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# Bacterial degradation of *N*-cyclopropylmelamine

## The steps to ring cleavage

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1. The *s*-triazine cyclopropylmelamine (*N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine) was degraded to about 6 mol of $NH_4^+$/mol of substrate by a mixture of two bacteria (strains A and D, both *Pseudomonas* spp.). 2. Only strain A grew with cyclopropylmelamine as sole and limiting source of nitrogen. The organism obtained 2 mol of nitrogen/mol of substrate and excreted a product that was identified as cyclopropylammelide [6-cyclopropylamino-1,3,5-triazine-2,4(1*H*,3*H*)-dione]. 3. Proteins in extracts from strain A were separated on a Sephadex G-200 column. Cyclopropylmelamine was found to be deaminated in two separable steps to cyclopropylammelide via cyclopropylammeline [4-amino-6-cyclopropylamino-1,3,5-triazine-2(1*H*)-one], which was identified. 4. Strain D could not utilize cyclopropylmelamine or cyclopropylammeline, but could utilize cyclopropylammelide (or homologue) as sole and limiting source of nitrogen and obtain about 4 mol of nitrogen/mol of substrate. 5. Proteins in cell extracts from strain D were separated on a DEAE-cellulose column. Alkylammelides were degraded quantitatively by one enzyme fraction to 1 mol of cyanuric acid plus 1 mol of alkylamine/mol of substrate. 6. The specific activities of enzymes in extracts of the two strains were as high as the activities observed during growth. 7. The three activities studied in the two strains were all active under aerobic and oxygen-free conditions. 8. The reactions appear to be hydrolytic, yielding 2 mol of $NH_4^+$ plus 1 mol of cyclopropylamine and 1 mol of cyanuric acid/mol of substrate.

*N*-cyclopropylmelamine (PAAT; Table 1 and Scheme 1) is a representive of the most highly aminated group of mono-*N*-alkylated *s*-triazines. Hydroxyanalogues and homologues (Table 1) are perhaps better known, because they are released into the environment as degradative products of *s*-triazine herbicides (e.g. Esser *et al.*, 1975).

The defined quantitative microbial reactions in the degradation of *s*-triazines are a single *N*-dealkylation in fungi (of CEET to CEAT; cf. Table 1; Kaufman *et al.*, 1965; Kearney *et al.*, 1965) and the successive hydrolytic deaminations of melamine (Table 1) in bacteria (Jutzi *et al.*, 1982). We now report not only further hydrolytic deaminations of *N*-alkyltriazines but also hydrolytic removal of the *N*-alkyl side chain as the alkylamine in a new degradative pathway.

Abbreviations used: those for *s*-triazines are defined in Table 1; h.p.l.c., high-pressure liquid chromatography; g.(l.)c., gas (–liquid) chromatography.

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## Experimental

### *Materials*

The *s*-triazines used, their abbreviations, purity and availability are shown in Table 1. Most of these materials were difficult to handle because they were sparingly soluble in neutral aqueous solvents and in organic solvents. PAAT (10 mM) was dissolved in water; 10 mM-tBOAT or 10 mM-sBOAT was dissolved in 0.1 M-HCl; 10 mM-tBOOT or 10 mM-MOOT was dissolved in 0.1 M-NaOH. Alkylamines (>99%) were supplied by Fluka (Buchs, Switzerland). Other materials were described elsewhere (Beilstein *et al.*, 1981; Cook & Hütter, 1981*a*; Jutzi *et al.*, 1982; Daughton *et al.*, 1979).

### *Apparatus, analyses and identification of metabolites*

H.p.l.c. was done with the apparatus of Beilstein *et al.* (1981). Spectrophotometric analyses, g.(l.)c., chemical derivatizations and experiments under anaerobic conditions were done with equipment

Table 1. *s-Triazines*

The common name is uninformative and often disadvantageous because it differs from that of an analogue in only the penultimate letter and the abbreviations from the common names are only of limited help (cf. Cook & Hütter, 1981*a*). We have introduced a semisystematic abbreviation based on the substituents of the ring: A, amino; B, butylamino; C, chloro; E, ethylamino; H, hydro; I, isopropylamino; M, methylamino; O, hydroxy; P, cyclopropylamino; T, triazine ring structure. The sequence of letters for substituents is usually in order of descending molecular mass, except for C, which always has priority. The system allows the structure to be deduced from the abbreviation and the abbreviation to be deduced from the structure. The availability of *s*-triazines was often limited and the column 'Supply' indicates whether, e.g., kg or μg amounts were available. The purities are from elemental analyses.

