CH_2–), 1.57 (s, =CCH_3–), 1.93 (br, –CH_2CH_2–), 2.10 (d, J = 1 Hz, COC=CCH_3–), 4.03 (q, J = 7 Hz, CH_3CH_2), 5.03 (br, –CH=), 5.52 (br, COCH=).

3,7,11,15,19,23,27-Heptamethyl-2,6,10,14,18,22,26-octacosaheptenol (45).—$LiAlH_4$ (21 mg, 0.56 mmol) and $AlCl_3$ (12.3 mg, 0.092 mmol) were weighed into a 1-ml centrifuge tube and the above crude C_{35} ester (**44**) dissolved in ether (0.5 ml) was added at −70°. After 1 hr at −10°, the reaction mixture was decomposed with wet ether and then partitioned between saturated NH_4Cl solution and petroleum ether. The crude yield of alcohol **45** was 136 mg: nmr δ 1.58 (s, =CCH_3–), 1.95 (br, –CH_2-CH_2–), 4.00 (d, J = 6 Hz, OCH_2–), 5.04 (br, –CH=), 5.34 (t, J = 6 Hz, OCH_2CH=).

3,7,11,15,19,23,27-Heptamethyl-2,6,10,14,18,22,26-octacosaheptenal (46).—The C_{35} alcohol **45** (above) was dissolved in chloroform (2 ml), MnO_2 (0.5 g) was added, the solution was sonicated for 15 min, and another portion of MnO_2 and chloroform was added and the sonication repeated. The MnO_2 was extracted exhaustively with chloroform to yield crude aldehyde (122 mg, 90%), which was chromatographed on silica gel yielding pure α,β-unsaturated aldehyde **46** as a viscous oil (83 mg, 50% overall yield from **15**): nmr δ 1.58 (br, =CCH_3–), 1.96 (br, –CH_2CH_2–), 2.13 (d, J = 2 Hz, trans COC=CCH_3–), 5.03 (br, –CH=), 5.75 (d, J = 8 Hz, COCH=), 9.85 (cis), 9.90 (trans) (d, J = 8 Hz, –CHO).

Dimethyl Ether of 1′-Oxomenaquinol-7 (17).—A suspension of **20** was prepared from 2-bromo-3-methyl-1,4-dimethoxynaphthalene (53 mg, 0.19 mmol), butyllithium (0.116 ml, 0.19 mol), and ether (0.5 ml). The C_{35} aldehyde **46** (above) (83 mg, 0.17 mmol) was dissolved in ether (0.5 ml) and added to the lithium reagent. After 10 min at room temperature, the mixture was partitioned between 2 N H_2SO_4 (0.2 ml) and petroleum ether. The crude product (120 mg) was oxidized with MnO_2 (1 g) in chloroform (5 ml) without further purification by sonicating for 15 min and then refluxing for 1 hr. Extraction of the MnO_2 with ether gave crude hydroquinone (108 mg) which was chromatographed to yield *cis*-**15** (20 mg) and *trans*-**15** (49 mg): overall yield from **46** was 60%; nmr, *trans*-**15**, δ 1.58 (s, =CCH_3), 1.95 (br, –CH_2CH_2–), 2.20 (d, J = 1 Hz, COC=CCH_3–), 2.23 (s, $ArCH_3$), 3.80 (s, $ArOCH_3$), 5.03 (br, –CH=), 6.27 (br, CO-CH=), 7.4, 8.0 (m, ArH); uv, *trans*-**15**, λ_{max} 220 sh (44,000), 232 sh (40,400), 325 (2500).

Anal. Calcd for $C_{46}H_{68}O_3$: C, 83.2; H, 9.9. Found: C, 83.1; H, 9.9.

1′-Oxomenaquinone-7 (15).—*trans*-**15** (52 mg, 0.06 mmol) was dissolved in dioxane (1 ml), 85% H_3PO_4 (0.1 ml) and AgO (42 mg, 0.34 mmol) were added, and the mixture was sonicated for 15 min. Extraction with ether gave crude product (42 mg) which was chromatographed on kieselgel to obtain pure *all-trans*-**5** (22 mg, 55%), mp 50° after crystallization from petroleum ether. *cis*-**15** (20 mg) was similarly treated to obtain $\Delta^{2'}$-mono-*cis*-**5**, mp 42°. Nmr is in Table I; uv, *all-trans*-**5**, λ_{max} 250 nm (ϵ 32,000), 245 sh (31,000), 255 sh (31,000), 265 sh (22,000), 325 (3000); $\Delta^{2'}$-mono-*cis*-**5**, 250 (30,800), 245 sh (29,700), 255 sh (29,700), 265 (21,400), 325 (2900); ir (neat), *all-trans*-**5**, 2960, 2940, 2910, 2850, 1660, 1610, 1595 cm^{-1} (C=O); mass spectrum m/e (rel intensity) 664 (M^+ +2, 7), 662 (M^+, 2), 241 (44), 201 (57), 200 (65), 81 (44), 69 (100), identical for $\Delta^{2'}$-mono-cis and all-trans.

Anal. Calcd for $C_{46}H_{62}O_3$: C, 83.3; H, 9.4. Found ($\Delta^{2'}$-mono-cis and all-trans): C, 83.1; H, 9.3.

Registry No.—*cis*-**4**, 32247-28-2; *trans*-**4**, 32304-12-4; *all-trans*-**5**, 32247-29-3; 2-mono-*cis*-**5**, 32247-30-6; *cis*-**6**, 32247-31-7; *trans*-**6**, 32247-32-8; **8**, 32247-33-9; **9**, 32247-34-0; **12**, 47827-40-6; **13**, 32247-36-2; **14**, 32247-37-3; **15**, 32304-17-9; 2-mono-*cis*-**17**, 32304-13-5; *all-trans*-**17**, 32247-38-4; *cis*-**18**, 32247-39-5; *trans*-**18**, 32247-40-8; **22**, 459-80-3; *cis*-**24**, 32247-42-0; *trans*-**24**, 32247-43-1; **25**, 32247-44-2; **26**, 32247-45-3; **29**, 3796-70-1; **30**, 3796-62-1; **32**, 32247-48-6; **33**, 32247-49-7; **34**, 32367-44-5; **35**, 24183-02-6; **36**, 32247-51-1; **38**, 3790-61-2; **39**, 32247-53-3; **43**, 32304-14-6; 2-mono-*cis*-**44**, 32304-15-7; *all-trans*-**44**, 32247-54-4; 2-mono-*cis*-**45**, 32247-55-5; *all-trans*-**45**, 32304-16-8; 2-mono-*cis*-**46**, 32247-56-6; *all-trans*-**46**, 32247-57-7.

