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Qualitative Analysis of Waste-Water from Ametryne Production†

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s-Triazines in waste-water from the synthesis of ametryne (2-(ethylamino)-4-[(l-methylethyl) amino]-6-(methylthio)-1, 3, 5-triazine) were tentatively identified co-chromatographically by HPLC and by UV-spectra. Alkylated s-triazines (e.g. hydroxyametryne, 4-(ethylamino)-6-(methylthio)-1, 3, 5-triazine-2(lH)-one, and N-ethylammelide) were isolated by preparative chromatography on a reversed phase support, and were identified by mass spectrometry. Putative cyanuric acid was desalted on activated charcoal and its identity confirmed as the silylated derivative by GLC.

KEY WORDS: s-triazines, analysis, herbicide synthesis, by-products, biodegradation.

INTRODUCTION

Any attempt to improve waste-water quality depends on reliable analyses of the wastes. This is especially important in wastes with low BOD and high TOC, which indicate that the wastes could pass largely undegraded through a conventional sewage treatment plant.

Wastes from the syntheses of s-triazine herbicides are effectively treated by adsorption on active charcoal, but we are exploring the use of specific biological catalysts[1] as an alternative process. In order to select the correct biological catalysts (i.e. enzymes, whether in growing cells, non-growing cells or in cell-free systems) to remove potential pollutants, we need reliable methods to identify and monitor individual chemicals in the waste-waters. We have developed methods to do such analyses with waste-water from the production of s-triazine herbicides.

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EXPERIMENTAL

Apparatus and chemicals

HPLC was done with the apparatus described by Beilstein *et al.*[2]. GLC was done with a 5480A chromatograph equiped with a flame ionization detector (Hewlett-Packard, Palo-Alto, CA, USA). Other apparatus was described elsewhere.[2,3] The authentic s-triazines used in this study were obtained from Ciba-Geigy AG (Basel, Switzerland) and are given in Table I. Wastes from ametryne production were supplied by Ciba-Geigy. Chemicals were of reagent grade or better.[2,3]

TABLE I
s-Triazines

Common name	Systematic name
Ametryne	N-ethyl-N'-(l-methylethyl)-6-(methylthio)-1, 3, 5-triazine-2, 4-diamine
Hydroxyametryne	4-(ethylamino)-6-[(l-methylethyl)amino]-1, 3, 5-triazine-2(lH)-one
—	4-[(1-methylethyl)amino]-6-(methylthio)-1,3,5-triazine-2(1H)-one
—	4-(ethylamino)-6-(methylthio)-1,3,5-triazine-2(1H)-one
N-isopropylammelide	6-[(1-methylethyl)amino]-1,3,5-triazine-2,4(1H,3H)-dione
N-ethylammelide	6-(ethylamino)-1, 3, 5-triazine-2, 4(lH, 3H)-dione
Cyanuric acid	1, 3, 5-triazine-2, 4, 6(1H, 3H, 5H)-trione.

Analyses

HPLC by gradient elution from a reversed-phase support was done essentially as previously described.[2] Vastly improved column lifetime was achieved by use of replaceable guard columns (40 × 4.6 mm; Knauer, Oberursel, FRG), and the separations, symmetry and flow characteristics were improved by using Nucleosil 7 C_{18} (Macherey–Nagel, Düren, FRG) as the stationary phase. HPLC with an amine bonded phase [(40 + 250) × 4.6 mm; Nucleosil 10] was done with 60% acetonitrile in water as described by Jessee *et al.*[4]

Silylation of non-alkylated s-triazines (e.g. cyanuric acid) and GLC of the derivatives were done as described by Stoks and Schwartz.[5] Amines were measured by GC with Chromosorb 103 (2 m × 2 mm) as the stationary phase.

UV-spectra of authentic and putative s-triazines were measured after separation by HPLC; the reference cuvette contained baseline eluate from the same experiment. The UV-spectra of s-triazines were very sensitive to changes in the methanol concentration or pH in the solvent.

