DEGRADATION OF 4-CHLORO-4′, 6-BIS(ISOPROPYLAMINO)-6′-ETHYLAMINO-DI(*s*-TRIAZINYL) SULPHIDE BY PLANT TISSUE

P. MILDNER, BRANKA MIHANOVIĆ and M. POJE
Laboratory of Biochemistry, Technological Faculty,
University of Zagreb, Zagreb, Croatia, Yugoslavia

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1. Introduction

The biochemical glucosidation of *s*-triazines in plant tissue has not been reported so far, although a considerable amount of work has been done to follow the metabolic fate of various biologically active *s*-triazines. Three main degradative pathways are evident: hydrolysis at C-atom 2, and to a minor extent *N*-dealkylation at C-atoms 4 and 6, as well as splitting of the triazine ring [1]. At present, only limited results concerning the biochemical hydrolysis of methylthiotriazines within the plant are known. The corresponding hydroxytriazines were detected as the sole metabolites in different plant species. Sulfoxide and/or sulfone derivatives were claimed to be hypothetical intermediates during the removal of the entire methylthio group [2,3], but all our attempts to obtain these compounds by chemical synthesis failed, and we could not even detect their appearance. We found that methylthiotriazines are relatively stable toward hydrolytic attack, but in the presence of an oxidant, hydrolysis proceeds faster with formation of corresponding hydroxytriazine and concomitant formation of dimethyldisulphide, instead of methylmercaptan, which was the main product when hydrolysis occurred in the absence of an oxidant [4]. The same course of the reaction could be followed during the hydrolysis of the di(*s*-triazinyl) sulphides*.

Studying the uptake of 4-chloro-4′, 6-bis(isopropylamino)-6′-ethylamino-di(*s*-triazinyl) sulphide by cucumber plants (*Cucumis sativus*) we found that this compound was transformed within the tissue into 2-ethylamino-4-*S*-(β-D-glucopyranosyl)-6-isopropylamino-*s*-triazine and 2,4-dihydroxy-6-isopropylamino-*s*-triazine, as main products. Moreover, we found traces of 2-mercapto-4-ethylamino-6-isopropylamino-*s*-triazine and the corresponding disulphide, as well as 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine. The *S*-glucoside was isolated by preparative TLC and its constitution confirmed by comparison of the unknown with the synthetic glucoside of 2-mercapto-4-ethylamino-6-isopropylamino-*s*-triazine, prepared in our laboratory.

2. Experimental

Seeds of cucumber were purchased from a commercial source. Plants were grown under controlled conditions. Ten day old seedlings were treated with a water suspension of 4-chloro-4′,6-bis(isopropylamino)-6′-ethylamino-di-(*s*-triazinyl) sulphide (0.1 g/ml) for 5 days. The treated seedlings showed no signs of chlorosis, but the turgor was greatly diminished in comparison with untreated plants. The plants (2860 g fresh wt.) were picked up, washed with tap water and ground with clean sand. The filtrate was added to an equal volume of

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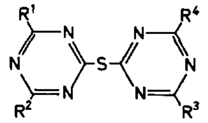
*This is a new class of *s*-triazine compounds of general formula: where R^1, R^2, R^3, and R^4 are alkylamino groups and chlorine, respectively. Their synthesis will be published elsewhere.



