90 % on a weight basis and 94 % by ultraviolet-light absorption The recoveries of the amino acids were quantitative by radioactivity measurement and by ultraviolet-light absorption for tryptophan The separations were equivalent for each of the amino acids

When the proteolipid and amino acids were mixed in aqueous media as described above, the binding of amino acids was also demonstrated by use of the starch column, as illustrated in Fig 2 The proteolipids in the incubation mediums were recovered in $CHCl_3-CH_3OH$, washed 7–9 times, applied to the column, and eluted with the same solvent

The presence or absence of the compounds, tested above for their effect on the distribution coefficients, had no effect on the congruence of the radioactivity and proteolipid elution peaks It was further seen that the addition of the following aqueous media to the eluant (1 vol to 10 vol of eluant) gave identical elution patterns water, 1 0 *M* formic acid, 0 1 *M* ammonium formate (pH 3 7), 0 01 *M* Tris–chloride (pH 7 57), 0 01 *M* $CaCl_2$ In each case the fraction with the maximum specific radioactivity also had the maximum dry weight, ultraviolet absorbancy and radioactivity

The two techniques used here demonstrate the existence of strong bonds between the amino acids and the proteolipid, which are not dissociated by the treatments used The intervention of enzymes in the binding seems to be excluded

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## On the transformation of the fungicide sodium dimethyldithiocarbamate into its alanine derivative by plant tissues

DEKHUIJZEN[1,2] reported that the fungicide sodium dimethyldithiocarbamate is transformed by plants 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 which were developed with propanol – water (85 15, v/v) and subsequently sprayed with the mould *Glomerella cingulata* in a nutrient solution A second, chemical, test for the demonstration of these compounds on the strip is the iodine–sodium azide reagent of FEIGL[3] which indicates the presence of C = S groups

In a previous publication[4] we reported the isolation of one of the compounds which is formed enzymically when potato slices are incubated in a solution of sodium dimethyldithiocarbamate This compound with the $R_F$ value of 0 58 proved to be the $\beta$-glucoside of dimethyldithiocarbamate

We have now succeeded in isolating and identifying the compound with $R_F = 0\ 27$ It was obtained together with the glucoside by incubation of 50 kg of potato slices in 50 l of water containing 100 g of sodium dimethyldithiocarbamate as previously described[4] The compound appeared to be mainly present in the sap pressed from the slices This sap was treated with lead acetate at pH 7 0 The precipitate was discarded and the excess of lead acetate removed with sodium phosphate The resulting filtrate was then concentrated *in vacuo* and submitted to a countercurrent distribution between butanol – ethanol – water (52 11 37, v/v/v) By this procedure the compound with $R_F = 0\ 27$ was purified and it was also separated from the glucoside The fractions which contained the compound with $R_F = 0\ 27$ were pooled and concentrated A further purification could be achieved by partition chromatography on a cellulose column with propanol – water (85 15, v/v) as a solvent The eluates containing the compound with $R_F = 0\ 27$ were taken to dryness and the residue dissolved in the minimum amount of boiling propanol – water (85 15, v/v) After standing for some hours compound I crystallized as glistening flakes It was recrystallized from the same solvent and dried *in vacuo* at 80° over $P_2O_5$ for 1 h Incubation of 50 kg potato slices with 100 g sodium dimethyldithiocarbamate dissolved in 50 l of water yielded 350 mg of the pure compound I, as well as 10 g of the glucoside Calc for $C_6H_{12}N_2O_2S_2$ C, 34 59, H, 5 82, N, 13 45, S, 30 79 Found C, 34 76, H, 5 67, N, 13 33, S, 30 0

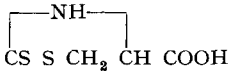
Compound I appeared to be a neutral, optically active compound giving a positive ninhydrin reaction Its empirical formula suggested that an amino acid with 3 carbon atoms might be attached to the dimethyldithiocarbamate structure In view of the fact that we had meanwhile demonstrated[5] that sodium dimethyldithiocarbamate is converted by microorganisms into $\gamma$-(dimethylthiocarbamoylthio)-$\alpha$-aminobutyric acid $(CH_3)_2$ N CS S $(CH_2)_2$ $CHNH_2$ COOH, we assumed that compound I might be the corresponding alanine derivative, *viz* $\beta$-(dimethylthiocarbamoylthio)-$\alpha$-aminopropionic acid To gain definite proof the DL-form of this compound was synthesized by Dr PLUIJGERS and Mr BERG at this Institute This synthesis will be reported elsewhere Infrared spectra, $R_F$ values as well as behaviour to hydrolysis proved to be identical We therefore conclude that compound I is $\beta$-(dimethylthiocarbamoylthio)-alanine, presumably the L-form

$$(CH_3)_2\ N\ CS\ S\ CH_2\ CHNH_2\ COOH \qquad (I)$$

From the mother liquors of compound I another compound (II) also giving a positive reaction with iodine–sodium azide could be isolated This compound is not fungitoxic and could therefore not be detected with the Glomerella technique

The peculiar behaviour of compound I to acid hydrolysis led us to the identification of compound II Although, according to expectation, serine and cystine were formed by acid hydrolysis of I, the greater part is converted into another compound which could be isolated and crystallized To our surprise this substance appeared to be identical with II as followed from melting point, $R_F$ value and analysis Further

investigation revealed the compound to be optically active thiazolidine-2-thione-4-carboxylic acid




(2)

We have reason to suppose that the presence of II in the mother liquors is due to non-enzymic decomposition of I

TABLE I

GROWTH-INHIBITING ACTIVITY OF SODIUM DIMETHYLDITHIOCARBAMATE AND ITS ALANINE DERIVATIVE

| | *Minimal conc giving complete growth inhibition*§ | |
|---|---|---|
| | *Sodium dimethyldithiocarbamate (p p m )* | *Compound I (p p m )* |
| *Glomerella cingulata** | 1 | 2 |
| *Aspergillus niger*** | 1 | 20 |
| *Cladosporium cucumerinum*** | 1 | 20 |
| *Botrytis allii** | 0 5 | 2 |
| *Hansenula anomala** | 1 | 10 |
| *Saccharomyces cerevisiae**** | 20 | >50 |
| *Bacillus subtilis** | 2 | 10 |
| *Bacterium coli** | >100 | >100 |

* Glucose mineral salts agar (pH 6 5)
** Idem + biotin
*** Malt agar (pH 6 5)
§ Incubation time 3 days

Comparable values for the activity of sodium dimethyldithiocarbamate and its synthetic DL-alanine derivative against various micro-organisms are given in Table I We assume that toxicity of the latter compound is due to enzymic cleavage leading to the liberation of dimethyldithiocarbamate ion The activity of the L-alanine derivative was found to be equal to that of the DL- derivative This does not exclude the possibility that the L- form is split more readily that the D- form, as the accuracy of the test used is insufficient to solve this problem

Plant tissues thus appear to be able to convert dimethyldithiocarbamate both into its $\beta$-glucoside and—to a much smaller extent—into its alanine derivative Aminotriazole is the only other foreign compound known to be converted into an alanine derivative by plant tissues[6] It is remarkable that also in this case the glucoside is found as well[7]

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