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Note

Tryptophyl β-D-glucopyranoside: chemical synthesis, metabolism, and growth-promoting activity*

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In earlier work on the biogenesis of the plant-growth hormone indol-3-ylacetic acid (IAA), in etiolated pea-seedlings (*Pisum sativum* L. cv. Lincoln), we found[1] that tryptophan and tryptamine, when supplied as precursors, yielded only very small quantities of this hormone. 2-(Indol-3-yl)ethanol (tryptophol) was formed instead and further converted into a metabolite identified as 2-(indol-3-yl)ethyl β-D-glucopyranoside[1] (**1**). Tryptophol is a naturally occurring, plant constituent[2–6]. It may be metabolized to IAA[3,7], and the balance involving the formation and the utilization of this alcohol has been proposed as one of the regulatory mechanisms controlling the endogenous level of IAA[3,8,9]. Any metabolic side-reaction of tryptophol must necessarily interfere with this regulatory function. We therefore undertook the synthesis of tryptophyl glucoside and performed some experiments on its metabolism and physiological activity.

The synthesis was accomplished in two steps, namely, a Koenigs–Knorr condensation of tryptophol with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide to yield the glycoside tetra-acetate (**2**), and its subsequent deacetylation to give **1**. Both **1** and its tetra-acetate were obtained as amorphous solids moderately sensitive to light and atmospheric carbon dioxide. The presence of a free imino-group evidenced by i.r. (ν_{max}^{KBr} 3400 cm^{-1} for **2**) and ^{1}H-n.m.r. spectroscopy (δ 8.38, disappearing on deuteration) indicated that the alcohol function of tryptophol is involved in the glycosidic linkage. The uncommon upfield-shift of one of the acetoxyl signals of **2** (δ 1.83) is paralleled by that reported for the AcO-2 group of 2,3,4,6-tetra-*O*-acetyl-1-*O*-(indol-3-ylacetyl)-β-D-glucopyranose[10], the structure of which is closely related to that of **2**. The coupling constant ($J_{1,2}$ 7 Hz) for the anomeric proton indicated the β-D configuration. The anomeric purity of **1** was demonstrated by the fact that it was completely digested by β-D-glucosidase, but not attacked by α-D-glucosidase.

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No difference between synthetic **1** and the isolated plant-metabolite could be detected in enzymic hydrolysis. The two samples were also indistinguishable when chromatographed in acidic, neutral, and alkaline solvents of various polarity. Comparison of the mass spectra of the fully *O*-acetylated plant-metabolite and the synthetic compound **2**, by the method of Hertz *et al.*[11], gave a similarity index of 0.74.

When incubated with etiolated pea-seedlings, ~50% of synthetic **1** was converted into tryptophol, and ~50% of **1** was recovered. Tryptophol was identified by t.l.c. in standard solvents[17] which clearly distinguish this compound from any other metabolite that might reasonably be expected (*e.g.*, IAA and indol-3-ylacetaldehyde). The glucoside **1** did not spontaneously hydrolyse under the conditions (pH 4.55) of the above experiment. As in former work on the biogenesis and metabolism of IAA in etiolated pea-seedlings[1,12], we could find no indication for a participation of contaminant micro-organisms in the cleavage of **1**. Our data indicate, therefore, that the previously reported[1] metabolic conjugation of tryptophol with glucose is reversible.

Since the main, indolic metabolite of **1**, tryptophol, is converted into IAA by plants[3,7], the glucoside should also promote plant growth. This was indeed observed, using the pea-stem, straight-growth test[13–16]. The effective concentrations were ~4 orders of magnitude larger than reported[13] for IAA. This would be expected for a "reserve auxin" undergoing slow conversion into the active growth-hormone IAA.

EXPERIMENTAL

General methods. — T.l.c. on silica gel (Merck) was performed with the conditions and solvent systems given by Stahl[17]. A 1:1 (w/w) mixture of Kieselgel H (Merck) and Celite (Hyflo Super Cel, C. Roth, Germany) was used for column chromatography. Optical rotation was determined on a Zeiss Kreispolarimeter. ^{1}H-N.m.r. spectra were recorded at 60 MHz (internal Me_4Si). Mass spectra (70 eV; trap current, 100 μA; ion-source temperature, 160°) were recorded on a Varian CH-7 mass spectrometer, with a solid-probe inlet system, at 140°. Enzymic hydrolysis was performed at 35° with β-D-glucosidase (B.D.H.) from sweet almonds (5 mg/ml in 0.1M sodium citrate buffer[18], pH 5.0) and α-D-glucosidase (Sigma), type I, from yeast (1 mg/ml in 0.067M potassium phosphate buffer, pH 7.0, containing 0.1 mg/ml of glutathione[19,20]), for 1.5 or 2.5 h, respectively. After precipitation of the enzymes with ethanol, the supernatant solutions were concentrated and examined by t.l.c.

