Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

CHEMICAL SYNTHESIS OF *p*-NITROPHENYL β-SOPHOROSIDE AND *p*-NITROPHENYL β-LAMINARABIOSIDE*

R. N. IYER† AND I. J. GOLDSTEIN‡

Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48104 (U. S. A.)

(Received March 21st, 1969)

ABSTRACT

Condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside followed by appropriate deblocking reactions gives rise to an approximately equimolar mixture of *p*-nitrophenyl β-sophoroside and *p*-nitrophenyl β-laminarabioside. The two glycosides are readily separated and isolated in pure form by cellulose-column chromatography.

INTRODUCTION

Our interest in carbohydrate–protein conjugates as model substrates for investigating the interaction of phytohemagglutinins[1,2] and for studying the immunogenicity of carbohydrates[3] prompted us to prepare the β-sophorosyl and β-laminarabiosyl-*p*-azophenyl–protein conjugates. Carbohydrate–protein conjugates are generally prepared by the diazo coupling of *p*-aminophenyl glycosides to tyrosyl, histidyl, and lysyl side chains of a protein carrier[4]. *p*-Nitrophenyl glycosides, precursors for the synthesis of *p*-aminophenyl glycosides, are generally prepared by the method of Helferich[5] or that of Koenigs and Knorr[6]. Since sophorose and laminarabiose are relatively rare oligosaccharides, the general methods for the preparation of the *p*-nitrophenyl glycosides of these oligosaccharides were found unsuitable. This communication describes a facile, simultaneous preparation of the *p*-nitrophenyl β-glycosides of sophorose and laminarabiose.

EXPERIMENTAL

Melting points were determined on a Fisher–Johns melting-point apparatus and are uncorrected. All evaporations were conducted *in vacuo* at 35–40° with a rotary evaporator. Silica gel (type G) for t.l.c. was purchased from Brinkmann

*This research was supported by Grant AM-10171 from the National Institutes of Health.
†Present address: Department of Pediatrics, Medical Center, Case-Western Reserve University, Cleveland, Ohio.
‡This work was done while this author was an Established Investigator of the American Heart Association. To whom enquiries concerning this paper should be sent.

Instruments; components were detected by spraying with 50% (v/v) ethanol-sulfuric acid followed by charring in an oven at 120°. Paper chromatography was carried out on Whatman No. 1 paper with butanone–water azeotrope as the solvent system. Localization of *p*-nitrophenyl glycosides was accomplished by examination of paper chromatograms under ultraviolet light.

p-*Nitrophenyl 4,6-O-benzylidene-β-D-glucopyranoside.* — To a solution of *p*-nitrophenyl β-D-glucopyranoside (10 g, 33.2 mmoles) in formic acid (50 ml) was added benzaldehyde[7] (60 ml). The reaction mixture was stirred vigorously for 5.5 min and was immediately neutralized with 30% aqueous potassium carbonate. Petroleum ether (b.p. 30–60°, 2 l) was added and the product that separated at the interface was filtered, and washed throughly with petroleum ether and subsequently with water. The product (10.8 g, 88.5%), was recrystallized from 95% ethanol; m.p. 184–185°, $[\alpha]_D^{25}$ −46.2° (*c* 1.0, acetone). Lit.[8] m.p. 184–185°, $[\alpha]_D^{20}$ −44.9° (*c* 2, acetone).

p-*Nitrophenyl 4,6-O-benzylidene-2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside.* — To a mixture of *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside (10 g, 25.2 mmoles), Drierite (15 g), and silver carbonate (15 g) in dichloromethane (150 ml, dried over molecular sieve), which had been stirred for 0.5 h, was added tetra-*O*-acetyl-α-D-glucopyranosyl bromide (13.4 g, 32.7 mmoles) and iodine (1.7 g). The reaction mixture, protected from light, was shaken on a Burrell Wrist-Action Shaker at room temperature. The progress of the reaction was followed by testing for ionizable bromide and by t.l.c. (solvent: benzene–methanol, 9:1, v/v). After 28 h no further ionizable bromide was detectable, and the reaction mixture was filtered through a thin layer of Norite and concentrated to a syrup. Purification of *p*-nitrophenyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (henceforth called sophorose adduct) by crystallization proved to be difficult inasmuch as the sophorose adduct had a strong tendency to co-crystallize with *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside. However a small amount of sophorose adduct (760 mg) was obtained as fine needles by dissolving the reaction mixture in hot ethanol and adding water until the first sign of turbidity. Crystallization ensued at room temperature, and the crystals that formed within 5 min were filtered immediately. The process was repeated (2 or 3 times) until the product could be shown to be chromatographically homogeneous; yield 760 mg, m.p. 170–171°.

