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Preparation of carbon-14 labelled guazatine

Harry R. Hudson*, Fatima Ismail and Lubomira Powroznyk
Division of Chemistry, School of Biological and Applied Sciences, University of North London, Holloway Road, London N7 8DB, UK

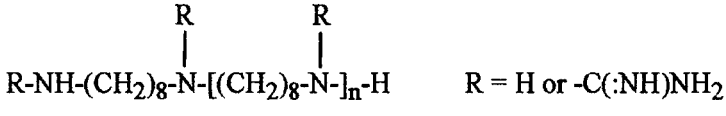
Summary

A method is described for small-scale laboratory simulation of the industrial process for the manufacture of guazatine, an agrochemical fungicide comprising a mixture of guanidated di-, tri-, and poly-amines derived from octamethylenediamine. The product has been prepared with carbon-14 labelling of the terminal positions of the octamethylene chains. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: carbon-14; agrochemical; fungicide; guanidine; polyamine

Introduction

Guazatine is a guanidine fungicide[1] which finds application in agriculture for the control *inter alia* of seed-borne diseases of cereal crops.[2,3] Having only low mammalian toxicity it is a useful replacement for the highly toxic organomercurial seed dressings.[4] The commercial product,[5] marketed as the acetate, is defined[3] as a mixture of guanidine derivatives (**1**) in which 77–83% of the amino groups in a mixture of 1,8-diamino-octane, 1,17-diamino-9-aza-heptadecane, and other higher polyamine oligomers derived from 1,8-diamino-octane, have been converted to guanidino groups:



(1) n = 0, 1, 2, 3 etc.

*Correspondence to: H. R. Hudson, 28 Green Dragon Lane, Winchmore Hill, London N21 2LD, UK.

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In an earlier paper we reported the synthesis of 1,17-bis[^{14}C]-guanidino-9-aza-heptadecane triacetate (**2**), an active component of guazatine,[†] in which the two guanidino groups were labelled with carbon-14:[6]

$$H_2N(H_2N{:})^{14}C\text{-}NH\text{-}(CH_2)_8\text{-}NH_2^{+}\text{-}(CH_2)_8\text{-}NH\text{-}^{14}C(:NH_2^{+})NH_2 \quad 3OAc^-$$

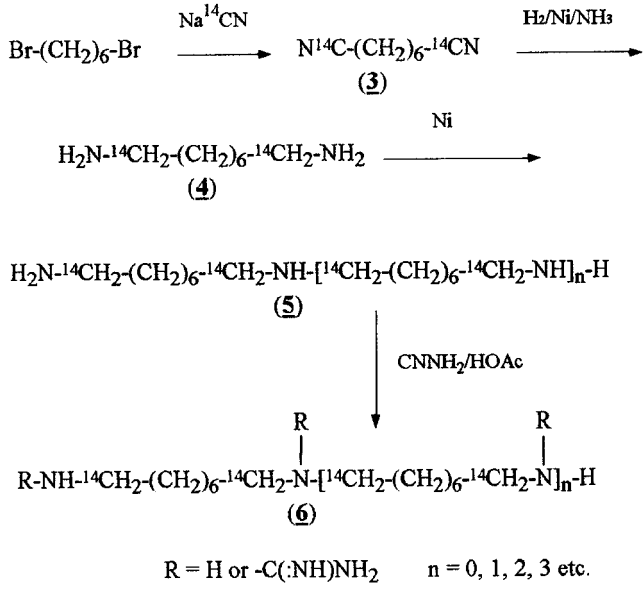
(2)

We now describe a procedure for small-scale laboratory simulation of the commercial process in order to obtain a mixture of guanidines (**1**) whose composition is within the defined product specification limits and with radio-labelling of the terminal carbon atoms in the octamethylene chains.

