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THE TRANSFORMATION OF HISTIDINE SIDE CHAIN TO NON-CODED ASPARAGINES

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Abstract: The transformation of the histidine side chain to that of N^{ω}-carbamoyl asparagine, N^{ω}-formyl asparagine, N^{ω}-benzoyl asparagine, β-cyano alanine and aspartic acid has been described, involving as the primary step, the Ru(VIII) mediated scission of imidizole 4,5-π bond.

The transformation of coded amino acid side chains, in a protein environment, to potentially interesting non-coded ones, would be useful illustrations of chemical approaches to protein engineering[1] Of equal importance is the characterization of α-amino acids, containing non-coded side chains, that are related either metabolically or otherwise to a coded precursor, since, they could be tried either as potential inhibitors or used in normal peptide synthesis to generate hormones of altered profile. From these perspectives, we have examined the highly intricate, yet rapid, oxidative conversion of histidine side chain to that of β-amino aspartic acid that takes place in liver and bacteria involving imidazole-4-hydroxylation[2] We have recently been able to demonstrate a related chemical functionalization by treatment of protected histidine with 4-^{t}butyl iodylbenzene which led to γ-formamido glutamine side chain[3]

We report in this communication that *in situ* generated Ru^{VIII} consistently cleaves, first the imidazole 4,5-π bond of the histidine side chain leading to the generation of an asparagine unit.

N^{α}-Benzyloxycarbonyl histidine methyl ester [N^{α}-Z-His-OMe, (1)][4] with 2 2 mol percent of the reagent – generated from $RuCl_3 \cdot 3H_2O$ (Cat.) and 18 equiv of periodate in H_2O MeCN CCl_4 – when shaken in a sealed vessel for 60 h, afforded N^{α}-benzyloxycarbonyl-N^{ω}-carbamoyl asparagine methyl ester [N^{α}-Z-N^{ω}-$CONH_2$-Asn-OMe, (2), mp 168-169°C, 22%][5] and N-benzyloxycarbonyl aspartic acid-α-methyl ester [Z-Asp(β-OH)-OMe, (3), 25%)[6] Fortuitously, product from primary oxidation, namely, N^{α}-benzoyl-N^{ω}-formyl asparagine methyl ester [N^{α}-Bz-N^{ω}-Formyl-Asn-OMe, (5), mp 218-220°C] could be isolated in 31% yields in addition to N-benzoyl aspartic acid-α-methyl ester [Bz-Asp(β-OH)-OMe, (6),

mp 127–128°C, (34%)], when N^{α}–benzoylhistidine methyl ester [N^{α}–Bz–His–OMe, (4)], was reacted with Ru^{VIII} and the stirred reaction mixture worked up, similar to that described previously[6], soon after the disappearance of (4) [tlc, 48 h]. Interestingly, treatment of (5) with benzoyl chloride –pyridine yielded, N–benzoyl–β–cyano alanine methyl ester [Bz–Ala(β–CN)–OMe, (7), mp 164°C, 37%] and N^{α}–N^{ω}–dibenzoyl asparagine methyl ester [N^{α}–N^{ω}–di Bz–Asn–OMe, (8), mp 143°C, (29%)].

Finally the general nature of the imidazole 4,5–π bond rupture was demonstrated with tetrahydrobenzimidazole (9)[7] Compound (9), under conditions described for the (4) → (5) + (6) change, afforded, as the sole isolable product, mono N–formyl adipic acid amide [(10), mp 163–164°C, (25%)]

A fortuitous outcome of the present study is that, as rationalized in Scheme 1 products arising from cleavage of *each of the 5 bonds* of the imidizole ring have made their appearence

from the vantage of reaction mechanisms, the profile of the intermediate arising from path a, Scheme 1, is noteworthy In the present study, this highly electrophilic substance yields products by water addition In a protein environment such an intermediate can well give rise to cross linking by interaction with proximate basic residues For example, eye lens protein deterioration has been shown to be associated with such cross linking involving intermediates from histidine side chain oxidation[8,9]

Above all, this communication has endeavoured to project the chemical modification along metabolic pathways as attractive routes to the generation of non–coded amino acid side chains having biological interest Histidine has proved its versatility here also, since it has been possible to transform its side chain, in a peptide environment, to that of γ–formamidoglutamine[3], N^{ω}–carbamoyl asparagine, N^{ω}–formyl asparagine, N^{ω}–benzoyl asparagine, β–cyano alanine and aspartic acid. They could either, after de–protection[10] be used in normal peptide synthesis or could be generated, in selective cases, by direct protein modification