| Common name | Abbreviation | Supply | Purity (%) | Substituents on ring | | |
|---|---|---|---|---|---|---|
| *N*-Cyclopropylmelamine | PAAT | g | 98 | Cyclopropylamino | Amino | Amino |
| *N*-t-Butylammeline | tBOAT | mg | 98 | t-Butylamino | Hydroxy | Amino |
| *N*-s-Butylammeline | sBOAT | mg | 92 | s-Butylamino | Hydroxy | Amino |
| *N*-Cyclopropylammeline | POAT | μg | Low | Cyclopropylamino | Hydroxy | Amino |
| *N*-Isopropylammeline | IOAT | g | 98 | Isopropylamino | Hydroxy | Amino |
| *N*-Ethylammeline | EOAT | g | 98 | Ethylamino | Hydroxy | Amino |
| *N*-t-Butylammelide | tBOOT | mg | 98 | t-Butylamino | Hydroxy | Hydroxy |
| *N*-s-Butylammelide | sBOOT | – | – | s-Butylamino | Hydroxy | Hydroxy |
| *N*-Cyclopropylammelide | POOT | μg | Low | Cyclopropylamino | Hydroxy | Hydroxy |
| *N*-Isopropylammelide | IOOT | g | 98 | Isopropylamino | Hydroxy | Hydroxy |
| *N*-Ethylammelide | EOOT | g | 94 | Ethylamino | Hydroxy | Hydroxy |
| *N*-Methylammelide | MOOT | mg | 96 | Methylamino | Hydroxy | Hydroxy |
| Cyanuric acid | OOOT | kg | 99 | Hydroxy | Hydroxy | Hydroxy |
| Melamine | AAAT | kg | 98 | Amino | Amino | Amino |

previously described (Jutzi *et al.*, 1982). Tentative identification of an organic compound was confirmed by mass spectrometry of purified material (Beilstein *et al.*, 1981), by multiple independent chromatographic methods or by mass spectrometry interfaced with a gas chromatograph.

*s*-Triazines in neutralized solutions were quantified routinely and identified tentatively by h.p.l.c. (Cook *et al.*, 1983*a*; Beilstein *et al.*, 1981). Isocratic elution was used. OOOT was measured with 100mM-potassium phosphate buffer, pH6.7 (17μl/s) as eluent. Alkylated triazines were eluted with a mixture of the same buffer and methanol (3:1, v/v). OOOT and IOOT could also be separated and identified by partition chromatography. We extended our observations (Jutzi *et al.*, 1982; Cook *et al.*, 1983*a*) that OOOT exhibits varying capacity ratios on amino-phases (Nucleosil, LiChrosorb and Spherosil) and a diol-phase (Nucleosil) with 60% (v/v) acetonitrile (in water) as the mobile phase. Stable retention times could be obtained for several hours with acetonitrile/10mM-potassium phosphate buffer, pH6.7 (4:1, v/v), at the cost, however, of damaging the stationary phase.

$NH_4^+$ was measured by the Berthelot reaction (Weatherburn, 1967). The protein assay of Kennedy & Fewson (1968) was used as described by Cook & Hütter (1981*a*). Propene was measured by g.c with a Durapak column, isopropyl alcohol was measured by g.c with a Chromosorb 101 column (Daughton *et al.*, 1979) and acetone was measured by g.c. with a Porapak N column (Dave, 1969). Alkylamines ($C_1$–$C_4$) were identified tentatively and determined by g.c. using direct aqueous injection on a 1.8m × 2mm-i.d. (internal diameter) glass column packed with 80/100 mesh Chromosorb 103. G.c. was done isothermally between 80 and 110°C (depending on the analyte) with a gas flow rate of 0.8ml of $N_2$/s: the injector and flame-ionization-detector temperatures were 200 and 225°C respectively. The identity of the alkylamine was further tested by g.l.c. using direct aqueous injection on a 1.8m × 2mm-i.d. glass column packed with 4% Carbowax 20M plus 0.8% KOH on 60/80 mesh Carbopak B (Supelco, Bellefonte, PA, U.S.A.), according to the manufacturer's instructions. The eluent from a Chromosorb 103 column (2m × 2mm; 0.5ml of He/s at 150°C) was examined in a mass spectrometer with electron-impact ionization at 40eV.

