Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIII, No. 10

Synthesis of the β-D-Glucosyl Ester of [*carbonyl*-^{13}C]-Indole-3-Acetic Acid

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SUMMARY

An efficient, operationally simple synthetic approach to 1-*O*-([*carbonyl*-^{13}C]-indole-3'-ylacetyl)-β-D-glucopyranose is described. The synthesis was carried out by fusing a fully benzylated 1-*O*-glucosylpseudourea intermediate with [*carbonyl*-^{13}C]-indole-3-acetic acid, followed by hydrogenolytic removal of the protective groups.

KEY WORDS: ^{13}C-labeling, indole-3-acetic acid, glucosyl ester

INTRODUCTION

1-*O*-(indol-3'-ylacetyl)-β-D-glucopyranose (IAA-glc) is a key intermediate in the metabolic conjugation of the plant hormone, indole-3-acetic acid (auxin, IAA), a process which participates in auxin transport and storage (1) and also affects the response to exogenously supplied IAA under manipulative conditions, such as plant tissue culture (*e. g.* 2). In naturally occuring conjugates, the carbonyl group of IAA may be linked *1) via* an amide bond to an amino acid or peptide, or *2) via* an ester bond to carbohydrate and/or inositol molecules (1, 3, 4). The involvement of IAA-glc in the formation of amide-type conjugates is plausible on chemical grounds (5, 6); its role in the biogenesis of the *O*-(indol-3'-ylacetyl)-*myo*-inositols has been demonstrated by detailed enzymatic studies (7 - 13). Transgenic plants overexpressing the *iaaM*

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0362–4803/93/100933–07$08.50
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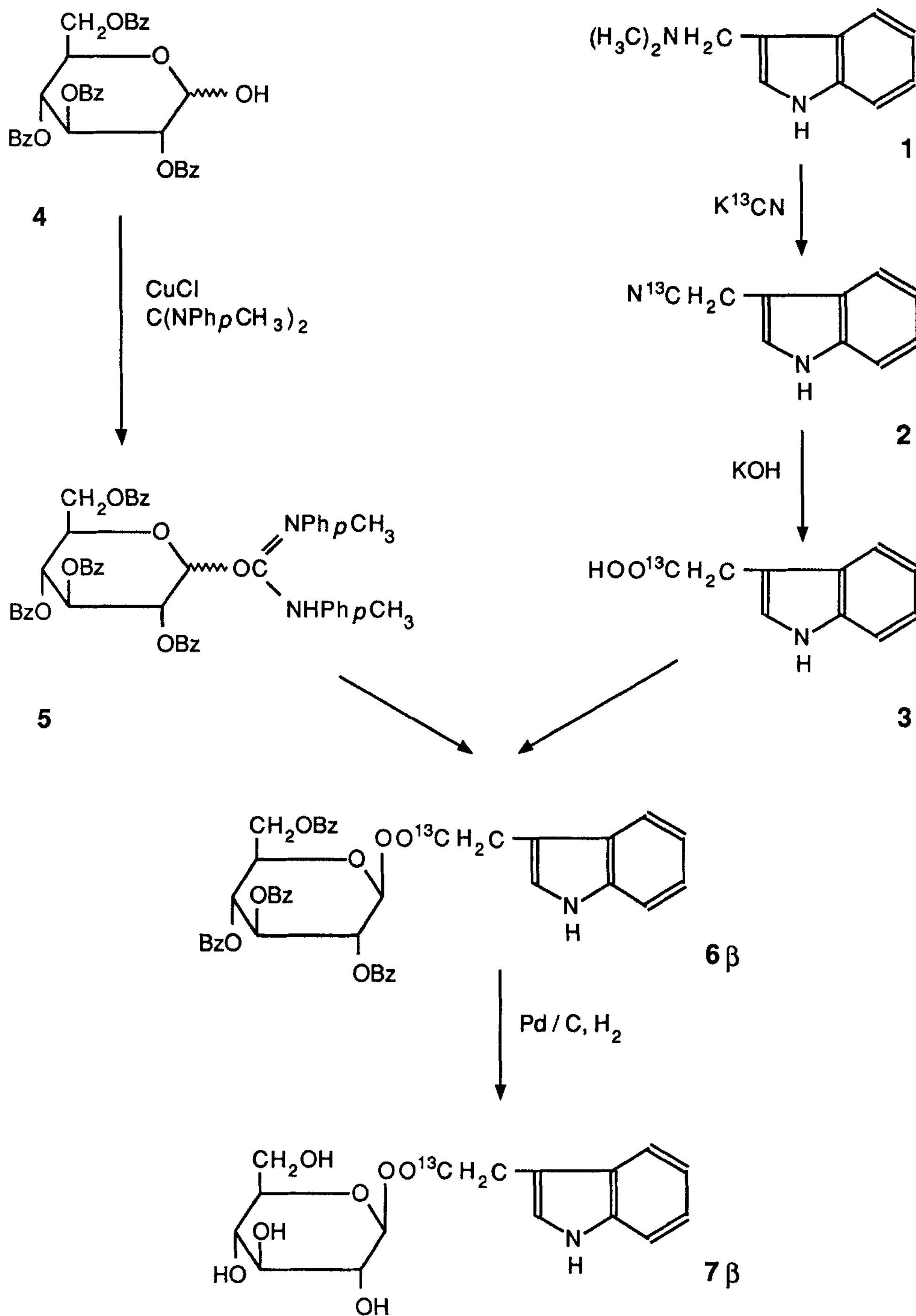
Received 15 April, 1993
Revised 10 May, 1993

