triphosphate there was to bind amines, as it is believed to be in other amine-storage particles. The lack of effect on the content of adenosine triphosphate of doses of reserpine which effectively depleted the brain of 5-hydroxytryptamine and the lack of correlation in the regional distributions of adenosine triphosphate and pressor amines are further reasons for thinking that the 'stable' fraction of adenosine triphosphate studied is not mainly concerned with amine storage.

We are most grateful to Professor G. V. R. Born for helpful advice on the estimation of ATP.

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The Formation of 2,6-Dichlorobenzonitrile from Related Compounds in Plants

By B. V. MILBORROW

Woodstock Agricultural Research Centre, 'Shell' Research Ltd., Sittingbourne, Kent

(Received 7 November 1962)

The discovery of the herbicidal properties of 2,6-dichlorobenzonitrile (Barnsley & Rosher, 1961; Koopman & Daams, 1960) led to the synthesis of a number of related compounds, containing a 2,6-dihalogenated phenyl nucleus, which had the same highly specific toxicity symptoms (Milborrow, 1963) as 2,6-dichlorobenzonitrile when applied to plants. The similarity of the plant response obtained with 2,6-dichlorobenzonitrile, 2,6-dichlorobenzaldoxime and α -amino-2,6-dichlorobenzaldoxime suggested that the last two compounds were converted into 2,6-dichlorobenzonitrile. A similar reaction has been described by Ahmad & Spenser (1960), who suggested that the indolylacetonitrile precursor obtained by Housley & Bentley (1956) was the oxime of indolylpyruvic acid as synthetic indolylpyruvic acid oxime was readily converted into indolylacetonitrile under mild chemical conditions. The identity of the metabolite produced in cultures of *Pseudomonas putrefaciens* and field soil, to which the 2,6-dichlorobenzaldoximes had been added, has therefore been investigated.

MATERIALS AND METHODS

Chemicals. 2,6-Dichlorobenzonitrile was purified (m.p. 145°) by repeated recrystallization from acetone. The 2,6-dichlorobenzaldoximes were obtained from 'Shell' Research Ltd.

Analytical methods. The solutions were analysed for 2,6-dichlorobenzonitrile by gas-liquid chromatography (Goodwin, Goulden & Reynolds, 1961) but with a 122 cm. column of 5% 'Epikote' resin 1001 (Shell Chemical Co. Ltd.) on 100-120 mesh Celite (J. Jay's, Ewell, Surrey) at 163°, and also by reversed-phase paper chromatography with phenoxyethanol as stationary phase and iso-octane as the mobile one. The compounds were detected by exposure to ultraviolet light after spraying with 5% (w/v) silver nitrate in phenoxyethanol (Mitchell, 1958). Nitrogen was determined by a Kjeldahl method (Knowles & Watkin, 1950).

Soil-incubation experiments. α -Amino-2,6-dichlorobenzaldoxime solutions (1 ml.) were added to boiling tubes containing 20 g. of a brick-earth field soil and incubated at 25°. After appropriate intervals the contents of each tube were mixed with 50 g. of anhydrous sodium sulphate to form a dry powder which was extracted with 25 ml. of

redistilled hexane. The 2,6-dichlorobenzonitrile formed was measured by gas-liquid chromatography.

Experiments with Pseudomonas putrefaciens. Flasks of 2% (w/v) bacteriological peptone broth were inoculated with *P. putrefaciens* and allowed to grow for 4 days. Then 5 mg. of α -amino-2,6-dichlorobenzaldoxime, 2,6-dichlorobenzaldoxime or 2,6-dichlorobenzonitrile in 1 ml. of ethanol-acetone-water (1:1:2, by vol.) was added/l. of medium. Samples were withdrawn after 24 hr. and extracted with hexane either directly or after steam-distillation.

Experiments with Salvinia auriculata. This aquatic fern was grown in 500 ml. of Steinberg solution (Clatworthy & Harper, 1962) in beakers, pairs of which contained 10 mg. of α -amino-2,6-dichlorobenzaldoxime. Field soil (5 g.) was added to one beaker and 1 mg. of penicillin and 1 mg. of streptomycin to the other.

