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Note

Synthesis of 3-(β-D-ribofuranosyl)-DL-alanine*

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In continuation of our studies on the synthesis of carbohydrate α-amino acids[1], we now report the synthesis of 3-(β-D-ribofuranosyl)-DL-alanine (**7**). Our interest in this type of amino acid stemmed not only from the fact that it could be regarded as an analogue of the sugar moiety of the polyoxins[2], but also that it could be utilized as an intermediate in the synthesis of *C*-nucleosides. In a number of recently published *C*-nucleoside syntheses[3–5], the crucial step was the preparation of an appropriately functionalized, β-oriented, *C*-glycosyl derivative, which could then be elaborated into the *C*-nucleoside. For this reason, β-oriented *C*-glycosyl α-amino acids have potential value in the synthesis of *C*-nucleosides. As it was conceivable that these glycosyl α-amino acids might have interesting biological properties themselves, this served as a further incentive for their synthesis.

Although the classical Erlenmeyer azlactone synthesis[6] is one of the oldest known methods for the synthesis of amino acids[7], it has received surprisingly little attention in carbohydrate chemistry. In a previous communication[8], we described the use of this reaction in the preparation of glycos-3-yl α-amino acids and related compounds, from ketoses and aldehydo nucleosides. This work has now been extended to include the azlactone synthesis with an anhydroaldose.

In the earlier versions of the Erlenmeyer synthesis[9], the reaction was conducted by heating the carbonyl compound with hippuric acid, acetic anhydride, and sodium or lead(II) acetate. In later years, it was found that the usually low yields obtained in the classical method[9] could be markedly improved by the direct use of preformed 2-phenyloxazolin-5-one[10] (**2**) with the carbonyl compound and catalyst. Thus, when 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allose[4,11] was treated with an equimolar amount of **2** in anhydrous 1,2-dimethoxyethane in the presence of a catalytic amount of lead(II) acetate, 4-(2,5-anhydro-3,4,6-tri-*O*-benzoyl-1-deoxy-D-allitol-1-ylidene)-2-phenyloxazol-5-one (**3**) was formed in 56% yield (Scheme 1).

Although it is known that, in some cases, only one of the two possible geometric isomers is obtained by the azlactone synthesis[12], both isomers were obtained when **2** was treated with 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose[8], and thus

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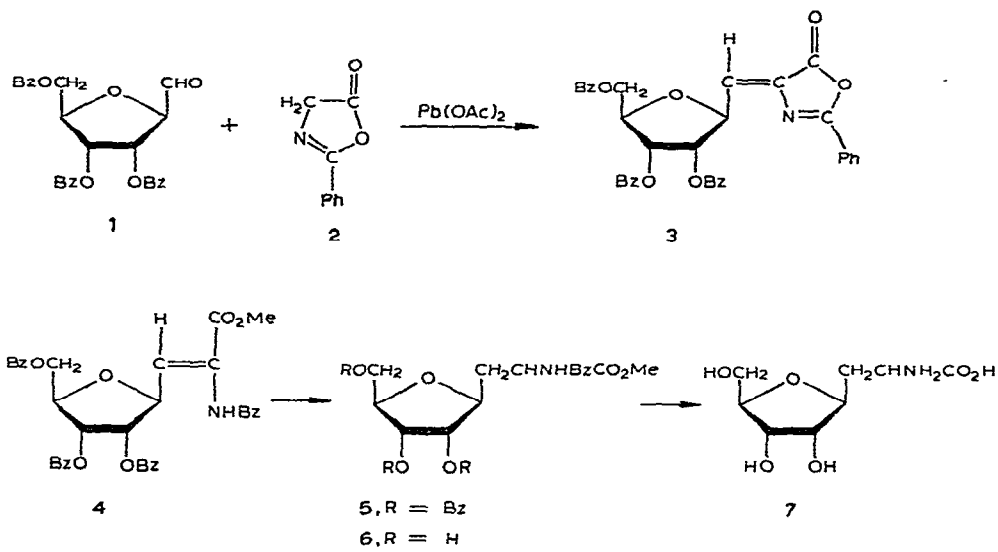

Scheme 1

it seemed very likely that both isomers would be formed in this instance. Compound **3** was, however, obtained as a crystalline material having a sharp melting-point and although the possibility of co-crystallization of the *E* and *Z* isomers could not be excluded, no evidence for the existence of more than one isomer could be detected by t.l.c. in a variety of solvent systems. The n.m.r. spectra of a number of isomeric azlactones, and the acrylic esters derived from the corresponding azlactones, have been investigated[13–15]. In each case it was found that a difference in chemical shift of the groups attached to the unsaturated carbon atom could be observed because of the deshielding effect of the carbonyl and benzamido groups. It is thus to be expected that, if compound **3** consisted of a mixture of isomers, its n.m.r. spectrum should show two signals for both the vinylic proton and H-2 of the carbohydrate moiety. The presence of only one set of signals for each of these protons in the n.m.r. spectrum of **3** was verified by decoupling experiments, thus providing conclusive evidence for the existence of only one isomer.

