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STEREOCHEMICAL COURSE OF THE ENZYMIC AMINATION OF CHLORO- AND BROMO- FUMARIC ACID BY 3-METHYLASPARTATE AMMONIA-LYASE

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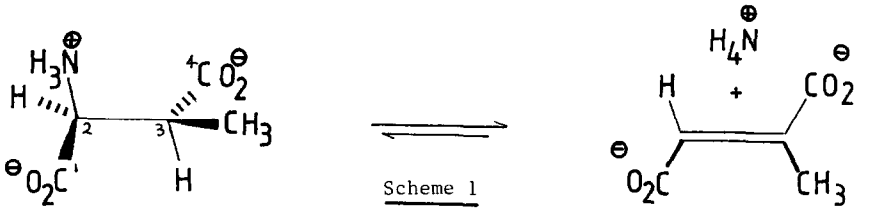
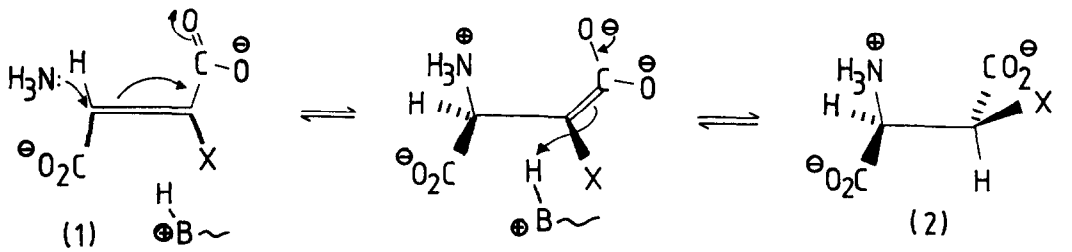
Abstract Enzymic amination of chloro- and bromo- fumaric acid using 3-methylaspartate ammonia-lyase in the presence of ammonia leads to the formation of 3-chloro- and 3-bromo-aspartic acid respectively; the absolute configuration of each product is (R) at C-2 and (S) at C-3.

3-Methylaspartate ammonia-lyase (EC 4.3.1.2) catalyses the reversible α,β-elimination of ammonia from (2S,3S)-3-methylaspartic acid to give mesaconic acid,[1] Scheme 1. It is known that the enzyme is also capable of catalysing the deamination of (2S)-aspartic acid and some other 3-alkylaspartic acids.[2]



Scheme 1

We first became interested in using the enzyme in the synthesis of potential suicide inhibitors and thus our initial objective was to assess the possibility of enzymically aminating α,β-unsaturated dioic acids (e.g. 3-substituted fumaric acids,numbered with respect to the products) using ammonia in a retro-physiological reaction direction. It was expected that the reactions would proceed stereospecifically via anti-addition of ammonia to the C-2 si-face* of the substrates thus introducing two chiral centres, Scheme 2.



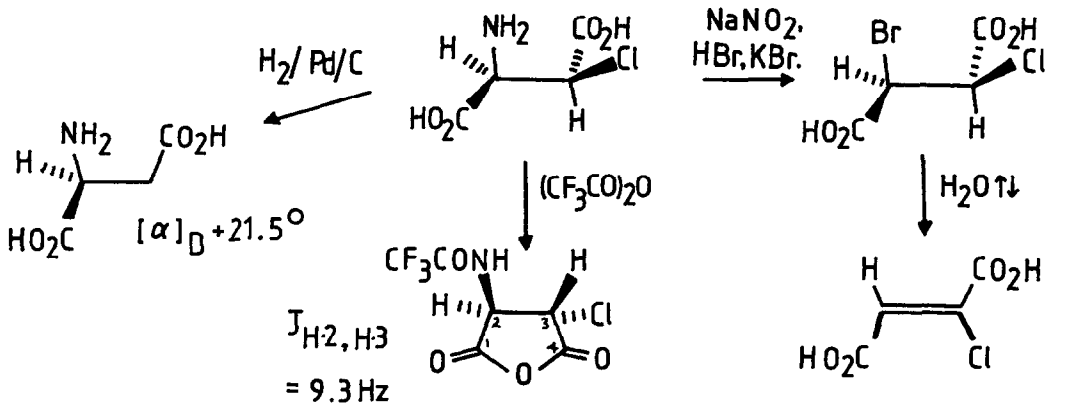
Scheme 2

* This is the C-2 re-face when the substituent at C-3 possesses a higher priority than oxygen.

