Semi-natural products and related substances as alleged botanical pesticides†

István Ujvary*

Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary

Abstract: Plants are a virtually inexhaustible source of structurally diverse biologically active substances. In some cases, however, xenobiotics or their biotransformed derivatives can also be found in extracts from botanical sources. Whilst it is not improbable that plants are able to biosynthesise such compounds, the fact that they have similar, or even identical, structures to those of synthetic materials used in practice indicates a problem in determining their origin and blurs the borderline between natural and man-made substances. The recent identification of a herbicide-like 1,3,5-triazine (halimedin) from an algal source illustrates the difficulty of separating a natural from a semi-natural product, the latter being defined as substance of apparently man-made origin that is re-isolated from natural sources. Additional literature reports shown here demonstrate that pesticide contamination can turn up in unexpected locations. Although these cases appear to be only curiosities, they have the potential of toxicological or ecological risks.

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1 INTRODUCTION

Nature’s chemical diversity continues to be intensively exploited to find new pest control agents with favourable biological and environmental properties. In some cases, however, natural product research has turned up familiar structures with a striking resemblance to established synthetic pesticides thought to have unique structural features. It has also been recognised that pesticidal and other biologically active substances, either of natural or synthetic origin, can pollute remote, non-target areas through run-off, leaching, spray drift, and by long-range transport in the atmosphere. The problem of environmental contamination might be compounded by the extreme biological activity of some recently developed substances capable of affecting non-target organisms even in trace amounts. This communication reports studies examining the possible origin of a recently reported triazine derivative that is remarkably similar to the widely used 1,3,5-triazine herbicides. Additional literature examples reporting common pesticides or related compounds isolated from natural sources are also presented.

A 1,3,5-triazine derivative, named halimedin (1, Fig. 1) has recently been isolated in 0.0028% yield from the alga Halimeda xishaensis Tseng & Deng, a new species collected from the Xisha (Paracel) Islands in the South China Sea. The biological activity of 1, alleged to be the ‘first cyano-s-triazine derivative isolated from a natural source’, was not characterised and no reference was made to the photosynthesis-inhibiting properties of such triazines, and especially to cyanazine (2), a herbicide heavily used in corn, sorghum, cotton and barley, and frequently implicated in groundwater and surface water contamination. The authors also failed to mention that this newly discovered ‘natural product’ (CAS RN [21725-68-8]) had already been described as a herbicide and a depressant.

2 MATERIALS AND METHODS

2.1 General

Cyanazine (approximately 95% pure) was obtained from the herbicide collection of the Plant Protection Institute of the Hungarian Academy of Sciences. Silica gel (0.063–0.20 mm diameter) and analytical grade chemicals were from Reanal (Budapest, Hungary) and used as received. Analytical and preparative thin layer chromatography (TLC) was carried out on 0.25 mm and 1.0 mm thick silica gel plates (E Merck, Darmstadt, Germany), respectively, using disopropyl ether for development and UV (254 nm) light for visualisation. [1H]NMR spectra were obtained in pyridine-d5 at 200 MHz with a Varian spectrometer. Mass spectro-

* Correspondence to: István Ujvary, Chemical Research Centre, Institute of Chemistry, Hungarian Academy of Sciences, PO Box 17, H-1525, Budapest, Hungary
E-mail: ujvary@matavnet.hu
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NMR in mixture B after two months. The six-month-old materials. Mixture A yielded only unchanged cyanazine (Rf 0.32) but a more polar spot (Rf 0.14) appeared in mixture B after two months. The six-month-old mixture B was concentrated and separated by preparative TLC (section 2.1). The two UV-active bands were removed from the plates and eluted with methanol to give cyanazine (32 mg) and a conversion product (10 mg) identified from NMR and MS spectral data as the methanolysis product 1 (Fig 1). \([(^1H)\text{NMR} \delta 1.10(t, 3H), 1.72(s, 3H), 1.79(s, 3H), 3.45(m, 2H), 3.80(s, 3H), 8.10(\text{broad}s, 1H), 8.60(\text{broad}s, 1H). \text{MS (FAB) m/z (relative intensity): 237 (100\%) [M + H\(^+\)], 210 (38\%) [M\(^+\) – HCN], 115 (64\%)}\].

Figure 1. Structures of the algal metabolite halimedin (1), the herbicide cyanazine (2) and a potential cyanazine metabolite 3.

2.2 Cyanazine stability studies

2.2.1 Slow column chromatography

Cyanazine (165 mg) was chromatographed slowly over the course of 8h on a silica gel column (60 g; 60 cm long and 3 cm in diameter) using gradient elution with methanol (5–50% by volume) in chloroform. The recovery of cyanazine was essentially quantitative.