and *iaaH* genes from *Agrobacterium tumefaciens* increase IAA conjugation in an attempt to down-regulate the auxin pool (14). IAA-glc is among the conjugates formed, and detailed studies of this process would be facilitated by the availability of an adequately labeled form of the glucoside. The method which permitted access to the unlabeled sugar conjugate (15) has already been used to prepare the radioactive [*carbonyl*-^{14}C]-IAA-glc (16). Its application in biological systems has, however, been limited by the laborious synthesis and by the general sensitivity of indolic compounds to radiolytic decomposition. Here we present an improved method for the preparation of IAA-glc; its application for the synthesis of the [*carbonyl*-^{13}C]-labeled glucoside (7β) is outlined in Scheme 1. Gramine [3-(*N,N*-dimethylaminomethyl)indole] (**1**) was reacted with [^{13}C]-KCN and the resulting nitrile **2** was saponified to yield [*carbonyl*-^{13}C]-IAA (**3**). Coupling to tetrabenzylglucose **4** was achieved *via* an *O*-glucosylpseudourea intermediate (**5**); the resulting fully benzylated glucoside **6β** was, after removing minor contamination by the α-anomer, deprotected to yield **7β**. This approach, in addition to its simplicity, required less time and afforded higher yields of the desired β-anomer than the previously reported method (15).

EXPERIMENTAL

General.

