking biochemical effects of IAA is the enhancement of RNA synthesis. The mechanism whereby IAA enhances the synthesis of RNA still remains to be determined, although it has been shown that ^{14}C labeled IAA is incorporated into RNA [8-10]. It was reported that IAA couples directly with RNA, while some IAA is degraded with the products of degradation being utilized in RNA synthesis [9]. The radioactivity was found in all types of RNA [8]. However, it is not clear how and in what form the IAA (or degradation products) is incorporated into RNA although it has been shown in some cases that the cytidine and adenine moieties are labeled [8]. Thus, as a part of our efforts to study the mode of action of IAA, we report the synthesis of 1-(β-D-ribofuranosyl)indol-3-acetic acid (**9**).

Although various indole nucleosides have been reported [11-18], the most common indole nucleosides prepared contain the glucose moiety [11,12,14,16] instead of ribose. Methyl 1-(β-D-glucopyranosyl)-3-indolacetate (**1**) was synthesized in an effort to study the mode of action of IAA [15]. However, the ester **1** could not be hydrolyzed to its acid at the final step. Furthermore, no indol-3-acetic acid riboside has previously been synthesized. Thus, it was of interest to prepare 1-(β-D-ribofuranosyl)indol-3-acetic acid for biological tests.



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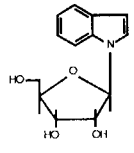
The most common method of synthesis of indole nucleosides has been the condensation of indolines with carbohydrates, which were then aromatized to the indole ring system [11-17]. Synthesis of **9** followed the same approach, in which the indoline derivative **4** was condensed with the ribosyl acetate **5**. Since ethyl indol-3-acetate (**3**) is a relatively expensive reagent as a starting material, it was of interest to develop an efficient preparative method for **3** from indol-3-acetic acid (**2**), which is readily available and cheap. Several methods of esterification using acids such as hydrogen chloride, sulfuric acid, *etc.*, as a catalyst are available [18,19]. However, we developed an efficient esterification procedure, in which simple refluxing of the acid **2** and absolute ethanol with a strong cationic resin such as Dowex 50 (H^+) gave a quantitative yield of **3**. The reduction of the indole **3** to indoline **4** was readily achiev-

ed by borane-pyridine complex [20,21] in high yield (95%). The indoline **4** was previously prepared in low yield from indolin-3-acetic acid [22]. The indoline **4** was then treated with the ribose acetate **5** under acidic conditions to obtain the indoline-nucleoside **6** in high yield (92%). Due to the complexity of pmr spectrum, the anomeric configuration of **6** could not be determined. The anomeric configurations of these types of compounds have never been assigned. However, it is interesting to mention that no α-isomer has been reported from similar reactions. This was also found to be the case (*vide infra*). Oxidation of indoline **6** to the indole-nucleoside **7** was accomplished by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in low yield (29%) after silica gel column chromatography. Again, the assignment of the anomeric configuration of **7** was impossible to make, due to the virtual coupling of other than first-order $H_{1',2'}$ coupling in the pmr spectrum.

In order to obtain the free nucleoside **9**, the benzoyl protected nucleoside **7** was treated with barium hydroxide in methanol, from which the methyl ester **8** was obtained instead of the acid **9**. A similar reaction was also observed by Franklin and Sell [15] in which they also obtained **1** instead of **11**. Furthermore, they were unable to obtain the free nucleoside **11** from **1**.



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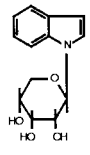

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However, the fully deblocked nucleoside **9** was successfully obtained by treating **8** with barium hydroxide in water resulting in a moderate yield (43%). A partial formation of barium salt with **9** made it difficult to obtain an analytical sample. However, by using a strong cationic resin as a neutralizing agent we overcame this problem. The structural determination of **9** was made on the basis of the pmr spectrum in which the coupling constant of $H_{1',2'}$, (J = 6.1 Hz) was similar to that of (β-D-ribofuranosyl)indole **12** ($J_{1',2'}$ = 5.0 Hz) [13,14]. Additionally, the spectral pattern of the carbohydrate moiety **9** was very similar to that of **12**, which is quite different from that of the pyranose form **13** [13]. The biological evaluation of **9** is in progress and will be reported elsewhere.

