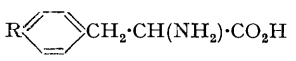
20. *Bacteriostasis in the Amino-acid Series. Part I. Derivatives of Alanine.*

By D. F. Elliott, A. T. Fuller, and C. R. Harington.

It is known that inhibition of bacterial growth can be effected by structural analogues of naturally occurring amino-acids; it is also known that all natural aromatic and heterocyclic amino-acids are β-substituted alanines. These considerations led to a search for antibacterial activity among alanines substituted in the β-position by pyridine, quinoline, and basic derivatives of benzene. The compounds so far studied are *p*-aminophenyl-, ω-amino-*p*-tolyl-, and *p*-dimethylaminophenyl-alanine, β-pyridyl(4)alanine, β-quinolyl(4)alanine, and the 6-methoxy-derivative of the last. Significant inhibitory actions have been observed with three only of these compounds, namely β-ω-amino-*p*-tolylalanine, β-6-methoxyquinolyl(4)alanine, and β-pyridyl(4)alanine; these inhibit hæmolytic streptococcus in broth in the range 1 : 2000 to 1 : 5000, and the last shows some action against *B. coli* in synthetic medium.

One of the main requirements of a satisfactory chemotherapeutic agent is that it should readily penetrate or be absorbed by the micro-organism against which it is directed. It occurred to us that such penetration might be facilitated if the drug were to contain an α-amino-acid grouping; moreover, since all naturally occurring aromatic and heterocyclic amino-acids are β-substituted alanines it was to this particular series of compounds that our attention was first directed. It is already known that some degree of inhibition of bacterial growth, presumably of a competitive character, can be effected by close structural analogues of essential natural amino-acids; examples are the antagonism of phenylalanine by β-thienyl(2)alanine (Dittmer, Ellis, McKennis, and du Vigneaud, *J. Biol. Chem.*, 1946, **164**, 761) and that of methionine by methoxinine (Roblin, Lampen, English, Cole, and Vaughan, *J. Amer. Chem. Soc.*, 1945, **67**, 290). Our own aim has been not so much to develop this type of competitive growth inhibition by closely related analogues of amino-acids but rather to study the effect of compounds in which the characteristic α-aminopropionic acid side-chain is attached through its β-carbon atom to benzene carrying a basic substituent or basic heterocyclic nuclei.

As a preliminary exploration of this idea we have studied *p*-aminophenylalanine (I; R = NH_2), β-ω-*amino*-p-*tolylalanine* (I; R = $NH_2 \cdot CH_2$), β-p-*dimethylaminophenylalanine* (I; R = NMe_2), β-pyridyl(4)alanine (II), β-*quinolyl*(4)*alanine* (III; R = H) and β-6-*methoxyquinolyl*(4)*alanine* (III; R = MeO). Extension to the *p*-guanidino- and *p*-amidino-derivatives of phenylalanine is projected.



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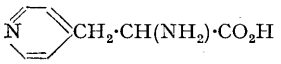

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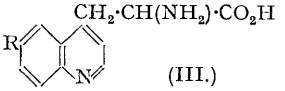

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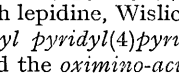
p-Aminophenylalanine was prepared according to Erlenmeyer and Lipp (*Annalen*, 1883, **219**, 219). For the synthesis of ω-amino-*p*-tolylalanine, *p*-cyanobenzyl chloride (Mellinghoff, *Ber.*, 1889, **22**, 3208) was condensed with ethyl acetamidomalonate, and the product hydrogenated by the method of Hartung (*J. Amer. Chem. Soc.*, 1928, **50**, 3370) to give *ethyl acetamido*-p-*aminomethylbenzylmalonate hydrochloride*, hydrolysis of which afforded the desired amino-acid. p-*Dimethylaminophenylalanine* was prepared by the Erlenmeyer synthesis as modified by Harington and McCartney (*Biochem. J.*, 1927, **21**, 852), starting with *p*-dimethylaminobenzaldehyde.