Anal. Calc. for $C_{33}H_{37}NO_{17}\cdot H_2O$: C, 53.7; H, 5.29; N, 1.9. Found: C, 53.6; H, 5.69; N, 1.51.

p-*Nitrophenyl 2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside.* — The sophorose adduct (100 mg) was dissolved in 50% aqueous acetic acid (5 ml) and refluxed for 20 min. Acetic acid and benzaldehyde were removed by repeated evaporation under diminished pressure with frequent additions of water. The white crystalline solid that formed was recrystallized from 95% ethanol; yield 40 mg (46%), m.p. 207–208°.

Anal. Calc. for $C_{26}H_{33}NO_{17}$: C, 49.5; H, 5.24; N, 2.2. Found: C, 49.29; H, 5.33; N, 2.09.

p-*Nitrophenyl β-sophoroside and* p-*nitrophenyl β-laminarabioside.*—The reaction was effected as described earlier and the reaction mixture was filtered through charcoal–Celite and concentrated to a syrup. No attempt was made to crystallize the sophorose adduct at this stage. The syrup was dried *in vacuo* for 8–10 h at 40° in a vacuum oven (yield 21 g) and dissolved in anhydrous methanol (50 ml). Sodium methoxide in methanol (1%, 1 ml) was added, and the mixture was kept for 90 min at room temperature. The reaction mixture was neutralized (acetic acid) and concentrated to a syrup. The deacetylated product was debenzylidenated by dissolving it in 50% aqueous acetic acid (100 ml) and refluxing the solution for 30 min. Acetic acid and benzaldehyde were removed by repeated evaporation in the presence of water, and the reaction product was concentrated to a syrup. The syrup was dissolved in water (100 ml) and extracted with chloroform; the chloroform layer was discarded. Cations were removed from the aqueous phase by treatment with Amberlite IR-120 (H^+) resin and the water was removed by evaporation to afford a thick syrup (10.2 g) (*p*-nitrophenyl *β*-laminarabioside and *p*-nitrophenyl *β*-sophoroside may be co-crystallized at this stage from aqueous ethanol). Paper-chromatographic examination (butanone–water azeotrope) showed the presence of *p*-nitrophenyl *β*-D-glucopyranoside (R_F 0.47), *p*-nitrophenyl *β*-laminarabioside (R_F 0.16), *p*-nitrophenyl *β*-sophoroside (R_F 0.11), and glucose (at the origin). The *p*-nitrophenyl glycosides were readily detected by examination of the chromatogram under ultraviolet light; D-glucose was visualized by an alkaline silver nitrate spray.

Separation of p-*nitrophenyl β-sophoroside and* p-*nitrophenyl β-laminarabioside by chromatography on cellulose.* — The deblocked reaction mixture (0.5 g) was applied to a cellulose powder (Whatman cellulose powder, standard grade) column (48 cm × 1 cm), and the column was developed with butanone–water azeotrope at a flow-rate of 20 ml per h. Fractions (5 ml) were collected and monitored for the presence of carbohydrate by paper chromatography. The following components were identified: *p*-nitrophenyl *β*-D-glucopyranoside (fractions 10–29), *p*-nitrophenyl *β*-laminarabioside (fractions 35–56), *p*-nitrophenyl *β*-laminarabioside and *p*-nitrophenyl *β*-sophoroside (fractions 58–62), *p*-nitrophenyl *β*-sophoroside (fractions 63–80).

p-*Nitrophenyl β-laminarabioside.*—Fractions (35–56), which contained *p*-nitrophenyl *β*-laminarabioside (R_F 0.16), were pooled and concentrated to afford a crystalline mass. Recrystallization from 90% ethanol gave the pure product (105 mg), m.p. 235–236°, $[\alpha]_D^{25}$ −87° (*c* 0.5, water).