Melting points were determined in open capillaries on a Tottoli (Büchi) apparatus and are reported uncorrected. Optical rotations were measured on a digital polarimeter (Anglia Instruments Ltd., model AA-10). Thin-layer chromatography was on glass plates coated with silica gel 60 F_{254} (Merck) using the following solvent systems: A, 2-propanol/ethyl acetate/NH_4OH (35:45:20; by vol.); B, dichloromethane/methanol/acetic acid (5:1:0.2; by vol.); C, light petroleum/benzene/ethyl acetate (5:5:1; by vol.) and D, ethyl acetate/acetic acid/water (70:2:2; by vol.). Indolic compounds on chromatograms were detected by Ehmann's reagent (17) or, together with sugar derivatives, by charring with sulfuric acid. Column chromatography was on silica gel 60 (Merck), particle size 70 - 230 mesh (solvent flow by gravity) or 230 - 400 mesh (flash chromatography). The homogeneity of glucoside **7β** was checked on a Varian 9010 liquid chromatograph, using a 5 μm, 250 x 4.6 mm i. d. octadecyl-Si 100 reversed-phase column (Serva) equilibrated with 40% (by vol.) aqueous methanol containing 0.1% trifluoroacetic acid and eluted (0.5 ml/min) raising the methanol concentration to 75% (by vol.; linear gradient, 20 min). The structures of the prepared compounds were verified by [^{1}H]-NMR spectra (Varian Gemini 300) and by combined high pressure liquid chromatography-mass spectrometry with ionization by fast atom bombardment (FAB HPLC/MS). Details of the latter method have been described previously (18, 19). In brief, the sample was passed through a 250 x 0.32 mm i. d. capillary HPLC column packed with C_{18}-reversed phase silica gel (LC Packings, Amsterdam, The Netherlands), particle size 5 μm, and eluted (4 μl/min) with 30% (by vol.) aqueous methanol containing 1% (by vol.) each of acetic acid and glycerol (matrix for FAB). The effluent was introduced, *via* a frit-FAB HPLC/MS interface (JEOL Ltd., Tokyo, Japan), into the ion source (kept at 50° C) of a double focusing JEOL JMS SX 102 mass spectrometer. Ions were generated with a beam of 5 kV xenon atoms at an emission current of 20 mA, and positive-ion mass spectra were



Scheme 1

acquired at a rate of 3.4 s per scan, with a cycling time of 3.6 s, for a mass range of 20 - 2000 atomic mass units. Daughter ion spectra were obtained by tandem mass spectroscopy (JEOL JMS SX/SX 102A) using *B/E* linked scanning on ions formed in the third field free region.

[*carbonyl*-^{13}C]-Indole-3-acetic acid (3).

A solution of 1.22 g gramine (**1**, 7 mmol, Aldrich) and 2.31 g [^{13}C]-KCN (35 mmol, 99 atom % ^{13}C, Larodan AB, Malmö, Sweden) in 13.2 ml 95% ethanol and 3.3 ml water was boiled (7 days) until complete consumption of **1** was indicated by thin-layer chromatography in solvents A (R_F = 0.8) and B (R_F = 0.3). Simultaneously, the primary product, nitrile **2**, underwent partial hydrolysis to yield (labeled) indole-3-acetamide (R_F = 0.85; solvent B) and **3** (R_F = 0.35; solvent B). The saponification was completed by adding 3.69 g KOH and 2.83 ml water and boiling (7.5 h). The cooled mixture was diluted with 10 ml water and partitioned against 3 x 40 ml diethyl ether to remove non-acidic products. The organic phase was extracted with 4 x 10 ml of 10% (by weight) aqueous K_2CO_3 which was added to the aqueous phase. This was then acidified to pH 2 (in a fume hood!) and partitioned against 5 x 50 ml diethyl ether. The extract was washed with 2 x 10 ml of brine, dried over anhydrous Na_2SO_4, and evaporated to yield 1.08 g (87%) of crude **3** (light brown crystals). Recrystallization from chloroform/cyclohexane (about 10:1, by vol.) gave white platelets, m. p. 168° C. Lit. (20) (unlabeled): 168 - 169° C.

2,3,4,6-Tetra-*O*-benzyl-1-*O*-([*carbonyl*-^{13}C]-indol-3'-ylacetyl)-β-D-glucopyranose (6β).