The alanine derivatives in the pyridine and quinoline series were synthesised by a method which seems to be generally applicable to amino-acids of this type, and which may be exemplified by the preparation of β-pyridyl(4)alanine. γ-Picoline was condensed with ethyl oxalate (cf. the condensation with lepidine, Wislicenus and Kleisinger, *Ber.*, 1909, **42**, 140) to give the potassium derivative of *ethyl pyridyl*(4)*pyruvate* (IV); this was converted successively into the *oxime* (V; R = Et) and the *oximino-acid* (V; R = H), which was finally reduced to give the desired



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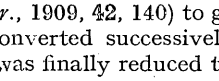

(V.)

amino-acid (II). The initial condensation with ethyl oxalate proceeded satisfactorily with lepidine and 6-methoxylepidine, but with γ-picoline the yield obtained was very poor, even when pyridine was added to the reaction mixture (cf. Grundmann, *Ber.*, 1937, **70**, 1148; Elks, Elliott, and Hems, *J.*, 1944, 629; Blout, Fried, and Elderfield, *J. Org. Chem.*, 1943, **8**, 37). For the final reduction of the oximino-acids catalytic methods were first tried, but without great success, the process being incomplete with palladium black in aqueous ammonia and very slow with Raney nickel. The latter case was complicated by the passage of nickel ions into solution and by the formation of amorphous by-products. The well-known method of reduction using palladised charcoal in alcoholic hydrogen chloride was also unsuccessful. The preparative problem was satisfactorily solved by reduction with stannous chloride in cold hydrochloric acid solution. The formation of amorphous products is probably due to polymerisation of dihydro-derivatives formed by partial reduction of the heterocyclic ring (see Bergstrom, *Chem. Reviews*, 1944, **35**, 162). This phenomenon was particularly marked with the pyridine derivative which, in presence of Raney nickel, absorbed 1 mol. of hydrogen only to give a completely amorphous product (cf. Adkins, Kuick, Farlow, and Wojeik, *J. Amer. Chem. Soc.*, 1934, **56**, 2425).

The pyridyl and quinolyl derivatives of alanine behave abnormally with ninhydrin. It was stated by Niemann, Lewis, and Hays (*J. Amer. Chem. Soc.*, 1942, **64**, 1678), who synthesised β-pyridyl(4)alanine in minute yield by a method different from that described in this paper, that the colour given by this amino-acid on heating with ninhydrin was red. We have confirmed this observation and have found that it also applies to the quinoline derivatives if the test is carried out in the ordinary way, *i.e.*, in aqueous or aqueous pyridine solution. If on the other hand pure pyridine or pyridine containing only a trace of water is used the normal blue colour is given by the quinolylalanines, although even under these conditions the colour given by pyridylalanine remains red [cf. the behaviour of proline (Grassman and von Arnim, *Annalen*, 1934, **509**, 288)].

Biological Results.

The amino-acids mentioned above have been tested for bacteriostatic activity by the methods usually employed in this laboratory, and the results are summarised in the Table. It will be seen that significant activity against *Strep. pyogenes*, Group A in broth is shown by ω-amino-*p*-tolylalanine, β-6-methoxyquinolyl(4)alanine, and β-pyridyl(4)alanine; the last compound is also fairly active against *B. coli* in synthetic medium. The inhibitory activity of *p*-aminophenylalanine is antagonised not only by phenylalanine, which is a possible direct

competitor, but by other amino-acids such as asparagine, lysine, and a mixture of glycine, glutamic acid, and arginine; this probably accounts for the fact that *p*-aminophenylalanine is apparently more active against *B. coli* than against *Staph. aureus*, since the synthetic medium in the latter case contained amino-acids (hydrolysed casein) and in the former did not.

Minimum inhibitory concentrations (*mg.*/100 *c.c.*).