2.2.2 Methanolysis of cyanazine in the presence of silica gel or triethylamine

Cyanazine (50 mg) in methanol (1 ml) was mixed with either silica gel (100 mg) or triethylamine (30 µl), to give mixtures A and B, respectively, and the mixture was stirred at ambient temperature for two weeks, after which it was left to stand at ambient temperature for six months. The mixtures were sampled at regular intervals and analysed by TLC for the presence of new materials. Mixture A yielded only unchanged cyanazine (Rf 0.32) but a more polar spot (Rf 0.14) appeared in mixture B after two months. The six-month-old mixture B was concentrated and separated by preparative TLC (section 2.1). The two UV-active bands were removed from the plates and eluted with methanol to give cyanazine (32 mg) and a conversion product (10 mg) identified from NMR and MS spectral data as the methanolysis product 1 (Fig 1). \([(^1H)\text{NMR} \delta 1.10(t, 3H), 1.72(s, 3H), 1.79(s, 3H), 3.45(m, 2H), 3.80(s, 3H), 8.10(\text{broad}s, 1H), 8.60(\text{broad}s, 1H). \text{MS (FAB) m/z (relative intensity): 237 (100\%) [M + H\(^+\)], 210 (38\%) [M\(^+\) – HCN], 115 (64\%)}\].

3 RESULTS AND DISCUSSION

The striking structural similarity of halimedin (1) and cyanazine (2) prompted the author to consider the algal product as a possible artefact arising from methanolysis during reported chromatographic purification of cyanazine-containing sample specimens. Solvolysis of 6-chloro-1,3,5-triazine herbicide residues during sample clean-up and chromatography had been suggested earlier.8,9 Furthermore, slow conversion of simazine and, to some extent, atrazine in methanol into their respective 6-methoxy derivatives had been demonstrated upon storage over several months.10 Recent reports have shown that the unexpected isolation of pesticides or related substances from natural, mostly botanical, sources is not uncommon. One of the early examples is the insect repellent deet that was isolated from females of the pink bollworm moth, Pectinophora gossypiella Saunders.18 The highly active juvenoid, fenoxycarb, presumably can drift considerable distances from the site of application, and is the likely cause of the ‘non-spinning syndrome’ in the highly sensitive Bombyx mori L larvae fed with contaminated mulberry leaves in Italy, Japan and France.19,20 The related juvenoid diofenolan is suspected to be causing similar problems in South Africa.21

Disturbingly, synthetic pesticides have recently been identified as the bioactive principles of some ‘botanical preparations’ sold in China and Japan. Thus, the neurotoxic pyrethroid insecticide cypermethrin was the principal active ingredient of the plant extract ‘Muso’.22 Further compounds and the ‘natural plant extract formulations’ also reported23 were the insect growth regulator diflubenzuron in ‘Sin-Muso’; cypermethrin in ‘Musikoro’ and ‘Nurse Green’, deltamethrin in ‘New Mushigie’, fenvalerate in ‘Mushigie’, the fungicide triadimefon in ‘Kensogen-Ku’ and the herbicide oxadiazon in ‘Kensogen-Chi’.

Some xenobiotics have also been shown to bind covalently to plant constituents.24 The structural aspects of the co-polymerisation of chloroaniline metabolites of acetanilide or ary lurea herbicides and the insect control agent diflubenzuron into lignin have been well studied.24–27
An interesting case of natural pesticide-like molecules is that of the South Asian arrow poison with metaquat (1,1′-dimethyl-3,3′-bipyridinium) as the main active ingredient with curare-like activity. This genuine plant-derived muscle relaxant is an isomer of the widely used bipyridyl herbicide paraquat which, if manufactured from insufficiently purified bipyridyl intermediates, could contain synthetic metaquat as a contaminant.

4 CONCLUSIONS

In summary, the ‘unnatural’ origin of the alleged natural product halimedin is a most surprising finding. Although definitive proof is lacking I suggest that this algal triazine derivative is an environmental contaminant originating from the widely used herbicide, cyanazine. This example illustrates the difficulties of separating a natural product from a semi-natural one, the latter being defined as a man-made substance (re)isolated from natural sources. Furthermore, both persistent pesticides and several widely used modern and safe pest control products can become environmental pollutants and turn up at unexpected locations. Trace amounts of these synthetics or their metabolites or degradation products can contaminate not only food crops but also plants that are used to make botanical pesticides and thus contribute to or be themselves the sources of the biological activity of these ‘natural products’. The borderline separating the realms of the natural and the man-made has become blurred and it is often difficult to tell what is natural and what is not. Although the cases presented appear to be curiosities, they have the potential of toxicological or ecological risks.

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REFERENCES