A mixture of 1080 mg of **4** (2.0 mmol) prepared according to (21), 444 mg 1,3-di-*p*-tolylcarbodiimide (2.0 mmol) and 10 mg Cu(I)Cl (0.1 mmol) was fused at 80° C for 10 min. To the melt 175 mg of **3** (1.0 mmol) were added and the mixture was heated at 80° C for 30 min. The crude product was dissolved in 10 ml dichloromethane and left at 0° C for 3 h. The 1,3-di-*p*-tolylurea was filtered off, the filtrate evaporated and the residue passed through a column of silica gel eluted with solvent C. The fractions containing the β-anomer (**6β**, R_F = 0.37; contaminant α-anomer: R_F = 0.44; solvent C) were pooled and the residual oil crystallized from diisopropyl ether to yield 299 mg of pure **6β** (43%), m. p. 105 - 106° C, $[\alpha]^{22}_D$ +1.03 (c 1, $CHCl_3$); lit. (15) (unlabeled): m. p. 99 - 100° C, $[\alpha]^{22}_D$ -1.7±1° (c 1, $CHCl_3$). The [^{1}H]-NMR spectrum (CD_3OD) of *unlabeled* **6β** prepared by our method showed the signal of the anomeric proton at δ = 5.61 ppm (d, $J_{1,2}$ = 8.0 Hz).

1-*O*-([*carbonyl*-^{13}C]-indol-3'-ylacetyl)-β-D-glucopyranose (7β).

To a solution of 100 mg of **6β** (0.143 mmol) in 10 ml 2-methoxyethanol and 1 ml acetic acid, 100 mg of 10% palladium on charcoal (Fluka, puriss.) were added and the mixture was hydrogenated at room temperature and pressure for 3 h. The catalyst was removed by centrifugation and rinsed with 2-methoxyethanol. The combined supernatants were evaporated to dryness and subjected to flash chromatography using solvent D. The fractions containing unprotected glucoside 7β (R_F = 0.35; solvent D) were pooled, evaporated, and the residue was dissolved in 5 ml of dry ethyl acetate by gentle heating. Light petroleum, added gradually at 0° C, precipitated 30 mg (61%) of white needles containing at least 98% (HPLC; retention time: 9.14

min) of 7β, m. p. 172 - 174° C, $[\alpha]^{22}_D$ +3.06 (c 1, methanol). Lit. (15) (unlabeled): m. p. 172 - 173° C, $[\alpha]^{22}_D$ +5±2° (c 1, methanol).

An *unlabeled* sample of 7β prepared by our method was further characterized as follows. [^{1}H]-NMR data (CD_3OD): δ 7.54 (1 H, d, $J_{4',5'}$ = 7.8 Hz, H-4'), 7.34 (1 H, d, $J_{6',7'}$ = 8.2 Hz, H-7'), 7.20 (1 H, s, H-2'), 7.09 (1 H, dd, $J_{5',6'}$ = 7.0 Hz, H-6'), 7.01 (1 H, dd, H-5'), 5.51 (1 H, d, $J_{1,2}$ = 7.3 Hz, H-1) ppm. *Analysis.* Found: C, 57.13; H, 5.50; N, 4.24. $C_{16}H_{19}NO_7$ requires C, 56.97; H, 5.68; N, 4.15.

RESULTS AND DISCUSSION

The synthetic route adopted for the preparation of the β-D-glucopyranosyl ester of [*carbonyl*-^{13}C]-IAA (7β, Scheme 1) follows a general method for *O*-acylation of carbohydrates *via* the corresponding *O*-glycosylpseudoureas which is based on the work of Tsutsumi and Ishido (22).

The labeled starting compound, [*carbonyl*-^{13}C]-IAA (3), was prepared from gramine and [^{13}C]-KCN by slight modification of a classical method (20). Coupling to the carbohydrate moiety was preceded by fusion of the fully benzylated 1-*O*-unprotected sugar 4 with 1,3-di-*p*-tolylcarbodiimide in the presence of a catalytical amount of copper(I)chloride, to generate the *O*-glucosylpseudourea derivative 5 which was not isolated. Further fusion of this activated carbohydrate with 3 afforded glucosyl ester 6β along with exceptionally small amounts of the corresponding α-anomer. After silica-gel column chromatography and repeated crystallization, pure 6β was obtained in 42 - 43% yield. β-Configuration was confirmed by comparison with an authentic (unlabeled) sample synthesized according to Keglevic and Pokorny (15). In particular, in the [^{1}H]-NMR spectrum, the anomeric proton was presented by a single 8 Hz ($J_{1,2}$) doublet at the appropriate chemical shift (measurements performed with an unlabeled sample prepared by our method to avoid possible ambiguities caused by heteronuclear coupling between H-1 and *carbonyl*-^{13}C).

