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SYNTHESIS OF *O*-GLYCOSYLTHREONINES

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ABSTRACT

A synthesis of *N*-(2,4-dinitrophenyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-threonine methyl ester and *O*-(β-D-glucopyranosyl)-L-threonine methyl ester is described. Attempts at glycosylation of L-threonine derivatives by the Koenigs–Knorr method or by the orthoester method gave the corresponding 1,2-orthoacetates of D-glucose, which were converted into isomeric D-glucosides.

INTRODUCTION

Recent, rapid progress in the development of glycoprotein chemistry necessitates the synthesis and study of simple model compounds corresponding to portions of these biopolymers[1]. The identification of glycosidic bonds involving oxyamino acids as a key protein–carbohydrate linkage in a number of glycoproteins[2–7] has stimulated the synthesis of some *O*-glycosylserines[8–13]. However, the synthesis of an *O*-glycosylthreonine has only recently been accomplished[13a], although there is no doubt of the importance of this type of linkage in natural biopolymers. Moreover, in some glycoproteins[7], L-threonine is detected as the only oxyamino acid involved in the protein–carbohydrate glycosidic linkage.

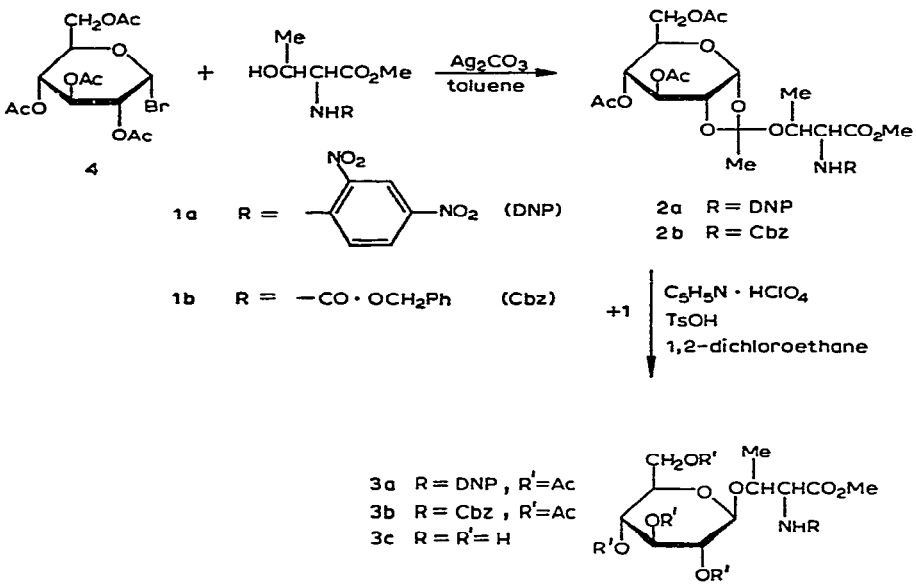
This paper deals with the first synthesis of *N*-(2,4-dinitrophenyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-threonine methyl ester (**3a**) and *O*-(β-D-glucopyranosyl)-L-threonine methyl ester (**3b**).

RESULTS AND DISCUSSION

The first approach to the synthesis of *O*-glycosylthreonines involved attempts at glycosylation of *N*-(2,4-dinitrophenyl)-L-threonine (**1a**) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4**) by the Koenigs–Knorr method (using silver carbonate as the acid acceptor) and also by the orthoester method[14]. In both cases, the orthoester **2a** was the sole product.

A successful synthesis was accomplished by a new, two-step procedure involving the orthoester method[15]. 3,4,6-Tri-*O*-acetyl-α-D-glucopyranose 1,2-[2-(2,4-dinitrophenyl)amino-2-methoxycarbonyl-1-methylethyl orthoacetate] (**2a**) and the

analogous *N*-benzyloxycarbonyl derivative (**2b**) were prepared, in *ca.* 70% yield, by application of the Koenigs–Knorr procedure for orthoester synthesis.



When compound **2a** in dry 1,2-dichloroethane containing a small proportion of L-threonine methyl ester **1a** and a mixture of toluene-*p*-sulphonic acid and pyridinium perchlorate as catalyst were heated in a sealed tube, the L-threonine glycoside **3a** was formed in 30–40% yield.

