

Formation of Monomeric Alkyl Cyanates by the Decomposition of 5-Alkoxy-1,2,3,4-thiatriazoles

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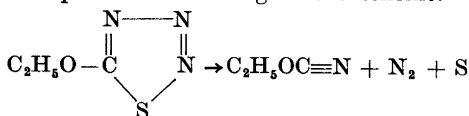
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It has generally been taken for granted that simple alkyl or aryl cyanates, RO-CN, cannot be isolated. Mulder,¹ Ponomareff² and others who investigated the reaction between cyanogen bromide and sodium ethoxide were only able to isolate the trimeric product, triethyl cyanurate, together with diethyl iminocarbonate. Houben tried to prepare alkyl cyanates by elimination of water from hydroximic esters,³ or by elimination of alcohol from diethyl iminocarbonate⁴ but obtained only esters of cyanuric or isocyanuric acid.

Recently Stroh and Gerber⁵ have succeeded in preparing some cyanic esters of phenols in which both *ortho*-positions are substituted with *tert*-butyl groups. These esters are quite stable. They may be distilled without polymerisation or isomerisation and are hydrolysed only on heating with sodium hydroxide at 180°C. However, no general conclusions can be drawn from the existence of such sterically hindered compounds.

We now report on the formation of alkyl cyanates by the spontaneous decomposition of 5-alkoxy-1,2,3,4-thiatriazoles. Although the alkyl cyanates are metastable and may undergo exothermic isomerisation and polymerisation they can be kept for some time in the absence of catalysts so that their physical and chemical properties may be investigated.

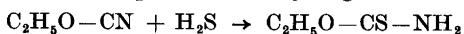
As mentioned in the preceding paper⁶ 5-ethoxy-1,2,3,4-thiatriazole decomposes at room temperature with the formation of sulfur and nitrogen. When the decomposition takes place in ether solution at controlled temperature it is possible to isolate ethyl cyanate as a pure compound in almost quantitative yield so that the decomposition proceeds according to the scheme:



Under less well defined conditions triethyl isocyanurate is formed. Thus from 5-ethoxy-1,2,3,4-thiatriazole which had been kept for 48 h at room temperature it was possible to isolate triethyl isocyanurate by extraction with boiling water in about 25% yield (identity with an authentic sample of triethyl isocyanurate proved by m.p. and infrared spectrum).

After the evaporation of the ether, ethyl cyanate was isolated as a colourless liquid. Its vapours are lachrymatory but the smell is distinctly different from that of ethyl isocyanate and it does not chemically behave as ethyl isocyanate. By the aid of gas chromatography it was shown that the crude liquid immediately after preparation consists of four components, a main component amounting to about 97%, a low boiling component in the amount of 2% and less than 1% of two other low boiling compounds. By addition of ethyl isocyanate the peak corresponding to the 2% constituent increased. The identity of this component with ethyl isocyanate was also shown by analysis and IR spectrum of an isolated sample.

At the column temperature used, 60°C, the gas chromatographic analysis does not exclude the possibility that a trimeric compound might be present. However, determination of the molecular weight in connection with elementary analyses shows that the main component is an isomer of ethyl isocyanate. It therefore can hardly be anything but ethyl cyanate. This conclusion is substantiated by the isolation of O-ethyl thiocarbamate in fairly good yield as the reaction product of this compound with hydrogen sulfide:



The infrared spectrum of a sample purified by gas chromatography was substantially coincident with the infrared spectrum of the unpurified product, thus excluding the possibility that the main component should have been formed during the gas chromatographic procedure. In later experiments ethyl cyanate was prepared with as little as 0.5% contamination of ethyl isocyanate.

It has not been possible to determine the boiling point of ethyl cyanate because on heating it is transformed into ethyl isocyanate and triethyl isocyanurate. It has, however, been possible to distil it in a vacuum with 85% recovery. This distillation is useful to purify ethyl cyanate from higher boiling compounds but no separa-

tion from the lower boiling impurities is attained.

