

3-*O*-Substituted D-Glucosamine; Oxazoline and N-Benzoyl Derivatives

BERNT LINDBERG and HUBERT AGBACK

Research Division, AB Pharmacia, Uppsala, Sweden

From D-glucosamine a series of 2-phenyl-4,5-(3-*O*-carboxyalkyl-5,6-isopropylidene-D-glucofurano)-*A*²-oxazolines and related derivatives have been prepared and tested for potential antimicrobial activity.

Muramic acid has been isolated from bacterial cell walls¹ and it has been shown by Strange and Kent² to be 3-*O*-(1-carboxyethyl)-D-glucosamine. In seeking potential muramic acid antagonists we have prepared the compounds listed in Table 1 and Fig. 1. They have been tested for antimicrobial action in a serial dilution test (highest concentration used 200 $\mu\text{g/ml}$) against a human strain of *Mycobacterium tuberculosis*, and *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus β -haemolyticus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Tricophyton mentagrophytes*. None of the compounds caused any inhibition of growth.

Strange and Kent synthesized muramic acid from methyl 4,6-benzylidene-*N*-acetyl-D-glucosaminide.² Gigg and Carroll have reported a more convenient method for the synthesis³ using 2-phenyl-4,5-(5,6-isopropylidene-D-glucofurano)-*A*²-oxazoline, *I*, which has proved to be a useful intermediate for the substitution of the 3-position in D-glucosamine.⁴ They have also used this compound for the preparation of the 3-*O*-carboxymethyl and 3-*O*-methyl ethers of D-glucosamine.

The oxazoline derivative, *I*, was first prepared by Konstas *et al.*⁵ by treating *N*-benzoyl-D-glucosamine with hydrochloric acid in acetone. Reckendorf and Bonner⁴ improved and simplified the isolation of the compound. It is very sensitive to acid hydrolysis, and we have found that the yield is dependent on how the final neutralization of the excess hydrochloric acid used in the synthesis is performed. If the neutralization is carried out in an aqueous medium, the reaction mixture should be added to the base. The best results were obtained with water-free neutralization using triethylamine as a base.

The substitution of *I* is performed by treating it with sodium hydride in a suitable solvent and adding the ester of the appropriate halogen carboxylic acid. Gigg and Carroll used tetrahydrofuran as a solvent.³ When preparing

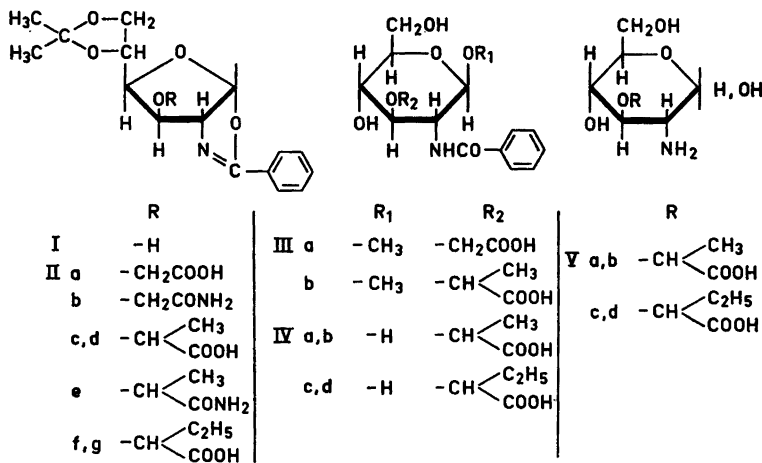


Fig. 1. Derivatives of D-glucosamine.

the 3-*O*-carboxymethyl ether, *IIa*, we found, however, that the yield increases if tetrahydrofuran is replaced by dimethylformamide. Reactions of this type are favoured by aprotic dipolar solvents, and Parker⁶ has shown that dimethylformamide is a good solvent for S_N2 reactions between anions and alkyl halides.

