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CONVERSION OF α[^{15}N]AMINO-2,6-DICHLOROBENZALDOXIME INTO [^{15}N]2,6-DICHLOROBENZONITRILE BY A SOIL CULTURE

B. V. MILBORROW* and P. A. HARTHOORN†

* Shell Research Limited, Milstead Laboratory, Sittingbourne Research Centre, Sittingbourne, Kent, ME9 8AG;
† Shell Research Limited, Woodstock Laboratory, Sittingbourne Research Centre
Sittingbourne, Kent, ME9 8AG, England

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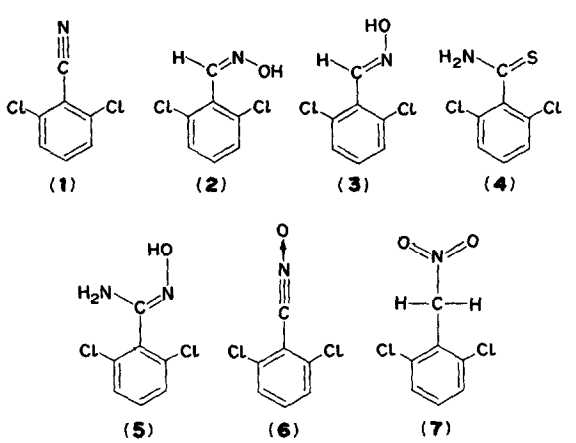
Key Word Index—α-Amino-2,6-dichlorobenzaldoxime; 2,6,-dichlorobenzonitrile; 2,6,-dichloro-α-nitrotoluene; [^{15}N]; wheat; microbiological conversion; phytotoxicity; MS; herbicide.

Abstract—α-[^{15}N]amino-2,6-dichlorobenzaldoxime was shown by mass spectrometry to be converted by a culture of soil microorganisms into [^{15}N]2,6-dichlorobenzonitrile. Thus the oxime nitrogen atom is lost and the α-amino nitrogen is retained as the nitrogen atom of the nitrile group. No 2,6-dichlorobenzonitrile could be detected in extracts of soil or wheat seedlings incubated with 2,6,-dichloro-α-nitrotoluene although this compound and 2,6-dichlorobenzonitrile produce identical toxicity symptoms in plants.

INTRODUCTION

The characteristic symptoms of phytotoxicity induced in higher plants by 2,6-dichlorobenzonitrile [1,2] are also produced by several classes of 2,6-dihalogenated, substituted-phenyl compounds [3]. 2,6-Dichlorobenzaldoxime (**2**, **3**) and some α-substituted derivatives, though inactive *per se*, are converted by micro-organisms into (**1**); higher plants cannot effect this transformation [4]. It was presumed that the nitrogen atom of 2,6-dichlorobenzaldoxime becomes the nitrile nitrogen. In 2,6-dichlorothiobenzamide (**4**) the amino group can become part of the nitrile group of (**1**) even under mild basic conditions [5]. With α-amino-2,6-dichlorobenzaldoxime (**5**) either nitrogen could *a priori* serve as the source of nitrile nitrogen. This was investigated by MS analysis of the 2,6-dichlorobenzonitrile produced by soil micro-organisms from α-[^{15}N]amino-2,6-dichlorobenzaldoxime.



RESULTS AND DISCUSSION

α-Amino-2,6-dichlorobenzaldoxime formed from [^{15}N]ammonia (^{15}N, 97 atoms %) and 2,6-dichlorobenzonitrile *N*-oxide (**6**) [6] contained the [^{15}N] in the α-amino group. The 2,6-dichlorobenzonitrile formed from this material on incubation with non-sterile soil also contained 97 atoms % [^{15}N], measured as the M^+ at *m/e* 172 (Cl = 35) and most of the fragment ions were one a.m.u. heavier than the corresponding peaks of [^{14}N] material (Fig. 1). Only the nitrogen of