*Organisms, cell suspensions and enzyme assays*

Two strains of bacteria (*Pseudomonas* spp.) were used, strains A and D, which were deposited as N.R.R.L. [Northern Regional Research Laboratory (Peoria, IL, U.S.A.)] B-12227 and B-12228 respectively (Cook & Hütter, 1981*a*). They were grown as previously described (Jutzi *et al.*, 1982). Cell suspensions, cell-free extracts and enzyme assays were as previously described (Jutzi *et al.*, 1982). Several variants of the enzyme assays were also used. When the quantitative conversion into a product was being studied, the enzyme concentra-

tion was increased 10-fold and the substrate was sometimes added as a solid. The anaerobic reaction mixtures were gassed in two portions with oxygen-free $N_2$ before being passed into the glovebox, where the reaction was started by addition of enzyme; the reactions in portions of the mixture were stopped by addition of acid before removal from the glovebox. The conversion of PAAT into POAT and POOT, and the subsequent purification of these products, were analogous to the experiments described by Cook & Hütter (1981*a*).

The separation of enzyme activities by column chromatography with DEAE-cellulose was as described by Jutzi *et al.* (1982), and with Sephadex G-200 it was according to the manufacturer's (Pharmacia, Uppsala, Sweden) instructions. Initial screening for enzyme activity was done by monitoring release of $NH_4^+$ from *s*-triazines in reactions stopped with trichloroacetic acid (0.5M final conc.), which, on neutralization had no effect on the determination of $NH_4^+$; in the case of IOOT as substrate, whole cells of strain A (1mg of protein/ml) were present in the test to couple OOOT formation with reactions yielding $NH_4^+$. Detailed examination of fractions was analogous to that described in Jutzi *et al.* (1982), and the concentration of *s*-triazine was routinely 0.5mM.

## Results

### *Quantitative aspects of growth*

*Pseudomonas* sp. strain A, which utilized $NH_4^+$ quantitatively as sole and growth-limiting source of nitrogen with a yield of about 60g of protein/mol of nitrogen, grew with PAAT as nitrogen source (136g of protein/mol) and thus obtained 2mol of nitrogen/mol of substrate. PAAT ($\lambda_{max.}$ 208nm) was utilized quantitatively, yielding a single product that was tentatively identified as POOT by co-chromatography (h.p.l.c.) with authentic material and by u.v. spectrophotometry ($\lambda_{max.}$ 202nm; $\lambda_{min}$ 210nm). Strain A also grew with POAT as nitrogen source, and POOT was the product [cf. growth with EOAT and IOAT (Cook & Hütter, 1981*a*)]. Strain A utilized PAAT as a nitrogen source with a specific growth rate ($\mu$) of $0.15\,h^{-1}$, which, together with the growth yield, allowed the specific utilization rate of PAAT to be calculated as 0.3mkat/kg of protein.

*Pseudomonas* sp. strain D could not utilize the *s*-triazine substrates used by strain A (see above), but could utilize short-chain alkylaminotriazinediones (MOOT, EOOT, IOOT and POOT) quantitatively for growth [an extension of the data of Cook & Hütter (1981*a*)]. These latter compounds, which contain 4 atoms of nitrogen/mol, supplied 4mol of nitrogen for growth/mol of substrate.

### *Identification and quantification of intermediates*

*Strain A.* Two enzymic activities involved in the conversion of bis(amino)alkylaminotriazine to alkylaminotriazinedione were observed after separation on a Sephadex G-200 column (Fig. 1). One reaction converted PAAT into putative POAT (which was not quantified), releasing 1.1mol of $NH_4^+$/mol of PAAT (Table 2); no homologues were available to test enzyme specificity. The other reaction released $NH_4^+$ from tBOAT, sBOAT, POAT, IOAT and EOAT, and it was studied quantitatively in the conversion of the readily available IOAT to putative IOOT (Fig. 1). The reaction was stoichiometric, yielding 1mol of IOOT and 1mol of $NH_4^+$/mol of IOAT (Table 2).

