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# SYNTHESIS, IDENTIFICATION AND PROPERTIES OF SOME β-AMINOALANINE DERIVATIVES

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**Abstract**—A number of new β-(*N*-alkylamino) alanines have been synthesized from acetyldehydroalanine. Using keratin as a cystine-containing protein, it has been shown that, on treatment with a series of amines, β-(*N* alkylamino) alanine residues are formed in varying amounts in the protein, depending on the structure of amines applied.

Dehydrolanine has long been postulated as a product in degradation of cystine residues in proteins during treatment with alkali. The formation of this intermediate has been used to account for the subsequent isolation from such treated proteins of amino acids such as lanthionine,[1] lysinoalanine,[2] ornithinoalanine[3] and β-aminoalanine.[4] More recently the formation of this intermediate has been clearly established during alkali treatment of oxidised glutathione.[5] It has also been further established that the presence of free organic bases or thiols in the alkali treatment can result in a variety of reaction products, the amounts of which depend on the structure of the amine or thiol applied.[6-8] The absolute identification of these products depends on the synthesis of suitable model compounds. Both acetyldehydrolanine and its ethyl ester have been used to synthesize a variety of such products, thus lysinoalanine,[2] lanthionine,[9] some β-sulphoamino acids[10,11] and some substituted β-aminoalanine have been prepared.[12-14]

The purposes of this paper are to report further syntheses of new *N*-substituted β-aminoaline derivatives and to give information on their electrophoretic properties. Using these synthetic products as standards, it is possible to assess the extent of reaction which occurs under standard conditions, between specific amines and the disulphide bonds in keratin.

## RESULTS AND DISCUSSION

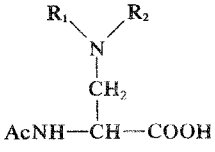
Synthesis of acetyldehydrolanine has been previously reported[15] and the one step procedure of Kil'disheva[16] has been found to give excellent yield. Treatment of acetyldehydroalanine with amines results in the formation of the *N*(α)-acetyl β-aminoalanine derivatives in good yield in all cases. Isolation of the products in crystalline form is impossible unless all traces of the excess amine are removed prior to crystallization. All the amines, which are volatile, were removed by repeated rotary evaporation. Table 1 gives the melting points and analysis of the new amino acid derivatives.

These products do not give a clear band on high voltage electrophoretograms, only giving a faint yellow colour when treated with cadmium–ninhydrin solution, indicating the β-(*N*-alkyl-amino) group is not particularly reactive to ninhydrin.

Subsequent hydrolysis of the acetyl derivatives should yield the β(*N*-alkylamino) alanines. In most cases these products could be isolated easily. β-(*N*-t-butylamino-*N*-(α)-acetylalanine on hydrolysis of the acetyl group apparently decomposed and the β-aminoalanine product was not obtained. This instability to the hydrolysis of the acetyl group has been previously noted in the case of β-(*N*-diethylamino)*N*-(α)acetylaminoalanine.[17] This product also could not be obtained in a pure form by this method. The series of the new amino acids has been successfully synthesized and their m.p. are given in Table 2.

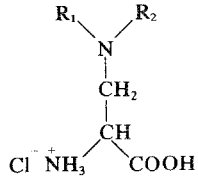
Clear electrophoretic separation of the *N*-substituted β-aminoalanine derivatives from one another can be effected over a wide range of pH. Typical Rm values for the series are given in Table 3.

Table 1. New β-(*N*-alkylamino)-*N*(α)-acetylalanines




| | | | | Required % | | | Found % | | |
|---|---|---|---|---|---|---|---|---|---|
| $R_1$ | $R_2$ | Yield % | m.p. °C | C | H | N | C | H | N |
| Isopropyl | H | 48 | 178–179 | 51.0 | 8.5 | 14.9 | 50.8 | 8.6 | 14.8 |
| Isobutyl | H | 50 | 164–165 | 53.4 | 8.9 | 13.7 | 53.4 | 8.9 | 13.6 |
| *t*-Butyl | H | 62 | 156–157 | 53.4 | 8.9 | 13.7 | 53.3 | 8.85 | 13.7 |
| Isopentyl | H | 42 | 166–167 | 60.0 | 10.0 | 14.0 | 55.1 | 9.4 | 12.85 |

Table 2. New β-(*N*-alkylamino) alanines



| | | | Required % | | | | Found % | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $R_1$ | $R_2$ | m.p. °C | C | H | N | Cl | C | H | N | Cl |
| Isopropyl | H | 173–174 | 38.8 | 8.1 | 15.1 | 19.45 | 39.3 | 8.3 | 15.2 | 19.4 |
| Isobutyl | H | 167–168 | 42.8 | 8.65 | 14.25 | 18.05 | 42.9 | 8.7 | 14.4 | 18.2 |
| Isopentyl | H | 170–171 | 45.6 | 9.0 | 13.3 | 16.85 | 45.4 | 9.1 | 13.2 | 7.0 |

Table 3. Rm values of β-N-alkyl-aminoalanines relative to glycine at pH 1.85

| Amino acid | Rm (to glycine) | Incremental decrease of Rm values |
|---|---|---|
| β-(*N*-methylamino)-alanine | 1.20 | — |
| β-(*N*-ethylamino)-alanine | 1.13 | 0.7 |
| β-(*N*-propylamino)-alanine | 1.07 | 0.6 |
| β-(*N*-butylamino)-alanine | 1.00 | 0.7 |
| β-(*N*-pentylamino)-alanine | 0.95 | 0.5 |
| β-(*N*-hexylamino)-alanine | 0.91 | 0.4 |
| β-(*N*-heptylamino)-alanine | 0.86 | 0.5 |
| β-(*N*-isopropylamino)-alanine | 0.99 | — |
| β-(*N*-isobutylamino)-alanine | 0.94 | 0.5 |
| β-(*N*-isopentylamino)-alanine | 0.88 | 0.6 |

In Table 3 the Rm values of the previously synthesized straight chain alkyl derivatives[17] are also included for comparison. The incremental fall in Rm values with the addition of a $-CH_2$ group or a side chain $-CH_3$ group is very similar indicating that there is no massive change either in aggregation or structural conformation when the side chain group is present.