At room temperature ethyl cyanate is rather rapidly transformed into ethyl isocyanate. After 26 h at room temperature a gas chromatogram showed that the lower boiling part now consisted of 14 % ethyl isocyanate and 86 % ethyl cyanate. After 44 h all the cyanate had disappeared and the liquid partly solidified to a mixture of ethyl isocyanate and triethyl isocyanurate (proved by IR spectroscopy). Preliminary experiments suggest that the isomerisation is autocatalytic. At the beginning the isomerisation proceeds very slowly but after some time the rate increases rapidly.

The formation of triethyl isocyanurate is unexpected since the reaction between cyanogen bromide and sodium ethoxide gives triethyl cyanurate, probably with ethyl cyanate as an intermediate. It seems possible that the direct trimerisation is caused by base catalysis. This needs further investigation, but we have found that addition of a few drops of pyridine to ethyl cyanate results in a violent reaction. In one experiment the content was thrown out of the flask after a few seconds.

In the absence of catalysts, however, ethyl cyanate is slowly transformed into ethyl isocyanate and this in turns is partly trimerised to triethyl isocyanurate. At -15°C there is no apparent isomerisation even after a week. Heating of the ethyl cyanate to boiling for only 20–30 sec results, however, in complete transformation into the isocyanate with considerable evolution of heat.

The ethyl isocyanate was identified by the formation of N-ethyl-N'-phenylurea by the reaction with aniline and the formation of ethylurea by the reaction with ammonia. Ethyl cyanate on the other hand does not form N-ethyl-N'-phenylurea with aniline but only phenylurea and the same was found to be the case with the higher homologues propyl and butyl cyanate. How this product is formed is not yet quite clear. Although we have tried to exclude water rigorously it seems possible that it is formed by hydrolysis of an O-alkyl-N-phenylisourea, RO-C(=NH)NHPH , as the primary product.

The chemical reactions of the alkyl cyanates are complicated by the isomerisation and polymerisation to isocyanates and isocyanurates. The above mentioned reaction between ethyl cyanate and hydrogen sulfide shows, however, that it is possible

to isolate compounds directly derived from the cyanate. Further experiments in this direction are in progress.

Preliminary experiments have shown that the homologues of 5-ethoxy-1,2,3,4-thiazotriazole similarly decompose to form alkyl cyanates. Further details will be given in a forthcoming paper, in which also the infrared spectra of the alkyl cyanates will be discussed.

Experimental. Ethyl cyanate. 5-Ethoxy-1,2,3,4-thiazotriazole (1.000 g, 0.00763 mole), dried at 1 mm Hg for 2–3 min at room temperature (not exceeding 25°C) was dissolved in 5 ml of dry ether. The solution was placed in a small, closed flask provided with a valve to equalize the pressure and kept in a thermostat at 20°C for 16 h. During the reaction nitrogen was evolved and sulfur precipitated. The ethereal solution was decanted from the sulfur and the solvent removed by evaporation *in vacuo*. When the pressure had diminished to 12 mm Hg the flask was placed in a bath at 25°C for 2–3 min in order to remove the last traces of ether. Yield 0.465 g = 84 % of a colourless liquid. This was found by gas chromatography to contain 97 % ethyl cyanate, 2 % ethyl isocyanate and less than 1 % of two other compounds. (Found: C 49.62; H 7.15; N 19.72. Calc. for $\text{C}_3\text{H}_5\text{NO}$: C 50.69; H 7.09; N 19.71. Mol.wt. 74.3 ± 3 (calc. 71), determined by measuring the heat of evaporation of a chloroform solution). The precipitated sulfur was purified by washing with boiling water and dried. Yield 0.247 g = 98 % of the calculated amount (m.p. 115°C).

In later experiments it was possible to obtain yields as high as 91 % of ethyl cyanate containing less than 0.5 % of ethyl isocyanate.

As some heat is evolved during the reaction the preparation should not be carried out with larger than 4 g portions. It proved necessary to use rigorously purified 5-ethoxy-1,2,3,4-thiazotriazole for the preparation. In one experiment with a less pure product the ethyl cyanate isomerised with vigorous boiling a few seconds after the removal of the ether.