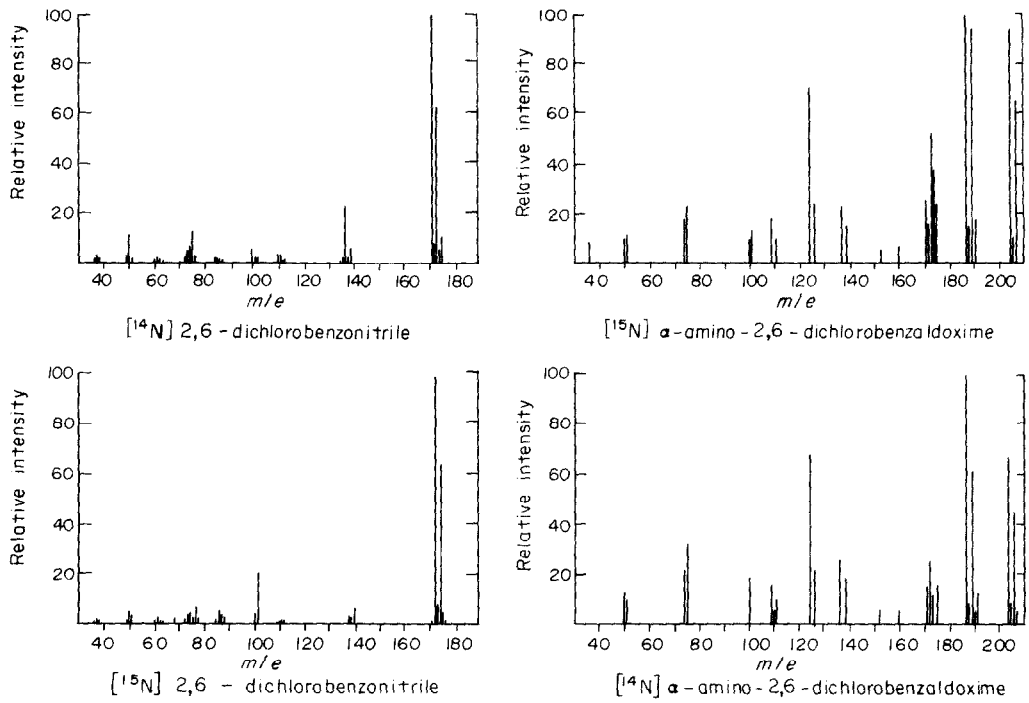


Fig. 1. Mass spectra of **3**, [^{15}N]α-amino **3**, **1** and [^{15}N] labelled **1**.

the α-amino group of (**5**), therefore, is converted into the nitrile group, and the nitrogen of the oximino group does not contribute.

Conversion of oximes into nitriles by biological systems is known [7–9], but this is believed to be the first example of the conversion of the α-substituent of an oxime into a nitrile group with the loss of the oxime group. The elements of hydroxylamine are lost from α-amino-2,6-dichlorobenzaldoxime but whether they are lost in that form is not known because no nitroprusside colour reaction [10] was given by a leachate of field soil in which **1** had been formed from **5**, nor was 4-hydroxybenzaldoxime formed (which could be detected by GLC) when 4-hydroxybenzaldehyde was added to the soil.

Earlier work [4] with the isomeric 2,6-dichlorobenzaldoximes has shown that there was no consistent difference between the phytotoxicity of the *anti* (*Z*) (**2**) and *syn* (*E*) (**3**) forms [11], nor was there any difference between the amount of 2,6-dichlorobenzonitrile formed from the two isomers. PMR analysis of the sample of **5** used showed that it consisted of one isomer only; indeed only one isomer of an α-aminobenzaldoxime has ever been reported, no matter how synthesised [12], and the configuration has never been defined. The broadening of the amino and hydroxyl hydrogen PMR signals of **5** and their having identical profiles when in dilute or concentrated solution suggest that there is interaction between these groups and so the compound is probably the *Zusammen* isomer shown. The closer similarity between the IR spectra of **2** and **5** compared with **3** and **5** also suggests that compound **5** has a *Z* configuration.

In contrast to (**5**), α-nitro-2,6-dichlorotoluene (**7**) [12] is toxic to higher plants, even under sterile conditions (Fig. 2), so microbiological conversion is not involved and the physiological effects are identical with those caused by **1**. It is possible that **7** is reduced to 2,6-dichlorobenzonitrile by the plants but three attempts to identify 2,6-dichlorobenzonitrile in samples of wheat seedlings (50 g) and field soil (100 g) that had both been treated with (**7**) (0·5 mg) gave no detectable 2,6-dichlorobenzonitrile by bioassay of eluates of the relevant areas of silica gel TLC plates or by GLC analysis of hexane extracts. The starting material was recovered from extracts of seedlings and soil.