Ammonium ion was measured by the method of Weatherburn.[6]

Isolation of s-triazines

s-Triazine-ones are frequently only sparingly soluble in both organic and aqueous solvents, but they also tend to supersaturation with irreproducible crystallisation properties. This supersaturation of hydroxyparents (e.g. hydroxyametryne) led us to give solubility values[2] that were too high. Ramsteiner and Hörmann[7] presented better solubility data for hydroxyparents.

Hydroxyametryne normally precipitated immediately on neutralization of waste-water. The precipitate was dissolved in 0.1 M HCl and precipitated by neutralization. Recrystallization from water failed to remove an impurity, and the s-triazine was purified by reversed phase chromatography.[8]

The 4-alkylamino-6-thiomethyl-triazine-ones crystallized slowly from the neutralized waste-water. Harvested crystals were dissolved in 50% (v/v) methanol and the triazines separated on a reversed phase column (Lobar RP-8, 310 × 25 mm; Merck, Darmstadt, FRG) with 50% methanol in water as the mobile phase. Fractions were taken to dryness and recrystallized from water.

N-Alkylammelides remained in solution after neutralization of the waste-water and were separated on the Lobar column with 5% methanol in water as the mobile phase. Fractions were taken to dryness and recrystallized from water.

The first peak from the separation of N-alkylammelides was used to obtain non-alkylated s-triazines, which were desalted on columns of activated charcoal.[9]

RESULTS AND DISCUSSION

Waste-water from the production of ametryne was examined by HPLC and the components (except three unidentified traces, which eluted before cyanuric acid) tentatively identified by co-chromatography (Fig. 1). In isocratic analyses of waste-water, portions of the eluates were examined by UV-spectrophotometry (Table II). The UV-spectra of the unknowns (I–VII) supported the tentative identifications made by co-chromatography.

The chromatogram (Fig. 1) illustrates that the method is effective for the analysis of waste-water from herbicide synthesis. The uncomplicated analysis, without interference over a wide range of polarity, shows the high quality of the raw material used in the synthesis of ametryne and suggests that there is only a low risk of poisoning biological catalysts with unknown organic impurities.

Ametryne (unknown VII) was anticipated in the waste-water, so no attempt was made to isolate the traces of his compound for conclusive

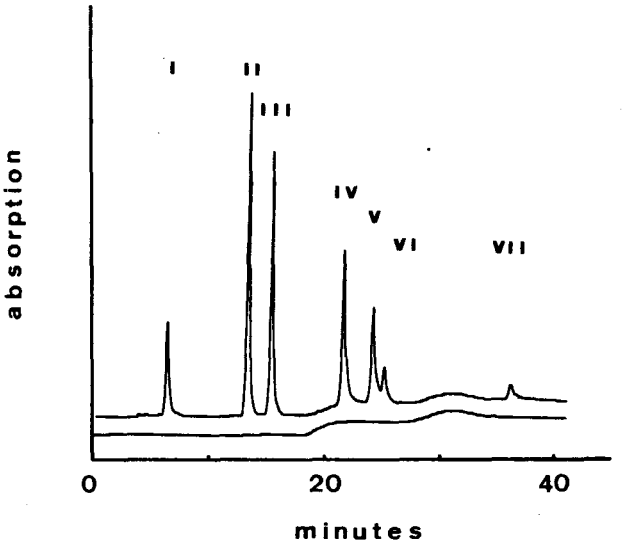

FIGURE 1 Co-chromatography of s-triazines in diluted waste-water with authentic s-triazines. The experiment was done at room temperature at a flow-rate of 0.8 ml/min. A stepped gradient was used. The first solvent (100 mM potassium phosphate buffer, pH 6.7) was delivered for 4 minutes, when a linear gradient to 70% of the second solvent (70% methanol, 30% 10 mM potassium phosphate buffer, pH 6.7) over 10 minutes was applied. After a further 10 minutes, the gradient was resumed to give 100% of the second solvent over 5 minutes. The lower line in the figure is the blank, obtained without injection of sample.

identification. These present data supersede earlier assays[8] with LiChrosorb RP-18 as the stationary phase, where ametryne was not detected. The bulk of the s-triazines present in the waste-water consists of by-products.