The hydrolysis of the 3-*O*-carbalkoxy substituted oxazoline derivative can be carried out in several steps. Alkaline hydrolysis only attacks the ester linkage and yields the corresponding free acid after acidification, *II a, c, d, f, g*. Hydrolysis in the pH range of the autoacidity of the carboxyl group of the substituent, or in other cases with very dilute hydrochloric acid,⁵ opens the oxazoline ring, removes the isopropylidene group, rearranges the furanose to pyranose and yields the corresponding 3-*O*-substituted 2-benzamido-2-deoxy-D-glucopyranose, *IV a-d*.

If the hydrolysis is carried out in a methanolic hydrochloric acid medium, the oxazoline ring is opened stereospecifically with subsequent removal of the isopropylidene group and rearrangement from furanoside to pyranoside yielding the methyl β -D-glucopyranoside,^{4,5} *III a, b*.

Hydrolysis with stronger hydrochloric acid removes the benzoyl group yielding the 3-*O*-substituted D-glucosamine, *V a-d*. The isolation and characterization of the fully hydrolyzed compounds was, however, abandoned because of very poor crystallization properties.

Separation of stereoisomers is best effected after the alkaline hydrolysis. In the case of muramic acid, the oxazoline derivative could easily be separated from the stereoisomer by crystallization from benzene,³ and we succeeded in separating the carboxypropyl homologues in the same manner.

The lactic acid moiety of muramic acid was assumed by Strange and Kent² to have the D-configuration because of the high dextrorotation of muramic acid; Matsushima⁷ has shown this to be the case by stereospecific synthesis. From Table 1 it is seen that the physical properties of the homologous pairs *II f, g* and *II c, d* are entirely analogous. As the replacement of the methyl group

Table 1. Physical and analytical data of the synthesized 3-O-substituted D-glucosamine derivatives.

Compound		m.p.	[α] _D ²⁰	IR data		Elementary analyses			Found/Calc.	
Type	3-O-substituent			No.	$\nu_{C=O}$ substituent	$\nu_{C=N}$ oxazoline	Formula	C	H	N
Oxazoline derivatives	carboxymethyl	IIa	182-184°	-23°	1728	1630	C ₁₈ H ₃₁ NO ₇	59.2	5.6	3.5
	carbonylmethyl	b	97-98.5°	+ 0.2°	1692	1648	C ₁₈ H ₃₁ N ₂ O ₆	59.5	5.83	3.85
	D-1-carboxyethyl	c	163-164°	+ 63°	1725	1635	C ₁₉ H ₃₃ NO ₇	59.7	6.12	7.73
	L-1-carboxyethyl	d	207-208°	-38°	1705	1635	C ₁₉ H ₃₃ NO ₇	60.5	6.14	3.71
	L-1-carbamylethyl	e	164.5-166°	+ 6.4°	1679	1647	C ₁₉ H ₃₃ N ₂ O ₆	60.5	6.14	3.71
	D-1-carboxypropyl	f	192-193°	+ 73°	1720	1635	C ₂₀ H ₃₅ NO ₇	60.6	6.43	7.44
	L-1-carboxypropyl	g	217-217.5°	-34°	1708	1635	C ₂₀ H ₃₅ NO ₇	61.4	6.69	3.58
Methyl N-benzoyl-glucosaminide derivatives	carboxymethyl	IIIa	135°dec.	-23°	1748		C ₁₆ H ₂₁ NO ₆	53.2	6.3	3.9
	D-1-carboxyethyl	b	179-181°d.	+ 7.6°	1752		C ₁₇ H ₂₃ NO ₆	54.1	5.96	3.94
N-Benzoyl-glucosamine derivatives	D-1-carboxyethyl	IVa	170-172°d.	+ 37°	1730		C ₁₆ H ₂₁ NO ₆	54.0	6.1	3.9
	L-1-carboxyethyl	b	189-192°d.	+ 13°	1719		C ₁₇ H ₂₃ NO ₆	54.1	5.96	3.94
	D-1-carboxypropyl	c		+ 35°	1710		C ₁₇ H ₂₃ NO ₆	53.7	6.1	3.6
	L-1-carboxypropyl	d		+ 16°			C ₁₇ H ₂₃ NO ₆	55.3	6.28	3.79
Glucosamine hydrochloride derivatives	D-1-carboxyethyl	Va	105°	+ 105°			C ₁₀ H ₁₆ ClNO ₇	40.1	6.7	4.3
	L-1-carboxyethyl	b	74°	+ 74°				39.8	6.68	4.64
	D-1-carboxypropyl	c	120°dec.	+ 104°	1727					
	L-1-carboxypropyl	d		+ 69°						