Anal. Calc. for $C_{18}H_{25}NO_{13}$: C, 46.7; H, 5.41; N, 3.02. Found: C, 46.7; H, 5.53; N, 3.13.

The compound was characterized as *p*-nitrophenyl *β*-laminarabioside on the basis of the following experiments: partial acid hydrolysis (0.33N H_2SO_4 for 30 min at 100°) gave products migrating with mobilities corresponding to laminarabiose, *p*-nitrophenyl *β*-D-glucopyranoside, D-glucose, *p*-nitrophenol, and unhydrolyzed product. Treatment with almond emulsin gave glucose, *p*-nitrophenol, and a trace of *p*-nitrophenyl *β*-D-glucopyranoside.

p-*Nitrophenyl β-sophoroside.* — Tubes (63–80), containing the slower-moving

component (R_F 0.11), were combined and concentrated to yield a crystalline product. The compound was recrystallized from 90% aqueous ethanol giving the pure substance (87 mg), m.p. 261–262°, $[\alpha]_D^{25}$ −67.9° (*c* 1, water).

Anal. Calc. for $C_{18}H_{25}NO_{13}$: C, 46.7; H, 5.41; N, 3.02. Found: C, 46.53; H, 5.65; N, 2.85.

The product was identical by m.p., mixed m.p., and specific rotation with a sample of *p*-nitrophenyl β-sophoroside prepared by condensing hepta-*O*-acetylsophorosyl bromide and *p*-nitrophenol. Partial acid hydrolysis (0.33N H_2SO_4 for 30 min at 100°) gave products migrating with sophorose (neither the reference nor the sample reacted with triphenyltetrazolium chloride reagent[9]), D-glucose, *p*-nitrophenol, *p*-nitrophenyl β-D-glucopyranoside, and the unhydrolyzed product. Treatment with almond emulsin gave D-glucose and *p*-nitrophenol.

p-*Aminophenyl β-sophoroside.* — *p*-Aminophenyl β-sophoroside was prepared by catalytic hydrogenation of *p*-nitrophenyl β-sophoroside. Platinum oxide (100 mg) was added to a solution of *p*-nitrophenyl β-sophoroside (500 mg) in 50% aqueous methanol (150 ml). The reduction was conducted at atmospheric pressure for 1 h. The catalyst was filtered and washed, and the filtrate was concentrated to a syrup under diminished pressure. The syrup was dissolved in a minimum quantity of water, abs. ethanol was added, and crystallization was allowed to proceed in the cold; yield, 380 mg; m.p. 211–212°.

Anal. Calc. for $C_{18}H_{27}NO_{11}$: C, 47.9, H, 6.45; N, 3.1. Found: C, 47.66; H, 6.63; N, 2.87.

Determination of the ratio of p-*nitrophenyl β-sophoroside to* p-*nitrophenyl β-laminarabioside.* — Unfractionated, deblocked reaction mixture (1 mg) was applied to Whatman No. 1 paper, and the chromatogram was developed with butanone–water azeotrope. The regions corresponding to *p*-nitrophenyl β-sophoroside and *p*-nitrophenyl β-laminarabioside were excised and equilibrated with 10 ml of water. Sugar concentrations were determined by the phenol–sulfuric acid procedure[10], employing *p*-nitrophenyl β-sophoroside and *p*-nitrophenyl β-laminarabioside as standards. The ratio of *p*-nitrophenyl β-laminarabioside to *p*-nitrophenyl β-sophoroside was found to be 1:1.

