PREPARATION OF PERBENZYLATED 2-AZIDO-2-DEOXY-D-ALLONO-1,5-LACTONE AND ITS CONDENSATION WITH AN AMINO ACID ESTER*

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(Received January 26th, 1972)

ABSTRACT

Oxidation of 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-altropyranose (**4**) with a mixture of methyl sulfoxide and acetic anhydride gave 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-allono-1,5-lactone (**5**) with concomitant inversion of the azido group. Compound **5** was readily condensed with L-alanine benzyl ester to afford *N*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-allonoyl)-L-alanine benzyl ester (**12**) without any racemization of the L-alanine moiety.

INTRODUCTION

Our project[1] directed towards the synthesis of polyoxins[2], a novel group of antibiotics, necessitated an examination of the possibility of condensing a fully protected 2-azido-2-deoxy-aldono-1,5-lactone with an ester of an amino acid. The general usefulness of perbenzylated aldono-1,5-lactones as synthetic intermediates had been shown with 2,3,4,6-tetra-*O* benzyl-D-glucono-1,5-lactone[3]. Furthermore, the preparation of perbenzylated 2-acetamido-2-deoxy-D-glucono-1,5-lactone[4] was reported recently. A perbenzylated 2-azido-lactone obtainable from methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside[5] (**1**) was, therefore, chosen as the model for the condensation, as the latter 2-azido glycoside is the most readily available one of this class. The present paper deals with the synthesis of the 2-azido lactone and its successful condensation with L-alanine benzyl ester, a typical amino acid ester.

RESULTS AND DISCUSSION

Compound **1** (ref. 5) was obtained by cleavage of the epoxide ring of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside with azide ion. The benzylidene group of **1** was removed in aqueous *p*-dioxane with a trace of sulfuric acid, affording the known methyl 2-azido-2-deoxy-α-D-altropyranoside[5] (**2**). Complete benzylation of **2** to give **3** was attained in one step by use of benzyl chloride and powdered potassium hydroxide, with *p*-dioxane as a diluent. The glycosidic linkage of the crude

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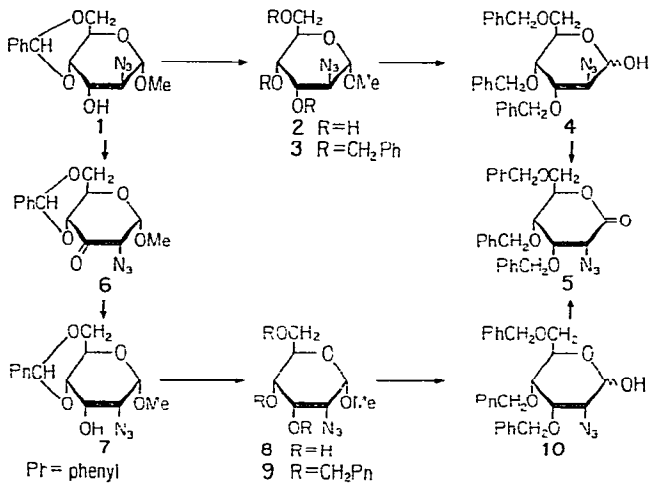
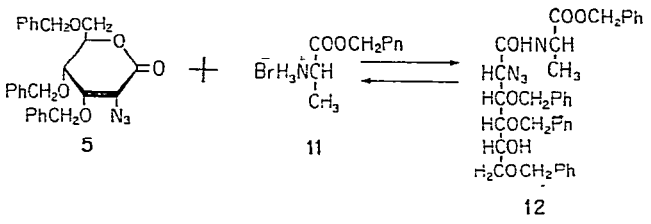
tri-*O*-benzyl compound **3** was hydrolyzed with aqueous sulfuric acid in acetic acid and, after chromatographic purification, analytically pure 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-altropyranose (**4**) was obtained. Oxidation of **4** with a methyl sulfoxide–acetic anhydride gave a single product in high yield. This product was proved to be the expected 2-azido lactone **5** on the basis of its i.r. spectrum and the observation that it gave a positive hydroxamic acid test for esters[6].