Methanolysis of **3** with a catalytic amount of sodium acetate in methanol, which should not change the geometric configuration[12,15], gave a quantitative yield of methyl 4,7-anhydro-2-benzamido-2,3-dideoxy-5,6,8-tri-*O*-benzoyl-D-*allo*-oct-2-enoate (**4**), obtained crystalline. The deshielding effect exerted by the methoxycarbonyl and benzamido groups on the vinylic substituents is even more pronounced in this compound than in the corresponding azlactone[13]; thus the presence of only one set of resonances for both H-3 and H-4 in the n.m.r. spectrum of **4** confirmed the isomeric purity of both **3** and **4**. In order to determine the stereochemistry about the double bond by means of the chemical shift of the vinylic proton, it was necessary to consider the deshielding influences of the various substituents on this proton. Morgenstern *et al.*[16] very elegantly proved that a vinylic proton *cis* to the benzamido

group is more deshielded than a vinylic proton *cis* to the carboxyl group. Although it would have been preferable to have both isomers to permit an unequivocal assignment, the chemical shift of H-3 of **4** (τ 3.73) corresponded very well with the chemical shift of the vinylic proton *cis* to the methoxycarbonyl group in methyl 2-benzamidoacrylate[16]. The thermodynamically more stable[15] *Z* stereochemistry was thus assigned to both **3** and **4**.

Hydrogenation of **4** over 5% palladium on charcoal was non-specific and gave the stereoisomeric mixture of protected glycosyl α-amino acids **5**. The presence of both isomers was verified by the observation of two methoxyl resonances in the n.m.r. spectrum of **5**, but unfortunately it was not possible to separate the isomers. Instead of hydrolyzing the mixture directly to the fully deprotected glycosyl α-amino acids, the benzoate groups were saponified with sodium methoxide in methanol in the hope that product **6** might be resolved. All attempts to resolve this mixture failed, and thus it was hydrolyzed with concentrated aqueous barium hydroxide to give 3-(β-D-ribofuranosyl)-DL-alanine (**7**) in 52% yield.

EXPERIMENTAL

General methods. — N.m.r. spectra were determined with a Varian XL-100 or T-60 spectrometer with tetramethylsilane as the internal standard. Optical rotations were recorded with a Perkin–Elmer Model 141 automatic polarimeter and i.r. spectra with a Perkin–Elmer 337 spectrometer. Column chromatography was performed on t.l.c.-grade silica gel (Merck Silica Gel H) under a pressure of 8–10 $lb \cdot in^{-2}$. Melting points were determined on a Leitz Model 350 microscope heating-stage and are corrected. Chemical analyses were performed by Mr. P. Borda, Microanalytical Laboratory, University of British Columbia.

(Z)-*4-(2,5-Anhydro-3,4,6-tri-*O*-benzoyl-1-deoxy-*D*-allit-1-ylidene)-2-phenyloxazol-5-one* (**3**). — Anhydrous lead(II) acetate (0.5 g) was added to a solution of **1** (7.4 g, 15.6 mmoles) and **2** (2.52 g, 15.6 mmoles) in dry 1,2-dimethoxyethane (200 ml). The mixture was refluxed for 4 h in an apparatus equipped with a Kontes cylindrical funnel (filled with type 4A molecular sieves) and a pressure-equalizing line. The solvent was removed *in vacuo* and the residue dissolved in benzene (200 ml). The benzene solution was washed with water (2 × 100 ml), dried (sodium sulfate), and concentrated to a syrup. Chromatography of this mixture on silica gel with 7:3 hexane–acetone gave crystalline **3** (4.65 g, 56%). Recrystallization from benzene–hexane afforded **3** as colourless needles, m.p. 140–141°, $[\alpha]_D^{28}$ −4.6° (*c* 0.65, chloroform); τ_{CDCl_3} 1.82–2.75 (m, 20, aromatic protons), 3.31 (d, 1, $J_{1,2}$ 7.5 Hz, H-1), 4.18 (dd, 1, $J_{3,4}$ 5.5 Hz, $J_{2,3}$ 5.5 Hz, H-3), 4.39 (q, 1, H-2), 5.11–5.44 (m, 3, H-5,6,6').

