

## Synthesis of Saccharopine and Pyrosaccharopine

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The isolation and structure elucidation of L-saccharopine ((2*S*,2'*S*)-*N*<sub>6</sub>-(2-glutaryl)-lysine) from *Saccharomyces* species has previously been reported. Synthesis was reported at the same time of a diastereoisomeric mixture of L-saccharopine and D-allosaccharopine (with *R*-configuration at C<sub>2</sub> in the glutamic acid moiety) which was separated by fractional crystallization. Conversion of the diastereoisomeric mixture into a mixture of lactams (*N*-(5-amino-5-carboxypentyl)-2-pyrrolidon-5-carboxylic acids) was also described.<sup>1,2</sup> The name pyrosaccharopine is proposed for that lactame isomer which bears the same structural relationship to saccharopine as pyroglutamic acid to glutamic acid.

L-Saccharopine has subsequently been identified as a key intermediate in the biosynthesis of lysine in yeasts and fungi (see Ref. 3 and references cited therein) and as a degradation product of lysine in mammals (see Ref. 4 and references cited there). Recently, both saccharopine and pyrosaccharopine have been identified in a higher plant (buckwheat, *Fagopyrum esculentum*).<sup>5</sup> The growing interest in saccharopine and pyrosaccharopine prompts us to report in more detail a simplified synthesis of saccharopine, as well as the synthesis and properties of homogeneous pyrosaccharopine. The nuclear magnetic resonance spectra of the two compounds are also discussed.

Strecker synthesis involving unprotected L-lysine, 2-ketoglutaric acid, and cyanide affords L-saccharopine and D-allosaccharopine, easily isolated from the reaction mixture by ion-exchange chromatography. The optical rotation value of the isolate indicates a nearly 1:1 composition with regard to the two diastereoisomers. The infrared spectrum of the isolate was identical with that of the previously described mixture of the two isomers.<sup>2</sup> The NMR-spectrum of the isolate was identical with that of L-saccharopine (see below). On paper chromatograms, the isolate gave negative reaction with a colour

reagent developed for distinguishing  $\alpha$ -monoamino acids from other amino compounds,<sup>6</sup> ascertaining that no significant amount was present of the positional isomer of saccharopine, with substitution at the  $\alpha$ - rather than the  $\epsilon$ -amino group of the lysine moiety. The previously employed protection of the  $\alpha$ -amino group (involving three synthetic steps)<sup>2</sup> hence appears superfluous.

It was reported previously that saccharopine was transformed into pyrosaccharopine by heating in the dry state or in water at 125°. Transformation of saccharopine into pyrosaccharopine can also be achieved by refluxing of an aqueous solution. Paper chromatographic examination of the reaction mixture indicates a "half life" of saccharopine under these conditions of between one half and one hour. The transformation into pyrosaccharopine closely resembles the conversion of glutamic acid into pyroglutamic acid.<sup>7</sup> Pure L-pyrosaccharopine has now been prepared from L-saccharopine by refluxing an aqueous solution of the latter.

The NMR-spectra of L-saccharopine and L-pyrosaccharopine have been recorded in trifluoroacetic acid and in deuterium oxide containing sodium hydrogen carbonate. The spectra of L-lysine, L-glutamic acid, and L-pyroglutamic acid were recorded for comparison. In trifluoroacetic acid the spectra consist of broad, poorly resolved peaks. The  $\alpha$ -protons occur at  $\delta$  4.2–4.8 ppm in all of the compounds. The two protons at C<sub>6</sub> in the lysine moiety occur at  $\delta$  3.1–3.6 ppm in lysine, at  $\delta$  3.2–3.6 ppm in saccharopine, and at  $\delta$  3.3–4.0 ppm in pyrosaccharopine. The rest of the protons attached to carbon occur as complex patterns at  $\delta$  1.6–3.1 ppm. The protons attached to nitrogen occur as single, though broadened peaks in this solvent. In lysine the protons on N<sub>2</sub> occur at  $\delta$  7.5 ppm, those on N<sub>6</sub> at  $\delta$  6.9 ppm. In glutamic acid the protons on N occur at  $\delta$  7.6 ppm, in pyroglutamic acid at  $\delta$  8.4 ppm. Saccharopine shows two peaks for N-protons, one at  $\delta$  7.5 ppm (protons at N<sub>2</sub> in the lysine portion), and one at  $\delta$  8.3 ppm. As expected, pyrosaccharopine shows only one peak for N-protons at  $\delta$  7.4 ppm. In deuterium oxide most of the protons occur as multiplets. Most indicative are the  $\alpha$ -protons, appearing in saccharopine as two triplets at  $\delta$  3.73 ppm (corresponding value for lysine  $\delta$  3.73 ppm) and  $\delta$  3.58 ppm (corresponding value for glutamic acid  $\delta$  3.74 ppm). In pyrosaccharopine the