Synthesis and Properties of α-Cyanoamino Acids. α-Cyanoglycine, L-β-Cyano-β-alanine, and L-γ-Cyano-γ-aminobutyric Acid[1a]

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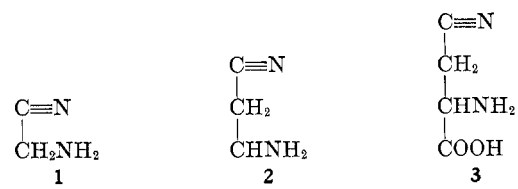
Syntheses of α-cyanoamino acids in the free state are reported for the first time. Enzymic deacylation of acetamidocyanoacetic acid gave α-cyanoglycine. *p*-Methoxybenzyloxycarbonyl-L-isoasparagine was dehydrated to *p*-methoxybenzyloxycarbonyl-L-β-cyano-β-alanine and treated with trifluoroacetic acid to give L-β-cyano-β-alanine. *p*-Methoxybenzyloxycarbonyl-L-isoglutamine was first converted to the methyl ester that was dehydrated and deprotected to give L-γ-cyano-γ-aminobutyric acid. Overall yields were 43–63%. Also synthesized were *p*-methoxybenzyloxycarbonyl-L-β-cyanoalanine, and, from it, L-β-cyanoalanine, and benzyloxycarbonyl-L-β-cyano-β-alanine and benzyloxycarbonyl-L-γ-cyano-γ-aminobutyric acid and their methyl esters. Characteristic physical properties and reactions of α-cyanoamino acids are given including hydration to amino acid amides and reductive cleavage of the cyano group as well as the kinetics of decomposition in aqueous solution.

Osteolathyrogens produce skeletal defects in experimental animals by inhibiting the maturation of collagen.[2] By contrast, the lathyrogens more recently isolated from legumes act as convulsants.[2] As part of an attempt to elucidate structure–activity relationships in the lathyrogens, it was desired to synthesize compounds that would incorporate structural features of both types. Such compounds would thus contain the α- or β-aminonitrile moiety of the osteolathyrogens, *viz.*, α-aminoacetonitrile (**1**) or β-aminopropionitrile (**2**), and the carboxyl group characterizing the neurolathyrogens, *viz.*, β-cyanoalanine (**3**). All these structural features

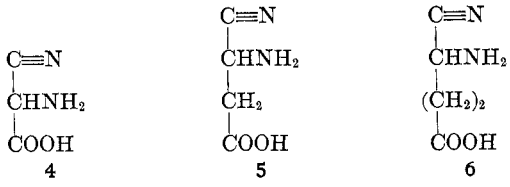


1 **2** **3**

would be present in α-cyanoamino acids such as α-cyanoglycine (**4**), L-β-cyano-β-alanine (**5**), and L-γ-cyano-γ-aminobutyric acid (**6**), a class of compounds

(1) (a) Aided by U. S. Public Health Service Grant NS 04316 and by Muscular Dystrophy Associations of America; (b) Visiting Research Fellow, 1967–1969; (c) 1964–1965; (d) 1961–1962.

(2) For reviews see K. A. Piez, *Annu. Rev. Biochem.*, **37**, 563 (1968); C. Ressler, *Fed. Proc.*, **23**, 1350 (1964).

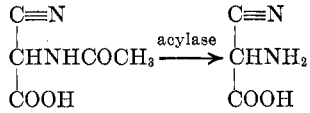


4 5 6

previously unavailable in the free state. It may be noted that L-β-cyano-β-alanine would be a structural isomer of L-β-cyanoalanine, the neurotoxic principle of *Vicia sativa* (common vetch),[3] and that L-γ-cyano-γ-aminobutyric acid would be a structural isomer of γ-cyano-α-aminobutyric acid, the neurotoxic product of cyanide fixation of *Chromobacterium violaceum*.[4] In the isomers the carboxyl and the cyano groups would remain approximately the same distance apart but the amino group would now be adjacent to the cyano rather than the carboxyl group.

The present paper describes the synthesis and properties of α-cyanoglycine, L-β-cyano-β-alanine, and L-γ-cyano-γ-aminobutyric acid. The three α-cyanoamino acids are strong inhibitors of bacterial L-glutamate decarboxylase,[5] an enzyme thought to modulate neuronal activity in higher species.

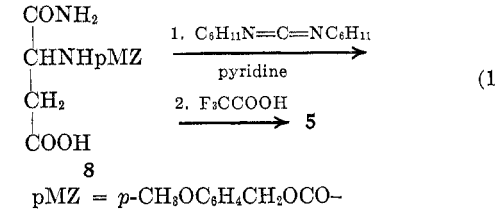
Syntheses.—Although free α-cyanoamino acids were not known, a variety of unisolated alkylated intermediates arising in the synthesis of amino acids by the acylaminocyanoacetic ester route were potentially useful precursors. For α-cyanoglycine commercial ethyl acetamidocyanoacetate served as the starting material. It was hydrolyzed in alkali to known acetamidocyanoacetic acid (**7**). The reported procedure[6] gave variable results in our hands. Frequently, from concentrated hydrolysis mixtures acidified to pH 1–2, a new substance crystallized out that analyzed interestingly as a hemipotassium salt of **7**. By further acidification to pH 0.5 it could be converted into **7**. Since it decarboxylates when heated in aqueous solution,[6] **7** was deacylated at 20° enzymically with hog kidney acylase.[7] The digest



7 4

was passed promptly through a column of CG-120 H^+ resin at 5°. Probably because of its acidic character, α-cyanoglycine appeared in early effluents but was retarded sufficiently to be freed of most of the salts in the digest. α-Cyanoglycine crystallized from the concentrated column effluents after addition of ethanol and was then recrystallized. Even though hydrolysis of **7** seemed to be largely asymmetric as judged by the yield of **4**, it is uncertain that the isolated α-cyanoglycine has the L configuration. Its specific rotation was close to 0. Moreover, carbethoxyacetamidoacetic acid in acid solution racemizes with a half-life at pH 3.85 of 20 min.[8]

An attempt to synthesize free β-cyano-β-alanine (**5**) by hydrogenolysis of *N*-benzyloxycarbonyl-L-β-cyano-β-alanine[9] was unsuccessful, although this procedure has preparative value for obtaining L-β-cyanoalanine and L-γ-cyanoaminobutyric acid from their *N*-benzyloxycarbonyl derivatives.[4] A variety of products formed; probably some reductive fission took place as it does with the Birch reagent.[9] The *p*-methoxybenzyloxycarbonyl (pMZ) protecting group removable under mild hydrolytic conditions[10] proved to be a more favorable approach in the route outlined as (1).



(1)

8

pMZ = *p*-$CH_3OC_6H_4CH_2OCO$–

pMZ-L-Isoasparagine (**8**) was dehydrated with *N*,*N*′-dicyclohexylcarbodiimide (DCCI) in pyridine[9,11] to pMZ-L-β-cyano-β-alanine. The latter was unusually susceptible to hydration back to the amide and it was desirable to treat the crude dehydration product directly with trifluoroacetic acid (TFA) to remove the protecting group. L-β-Cyano-β-alanine was then isolated by crystallization from water–ethanol in an overall yield of 50–60%. Such products usually contained 4–9% isoasparagine. Products having larger amounts of the latter were purified at 5° on a column of Dowex-1 (acetate) resin which retained only the slightly acidic cyanoamino acid.

pMZ-L-Asparagine likewise was dehydrated with DCCI in pyridine. pMZ-L-β-Cyanoalanine was isolated and then deprotected with TFA to give β-cyanoalanine that required little purification and was identical with authentic material.[11] Although this sequence was carried out on a microscale, it appears to offer a satisfactory alternate route to L-β-cyanoalanine[4,9,11–13] that avoids losses due to side reduction of the cyano group on hydrogenolysis of the benzyloxycarbonyl (Cbz) group.

