Purines, Pyrimidines, and Imidazoles. Part XXXIX.[1] Formation of Some 5-Aminoimidazole-4-carboxylic Acid Derivatives from Ethyl α-Amino-α-cyanoacetate

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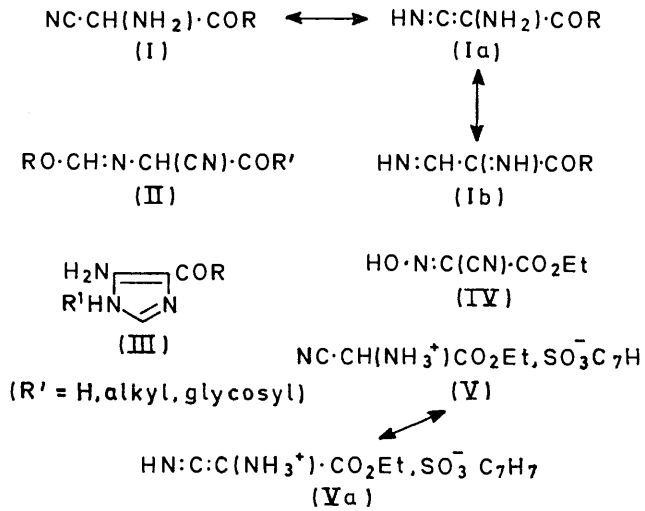
Dimerisation of ethyl α-amino-α-cyanoacetate has been found to yield diethyl 5-aminoimidazole-2,4-dicarboxylate. The amino-ester also reacts with triethyl orthoformate to produce, according to the conditions, ethyl *N*-(α-cyano-α-ethoxycarbonylmethyl)formimidate, ethyl 5-amino-1-(α-cyano-α-ethoxycarbonylmethyl)imidazole-4-carboxylate or diethyl 2-ethoxymethylidenamino-3*H*-imidazo[1,5-*a*]imidazole-3,7-dicarboxylate.

Mass spectral, n.m.r., i.r. and other analytical evidence is presented to support the structures assigned and the mechanisms of formation of the different compounds are discussed.

In recent years there has been considerable interest in the formation of purines and related, precursor 5-aminoimidazole-4-carboxylic acid derivatives from small molecules including ammonia, hydrogen cyanide, and cyanoacetylene.[2] These various types of reactions have been frequently forwarded as models for possible abiogenic synthesis of the nucleic acid bases on a primordial earth.[2] In earlier publications in this series we have described the use of substances such as ethyl aminocyanoacetate[3] (I; R = OEt) and related amide[4] or nitrile[5] derivatives for the preparation of a variety of 5-aminoimidazoles by condensation of the appropriate amino-acid derivative with ethyl formimidate hydrochloride to produce a more complex formimidate such as the ester (II; R′ = OEt, R = Et), and further condensation of this type of compound with either ammonia or primary amines to afford directly the appropriate aminoimidazole derivative (III; R = OEt). The reactions have also been extended to include syntheses of 5-aminoimidazole nucleosides and nucleotides identical with or related to various intermediates in the biosynthesis *de novo* of purine nucleotides.[3,4,6]

The amine (I; R = OEt) may be obtained by reduction of the corresponding oximino-derivative (IV) with aluminium amalgam and water,[3] but normally deteriorates during storage, and has usually to be prepared afresh for each experiment. However, we now find that it may be readily obtained as the stable crystalline toluene-*p*-sulphonate monohydrate (V) which is readily stored. In addition to the strong band at 1760 cm^{-1} (C=O), the i.r. spectrum of (I; R = OEt) shows only a weak peak at 2260 cm^{-1} (C≡N) and a fairly broad strong peak at 1610 cm^{-1} (C=N). This suggests that at room temperature the amine is a tautomeric mixture of forms such as (I), (Ia), and (Ib) (R = OEt). The i.r. spectrum of the amine toluene-*p*-sulphonate (V), however, shows a strong band at 1770 cm^{-1} (C=O) and a broad, weak peak at 2100 cm^{-1} (N=C=C). This suggests that the ketenimine structure (Va) more accurately represents the structure of the salt. The corresponding methyl α-amino-α-cyanoacetate toluene-*p*-sulphonate was obtained as anhydrous crystals. The i.r. spectrum shows a strong band at 1770 cm^{-1} (C=O), and the broad, weak peak at 2030 cm^{-1} (N=C=C).



