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THE NITRILE GROUP IN PEPTIDE CHEMISTRY—VII*

AN ALTERNATIVE SYNTHESIS OF THE NEUROTOXIC AMINO ACID β-CYANO-L-ALANINE FROM L-ASPARAGINE

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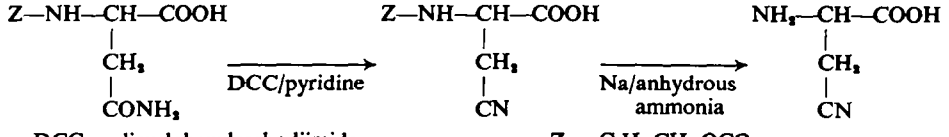
Abstract—The neurotoxic amino acid, β-cyano-L-alanine, has been synthesized by the dehydration of trityl-L-asparagine by means of dicyclohexylcarbodiimide and removal of the trityl group in 50% acetic acid. The racemate has been prepared analogously.

THE neurotoxic amino acid β-cyano-L-alanine occurs in seeds of *Vicia sativa* and *V. angustifolia* both free[1] and combined as γ-L-glutamyl-β-cyano-L-alanine,[2,3] and has recently been isolated in pure crystalline form from common vetch (*V. sativa*).[1] It is of current interest not only because of its neurotoxic properties,[1,2,4] but also because of structural and possible biogenetic relationship to the active principles of the lathyrogenic factors, β-aminopropionitrile (in *Lathyrus odoratus*) and α,γ-diaminobutyric acid (in *L. latifolius* and *L. sylvestris Wagneri*).[5] Of special interest is the possible metabolic relationship of β-cyano-L-alanine to the amino acid of widespread distribution in animal and plant tissues —L-asparagine.[4]

The metabolism of β-cyano-L-alanine is of added interest in view of the evidence for the assimilation of cyanide ion in species of *Vicia* and *Lathyrus*.[3,4] The studies of Ressler *et al.* and of Tschiersch have established that in both plants the radioactive cyanide carbon can serve as an excellent precursor of the cyano carbon of β-cyano-L-alanine and of the amide carbon of L-asparagine.[3,4]

As a part of the programme of the synthetic work in the β-cyanoalanine field, which has been in progress in this laboratory,[6–9] attention has been focussed on the development of new approaches to the synthesis of free β-cyano-L-alanine from L-asparagine.[10] The only prior report of its preparation is that of Ressler and Ratzkin



DCC = dicyclohexylcarbodiimide Z = $C_6H_5 \cdot CH_2 \cdot OCO-$

* Part VI; *Roczniki Chem.* **39**, 369 (1965).

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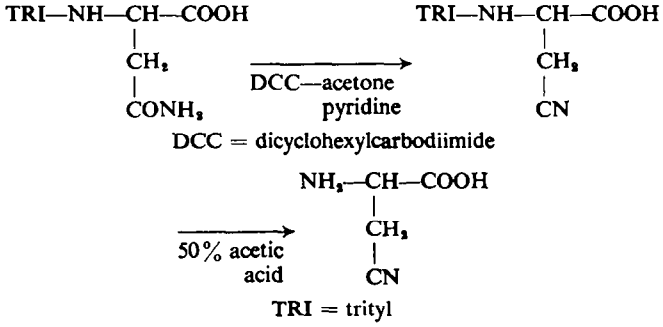
who prepared β-cyano-L-alanine from benzyloxycarbonyl-L-asparagine by the above sequence of reactions.[10]