Anal. Calc. for $C_{36}H_{27}NO_9$: C, 70.01; H, 4.41; N, 2.27. Found: C, 69.85; H, 4.49; N, 2.10.

Methyl (Z)-*4,7-anhydro-2-benzamido-2,3-dideoxy-5,6,8-tri-*O*-benzoyl-*D*-allo-oct-2-enoate* (**4**). — A suspension of compound **3** (140 mg) in a solution of sodium acetate (20 mg) in methanol (20 ml) was briefly heated on a waterbath to effect

dissolution. After 30 min at room temperature, no more starting material could be detected by t.l.c. (7:3 hexane–acetone) and the mixture was concentrated to a syrup that was partitioned between water (25 ml) and chloroform (25 ml). The chloroform layer was dried (sodium sulfate) and evaporated to a crystalline residue. Recrystallization from methanol gave **4** (140 mg, 95%) as colourless needles, m.p. 134–135°, $[\alpha]_D^{28}$ +30° (*c* 0.82, chloroform); τ_{CDCl_3} 1.47 (s, 1, exchanges with D_2O, NH), 1.87–2.68 (m, 20, aromatic protons), 3.73 (d, 1, $J_{3,4}$ 6.5 Hz, H-3), 4.12–4.22 (m, 2, H-5,6), 4.75 (q, 1, $J_{4,5}$ 3.8 Hz, H-4), 5.10–5.54 (m, 3, H-7,8,8'), 6.23 (s, 3, OMe).

Anal. Calc. for $C_{37}H_{31}NO_{10}$: C, 68.41; H, 4.81; N, 2.16. Found: C, 68.13; H, 4.83; N, 2.00.

*Methyl 4,7-anhydro-2-benzamido-2,3-dideoxy-5,6,8-tri-*O*-benzoyl-*D-glycero-D-allo(*and* D-altro)*-octonate* (**5**). — Compound (**4**) (356 mg) was dissolved in ethyl acetate (20 ml) and hydrogenated over a catalytic amount of 5% palladium-on-charcoal catalyst at atmospheric pressure for 24 h. The catalyst was removed by filtration and the solvent was removed under vacuum to give **5** as an oil (351 mg, 98%).

Anal. Calc. for $C_{37}H_{33}NO_{10}$: C, 68.20; H, 5.10; N, 2.15. Found: C, 68.04; H, 5.24; N, 2.00.

*Methyl 4,7-anhydro-2-benzamido-2,3-dideoxy-*D-allo (*and* D-altro)*-octonate* (**6**). — Compound **5** (500 mg) was dissolved in dry methanol (5 ml) and treated with sodium methoxide (1 ml of a solution prepared from 0.5 g of sodium in 100 ml of methanol). The mixture was kept for 20 h at room temperature and then neutralized by stirring with an excess of Amberlite IR-120 (H^+). After filtration, the mixture was concentrated to an oil that was purified by chromatography on silica gel with 4:1 ethyl acetate–methanol as developer. Compound **6** was obtained as an oil (235 mg, 90%).

Anal. Calc. for $C_{16}H_{21}NO_7$: C, 56.63; H, 6.24; N, 4.13. Found: C, 56.47; H, 6.50; N, 4.21.

*2-Amino-4,7-anhydro-2,3-dideoxy-*D-glycero-D-allo(*and* D-altro)*-octonic acid* [3-(*β*-D-*ribofuranosyl*)-DL-*alanine*] (**7**). — Compound **6** (0.3 g) was refluxed with aqueous concentrated barium hydroxide (50 ml) for 48 h. The solution was neutralized with dilute sulphuric acid and the precipitated barium sulphate removed by filtration. The solution was then concentrated *in vacuo* to a small volume, and was deposited on a column of Dowex-203 (OH^-) resin. The column was eluted with distilled water and the ninhydrin-positive fractions were combined and evaporated to an amorphous residue. This residue was further purified on a column of Dowex 50W-X8 (acid form)[11]. The product so obtained was recrystallized from ethanol–water to give the amino acid **7** (120 mg, 52%), m.p. 108–109° $[\alpha]_D^{27}$ −12.1° (*c* 0.87, methanol); c.d. $\Delta\varepsilon_{209}$ 0.0 (*c* 1, water).

Anal. Calc. for $C_8H_{15}NO_6 \cdot 0.5\ H_2O$: C, 41.74; H, 6.95; N, 6.09. Found: C, 41.54; H, 6.75; N, 6.21.

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