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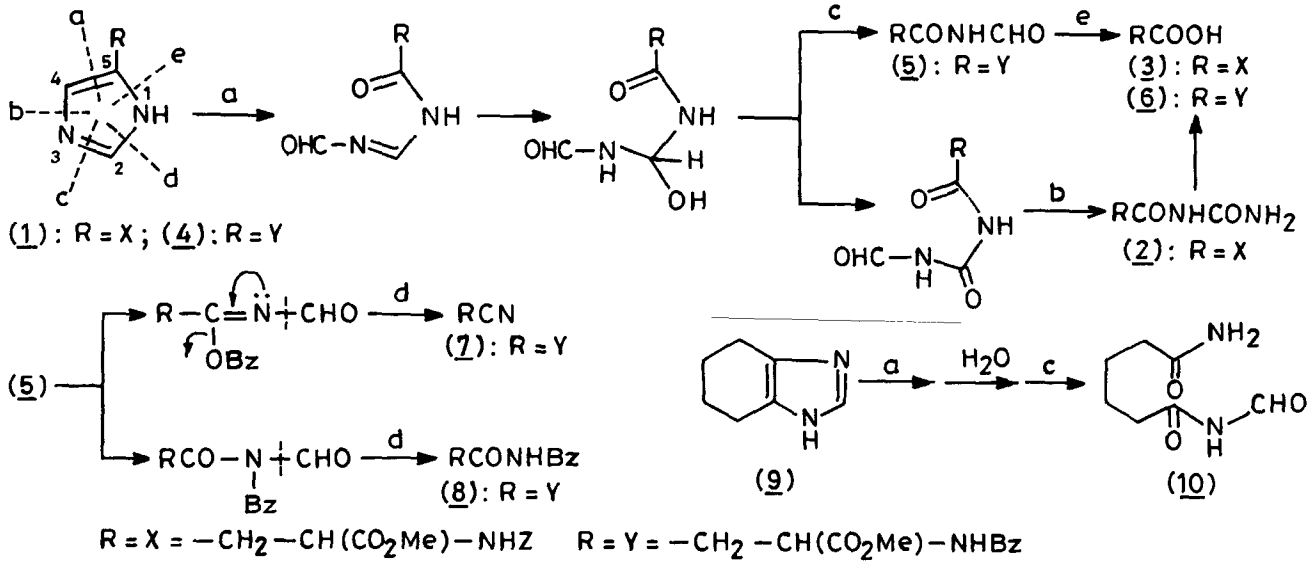
Scheme 1


(1): R = X; (4): R = Y
(5): R = Y
(3): R = X
(6): R = Y
(2): R = X
(7): R = Y
(8): R = Y
(9)
(10)
R = X = −CH2−CH(CO2Me)−NHZ
R = Y = −CH2−CH(CO2Me)−NHBz

2. Fournier, J P , Bouthiller, L P , J Am. Chem Soc., 1952, 74, 5210, Tabor, H., Mehler, A , J Biol Chem , 1954, 210, 559

3. Ranganathan, S., Ranganathan, D , Singh, S.K., Bhattacharyya, D , J. Chem Soc Chem Commun , 1987, 1887

4 All amino acids used were of the L configuration. Starting materials as well as products were fully characterized (ir, nmr, ms) Elemental analysis, performed on all new compounds, were in good agreement with expected values

5 Several attempts to prepare (2) from Asp or Asn failed In view of the potential of (2) for use in peptide synthesis, detailed procedure for the (1) $\longrightarrow$ (2) change is given below

6 A mixture of (1) (5 mmol), MeCN CCl_4.H_2O (1 1 2, 80mL), $NaIO_4$ (90 mmol) and $RuCl_3 \cdot 3H_2O$ (2.2 mol%), was sealed, left shaken for 60 h, filtered, residue washed with CCl_4 (3x10 mL), the aqueous layer extracted with EtOAc (3x30 mL), the combined organic extracts dried, evaporated,digested with satd. $NaHCO_3$ (40 mL), extracted with EtOAc (3x30 mL),dried, evaporated and chromatographed over silica gel. Elution with PhH EtOAc (3.7) gave 22% of (2), mp 168–169°C, ir ν_{max} KBr cm^{-1} 3320, 1740, 1690, 1660,1530; ^1H nmr δ ($CDCl_3$ + DMSO–d_6), 2 8(d, 2H), 3 65(s, 3H), 4 3–4 7(m, 1H), 5 05(s, 2H), 6 7–7 1 (br, 2H), 7 3(s, 5H), 7 8–8 1(m, 1H), 10 1(s, 1H), ms m/z 323 (M^+) The bicarbonate extract was adjusted to pH ≈ 3 (2N H_2SO_4), saturated with NaCl, extracted with EtOAc (3x30 mL), dried, evaporated, treated with etheral diazomethane, evaporated and chromatographed on silica gel Elution with PhH.EtOAc (7 3) afforded 25% of Z–Asp–di OMe

7 Bredereck, H , Gompper, R , Schuh, H.G V , Theilig, G. "Neurere Methoden der Preparativen Organischen Chemie", Verlag Chemie, Vol III, p. 163

8 We are grateful to Professor D Balasubramanian, Centre for Cellular and Molecular Biology Hyderabad, India, for sharing his findings on eye lens protein deterioration.

9 So far, we have been unable to generate a test system to show the cross linking involving the ω–amino group of lysine and side chain oxidation products of histidine present, however efforts in this direction are continuing

10 The amino acid, N^{ω}–carbamoyl asparagine, arising from de–protection of (2), is structurally similar to the insecticidal and nitrogen storing non–coded plant amino acid L–alibizzine (N^{β}–carbamoyl–β–amino alanine) and has potential as a competitive antagonist of asparagine (Rosenthal, G A "Plant Nonprotein Amino and Imino Acids", Academic Press, 1982, p 197)

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