The configuration of the carbon atom bearing the azido group in lactone **5** was uncertain, because epimerizations accompanying oxidations in methyl sulfoxide had been reported by several workers[7–9]. For example, Ali and Richardson[8] reported that oxidation of **1** with methyl sulfoxide–acetic anhydride gives methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hex-3-ulopyranoside (**6**) with inversion of the vicinal azido group from axial to equatorial attachment. The n.m.r. spectrum of the 2-azido lactone **5** obtained afforded no information concerning the configuration at C-2, because the H-2, H-3 signals could not be assigned by interference with the methylene protons of benzyl groups. In order to clarify the configuration at C-2, we attempted to prepare the epimer of **4**, namely, 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-allopyranose (**10**) in order to examine its oxidation product. Compound **6** was known to give methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-allopyranoside[8] (**7**) by restricted reduction with sodium borohydride. In a manner similar to that used for the preparation of **4**, compound **7** was debenzylidenated to methyl 2-azido-2-deoxy-α-D-allopyranoside (**8**), which was then perbenzylated to afford methyl 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-allopyranoside (**9**). Without characterization of **9**, its glycosidic linkage was hydrolyzed with acid to give **10**. Although **4** and **10** should be capable of mutarotation, neither compound showed mutarotation, even in aqueous media. Compound **10** (like **4**) was oxidized with methyl sulfoxide–acetic anhydride to a single product, a fully benzylated, azido lactone that was found to be identical with the oxidization

product of **4** (by comparison of their physical properties, chromatographic behavior, and i.r. spectra). The fact that **4** and **10** gave the same oxidation product indicated that either **4** or **10** had been epimerized during the oxidation. It was quite reasonable to assume that the axially attached azido group of **4** had undergone inversion (rather than the equatorially attached azido group of **10**), because, in all similar cases reported, inversion from the axial to the equatorial position had occurred. Therefore, the oxidation product from **4** was considered to be 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-allono-1,5-lactone (**5**).

A 2-azido lactone such as **5** may be regarded as a precursor of an amino acid ester, because an azido group is readily reduced to an amino group. Furthermore, condensation of such a 2-azido lactone with an amino acid ester to afford an *N*-(2-azido-aldonoyl)amino acid ester would constitute a potential method of dipeptide synthesis. When a mixture of **5** with L-alanine benzyl ester hydrobromide[10] (**11**; chosen as a model amino acid ester) and triethylamine (1.3:1.0:1.0 in molecular ratios) was kept at room temperature, condensation of **5** with the amino acid ester occurred and was complete within 3 days. The resulting *N*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-allonoyl)-L-alanine benzyl ester (**12**) was isolated in 45% yield by column chromatography. It was proved that no racemization of the L-alanine moiety had occurred during the reaction, because treatment of **12** with anhydrous hydrogen bromide gave lactone **5** together with **11** having almost the same optical rotation as authentic **11**. This cleavage apparently involved participation of the 5-hydroxyl group of the 2-azido-2-deoxy-D-allonoyl group.



Considering that peptide synthesis generally requires activation of the carboxyl group of one of the constituent amino acids, it is interesting that the preparation of **12**, a precursor of a peptide, was achieved in such a simple way.

EXPERIMENTAL

General methods.—All melting points are uncorrected. Specific rotations were measured with a Perkin–Elmer Model 141 polarimeter and a 1-dm tube. The i.r. spectra were recorded with a Shimadzu IRS-27 i.r. spectrometer. T.l.c. was performed with Kieselgel G (E. Merck, Darmstadt) and the solvent system specified.

Methyl 2-azido-2-deoxy-α-D-altropyranoside (**2**).—Aqueous sulfuric acid (1.5% w/w, 100 ml) was added to a solution of **1** (21 g) in *p*-dioxane (150 ml). The

mixture was kept overnight at 55° and then made neutral at 60° with an excess of barium carbonate. After filtration of the suspension, the filtrate was evaporated *in vacuo* to afford syrupy **2** (15 g, quantitative yield); on agitation in a small volume of ethanol, it crystallized. It was recrystallized from acetone; m.p. 135–138° (lit.[5] m.p. 140–141°); $[\alpha]_D^{19}$ +61.5° (*c* 2.44, water); ν_{max}^{KBr} 3500, 3360, 3240 (OH), and 2100 cm^{-1} (N_3).

Anal. Calc. for $C_7H_{13}N_3O_5$: C, 38.35; H, 5.98; N, 19.17. Found: C, 38.57; H, 5.79; N, 19.30.

*2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-*D*-altropyranose* (**4**) via *its methyl* α-D-*glycoside* (**3**).—Powdered potassium hydroxide (38 g) was added to a solution of **2** (7.5 g) in *p*-dioxane (70 ml), and the mixture was heated at 90° with vigorous stirring. Benzyl chloride (35 ml) was added dropwise to the mixture, giving a sticky mass. Stirring was continued at 90–100° for a further 3 h. After being cooled, the mixture was poured into water (~600 ml) and the mixture was extracted with ether (700 ml). The extract was washed with water, dried (sodium sulfate), and evaporated *in vacuo* (finally, at <1 torr), to afford a brown syrup (16 g) which, on t.l.c. with 25:1 (v/v) benzene–ether, was found to contain one major component (**3**), with several minor components. The syrupy product was chromatographed on silica gel (1 kg) with 26:1 (v/v) benzene–ether as eluant to give slightly colored **3** (7 g, 42%); ν_{max}^{film} no OH absorption, 2100 (N_3), and 1610, 1590, 1500 cm^{-1} (benzene ring).