When each of the fumaric acids (1,X=H,CH_3,F,Cl and Br) were incubated with the enzyme, the expected aminated products were obtained.[3] Since the aspartic acids (2,X=H[4],CH_3[5] and F[6]) were known compounds it was possible to confirm that each reaction had occurred to give the expected stereochemical outcome, Scheme 2. However, in the light of the known ability of the enzyme to deaminate both L-threo- and L-erythro-3-methylaspartic acids[7,8] [i.e. the (2S,3S)- and (2S,3R)- diastereomers respectively] our absolute configurational assignment for chloro- and bromo- aspartic acids [as (2R,3S)†] based on analogy, needed to be verified. Here we present the results of stereochemical determinations for 3-chloro- and 3-bromo- aspartic acid. Chloroaspartic acid {$[\alpha]_D$ - 38.8° (c=0.5,0.1 MHCl)[3]} was subjected to catalytic hydrogenolysis to yield, after purification, aspartic acid with identical chromatographic and nmr spectral parameters to an authentic sample. The optical rotation {$[\alpha]_D^{20}$ + 21.5° (c=1, 6 MHCl), lit[4] $[\alpha]_D$ + 24.6° (6 MHCl)} revealed that the product was the (2S)-antipode and thus the absolute stereochemistry of chloroaspartic acid was (R)- at C-2, Scheme 3.

In order to relate the configuration of C-3 to the now established configuration of C-2, N-trifluoroacetyl-3-chloroaspartic anhydride was prepared through treatment of the diacid with trifluoroacetic anhydride in THF. {$^m/z$ (EI) 209 ($[M-HCl]^+$); ν_{max} 1850, 1790 (cyclic anhydride carbonyl groups) . The 360 MHz ^{1}H-nmr spectrum of the anhydride in d_6-DMSO showed a vicinal coupling constant of 9.3 Hz. This value, although indicative of a H-2, H-3 dihedral angle of near 180° was too small to allow us to rule out the possibility of a vicinal dihedral angle of about 0° which could occur in the other diastereomer. Due to our inability to unambiguously determine the absolute configuration at C-3, an alternative procedure was investigated.

Diazotization of chloroaspartic acid in the presence of bromide ion gave 2-bromo-3-chlorosuccinic acid. {$[\alpha]_D^{20}$ - 18.2° (c=0.8, H_2O); $^m/z$ (EI) 152 and 150 ($[M-HBr]^+$, Cl isotopes), 82 and 80 ($[Br]^+$), 38 and 36 ($[HCl]^+$, Cl isotopes), ^{1}H-nmr (D_2O), δ, 5.12 (2H,s,H-2 and 3)}. This type of reaction is known to occur with overall retention of configuration at the amino-bearing carbon via two inversion processes involving the transient formation of an α-lactone.[9] The resulting 2-bromo-3-chlorosuccinic acid was refluxed with water to yield only trans-chlorobut-2-enedioic acid (chlorofumaric acid) identical in all respects to an authentic sample, Scheme 3.