A more-convenient procedure avoids the isolation of the intermediate threonine orthoester **2a**, and the preparation of threonine glycoside **3a** involves preparation of the orthoester (**2a**), under the conditions of the Koenigs–Knorr reaction, and the further conversion into the threonine glycoside under the conditions[15] of orthoester "rearrangement".

Methanolysis of glucoside **3a** gave *N*-(2,4-dinitrophenyl)-L-threonine methyl ester and methyl D-glucoside, which were identified by paper chromatography. The threonine derivative had an unchanged optical rotation, proving that no racemisation had occurred during the glycosylation. The structure of the orthoester (**2a**) and the glucoside (**3a**) are supported by n.m.r. data. Compound **2a** gave signals at τ 8.23 (orthoester, *C*-methyl protons) and τ 7.92 (three *O*-acetyl groups). The presence of a signal at τ 8.23 indicates that compound **2a** is an *endo*-isomer[16,17]. The glucoside **3a** gave a signal at τ 7.93 (twelve protons of four *O*-acetyl groups) but no signal at τ 8.20. The signal for the anomeric proton has not been identified, but the marked stereospecificity of the orthoester method[14,15], which, in all known examples, produces only 1,2-*trans* isomers, suggests the β-D configuration for the glucoside **3a**.

The *N*-benzyloxycarbonyl-L-threonine D-glucoside **3b** was prepared analogously. Treatment of *N*-benzyloxycarbonyl-L-threonine methyl ester (**1b**) with

tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of silver carbonate gave the L-threonine orthoester **2b** which was converted directly, without purification, into compound **3b** (35–40%) by treatment with a small proportion of compound **1b** in the presence of catalyst as described above.

A preliminary investigation of compounds **3a** and **3b** shows that the stability of *O*-D-glucosyl derivatives of L-threonine in an acid medium is comparable with that of glucosides derived from aliphatic alcohols; the L-threonine orthoesters **2a** and **2b**, on the contrary, are very labile and are completely cleaved after treatment with 0.5N sulphuric acid in 90% acetone for 5 min at room temperature.

Deacetylation of compound **3b** was effected by treatment with 4% methanolic methylamine at room temperature, and subsequent, direct hydrogenolysis of the *N*-benzyloxycarbonyl group gave *O*-(β-D-glucopyranosyl)-L-threonine methyl ester, which was isolated as the hydrochloride.

EXPERIMENTAL

Paper chromatography was performed on paper "M" (Leningrad factory No. 2), and thin-layer chromatography (t.l.c.) on neutral alumina (activity, Brockmann grade III) and silica KSK with the solvent systems: chloroform–acetone, 95:5 (*A*); benzene–methanol, 85:15 (*B*); *tert*-pentyl alcohol–propyl alcohol–water, 8:2:3 (*C*); chloroform–acetone, 97:3 (*D*). Electrophoresis was performed in pyridine–acetate (pH 4.5) buffer at 40 volts/cm, for 15–20 min. The following spray reagents were used: silver nitrate, Bonner reagent, benzidine–chlorine reagent, hydroxylamine–ferric chloride, and (for t.l.c.) sulphuric acid and morin (on alumina). The solvent evaporation was performed *in vacuo* at <40°.

N-(*2,4-Dinitrophenyl*)-L-*threonine methyl ester* (**1a**). — *N*-(2,4-Dinitrophenyl)-L-threonine[18], m.p. 144.5–145°, was converted into the methyl ester **1a** by treatment with diazomethane. The product (95%) had m.p. 119.5–120° (from aqueous ethanol); $[\alpha]_D^{20}$ −63.3° (*c* 1.09, chloroform); ν_{max} 1068 (C–O–C), 1370 (OAc), 1345, 1528 (−NO_2), 1590 (C=C–C=C), 1732 (COOR), 1240, 3350 (NH), and 3435 cm^{-1} (OH) (Found: C, 44.04; H, 4.38; N, 14.30. $C_{11}H_{13}N_3O_7$ calc.: C, 44.15; H, 4.38; N, 14.04%).

