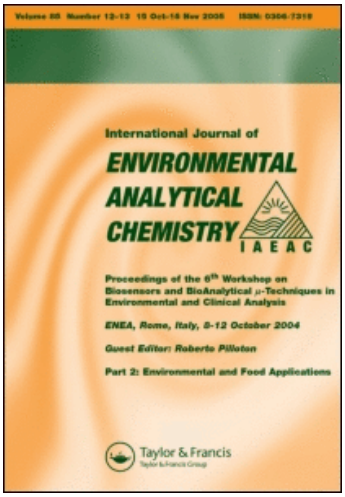

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Evidence for Thiamine Cleavage in SO_2-polluted Plants

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KEY WORDS: Thiamine cleavage, SO_2, plants, vitamin B_1.

Treatment of thiamine or vitamin B_1 with HSO_3^- ions results in the formation of 4-methyl-5-β-hydroxy-ethyl-thiazole and 2-methyl-4-amino-sulfomethyl-pyrimidine(pyrimidinesulfonic acid). A method is described to separate these cleavage products by TLC. Pyrimidinesulfonic acid was identified with fluoresceine-$AgNO_3$. Fumigation of lettuce, pea and maize seedlings with SO_2 induces the formation of pyrimidinesulfonic acid. From this it is concluded that SO_2-pollution leads to cleavage of thiamine in plants.

INTRODUCTION

Thiamine or vitamin B_1 is destroyed in aqueous solution by bisulfite ions.[1–4] According to Leichter and Joslyn[4] the cleavage of the thiamine molecule by HSO_3^- is caused by a nucleophile mechanism of reaction. In this procedure 4-methyl-5-β-hydroxy-ethyl-thiazole and 2-metyhl-4-amino-sulfomethylpyrimidine(pyrimidinesulfonic acid) appear as cleavage products (Figure 1).

According to Leichter and Joslyn[4] and the studies of the authors of this paper the rate of cleavage depends on the concentration of bisulfite, the pH value and the temperature. In wet foods enriched by caseine containing SO_2 a loss of thiamine could also be demonstrated, the reason of which is supposed to be the destruction of the vitamin by bisulfite ions.[5] Rats, fed with a normally sufficient quantity of vitamin, equally showed reduced growth rates if dissolved sodium bisulfite was injected intraperitoneally.[6] Cremer and Hötzel[6] attribute these effects to a secondary lack of thiamine and believe that thiamine seems to be involved in the detoxification of SO_2.

In experiments with SO_2-polluted plants we were able to demonstrate a decrease of the content of vitamin B_1, when externally visible SO_2-damages (nekrosis) appear.[7] We suppose that this decrease of vitamin B_1 is due to a cleavage of thiamine molecules by bisulfite. The latter is known to occur in SO_2 treated plants.[8,9] The purpose of the present paper was to demonstrate the cleavage of vitamin B_1 in SO_2-polluted plants. We proved this idea by isolating and identifying the pyrimidinesulfonic acid, as the thiazole compound can appear as such in the plants (cf. the biosynthesis of the vitamin B_1).[10,11]

In the present paper we give a report of the cleavage of thiamine in SO_2-polluted plants and describe a TLC procedure and a color reagent to detect the pyrimidinesulfonic acid.

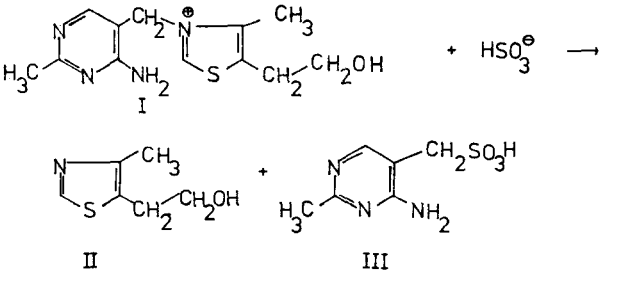

FIGURE 1

Scheme of the thiamine cleavage by bisulfite ions
I = Thiamine
II = 4-methyl-5-β-hydroxy-ethyl-thiazole
III = 2-methyl-4-amino-sulfomethyl-pyrimidine

MATERIAL AND METHODS

1) Plant material: Seedlings of lettuce, pea and maize were cultivated and fumigated as described elsewhere.[12,13]

2) Thin layer chromatography (TLC): Sheets (20 × 20 cm) were coated with silica gel (Merck) G and HF_{254} (9:1) and activated at 40°C.