Whilst the branched chain β-(*N*-alkylamino)-alanine derivatives separate from one another by high-voltage electrophoresis, these products in protein hydrolysates may overlap the natural basic amino acids (e.g. lysine) on the electrophoretogram. In all cases, separation from the natural occurring amino acids could be achieved by careful selection of pH values of the electrophoresis buffer solution between pH1 and 2 (e.g. pH 1.85 for methyl, ethyl and propyl products).

The β-(*N*-*t*-butylamino) alanine could not be produced by hydrolysis of the acetyl derivative with acid. No product was obtained which gave a colour with ninhydrin. The failure to synthesise this amino acid is difficult to explain, as hydrolysis of keratin, previously treated with *t*-butylamine, yields a new basic band on the electrophoretogram. The Rm value of this bond is that to be expected for β-(*N*-*t*-butylamino) alanine, by comparison with the mobilities of other β-*N*-alkylamino-alanine derivatives.

Treatment of keratin, under standard conditions, with alkylamines gave varying amounts of β-(*N*-alkylamino)-alanine derivatives products, the amount obtained depending on the structure of the alkylamine applied. The results are given in Table 4.

It can be seen that the pK values of the amines do not vary greatly and hence the differing reactivities cannot be ascribed to differing pH values of the amine solutions. It is noticeable that with the straight chain β-(*N*-alkylamino) alanine derivatives, the extent of reaction of the amine increases with increasing chain length, the only exceptions being the hexylamine and heptylamine. In these cases the insolubility of the amines obviously influences the extent of reaction. The branched chain amines equally follow a series in which the larger is the chain the greater is the extent of reaction, though in all cases, these react to a less extent than the corresponding straight chain amines. The reasons for these differences in extent of reaction are difficult to explain. As the

Table 4. Amounts of β-(*N*-alkylamino) alanines found in wool keratin hydrolysates after treatment of the protein with solutions of different amines for 30 min at 25°C

| Amine reacted | μ mole $gm^{-1}$ wool of RNH—$CH_2$—CH(COOH)($NH_2$) formed | $pK_1$ of amine | pH value of Electrophoretic separation of hydrolysates |
|---|---|---|---|
| Methylamine | 131 | 10.63 | 1.85 |
| Ethylamine | 206 | 10.63 | 1.85 |
| Propylamine | 230 | 10.58 | 1.85 |
| Butylamine | 292 | 10.61 | 1.1 |
| Pentylamine | 338 | 10.63 | 1.1 |
| Hexylamine | 151 | 10.60 | 1.1 |
| Heptylamine | 53 | 10.62 | 1.1 |
| Isopropylamine | 135 | 10.63 | 1.5 |
| Isobutylamine | 187 | 10.48 | 1.1 |
| Isopentylamine | 229 | 10.60 | 1.1 |

reaction is heterogeneous, the keratin being the insoluble phase, it may be the larger side chain amines penetrate the insoluble protein more easily by enhancing the swelling of the protein to a greater extent in aqueous solution. Further studies are being carried out on water-soluble proteins.

## EXPERIMENTAL

M.ps were taken using a hot-stage microscope and are uncorrected. *Acetyldehydroalanine* was prepared by the method of Kil'disheva *et al.* m.p. 198–200° (lit.[16] 198–200°).

*Preparation of β-(N-alkylamino)-acetyl alanines*

Acetyldehydroalanine (5 g) was dissolved in a 30% solution (50 ml) of the amine in water and kept at 40–50°C for 72 h. The excess amine was removed by rotary evaporation and dissolution in water followed by rotary evaporation. The yellow oils were treated with acetone and chilled to precipitate the amino acid derivatives which were recrystallized from MeOH/$Et_2O$. Yields and m.p.s are in Table 1.

*Preparation of β-(N-alkylamino) alanines*

The $N(\alpha)$-acetyl derivatives were refluxed with 15 fold excess of 2 M HCl for 3 h to ensure complete removal of the acetyl group. The solution was evaporated at 40°C to dryness, water washed repeatedly and evaporated again. The residue (after taking up in little water) was applied to a column of Amberlite 1R 4B to remove excess HCl and the eluate evaporated to small bulk. The amino acids were precipitated as monohydrochlorides by addition of acetone and chilling followed by recrystallization from water and acetone. (The β-(*N*-isopropylamino) alanine was re-crystallized from MeOH/$Et_2O$).

*Purification of keratin*

Wool "Merino 64s" was degreased by successive extraction with ether and ethanol followed by washing with water, the tips and roots being removed and the vegetable matters removed by hand prior to drying over phosphorous pentoxide.

*Treatment of keratin*

Amine solutions were made up in distilled water and titrated against 1 M HCl using methyl orange as indicator until they were 1 M (±1%). Treatments were followed on samples (50 mg) in freeze drying tubes (liquor to wool ratio 100:1) after which the samples were repeatedly washed with distilled water, freeze dried and reweighed. Hydrolysis was carried out using 6 M HCl in sealed freeze drying tubes for 20 h at 105°C.

*Estimation of β-(N-alkylamino) alanines in keratin hydrolysates*

Hydrolysates were examined by high-voltage electrophoresis at pH values listed in Table 4 using the technique of Atfield and Morris[18] to determine the quantities present.

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