*2-(Indol-3-yl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside* (**2**). — 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1.23 g) in dichloromethane (8 ml) was added to a solution of tryptophol (0.48 g) in ether (15 ml) containing Drierite (1.5 g) and freshly prepared silver oxide (0.48 g), and the mixture was agitated overnight. After centrifugation, the supernatant solution was concentrated *in vacuo* and immediately passed through a column of silica gel–Celite (600 g) with dichloromethane–ether (10:1.5), to yield analytically pure **2** (704 mg), m.p. 45–47°, and a second crop containing minor impurities (389 mg; total yield, 74%). ^{1}H-N.m.r. data ($CDCl_3$):

δ 1.83, 1.93, 1.98, 2.02 (4 s, 4 AcO), 3.02 (t, J 6–7 Hz, Ar–CH_2-CH_2O), 4.50 (d, $J_{1,2}$ 7 Hz, H-1), 6.95–7.70 (m, 5 H, indolic CH), and 8.38 (s, broad, indolic NH).

Anal. Calc. for $C_{24}H_{29}NO_{10}$: C, 58.63; H, 5.95; N, 2.85. Found: C, 58.48; H, 5.99; N, 2.86.

2-(Indol-3-yl)ethyl β-D-glucopyranoside (**1**). — **2** (250 mg), dissolved in methanol (5 ml), was deacetylated with sodium methoxide (2 mg; monitoring by t.l.c., dichloromethane–methanol, 9:1). After neutralization (Dowex 50W-X8), immediate filtration, and evaporation, the crude glucoside was passed through a column of silica gel–Celite (70 g). Gradient elution was applied, changing the ratio of dichloromethane–acetone–methanol from 5:1:0 to 5:1:2 (total volume, 1500 ml). The final fractions gave **1** (120 mg) as an amorphous, white solid (no sharp m.p.) which contained bound acetone and methanol (~10 mg; estimated by ^{1}H-n.m.r. spectroscopy and weight loss on heating to 130°). In order to remove the bound solvents, the product was sublimed at 140°/10^{-6} Torr; $[\alpha]_D$ −21° (*c* 1.4, water). ^{1}H-N.m.r. data (D_2O): δ 7.00–7.75 (m, 5 H, indolic CH), 4.33 (d, $J_{1,2}$ 7 Hz, H-1), and 3.02 (t, J 7 Hz, Ar-CH_2-CH_2O).

Anal. Calc. for $C_{16}H_{21}NO_6$: C, 59.43; H, 6.55; N, 4.13. Found: C, 59.39; H, 6.31; N, 4.26.

Comparison of the mass spectra of **2** *and the fully* O*-acetylated tryptophol conjugate*[1] *of plant origin.* — According to the method of Hertz *et al.*[11], the mass spectrum of **2** was "abbreviated" as follows. Beginning at 6 a.m.u., it was divided into successive arrays of 14 a.m.u., and the mass numbers and relative intensities (in parentheses) were determined. In the following listing, arrays are separated by "/", and arrays below 49 a.m.u., containing ions of small discriminating value, are omitted: 55 (7.5), 57 (7.5)/ 69 (7.0,) 73 (6.6)/ 77 (4.3), 81 (5.9)/ 97 (5.9), 103 (7.0)/ 115 (12.9), 117 (21.5)/ 130 (89), 131 (9.7)/ 143 (64.5), 144 (100.0)/ 157 (3.1), 160 (2.1)/ 161 (3.2), 169 (6.5)/ 186 (1.7), 187 (1.1)/ 189 (1.9), 200 (1.0)/ 203 (0.6), 211 (0.9)/ 226 (0.9), 229 (0.9)/ 242 (0.6), 243 (0.4)/ 245 (0.4), 256 (0.4)/ 259 (0.4), 270 (0.3)/ 286 (0.9)/ 287 (0.4), 298 (0.9)/ 311 (0.8), 312 (0.4)/ 331 (0.8), 332 (0.2)/ 418 (0.3)/ 431 (0.3), 432 (0.2)/ 449 (0.4), 450 (0.2)/ 491 (M^+, 19.3), 492 (5.4).

The mass spectrum of the fully *O*-acetylated tryptophol conjugate of plant origin[1] was treated in the same manner, and the similarity index, with regard to the spectrum of synthetic **2**, was determined as the average weighted-ratio of corresponding ion intensities, divided by the fraction of unmatched intensities plus one[11].

Metabolic experiments. — Etiolated pea-seedlings (30 g, 1–2-cm sections) were *vacuum* infiltrated with a solution of **1** (30 mg) in 0.067M KH_2PO_4 (pH 4.55; 25 ml) and incubated (22°, 6 h)[1,12]; the plant material was then extracted with methanol (2 × 45 ml). Partial purification of the extract was achieved by successive concentrations to 10 and 3 ml, addition of equal volumes of acetone, and centrifugation. After evaporation of the final supernatant solution, the residue was dissolved in water (5 ml), and the solution was passed through a column of Sephadex G-10 (14 × 2.5 cm; 70 ml). The elution of various plant-constituents and unchanged conjugate **1** was accomplished with 0.05M NaCl (470 ml). The column was then washed with water

(70 ml), followed by a methanol–water gradient (open mixing-chamber, initially containing 100 ml of water; initial content of the reservoir, 350 ml of methanol) which eluted tryptophol.

When a mixture of authentic indolic compounds was separated by this method, IAA was eluted by the water, and tryptophol with the gradient.

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