	Strep. pyogenes Group A.		*Staph. aureus.*		*B. coli.*	
	Blood.	Broth.	Synthetic.	Broth.	Synthetic.	Broth.
p-Aminophenylalanine	>500	>1000	1000	>1000	200	>1000
p-Dimethylaminophenylalanine	>500	500	1000	>1000	1000	>1000
ω-Amino-*p*-tolylalanine dihydrochloride	>500	50	>1000	>1000	1000	>1000
β-Quinolyl(4)alanine	500	500	1000	>1000	500	>1000
β-6-Methoxyquinolyl(4)alanine	200	35	>1000	>1000	1000	>1000
β-Pyridyl(4)alanine dihydrochloride	200	20	>1000	>1000	75	1000

The compounds were also tested against acid-fast organisms but showed no activity. The two quinolylalanines were tested in addition against *E. histolytica*, but again showed no activity. We are indebted to Dr. P. D'Arcy Hart and Dr. J. D. Fulton respectively for carrying out these tests.

Experimental.

Ethyl Acetamido-p-*cyanobenzylmalonate.*—Sodium (1·15 g.; 1/20 atom) was dissolved in dry alcohol (25 c.c.); the solution was cooled somewhat and treated with ethyl acetamidomalonate (10·9 g.; 1/20 mol.) followed by *p*-cyanobenzyl chloride (Mellinghoff, *loc. cit.*) (8 g.; 5% excess over 1/20 mol.). The mixture was refluxed for 2 hours; the product had then separated as a crystalline mass. After addition of water (200 c.c.) to the cooled product it was filtered off and dried in a desiccator. Yield, 15·6 g. (94%). The *ester* formed long colourless needles from alcohol or acetone, m. p. 167° (Found: N, 8·7. $C_{17}H_{20}O_5N_2$ requires N, 8·4%).

Ethyl Acetamido-p-*aminomethylbenzylmalonate Hydrochloride.*—The cyano-compound (10 g.) was suspended in a mixture of absolute alcohol (200 c.c.) and absolute alcoholic hydrogen chloride (2·7N; 35 c.c.) and hydrogenated at room temperature and 100 atmospheres' pressure in the presence of palladium charcoal catalyst (10 g.) prepared according to Iwamoto and Hartung (*J. Org. Chem.*, 1944, **9**, 514). After 5 hours no further absorption of hydrogen took place. After removal of the catalyst the filtrate was evaporated to small bulk under reduced pressure. Dry ether (500 c.c.) was added to the crystalline mass. After 12 hours at room temperature the *hydrochloride* (yield, 6·5 g.; 70% on cyano-compound consumed) was filtered off, washed with dry ether, and crystallised from alcohol, from which it separated in elongated rectangular plates, m. p. 211—212° (decomp.) (Found: C, 54·45; H, 6·8. $C_{17}H_{25}O_5N_2Cl$ requires C, 54·8; H, 6·8%). Unchanged cyano-compound (1·6 g.) was recovered from the ethereal filtrate by evaporation and also from the catalyst by extraction with acetone. The catalyst could be used several times for this reduction. Treatment of a concentrated aqueous solution of the hydrochloride with sodium acetate yielded the *acetate* which crystallised from alcohol in fine needles, m. p. 163—164° (Found: N, 7·25. $C_{19}H_{28}O_7N_2$ requires N, 7·1%).

Ethyl Acetamido-p-*aminomethylbenzylmalonate* was liberated from the hydrochloride by treatment with two equivalents of ice-cold N-sodium hydroxide and extraction with chloroform. It had a strongly alkaline reaction and formed long rectangular plates, m. p. 86—87° (Found: N, 8·5. $C_{17}H_{24}O_5N_2$ requires N, 8·3%).