Seed germination experiments. Seeds of *Pisum sativum*, *Zea mays*, *Linum usitatissimum* and *Sinapis alba* were sown in plastic dishes (20 cm. $\times$ 40 cm. $\times$ 5 cm.) of loam, lightly pressed into the surface and covered with fine gravel. The compounds to be assayed were sprayed on the gravel at doses of 117, 73, 40.5 or 9.8 $\mu\text{g./cm.}^2$ and the seeds allowed to germinate in a glasshouse for 10 days at 22°. The plants were then cut at soil level and weighed; the amount of compound given a 50% decrease in fresh weight in 10 days was interpolated from the probit percentage growth decrease/log (dose lines) (Finney, 1947).

Seeds of *Avena sativa* were soaked in '20 volume' hydrogen peroxide solution for 10 min., rinsed in sterile water and then allowed to germinate on sterile filter paper pads in Petri dishes.

Plant homogenates. Fresh cauliflower curd (*Brassica oleracea*) (50 g.) was washed in 2% (w/v) calcium hypochlorite for 1 min., thoroughly rinsed in sterile water, chilled to 10° and homogenized with 100 ml. of phosphate buffer to give a final concentration of 15 mM- Na_2HPO_4 and 53 mM- KH_2PO_4 at pH 6.2, and of 46 mM- Na_2HPO_4 and 27 mM- KH_2PO_4 at pH 7.0. α -Amino-2,6-dichlorobenzaldoxime (5 mg.) was added to 20 ml. portions that were either treated immediately or incubated for 4 hr. at 25°. Streptomycin (10 $\mu\text{g.}$) was added to each flask to diminish bacterial contamination, and some samples were boiled and cooled before the α -amino-2,6-dichlorobenzaldoxime was added. Each 20 ml. sample was cooled to 0° and mixed with anhydrous sodium sulphate to form a dry powder that was extracted with 50 ml. of redistilled hexane at room temperature.

Experiments with vinegar eelworms. Vinegar eelworms (*Turbatrix aceti*) were washed and bacterial contamination

was decreased by repeated centrifuging (at 1000 g) in a solution containing 2 $\mu\text{g.}$ of each of penicillin and streptomycin/ml. A plug of glass wool was placed in the bottom of each centrifuge tube because without it the pellet of living worms was broken up by their swimming movements before the supernatant could be poured off. After the final centrifuging active worms redistributed themselves throughout the liquid; moribund worms remained trapped in the glass wool. After the addition of 300 $\mu\text{g.}$ of α -amino-2,6-dichlorobenzaldoxime/20 ml. of suspension containing 10 mg. dry wt. of nematodes, the mixture was incubated at 25° for 15 hr.

RESULTS

Identification of 2,6-dichlorobenzonitrile. Comparison of retention times and R_F values of a compound in hexane extracts of *P. putrefaciens* and field soil, to which α -amino-2,6-dichlorobenzaldoxime or other 2,6-dichlorobenzaldoximes had been added, with those of authentic 2,6-dichlorobenzonitrile confirmed that the product formed was 2,6-dichlorobenzonitrile. Steam-distillation of the cultures of *P. putrefaciens* gave a volatile compound that had the same retention time and R_F (0.2) as 2,6-dichlorobenzonitrile; α -amino-2,6-dichlorobenzaldoxime is non-volatile. The ultraviolet-absorption spectra between 270 and 312 $m\mu$ of steam-distillates of a culture of *P. putrefaciens* to which α -amino-2,6-dichlorobenzaldoxime had been added and of 2,6-dichlorobenzonitrile both had characteristic absorption maxima at 294 and 284 $m\mu$. Crystals separated from the steam-distillate and had m.p. 143°, undepressed by admixture with authentic 2,6-dichlorobenzonitrile (m.p. 145°).

Formation of 2,6-dichlorobenzonitrile in field soil. Breakdown of α -amino-2,6-dichlorobenzaldoxime was followed in a brick-earth soil (1.7 mg. of nitrogen/g.) by measuring the 2,6-dichlorobenzonitrile formed (Table 1). The measurement of the breakdown product was considered justifiable as it had been found that the conversion was stoichiometric.

Breakdown of cis- and trans-isomers of 2,6-dichlorobenzaldoxime. The *cis*- and *trans*-forms of 2,6-dichlorobenzaldoxime (10 mg./l.) were added to growing cultures of *P. putrefaciens*. There was no difference between the two isomers in the amount of 2,6-dichlorobenzonitrile formed after 24 hr., by which time one-fifth of the possible amount had been formed. Seed-germination tests were carried out, as described by Milborrow (1963), with both isomers but measurements of the germination of *Pisum sativum*, *Zea mays*, *Linum usitatissimum* and *Sinapis alba* failed to show any significant difference in phytotoxicity between the two isomers at the P 0.01 level (Table 2).