Putative POAT was co-eluted (h.p.l.c.) with authentic POAT and had a u.v. spectrum identical with that of authentic POAT ($\lambda_{max.}$ 208nm; $\lambda_{min}$ 227nm). The identity of the putative POAT or POOT was confirmed by mass spectrometry of the isolated material. In addition to observing the putative molecular ion ($M^+$) (POAT, 167; POOT, 168) the disintegration pattern below *m/z* 110 confirmed the *s*-triazine nature of the product, and the pattern above *m/z* 110 confirmed the structure when compared with standard spectra (Safe & Hutzinger, 1973).

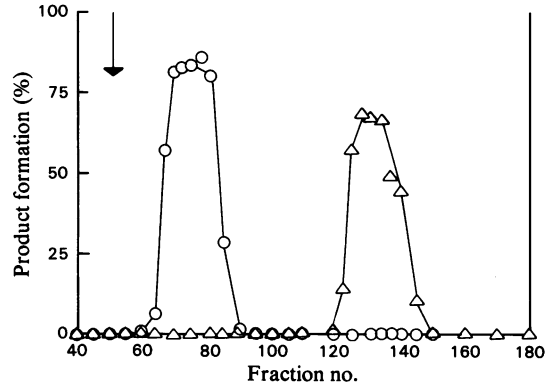



Fig. 1. *Chromatography of enzymes of PAAT-degradation from strain A on Sephadex G-200*

Crude extract [about 250mg of protein in 10mM-potassium phosphate buffer, pH7.2, containing 0.25mM-$MgSO_4$ (elution buffer)] of strain A, which had been grown with $NH_4^+$ as sole nitrogen source, was centrifuged (110000*g* for 2h at 4°C). The supernatant fluid was rebuffered on Sephadex PD-10 columns into fresh elution buffer, and 18ml of the solution was applied to the column (93cm × 2.6cm, at 4°C). Proteins were eluted at a flow rate of 19ml/h and 2.9ml fractions were collected. The arrow indicates the void volume. Activity was assayed as product ($NH_4^+$) formation from PAAT (○) or IOAT (△).

Table 2. *Quantitative conversions in the degradation of mono N-alkylated s-triazines*
In all reactions, the substrate was no longer detectable at the end of the reaction. Abbreviations: n.a., not assayed; n.d., not detectable.

| Substrate | Concn. (mM) | Catalyst | Source of catalyst (*Pseudomonas* strain) | Products of reaction and yield (mol/mol of substrate): Triazine 77 Type | Triazine 77 Yield | $NH_4^+$ (mM) | $NH_4^+$ Yield | Alkylamine (mM) | Alkylamine Yield |
|---|---|---|---|---|---|---|---|---|---|
| PAAT | 0.25 | Enzyme* | A | POAT | n.a. | 0.27 | 1.1 | | |
| PAAT | 0.5 | Extract | A | POOT | n.a. | 1.0 | 2.0 | | |
| PAAT | 0.5 | Extracts | A+D | n.d. | | 2.5 | 5.0 | | |
| PAAT | 2.0 | Extracts | A+D | n.d. | | | | 2.0 | 1.0 |
| PAAT | 0.4 | Cells | A+D | n.d. | | 2.6 | 6.4 | | |
| tBOAT† | 0.5 | Extract | A | tBOOT | 1.0 | 0.5 | 1.1 | | |
| IOAT | 0.5 | Enzyme | A | IOOT | 1.0 | 0.5 | 1.0 | | |
| IOOT | 0.5 | Extract | D | n.d. | | 1.5 | 3.0 | | |
| IOOT | 0.4 | Cells | D | n.d. | | 1.5 | 3.8 | | |
| IOOT | 2.0 | Extract | D | n.d. | | | | 1.8 | 0.9 |
| IOOT | 0.5 | Enzyme | D | OOOT | 1.0 | | | | |
| EOOT | 0.4 | Cells | D | n.d. | | 1.6 | 4.0 | | |
| EOOT | 10 | Extract | D | n.d. | | 28 | 2.8 | 9.6 | 1.0 |
| MOOT | 10 | Extract | D | n.d. | | 30 | 3.0 | 9.0 | 0.9 |

* The word 'enzyme' denotes a portion from a fractionation using column chromatography.
† Similar data were obtained for IOAT, EOAT (Cook & Hütter, 1981*a*) and for sBOAT; products were identified, at least tentatively, in each case.