Chemicals: As standards we used thiamine, thiochrome, 4-methyl-5-β-hydroxy-ethyl-thiazole and 2-methyl-4-amino-sulfomethyl-pyrimidine. Thiochrome was prepared by oxydizing thiamine with $K_3Fe(CN)_6$ in alkaline solution, pyrimidinesulfonic acid and the thiazole compound were obtained by cleavage of thiamine with $NaHSO_3$. After initial crystallization pyrimidinesulfonic acid was separated and purified by repeated crystallization in water. After alkalization of the solution with NaOH the thiazole

compound was extracted with chloroform and purified by alternating shaking with chloroform and hydrochloric acid. The chromatograms were developed in the following solvents:

a) Pyridine: isobutanol: water (4:1:1)[14]
b) Acetonitrile: water: formic acid (4:1: pH adjusted to 2.54)[15]
c) Acetonitrile: water: formic acid (4:1: pH adjusted to 4.03)[15]
d) Acetonitrile: water: acetic acid (4:1: pH adjusted to 4.03)†
e) Acetonitrile: water: NH_3: acetic acid (4:1: pH 8.5: pH 7.85)†

The detection of the compounds was accomplished by UV-light at 254 nm (for absorbing compounds) and at 366 nm (for fluorescent compounds) as well as by color reaction with the following two spray reagents:

a) A mixture of 50 ml fluoresceine-Na solution (0.2 g in 100 ml ethanol) and 10 ml of a 10% aqueous solution of $AgNO_3$.[16]

b) 2% aqueous solution of $AgNO_3$. To the $AgNO_3$ solution ammonia is added prior to spraying until precipitation is just resolved.

3. Extraction and TLC of plant material: Homogenized plant material was heated shortly and centrifuged. The residue was reextracted with some hot water and the combined extracts were adjusted to a defined volume. Aliquote volumes of the extracts of polluted and control plants were spotted on a thin layer plate and separated with the indicated developing solvents. In order to clearly identify the pyrimidinesulfonic acid, a sample of plants enriched with pyrimidinesulfonic acid and two pure standards were separated and developed on the same plate.

RESULTS AND DISCUSSION

As was shown by the separation of the standards (as for the preparation see materials and methods) the developing solvent pyridine/water/isobutanol is not satisfactorily suited for the resolution, because the Rf-values differ too much from one another. Different Rf-values are indicated in the literature, too.[15] More uniform Rf-values can be obtained by buffering the solvent with a mixture of acetic acid and sodium acetate (1 M, 1:1). Thus the Rf-value of thiamine increases, but the detection in UV-light and the color detection of the standards remain still difficult.

Satisfactory results were achieved by using the developing solvent indicated by Waring *et al.*[15] But we could find that in the alkaline range the solvent should be adjusted with ammonia to pH 8–9 and be retitrated with formic acid or acetic acid to pH 7.85. The Rf-values agree fairly well with those quoted in the literature. In using formic acid we could find a higher

† Modified according to Waring *et al.*[15]

TABLE I

Rf-values, UV-detection and color of the standards

Solvent pH	2.54	4.03	7.85[a]	UV	Fluorescein-$AgNO_3$	$AgNO_3-NH_3$
Thiamine	0.16	0.05	0.08	A_{254}	brown	white to yellow
Thiochrome		0.26	0.33	F_{366}	brown	faint white
Pyrimidine-sulfonic acid	0.45	0.37	0.40	A_{254}	yellow	blue-grey
Thiazole compound	0.81	0.79	0.85	A_{254}	brown	white to yellow
Sulfanilic acid	0.61		0.60 + 0.68	A_{254}	yellow	grey

[a] = modified according to Waring *et al.*[15]
A_{254} = absorbing
F_{366} = fluorescent.