by an ethyl group in a number of known lactic acid derivatives has only a very slight influence on the rotation,⁸ there is no reason why this should not hold in this case. The assignments of configurations to the pair *II f, g* are therefore based on this analogy. *Vc* should then be highly dextrorotatory, which is the case. The optical rotation properties of the hydrolysis products of the pair *II f, g* compared with those of the pair *II c, d* are also entirely consistent with the assignments made.

The amides, *II b, e*, have been prepared from the corresponding acids by the mixed anhydride method with isobutyl chloroformiate.^{9,10} The amides are probably best prepared from the separated acid stereoisomers, as difficulties were encountered in the separation of stereoisomers when the oxazoline derivative *I* was substituted with 1-bromopropionamide.

EXPERIMENTAL

N-Benzoyl-D-glucosamine was prepared according to Konstas *et al.*⁵

2-Phenyl-4,5-(5,6-isopropylidene-D-glucosufurano)-Δ²-oxazoline,^{4,5} *I*. Hydrogen chloride (77.5 g) was absorbed in dry acetone (3500 ml). *N-Benzoyl-D-glucosamine* (110 g) was added to the cooled solution, the mixture was stirred for 3 h and was then left for 20 h at room temperature. Undissolved material (15 g) was removed by filtration, and the filtrate was added to a solution of triethylamine (345 ml) in acetone (1000 ml) with cooling and stirring. The solution was filtered, dried, treated with activated carbon, and evaporated. *I* (120 g) was obtained and recrystallized from methanol, m.p. 160–161.5°.

(a) *Oxazoline derivatives*. *2-Phenyl-4,5-[3-O-(carboxymethyl)-5,6-isopropylidene-D-glucosufurano]-Δ²-oxazoline*,³ *IIa*. *I* (23.2 g, 0.076 mole) was dissolved in dimethylformamide (200 ml). Sodium hydride (2.0 g, 0.083 mole) was added and the mixture was kept at 40° for one hour. Ethyl chloroacetate (16.0 g, 0.13 mole) was added, and the temperature was kept at 70° for 20 h. 120 ml of dimethylformamide were removed by evaporation *in vacuo*, the rest was poured into water (500 ml), and the mixture was extracted with ethyl acetate. The extract was dried and evaporated. The material obtained was hydrolyzed with sodium hydroxide (5.4 g) in a mixture of water (25 ml) and methanol (25 ml) for 10 min at 50°. Water (250 ml) was added, and unreacted *I* (8.0 g) was removed by filtration. The filtrate was washed with ether, and from the ether solution a further yield of 0.9 g of *I* was isolated. The crude product (14.5 g) was precipitated from the water phase by acidification to pH 4. Yield corrected for recovered *I*, 86 %. Heating for 2.5 h instead of 20 h gives a corrected yield of 79 %. When tetrahydrofuran was used as a solvent with heating for 3 h, a corrected yield of 57 % was obtained. The product was recrystallized from ethyl acetate.

2-Phenyl-4,5-[3-O-(carbamylmethyl)-5,6-isopropylidene-D-glucosufurano]-Δ²-oxazoline, *IIb*. *IIa* (3.6 g, 0.01 mole) and triethylamine (1.1 g, 0.011 mole) were dissolved in chloroform (75 ml), and the solution was cooled to –5°. A solution of isobutyl chloroformiate (1.4 g, 0.01 mole) in chloroform (25 ml) was added, keeping the temperature below –5°. After 5 min, a 0.16 N solution of ammonia in ether (80 ml) was added with cooling, the temperature being kept below 10°, and the solution was left for 3 h at room temperature. After evaporation a semi-solid residue was obtained, which crystallized when rubbed with water. The crude product (3.6 g) was obtained in a nearly quantitative yield. Recrystallization from benzene-cyclohexane followed by recrystallization from water-ethanol yielded 2.5 g. The IR spectrum in chloroform, 2 % w/v, showed $\nu_{C=O}$ (amide I) 1687s, $\nu_{C=N}$ (oxazoline) 1642s, $\nu_{amide II}$ 1603s, 1585m.