Table 3. *Specific rates of enzyme activities involved in the degradation of mono N-alkylated s-triazines*

| Substrate in test | Source of enzyme (*Pseudomonas* strain) | Specific activities in extracts from cells grown with the named source of nitrogen (mkat/kg of protein): PAAT | IOOT | AAAT | OOOT | $NH_4^+$ |
|---|---|---|---|---|---|---|
| PAAT | A | 0.2 | | 0.1 | 0.3 | 0.3 |
| POAT | A | | | | 0.7 | |
| tBOAT | A | 0.5 | | 0.5 | 0.7 | |
| sBOAT | A | 0.9 | | 0.7 | 1.2 | |
| IOAT | A | 0.6 | | 0.6 | | |
| EOAT | A | 0.5 | | 0.5 | | 0.9 |
| IOOT | D | | 1.4 | | 1.1 | 1.4 |

The specific activities of the two enzymes of PAAT degradation in strain A were measured in extracts of cells grown with different nitrogen sources, and synthesis of these enzymes was apparently constitutive (Table 3). The specific activity of PAAT degradation (Table 3) was similar to that observed in cells utilizing PAAT (0.3 mkat/kg of protein); similarly, the specific activity of deamination of POAT and IOAT was as high as the specific activity observed in cells utilizing IOAT for growth (0.5 mkat/kg of protein; Cook & Hütter, 1981*a*). The enzyme deaminating POAT and its homologues appeared to have a similar specific activity for all tested substrates (Table 3).

*Strain D.* The degradation of POOT (and homologues) by strain D had been predicted, from studies in fungi, plants and animals, to be initiated by *N*-dealkylation (e.g. Esser *et al.*, 1975), but, in cell-free studies with strain D and the more readily available IOOT, no acetone, isopropyl alcohol or propene was observed, although the putative products were stable and readily measurable under the reaction conditions. In cell-free assays with complete disappearance of 0.5 mM-IOOT, only about 1.5 mM-$NH_4^+$ was released, whereas 2 mM-$NH_4^+$ was released by whole cells (Table 2). We deduced that the $NH_4^+$ released in cell-free assays, which contained no added cofactors, represented ring-nitrogen and that the alkylamino bond of

the side chain was not cleaved. Isopropylamine was tentatively identified by co-chromatography (g.c.) as a product from the degradation of IOOT. The identification of isopropylamine was supported by co-chromatography (g.l.c.) and was confirmed by mass spectrometry interfaced with a gas chromatograph [$M^+ = 59$; $M^+/(M-1)^+ = 0.33$ in unknown and in authentic isopropylamine]. No isopropylamine was detected in reaction mixtures without substrate or without cell extract. Methylamine, ethylamine, cyclopropylamine and *sec*-butylamine were detected co-chromatographically by both g.c. and g.l.c. as products from MOOT, EOOT, POOT and sBOOT respectively. Crude extract of strain D yielded 1 mol of alkylamine and 3 mol of $NH_4^+$/mol from POOT analogues (Table 2).

The nature of the ring moiety formed from IOOT on removal of the side chain could not be determined in crude extract, because the moiety was degraded to $NH_4^+$ with slight transient accumulation of OOOT. The 110000*g* supernatant fluid of desalted crude extract was fractionated on DEAE-cellulose, and fractions were obtained that degraded IOOT (Fig. 2). The *s*-triazine product co-chromatographed with OOOT (h.p.l.c.) and had a u.v. spectrum identical with that of OOOT ($\lambda_{max.}$ 214nm). The identification was confirmed by co-chromatography (h.p.l.c.) of the unknown with authentic OOOT on an amino- and on a diol-phase.