The use of ethyl formimidate hydrochloride or the conversion of (I; R = OEt) into (II; R′ = OEt or NH_2, R = Et) is to some extent inconvenient since its preparation involves the use of hydrogen cyanide. We have consequently investigated the alternative use of triethyl orthoformate in this reaction and this has resulted in the isolation of some new aminoimidazoles and a new sequence of reactions which we now report.

When the amine (I; R = OEt) was set aside at 35° for a few days a crystalline compound slowly separated. We assign the aminoimidazole structure (VI) to this compound. Evidence in support of the structure includes elemental analysis, mass spectrum (M^+ = 227), ready formation of a coloured dyestuff in the Bratton–Marshall[7] assay and ready solubility of the compound

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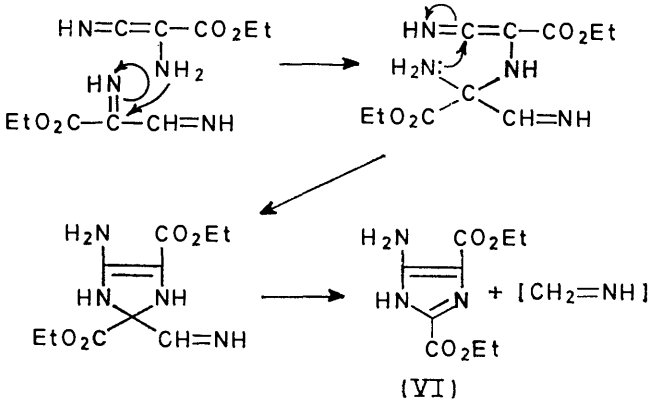
in alkali which suggested the presence of the unsubstituted NH group. In addition the i.r. spectrum shows strong bands at 1720 cm^{-1} (CO_2Et) and the n.m.r. spectrum recorded in the solvent $(CD_3)_2SO/D_2O$ showed the presence of methyl and methylene protons only (see Experimental section), indicating substitution at the 2-position of the imidazole ring. The compound may be envisaged to form by the following route.

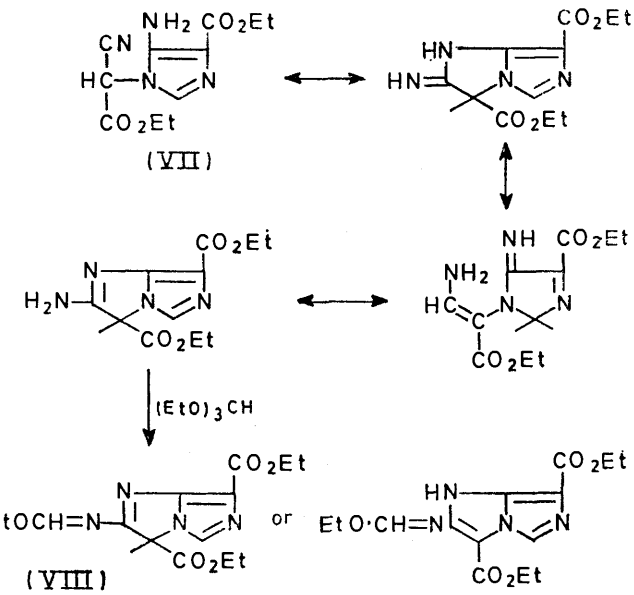


(VI)