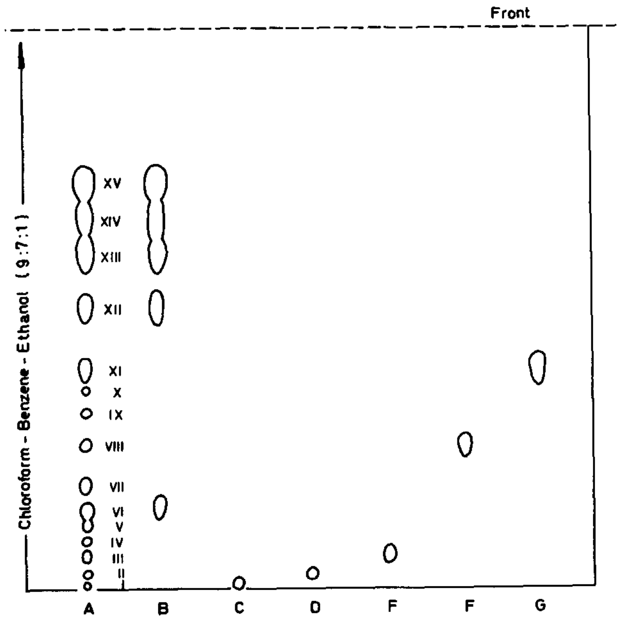


Fig. 1. Thin-layer chromatogram of extracted plant material (A). Layer: silica gel. Visualisation: UV (254 nm). B: extract of untreated plants; C: 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine; D: 2,4-dihydroxy-6-isopropylamino-*s*-triazine; E: 2-mercapto-4-ethylamino-6-isopropylamino-*s*-triazine; F: 4,4'-bis(ethylamino)-6,6'-bis(isopropylamino)-di(*s*-triazinyl) disulphide; G: 4-chloro-4',6-bis (isopropylamino)-6'-ethylamino-di(*s*-triazinyl) sulphide.

methanol and kept for 24 hr in the refrigerator. The deproteinized filtrate was concentrated in vacuo and extracted with chloroform. The combined extracts were dried and solvent evaporated, yielding 0.93 g of a highly hygroscopic material (A). Using TLC technique we could demonstrate the presence of 15 components (fig.1).

The identification of the unknown spot VII was carried out applying a novel two-dimensional TLC technique. The chromatogram was first run in one direction, and after drying a hydrolytic agent applied along the line of the spots. Finally, chromatography was performed at a right angle to the first direction (see fig.2). Spot VII gave on hydrolysis 2-mercapto-4-ethylamino-6-isopropylamino-*s*-triazine. Repeating the described procedure, but this time changing the solvent system in second direction, glucose was detected as the second product of hydrolysis of VII (see fig. 3).

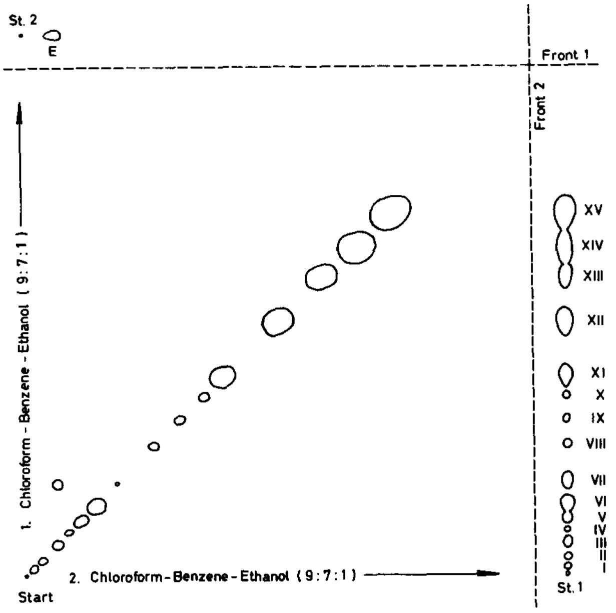


Fig.2. Two-dimensional separation of extracted plant material (A) on 0.25 mm thick silica gel layer. After development in the first direction the plate has treated with 10% H_2SO_4, then with 10% NaOH along the line of the spots, and kept for 1 hr at 70°. Visualisation: UV (254 nm). E: 2-mercapto-4-ethylamino-6-isopropylamino-*s*-triazine.

This led us to believe that the unknown substance might be a glucoside of 2-mercapto-4-ethylamino-6-isopropylamino-*s*-triazine. To isolate this metabolite, the deproteinized, dried extract (A) from treated plants (0.9 g) was dissolved in anhydrous ether and submitted to preparative chromatography on 1.0 mm thick layer of silica gel with chloroform–benzene–ethanol (9:7:1) as the solvent. The position of glucoside was detected in UV light, the spot scraped off and percolated in absolute methanol. After removal of the solvent a highly hygroscopic crystalline product was separated, m.p. 199–201° (dec.).

Anal. $C_{14}H_{25}N_5O_5S$ Calc'd. : C 44.79 ; H 6.71
Found : C 44.58 ; H 6.81

By the reaction of α-acetobromoglucose and sodium 2-mercapto-4-ethylamino-6-isopropylamino-*s*-triazine, followed by saponification of tetraacetate, 2-ethylamino-4-*S*-(β-D-glucopyranosyl)-6-isopropylamino-*s*-triazine was obtained. The identity of the synthetic compound and isolated metabolite was proved by