The analogous route did not appear feasible for **6**. Although Cbz-L-glutamine is dehydrated to the γ-cyanoamino acid derivative with DCCI,[11] Cbz-L-isoglutamine[14] gave evidence of reaction but yielded no product having the expected acidic character.[15] Preparation of **6** was therefore undertaken by the route outlined as (2).

pMZ-L-Isoglutamine (**9**) was prepared by condensation of *p*-methoxylbenzyoxylcarbonyl azide (pMZ azide)[10] and L-isoglutamine[16] in the same manner as the asparagine compounds. Attempted liberation of **9** by acidification with 20% citric acid yielded largely an insoluble sodium salt that could be converted with 2 *N* HCl into **9** which was extractable. In both forms, **9** was esterified with diazomethane to give **10**. Treatment of **10** with the dehydrating agent dimethylform-

(3) C. Ressler, *J. Biol. Chem.*, **237**, 733 (1962).
(4) M. Brysk and C. Ressler, *ibid.*, **245**, 1156 (1970).
(5) C. Ressler and T. Koga, *Biochim. Biophys. Acta*, **242**, 473 (1971).
(6) N. F. Albertson, *J. Amer. Chem. Soc.*, **68**, 450 (1946).
(7) J. P. Greenstein, "Methods in Enzymology," Vol. 3, S. P. Colowick and N. O. Kaplan, Ed., Academic Press, New York, N. Y., 1957, p 558.
(8) S. G. Cohen and L. H. Klee, *J. Amer. Chem. Soc.*, **82**, 6038 (1960).
(9) C. Ressler and D. V. Kashelikar, *ibid.*, **88**, 2025 (1966).
(10) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).
(11) C. Ressler and H. Ratzkin, *J. Org. Chem.*, **26**, 3356 (1961).
(12) B. Liberek, Cz. Buczel, and Z. Grzonka, *Tetrahedron*, **22**, 2303 (1966).
(13) M. Wilchek, S. Ariely, and A. Patchornik, *J. Org. Chem.*, **33**, 1258 (1968).
(14) C. Ressler, *J. Amer. Chem. Soc.*, **82**, 1641 (1960).
(15) Recently instances were cited in which isoglutamine derivatives, related as potential precursors to thalidomide, on treatment with several dehydrating or peptide-forming agents cyclized to this glutarimide much more readily than the corresponding glutamine derivatives: Y. F. Shealy, C. E. Opliger, and J. A. Montgomery, *J. Pharm. Sci.*, **57**, 757 (1968).
(16) M. Bergmann and L. Zervas, *Chem. Ber.*, **65**, 1192 (1932).

TABLE I

SYNTHESES, PROPERTIES, AND ANALYSES OF α-CYANOAMINO ACID DERIVATIVES

Derivative	Scale, mmol	Reaction time, hr	Yield,[a] %	Crystn solvent[b]	Mp, °C	[α]D, in methanol
Cbz-L-β-Cyano-β-alanine methyl ester[c]	1	3	70, 54	A	60–61.5	$[\alpha]^{28}$ −35.7° (*c* 1.1)
Cbz-L-γ-Cyano-γ-aminobutyric acid methyl ester[d]	1.5	2.5	81, 35	B	51–52.5	$[\alpha]^{25}$ −47.8° (*c* 0.9)
pMZ-L-γ-Cyano-γ-aminobutyric acid methyl ester (**11**)[e]	4.5	1	79, 65	C	84.5–85.5	$[\alpha]^{24}$ −44.1° (*c* 1.1)
Cbz-L-β-Cyano-β-alanine	0.5	1.75	61, 32	B	88.5–90[f]	
Cbz-L-γ-Cyano-γ-aminobutyric acid[g]	0.5	.75	69, 47	B, C	110.5–112	$[\alpha]^{25}$ −49.7° (*c* 0.8)
pMZ-L-γ-Cyano-γ-aminobutyric acid (**12**)[h–i]	3.0	2.3	93	C	117–119	$[\alpha]^{24}$ −45.5° (*c* 1)

[a] Yield of crude and purified product melting within 1° of analytical material. [b] A, ether; B, ether–petroleum ether; C, ethyl acetate–petroleum ether. [c] *Anal.* Calcd for $C_{13}H_{14}N_2O_4$: C, 59.5; H, 5.38; N, 10.7. Found: C, 59.5; H, 5.32; N, 10.8. [d] *Anal.* Calcd for $C_{14}H_{16}N_2O_4$: C, 60.9; H, 5.84; N, 10.1. Found: C, 60.5; H, 5.86; N, 10.2. [e] *Anal.* Calcd for $C_{15}H_{18}N_2O_5$: C, 58.8; H, 5.92; N, 9.15. Found: C, 58.6; H, 6.07; N, 9.13. [f] Lit.[9] mp 87.5–89°. [g] *Anal.* Calcd for $C_{13}H_{14}N_2O_4$: C, 59.5; H, 5.38; N, 10.7. Found: C, 59.6; H, 5.38; N, 10.7. [h] *Anal.* Calcd for $C_{14}H_{16}N_2O_5$: C, 57.5; H, 5.52; N, 9.59. Found: C, 57.5; H, 5.58; N, 9.68. [i] Mass spectrum (70 eV) *m/e* (rel intensity) 292 (3) (p^+), 274 (1) ($p^+ - H_2O$), 265 (1) ($p^+ - HCN$), 230 (1) ($p^+ - COOH, - NH_3$), 223 (3), 219 (3), 210 (4), 203 (2) ($p^+ - COOH, - NH_3, - HCN$), 185 (7), 137 (42) ($CH_3OC_6H_4CH_2O$), 127 (8), 121 (100) ($CH_3OC_6H_4CH_2$), 109 (35) ($CH_3OC_6H_5$), 107 (18) ($C_6H_5CH_2O$), 94 (14), 91 (14) ($C_6H_5CH_2$), 84 (26), 77 (30) ($C_6H_5$), 55 (30) ($CHNH_2$-C≡N). [j] The acids showed small cyano bands and the esters, small or barely detectable cyano bands near 4.4 μ in ir.

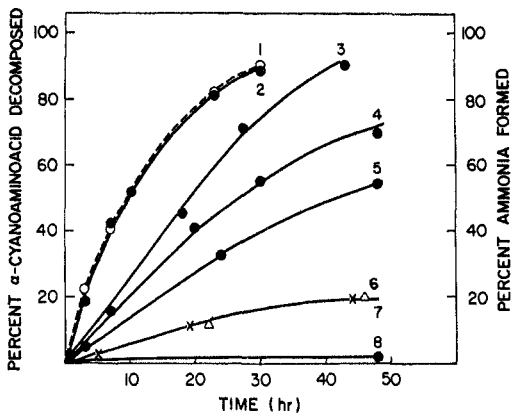
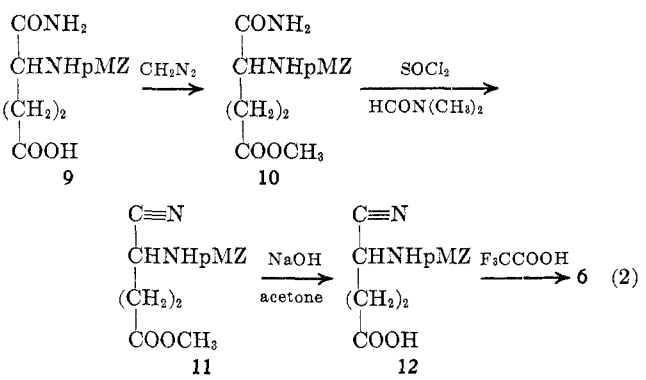