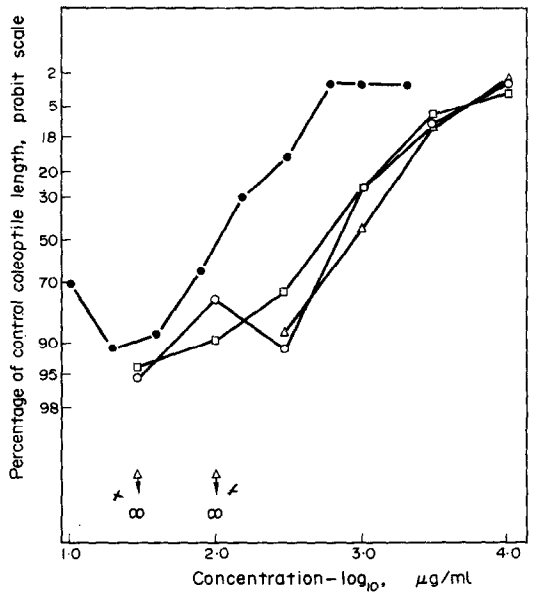


Fig. 2. Mean lengths of wheat coleoptiles measured after 4 days plotted as probit percentage of the control values against $\log_{10}$ dosage. There is no significant difference in the effectiveness of compound **6** between applications under sterile conditions (⊙); as before but with 1 g field soil added (△); or unsterilised VI ⊡. The dosage/response curves are parallel to that of compound **1** (●).

The sensitivity of detection of **1** by GLC limits the maximum observable conversion of **7** into **1** to less than 0·04% of the amount added and this proportion is far too small to account for the 10-fold difference in phytotoxicity that is observed between **1** and **7**.

If the plants converted **7** into **1** with facility then some indication of the presence of **1** could be expected by GLC. On the other hand if the formation of **1** were extremely slow then the higher concentrations of **7** would be expected to saturate the metabolism and stepwise increases in dosage at higher concentrations would fail to produce a corresponding increase in phytotoxicity. The log dose/probit response curves were linear and parallel to those of **1** (Fig. 2) so this explanation is discounted and it appears, therefore, that **7** is itself phytotoxic and is not converted into (**1**). Because **1** and **7**, and certain analogues af each, produce identical symptoms, the nitromethyl group, with 2,6-dihalophenyl substitution, probably affects the same metabolic site in higher plants as does a nitrile group when present on an aromatic nucleus with a favourable pattern of substituents.

EXPERIMENTAL

Preparation of compounds. α-[^{15}N]amino-2,6-dichlorobenzaldoxime (**2**) was prepared by treatment of the 2,6-dichlorobenzonitrile *N*-oxide[6] (**6**) (474 mg) in EtOH (10 ml) with [^{15}N]NH_3 (97 atoms % [^{15}N]), (110 mg). This gave **5** (518 mg). The hydrochloride of a sample of this material (249 mg) in H_2O (0·5 ml) was washed with Et_2O (3 × 0·5 ml) and then made alkaline with 3 N-NaOH. The ppt. of **3** was extracted with Et_2O and dried to give 163 mg product (mp 164–167°). It was recrystallised from C_6H_6 and compared with authentic **5** (mp 165–167°) by co-chromatography in system "A"; and by reverse-phase chromatography in system "B". Respective R_f's were for **1**, 0·30 and 0·40; for **5**, 0·10 and 0·90; for **7**, 0·25 and 0·70. In system "A" Si gel TLC plates were developed in toluene–hexane–Me_2CO (3:2:1). In system "B", a reverse phase chromatographic system, Me_2CO and H_2O satd liquid paraffin was supported on a Si gel stationary phase. The mobile phase consisted of H_2O saturated with liquid paraffin and Me_2CO (4:1). Mmps gave no depression.

