SYNTHESIS OF CARBOXYL-^{14}C-LABELLED 1-O-(3'-INDOLYLACETYL)-β-D-GLUCOPYRANOSE

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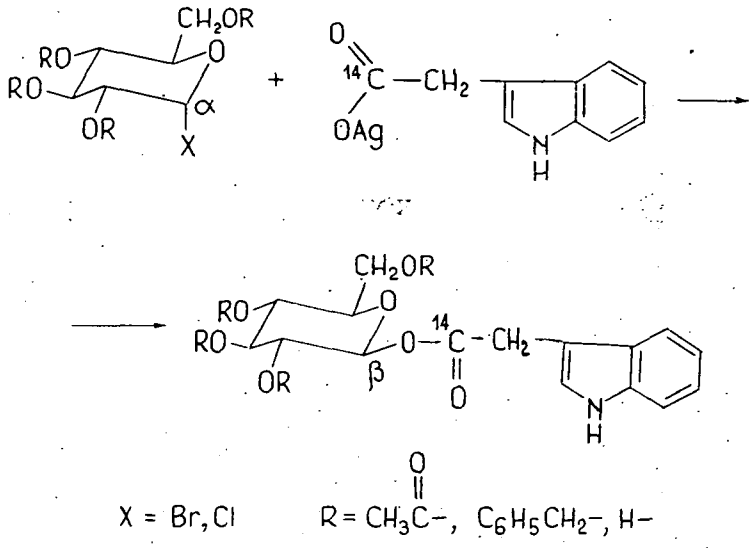
For studying indole derivatives with hormonal activity occurring in plants, it is necessary to synthesize ^{14}C-labelled compounds. The paper describes the syntheses of carboxy-^{14}C-labelled 2,3,4,6-tetra-O-benzyl-1-O-(3'-indolylacetyl)-β-D-glucopyranose, 2,3,4,6-tetra-O-benzyl-1-O-(3'-indolylacetyl)-β-D-glucopyranose and 1-O-(3'-indolylacetyl)-β-D-glucopyranose.

In recent years a great number of papers dealing with the metabolism of 3-indolylacetic acid in plants were published. At the same time various 3-indolylacetic acid conjugates, drawing attention to the different sugar conjugates of 3-indolylacetic acid [7, 11, 12] and its amino acid conjugates [1, 6] were also detected. Studies on 3-indolylacetic acid conjugates have also demonstrated that, besides the amino acid or sugar components, phosphoric acid, too, may be linked to 3-indolylacetic acid [8], while the existence of complexes in which 3-indolylacetic acid is bound to nucleic acids [3] permits the conclusion that the 3-indolylacetic acid—nucleotide conjugates are also of importance in the metabolism of 3-indolylacetic acid.

The β-D-glucopyranoside of 3-indolylacetic acid was detected in 1961, on the basis of its qualitative reactions [7, 12]. Similar types of conjugates are formed by various groups of plant materials possessing hormonal activity, *e.g.* gibberellins, cytokinines and abscisic acid. Investigation of the physiological importance of the individual conjugates demands the preparation of the chemically pure compounds both in inactive and radioactive forms. In a great number of cases research workers failed to prepare the authentic conjugate desired and, therefore, experiments concerning their effects and mechanisms of action are virtually still lacking. In the absence of such experiments, numerous authors assume that the various conjugates have no physiological importance [7, 12], and are formed only as detoxification products. The simplest means of deciding wether the 3-indolylacetic acid in the conjugates appears under *in vivo* conditions in the free 3-indolylacetic acid, in a metabolite, or in the other conjugates, is investigation with isotope technique. For this purpose the synthesis of conjugates labelled in the 3-indolylacetic acid seems justified. The *in vivo* formation of 3-indolylacetic acid-β-D-glucopyranose cannot be utilized for the isolation of greater amounts of the compound, nor is it possible to ensure

the specific activity necessary for the biological experiments. On the basis of analogous reactions, carboxy-^{14}C-1-O-(3-indolylacetyl)-β-D-glucopyranose was prepared by synthesis of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl chloride, followed by its coupling with the silver salt of carboxy-^{14}C-3-indolylacetic acid.