Figure 1.—Lability of α-cyanoamino acids in water: (2) L-γ-cyano-γ-aminobutyric acid at 36°; (5) at 25°; (3) L-β-cyano-β-alanine at 38°; (6) at 25°; (4) α-cyanoglycine at 38°; (7) at 25°; (8) L-β-cyanoalanine at 38°—all in 0.01 *M* solution (see Experimental Section). Release of ammonia during decomposition of γ-cyano-γ-aminobutyric acid at 36° is shown as 1 with the broken line. At the terminal periods for 2, 3, and 4, formation of free cyanide was 0.4, 7, and 12%.

amide–thionyl chloride[9,17] converted it in about 70% yield to pMZ-L-γ-cyano-γ-aminobutyric acid methyl ester (**11**) that was purified by recrystallization. This



was then hydrolyzed in the presence of 1 equiv of sodium hydroxide almost quantitatively to pMZ-L-γ-cyano-γ-aminobutyric acid (**12**). Deprotection gave in 75% yield crude L-γ-cyano-γ-aminobutyric acid (**6**) that was homogeneous on paper electrophoresis and required little purification. It was recrystallized from water–ethanol.[18]

As models for the latter dehydration route, the more accessible benzyloxycarbonyl derivatives were prepared. These included Cbz-L-isoglutamine methyl ester,[19] which was dehydrated to Cbz-L-γ-cyano-γ-aminobutyric acid methyl ester, that was hydrolyzed to Cbz-L-γ-cyano-γ-aminobutyric acid; and Cbz-L-isoasparagine methyl ester[17] which was dehydrated to Cbz-L-β-cyano-β-alanine methyl ester, that was hydrolyzed to Cbz-L-β-cyano-β-alanine. The latter agreed in melting point and ir spectrum with a sample of this material prepared by direct dehydration of Cbz-L-isoasparagine with DCCI.[9] Yields in each step were satisfactory and the new compounds were well characterized. Pertinent reaction conditions and results are summarized in Table I.

Properties—Purity of the three α-cyanoamino acids was established by elemental analysis, chromatography on the automatic amino acid analyzer,[20] and paper electrophoresis. The latter was particularly convenient for detecting the presence of isoasparagine and isoglutamine in **5** and **6**.

When stored in the solid state for several years in the cold under anhydrous conditions, the α-cyanoamino acids appeared to be stable. In dilute aqueous solution, however, they decomposed readily with the kinetics shown in Figure 1. To obtain these amino acids from aqueous solution, it is essential to isolate them promptly. γ-Cyano-γ-aminobutyric acid was the most unstable with a half-life at 36° of 9.5 hr. It decomposed with the formation of stoichiometric amounts

(17) H. Eilingsfeld, M. Seefelder, and H. Weidinger, *Angew. Chem.*, **72**, 836 (1960).

(18) Since this work was undertaken, **6** was reported to be an intermediate in glutamate biosynthesis for an unidentified basidiomycete, and it was synthesized in 1% yield by the Strecker reaction: G. A. Strobel, *J. Biol. Chem.*, **242**, 3265 (1967). The melting points and ir spectra of the natural and synthetic compounds, both of which were unanalyzed and obtained on only a 1-mg scale, differ from those of **6** synthesized here.

(19) E. Sondheimer and R. W. Holley, *J. Amer. Chem. Soc.*, **76**, 2467 (1954).

(20) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

of NH_3 (Figure 1). β-Cyano-β-alanine gave similar results with more scatter. No other ninhydrin-positive product was detected. In the decomposition of γ-cyano-γ-aminobutyric acid and β-cyano-β-alanine, presumably the amino group α to the cyano group is eliminated as NH_3. Little free cyanide was present in decomposition mixtures, and the other products, which may include cyanohydrins, remained to be elucidated.

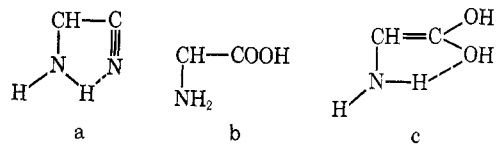
Despite their tendency to lose NH_3 in aqueous solution, β-cyano-β-alanine and γ-cyano-γ-aminobutyric acid hydrolyzed quantitatively in acid to the respective dicarboxylic acids, aspartic acid and glutamic acid, and 1 equiv of NH_3. α-Cyanoglycine gave 1 equiv of glycine and NH_3, presumably *via* decarboxylation and hydrolysis.

Hydrobromic acid–acetic acid hydrated β-cyano-β-alanine and γ-cyano-γ-aminobutyric acid almost quantitatively to isoasparagine and isoglutamine and appeared to decarboxylate α-cyanoglycine. This reaction was known to convert derivatives of β-cyanoalanine into asparagine compounds,[21] and it recently proved useful in identifying γ-cyano-α-aminobutyric acid isolated from certain culture filtrates of *Chromobacterium violaceum*.[4] It appears to be equally useful for characterizing α-cyanoamino acids of $n > 1$.

When treated with a slight excess of sodium in liquid ammonia, α-cyanoglycine gave glycine, L-β-cyano-β-alanine and its benzyloxycarbonyl derivative gave β-alanine, and L-γ-cyano-γ-aminobutyric acid and its benzyloxycarbonyl derivative gave γ-aminobutyric acid, all in yields of 90% or more.[22]

α-Cyanoglycine had the expected ir spectrum. Resembling L-β-cyanoalanine and L-γ-cyano-α-aminobutyric acid in the 4–5.5-μ range, it had a very sharp cyano band near 4.4 μ and a smaller, broader band at 4.9 μ that is attributed to the NH stretching vibration of the charged NH_3^+ group found in many amino acids.[23] By contrast, β-cyano-β-alanine and γ-cyano-γ-aminobutyric acid showed only a single small broad band at 4.5 and 4.6 μ (see Figure 2). It is uncertain if this band is a composite of the cyano and NH stretching vibration or if one of them is absent. In the ω-amino acids, β-alanine and γ-aminobutyric acid, NH stretching vibration is present near 5 μ.[23] When adjacent to an amino group that is separated from the carboxyl group by one or more methylene groups, the cyano group perhaps tends to suppress NH_3^+ formation. This possibility is consistent with the less polar nature of these amino acids suggested by their low melting points, which were all below 135°.

Mass spectra of the α-cyanoamino acids were obtained at low reservoir temperatures of 110–130°. As with EI spectra of most amino acids, the parent peak was absent or slight. Loss of carboxyl and HCN characterized their fragmentation. pMZ precursor **12** of γ-cyano-γ-aminobutyric acid showed a small parent ion and prominent expected aromatic fragments as well as loss of carboxyl and HCN. In all cases a very prominent ion was present at *m/e* 55; for α-cyanoglycine and β-cyano-β-alanine this was the base peak above *m/e* 50. The 55 peak may represent the fragment a, which might



a b c

be expected to be stable and may correspond to the less abundant masses 74 and 75 representing b and c of α-amino acids.[24] Its intensity may make the *m/e* 55 peak helpful in detecting the α-aminoacetonitrile structure.

Experimental Section[25]

Reductive fission was carried out by treatment of the sample (1–3 mg) in 2 ml of liquid NH_3 with a small excess of sodium. A few crystals of NH_4Cl were then added. The residue was taken up in water, adjusted to pH 2, and then determined on the amino acid analyzer. System C was used for β-alanine and γ-aminobutyric acid; system D for α-aminoacetonitrile.

Hydration was carried out by treatment of the sample (15–30 μmol) with 30–32% hydrobromic acid–acetic acid (Eastman) (25–50 μl) under anhydrous conditions for 15 min at room temperature. The mixture was frozen and lyophilized over P_2O_5 and KOH. The residue was dissolved in water, adjusted to pH 5, and determined on the analyzer.