β-ω-Amino-p-*tolylalanine Derivatives.*—The above-described amine acetate (3 g.) was refluxed for 4 hours with 3N-hydrochloric acid (50 c.c.) and the solution evaporated to dryness under reduced pressure. The solid residue was dissolved in hot water (15 c.c.) and neutralised to pH 5 with concentrated ammonia solution.

β-ω-Amino-p-*tolylalanine monohydrochloride* separated on cooling in clusters of stout prisms. A second crop of crystals was obtained by evaporation of the mother liquors. Yield, 1·2 g. (71%). The compound was extremely stable to heat; it did not melt at 360° and even at this temperature turned only slightly brown (Found: N, 12·0; 12·2; Cl, 15·8. $C_{10}H_{15}O_2N_2Cl$ requires N, 12·15; Cl, 15·4%).

β-ω-Amino-p-*tolylalanine dihydrochloride*, obtained by treating a hot concentrated solution of the monohydrochloride with concentrated hydrochloric acid and allowing it to cool, separated in hydrated form and had m. p. 266—268° (with foaming). It was dried at 100°/20 mm. before analysis (Found: N, 10·2. $C_{10}H_{16}O_2N_2Cl_2$ requires N, 10·5%). When ethyl acetamido-*p*-aminomethylbenzylmalonate hydrochloride (2·7 g.) was refluxed for 5 hours with 2N-sulphuric acid (25 c.c.) and the solution evaporated to small bulk under reduced pressure at low temperature, *β-ω-amino*-p-*tolylalanine bis*(*hydrogen sulphate*) (1·5 g.) separated in colourless plates, m. p. 209—210° (decomp.) (Found: N, 7·1. $C_{10}H_{18}O_{10}N_2S_2$ requires N, 7·2%). The sulphate was dissolved in water and sulphuric acid removed with several times recrystallised barium hydroxide (cf. Vickery and Leavenworth, *J. Biol. Chem.*, 1928, **76**, 437). The filtrate, which was strongly alkaline, was allowed to evaporate in a desiccator, leaving *β-ω*-amino-*p*-tolylalanine as a crystalline mass which resisted attempts at recrystallisation.

2-*Phenyl*-4-p-*dimethylaminobenzylidene*-5-*oxazolone.** *p*-Dimethylaminobenzaldehyde (15 g.), hippuric acid (18 g.), freshly fused sodium acetate (8·2 g.), and acetic anhydride (30 c.c.) were mixed and well stirred by hand while being heated at 100° for 20 minutes. The red mass was cooled, rubbed with

* Hellerman, Porter, Lowe, and Koster (*J. Amer. Chem Soc.*, 1946, **68**, 1890) refer to a red azlactone, and the corresponding cinnamic acid, identical with ours, prepared by a method as yet unpublished.

cold water, filtered, and the residue washed with spirit to remove tarry material and finally washed with ether. The bright red *oxazolone* (16·8 g.; 57·5%) crystallised from benzene in long red needles, m. p. 213—214° (Found: C, 73·8; H, 5·3; N, 9·7. $C_{18}H_{16}O_2N_2$ requires C, 74·0; H, 5·5; N, 9·6%). The azlactone was soluble in cold concentrated hydrochloric acid and insoluble in dilute hydrochloric acid (3N) in the cold, but it rapidly dissolved on boiling. When this solution was neutralised with ammonia until just acid to litmus, *α-benzamido-p-dimethylaminocinnamic acid* separated as a yellow precipitate. It formed bright yellow prisms from alcohol, m. p. 225—226° (decomp.) (Found: C, 69·8; H, 5·2; N, 9·25. $C_{18}H_{18}O_3N_2$ requires C, 69·7; H, 5·8; N, 9·0%). The cinnamic acid was easily soluble in acid and alkali.