We have evaluated the reported methods for amino protection and found the trityl protecting group the most valuable one for our purpose. It is well known that acyl-asparagines can be dehydrated to the corresponding acyl-β-cyanoalanines,[11–14] and benzyloxycarbonyl-L-asparagine readily gives benzyloxycarbonyl-β-cyano-L-alanine.[7,10,15,16] We have found that N-alkylasparagines can be dehydrated analogously. Treatment of trityl-L-asparagine with dicyclohexylcarbodiimide in acetone in the presence of pyridine led to high yields of the desired trityl-β-cyano-L-alanine.[7,8,10] The trityl amino protecting group can be removed smoothly by heating at reflux temperature in 50% acetic acid for 2–3 minutes,[17,18] without affecting the cyano group. In Ressler's method, removal of the benzyloxycarbonyl group was achieved by the action of sodium in anhydrous ammonia,[10] which may partly reduce the cyano group, especially if excess sodium is used or if the reaction is carried out in the presence of a trace of water or methanol.[10,11] This applies also to hydrogenation in the presence of a catalyst.[19] After the acetic acid treatment, simple filtration of the insoluble triphenylcarbinol and removal of the solvent in the vacuum rotary evaporator afforded crystalline β-cyano-L-alanine. Recrystallization from dioxan–water[10] gave pure β-cyano-L-alanine of m.p. 217°. Analogously, trityl-DL-asparagine gave β-cyano-DL-alanine.



The synthetic amino acids were identified by paper electrophoresis at pH 8·6,[1,10] and by conversion to their benzyloxycarbonyl derivatives and thence to the *p*-nitrophenyl esters.[6,8,20] Both amino acids gave a characteristic bright green colouration with ninhydrin[1,10] and showed IR absorption at 2250 cm^{-1} (C≡N)[1]. Hydrolysis by 6N HCl gave the calculated quantity of ammonia, together with aspartic acid, identified by chromatography.[1]

This method for the preparation of β-cyanoalanine from tritylasparagine is a very simple one and affords a high overall yield. An additional advantage of our procedure

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is the ease of isolation of the product. A disadvantage is the rather low yield obtained in the direct tritylation of asparagine.[21] It seems, however, that this yield might be considerably improved by proceeding through the easily accessible trityl ester of tritylasparagine.[22–25]

EXPERIMENTAL

M.ps are uncorrected. Trityl-L-asparagine was prepared according to Zervas *et al.*[21]

Trityl-DL-asparagine (I). This compound was prepared from DL-asparagine in the way described by Zervas *et al.*[21] for the L-isomer. The washing of the chloroform solution of trityl-DL-asparagine led to separation of the product in the form of a fine suspension, which was filtered off. The second crop of crude trityl-DL-asparagine was obtained after evaporation of the chloroform solution, yield: 36–40%, m.p. 159–162°. The analytical sample was recrystallized several times from tetrahydrofuran, m.p. 173–174°. (Found: N, 7·65. Calc. for $C_{23}H_{22}N_2O_3$: N, 7·49%.)

Trityl-β-cyano-L-alanine (II). Trityl-L-asparagine (crude product 1·87 g, 5 mmoles) was dissolved in dry pyridine (5 ml). To this solution dicyclohexylcarbodiimide (1·04 g; 5 mmoles) in dry acetone (10 ml) was added portionwise with stirring. After 2 hr the separated dicyclohexylurea (1·07 g, 96%) was filtered off and washed with acetone. The filtrate was evaporated under reduced press and the residue treated with 8 ml water and 7 ml AcOH. The mixture was extracted twice with 15 ml $CHCl_3$. The $CHCl_3$-solution was dried 3 min over $MgSO_4$ and evaporated, yield: 1·69 g (95%); m.p. 112–117°. Recrystallization from AcOEt–pet. ether gave 1·43 g (80·5%) of a material m.p. 118–122°. The analytical sample was recrystallized additionally twice from AcOEt–pet. ether, m.p. 124–125°; $[\alpha]_D^{20}$ +23·5° (c = 2·0 in acetone). (Found: N, 7·78. Calc. for $C_{23}H_{20}N_2O_2$: N, 7·86%.)

Trityl-β-cyano-DL-alanine (III). Trityl-DL-asparagine (crude product 1·87 g; 5 mmoles) was dissolved in dry pyridine (5 ml) by warming. The solution was cooled rapidly to room temp and treated with dicyclohexylcarbodiimide in acetone as described for the L-isomer. The crude product, 95% yield, (1·69 g) melted at 113–116°. Recrystallization from AcOEt afforded 1·24 g (73%) of a material m.p. 122–125°. The analytical sample was recrystallized additionally twice from AcOEt, m.p. 125–126°. (Found: N, 7·93. Calc. for $C_{23}H_{20}N_2O_2$: N, 7·86%.)

