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Synthesis of the Potassium Salt of 2,3,4,5-Tetrahydrodipicolinic Acid, a Key Intermediate in the Diaminopimelate Pathway to L-Lysine

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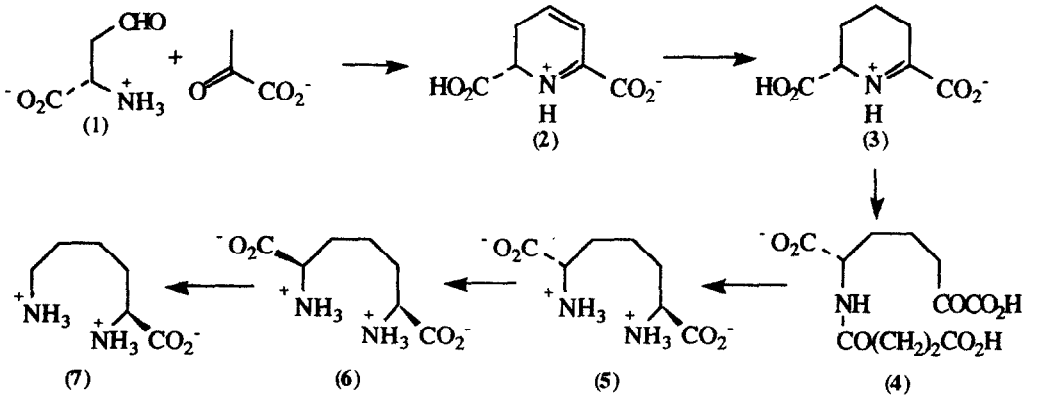
Abstract: The potassium salt (13) of 2,3,4,5-tetrahydrodipicolinic acid, a key intermediate in the diaminopimelate (DAP) pathway to L-lysine (7), has been prepared by elimination of *p*-toluenesulphinic acid from the *N*-toluenesulphonyl derivative (12) of dimethyl *cis*-piperidine-2,6-dicarboxylate with simultaneous cleavage of the ester groups. 2,3,4,5-Tetrahydrodipicolinic acid exists in solution in equilibrium with the corresponding enamine (15) and an open chain form (14), and serves as a substrate for *meso*-DAP dehydrogenase on the biosynthetic pathway to L-lysine (7).

Two distinct pathways to L-lysine (7) exist in bacteria and fungi. The α-aminoadipate route is characteristic of yeast and fungi,[1] whereas the diaminopimelate (DAP) pathway (Scheme 1) occurs in bacteria and higher plants.[2] In the DAP pathway, the condensation of L-aspartic semialdehyde (1) with pyruvate is catalysed by dihydrodipicolinate synthase and gives L-2,3-dihydrodipicolinic acid (2). The action of a reductase then generates L-2,3,4,5-tetrahydrodipicolinic acid (THDPA) (3). These enzymes have been characterised from *E. coli*[3] and from plants.[4] Further enzyme catalysed reaction of THDPA leads to the succinyl derivative (4) *en route* to LL-DAP (5), DL-DAP (6), and L-lysine (7). Very little chemical evidence is available for the widely accepted products (2) and (3) of the first two enzymic reactions in this pathway, and no literature methods are available for the synthesis of 2 or 3 in forms which can be isolated and characterized. A form of THDPA (3) which can be isolated, characterized and stored, is urgently required in order to set up an assay for the reductase enzyme and as a substrate for the succinylase enzyme. We now report the synthesis of the solid, stable, potassium salt of THDPA (3).

Shapshak[5] claimed the formation of the D-isomer of THDPA by treatment of DL-DAP (6) with the L-amino acid oxidase from *Neurospora crassa*, but no chemical or

spectroscopic evidence for the product was provided. In our work DL-DAP was separated from the commercial mixture of DD-, LL-, and DL-isomers by crystallisation from water and ethanol.[6] Treatment of DL-DAP (6) with L-amino acid oxidase (EC 1.4.3.2) or D-amino acid oxidase (EC 1.4.3.3) in D_2O at pD 6 gave no reaction at 18 °C or 40 °C after 2 weeks as judged by ^1H NMR spectroscopy. Attempted chemical transamination of DL-DAP (6) with isonicotinaldehyde in DMF with DBU as base also failed to produce any of the desired imine (3).