value for thiamine. The pyrimidinesulfonic acid separated by this treatment can be identified undoubtedly with fluoresceine-$AgNO_3$ (yellow spot on a ground which was faint brown to pink). The spots of thiamine are brownish and those of the thiazole compound are similar but relatively brighter by far. The detection with $AgNO_3$-NH_3 is less sensitive, but it may be sensitized by exposing the sprayed plates to UV-light. Then blue-grey zones arise in the range of the pyrimidinesulfonic acid. The UV-detection showed that the range of the pyrimidinesulfonic acid does not exactly agree with the one of the fluoresceine-$AgNO_3$. Therefore impurities seemed to be involved. Experimenting with sulfanilic acid, similar effects could be obtained, so that the observed effect might be due, therefore, to an effect of concentration or a partial masking of the substance.

In Table I the results are given which were achieved with pure standards. These results indicate that pyrimidinesulfonic acid can well be separated by the modified solvent according to Waring *et al.*[15] and as well be localized by fluoresceine-$AgNO_3$.

The studies of fumigated and control plants gave the following results: The detection of the pyrimidinesulfonic acid with all chromatograms was positive as well with $AgNO_3$-NH_3 as with fluoresceine-$AgNO_3$. With lettuce only a slight difference in the content of pyrimidinesulfonic acid of fumigated and control plants became obvious. The leaves of these fumigated plants were strongly damaged after a five days' exposure to SO_2. Pea seedlings exposed to SO_2 for 12 days showed a significantly higher content of pyrimidinesulfonic acid than the corresponding controls, whereas plants after a fumigation of 14 days only showed a slight but clear increase towards the controls. Seedlings of maize, too, after an exposure of 20 days to SO_2 had significantly more pyrimidinesulfonic acid.

A remarkable result of our experiment is the fact that pyrimidinesulfonic acid could be detected in unfumigated plants, too. The reason for its origin is not yet known, but it may possibly be that thiamine or a similar compound is involved in the metabolism of sulfur. However, we may conclude from our results without any doubt that in SO_2-polluted plants a cleavage of thiamine takes place, because the detection of the pyrimidinesulfonic acid by the method described above is specific and as clearly more pyrimidinesulfonic acid appears in SO_2-polluted plants.

References

1. E. R. Buchman, R. R. Williams, and J. C. Keresztesy, *J. Amer. Chem. Soc.* **57**, 1849 (1935).
2. R. R. Willliams, R. E. Waterman, J. C. Keresztesy, and E. R. Buchman, *J. Amer. Chem. Soc.* **57**, 536 (1935).
3. R. R. Williams, E. R. Buchman, and A. E. Ruehle, *J. Amer. Chem. Soc.* **57**, 1093 (1935).

4. J. Leichter and M. A. Joslyn, *Biochem. J.* **113,** 615 (1969).
5. M. A. Joslyn and J. Leichter, *J. Nutrition* **96,** 89 (1968).
6. H. D. Cremer and D. Hötzel, *Bibl. Nutr. Dieta.* **8,** 126 (1966).
7. H.-J. Unzicker, H.-J. Jäger, and L. Steubing, *Angew. Bot.* (1974, in press).
8. J. Weigl and H. Ziegler, *Planta* **58,** 435 (1962).
9. N. Faller and W. Höfner, Z. Pflanzenernährung, Düngung u. Bodenkunde **121,** 111 (1968).
10. H. Mitsuda, T. Tanaka, Y. Takii, and F. Kawai, *J. Vitaminol.* **17,** 89 (1971).
11. H. Mitsuda, T. Tanaka, and F. Kawai, *J. Vitaminol.* **16,** 263 (1970).
12. H.-J. Jäger and L. Steubing, *Angew. Bot.* **44,** 209 (1970).
13. H.-J. Jäger, E. Pahlich, and L. Steubing, *Angew. Bot.* **46,** 199 (1972).
14. D. B. Johnson and T. W. Goodwin, *Biochem. J.* **88,** 62 (1963).
15. P. P. Waring, W. C. Goad, and Z. Z. Ziporin, *Anal. Biochem.* **24,** 185 (1968).
16. E. Merck, Anfärbereagenzien für Dünnschicht- und Papierchromatographie (Mittlg. d. Fa. E. Merck A.G.), Darmstadt (1970).