2-Phenyl-4,5-[3-O-(D,L-1-carboxyethyl)-5,6-isopropylidene-D-glucosufurano]-Δ²-oxazoline, *II c, d* were prepared and the stereoisomers separated according to Gigg and Carroll.³ Yield of *IIc* 41 % and of *II d* 28 %, starting with 11.6 g of *I*.

2-Phenyl-4,5-[3-O-(L-1-carbamylethyl)-5,6-isopropylidene-D-glucosufurano]-Δ²-oxazoline, *IIe* was prepared from *II d* in the same manner as *II b*. 3.8 g of *II d* yielded 3.4 g of crude product, which was recrystallized from benzene-cyclohexane. Yield 2.4 g, 63 %. The IR spectrum in chloroform, 2 % w/v, showed $\nu_{C=O}$ (amide I) 1693s, $\nu_{C=N}$ (oxazoline) 1642s, $\nu_{amide II}$ 1583s, 1573m.

A product obtained in the same manner from *IIC* crystallized only with difficulty in very poor yield, m.p. 176–181°.

2-Phenyl-4,5-[3-O-(D,L-1-carboxypropyl)-5,6-isopropylidene-D-glucopyranano]-Δ²-oxazoline, II f, g. I (11.7 g, 0.038 mole) was dissolved in dry tetrahydrofuran (150 ml) and refluxed with sodium hydride (0.97 g, 0.04 mole) for 2 h. Under cooling and stirring, ethyl 1-bromobutyrate (12.0 g, 0.062 mole) was added, and refluxing was continued for another hour. After cooling, sodium bromide was removed by filtration, and solvent as well as excess ester were removed by evaporation *in vacuo* at room temperature. The product obtained was hydrolyzed with sodium hydroxide (2.7 g) in a mixture of water (11.7 ml) and methanol (11.7 ml) for 10 min at 50°. Water (117 ml) was added and the solution was saturated with potassium chloride. Unreacted *I* was removed by extraction with ether, and the acid *II f, g* was precipitated by acidification with hydrochloric acid to pH 4 at 2–3°. The crude material (11.3 g) was dissolved in hot benzene (120 ml) and when left overnight, the high melting stereoisomer (3.1 g), *IIg*, crystallized as fine needles, yield 21%. The filtrate was concentrated to 60 ml and when left overnight, the low melting stereoisomer (4.8 g), *IIf*, crystallized as fine needles, yield 32%. The optical rotation of the oxazoline derivatives was measured in chloroform, *c* 3% w/v.

(b) *Hydrolysis products. Methyl 2-benzamido-3-O-(carboxymethyl)-2-deoxy-β-D-glucopyranoside, IIIa. IIIa* (1.5 g) was dissolved in a 0.1 N solution of hydrogen chloride in dry methanol (50 ml) and the solution was left for 4.5 h at room temperature and was neutralized to pH 5 with silver carbonate, filtered, and evaporated. The residue was crystallized by rubbing with cyclohexane-ether. The crude material obtained (1.2 g) was recrystallized from isopropanol.

Methyl 2-benzamido-3-O-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranoside, IIIb. From *IIC*, (1.5 g) *IIIb* (1.2 g) was obtained in the same manner as *IIIa*. The product was recrystallized from ethyl acetate. The optical rotation of *IIIa, b* was measured in methanol, *c* 3% w/v.

2-Benzamido-3-O-(L-1-carboxyethyl)-2-deoxy-D-glucopyranose, IVb. IId (1 g) was hydrolyzed by refluxing with water (100 ml) for 4 h. The solution was washed with ether, treated with activated carbon, and evaporated. The residue was recrystallized from water; yield 0.7 g, 74%. The product was shown to be chromatographically uniform by thin-layer chromatography in water. It mutarotated from +23° (4 min) to the value given in Table 1 (17 h), *c* 3% w/v in water.