Stability was examined in water in the presence of several drops of toluene in stoppered, 3-ml test tubes. Samples, taken

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(22) Reductive cleavage of the cyano group α to the amino group, in somewhat lower yields, had been observed previously in the analytical study[9] when dehydrated isoasparagine–oxytocin, dehydrated isoglutamine–oxytocin, and a few model compounds were treated with the Birch reagent. Although it is now clear that cleavage does not require methanol, when it is sought to distinguish the α-cyanoamino acid (isoasparaginyl and isoglutaminyl) from the ω-cyanoamino acid (asparaginyl and glutaminyl) structure, it may be desirable to include it in order to convert the ω-cyano group to the recognizable ω-aminomethyl group.[9]

(23) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, Wiley, New York, N. Y., 1961, pp 1696–1705.

(24) K. Biemann and J. A. McCloskey, *J. Amer. Chem. Soc.*, **84**, 3192 (1962); G. Junk and H. Svec, *ibid.*, **85**, 839 (1963).

(25) Ethyl acetamidocyanoacetate was purchased from Aldrich Chemical Co., Milwaukee, Wis.; porcine kidney acylase, from Calbiochem, Los Angeles, Calif. *p*-Methoxybenzyl carbazate was prepared as described[10] and was also purchased from the Protein Research Co., Institute for Protein Research, Osaka University, Osaka City, Japan.

Infrared spectra were taken on a Perkin-Elmer Model 137 spectrophotometer on KBr disks containing 0.3% of sample. Medium, strong, and significant absorption bands are recorded. Mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6E spectrometer. Samples were inserted into the direct inlet system at 110–140°. Elemental analyses were performed by Micro-Tech Laboratories, Inc., Skokie, Ill., and by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Optical rotations were taken in a 2-dm cell in a Rudolph polarimeter, Model 80, or in a Rudolph photoelectric spectropolarimeter system, Model 80Q6-34402. Melting points were taken in capillaries and are corrected. Some varied with the rate of heating. Capillaries were inserted in a bath usually preheated to 30° below the melting point and heated at a rate of 2.5 or 3° per min. Dimethylformamide for amide dehydration was stored over Linde 4A Molecular Sieves. Evaporations were under reduced pressure unless otherwise indicated. Compounds liberated with HCl were washed on the filter until free of Cl^-. Acid hydrolyses were in 6 *N* HCl in sealed tubes under N_2 at 115° for 16 hr. AG 1-X4 resin (chloride, 100–200 mesh) was Dowex 1-X4 anion-exchange resin, analytical grade, purchased from Bio-Rad Laboratories, Richmond, Calif. The resin column was washed with 5 vol of 2 *N* sodium acetate until free of Cl^-, then with 2 vol of 0.5 *N* acetic acid, and, before use, with water until the effluent had pH 4.7.

Amino acid and ammonia analyses were performed on a Beckman–Spinco automatic amino acid analyzer, Model 120.[20] System A refers to the 150-cm resin column, pH 3.25 at 30° described for physiological fluids; system B, to the 50-cm column with type 50A resin at pH 4.26 and 30°; system C, at 50°; system D, the 15-cm column at pH 5.28 and 30°. Electrophoresis was on strips of Whatman No. 1 paper at 9–10 V/cm in sodium barbital buffer of pH 8.6 or pyridinium acetate buffer of pH 5.7 for 2.5 or 3 hr. Strips were sprayed with 0.15% ninhydrin in acetone and heated at 105°. Thin layer chromatography (tlc) was carried out on plates of silica gel G in system 1, *n*-butyl alcohol–acetic acid–5% NH_3 (11:6:3); system 2, *n* butyl alcohol–acetic acid–water (3:1:1); or system 3, *n*-propyl alcohol–concentrated NH_3 (67:33). For detection of pMZ derivatives, the plates were dried at 120° for 15 min and were then sprayed with dichromate–sulfuric acid and heated at 120° ("Thin-layer Chromatography, a Laboratory Handbook," E. Stahl, Ed., Academic Press, New York, N. Y., 1965, p 488). Ascending paper chromatography was on sheets of Whatman No. 1 paper in system 4, *n*-butyl alcohol–pyridine–acetic acid–water (15:10:3:12); in system 5, ethanol–concentrated NH_3–water (18:3:1), or descending in system 6, *n*-butyl alcohol–acetic acid–water (4:1:5).

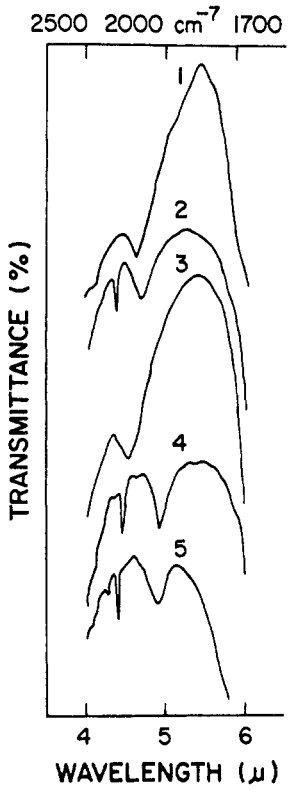

Figure 2.—Infrared spectra of α- and ω-cyanoamino acids in potassium bromide disks within 1700 and 2500 cm^{-1}; (1) L-γ-cyano-γ-aminobutyric acid; (2) L-γ-cyano-α-aminobutyric acid; (3) L-β-cyano-β-alanine; (4) L-β-cyanoalanine; (5) α-cyanoglycine.

at the times indicated in Figure 1, were placed directly on the analyzer, or frozen and analyzed soon after. For cyanide analysis, the solutions were kept in sealed ampoules and then placed in microdiffusion vessels. HCN was distilled into 1 *N* NaOH and determined as described.[5]

Acetamidocyanoacetic Acid Hemipotassium Salt.—Ethyl acetamidocyanoacetate, 34 g, was hydrolyzed as described[6] except that the reaction time was extended to 65 hr. The aqueous solution was concentrated to 30 ml, cooled, adjusted to pH 2 with concentrated HCl, and allowed to stand overnight in the cold. The white crystalline solid, wt 14.9 g (51%), mp 127–133°, was recrystallized from water–ethanol to give 8.32 g of clusters of needles, mp 133–134°, which was 2° below analytical material.

Anal. Calcd for acetamidocyanoacetic acid, $C_5H_6N_2O_3$ (142.1): C, 42.3; H, 4.26; N, 19.7. Calcd for potassium acetamidocyanoacetate, $C_5H_5N_2O_3K$ (180.2): C, 33.3; H, 2.79; N, 15.6. Calcd for acetamidocyanoacetic acid–potassium acetamidocyanoacetate, $C_5H_6N_2O_3 \cdot C_5H_5N_2O_3K$ (322.3): C, 37.3; H, 3.44; N, 17.4. Found: C, 37.3 (V_2O_5 used in combustion); H, 3.52; N, 17.8; neut equiv, 348 [determined by titration with 0.1 *N* NaOH (phenolphthalein)], 330 (electrometric).

Acid hydrolysis gave Gly, 1.04, and NH_3, 1.00, with quantitative recovery based on mol wt 322.