DISCUSSION

Although sophorose occurs in Nature (pods of *Sophora japonica*[11]) and as residues in a (1→2)-β-D-glucan[12], it is more readily prepared by chemical synthesis[13–15]. Laminarabiose, also a rare saccharide, has been prepared by the partial acid hydrolysis of laminaran[16] and by the Koenigs–Knorr condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with 4,6-*O*-benzylidene-1,2-*O*-isopropylidene-D-glucose[17], 1,2,4,6-tetra-*O*-acetyl-β-D-glucose[18], or 5,6-di-*O*-acetyl-1,2-*O*-isopropylidene-D-glucose[19], with subsequent removal of protecting groups.

A comparative study of the protecting groups available for the synthesis of sophorose was recently reported by Koeppen[20]. This author claimed that the Hel-

ferich and Zirner procedure[14], of condensing 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose with tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of a suitable catalyst, is the method of choice despite the lower yields, because of the advantage of producing a peracetylated glycosyl halide as an intermediate, useful for the synthesis of glycosides. As shown herein, the Freudenberg[21,22] procedure is quite versatile inasmuch as use of the preformed glucoside obviates a further condensation step in the synthesis of the *p*-nitrophenyl glycoside of sophorose. Furthermore the method has the advantage of yielding at the same time the *p*-nitrophenyl glycoside of laminarabiose.

In a previous report[13] on the synthesis of sophorose from the condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, no mention was made concerning the possibility that laminarabiose was also formed. However, when the corresponding benzyl glucoside was employed as one of the reactants, laminarabiose as well as a trisaccharide [3,6-di-*O*-(β-D-glucopyranosyl)-D-glucopyranose] was formed[15]. In the present synthesis we have shown that equivalent amounts of *p*-nitrophenyl laminarabioside and sophoroside are formed, and it is quite probable that the aglycon moiety has a directive influence in affording the equimolar mixture of these two glycosides.

REFERENCES

1 I. J. GOLDSTEIN AND R. N. IYER, *Biochim. Biophys. Acta*, 121 (1966) 197.
2 I. J. GOLDSTEIN, R. N. IYER, E. E. SMITH, AND L. L. SO, *Biochemistry*, 6 (1967) 2373.
3 P. Z. ALLEN, I. J. GOLDSTEIN, AND R. N. IYER, *Biochemistry*, 6 (1967) 3029.
4 W. F. GOEBEL, O. T. AVERY, AND F. H. BABERS, *J. Exp. Med.*, 60 (1934) 599.
5 B. HELFERICH AND E. SCHMITZ-HILLEBRECHT, *Ber.*, 66 (1933) 378.
6 W. KOENIGS AND E. KNORR, *Ber.*, 34 (1901) 957.
7 J. G. BUCHANAN AND J. C. P. SCHWARZ, *J. Chem. Soc.*, (1962) 4770.
8 M. A. JERMYN, *Aust. J. Chem.*, 10 (1957) 448.
9 K. WALLENFELS, *Naturwissenschaften*, 37 (1950) 491.
10 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
11 M. J. CLANCY, *Methods Carbohyd. Chem.*, 1 (1962) 345.
12 F. C. MCINTIRE, W. H. PETERSON, AND A. J. RIKER, *J. Biol. Chem.*, 143 (1942) 491.
13 B. COXON AND H. G. FLETCHER, JR., *J. Org. Chem.*, 26 (1961) 2892.
14 B. HELFERICH AND J. ZIRNER, *Ber.*, 2 (1963) 2604.
15 A. KLEMER AND K. HOMBERG, *Ber.*, 94 (1961) 2747.
16 V. C. BARRY AND J. E. MCCORMICK, *Methods Carbohyd. Chem.*, 1 (1962) 328.
17 P. BACHLI AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1952) 1243.
18 K. MATSUDA, *Chem. Ind.* (London), (1958) 1627.
19 K. FREUDENBERG AND K. V. OERTZEN, *Ann.*, 574 (1951) 37.
20 B. H. KOEPPEN, *Carbohyd. Res.*, 7 (1968) 410.
21 K. FREUDENBERG AND H. KNAUBER, *Naturwissenschaften*, 34 (1947) 344.
22 K. FREUDENBERG, H. KNAUBER, AND F. CRAMER, *Ber.*, 84 (1951) 144.