β-p-Dimethylaminophenylalanine.—The azlactone (5 g.) was refluxed 1½ hours with a mixture of acetic anhydride (25 c.c.) and hydriodic acid (*d* 1·7; 25 c.c.) (previously prepared by adding the anhydride slowly to the hydriodic acid with cooling) and red phosphorus (5 g.). After cooling, the solution was diluted, filtered, and evaporated to dryness under reduced pressure. The residual syrup rapidly crystallised and was dissolved in hot water (50 c.c.) and made faintly alkaline with concentrated ammonia solution. The *amino-acid* separated as colourless plates (2·9 g.; 82%). After recrystallisation from hot water, in which it was not very soluble, the product had m. p. 256—258° (decomp.). It was dried at 100°/20 mm. for 3 hours before analysis (Found: C, 63·7; H, 7·6; N, 13·4. $C_{11}H_{16}O_2N_2$ requires C, 63·4; H, 7·7; N, 13·5%).

α-Oximino-β-pyridyl(4)propionic Acid.—Dry potassium ethoxide was prepared by adding clean potassium (3·9 g.; 1/10 atom) to the minimum quantity of dry alcohol and evaporating the solution to dryness under reduced pressure over a cool flame with swirling of the flask to spread the solid evenly over the walls. The flask was then immersed up to the neck in an oil-bath at 180°, and heating continued for 15 minutes at 20 mm. After cooling in a vacuum, dry ether (250 c.c.) was added, followed immediately by ethyl oxalate (14·6 g.; 1/10 mol.), the mixture being gently shaken. The solution of the ethoxide was treated with γ-picoline (9·3 g.; 1/10 mol.) and dry pyridine (50 c.c.), and the mixture refluxed in a nitrogen atmosphere for 48 hours in a bath at 45—50°. The yellow potassium salt, which was very hygroscopic, was collected, washed with dry ether, and dried in a desiccator. Yield, 4 g. (17·3%). The salt was added to ice-cold, oxygen-free N-hydrochloric acid (20 c.c.) and then a mixture of hydroxylamine hydrochloride (1·2 g.) and potassium acetate (2·0 g.) in a small quantity of water was added in small portions with warming to about 50° for a few minutes. On cooling, crystals of *ethyl α-oximino-β-pyridyl(4)propionate* separated, and further crops were obtained on evaporation of the mother liquors at low temperature. Yield, 2·5 g. (12% calculated on γ-picoline). The ester formed orange prisms from ethyl acetate, m. p. 146—147° (Found: N, 13·3. $C_{10}H_{12}O_3N_2$ requires N, 13·5%). It dissolved easily in 1 equiv. of N-sodium hydroxide in the cold and was reprecipitated unchanged on neutralisation with acid. When the ester (3·8 g.) was boiled for 5 minutes with N-sodium hydroxide (21·4 c.c.; 2 equivs.), neutralised while warm with 5N-hydrochloric acid (4·3 c.c.), filtered rapidly from a small amount of red pigment, and kept 2 days at 0°, *α-oximino-β-pyridyl(4)propionic acid* separated, and a further crop was obtained from the mother liquors on evaporation. Yield, 2·2 g. (67%). The oximino-acid formed buff-coloured plates from hot water, m. p. 178° (decomp.) (Found: N, 15·5. $C_8H_8O_3N_2$ requires N, 15·6%).

β-Pyridyl(4)alanine Dihydrochloride.—The oximino-acid (0·9 g.) was added to a solution of stannous chloride (2·5 g.) in concentrated hydrochloric acid (10 c.c.). The oxime rapidly dissolved and gave a light brown solution. After 24 hours the solution was diluted to 300 c.c. and tin removed with hydrogen sulphide. The filtrate afforded a mass of heavy crystals on evaporation (0·82 g.; 68%). The *β-pyridyl(4)alanine dihydrochloride* separated in massive prisms when a hot concentrated solution in 3N-hydrochloric acid was treated with several parts of boiling alcohol. It had m. p. 229—230° (decomp.) (Found: C, 40·6; H, 5·1; N, 11·6. $C_8H_{12}O_2N_2Cl_2$ requires C, 40·2; H, 5·1; N, 11·7%).

