On the transformation of dimethyldithiocarbamate into its β -glucoside by plant tissues

In a recent publication Dekhuijzen¹ reported that after uptake of the fungicide sodium dimethyldithiocarbamate by the roots of cucumber plants this compound is transformed in the tissues into three other fungitoxic agents which can be distinguished by their R_F values of 0.03, 0.27 and 0.58 on paper chromatograms developed with propanol–water (85:15, v/v).

These compounds could be detected on the paper by direct spraying of the strips with a spore suspension of the mould Glomerella cingulata and subsequent incubation. The toxic action of all three unknown compounds could be antagonized by the addition of L-histidine or sodium dibutyldithiocarbamate to the spore suspension before spraying, which indicates that the three compounds have the same mode of action as dimethyldithiocarbamate itself². This led us to believe that the unknown substances might be derivatives of dimethyldithiocarbamate which have been formed enzymically and which, in contact with mould enzymes, can again liberate certain amounts of the toxic dithiocarbamate ion. The three compounds were also found in broad-bean plants after uptake of sodium dimethyldithiocarbamate. They were formed as well by incubation of sliced pods of broad beans in a solution of dimethyldithiocarbamate. When potato chips were incubated in a solution of dimethyldithiocarbamate only the compounds with $R_F = 0.27$ and $R_F = 0.58$ were found.

By this latter method we have now succeeded in isolating and characterizing the compound with the R_F value of 0.58. To this end 50 kg of potato chips were incubated at 18–20° under continuous stirring and aeration in a vessel containing 100 g of sodium dimethyldithiocarbamate dissolved in 50 l of water. After an incubation period of 2 days the juice pressed from the frozen and thawed chips was treated with an excess of lead acetate at pH 7.0. The precipitate was discarded and the excess lead acetate removed with sodium phosphate. The resulting filtrate was then concentrated *in vacuo* and extracted five times with an equal volume of butanol. After concentrating these butanol extracts the resulting syrup was submitted to a 200-cycles Craig partition between butanol and water. The presence of the dithiocarbamate derivative was detected in the fractions by the use of the iodine–sodium azide reagent of Feigl³.

The positive fractions were pooled and concentrated again *in vacuo*. After dissolving the resulting syrup in an equal volume of water and chilling, needle-shaped crystals which contained water of crystallization began to separate.

Paper chromatography of this crystalline product with propanol-water (85:15, v/v) as a solvent followed by incubation of the strip with the mould *Glomerella cingulata* showed a fungitoxic compound at $R_F = 0.58$. On similar chromatograms the reagent of Feigl was decolorized and ammoniacal AgNO₃ was reduced at this same R_F value of 0.58.

On acid hydrolysis the active compound yielded a reducing substance with the R_F value of glucose. This finding suggested that the compound might be a glucoside of dimethyldithiocarbamate. This could readily be confirmed by comparison of the

$$CH_3$$
 $N-C-S-CH-(CHOH)_3-CH-CH_2OH$
 CH_3

unknown compound with the available synthetic glucoside of dimethyldithiocarbamate prepared by Dr. Pluijgers of this Institute by the reaction of acetobromoglucose and sodium dimethyldithiocarbamate, followed by saponification of the tetraacetate. This synthesis is strongly indicative for a β -configuration of the glucoside. The identity of the synthetic compound and the biochemically produced compound was proved by comparison of their R_F values in different solvent systems, ultraviolet and infrared spectra as well as their optical rotation.

The dimethyl dithiocarbamate-glucoside is only a weak fungicide, its minimal growth-inhibiting concentration being approx. 0.05 % for G. cingulata and other moulds tested.

In the large-scale experiment approx. 10 % of the sodium dimethyldithiocarbamate was converted into the glucoside, a much smaller percentage was transformed into the fungitoxic compound with $R_F = 0.27$ whereas most of the originally added compound had been chemically decomposed in the outer medium as a result of its slightly acid reaction.

The amount of dithiocarbamate-glucoside present in the sap pressed from hypocotyls and cotelydons of cucumber plants which had stood for 2 days with their roots in 0.015 % of sodium dimethyldithiocarbamate, will be approx. 0.001-0.002 %. If instead of sodium dimethyldithiocarbamate the glucoside was fed to the roots, the presence of the fungitoxic compounds with R_F values of 0.05 and 0.27 could be demonstrated as well. This observation suggests interconvertibility of the compounds within the plant.

Though the biochemical glucosidation of dithiocarbamates was unknown up till now, it is strongly reminiscent of the glucosidation of phenolic compounds^{4,5} and of aminotriazole⁶ in the plant.

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