Because of the sensitivity of the molecule, the deacetylation may be questionable, therefore, 2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl chloride was used for the preparation of the free glycoside, and, after the separation of the anomers, the benzyl groups could be succesfully removed by reduction on 10% palladium on charcoal, on the analogy of the methods applied in the chemical synthesis of 1-O-(3-indolylacetyl)-β-D-glucopyranose [5].

Chromatographic comparison of the synthetized product indicated that it is not identical with the compound isolated from pea roots, the properties of which have been described [9].

Experimental

Carboxy-^{14}C-3-indolylacetic acid

was prepared from 10.5 mmole gramine and 40 mmole 20 mCi K^{14}CN by the method of GORDON *et al.* [4], by the reaction path indole, gramine, 3-indolylacetonitrile-^{14}C, 3-indolylacetic acid-^{14}C. Yield: 70%, 1225 mg, molar activity: 500 μCi/mmole. The radioactive purity was checked chromatographically and autoradiographically.

2,3,4,6-*Tetra*-O-*acetyl*-α-D-*glucopyranosyl chloride*

was synthetized by the method of REDMANN and NIEMANN [10]. Yield: 5%; m.p.: 70—71°C (decomp).

2,3,4,6-*Tetra*-O-*acetyl*-1-O-*indolylacetyl-carboxy*-^{14}C-β-D-*glucopyranose*

2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl chloride (1.12 g, 3.0 mmole) was dissolved in 25 ml abs. benzene and the freshly prepared silver salt of 3-indolylacetyl-carboxy-^{14}C (843 mg, 3 mmole, 750 μCi) was added. The reaction mixture was shaken in the dark at room temperature for 36 hours. The precipitate was separated by centrifugation, and washed once with 5 ml benzene. The combined supernatants were evaporated to dryness. The residue was fractionated on a silica gel column (1 cm in diameter, 40 cm high, containing 8 g silica gel), the solvent being a 1:1 mixture of benzene:ethyl acetate. Dried above concentrated sulfuric acid in vacuum, the chromatographically homogeneous syrup solidified within 24 hours. The 2,3,4,6-tetra-O-acetyl-1-O--(3-indolylacetyl)-carboxy-^{14}C-β-D-glucopyranose was recrystallized from abs. ether—pentane. Yield: 0.64 g, 42%. M.p.: 119—121°C (uncorrected) (lit.: 121—122°C [5]), $[\alpha]_D = -27°$C, c=1, in chloroform (lit. $[\alpha]_D = -26° \pm 0.2°$). Activity: 250 μCi/mmole.

2,3,4,6-*Tetra*-O-*benzyl*-α-D-*glucopyranosyl chloride*

was synthesized from 2,3,4,6--tetra-O-benzyl-α-D-glucopyranose with thionyl chloride by the method of AUSTIN, HARDY *et al.* [2]. The product was purified by chromatography on a silica gel column, 2:1 petroleum ether—ether mixture being used for elution. The product was a pale-yellow oil. Yield: 55%. The purity was checked by thin-layer chromatography.

2,3,4,6-*Tetra*-O-*benzyl*-1-O-*(3-indolylacetyl)-carboxy*-^{14}C-β-D-*glucopyranose*