Hydrolysis of *IIC* in the same manner yielded an impure amorphous product, *IVa*. The optical rotation of *IVa* was therefore measured on the hydrolyzed water solution after 40 h, pH 2.8, *c* 4% w/v.

2-Benzamido-3-O-(L-1-carboxypropyl)-2-deoxy-D-glucopyranose, IVd. A fraction of *IIg* with m.p. 208–210° (1.8 g) was hydrolyzed in the same manner as above. The product obtained was recrystallized from water; yield 0.72 g. This product mutarotated from +34° (4 min) to +15.9° (30 h), *c* 3% w/v in water. For comparison a hydrolyzed solution of *IIg* was also measured and showed an optical rotation of +15.5° after 40 h, pH 2.2, *c* 3.5% w/v.

Hydrolysis of *IIf* in the same manner yielded an impure product, which could not be crystallized. The optical rotation of *IVc* was therefore measured on the hydrolyzed water solution after 24 h, pH 2.5, *c* 3% w/v.

2-Amino-3-O-(D-1-carboxypropyl)-2-deoxy-D-glucopyranose, Vc. IIIf (1 g) was hydrolyzed by refluxing with 2.7 N hydrochloric acid (20 ml) for 4 h. After washing with ether and removal of the excess hydrochloric acid by evaporation, the residue was purified by chromatography on a silica gel column. A chromatographically uniform product (0.6 g) was obtained in the form of the hygroscopic hydrochloride. (Found: Cl 11.1. Calc. for C₁₀H₂₀ClNO₅: Cl 11.8). It mutarotated from ~0° (4 min) to the value given in Table 1 (24 h), pH 2.1, *c* 3% w/v in water. The IR spectrum showed a strong band in the region 3300–2950 mμ, and two medium peaks of 1605 and 1505 mμ.

Hydrolysis of *IIg* in the same manner did not yield a chromatographically pure product. The optical rotation of *Vd* was therefore measured on the 2.7 N hydrochloric acid hydrolysis solution, which was decanted from separated benzoic acid, *c* 2% w/v. For comparison a solution of *IIf*, hydrolyzed in the same manner, was also measured and yielded the same optical rotation as the isolated hydrochloride at pH 2.1, *c* 2% w/v.

The optical rotations and concentration data of all the glucosamine derivatives have been corrected to the free amine formula.

Table 2. The dependence of optical rotation on pH for 3-O-substituted glucosamines.

pH \ Compound	2.7 N HCl	0.7	2.1	3.3	5.8	6.8	Ref. 2
Va D	+ 105°	—	—	+ 106°	+ 110°	+ 95°	+ 109°
Vc D	+ 104°	+ 104°	+ 104°	+ 112°	+ 110°	+ 80°	—
Vb L	+ 74°	+ 75°	—	+ 55°	—	—	+ 52°
Vd L	+ 69°	—	~ + 70°	+ 48°	—	—	—

Muramic and isomuramic acid hydrochlorides, Va, b, IIc, d were hydrolyzed in 2.7 N hydrochloric acid in the same manner as above, and the optical rotations of the solutions were measured, *c* 2 % w/v.

Dependence of optical rotation on pH for 3-O-substituted glucosamine derivatives. Due to protolytic changes, the optical rotation of the glucosamine derivatives is pH dependent. Amino acids behave similarly, and their rotations undergo abrupt changes when passing from acid to alkaline medium.¹¹ Table 2 shows some measurements of the optical rotations of hydrolyzed solutions, which were buffered to various pH values by using sodium citrate and sodium hydroxide. The measurements were made at 20°, *c* 0.5–3% w/v. The rotation undergoes changes in the pH regions 2–3.5 and 5.5–7. In the first interval it increases for the D derivatives and decreases for the L derivatives. In the second interval it decreases for the D derivatives. The rotations in Table 1 are given for 2.7 N hydrochloric acid solutions.

Optical rotation. A Perkin Elmer Model 141 automatic recording polarimeter was used.

Infrared spectra. A Unicam SP 100 spectrophotometer with sodium chloride optics was used. The substances were examined with the potassium bromide disc technique.

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