A solution of 300 mg in 1 ml of water was adjusted with 6 *N* HCl from pH 1.7 to pH 0.5. The solution became turbid and crystallization soon started, wt 135 mg, mp 110–112° dec. Recrystallization as for **7** raised the melting point to 114–115°. Admixture with **7** caused no depression in melting point.

Acetamidocyanoacetic Acid (7).—Ethyl acetamidocyanoacetate (17–34 g) was hydrolyzed as described[6] except that the pH of the concentrated aqueous solution was carefully adjusted to 0.5. The yield of crude product, mp 114–116°, was similar to that reported.[6] Recovery of unreacted ester was lower, however, making the overall yield 45–65%. Crude **7** was recrystallized by addition of ether and petroleum ether (bp 30–60°) to a solution in hot acetone and allowing it to stand at 25°.

α-Cyanoglycine (4).—A solution of 4.26 g (30 mmol) of **7** in 200 ml of distilled water was adjusted to pH 7 with 14.9 ml of 2 *N* LiOH. Porcine kidney acylase, 100 mg, 90 EU/mg, was added. The solution was stirred magnetically at room temperature and maintained at pH 7 by periodic addition of 1 *N* LiOH. Usually 13 ml was taken up within 1 hr, when 10 mg of enzyme was added. After a total of 2.5 hr, when 21 ml of base had been added, the uptake of base was slow. The solution was then stirred vigorously with 650 mg of activated charcoal and after 10 min, was filtered through a thin layer of wet charcoal. The yellow or red concentrate was adjusted to pH 1.9 with cold concentrated HCl and applied to a 1 × 50 cm column of Amberlite CG-120 H^+ resin maintained at 5°. The column was washed with water and the effluent was collected in fractions of several milliliters. These were tested for Cl^-, and for ninhydrin-reactive material by spot test on paper. The major ninhydrin-positive fractions, 7–14, were salt free and were immediately concentrated to a small volume. Crystallization started and was completed by addition of ethanol and cooling. After 30 min the product was collected on the filter, dried, and stored in the cold under vacuum, wt 1.14 g, mp 121.5° dec. Fractions 5 and 6, which contained some salt, yielded 0.55 g of Cl^--free product, mp 121° dec. α-Cyanoglycine of similar melting point could be obtained in comparable yield by crystallization without use of resin. Such products, however, frequently retained color or tended to darken.

For analysis a solution of 438 mg of crude α-cyanoglycine in 10 ml of water was treated with charcoal and was then diluted cautiously with 30 ml of ethanol. The colorless plates were collected, washed with ethanol and ether, and dried: wt 270 mg (63%); mp 124° dec; $[\alpha]^{24}D$ −0.05° (*c* 2, 1 *N* acetic acid); ir absorption at 3040, 2285 ($W_{1/2}$ = 0.04 μ), 2040 ($W_{1/2}$ = 0.23 μ), 1660, 1490, 1360, 1140, 1075, 934, 878, and 784 cm^{-1}; mass spectrum (70 eV) *m/e* (rel intensity) 113 (5), 112 (3), 73 (8) (p^+ − HCN), 55 (100) (p^+ − COOH, $CHNH_2C{\equiv}N$), 44 (136) (CO_2).

Anal. Calcd for $C_3H_4N_2O_2$: C, 36.0; H, 4.03; N, 28.0. Found: C, 36.3; H, 4.06; N, 27.8.

Paper electrophoresis at pH 8.6 gave a single purple-yellow ninhydrin spot 10 cm from the origin toward the anode: amino acid analysis in system A, elution vol 80 ml; ninhydrin color yield constant (*c*) 15.5.

***p*-Methoxybenzyloxycarbonyl-L-isoasparagine (8).**—L-Isoasparagine hydrate, prepared in quantitative yield by hydrogenolysis[16] of Cbz-L-isoasparagine,[26] was condensed with pMZ azide.[10] The general procedure of Weygand and Hunger[10] for the synthesis of pMZ amino acids was modified so that 2 mol of $NaHCO_3$ replaced MgO, 2 mol of pMZ azide were used, the reaction was run for 70 hr and reduced to half its volume, and the product was liberated with cold 6 *N* HCl (61%), mp 156°, $[\alpha]^{26}D$ −25.4° (*c* 1, dimethylformamide), with the expected elemental analysis. After it had been synthesized, **8** with the same optical rotation but lower melting point, 144–146°, was reported[27] as prepared by the general procedure.

***p*-Methoxybenzyloxycarbonyl-L-asparagine.**—Prepared from asparagine as described for **8**, this product agreed well in yield and properties with this compound prepared independently with pMZ azide and MgO[27] and recently by acylation with *p*-methoxybenzyl chloroformate.[28]

L-β-Cyano-β-alanine (5).—To a solution of 1.0 g of **8** in 6.8 ml of pyridine held at 19–21° was added with magnetic stirring over a period of 25 min a solution of 0.87 g of DCCI in 5.5 ml of pyridine. Stirring was continued for an additional 3 hr at 21–23°. The dicyclohexylurea was then filtered off and washed with pyridine, and the filtrate and washings were concentrated to a syrup at 1 Torr. The residue was taken up in 8 ml of 5% $NaHCO_3$, and the mixture was extracted with three 15-ml portions of ether. The aqueous layer was cooled and adjusted to pH 3 with 4 *N* HCl. The liberated oil was extracted with 32 ml of ether. The extract was washed with three small portions of water and dried ($MgSO_4$) for 3.5 hr in the cold. It was then concentrated at atmospheric pressure (bath 40–45°). The last few milliliters of solvent were removed with a current of dry N_2 at room temperature. To the liquid residue 0.67 ml of anisole was

(26) C. Ressler, H. Malodeczky, and D. V. Kashelikar, *Biochem. Prep.*, **10**, 83 (1963).

(27) E. Schröder and E. Klieger, *Justus Liebigs Ann. Chem.*, **673**, 208 (1964).

(28) S. Sakakibara, I. Honda, M. Naruse, and M. Kanaoka, *Experientia*, **25**, 576 (1969).

added. The mixture was cooled in an ice bath and cold TFA (3.7 ml) was added. The light pink solution was allowed to remain at 0° for 8 min and then was evacuated promptly at 0.025 Torr at 0° for 8 min. The residue was triturated twice with 10 ml of ether and was then dissolved in water and adjusted to pH 4.7 with 4 *N* NH_3. The solution was again extracted with ether and then concentrated. Ethanol was added and the mixture was stored in the cold. The crystalline solid was collected by filtration, washed with ethanol, and dried, wt 238 mg (overall yield 62%), mp 121.5–123° dec. It contained 96% **5** and 4% isoasparagine; similar products had 5–9% isoasparagine.

Earlier obtained products containing larger amounts of isoasparagine could be purified as follows. A solution of 700 mg in 11 ml of water was applied to a 44 × 1.5 cm column of AG 1-X4 (acetate) resin,[25] and the column was washed with water, all at 5°. At effluent volume 225 ml, pyridinium acetate buffer, pH 4.0 (15 ml of pyridine/1 l. of 1 *N* acetic acid), was substituted. Ninhydrin-positive material was present in fraction 1 (effluent volume 45–133 ml) and fraction 2 (66–159 ml after the change of eluent). Both fractions were concentrated almost to dryness and then were diluted with ethanol. The crystalline materials were collected and examined by paper electrophoresis at pH 5.7.[25] (In 2.5 hr, **5** travels 36 mm toward the anode; isoasparagine remains near the origin.) Fraction 1 yielded 222 mg, mp 116–118°, containing similar amounts of **5** and isoasparagine and presumably was material not adsorbed onto the column. Fraction 2 yielded 410 mg of **5**, mp 123–124° dec, having only a trace of isoasparagine.