The presence of hydroxyametryne, 4-[(l-methylethyl)amino]-6-(methylthio)-1, 3, 5-triazine-2(lH)-one, 4-(ethylamino)-6-(methylthio)-1, 3, 5-triazine-2(lH)-one, N-isopropylammelide or N-ethylammelide, each postulated from co-chromatography (Fig. 1) and UV-spectra (Table II), was confirmed by mass spectrometry. Representative mass spectra with literature references can be found elsewhere.[3,8] Alkylated s-triazines could thus be isolated from waste-water and identified with relative ease, similar to studies on the biodegradation of s-triazines.[3,8] Most of the compounds identified are known to be subject to ready, quantitative biodegradation,[3,8] the major exception being hydroxyametryne,[1] which, however, does not accumulate in soil,[10] and whose biodegradation is under study in this laboratory.

TABLE II

Data from UV-spectra of Substances in Waste-Water and of Authentic Materials

Substance	Wavelength, nm					
	Authentic material			Unknown		
	max	min	max	max	min	max
Ametryne[a]	223					
Unknown VII[a]				222		
Hydroxyametryne[b]	218					
Unknown VI[b]				218		
Isopropylamino-methylthio-triazineone[b]	209	223	254[c]			
Unknown V[b]				209	223	254[c]
Ethylamino-methylthio-triazineone[b]	208	223	253[c]			
Unknown IV[b]				208	223	253[c]
N-isopropylammelide[d]	197	210	226[c]			
Unknown III[d]				197	210	226[c]
N-ethylammelide[d]	197	211	226[c]			
Unknown II[d]				197	210	226[c]
Cyanuric acid[e]	214					
Unknown I[e]				214		

[a]The solvent was 70% methanol and 30% 10 mM potassium phosphate buffer, pH 6.7.
[b]The solvent was 55% methanol and 45% 10 mM potassium phosphate buffer, pH 6.7.
[c]This peak in the spectrum had the higher molar absorption coefficient.
[d]The solvent was 25% methanol and 75% 10 mM potassium phosphate buffer, pH 6.7.
[e]The solvent was 100 mM potassium phosphate buffer, pH 6.7.

Tentative identification of cyanuric acid in salt solution was simple (Fig. 1, Table II), but neither mass spectrometry nor silylation was feasible in the presence of salt(s), and purification from salt solutions presented problems. The mobile phase for the reversed phase HPLC analysis had to be 100 mM to achieve separation, so only a change of salt was possible. We were unable to recover cyanuric acid from an ion retardation resin (AG 11–A8; Bio-Rad, Richmond, CA, USA). HPLC with the amine bonded phase did give baseline separation of melamine, ammeline, cyanuric acid and ammelide, in that order of elution, but whereas melamine or ammeline was eluted with a reproducible capacity ratio (0.2 and 0.6, respectively), the capacity ratio for cyanuric acid (2.1–4.0) or ammelide (2.3–5.6) varied enormously during any one day. Our lowest

capacity ratio for cyanuric acid, about 2.1, corresponds to the figure shown by Jessee *et al.*,[4] but not to their tabulated value (0.4). A further problem with the amine bonded phase in our hands was that we did not succeed in desalting ammelide.

Successful desalting of cyanuric acid, ammelide, ammeline or melamine was achieved by treatment with activated charcoal. Desalted material can be examined by HPLC, but the lowered aqueous solubility of mixtures of cyanuric acid, ammelide, ammeline and melamine (caused by co-precipitation) does not allow easy isolation from mixtures; cyanuric acid was identified by GLC of the tri(trimethylsilyl) derivative. Identification of silylated derivatives by mass spectrometry interfaced with the GLC is then possible.[5]

We found that the amount of nitrogen in the identified components in the waste-water accounted for the total nitrogen measured by the manufacturer.

Standard apparatus and simple methodology thus allow qualitative (and quantitative) analysis of wastes from production of a s-triazine herbicide.

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