To a solution of the chromatographed **3** (6.5 g) in acetic acid (120 ml) was added aqueous sulfuric acid (2M, 60 ml) dropwise. The mixture was heated for 8 h at 90° with stirring, cooled, poured into water, and the mixture extracted with chloroform (300 ml). The extract was successively washed with aqueous sodium carbonate and water, dried (sodium sulfate), and evaporated *in vacuo* to afford a yellow syrup (5.5 g) which was chromatographed on silica gel (400 g) with 15:1 (v/v) benzene–ether as the eluant. Pure **4** (3.5 g; 56% from **3**) was obtained; $[\alpha]_D^{19}$ +0.3° (*c* 2.73, chloroform), ν_{max}^{film} 3450 (OH), 2100 (N_3), and 1500 cm^{-1} (benzene ring).

Anal. Calc. for $C_{27}H_{29}N_3O_5$: C, 68.19; H, 6.15; N, 8.84. Found, C, 67.91; H, 5.78; N, 8.88.

*Methyl 2-azido-2-deoxy-*α*-*D*-allopyranoside* (**8**). — Compound **7** (9 g) was hydrolyzed in a mixture of *p*-dioxane (75 ml) and aqueous sulfuric acid (1.5% w/w, 50 ml), as described for the preparation of **2**, to give crude, crystalline **8** which was then chromatographed on silica gel with 19:1 (v/v) chloroform–methanol as the eluant. Recrystallization from ethyl acetate gave cubic crystals; yield 3.4 g (63%), m.p. 114–116°, $[\alpha]_D^{18}$ +116.7° (*c* 1.33, water); ν_{max}^{KBr} 3200–3600 (OH) and 2100 cm^{-1} (N_3).

Anal. Calc. for $C_7H_{13}N_3O_5$: C, 38.35; H, 5.98; N, 19.17. Found, C, 38.42; H, 6.02; N, 19.47.

*2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-*D*-allopyranose* (**10**) via *its methyl* α-D-*glycoside* (**9**). — Compound **8** (3.5 g) in *p*-dioxane (45 ml) was treated with benzyl chloride (20 ml) and powdered potassium hydroxide (20 g), as described for the preparation of **3**, to afford a brown syrup (8.2 g) which was chromatographed on

silica gel with 8:1 (v/v) hexane–ethyl acetate as the eluant. Slightly colored **9** was obtained; yield 5.7 g (73%); ν_{max}^{film} 2100 (N_3) and 1600, 1500 cm^{-1} (benzene ring).

The chromatographed **9** (5 g) was hydrolyzed in acetic acid (96 ml) and aqueous sulfuric acid (2M, 48 ml), as described for the preparation of **4**. The resulting syrup was chromatographed on silica gel with 15:1 (v/v) benzene–ether as the eluant, to give crystalline **10**; yield 3.2 g (66%); m.p. 63–67°; $[\alpha]_D^{25}$ +74.9° (*c* 1.33, chloroform), $[\alpha]_D^{25}$ +76.1° [*c* 1.33, 4:1 (v/v) *p*-dioxane–water; no mutarotation]; ν_{max}^{KBr} 3450 (OH) and 2100 cm^{-1} (N_3).

Anal. Calc. for $C_{27}H_{29}N_3O_5$: C, 68.19; H, 6.15; N, 8.84. Found: C, 68.25; H, 6.19; N, 8.66.

*2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-*D*-allono-1,5-lactone* (**5**).—*Method A. From compound* **4**. Acetic anhydride (1.5 ml) was added to a solution of **4** (500 mg) in methyl sulfoxide (2 ml). The mixture was kept at room temperature for 24 h, and poured into water (~25 ml). The syrup which was precipitated was suspended in fresh water, and the aqueous layer was discarded. This washing was repeated six times, the resulting syrup was extracted with ether (~50 ml), and the extract was dried (sodium sulfate) and evaporated *in vacuo* to afford a pale-yellow syrup (460 mg, 93%) which soon crystallized. Recrystallized from cyclohexane, it had m.p. 88–90°; $[\alpha]_D^{19}$ +116.1° (*c* 1.21, chloroform); ν_{max}^{KBr} 2100 (N_3), 1750 (C=O), and 1500 cm^{-1} (benzene ring). This compound gave a positive hydroxamic acid test.