For analysis 95 mg of **5** was dissolved in 1.8 ml of water at 25°, then diluted with alcohol and cooled: colorless needles; mp 122.5° dec; $[\alpha]^{26}D$ −12.1° (*c* 0.57, water); ir bands at 3040–2570 (b), 2220 ($W_{1/2}$ = 0.2 μ), 1645, 1575, 1520, 1410, 1335, 1298, 1150, 1063, 1020, 987, 972, and 714 cm^{-1}; mass spectrum (70 eV) *m/e* (rel intensity) 87 (51) (p^+ − HCN), 84 (24), 69 (52) (p^+ − COOH), 60 (23), 55 (100) ($CHNH_2C{\equiv}N$).

Anal. Calcd for $C_4H_6N_2O_2$: C, 42.1; H, 5.30; N, 24.6. Calcd for $C_4H_6N_2O_2$ containing 3.4% isoasparagine: C, 41.9; H, 5.33; N, 24.4. Found: C, 42.2; H, 5.41; N, 24.0.

Amino acid analysis in system B, elution vol 37 ml (*c* 24.5); 3.4% isoasparagine at 55 ml. In system A, elution vol 398 ml in the position of valine. In system C, 15–22% conversion to isoasparagine took place.

β-Cyanoalanine (3).—pMZ-L-Asparagine, 0.59 g, was treated with DCCI as described under **5**. The dried ether extract was concentrated to 10 ml and the product was precipitated with *n*-hexane and was reprecipitated in a similar way. Crude **pMZ-L-β-cyanoalanine**, 0.42 g (76%), mp 75–91° dec, was recrystallized from ether–petroleum ether (bp 30–60°): mp 93.5–94.5°; $[\alpha]^{25}D$ −13.8° (*c* 1, methanol); tlc R_{f1} 0.67, single spot; ir band at 2280 cm^{-1}.

Anal. Calcd for $C_{13}H_{14}N_2O_5$: C, 56.1; H, 5.07; N, 10.1. Found: C, 56.1; H, 5.16; N, 10.1.

Crude pMZ-L-βCNala, 0.137 g, was deprotected with 0.5 ml of TFA as described for **5**. The product in 0.5 ml of water at pH 5 was diluted with 2 vol of ethanol, wt 53 mg. Two recrystallizations yielded 30 mg (53%) of fine needles homogeneous on paper electrophoresis at pH 5.6 and showing 98.8% **3**[11] and 1.2% asparagine on amino acid analysis in system A. Admixture with **3**[11] caused no depression in melting point.

***p*-Methoxybenzyloxycarbonyl-L-isoglutamine (9).**[29]—Cbz-L-Isoglutamine was prepared by amidation of Cbz-L-glutamic anhydride[16] as modified by Shealy, *et al.*[15] For a large scale this was more convenient than the mixed anhydride procedure.[14] L-Isoglutamine, 7.24 g, obtained by hydrogenolysis of Cbz-L-isoglutamine, was treated with 20.5 g of pMZ azide and 8.33 g of $NaHCO_3$ as described for **8**. The concentrated aqueous layer, 80 ml, was cooled and acidified with 20% citric acid. The gelatinous precipitate was collected by filtration, wt 9.6 g. After two recrystallizations from ethanol this melted at 162.5–164.5° dec. It contained 7.1% residue (calcd for Na salt, 6.9%) and yielded 87% isoglutamine on hydrogenolysis. A suspension of 1.2 g in 30 ml of water was adjusted with 2 *N* HCl from pH 4.5 to pH 2. The solid was extracted with 100 ml of ethyl acetate. The dried ($MgSO_4$) extract yielded 0.95 g of residue melting at 118–123°. Two recrystallizations from tetrahydrofuran–petroleum ether raised the melting point to 127.5–130.5° dec, $[\alpha]^{26.5}D$ −5.5° (*c* 1.1, methanol).

Anal. Calcd for $C_{14}H_{18}N_2O_6$: C, 54.2; H, 5.85; N, 9.03. Found: C, 54.6; H, 6.05; N, 8.74.

***p*-Methoxybenzyloxycarbonyl-L-isoglutamine Methyl Ester (10).**—Crude **9** was extracted with hot ethanol, 40 ml per gram. The extract was filtered and gave 8.67 g of residue that was divided into three batches, each in 40 ml of methanol, and treated with a slight excess of diazomethane in ether for 10 min. The solutions were promptly filtered and taken to dryness. The combined product, 8.58 g, mp 104–108°, was recrystallized from methanol–water to give 6.51 g of needles, mp 117–119° (86%). Recrystallization raised the melting point to 119–120.5°, $[\alpha]^{24}D$ −5.5° (*c* 0.9, methanol).

Anal. Calcd for $C_{15}H_{20}N_2O_6$: C, 55.6; H, 6.22; N, 8.64. Found: C, 55.9; H, 6.42; N, 8.67.

Purified **9**, 70 mg in 5 ml of methanol, yielded 52 mg of **10** of the same melting point.

Methyl Esters of *N*-Benzyloxycarbonyl-L-β-cyano-β-alanine, *N*-Benzyloxycarbonyl-L-γ-cyano-γ-aminobutyric Acid, and *N*-*p*-Methoxybenzyloxycarbonyl-L-γ-cyano-γ-aminobutyric Acid (11).—Cbz-L-Isoasparagine methyl ester,[16] Cbz-L-isoglutamine methyl ester,[19] and pMZ-L-isoglutamine methyl ester (**10**) were starting materials. These were dehydrated with dimethylformamide–thionyl chloride as described previously with Cbz-L-asparagine methyl ester,[9] except that 3 mol of $SOCl_2$ per mol of amide were used. The reaction mixture of Cbz-L-γ-cyano-γ-aminobutyric acid methyl ester was processed in the cold room because of the low melting point of this product.

***N*-Benzyloxycarbonyl-L-β-cyano-β-alanine, *N*-Benzyloxycarbonyl-L-γ-cyano-γ-aminobutyric Acid, and *N*-*p*-Methoxybenzyloxycarbonyl-L-γ-cyano-γ-aminobutyric Acid (12).**—Cbz-L-β-Cyano-β-alanine methyl ester and **11** were dissolved in acetone–water (2:1), 1 mmol/ml, and 1 equiv of 1 *N* NaOH was added dropwise to maintain pH 8–9. Cbz-L-γ-Cyano-γ-aminobutyric acid methyl ester was dissolved in 0.5 ml of acetone and 1 equiv of 0.1 *N* LiOH was added initially. After the reaction periods indicated in Table I, the solutions were adjusted to pH 7 and acetone was evaporated off. The aqueous solutions were extracted with ethyl acetate and then acidified with 2 *N* HCl and extracted with ethyl acetate. For **12**, the solution was adjusted to pH 4.5 with 1 *N* citric acid. The ethyl acetate extracts were washed with water and dried ($MgSO_4$), and the solvent was removed. Table I gives the yields and properties of the acids and the foregoing esters.