When the amine (I; R = OEt) and a slight excess (1·1 mol) of triethyl orthoformate were heated together in acetonitrile an excellent yield of the formimidate (II; R′ = OEt, R = Et) was obtained. The identity of this formimidate was confirmed by its ability to condense and cyclise with cyclohexylamine to give ethyl 5-amino-1-cyclohexylimidazole-4-carboxylate (III; R′ = C_6H_{11}, R = OEt) which was identical (m.p. and mixed m.p., i.r., u.v.) with a sample earlier prepared by Shaw *et al.*[3]

By a similar method reaction of α-amino-α-cyanoacetamide (I; R = NH_2) with triethyl orthoformate gave the corresponding formimidate (II; R′ = NH_2, R = Et) as a crystalline product in 65% yield which was shown to be identical with material prepared using ethyl formimidate hydrochloride (m.p., mixed m.p., i.r.). As an extension to this synthesis, other trialkyl orthoformates were investigated. Spectrophotometric assay suggested that the action of trimethyl orthoformate on α-amino-α-cyanoacetamide produced the corresponding formimidate (II; R′ = NH_2, R = Me) in poor yield (≤30%). However, similar reaction of tri-isobutyl orthoformate with the aminoamide gave the corresponding formimidate (II; R′ = NH_2, R = Bu) in good yield 63%). The identity of this formimidate was confirmed by elemental analysis, mass spectrum [no molecular ion, but a peak at $M - 43$ $(M - CONH)^+$, characteristic of these formimidates], and its ability to condense and cyclise with cyclohexylamine to give 5-amino-1-cyclohexylimidazole-4-carboxamide (III; R′ = C_6H_{11}R = NH_2) in good yield which was shown to be identical (m.p., mixed m.p., i.r., u.v.) with material described earlier.[4]

When the amine (I; R = OEt) was heated with a half equivalent of triethyl orthoformate, an excellent yield of the aminoimidazole (VII) was obtained. Evidence for the structure came from elemental analysis, mass spectrum ($M^+ = 266$), the presence of a band at 2150 cm^{-1} (C≡N) in the i.r. spectrum, a positive Bratton–Marshall assay and its identity with material obtained as a by-product from the reaction of ethyl formimidate and (I; R = OEt) and a ribosylamine derivative and reported earlier.[3] However, the n.m.r. spectrum in $(CD_3)_2SO$ failed to show the characteristic singlet proton at $\tau \doteq 2{\cdot}5$ corresponding to 2-H, but as well as the methyl and methylene peaks of the ethyl groups, showed two broad low field bands, both of which disappear in a $(CD_3)_2SO/D_2O$ solvent (see Experimental section). This suggests that the proton at C-2 takes part in a tautomeric system, *e.g.*



(VII) (VIII)

Further evidence for tautomeric structures of these types comes from the observation that diazotisation of the compound is difficult and requires prior warming with dilute aqueous acid before it will produce, after diazotisation and coupling, a maximum colour yield. In addition, when the amine (I) was heated with a 10% molar excess of triethyl orthoformate a high yield of the crystalline imidazole (VIII) was obtained. The evidence for the assigned structure came from elemental analysis, mass spectrum ($M^+ = 322$), absence of a Bratton–Marshall test which however became positive if the compound was first heated for 5 min with dilute aqueous acid. The n.m.r. spectrum of (VIII) in $CDCl_3$ showed the presence of three sharp low-field singlets (see Experimental section) which suggest that little or no tautomerism is taking place, and is in full accord with the structure. The compound is readily seen to be derived from one of the tautomeric forms of (VII) and triethyl orthoformate, and the u.v. absorption spectra of (VII) and (VIII) are very similar, further confirming the relationship which exists between the compounds.