A mixture of the halogen sugar (2.57 g, 4.61 mmole), Ag salt of 3-indolylacetic acid-carboxy-^{14}C (1.3 g, 4.6 mmole) and 1 g anhydrous $CaSO_4$ protected by aluminium foil was refluxed in 100 ml absolute benzene for 8 hours, with magnetic stirring, under exclusion of moisture. The reaction was checked by thin-layer chromatography in a 3:2 mixture of ether—petroleum ether. The precipitate was separated by centrifugation and washed with 2×5 ml benzene. The combined supernatant was evaporated in vacuum, and the residue chromatographed on a silica gel column, with 3:2 ether—petroleum ether as eluent; 4 ml fractions were collected. The unchanged chloride was eluted first, then the desired ester product (2.26 g) with a little colouring matter, and finally, by chloroform elution, the substituted tetrabenzyl-glucose. The anomer composition can be checked by thin-layer chromatography in a 3:2 ether—petroleum ether mixture. A violet double spot was obtained with 10% sulfuric acid. The reaction mixture was separated in layers, and the more slowly moving β-anomer fractions were collected, dissolved in abs. benzene, and evaporated to dryness. The residual oil was dissolved in abs. benzene, petroleum ether was added, and after 8 hours at 0°C the β-anomer had crystallized (0.8 g); m.p.: 98—100°C; $[\alpha]_D = -1.7°$, c=1.0, in chloroform. The physical constants agree with those found in literature [5]. The α-isomer could not be isolated as crystals. Activity: 0.25 mCi/mmole. Chromatographically homogeneous.

1-O-*(3-indolylacetyl)-carboxy*-^{14}C-β-D-*glucopyranose*

400 mg 2,3,4,6-tetra-O-benzyl-1-O-3-indolylacetyl-carboxy-^{14}C-glucopyranose was dissolved in 15 ml 2-methoxyethanol, and reduced for 30 hours at room temper-

ature with 500 mg 10% palladium on charcoal in the presence of 0.4 ml acetic acid. The reaction mixture was checked chromatographycally in a 6:2:1 mixture of ether—petroleum ether—methanol, with 10% sulfuric acid as developer. The debenzylated glycoside had an R_f-value of ca. 0.1 and was reddish-violet. The catalyst was removed by centrifugation and washed with 2-methoxyethanol, and the solution was evaporated in vacuum at 35°C. The remaining 200 mg light-violet oil was dissolved in a 55:30:11 mixture of isopropanol—petroleum ether—water, and chromatographed with this solvent on a column containing 25 g cellulose. The individual fractions were checked by layer chromatography, and the eluates containing the glycoside were collected and evaporated in vacuum. The oily residue was extracted with 3×10 ml ethyl acetate. The extract was evaporated to 1/3 volume and petroleum ether was added until the first signs of turbidity. The glass-like material at 0°C, after 24 hours, solidified over concentrated sulfuric acid, and was pulverized to a pink powder (80 mg). This was extracted with a 20:1 mixture of ethyl acetate—isopropanol, a few drops of petroleum ether were added, and the filtered solution was left to stand at 0°C to separate the remaining coloured material. The practically colourless supernatant was put in a centrifuge tube and precipitation was promoted with petroleum ether. The 1-O-(3-indolylacetyl)-carboxy-^{14}C-β-D-glucopyranose crystallized in the form of very pale pinkish-white needles. Yield: 40 mg. M.p.: 168—172°C (lit. m.p.: 172—173°C [5]). Paper-chromatographic R_f-value in 12:3:5 butanol—acetic acid—water: 0.7. Chromatographycally homogeneous. Activity: 0.25 mCi/mmole.

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СИНТЕЗ 1-О-(ИНДОЛ-3'-ИЛАЦЕТИЛ)-β-D-ГЛЮКОПИРАНОЗЫ МЕЧЕНОЙ ПО КАРБОКСИ-^{14}С

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Синтез ^{14}С меченных соединений, необходим к изучению индолных веществ растений, имеющих гормональное действие. В работе описан синтез 2,3,4,6-тетра-О-ацетил-1-О-(индол-3'-илацетил)-β-D-глюкопиранозы, 2,3,4,6-тетра-О-бензил-10-(индол-3'-илацетил)-β-D-глюкопиранозы и 1-О-(индол-3'-илацетил)-β-D-глюкопиранозы, меченых по карбоксил-^{14}С.