EXPERIMENTAL

^{1}H nmr spectra were recorded on a JEOL FX 90Q spectrometer. Tetramethylsilane was the internal standard. Chemical shifts are reported in parts per million (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), b (broad), m (multiplet). Ultraviolet spectra were recorded on a Bausch and Lomb Spectronic 2000 spectrometer. The mass spectrum was obtained on a Hewlett-Packard 5985 GC/MS system. Thin-layer chromatography was performed on Uniplates purchased from Analtech Co. or Pre-coated tlc sheets [silica gel 60 (F-254)] from EM Laboratories. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

Ethyl Indol-3-acetate (**3**).

A mixture of indol-3-acetic acid (10.0 g, 57 mmoles), absolute ethanol (50 ml) and Dowex 50 (H^+) (20-50 mesh, 10 g) was refluxed for 24 hours (the reflux condenser was attached with a Dean-Stark receiver containing 20 ml of molecular sieve type 3A, (8-12 mesh). The reaction mixture was filtered and the filtrate was evaporated to dryness to give a chromatographically pure brownish syrup (11.6 g, quantitative). The syrup was triturated with 30% ethanol and then stored in a refrigerator for overnight. The resulting solid was filtered and dried to give a pale brownish powder (11.2 g, 97%), mp 41-43° (42-43° reported) [23].

Ethyl Indolin-3-acetate (**4**).

To a mixture of ethanol and concentrated hydrochloric acid (1:1) (50 ml) and ethyl indol-3-acetate (**3**) (10.2 g, 50 mmoles), a pyridine-borane complex (25 ml, 250 mmoles) was added dropwise with vigorous stirring while cooling in an ice-water bath. After the addition, the stirring was continued for 20 minutes under the same conditions. The mixture was then evaporated to a syrup to which a 10% sodium carbonate solution (400 ml) was added to adjust to *p*H 8. The resulting alkaline mixture was extracted with benzene (3 x 80 ml), which was washed (saturated sodium chloride solution, 80 ml), dried (sodium sulfate) and then evaporated to give **4** as a brownish syrup (9.8 g, 95%) [22], which was used in the next step without further purification.

Ethyl 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)indolin-3-acetate (**6**).

A mixture of 2,3,5-tri-*O*-benzoylribofuranose-1-acetate (**5**) (8.0 g, 15.9 mmoles), ethyl indolin-3-acetate (**4**) (9.8 g, 47.8 mmoles), glacial acetic

acid (9.8 ml), and absolute ethanol (100 ml) was heated at reflux for 5 hours. The reaction mixture was then evaporated to give a brownish syrup. The syrup was dissolved in benzene (120 ml), washed with a 10% sodium bicarbonate solution (2 x 60 ml), dried (sodium sulfate), and then evaporated to give a brownish syrup. The syrup was chromatographed on a short vacuum column (7 x 8 cm) using benzene-ethyl acetate (20:1) as the eluent. Evaporation of the solvents gave **6** as a white glassy solid (9.5 g, 92%); uv (methanol): λ max 231 nm 274 (sh), and 282; nmr (deuteriochloroform): δ 1.21 (t, 3H, CH_3), 2.26-2.94 (m, 2H, CH_2-$CO_2C_2H_5$), 3.35-4.25 (m, 5H, -CH_2CH_3 and indoline), 4.45-4.83 (m, 3H, $H_{4'}$, $H_{5'}$ and $H_{5''}$), 5.75-5.95 (m, 3H, $H_{1'}$, $H_{2'}$, and $H_{3'}$) and 6.65-8.20 (m, 19H, aromatic).

Anal. Calcd. for $C_{38}H_{35}NO_9$: C, 70.25; H, 5.43; N, 2.16. Found: C, 70.03; H, 5.46; N, 2.06.

Ethyl 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)indol-3-acetate (**7**).

A mixture of ethyl 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indolin-3-acetate (**6**) (9.5 g, 14.6 mmoles) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (3.3 g, 14.5 mmoles) in dry toluene (220 ml) was refluxed for 5 hours. After cooling to room temperature, the mixture was filtered and the dark-brown filtrate was evaporated to a syrup. The resulting syrup was chromatographed on a short vacuum silica gel column (7 x 8 cm) using benzene-ethyl acetate (20:1) as the eluent. The combined fractions were evaporated to give crude **7** as a greenish syrup (2.8 g, 30%). An analytical sample was obtained from preparative thin-layer chromatography using methyl ethyl ketone-*n*-hexane (1:4) as the eluent; uv (methanol): λ max 224 nm, 266, 272, 280, and 290; nmr (deuteriochloroform): δ 1.20 (t, 3H, CH_3), 3.61 (s, 2H, CH_2-$CO_2C_2H_5$), 4.11 (q, 2H, $CO_2CH_2CH_3$), 4.6-4.90 (m, 3H, $H_{4'}$, $H_{5'}$ and $H_{5''}$), 5.93-6.07 (m, 2H, $H_{2'}$ and $H_{3'}$), 6.39-6.50 (m, 1H, $H_{1'}$), and 7.10-8.20 (m, 20H, aromatic).