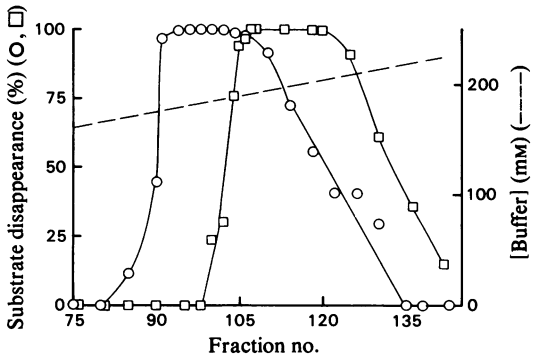



Fig. 2. *Chromatography of the IOOT-degradation activity from strain D on DEAE-cellulose*

Crude extract (about 500mg of protein in 100mM-potassium phosphate buffer, pH7.2) of strain D, which had been grown with OOOT as sole nitrogen source, was centrifuged (110000*g* for 90min at 4°C) and the supernatant fluid applied to the column. Proteins were eluted with 1 column vol. of 100mM-potassium phosphate buffer and a linear gradient (----) (to 250mM) of phosphate buffer, essentially as described by Jutzi *et al.* (1982), and 10ml fractions were collected. Activity was measured as substrate disappearance: ○, IOOT; □, OOOT.

IOOT (and homologues) were thus dealkylaminated to OOOT and the reaction was stoichiometric, yielding 1 mol of OOOT and 1 mol of alkylamine/mol of substrate (Table 2).

The activities responsible for IOOT, EOOT and MOOT degradation were co-eluted from the DEAE-cellulose column, so we presume a single enzyme to be active towards several homologues. The enzyme appears to be constitutive (Table 3). The specific activity observed in extracts (Table 3) was higher than that observed in cells utilizing IOOT [0.4mkat/kg of protein (Cook & Hütter, 1981*a*)].

The reactions of strain A deaminating PAAT to POOT and the dealkylamination of IOOT to OOOT by strain D proceeded quantitatively in crude extract with air or an anoxic mixture as the gas atmosphere. The reactions thus did not seem to involve molecular oxygen.

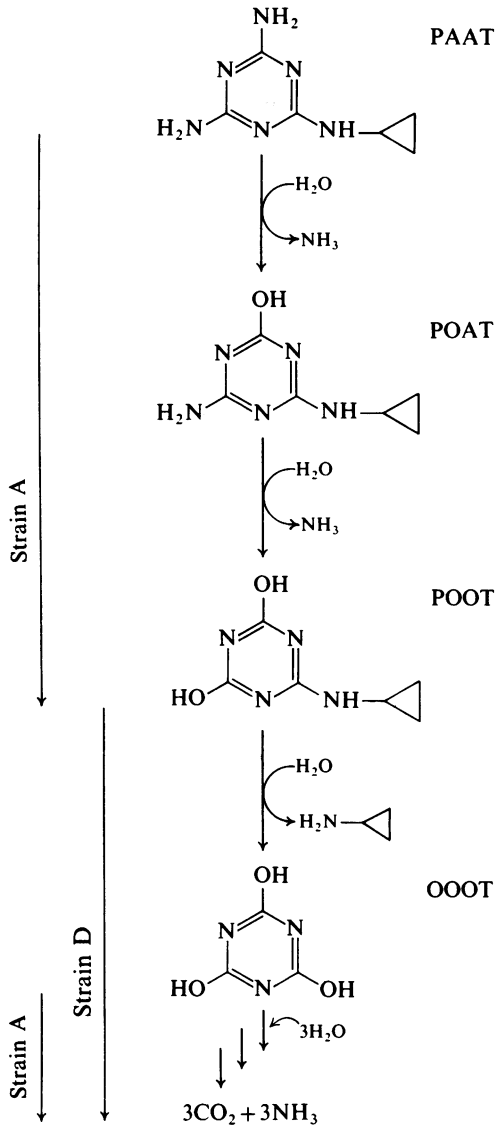
## Discussion

PAAT has been shown to be degraded quantitatively to $NH_4^+$ by a mixture of two organisms, the *Pseudomonas* spp. strains A and D (Table 2). The first two reactions were catalysed exclusively by strain A and were quantitative stoichiometric hydrolytic deaminations (Scheme 1). POOT and homologues were degraded exclusively by strain D in a quantitative stoichiometric hydrolytic dealkylamination to OOOT, which is further converted into $NH_4^+$ by both strains A and D (Cook *et al.*, 1983*b*). The reaction rates of the enzymes in crude extracts were high enough to explain the growth rates of the organisms. Thus, although there may be other degradative pathways for PAAT, there seems to be no need to hypothesize them in these organisms. The data (Scheme 1) confirm one of the two putative pathways we proposed (Cook & Hutter, 1981*b*), but leave the fate of the alkyl group unsolved.