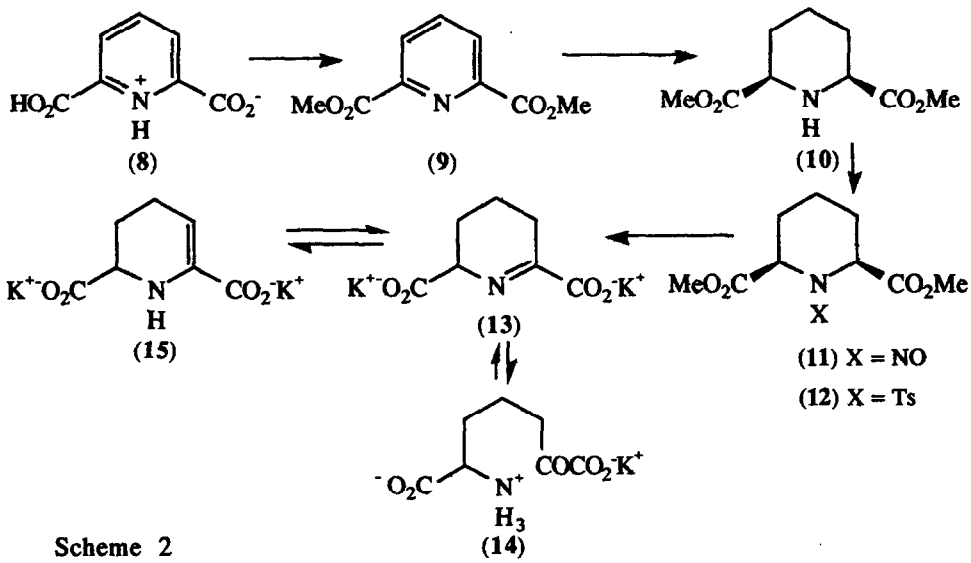
Scheme 1

Kimura and Sasakawa[7] reported the formation of dipicolinic acid (8) and THDPA (3) on cyclisation of αα'-dioxopimelic acid with ammonia. Since the reaction rate was not affected by the presence or absence of oxygen, they assumed that the presumed initial product, 1,4-dihydrodipicolinic acid, disproportionated to dipicolinic acid (8) and THDPA (3). Evidence for the formation of the products was provided by UV adsorption (dipicolinic acid) and by colour reactions with ninhydrin and *o*-aminobenzaldehyde (THDPA). In our work, treatment of αα'-dioxopimelic acid (prepared from diethyl oxaloacetate[8]) with liquid ammonia in a sealed tube gave a mixture of products. From ^1H and ^{13}C NMR data, dipicolinic acid was present together with a number of partially reduced forms, but separation of this mixture and identification of the products proved impossible.

Next we decided to investigate methods for oxidation of amines to the corresponding imines. The first involved heating the amine in dilute acetic acid with mercuric acetate, followed by removal of the mercury as HgS by flushing with H_2S.[9] When *cis*-piperidine-2,6-dicarboxylic acid, prepared by the hydrogenation of dipicolinic acid, was subjected to these conditions, starting material was recovered, probably because of the formation of a mercury complex with the amino diacid system. Use of the diester (10) in this procedure led to *cis*-piperidine-2,6-dicarboxylic acid by ester hydrolysis.

Another method for transforming amines into imines involves reaction of amines with electrophilic reagents such as *N*-bromosuccinimide, followed by elimination of HBr from the *N*-haloamines on treatment with base. This procedure was carried out

with dimethyl *cis*-piperidine-2,6-dicarboxylate (**10**) but led to a mixture of products (^{13}C NMR data). Other potential leaving groups on nitrogen were considered. Nitrosoamines are known to eliminate HNO on treatment with base to form imines.[10] The *N*-nitroso derivative (**11**) of the *cis*-diester was prepared using $NaNO_2$ in 1M HCl, but no elimination of HNO could be achieved with a wide variety of bases.