Anal. Calc. for $C_{27}H_{27}N_3O_5$: C, 68.48; H, 5.75; N, 8.87. Found: C, 68.35; H, 5.57; N, 9.10.

Method B. From compound **10**. Compound **10** (2.23 g) was treated with a mixture of methyl sulfoxide (9 ml) and acetic anhydride (6 ml), as described for the oxidation of **4**, to give crystalline **5** (2.18 g, quantitative yield); m.p. 88–90°; $[\alpha]_D^{20}$ +116.1° (*c* 1.37, chloroform); its i.r. spectrum was identical with that of **5** obtained from **4**.

N-(*2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-*D*-allonoyl*)-L-*alanine benzyl ester* (**12**).—Triethylamine (26.1 mg) was added to a suspension of **11** (73.1 mg) in *p*-dioxane (2.5 ml). After the mixture had been stirred for 15 min, it was added to a solution of **5** (161.4 mg) in *p*-dioxane (1 ml), and the mixture was kept at room temperature for 3 days. T.l.c.* showed that all of the **11** had reacted to give **12**. After filtration of the mixture, the filtrate was evaporated *in vacuo*, and the residue was chromatographed on silica gel with 15:1→7:1 (v/v) benzene–ether, to give syrupy **12** (101 mg; yield 45%); $[\alpha]_D^{21}$ −12.4° (*c* 3.04, chloroform); ν_{max}^{film} 3450 (OH), 2100 (N_3), 1750 (C=O of ester), 1680 (C=O of amide), 1610, 1590, 1500 (benzene ring), and 1520 cm^{-1} (NH of amide).

Anal. Calc. for $C_{37}H_{40}N_4O_7$: C, 68.08; H, 6.18; N, 8.58. Found: C, 67.83; H, 6.04; N, 8.67.

Acidic cleavage of **12** *to give* **5** *and* **11**.—A solution of dry hydrogen bromide in ether (54% w/w, 0.2 ml) was added to a solution of **12** (317 mg) in anhydrous ether

*Azido derivatives were found to be detectable by spraying with ninhydrin solution and then charring.

(2.5 ml). After the mixture had been stirred for 5 min at room temperature, it was diluted with anhydrous ether (20 ml) and kept for 15 min. The resulting crystals of **11** were filtered off, washed with ether, and dried; yield 108 mg (86%); m.p. 139–140°; $[\alpha]_D^{18}$ −5.7° (*c* 2.09, ethanol); for authentic **11**, $[\alpha]_D^{19}$ −6.6° (*c* 1.58, ethanol).

The filtrate and washings were combined and stirred with anhydrous sodium carbonate for 15 min at room temperature. The suspension was filtered, and the filtrate was evaporated *in vacuo* to give a brown syrup (**5**) which was agitated in cyclohexane to afford crystals; yield 164 mg (72%); m.p. 84–86°.

ACKNOWLEDGMENTS

The authors express their gratitude to Dr. K. Isono for helpful suggestions. Also, they are indebted to Dr. H. Homma and his collaborators for elemental analyses.

REFERENCES

1 H. OHRUI, H. KUZUHARA, AND S. EMOTO, *Tetrahedron Lett.*, 45 (1971) 4267.
2 K. ISONO, K. ASAHI, AND S. SUZUKI, *J. Amer. Chem. Soc.*, 91 (1969) 7490.
3 H. KUZUHARA AND H. G. FLETCHER, JR., *J. Org. Chem.*, 32 (1967) 2531.
4 N. PRAVDIĆ AND H. G. FLETCHER, JR., *Carbohyd. Res.*, 19 (1971) 353.
5 R. D. GUTHRIE AND D. MURPHY, *J. Chem. Soc.*, (1963) 5288.
6 M. ABDEL-AKHER AND F. SMITH, *J. Amer. Chem. Soc.*, 73 (1951) 5859.
7 B. R. BAKER AND D. H. BUSS, *J. Org. Chem.*, 30 (1965) 2308.
8 Y. ALI AND A. C. RICHARDSON, *Carbohyd. Res.*, 5 (1967) 441.
9 M. MATSUI, M. SAITO, M. OKADA, AND M. ISHIDATE, *Chem. Pharm. Bull.* (Tokyo), 16 (1958) 1294.
10 J. P. GREENSTEIN AND M. WINITZ, *Chemistry of the Amino Acids*, Vol. 2, John Wiley and Sons, New York, 1961, p. 938.