Further evidence is required to support the theory that the reactions are hydrolytic (cf. Jutzi *et al.*, 1982). Provisionally, we anticipate the deaminases (PAAT to POOT) and the dealkylamination to belong to EC 3.5.4.-. The types of catalysis are thus not new, but the pathway is. The deaminases could be postulated as analogous to plant and animal (cf. Esser *et al.*, 1975) or bacterial (Jutzi *et al.*, 1982), but the alkylamino hydrolase has never been observed before (Esser *et al.*, 1975; Cook & Hütter, 1981*b*).

A different method of removal of an amino (or possibly aminoalkyl) substituent on an aromatic *s*-triazine ring is suspected by Giardina *et al.* (1980). They identify 2-chloro-1,3,5-triazine-4-amine (CAHT) to be formed in low yield from atrazine (CIET) atrazine, which is partially metabolized in

Scheme 1. *Degradative pathway of PAAT*




a bacterial culture. Thus the amino (or alkylamino) substituent is replaced by –H and not by –OH as in our isolates. The claim of Giardina *et al.* (1980) that atrazine is serving as a carbon and nitrogen source is difficult to substantiate using the data provided and standard correlation factors (Cook & Hütter, 1981*b*). There would appear to be too little carbon (0.6 mM or about 4 $\mu$g of protein/ml) in the degraded atrazine to support the growth claimed (about 20 $\mu$g of protein/ml), though perhaps enough nitrogen is present (0.36 mM-nitrogen or about 18 $\mu$g of protein/ml; claimed, 42 $\mu$g/ml); no controls are described.

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## References

Beilstein, P., Cook, A. M. & Hütter, R. (1981) *J. Agric. Food Chem.* **29**, 1132–1135

Cook, A. M. & Hütter, R. (1981*a*) *J. Agric. Food Chem.* **29**, 1135–1143

Cook, A. M. & Hütter, R. (1981*b*) in *Microbial Degradation of Xenobiotics and Recalcitrant Compounds* (Leisinger, T., Cook, A. M., Nüesch, J. & Hütter, R., eds.), pp. 237–249, Academic Press, London

Cook, A. M., Beilstein, P. & Hütter, R. (1983*a*) *Int. J. Environ. Anal. Chem.* **14**, 93–98

Cook, A. M., Grossenbacher, H., Beilstein, P. & Hütter, R. (1983*b*) *Experientia* **39**, 1432

Daughton, C. G., Cook, A. M. & Alexander, M. (1979) *J. Agric. Food Chem.* **27**, 1375–1382

Dave, S. B. (1969) *J. Chromatogr. Sci.* **7**, 389–399

Esser, H. O., Dupuis, G., Ebert, E., Marco, G. J. & Vogel, C. (1975) in *Herbicides: Chemistry, Degradation and Mode of Action* (Kearney, P. C. & Kaufman, D. D., eds.), pp. 129–208, Marcel Dekker, New York and Basel

Giardina, M. C., Giardi, M. T. & Filacchione, G. (1980) *Agric. Biol. Chem.* **44**, 2067–2072

Jutzi, K., Cook, A. M. & Hütter, R. (1982) *Biochem. J.* **208**, 679–684

Kaufman, D. D., Kearney, P. C. & Sheets, T. J. (1965) *J. Agric. Food Chem.* **13**, 238–242

Kearney, P. C., Kaufman, D. D. & Sheets, T. J. (1965) *J. Agric. Food Chem.* **13**, 369–372

Kennedy, S. I. T. & Fewson, C. A. (1968) *Biochem. J.* **107**, 497–506

Safe, S. & Hutzinger, O. (1973) *Mass Spectrometry of Pesticides and Pollutants*, pp. 169–175, Chemical Rubber Co., Cleveland, OH

Weatherburn, M. W. (1967) *Anal. Chem.* **39**, 971–974