Scheme 2

N-Tosylsulphonamides undergo elimination under the influence of strong bases with elimination of *p*-toluenesulphinic acid.[11] Accordingly the *N*-tosyl derivative (**12**) of the dimethyl ester (**10**) was prepared. After treatment of **12** with KH or $KN(TMS)_2$ in DMSO the acidified organic extracts gave *p*-toluenesulphinic acid and the aqueous solution was freeze dried to give a mixture of products (NMR data). When the same diester (**12**) in CH_2Cl_2 was treated with KO^tBu under N_2, an exothermic reaction took place and a yellow solid precipitated after several hours. ^1H and ^{13}C NMR data indicated that formation of the desired imine had taken place by elimination of *p*-toluenesulphinic acid and cleavage of the ester groups. Attempts to purify this material using ion exchange chromatography under neutral or acidic conditions gave complex mixtures (^{13}C NMR data). The yellow solid was stirred in distilled water with the weak anion exchanger Amberlite IR-45 (hydroxide form) for 24 hours. The resin was filtered off and the filtrate was freeze dried. Methanol was added to the yellow solid and inorganic material was filtered off. Addition of diethyl ether gave a yellow hygroscopic solid. In the FAB MS of this solid an ion of *m/z* 171 could be due to the imine (**13**). Initial ^{13}C NMR data taken in D_2O were consistent with the presence of the potassium salt (**13**) of THDPA together with the open chain form (**14**) in *ca.* a 1:1 ratio *e.g.* two methine signals were present at δ 67.2 and 63.8. When a more detailed ^1H NMR spectroscopic study (250 MHz) in D_2O was subsequently carried out, evidence was obtained for the presence of three compounds

in solution, namely THDPA (13), the open chain form (14), and the enamine (15) in a 2.8:1:3.7 ratio, *e.g.* the enamine showed a dd at δ 5.20 due to the olefinic proton and the three separate signals for the sole proton α to an amino acid in compounds 13, 14 and 15 integrated in total for one proton. Samples of this mixture that had been left for some time also showed formation of dipicolinic acid (δ 8.34 in the ^1H NMR spectrum) in small amounts.

A compelling piece of evidence for the formation of an equilibrium mixture of 13, 14, and 15 is that hydrogenation of the mixture in water using PtO_2 as catalyst gave a single product, *cis*-piperidine-2,6-dicarboxylic acid, in 95% yield, identified by ^1H, ^{13}C NMR, m.p., and mixed m.p. with authentic material.

Finally the potassium salt of THDPA was shown to be a substrate for *meso*-DAP dehydrogenase by incubation with the enzyme and observation of the disappearance of NADPH. This dehydrogenase is used by some bacteria in the biosythesis of L-lysine (7),[12] and THDPA (3) is converted directly into *meso*-DAP (6) using NADPH.

The spectroscopic and enzymic data, as well as the extremely efficient conversion into the saturated diacid is convincing evidence for the formation of THDPA.

THDPA is stable if kept as the potassium salt. It rapidly decomposes in neutral or acid solution. This explains why methods which use neutral or acidic conditions to generate (3) have failed. THDPA is now available as a substrate for the succinylase step in the lysine pathway and to develop assays for the reductase step.

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References

1. Bhattacharjee, J.K. *Amino Acids Biosynthesis and Genetic Regulation;* Herrmann, K.M.; Somerville, R.L. Eds.; Addison-Wesley: Reading, Mass., 1983, Chap. 13, pp 229-244.
2. Patte, J.-C. Ref. 1, Chap. 12, pp 213-228.
3. Shedlarski, J.G.; Gilvarg, C. *J. Biol. Chem.* **1970**, *245*, 1362-1373; Tamir, H.; Gilvarg, C. *J. Biol. Chem.* **1974**, *249*, 3034-3040.
4. Kampaisal, R.; Hashimoto, T.; Yamada, Y. *Plant Physiol.* **1987**, *85*, 145-151; Tyagi, V.V.S.; Henke, R.R.; Farkas, W.R. *Plant Physiol.* **1983**, *73*, 687-691.
5. Shapshak, P. *Diss. Abstr. Int. B* **1970**, *31*, 521.
6. Wade, R.; Birnbaum, S.M.; Winitz, M.; Koegel, R.J.; Greenstein, J.P. *J. Am. Chem. Soc.* **1957**, *79*, 648-652.
7. Kimura, K.; Sasakawa, T. *J. Biochem.* **1975**, *78*, 381-390.
8. Cope, A.C.; Fournier, A. *J. Am. Chem. Soc.* **1957**, *79*, 3896-3899.
9. Leete, E.; Kim, S.H. *J. Chem. Soc., Chem. Commun.* **1989**, 1899-1900.
10. Oediger, H.; Moller, F.; Eiter, K. *Synthesis* **1972**, 591-598.
11. Negishi, E.; Day, A.R. *J. Org. Chem.* **1965**, *30*, 43-48.
12. Misono, H.; Soda, K. *J. Biol. Chem.*, **1980**, *255*, 10599-10605.

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