α-Oximino-β-quinolyl(4)propionic Acid.—The corresponding pyruvic ester was prepared by condensation of lepidine with ethyl oxalate essentially according to Wislicenus and Kleisinger (*loc. cit.*) except that the reaction mixture was kept for 48 hours instead of 24 hours as stated by these authors. The crude finely powdered ester (2·4 g.; 1/100 mol.) was added to a mixture of fused sodium acetate (1 g.; 1/80 mol.) and hydroxylamine hydrochloride (0·7 g.; 1/100 mol.) in water (2 c.c.), and following addition of alcohol (25 c.c.) the whole was refluxed for 30 minutes. After cooling and addition of water (50 c.c.) the *ethyl α-oximino-β-quinolyl(4)propionate* (2·15 g.; 84·5%) was collected and crystallised from spirit. It formed sheaves of colourless prisms, m. p. 183° (Found: N, 11·0. $C_{14}H_{14}O_3N_2$ requires N, 10·85%).

α-Oximino-β-quinolyl(4)propionic acid was prepared by refluxing the ester (1·2 g.) for ten minutes with N-sodium hydroxide (10 c.c.) and acidifying while hot with excess of glacial acetic acid. After cooling, the yellow crystals were filtered off and dried in a desiccator. Yield, 1·05 g. (98%); m. p. 204° (decomp.) (Found: N, 12·2. $C_{12}H_{10}O_3N_2$ requires N, 12·2%).

β-Quinolyl(4)alanine and Derivatives.—(*a*) The above oximino-acid (1·15 g.) was dissolved by warming with N-ammonia (50 c.c.) and hydrogenated at room temperature and pressure in the presence of Raney nickel (0·25 g.). Fresh catalyst (0·25 g.) was added after 4 hours, and shaking continued for 24 hours; the theoretical amount of hydrogen had then been absorbed. The greenish solution, in which was suspended some amorphous material found to be extremely tedious to remove by filtration or centrifugation, was decanted from the catalyst and evaporated to dryness under reduced pressure. The residue was dissolved in boiling water and nickel ions removed with hydrogen sulphide. On evaporating the filtrate to small volume *β-quinolyl(4)alanine* was obtained as a bulky mass of almost colourless needles (0·74 g.; 68·5%) which were filtered off and washed with absolute alcohol. The amino-acid had m. p. 248—250° (decomp.). It separated from hot water in varying degrees of hydration and was dried in the usual way for analysis (Found: C, 66·4; H, 5·9; N, 12·6. $C_{12}H_{12}O_2N_2$ requires C, 66·65; H, 5·6; N, 13·0%). The *dihydrochloride dihydrate* had m. p. 242—244° (decomp.) (Found: Cl, 21·1; loss at 60°/20 mm., 10·1. $C_{12}H_{14}O_2N_2Cl_2,2H_2O$ requires Cl, 21·8; loss for $2H_2O$, 11·1%). When the amino-acid (50 mg.) was added to 50% sulphuric acid (0·1 c.c.) it dissolved, and on standing overnight the *bis(hydrogen sulphate) monohydrate* (60 mg.) separated in aggregates of massive prisms, m. p. 202—204°

(decomp.) (Found: S, 15·1; loss at 100°/20 mm., 3·9. $C_{12}H_{16}O_{10}N_2S_2$ requires S, 14·9; loss for $1H_2O$, 4·3%).