*3,4,6-Tri-*O*-acetyl-α-D-glucopyranose 1,2-[2-(2,4-dinitrophenyl)amino-2-methoxycarbonyl-1-methylethyl orthoacetate*] (**2a**). — Compound **1a** (0.92 g, 3.1 mmoles) and silver carbonate (1.76 g, 6.2 mmoles) in dry toluene (30 ml) were boiled with vigorous stirring and slow distillation. When 5 ml of toluene had been distilled off, compound **4** (2.5 g, 6.1 mmoles) in toluene (30 ml) was added during 45 min, and distillation and addition of solvent were continued until the volume of distillate was 200 ml. The mixture was cooled and filtered, a drop of pyridine was added, and the mixture was evaporated *in vacuo*. The product **2a** (1.36 g, 70%), R_F 0.68, was isolated by t.l.c. (alumina, solvent *A*); $[\alpha]_D^{20}$ +28.2° (*c* 1.24, chloroform); ν_{max} 1050 (C–O–C), 1380 (OAc), 1345, 1530 (−NO_2), 1595 (C=C–C=C), 1750 (COOR), 1230, 3350 cm^{-1} (−NH) (Found: C, 48.62; H, 4.89; N, 6.51. $C_{25}H_{31}N_3O_{15}$ calc.: C, 48.94; H, 5.09; N, 6.85%).

N-(2,4-Dinitrophenyl)-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-threonine methyl ester (**3a**). — (*a*) A mixture of compounds **2a** (780 mg, 1.2 mmole) and **1a** (35 mg, 0.12 mmole), toluene-*p*-sulphonic acid (7 mg), and pyridine perchlorate (7 mg) in dry 1,2-dichloroethane (5 ml) was heated in a sealed tube in boiling xylene for 30 min. The cooled mixture was evaporated to dryness, 0.5N sulphuric acid in 90% aqueous acetone (4 ml) was added, and, after 10 min, the acid was neutralized with sodium hydrogen carbonate. The filtered solution was evaporated, and the residue was chromatographed (preparative t.l.c., alumina, solvent *A*) to give a product having R_F 0.6. Crystallisation from chloroform–ether–hexane gave compound **3a** (265 mg, 34%), m.p. 161–163°, $[\alpha]_D^{20}$ −8.9° (*c* 1.5, chloroform); ν_{max} 1050 (C–O–C), 1370 (OAc), 1345, 1530 (−NO_2), 1595 (C=C–C=C), 1755 (COOR), 1230, 3360 cm^{-1} (−NH) (Found: C, 48.81; H, 5.00; N, 6.72. $C_{25}H_{31}N_3O_{15}$ calc: C, 48.94; H, 5.09; N, 6.85%).

(*b*) A mixture of compound **1a** (1.38 g, 4.6 mmoles) and silver carbonate (2.64 g, 9.5 mmoles) in dry toluene (35 ml) was boiled with vigorous stirring and continuous distillation. When 5 ml of toluene had been distilled off, a solution of compound **4** (3.75 g, 9.1 mmoles) in dry toluene (50 ml) was added during 40 min, and slow distillation and addition of solvent were continued during 1 h. The mixture was cooled and filtered, and the filtrate was evaporated. Toluene-*p*-sulphonic acid (18 mg) and pyridine perchlorate (18 mg) were added, and the mixture was heated in a sealed tube and treated as described in the previous experiment. Preparative t.l.c. (alumina, 4-mm layer, solvent *A*) gave compound **3a** (200 mg, 41%), R_F 0.6. Crystallisation as above gave material having m.p. 160–162°, $[\alpha]_D^{20}$ −9.9° (*c* 1.6, chloroform).

Methanolysis of N-(2,4-dinitrophenyl)-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-threonine methyl ester (**3a**). — A solution of compound **3a** (98 mg) in 0.1N sulphuric acid in abs. methanol (5 ml) was refluxed for 35 min, cooled, neutralised ($BaCO_3$), and evaporated to dryness. Methyl D-glucoside was identified in the residue by paper chromatography (solvent *C*, R_F 0.6), and *N*-(2,4-dinitrophenyl)-L-threonine methyl ester {$[\alpha]_D^{20}$ −61° (*c* 1.0, chloroform)} was isolated from the residue (preparative t.l.c., alumina, solvent *B*, R_F 0.34).

N-Benzyloxycarbonyl-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-threonine methyl ester (**3b**). — A mixture of compound **1b** (1.1 g, 4.1 mmoles) and silver carbonate (2.2 g, 7.8 mmoles) in dry toluene (30 ml) was boiled with continuous, slow distillation. When 5–7 ml of solvent had been distilled off, compound **4** (3.0 mg, 7.3 mmoles) in dry toluene (25 ml) was added during distillation of solvent and treatment as above. The dry residue was dissolved in 1,2-dichloroethane (10 ml), and toluene-*p*-sulphonic acid (15 mg) and pyridinium perchlorate (15 mg) were added. The mixture was heated and treated as in the previous experiments. The chromatographically homogeneous product **3b** (890 mg, 37%) was isolated (preparative t.l.c., alumina, solvent *D*, R_F 0.35). After crystallisation from ether–light petroleum, the product had m.p. 55–56°, $[\alpha]_D^{20}$ −7.0° (*c* 1.15, chloroform) (Found: C, 53.97; H, 6.24; N, 2.51. $C_{27}H_{35}NO_{14}$ calc.: C, 54.27; H, 5.90; N, 2.34%).