Discussion

The overall procedure for the synthesis is shown in Scheme 1. 1,6-Di-[^{14}C]-cyanohexane (**3**) was first prepared by the interaction of 1,6-dibromohexane with a 10% excess of carbon-14-labelled sodium cyanide in dimethylsulphoxide[7,8] and then converted to the diamine (**4**) by hydrogenation with a Raney nickel catalyst in the presence of ammonia. The next stage, known as the reforming reaction, involves nickel catalyzed elimination of ammonia to form the triamine (**5**, $n = 1$) and other higher oligomers (**5**, $n = 2$, 3, etc.). Industrially, this stage is carried out under hydrogen pressure in order to control the rate of reaction and also to ensure the complete saturation of imine intermediates which may be formed. On the gram scale in the laboratory, however, we found it more convenient to carry out the reforming reaction under nitrogen at atmospheric pressure and to rely on hydrogen adsorbed on the surface of the nickel catalyst for saturation of imine intermediates. Progress of the reforming reaction was monitored conveniently by periodic removal of molten samples using a preheated Pasteur pipette and glc analysis. The reaction was continued until the diamine/triamine ratio corresponded with that of a

[†]The name guazatine was originally used to describe the single compound 1,17-bisguanidino-9-aza-heptadecane (cf. ref. 2) rather than the mixture of guanidines (**1**) which is produced commercially, and to which the name guazatine now applies.



Scheme 1.

sample of industrially produced 'technical triamine'. Conversion of amino groups to guanidine involved a standard procedure,[1,9] consisting of reaction with cyanamide in aqueous acetic acid. The final product (**6**, acetate), a viscous pale brown liquid, was analyzed for guanidino groups by potentiometric titration against perchloric acid in acetic anhydride/acetic acid (90 : 10)[10] and shown to contain 78% w/w active ingredient, calculated as 'guazatine' (9-aza-1,17-diguanidinoheptadecane) triacetate. TLC characteristics[11] and the Carbon-13 NMR spectrum of the product closely matched those of commercial guazatine and the measured radioactivity (7.365 μCi mg^{-1} of a.i.) agreed well with the predicted value (7.40 μCi mg^{-1} of a.i.) based on the activity of the sodium cyanide used as starting material.

Experimental

*Preparation of 1,6-di-([^{14}C]-cyano)hexane (**3**)*

Sodium carbon-14 cyanide (4.77 g, 0.0973 mol, 100 mCi) (supplied by Amersham International plc) was stirred with dimethylsulfoxide (80 ml) at 75°C and 1,6-dibromohexane (10.7 g, 0.04385 mol) was added dropwise (50 min) to give a clear solution which then was heated

further at 80–90°C (0.5 h). The mixture was cooled, diluted with ether, and filtered to remove sodium bromide and excess of sodium cyanide. Ether was removed under reduced pressure and the residue was distilled to give DMSO followed by 1,6-di-([^{14}C]-cyano)hexane (5.69 g, 0.04184 mol, 86.0 mCi), b.p. 120°C at 0.5 mm Hg, showing a single peak on glc.

*Preparation of 1,8-diamino-1,8-[^{14}C]$_2$-octane (**4**)*

The dinitrile (5.69 g, 0.04184 mol) was dissolved in absolute ethanol (170 ml) and placed in a 1 l Baskerville and Lindsay stainless-steel stirred autoclave together with Raney nickel (5 ml of wet solid after ethanol washing). The autoclave contents were cooled to 0°C, liquid ammonia (25 ml) was added, and the system was then sealed, purged with hydrogen and pressurized to 36 atm at room temperature. The temperature was raised to 110°C (41 atm) for 4 h with stirring. Stirring was continued while the contents cooled overnight. After separation of the clear solution from the nickel, which was washed with more ethanol, the solvent was removed under reduced pressure with gentle heating to a final maximum of 50°C to leave the carbon-14 labelled diamine (4.66 g, 77.3% yield, 66.5 mCi), showing a single peak on glc.[‡]

Reforming reaction

Raney nickel (1.0 ml of wet solid after washing with ethanol, followed by anhydrous ether) was added to the diamine (4.35 g), in a three-necked 50 ml flask under dry nitrogen. The flask was fitted with a reflux condenser and gas inlet tube and a magnetic stirrer was introduced. Ether was removed in a stream of nitrogen and the mixture was heated in an oil bath until molten (ca. 50°C). The temperature was then raised progressively, with vigorous stirring, during four short periods of time as indicated below. After each period of heating the mixture was cooled rapidly to below 100°C and, as the nickel settled, a small sample (ca. 0.06–0.09 g) was removed by pipette, weighed, dissolved in ethanol (4 or 5 ml) and analyzed by glc.