Breakdown of 2,6-dichlorobenzaldoxime in non-sterile and partially sterile culture solutions. When several substituted 2,6-dichlorobenzaldoximes

Table 1. Rate of formation of 2,6-dichlorobenzonitrile from α -amino-2,6-dichlorobenzaldoxime in boiling tubes containing 20 g. of a brick-earth soil at 25°

Experimental details are given in the text.

Initial concn. of α -amino-2,6-dichlorobenzaldoxime in soil ($\mu\text{g./g.}$ of damp soil)	Average rate of formation of 2,6-dichlorobenzonitrile ($\mu\text{g./g.}$ of damp soil/hr.)
0.4	2.7
1.0	4.0
10.0	16.0
25.0	21.0

Table 2. *Weights of cis- and trans-2,6-dichlorobenzaldoxime that reduce fresh weights of four test species by 50 % over 10 days*

Experimental details are given in the text. The means of the four species show no significant difference between the *cis*- and *trans*-forms at the *P* 0.01 level.

	50 % inhibition dose ($\mu\text{g./cm.}^2$)				Mean [antilog of log (mean)]
	<i>Pisum sativum</i>	<i>Zea mays</i>	<i>Linum usitatissimum</i>	<i>Sinapis alba</i>	
<i>cis</i> -2,6-Dichlorobenzaldoxime	26	44	26	23	29.4
<i>trans</i> -2,6-Dichlorobenzaldoxime	41	41	12	24	26.4

Table 3. *2,6-Dichlorobenzaldoximes converted into 2,6-dichlorobenzonitrile in non-sterile Steinberg solution but broken down slowly or not at all in a partially sterile solution*

Experimental details are given in the text. No symptoms of 2,6-dichlorobenzonitrile poisoning were developed during 4 days by *Salvinia auriculata* in the partially sterile solution (penicillin and streptomycin, each 2 $\mu\text{g./ml.}$).

2,6-Dichlorobenzaldoxime	M.p.
<i>cis</i> -	149°
<i>trans</i> -	174
Sodium salt	—
Acetate	106
α -Phenylhydrazino-	147
α -Amino-	167
α -Methylamino-	162
α -Dimethylamino-	122
α -Morpholino-	160
α -Ureido-	190
α -Nitro-	62
α -Cyano-	119

Table 4. *Toxicity of α -amino-2,6-dichlorobenzaldoxime and 2,6-dichlorobenzonitrile to Salvinia auriculata*

Experimental details are given in the text. To 500 ml. of Steinberg solution containing 10 mg. of the aldoxime and either 5 g. of field soil (non-sterile) or 1 mg. of penicillin, 1 mg. of streptomycin and 5 g. of boiled soil (partially sterile solution) were added.

	Non-sterile solution		Partially sterile solution	
	2 days	5 days	2 days	5 days
2,6-Dichlorobenzonitrile formed (mg.)	0.95	4.4	0.03	1.35
Phytotoxicity	Slight	Plants dead	None	Slight

(Table 3) were incubated in non-sterile or partially sterile culture solutions with *S. auriculata* the compounds were virtually non-toxic in the partially sterile solutions but were highly toxic to *S. auriculata* in the non-sterile solutions. 2,6-Dichlorobenzonitrile was formed rapidly in the non-sterile

Table 5. *Germination of surface-sterilized Avena sativa seeds in the presence of 2,6-dichlorobenzonitrile and α -amino-2,6-dichlorobenzaldoxime*

Experimental details are given in the text. Twenty seeds were placed on germination pads and mean coleoptile lengths recorded after 6 days at 25°.

Concn. of solution applied to pad ($\mu\text{g./ml.}$)	Coleoptile length (mm.)	
	2,6-Dichlorobenzonitrile	α -Amino-2,6-dichlorobenzaldoxime
10	0	40
5	0	50
2	0	38
1	0	60
0.5	0	65
0.2	1	59
0.1	5	58
0.08	15	60
0.06	17	69
0.04	30	69
0.02	32	65
0.01	40	60
0.0	68	68

and slowly, or not at all, in the antibiotic-sterilized solutions (Table 4).

Surface-sterilized seeds of *Avena sativa* germinated on pads wet with solutions containing up to 10 $\mu\text{g.}$ of α -amino-2,6-dichlorobenzaldoxime/ml. whereas solutions of 0.5 $\mu\text{g.}$ of 2,6-dichlorobenzonitrile/ml. prevented growth entirely (Table 5).