L-γ-Cyano-γ-aminobutyric Acid (6).—Deprotection of 0.73 g (2.5 mmol) of **12** was carried out as described for **5**. When the residue freed of TFA was dissolved in 5 ml of cold water and was adjusted to pH 5, the product separated. The mixture was shaken several times with ether and then concentrated to 2.5 ml and cooled overnight. The solid was collected by centrifugal filtration, wt 240 mg (75%), mp 131–133° dec. Paper electrophoresis at pH 8.5 showed a single ninhydrin-positive spot. For analysis 115 mg was dissolved in 10 ml of water at 25° and the solution was diluted with 30 ml of ethanol and cooled. Recrystallized in the same way, the prisms melted at 134.5–136.5° dec: $[\alpha]^{24}D$ +25.3° (*c* 0.8, water) (lit.[18] mp 188–190°); ir bands at 3130–2440, 2175 ($W_{1/2}$ = 0.12 μ), 1640, 1540–1420, 1180, 1150, 1075, 1040, 1015, 990, 888, 798, and 743 cm^{-1}; mass spectrum (70 eV) *m/e* (rel intensity) 128 (4) (p^+), 111 (7) (p^+ − NH_3 or − OH), 101 (20) (p^+ − HCN), 83 (59) (p^+ − COOH), 74 (58) (CH_3CH_2COOH), 57 (100) (p^+ − COOH, − CN), 55 (62) ($CHNH_2C{\equiv}N$).

Anal. Calcd for $C_5H_8N_2O_2$: C, 46.9; H, 6.29; N, 21.9. Found: C, 46.6; H, 6.42; N, 21.8.

Amino acid analysis in system B, elution vol 100 ml, 7 ml before isoglutamine. A mixture of the two was separable; *c* 24.5; ratio 1.94 of the absorbance of the ninhydrin product at 570 and 440 mμ. Tlc: R_{f2} 0.42, R_{f3} 0.55; R_{f4} 0.51, R_{f5} 0.31.

Registry No.—**4**, 6232-21-9; **5**, 31883-83-7; **6**, 31883-84-8; **8**, 31883-85-9; **9**, 31883-86-0; **10**, 31883-87-1; **11**, 31883-88-2; **12**, 31883-89-3; Cbz-L-β-cyano-β-alanine methyl ester, 31883-81-5; acetamidocyanoacetic acid hemipotassium salt, 31883-90-6; pMZ-L-β-cyanoalanine, 31883-91-7; Cbz-L-γ-cyano-γ-amino, butyric acid methyl ester, 31883-92-8; Cbz-L-β-cyano-

(29) No attempt was made to improve the procedure. In future preparations it may be preferable to acidify directly to pH 2 with 2 *N* HCl instead of with 20% citric acid and extract the product.

β-alanine, 7436-73-9; Cbz-L-γ-cyano-γ-aminobutyric acid, 31883-94-0.

Acknowledgments.—The authors thank Dr. Paul M. Gallop and Mr. Edward M. Henson for the mass spectra, Dr. Tomohide Koga for the cyanide analyses, and Mrs. Harriet R. Levie and Miss Christine Lauinger for assistance with the stability studies.

2,4-Dimethoxybenzyl as a Protecting Group for Glutamine and Asparagine in Peptide Synthesis

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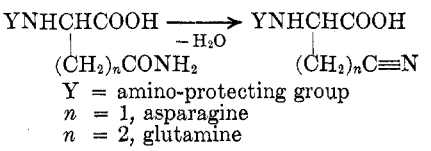
The properties of 2,4-dimethoxybenzyl (Dmb) as a protecting group for the amide side chain of glutamine and asparagine during peptide synthesis are described. 2,4-Dimethoxybenzylamine was prepared by the reduction of 2,4-dimethoxybenzyaldoxime with sodium bis(2-methoxyethoxy)aluminum hydride. The Dmb derivatives obtained by reaction of 2,4-dimethoxybenzylamine and either *N,N'*-dicyclohexylcarbodiimide or *N*-diethylamino-1-propyne with the appropriate amine acid derivatives are crystalline and the Dmb group can be removed by trifluoroacetic acid or anhydrous hydrogen fluoride to give the free amide. No formation of pyroglutamyl peptides or of other side reactions was detected with Dmb-protected glutamyl derivatives, even during saponification. On the contrary, use of alkali with either 2,4-dimethoxybenzyl- or bis(2,4-dimethoxybenzyl)-protected asparaginyl peptides resulted in a mixture of products and is not recommended.

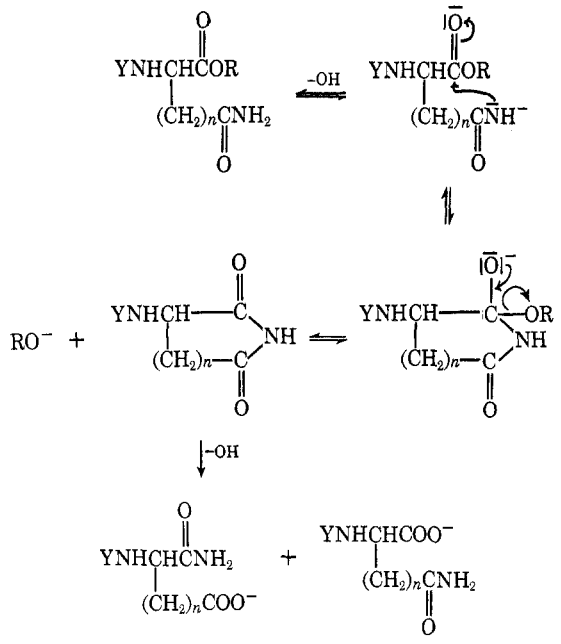
The amide groups of asparagine and glutamine undergo the following side reactions (eq 1–3) during peptide synthesis: (1) dehydration to the corresponding



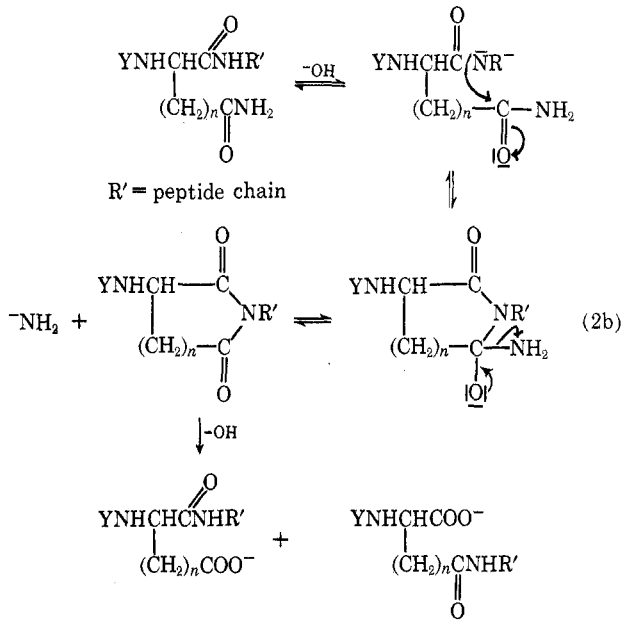
(1)

Y = amino-protecting group
n = 1, asparagine
n = 2, glutamine

cyano derivatives;[2–6] (2) formation of imides and subsequent hydrolysis[7–10] [N-protected asparagine or glutamine esters (a), asparaginyl or glutaminyl peptides (b). In this case, the loss of a proton by the action of alkali occurs in both position α and ω. The α site is more reactive because of the greater electrophilic strength of the α carbon atom as compared with that of the ω carbon atom. The subsequent release of the NH_2 group leads to formation of α and ω isomeric peptides, though the latter is obtained in greater amount. Reaction at the ω site causes cleavage of the peptide



(2a)



(2b)

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