O-(*β*-D-*Glucopyranosyl*)-L-*threonine methyl ester* (**3c**).— Compound **3b** (200 mg) was treated with 4% methanolic methylamine (5 ml) for 5 h at room temperature. The mixture was evaporated to dryness, and the residue was treated with dry ether (50 ml) containing 0.5 ml of abs. methanol. The filtered solution was evaporated, and the dry residue was hydrogenated in 75% aqueous methanol (1 ml) in the presence of 5% palladised barium sulphate (50 mg); the reaction was monitored by electrophoresis. The catalyst was removed by centrifugation, and compound **3c** (50 mg) was isolated by preparative paper electrophoresis, and converted into the salt by the addition of 0.1N hydrochloric acid in abs. methanol. The hydrochloride of compound **3c** had $[\alpha]_D^{20} - 5°$ (*c* 1.0, water) (Found: C, 44.50; H, 7.30; N, 4.50. $C_{11}H_{22}ClNO_8$ calc.: C, 44.74; H, 7.17; N, 4.74%).

REFERENCES

1 N. K. KOCHETKOV, V. A. DEREVITSKAYA, L. M. LIHOSHERSTOV, N. V. MOLODTZOV, AND S. G. KARA-MURZA, *Tetrahedron*, 18 (1962) 273.
2 K. TANAKA, M. BERTOLINI, AND W. PIGMAN, *Biochem. Biophys. Res. Commun.*, 16 (1964) 404.
3 B. ANDERSON, N. SENO, P. SAMPSON, J. RILEY, P. HOFFMAN, AND K. MEYER, *J. Biol. Chem.*, 239 (1964) PC2716.
4 N. K. KOCHETKOV, V. A. DEREVITSKAYA, AND S. G. KARA-MURZA, *Dokl. Akad. Nauk SSSR*, 163 (1965) 650.
5 N. K. KOCHETKOV, V. A. DEREVITSKAYA, AND S. G. KARA-MURZA, *Carbohyd. Res.*, 3 (1967) 403.
6 U. LINDAHL AND L. RODÉN, *J. Biol. Chem.*, 240 (1965) 2821.
7 J. ADAMS, *Biochem. J.*, 94 (1965) 368; 97 (1965) 345.
8 V. A. DEREVITSKAYA, M. G. VAFINA, AND N. K. KOCHETKOV, *Carbohyd. Res.*, 3 (1967) 377.
9 F. MICHEEL AND H. KÖCHLING, *Ber.*, 91 (1958) 673; 93 (1960) 2372.
10 J. K. N. JONES, M. B. PERRY, B. SHELTON, AND B. J. WALTON, *Can. J. Chem.*, 39 (1961) 1005.
11 B. LINDBERG AND B. G. SILWANDER, *Acta Chem. Scand.*, 19 (1965) 530.
12 J. R. VERCELLOTTI AND A. E. LUETZOW, *J. Org. Chem.*, 31 (1966) 825.
13a J. R. VERCELLOTTI, R. FERNANDEZ, AND C. J. CHANG, *Carbohyd. Res.*, 5 (1967) 97.
13 K. KUM AND S. ROSEMAN, *Biochemistry*, 5 (1966) 3061.
14 N. K. KOCHETKOV, A. YA. KHORLIN, AND A. F. BOCHKOV, *Tetrahedron Letters*, (1964) 289; *Tetrahedron*, 23 (1967) 693.
15 N. K. KOCHETKOV, A. F. BOCHKOV, AND V. SNYATKOVA, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 12 (1967) 2684.
16 A. S. PERLIN, *Can. J. Chem.*, 41 (1963) 399.
17 R. U. LEMIEUX AND A. R. MORGAN, *Can. J. Chem.*, 43 (1965) 2199.
18 K. R. RAO AND H. A. SOBER, *J. Am. Chem. Soc.*, 76 (1954) 1328.