[‡]Radioactive contamination was found to adhere tenaciously to the stainless-steel inner surface of the autoclave. Unless it is intended to reserve an autoclave spcifically for this and similar hydrogenations of radioactive material, it is recommended that a process of reduction that can be carried out at lower pressure in glass equipment should be used for this stage of the synthesis.

After 22 min at 60–140°C, 15 min at 110–150°C, 18 min at 130–156°C, and 10 min at 140–142°C, the glc trace of the carbon-14-labelled product was similar to that obtained for a sample of industrially produced 'technical triamine' at a similar concentration, showing the presence of diamine (**4**) (27.2%) and triamine (**5**) (44.1%).

A quantity of the reformed amine (0.367 g, 5.56 mCi) was removed directly from the upper layer of product and the remainder (1.337 g, 20.9 mCi) was separated from the nickel catalyst by ether extraction.

During the reforming process a sublimate of diamine (0.46 g, 6.58 mCi) collected on the upper cool parts of the reaction flask.

Guanidation

A mixture of the reformed amine (1.377 g, 14.5 meq), water (0.92 ml), and glacial acetic acid (0.687 g) was heated with stirring at 75–80°C, while cyanamide (0.64 g, 15.2 meq) in water (0.64 g) was added in several small portions over a period of 3 h. After one further hour at the same temperature the mixture was cooled and acetic acid (0.165 g) was added. The total final product thus obtained was a highly viscous pale brown liquid (3.41 g) containing 78% w/w active ingredient by titration, calculated as 'guazatine' (9-aza-1,17-diguanidinoheptadecane) triacetate; $\delta_C(D_2O)$ 183.8 (CO_2^-), 159.8 [$C(:NH_2)^+NH_2$], 158.7 [$C(:NH_2)^+NH_2$], 51.6, 50.3, 44.0, 42.4, 31.1, 31.0, 30.8, 29.7, 29.5, 28.6 (carbon chain), 26.5 ($\underline{C}H_3CO_2^-$) ppm; specific activity = 7.365 μCi mg^{-1} of active ingredient (calculated specific activity = 7.40 μCi mg^{-1} of active ingredient, based on the specific activity of the sodium cyanide used as starting material).

Analytical methods

Gas chromotography

Analyses were performed on a Pye isothermal instrument with flame ionization detector:

(a) Purity of the dinitrile was checked using a 2 m × 4 mm i.d. glass column packed with 10% Carbowax 20M on Chromosorb W-HP, 100–120 mesh, operating at 225°C, with an injection temperature of 250°C, detector temperature of 300°C and N_2 (carrier gas) pressure of 12 psi. Retention times: DMSO (2.0 min),

1,6-dibromohexane (3.5 min), 1-bromo-6-cyanohexane (6.9 min), 1,6-dicyanohexane (15.3 min).

(b) The monitoring of diamine and triamine content during the reforming reaction was based on a glc method described previously for the determination of 9-aza-1,17-diaminoheptadecane without derivatization.[12] Samples, dissolved in ethanol, were analyzed on a 2 m × 4 mm i.d. glass column packed with 5% Apiezon L on Chromosorb W-AW, 100–120 mesh, containing 6% sodium dimethylaminosuccinate and 1% NaOH. Injection and detector temperatures were set at 270 and 300°C, respectively. Initially, before quantitative determinations were made, 5 µl samples of an ethanolic solution containing 10–15 mg ml^{-1} of each amine were injected repeatedly until stable conditions were obtained. The column was maintained at 140°C for 3.6 min after injection, followed by ballistic temperature programming to 230°C. Under these conditions, retention times were: diamine (2.6 min), triamine (13.5 min). The method was shown to give a linear response in the concentration range under investigation. Analytical determinations were made by comparison of peak heights with those for standard solutions under identical conditions. The product was also compared with a sample of industrially produced 'technical triamine' to ensure similarity of composition.

Guanidine titration

Total guanidine content of the final product was determined by potentiometric titration against 0.1 M perchloric acid in acetic anhydride/acetic acid (90 : 10) as described previously for the analysis of dodecylguanidine.[10]

Nmr spectroscopy

Carbon-13 chemical shifts were determined in D_2O on a Bruker WP80 instrument operating at 20.12 MHz and with sodium trimethylsilylpropionate as internal standard.

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