(*b*) The oximino-acid (2·3 g.) was added, with shaking, to a solution of stannous chloride (5 g.) in concentrated hydrochloric acid (25 c.c.). The oxime dissolved slowly because of the formation of a sparingly soluble hydrochloride, and the solution was shaken mechanically at room temperature for 18 hours. The crystalline tin complex was filtered off, washed with a small quantity of cold concentrated hydrochloric acid and then with dry ether, and dissolved in water (300 c.c.). The yellow solution was treated with hydrogen sulphide to remove tin, and the filtrate evaporated to dryness under reduced pressure. The residual syrup rapidly solidified to a crystalline mass of the dihydrochloride dihydrate. Yield, 2·5 g. (76·9%). For comparison a small portion was warmed in a vacuum with excess of 50% sulphuric acid until most of the hydrogen chloride had been removed, and then cooled. The bis(hydrogen sulphate) monohydrate which separated was identical with the sample described in (*a*).

Ethyl 6-Methoxyquinolyl(4)pyruvate.—6-Methoxylepidine (Ainley and King, *Proc. Roy. Soc.*, 1938, **125**, *B*, 84) (17·3 g.; 1/10 mol.) dissolved in a minimum amount of dry ether was added to a mixture of ethyl oxalate (15 c.c.), potassium ethoxide (from 3·9 g. of potassium; 1/10 atom), dry pyridine (50 c.c.), and absolute ether (250 c.c.) prepared as previously described, and the solution was kept for 3—4 days at room temperature. The orange potassium salt was collected, washed with dry ether, and dried in a desiccator. Yield, 17 g. (55%). *Ethyl 6-methoxyquinolyl(4)pyruvate* was liberated from this salt by shaking it thoroughly with 1 equiv. of 0·5N-hydrochloric acid, filtering off the solid, and drying it in a desiccator. It formed orange prisms from dioxan, m. p. 186—187° (decomp.) (Found: N, 5·1. $C_{15}H_{15}O_4N$ requires N, 5·4%).

α-Oximino-β-6-methoxyquinolyl(4)propionic Acid.—The corresponding *ethyl* ester was prepared as previously described for the unsubstituted quinoline compound and formed colourless hexagonal plates from alcohol, m. p. 222—223° (yield, 93%) (Found: C, 62·6; H, 5·6; N, 9·8. $C_{15}H_{16}O_4N_2$ requires C, 62·5; H, 5·6; N, 9·7%). *α-Oximino-β-6-methoxyquinolyl(4)propionic acid*, prepared in quantitative yield by hydrolysis in the usual way with sodium hydroxide, formed pale yellow spear-shaped prisms, m. p. 213—214° (decomp.). The compound darkened in bright light (Found: N, 10·4. $C_{13}H_{12}O_4N_2$ requires N, 10·8%).

β-6-Methoxyquinolyl(4)alanine.—(*a*) The oximino-acid behaved similarly to the unsubstituted quinoline compound on hydrogenation with Raney nickel catalyst. *β-6-Methoxyquinolyl(4)alanine* was thus obtained in 57% yield and formed fine needles from hot water, m. p. 258—260° (decomp.). It was dried at 110°/20 mm. before analysis (Found: C, 63·1; H, 6·2; N, 11·4. $C_{13}H_{14}O_3N_2$ requires C, 63·4; H, 5·7; N, 11·6%).

(*b*) The oximino-acid (5·2 g.) was reduced with stannous chloride as previously described. This oxime dissolved more readily and gave a clear solution from which the tin complex suddenly separated after shaking for 15 minutes. After being shaken for 3 hours the solution was cooled overnight at 0° and the product isolated as before. The pale yellow *β-6-methoxyquinolyl(4)alanine dihydrochloride monohydrate* (4·5 g.; 67%) separated from dilute hydrochloric acid in irregular masses of hexagonal plates, m. p. 212—214° (decomp.). It was identical with a sample from the amino-acid prepared by method (*a*) [Found: N, 8·0; loss at 100°/20 mm., 5·9. $C_{13}H_{16}O_3N_2Cl_2,H_2O$ requires N, 8·3; loss for $1H_2O$, 5·3. Found (on dried sample): C, 49·1; H, 5·15. $C_{13}H_{16}O_3N_2Cl_2$ requires C, 48·9; H, 5·1%].

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