Cauliflower homogenate. α -Amino-2,6-dichlorobenzaldoxime (5 mg.) was added to 20 ml. of a buffered homogenate of cauliflower and incubated for 4 hr. The homogenate contained 25 mg. dry wt. and 1.2 mg. of nitrogen/ml. No 2,6-dichlorobenzonitrile was detectable in hexane extracts of the 4 hr. samples (less than 0.5 $\mu\text{g./ml.}$ of homogenate) nor in the boiled samples. If breakdown occurred it must have been less than 0.1 $\mu\text{g./mg.}$ of nitrogen/hr.

Vinegar eelworms. No 2,6-dichlorobenzonitrile was detectable in vinegar-eelworm cultures to which 15 $\mu\text{g.}$ of α -amino-2,6-dichlorobenzaldoxime/ml. had been added (less than 0.2 $\mu\text{g./ml.}$ of culture). If breakdown occurred it must have been less than 0.012 $\mu\text{g./ml.}$ of nitrogen/hr.

DISCUSSION

2,6-Dichlorobenzonitrile has been identified in extracts of field soil and cultures of *P. putrefaciens* to which α -amino-2,6-dichlorobenzaldoxime and other 2,6-dichlorobenzaldoximes had been added. It has been claimed that *trans*-2,6-dichlorobenzaldoxime inhibits germination of seeds more actively than does the *cis*-isomer (Belgian Patent, 1960). As inhibition of seed germination is one of the characteristic properties of 2,6-dichlorobenzonitrile (Barnsley & Rosher, 1961; Koopman & Daams, 1960) it was expected that 2,6-dichlorobenzonitrile would be formed at different rates from two isomers as Hantzsch & Werner (1890) found for the formation of benzonitrile from unsubstituted benzaldoxime. However, no difference in the rate of formation of 2,6-dichlorobenzonitrile was detected.

Indolylpyruvic acid oxime is active in the *Avena* straight-growth test (Dannenberg & Livermann, 1957), presumably by conversion via indolylacetonitrile into indolylacetic acid within the plant (Ahmad & Spenser, 1960), but neither *Avena sativa* nor any of the other seeds germinated in sterile culture converted 2,6-dichlorobenzaldoxime or α -amino-2,6-dichlorobenzaldoxime into 2,6-dichlorobenzonitrile. Breakdown of α -amino-2,6-dichlorobenzaldoxime and 2,6-dichlorobenzaldoxime was rapid in non-sterile Steinberg solution and slow in the partially sterile one. Growth of *Salvinia auriculata* was stopped immediately in the former but continued in the partially sterile solution for a few days. It is probable, therefore, that micro-organisms formed most, if not all, of the 2,6-dichlorobenzonitrile.

Rates of formation of 2,6-dichlorobenzonitrile/mg. of nitrogen in soil (12 μ g./mg. of nitrogen/hr.) were many times greater than the maximum values for breakdown in the cauliflower homogenate (less than 0.1 μ g./mg. of nitrogen/hr.) calculated from

the sensitivity of the detection apparatus. As only part of the soil nitrogen is present in living micro-organisms, it appears that the conversion of 2,6-dichlorobenzaldoximes is brought about by micro-organisms and not, or very slowly, by higher plants.

SUMMARY

1. Soil micro-organisms convert a range of 2,6-dichlorobenzaldoximes into 2,6-dichlorobenzonitrile.
2. Attempts to demonstrate conversion by plants and a free-living nematode failed.
3. *Pseudomonas putrefaciens* forms 2,6-dichlorobenzonitrile from α -amino-2,6-dichlorobenzaldoxime and 2,6-dichlorobenzaldoxime.
4. There is no significant difference between the rates of formation of 2,6-dichlorobenzonitrile from *cis*- and *trans*-2,6-dichlorobenzaldoxime.

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The Presence of Acetyl Groups in Histones

By D. M. P. PHILLIPS

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W. 3

(Received 13 November 1962)

The *N*-terminal amino acids of calf-thymus histones have been found to be chiefly proline and alanine (Phillips, 1957, 1958), and this has been confirmed by Luck, Rasmussen, Satake & Tsvetikov (1958) and by Bisert & Sautière (1958).

When the histones were fractionated with sodium chloride and ammonia, or by the method of Daly & Mirsky (1955), it was found that the proline end groups were associated with the slightly lysine-rich part and the alanine end groups with the arginine-