EXPERIMENTAL

Evaporations were carried out with a Büchi rotary evaporator, under water pump vacuum with a flask tem-

perature ⩽40 °C unless otherwise stated. U.v. absorption spectra were measured with a Unicam SP800 spectrophotometer, i.r. spectra with a Perkin-Elmer 157 spectrophotometer, n.m.r. spectra with a Varian A60 spectrometer using Me_4Si as internal standard, and mass spectra with an A.E.I. MS 902 spectrometer.

Ethyl Aminocyanoacetate.—To a suspension of dry (by washing with methanol and dry ether) aluminium amalgam (from 25 g aluminium foil cut into pieces approximately 2 × 2 cm) in dry ether (1 l) was added a solution of ethyl α-hydroxyimino-α-cyanoacetate (110 g) in dry ether (500 ml). Water (50 ml) was then added slowly to the stirred mixture to maintain reflux conditions. After *ca.* 30 min addition was complete and the mixture was then stirred for a further 30 min, cooled, and filtered through a pad of Supercel. The residue was extracted with ether (3 × 100 ml). To the combined filtrate and extracts was added a solution of toluene-*p*-sulphonic acid monohydrate (120 g) in ether (600 ml) and ethanol (100 ml). The *amine toluene-p-sulphonate monohydrate* (80 g) soon separated as plates which were washed with ether and dried. The compound was pure enough for most purposes and had m.p. 115—117° (Found: C, 45·4; H, 5·5; N, 8·95. $C_{12}H_{16}N_2O_5S,H_2O$ requires C, 45·3; H, 5·6; N, 8·8%). The corresponding methyl ester was obtained as anhydrous plates, m.p. 173—175° (decomp.) (Found: C, 46·1; H, 4·75; N, 9·8. $C_{11}H_{14}N_2O_5S$ requires C, 46·1; H, 4·9; N, 9·8%). The amine toluene-*p*-sulphonate (6 g) was covered with 2N-sodium hydroxide (10 ml) and the solution was extracted with chloroform (3 × 30 ml). The dried (Na_2SO_4) extracts were evaporated *in vacuo* using an oil pump and at a temperature not exceeding 30° to give the amino ester (1·8 g) as a pale yellow oil which was used as such in the subsequent reactions.

Diethyl 5-Aminoimidazole-2,4-dicarboxylate.— Ethyl aminocyanoacetate (1·8 g) was set aside at 35° for 7 days when it became brown and deposited a crystalline precipitate. The *di-ester* (0·3 g) recrystallised from aqueous dimethyl sulphoxide as cream coloured prisms, m.p. 204—205° (Found: C, 47·5; H, 5·8; N, 18·55. $C_9H_{13}N_3O_4$ requires C, 47·55; H, 5·75; N, 18·5%). The compound was insoluble in water but readily dissolved in aqueous alkali. The compound had $\lambda_{max.}$ 225 (ε 4600), 251 (8770), and 309 nm (13,060) in methanol. In the Bratton–Marshall assay it gave a coloured dyestuff with $\lambda_{max.}$ 513 nm. The n.m.r. spectrum in the solvent $(CD_3)_2SO$–D_2O, 4 : 1 showed signals at τ 5·75 (m, CH_2) and 8·72 (m, CH_3) only.

Ethyl N-(α-Cyano-α-ethoxycarbonylmethyl)formimidate.—A solution of ethyl α-amino-α-cyanoacetate (1·8 g) in acetonitrile (35 ml) and triethyl orthoformate (2·3 g, 10% molar excess) was boiled under reflux for 45 min. Preliminary experiments had indicated (from subsequent conversion to aminoimidazoles) that the reaction had proceeded to *ca.* 80% completion under these conditions. The *formimidate* may be isolated as a reactive oil by evaporation of the solvent but is most conveniently used *in situ*. Cyclohexylamine (1·1 g) was added to the foregoing solution which was then boiled under reflux for 15 min and then evaporated to about half volume when a crystalline precipitate was obtained. The mixture was set aside at 0° for a short time and the solid was collected. Ethyl 5-amino-1-cyclohexylimidazole-4-carboxylate (1·3 g) recrystallised from ethanol as plates, m.p. 225—226 °C.