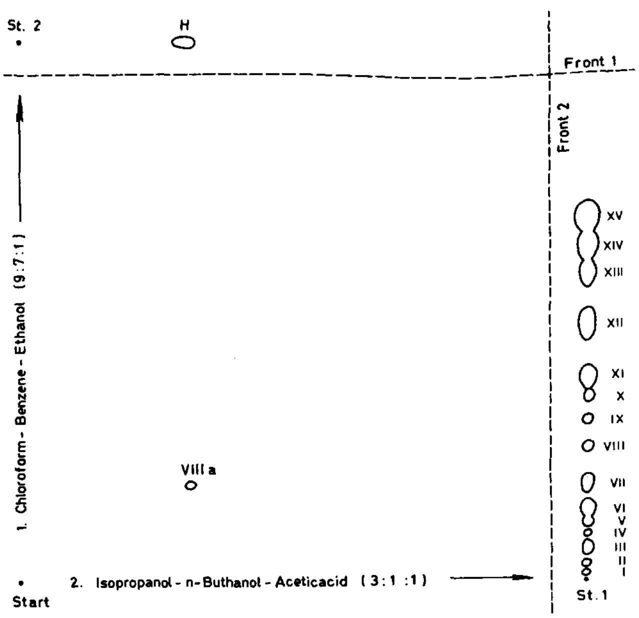


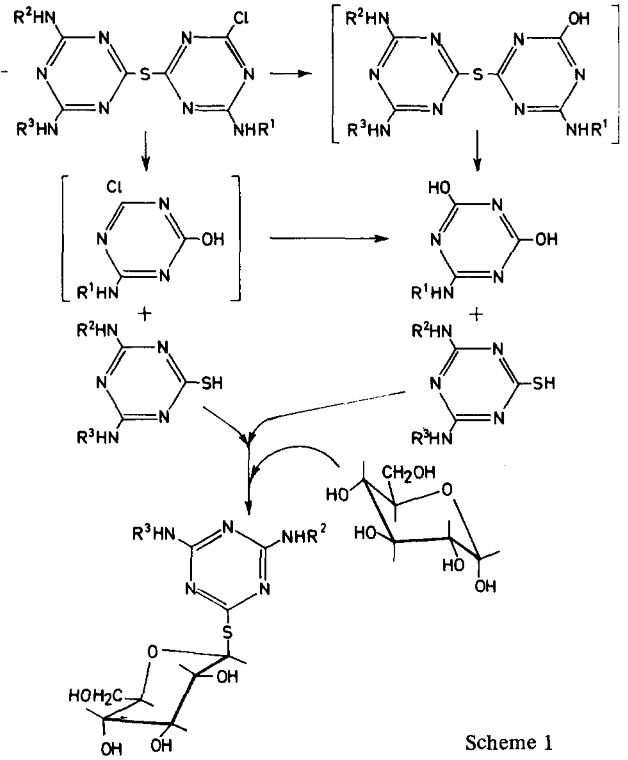
Fig. 3. Two-dimensional separation of extracted plant material (A). Adsorbent: silica gel. After development in the first direction, 10% H_2SO_4 followed by 10% NaOH is applied along the line of spots. Visualisation: UV (254 nm) and Tollens reagent for spot VIIIa and glucose (H).

comparison of their R_f values, melting points, as well as the infrared and NMR spectra.

3. Discussion

On the basis of the results obtained, the pathways for the degradation of di(*s*-triazinyl) sulphides in the plant tissue cound tentatively be proposed as in scheme 1.

Hydrolytic cleavage of the thioether bond in di(*s*-triazinyl) sulphide affords two triazine derivatives: 2-mercapto-4,6-bis(alkylamino)-*s*-triazine and 2,4-dihydroxy-6-alkylamino-*s*-triazine. Therefore the hydrolysis does not represent a complete detoxication mechanism, because the 2-mercaptotriazine retains an intermediate phytotoxicity. In the plant this metabolite is conjugated with glucose and the resultant *S*-glucoside seems to be the end product of the detoxication process.



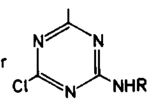
Scheme 1

The fact that the methylthiotriazines and di(*s*-triazinyl) sulphides reveal striking similarities in their chemical behaviour permits us to propose a working hypothesis for their metabolic degration:

$$R^1\text{-}S\text{-}R^2 \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} R^1SH + R^2OH$$

$$2\ R^1SH \xrightarrow{K_2} R^1SSR^1$$

$R^1, R^2 = -CH_3$ or



In this mechanism K_2 is the rate-determining step, and this would explain the increased rate of hydolysis observed in the presence of an oxidant. This standpoint is supported by our study on the behaviour of the thioether bond in *s*-triazine compounds; the sulphur-containing products were free mercapto derivatives, or the corresponding disulphides. No formation of sulphoxides and/or sulphones has ever been observed.

References

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