β-Cyano-L-alanine (IV). Trityl-β-cyano-L-alanine (recrystallized material of m.p. 118–122°, 1·78 g; 5 mmoles) was suspended in 50% AcOH (5 ml) and heated under reflux for 2–3 min. The contents of the flask were then cooled and treated with 5 ml water. The separated triphenylcarbinol was filtered off and washed with several ml water. The filtrate was evaporated to about 2 ml under reduced press at a temp not exceeding 40° (rotary vacuum evaporator), and then β-cyano-L-alanine was separated by the addition of 5 ml acetone and 20 ml anhydrous ether. The solid material was filtered off, yield: 450 mg (79%); m.p. 204°, (dec). Recrystallization from dioxan–water raised the m.p. to 213° (405 mg; 71%). Additional recrystallization from dioxan–water gave pure crystalline β-cyano-L-alanine (needles), m.p. 217° (dec). Because of the very low values reported for the specific rotation of β-cyano-L-alanine this physical constant was not measured. Reported, m.p. 218–218·5° (dec), $[\alpha]_D^{26}$ −2·9° (c = 1·4 in 1N AcOH) for synthetic β-cyano-L-alanine[10]; m.p. 214·5°, (dec), $[\alpha]_D^{23}$ −0·2° (c = 0·74 in 1N AcOH) for β-cyano-L-alanine isolated from seeds of common vetch (*V. sativa*).[1]

β-Cyano-DL-alanine (V). The removal of the trityl protecting group from trityl-β-cyano-DL-alanine was performed as described for L-isomer. The crude β-cyano-DL-alanine was obtained in 83% yield, m.p. 201°, (dec). Recrystallization from dioxan–water (twice) raised the m.p. to 213° (under the microscope dendritic aggregates readily distinguishable from the discrete needles[1] of the L-isomer; 93% yield from each recrystallization). (Found: N, 24·42. Calc. for $C_4H_6N_2O_2$: N, 24·55%.)

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Identification experiments

(a) *Electrophoresis.* Pure L- and DL-β-cyanoalanine was electrophoresed in barbital buffer, pH 8·6, μ 0·05, for 3 hr at 9 volts per cm. The strips developed with ninhydrin in acetone showed single bright green spots located 5·5–6·0 cm from the origin towards the anode. Reported,[10] single green spots located 5·5 cm towards the anode (barbital buffer, pH 8·5, μ 0·05, 9 volts per cm for 3 hr).

(b) *Hydrolysis.* β-Cyano-L-alanine in 6N HCl was heated under reflux for 12 hr, and the liberated ammonia was found to be in the ratio 1:1·02 to the starting β-cyano-L-alanine. After such treatment TLC showed only the presence of aspartic acid in solution.

(c) *Conversion to benzyloxycarbonyl-β-cyano-alanine* p-*nitrophenyl ester.* That the β-cyano-L-alanine prepared from L-asparagine posessed the L-configuration was established by the preparation of benzyloxycarbonyl-β-cyano-L-alanine, $[\alpha]_D^{20}$ −18·6° (c = 1·26 in MeOH). Reported, $[\alpha]_D$ −18·6° (c = 1·26 in MeOH) for carbobenzoxy-β-cyano-L-alanine.[7,8] This was converted to the *p*-nitrophenyl ester, m.p. 138°, $[\alpha]_D^{21}$ −78·9° (c = 0·73 in acetone). Reported, m.p. 137–138° $[\alpha]_D^{20}$ −81·8° (c = 2·0 in acetone).[6,8]

The crude *p*-nitrophenyl ester of benzyloxycarbonyl-β-cyanoalanine prepared from β-cyano-DL-alanine m.p. 112–114° showed no depression on admixture with a true sample of pure racemic benzyloxycarbonyl-β-cyanoalanine, m.p. 116–117° prepared previously.[20]

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