Anal. Calcd. for $C_{38}H_{33}NO_9$: C, 70.47; H, 5.14; N, 2.16. Found: C, 70.52; H, 5.18; N, 2.14.

Methyl 1-(β-D-Ribofuranosyl)indol-3-acetate (**8**).

A mixture of ethyl 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indol-3-acetate (**7**) (1.0 g, 1.5 mmoles) and barium hydroxide octahydrate (0.5 g, 1.6 mmoles) in methanol (60 ml) was stirred at room temperature for one hour. The reaction mixture was then neutralized with Dowex 50 (H^+) ion exchange resin. After filtration, the filtrate was evaporated to give a brownish syrup, which was chromatographed on a short vacuum silica gel column (5 x 10 cm) using chloroform-methanol (10:1) as the eluent. Evaporation of the combined fractions gave crude **8** as a yellowish solid (0.3 g, 50%). An analytical sample was obtained from a preparative thin-layer chromatography using chloroform-methanol (20:1) as the eluent; uv (water): λ max 221 nm, 272 (sh), 280, and 289 (sh); nmr (deuteriochloroform): δ 3.55 (s, 3H, CH_3), 3.54 (m, 2H, $H_{5'}$, $H_{5''}$), 3.62 (s, 2H, benzylic protons), 3.02-4.20 (m, 6H, OH, $H_{2'}$, $H_{3'}$ and $H_{4'}$), 5.72 (d, 1H, $H_{1'}$, $J_{1',2'}$ = 4.9 Hz), and 6.85-7.50 (m, 5H, aromatic).

Anal. Calcd. for $C_{16}H_{19}NO_6$: C, 59.81; H, 5.96; N, 4.36. Found: C, 59.67; H, 6.04; N, 4.36.

1-(β-D-Ribofuranosyl)indol-3-acetic Acid (**9**).

A mixture of methyl 1-(β-D-ribofuranosyl)indol-3-acetate (**8**) (0.21 g, 0.7 mmole), barium hydroxide octahydrate (0.15 g, 0.5 mmole), and water (15 ml) was stirred for 3 hours. The mixture was then neutralized with Dowex 50 (H^+), stirred for 30 minutes and then filtered. The filtrate was concentrated then freeze-dried to give **9** as a white solid (0.18 g, 81%). The product **9** was extremely hygroscopic [24]. Thus, we were unable to report the melting point; uv (water): λ max 220 nm (25,470), 270 (4,550), 278 (sh) (4,550), and 288 (sh) (3,470), (*p*H 1.0); 222 (24,890), 272 (sh) (4,060), 280 (4,330), and 289 (sh) (3,530), (*p*H 7.0); 223 (24,640), 272 (sh) (3,900), 281 (4,430), and 289 (sh) (3,690), (*p*H 13.0); nmr (dimethylsulfoxide-d_6): δ 3.64 (m, 4H, $H_{5'}$, $H_{5''}$ and benzylic protons), 3.90 (q, 1H, $H_{4'}$, $J_{3',4'}$ = 3.5 Hz), 4.06 (t, 1H, $H_{3'}$, $J_{2',3'}$ = 5.3 Hz), 4.25 (t, 1H, $H_{2'}$), 4.60-5.40 (b, 3H, OH, exchangeable) 5.84 (d, 1H, $H_{1',2'}$, J = 6.1 Hz), 6.96-7.10 (m, 5H, aromatic) and 12.20 (s, 1H, COOH); ms: 307 (M^+), 289 (M^+ -18), 263 (M^+ -44), 175 (M^+ -132), and 130 (M^+ -177).

Anal. Calcd. for $C_{15}H_{17}NO_6 \cdot ½ H_2O$: C, 56.96; H, 5.74; N, 4.43. Found: C, 57.11; H, 5.74; N, 4.40.

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