Ethyl cyanate (4 g) was distilled at 12 mm Hg, the distillation flask being kept in a bath at 30°C and the receiver cooled in a dry ice-acetone mixture. Within 10 min 3.4 g of ethyl cyanate was collected as a colourless, mobile liquid. Refraction index $n_D^{25} = 1.3788$ (ethyl isocyanate: $n_D^{25} = 1.3772$). (Found: C 50.85; H 7.18; Mol.wt. 72.7 ± 2 . Calc. for $\text{C}_3\text{H}_5\text{NO}$: C 50.69; H 7.09; M = 71). The proportion between ethyl cyanate and ethyl isocyanate was found, by gas chromatography, to be unchanged 98:2.

The gas chromatographic analyses were carried out on a two meter O column (Model Perkin Elmer 116). Column temp.: 60°C; carrier gas: He; flow rate of the carrier gas: 120 ml/min. Retention times: 2.8 min for ethyl isocyanate and 6.8 min for ethyl cyanate.

Reaction of ethyl cyanate with hydrogen sulfide. Hydrogen sulfide was bubbled through a solution of 1 g of ethyl cyanate in 5 ml of ether, cooled in an ice bath, for 4 h. After addition of 10 ml of ether the solution was filtered, washed successively with 2 ml portions of 2 N hydrochloric acid and water, dried over sodium sulfate and evaporated to dryness. The semi-solid residue (0.99 g) was crystallised from 20 ml of heptane and yielded 0.61 g (39 %) of O-ethyl thiocarbamate (xanthogenamide) with m.p. 40–41°C (lit. 40–41°C). (Found: N 13.25. Calc. for C₃H₇NOS: N 13.32). The infrared spectrum was identical in all details with the infrared spectrum of an authentic sample of O-ethyl thiocarbamate.

Reaction of ethyl cyanate with aniline. (a) 5-Ethoxythiazole (1 g) was dissolved in dry ether (5 ml) and 1 ml of aniline was added. An exothermic reaction took place and the solution had to be cooled to keep the temperature near 20°C. After 24 h the precipitate was isolated by centrifugation and washed with ether and with carbon disulfide (to remove sulfur). The infrared spectrum of the substance was identical with the infrared spectrum of phenylurea. M.p. after recrystallisation from ethanol 146–147°C (lit. 147°C). Yield 0.267 g = 26 %.

(b) Aniline (0.5 g) was added to ethyl cyanate (0.5 g) which had been kept for 48 h at room temperature. A reaction took place with heat evolution. After 24 h the reaction mixture was extracted with boiling hexane and the residue recrystallised from 20 % ethanol. Yield 0.25 g of N-ethyl-N'-phenylurea with m.p. 98–98.5°C (lit. 99°C), corresponding to 22 % of ethyl isocyanate in the starting material. The infrared spectrum of the product was identical with the infrared spectrum of an authentic sample of N-ethyl-N'-phenylurea.

Addition of aqueous ammonia to the same sample of ethyl cyanate yielded ethylurea, whose identity was proved by m.p. (91°C) and infrared spectrum.

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Formation of Serine and Threonine Dehydratases during the Growth Cycle of *Escherichia coli*. Evidence of two Different Enzymes

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Previous investigations carried out in this laboratory have shown that the total activities of tryptophan synthase¹ and certain transaminases² decrease sharply towards the end of the active cell growth phase to nearly the levels at the beginning of the growth cycle. This decrease in total activity has been demonstrated in many earlier papers that were briefly reviewed in one of ours.³

We have now studied the formation of serine and threonine dehydratases (EC 4.2.1.13 and EC 4.2.1.16) during the active growth phases of *Escherichia coli*. The effect of the pH of the growth medium on the formation of the enzymes was also estimated. Evidence for the existence of two separate enzymes is presented below.

Experimental. The activities of the serine and threonine dehydratases were determined by the hydrazone method as modified by Walker.³ The organism and its cultivation have been described earlier.^{4,5} The frozen and thawed cells were used as enzyme sources.¹ The amino acid medium (abbreviated AM) contained L-amino acids in the same ratios as these are present in the protein of *E. coli*.⁶ This AM medium is the same as the glucose-mineral salt medium⁵ except that the ammonium salts are replaced by amino acids (5.27 g per litre of growth medium).

Results and discussion. Fig. 1 shows the formation of threonine dehydratase by *E. coli* cells in the AM and MM (glucose-mineral salt medium) media. It will be