Ethyl N-(Carbamoylcyanomethyl)formimidate.—A solution of α-amino-α-cyanoacetamide (2·2 g) in acetonitrile (35 ml) and triethyl orthoformate (3·3 g, 10% molar excess) was boiled under reflux for 45 min. The solution was evaporated to dryness. The *formimidate* (2·0 g) recrystallised, from ethyl acetate. It had m.p. 86—88 °C; Shaw *et al.*[4] quotes 86—87 °C. Cyclohexylamine (1 g) and the formimidate (1·7 g) were refluxed in acetonitrile (10 ml) for 15 min. Evaporation and crystallisation from ethanol yielded 5-amino-1-cyclohexylimidazole-4-carboxamide (1·2 g) as colourless rods, m.p. 209 °C. *Isobutyl N-(carbamoylcyanomethyl)formimidate* was similarly obtained in good yield, by refluxing a mixture of α-amino-α-cyanoacetamide and triisobutyl orthoformate in acetonitrile. The *formimidate* recrystallised from ethyl acetate as colourless rods, m.p. 102—104 °C (Found: C, 52·4; H, 7·1; N, 22·9. $C_8H_{13}N_3O_2$ requires C, 52·2; H, 7·05; N, 23·0%).

Ethyl 5-Amino-1-(α-cyano-α-ethoxycarbonylmethyl)imidazole-4-carboxylate.—A solution of ethyl α-amino-α-cyanoacetate (1·8 g) in acetonitrile (20 ml) and triethyl orthoformate (1·0 g) was boiled under reflux for 1 h. The solution was evaporated to leave a solid residue. The aminoimidazole (1·7 g) recrystallised from acetonitrile as needles, m.p. 181—183° (Found: C, 49·75; H, 5·4; N, 21·15. Calc. for $C_{11}H_{14}N_4O_4$: C, 49·6; H, 5·3; N, 21·05%). Shaw *et al.*[3] give m.p. 175°. The compound had $\lambda_{max.}$ 237 (ε 19,700) and 270 nm (14,700) in methanol. The compound gave a coloured dyestuff in the Bratton–Marshall assay, $\lambda_{max.}$ 510 nm, but the colour yield was much increased if the product was first warmed for 1 min with dilute aqueous hydrochloric acid before diazotisation. The n.m.r. spectra in $(CD_3)_2SO$ showed signals at τ 5·80 (m, 2 × CH_2), 8·80 (m, 2 × CH_3), 1·98br (1H), and 3·62br (3H). The latter two signals are due to the four resonating protons (C–H, NH_2, and 2-H) and disappear on addition of 25% of D_2O to the $(CD_3)_2SO$.

Diethyl 2-Ethoxymethylidenamino-3H-imidazo[1,5-a]imidazole-3,7-dicarboxylate (VIII).—A mixture of ethyl α-amino-α-cyanoacetate (1·4 g) and triethyl orthoformate (1·8 g) was heated at 85° for 45 min. The cooled mixture soon crystallised and the solid was collected after trituration with cold ethyl acetate. The *imidazoimidazole* (1·3 g) recrystallised from acetonitrile as prisms, m.p. 183—185° (Found: C, 52·1; H, 5·7; N, 17·65. $C_{14}H_{18}N_4O_5$ requires C, 52·15; H, 5·65; N, 17·4%). The compound had $\lambda_{max.}$ 234 (ε 21,300) and 277 nm (16,100). The substance gave a Bratton–Marshall test but only after it had first been heated for 5 min with dilute aqueous hydrochloric acid. The coloured product had $\lambda_{max.}$ 513 nm. The n.m.r. spectra in $CDCl_3$ showed signals at τ 5·72 (m, 3 × CH_2), 8·70 (m, 3 × CH_3), and three singlet protons at 1·18, 2·40, and 3·65.

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