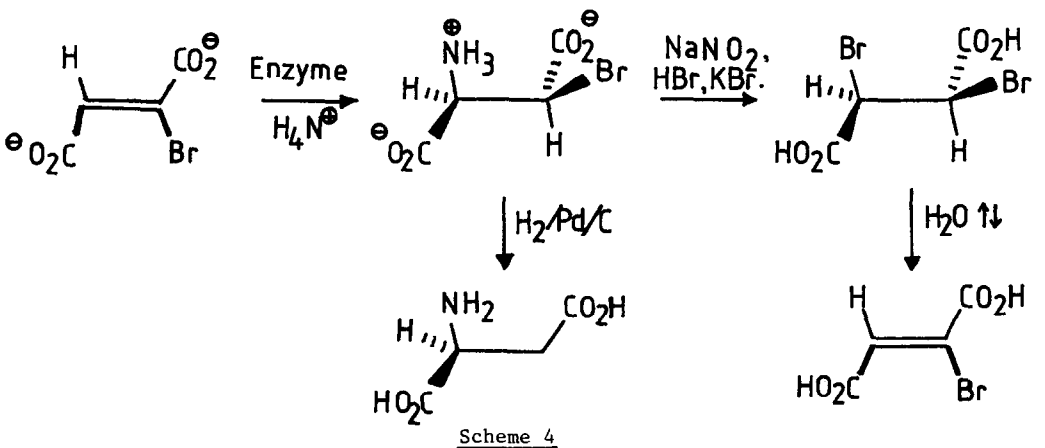
Scheme 3

† Note priority change at L-centre

Since dehydrobromination is expected to occur via anti-elimination, and since the absolute stereochemistry at C-2 is (R)- then the absolute stereochemistry of the 2-bromo-3-chlorosuccinic acid is (2R,3R). From this sequence it is thus established that enzymically synthesised chloroaspartic acid possesses (2R,3S)-stereochemistry.

In order to determine the absolute configuration of 3-bromoaspartic acid a similar reaction sequence was envisaged. However, the isolation of 3-bromoaspartic acid from incubations of bromofumarate and ammonia with β-methylaspartase is hampered by two complications. First, bromoaspartic acid inhibits the enzyme,[3] and; second, the compound itself rapidly cyclizes to give 2,3-aziridinedicarboxylic acid.

Reduction of the incubation mixture (after only partial amination of bromofumarate) using H_2/Pd/C yielded aspartic acid which after purification gave a positive optical rotation [≙75% (S)-aspartic acid]. This low rotational value does not reflect the presence of additional stereoisomers of the enzymic product (as was determined by ^{13}C-nmr spectroscopy) but rather the occurrence of side reactions (aziridine formation) and other complications during the chirality assessment. In order to relate the chirality of C-3 of bromoaspartic acid to the now known configuration at C-2, vis. (2R), the enzymic reaction was quenched, through chilling, after partial conversion of the bromofumaric acid. The excess ammonia was removed rapidly in a stream of N_2 and hydrobromic acid was added to prevent cyclization of the β-bromo amino acid to the aziridine. After thorough extraction of the acidified aqueous phase with ether to remove all traces of unreacted bromofumaric acid (verified by ^{1}H-nmr spectroscopy) the bromoaspartic acid was diazotized in the presence of KBr to yield (2R,3R)-2,3-dibromosuccinic acid {$[\alpha]_D^{20}$ - 130° (C=0.3,EtOAc), lit[4] $[\alpha]_D^{18}$ - 148° (c=5.8, EtOAc) ; other mass spectral and ^{1}H-nmr spectroscopic parameters were identical to an authentic sample of the racemic material}. On refluxing with water elimination of hydrogen bromide occurred[10] to give mainly bromofumaric acid (90% by ^{1}H-nmr spectroscopy, the remainder was bromomaleic acid) which was identical with an authentic sample, Scheme 4. The results of the above experiments indicate that the stereochemistry of enzymically synthesised 3-bromoaspartic acid is (2R,3S).



Scheme 4

Thus for both chloro- and bromo- fumaric acid, enzymic amination occurs via re-face attack at C-2 in an anti-fashion to give (2R,3S)-halogenoaspartic acids. This stereochemical assignment will aid the mechanistic rationalization of the observed K_{cat} inhibitory properties of these compounds with PLP-dependent enzymes[11] and with 3-methylaspartase.[3]

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10. In our laboratory bromofumaric acid is synthesized through bromination of maleic[12] acid (which gives racemic threo-2,3-dibromosuccinic acid viz the (2S,3S) and (2R,3R)-enantiomers) followed by dehydrobromination of the products. We have been unable to detect any bromomaleic acid in these samples and thus the dehydrobromination step occurs via anti-elimination only. Under identical conditions, dehydrobromination of commercial meso-dibromosuccinic acid yields exclusively bromomaleic acid.
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