In the final step, the protective groups in 6β were removed by hydrogenation in 2-methoxyethanol/acetic acid solution using palladium on charcoal as the catalyst. While the presence of acetic acid helps to prevent solvolysis of the glucosyl-ester bond, catalytic reduction of the indole nucleus is facilitated (15) and closely follows *O*-debenzylation. It was thus essential to monitor the progress of the deprotection reaction by thin-layer chromatography (solvents C and D). The free glucoside 7β was purified by flash silicagel chromatography, followed by crystallization, which afforded the pure compound in 61% yield. Its homogeneity was confirmed by thin-layer chromatography and analytical HPLC. These methods are appropriate to detect products of acyl migration, 2-*O*, 4-*O*, and 6-*O* esters of IAA and glucose, as the most critical contaminants (13, 23). Such impurities would also reveal themselves by additional [^{1}H]-NMR signals in the region characteristic for the anomeric sugar proton H-1 (23). The glucoside prepared by our method (*unlabeled* sample for the reasons stated above) showed only a single one-proton resonance for H-1; its chemical shift (5.51 ppm) and the coupling constant $J_{1,2}$ (7.3 Hz) unequivocally identify the compound as 1-*O*-(indol-3'-ylacetyl)-β-D-glucopyranose. The purity and the structure of 7β

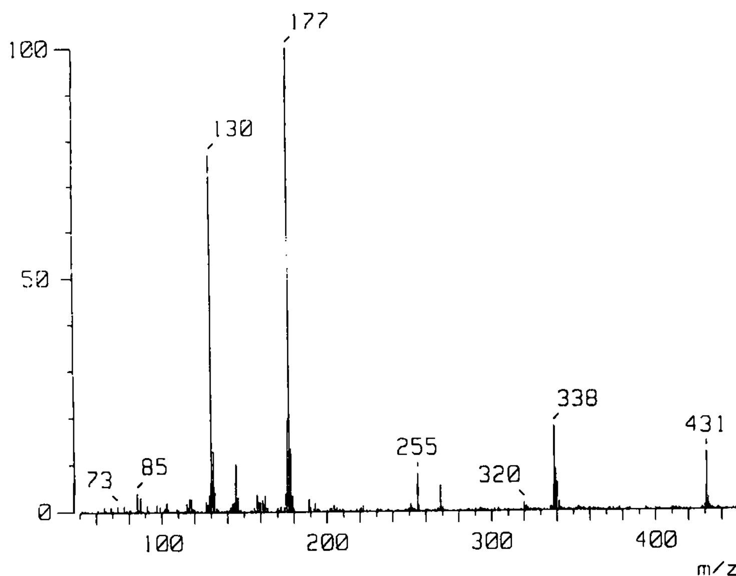


Fig. 1. Mass spectrum of 1-*O*-([*carbonyl*-^{13}C]-indol-3'-ylacetyl-β-D-glucopyranose (7β) obtained by positive ion FAB HPLC/MS using a glycerol matrix.

The units along the ordinate represent relative ion intensities in per cent of the base peak (*m/z* 177). For the *unlabeled* glucoside the following diagnostically important ions showed isotope shifts to yield *m/z* 430, 337, 319, 268, 176, and 175, while there were no such shifts for *m/z* 255 and 130. Further interpretations and details on the experimental conditions are given in the text.