MS. [^{15}N] and [^{14}N] labelled samples of **3** were subjected to high resolution MS by direct insertion on a probe. Analysis of the fragmentation pattern showed that the α-amino group of the former material contained 97 atoms % [^{15}N] and the oxime nitrogen was unlabelled because the loss of a 17 a.m.u. fragment (*m/e* 187) from the light material (*m/e* 204) was replaced by a loss of an 18 a.m.u. fragment (*m/e* 187) from the [^{15}N]labelled compound (*m/e* 205). The 2,6-dichlorobenzonitrile formed from the [^{15}N] labelled sample of **5** showed many MS peaks 1 a.m.u. greater than that of the unlabelled sample of **1**. The parent ions showed that 97% [^{15}N] was present (Fig. 1).

GLC procedures were carried out with a 200 × 6 mm glass column packed with OV 17, FID, and N_2 as carrier gas. At 110° the R_t were 3·3 min for **1** and 9·5 min for **7**.

PMR spectra (100 MHz) of **2** and **5** were obtained using TMS as an internal standard. (**3**) $CDCl_3$, 7·12δ–7·44δ *m* 3H (aromatic); 8·40δ *s* H (αH); 9·14δ *s* H (oxime OH), position concentration dependent, exchanges with D_2O. (**2**) $CDCl_3$, 7·12δ to 7·40δ in 3H (aromatic); 7·54δ H (αH); 7·66δ *s* H (oxime OH) position concentration dependent exchanges in D_2O. (**5**) $CDCl_3$ 7·8δ broad *s* 2H (α-amino); 7·15δ–7·44δ *m* 4H (3 aromatic and oxime OH). (III) DMSO-d_6 9·30δ broad *s* peak H (oxime OH) 7·25δ–7·56δ *m* 3H (aromatic) 5·85σ *s* 2H (α-amino); unchanged on dilution. *IR spectra* were obtained as mulls in liquid paraffin. (**2**) 3200 cm^{-1} *w*, 1575 *w*, 1560 *w*, 1420 *s*, 1310 *w*, 1095 *s*, 973 *m*, 856 *s*, 880 *s*, 783 *s*, 688 *m*. (**3**) 3200 *w*, 1570 *w*, 1550 *w*, 1535 *w*, 1290 *m*, 1180 *m*, 1085 *m*, 985 *s*, 918 *m*, 880 *w*, 782 *s*, 774 *w*, 719 *m*. (**5**) 3450 *w*, 3200 *m*, 1640 *s*, 1585 *s*, 1190 *s*, 1150 *w*, 1075 *s*, 926 *m*, 917 *m*, 822 *w*, 798 *w*, 785 *s*, 734 *m*. Mmps (corr.) **2**, 173–174 ; **3**, 148°; **5** 167°; **7** 40°.

Biological formation of 2,6-dichlorobenzonitrile. An aq soln of α-[^{15}N]amino-2,6-dichlorobenzaldoxime (1 ml of 5 mg/ml) was mixed with local brick-earth field soil (10 g) and incubated at 25°. The **1** formed was removed after 5 hr by steam distillation and purified by chromatography in the toluene: Me_2CO–hexane system. The crystalline product was identified by further TLC with an automatic standard in a number of solvents and by comparison of its UV, IR and MS. No conversion of **5** into **1** occurred in sterile soil. In a duplicate experiment, hexane extracts were monitored by GLC; after 2 days

incubation, 46% of the theoretical yield of **1** was observed, 68% after 5 days.

Extraction. The plant material or soil was ground with 10× its wt of anhydrous Na_2SO_4 and the mixture was extracted with a minimum vol of redistilled hexane.

Bioassay. About 20 wheat seedlings (1 g) (cv. Kloker) were sterilised by soaking in 30% satd NaOCl soln for 5 min, rinsed 2× in sterile H_2O and spread on a pad of 3 Whatman No. 1 filter paper discs (90 mm diam) in a sterile Petri dish. The pad was wetted with a soln (10 ml) of **7** added through a millipore filter. All manipulations were carried out in a laminar flow hood and no infection occurred. Coleoptile lengths were measured after 4 days growth at 22° in darkness. A similar batch was inoculated with damp field soil (2 g) and a further batch was left unsterilised.

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