proton at C<sub>2</sub> in the lysine moiety occurs at  $\delta$  3.70, whereas the proton at C<sub>2</sub> in the glutamic acid moiety occurs as a multiplet at  $\delta$  4.0–4.3 (corresponding value for pyroglutamic acid  $\delta$  4.0–4.3 ppm). These spectroscopic data lend further support to the structures of pyrosaccharopine and saccharopine.

*Experimental. Synthesis of a diastereoisomeric mixture of L-saccharopine and D-allosaccharopine.* A solution of 2-ketoglutaric acid (0.05 mol) and KCN (0.32 mol) in 1 N NaOH (100 ml) and adjusted to pH 11.4 with HCl (0.05 mol) in 1 N NaOH (75 ml), adjusted to pH 11.4 with NaOH. The mixture was kept at 50° for 22 h. Conc. hydrochloric acid (330 ml) was added, and the solution was refluxed for 18 h. The reaction mixture was concentrated to dryness. The residue was dissolved in water, and the amino acid fraction was isolated by binding to a strongly acid ion-exchange resin in the hydrogen form, followed by elution with ammonia. The individual amino acids were separated by ion-exchange chromatography on a strongly basic ion-exchange resin (Dowex 1  $\times$  8, 200–400 mesh, 3  $\times$  77 cm) in the acetate form, by elution with acetic acid (1 N). Fractions of 20 ml were collected. Lysine occurred in fractions Nos. 10–13, saccharopine in fractions Nos. 38–47, and pyrosaccharopine in fractions Nos. 65–78. The fractions containing saccharopine were evaporated to dryness. Crystallization from aqueous ethanol gave a paperchromatographically homogeneous mixture of the two diastereoisomers (2.6 g, 19%),  $[\alpha]_D^{20} + 15^\circ$  (c 1.9, 0.5 N HCl).

*Synthesis of L-pyrosaccharopine ((5S, 5'S)-N-(5-amino-5-carboxypentyl)-2-pyrrolidone-5-carboxylic acid).* L-Saccharopine of natural origin (262 mg) was refluxed in water (20 ml) for 5 h. Evaporation to dryness, and two recrystallizations from water-acetone afforded an analytical sample. (Found: C 50.65; H 7.17; N 10.79. Calc. for C<sub>11</sub>H<sub>18</sub>O<sub>5</sub>N<sub>2</sub>: C 51.15; H 7.03; N 10.85.)  $[\alpha]_D^{23} + 3.4^\circ$  (c 1, H<sub>2</sub>O),  $[\alpha]_D^{21} + 7.7^\circ$  (c 1, 1 N HCl). M.p. 164–167° (decomp.) (determined by inserting the sample in an oil bath preheated to 260°).

Rotations were determined in a 1 dm tube. NMR-spectra were measured on a JEOL C-60 HL instrument. TMS was used as an internal standard in trifluoroacetic acid, sodium 3-trimethylsilyl-2,2',3,3'-tetra-deuterio-propionate in deuterium oxide. Microanalyses were performed by Mr. G. Cornali and his staff.

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## The Molecular Basis for Some Physical Properties of Polyuronides

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The previous experimental work on the solution properties of alginate<sup>1,2</sup> has mostly dealt with one alginate sample prepared from *Laminaria digitata* with a ratio of 1.6 between D-mannuronic acid (M.A.) and L-guluronic acid (G.A.) residues. Alginate contains<sup>3-6</sup> long homopolymeric blocks of each monomer, together with blocks of the alternating sequence. In this work we investigate samples containing different amounts of the three types of structure, and we try to estimate their relative extension in the unperturbed state. Ion exchange experiments<sup>7</sup> have shown that in the gel state L-guluronic acid has a markedly higher affinity for calcium ions than has D-mannuronic acid. We shall here compare the ion exchange behaviour of the three types of blocks at conditions where they are all soluble. We then discuss the results in terms of the molecular structure of the polymer chains. The D-mannuronic and L-guluronic acids have