were further confirmed by FAB HPLC/MS, a method which affords diagnostically significant fragment ions otherwise difficult to obtain (Figure 1), and which is thus particularly useful for the identification of IAA-glc in plant extracts (14, 19). The labeled 7β was eluted from the capillary HPLC column in a single peak with a retention time of 5.6 min. The presence of *carbonyl*-^{13}C was evidenced by the fact that the following ions were shifted by +1 atomic mass unit with respect to the unlabeled glucoside: *m/z* 431 [M + glycerol + H]$^+$, *m/z* 338 [M]$^+$, *m/z* 320 [M - H_2O]$^+$, *m/z* 269 [IAA + glycerol + H]$^+$, *m/z* 177 [IAA + H]$^+$, and *m/z* 176 [IAA]$^+$, while there was no such shift for the quinolinium ion (*m/z* 130) formed by rearrangement of an [indol-3-ylmethylene]$^+$ fragment. The fragment *m/z* 255 was identified by daughter ion spectra as [glycerylglucose + H]$^+$. Fig. 1 also confirms high isotopic purity, although it should be noted that trace contamination by the unlabeled glucoside may be obscured by the relatively high level of background ions in FAB HPLC/MS. Our method for the preparation of 7β does, however, not permit carbon-isotope exchange and scrambling. The final product should thus be of the same isotopic purity as the initial source of label, [^{13}C]-KCN, *i. e.* 99 atom %.

ACKNOWLEDGMENT

This work was supported by research grants by the Croatian Ministry of Science, the Swedish Council for Forestry and Agricultural Research, and the Bo Rydin Foundation.

REFERENCES

1. Cohen, J. D. and Bandurski, R. S. — Annu. Rev. Plant Physiol. 33: 403 (1982)
2. Epstein, E., Kochba, J., and Neumann, H. — Z. Pflanzenphysiol. 85: 263 (1977)
3. Michalczuk, L., Baldi, B. G., and Cohen, J. D. — Plant Biol. 9: 93 (1990)
4. Bialek, K., and Cohen, J. D. — Plant Physiol. 80: 99 (1986)
5. Zenk, M. H. — Coll. Intern. Centre Nat. Rech. Sci. 123: 241 (1964)
6. Horvat, S. and Keglevic, D. — Carbohydr. Res. 108: 89 (1982)
7. Michalczuk, L. and Bandurski, R. S. — Biochem. Biophys. Res. Commun. 93: 588 (1980)
8. Michalczuk, L. and Bandurski, R. S. — Biochem. J. 207: 273 (1982)
9. Leznicki, A. J. and Bandurski, R. S. — Plant Physiol. 88: 1474 (1988)
10. Leznicki, A. J. and Bandurski, R. S. — Plant Physiol. 88: 1481 (1988)
11. Kowalczyk, S. and Bandurski, R. S. — Biochem. J. 279: 509 (1991)
12. Kesy, J. M. and Bandurski, R. S. — Plant Physiol. 94: 1598 (1990)
13. Kowalczyk, S. and Bandurski, R. S. — Plant Physiol. 94: 4 (1990)
14. Sitbon, F., Östin, A., Sundberg, B., Olsson, O., and Sandberg, G. — Plant Physiol. 101: 313 (1993)
15. Keglevic, D. and Pokorny, M. — Biochem. J. 114: 827 (1969)
16. Sirokman, F. and Köves, E. — Acta Phys. Chem. 20: 121 (1974)
17. Ehmann, A. — J. Chromatogr. 132: 267 (1977)
18. Östin, A., Moritz, T., and Sandberg, G. — Biol. Mass Spectrom. 21: 292 (1992)
19. Catalá, C., Östin, A., Chamarro, J., Sandberg, G., and Crozier, A. — Plant Physiol. 100: 1457 (1992)
20. Snyder, H. R. and Pilgrim, F. J. — J. Am. Chem. Soc. 70: 3770 (1948)
21. Perrine, T. D., Glaudemanns, C. P. J., Ness, R. K., Kyle, J., and Fletcher, Jr. H. G. — J. Org. Chem. 32: 664 (1967)
22. Tsutsumi, H. and Ishido, Y. — Carbohydr. Res. 111: 75 (1982)
23. Keglevic, D. — Carbohydr. Res. 20: 293 (1971)