bindung wurde chemisch durch Zusammenreiben von Malonylhalbchlorid mit D-Tryptophan unter Feuchtigkeitsausschluss in Anlehnung an die Darstellung von *S*-Malonyl-*N*-Caprylcysteamin[6] hergestellt, und verhielt sich in jeder Weise mit dem natürlichen Produkt identisch. Das im Konjugat vorliegende D-Tryptophan zeigt weite Verbreitung im Pflanzenreich und konnte natürlich vorkommend in Früchten oder vegetativem Gewebe folgender Arten chromatographisch nachgewiesen werden: *Astragalus baeticus, Caragana arborescens, Cotoneaster adpressa, Dryopteris filix-mas, Lathyrus platyphyllus, Malus silvestris, Myrrhis odorata, Papaver somniferum, Phaseolus coccineus, Pirus communis, Portulaca oleracea, Solanum tuberosum, Sorbus thianshanica, Vicia faba.*

Die natürliche Konzentration an Malonyl-Tryptophan im Apfel (Golden Delicious) beträgt zirka 0.8 μMol/kg Frischgewicht und hängt vom Reifegrad der Frucht ab.

Unseres Wissens handelt es sich hiermit um das erste natürliche Vorkommen einer D-Aminosäure in höheren Pflanzen und um das erste natürliche Vorkommen von D-Tryptophan in der Natur.

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SC 2219

Phenolic compounds in the cockroach ootheca

The hardening and darkening of the cockroach ootheca is probably the best understood example of sclerotization, the process in which proteins are stabilized by the tanning action of quinones. In the ootheca the *o*-quinone is derived from protocatechuic acid (3,4-dihydroxybenzoic acid)[1–3], although there is no definite chemical proof for the final step (cross-linking of the protein) in the reaction. DENNELL[4], KENNAUGH[5] and MALEK[6] have recently reported the identification of "aminophenols" in alkaline hydrolysates of hard insect cuticles and they have used this as evidence to support theories of *p*- or *o*-quinone tanning. The chemical basis for the identification of these "aminophenols" cannot be considered satisfactory. The cockroach ootheca should be an ideal material for such a study and this communication records attempts to detect and identify phenols in hydrolysates of oothecae.

Powdered, fully hardened oothecae from *Periplaneta americana* (for preparation see HACKMAN AND GOLDBERG[7]) were hydrolysed with alkaline stannite[8], the hydroly-

sate acidified and extracted with peroxide-free ether. Small amounts of white, unhardened ootheca and of very lightly coloured ootheca were also hydrolysed in the same manner. These small amounts of material were obtained from partially formed oothecae, which had been immersed in aqueous cyanide to inactivate phenol oxidases before being washed free of contents. The ethereal extracts were evaporated, the residues dissolved in 10% isopropanol and the solutions examined by paper chromatography using as solvents benzene–acetic acid–water (125:72:3, v/v) and butanol–acetic acid–water (77:6:17, v/v). The volume of 10% isopropanol used was in the proportion of 0.5 ml for each gram of oothecal material hydrolysed. Phenolic compounds were detected with $FeCl_3$, Folin–Ciocalteu, $AgNO_3$ and Pauly reagents, amino compounds with ninhydrin and indole-type compounds with Ehrlich reagent.

LUGG[8] showed that when tyrosine and tryptophan or proteins are hydrolysed by alkaline stannite, phenolic and indolic degradation products are formed which are removed from the aqueous solution by extraction with ether (or toluene). Hydrolysis of a protein, such as casein, readily confirmed this. The residue from the ethereal extract of a casein hydrolysate gave one phenolic compound which was detected with $FeCl_3$, Folin–Ciocalteu and $AgNO_3$ reagents. Many compounds were detected on the chromatograms with Pauly and Ehrlich reagents while with ninhydrin colour was developed at the origin. Interpretation of the results obtained on hydrolysis of the oothecal preparations is therefore complex.

Protocatechuic acid was the only ether-soluble phenol identified in hydrolysates of oothecae which did not appear in hydrolysates of casein. The white oothecal material contained the least and the fully hardened material the most but this is probably a reflection of the amount of preliminary washing which the materials received. In addition the ethereal extract from the hydrolysed, fully hardened material contained two compounds detected with Folin–Ciocalteu reagent (on being made alkaline) which did not appear in similar preparations from proteins (R_F 0.57 and 0.73 in benzene–acetic acid–water). The colour of the first spot was weak, that of the second very weak. Neither spot appeared in the ethereal extract from hydrolysed, white oothecae but the first was detected in that from the very lightly coloured oothecae. No phenolic material reacting positively with ninhydrin was detected in any of the extracts.

Aromatic material present in the ether-extracted, acidified hydrolysate from fully hardened oothecae (from 25 lots of 2 g) was adsorbed onto Darco G charcoal, prepared according to the method of SCHRAMM AND PRIMOSIGH[9]. (Preliminary experiments showed that tyrosine, tryptophan and phenylalanine were adsorbed from aqueous solution and could be recovered by this method.) The recovered aromatic compounds were examined by paper chromatography (solvents: butanol–acetic acid–water and phenol–water (20:80, w/v)) and by electrophoresis on paper (buffer pH 1.9, 1 M with respect to both formic and acetic acids). The only compound detected was one which behaved in a manner identical with that of tyrosine. Part of the material was purified by crystallization and its infrared and ultraviolet absorption spectra were identical in every respect with that of tyrosine (tyrosine content of ootheca 15%). The same results were obtained when the aromatic material was adsorbed onto Amberlite IR-120.

Attempts to degrade powdered, fully hardened oothecae enzymically (pepsin, trypsin or papain) to give quinone-amino acid fragments were not successful. A few

per cent only of the total nitrogen went into solution which was colourless indicating the absence of soluble quinonoid material. Complete solution of powdered ootheca was obtained in conc. HCl at 37° in 4 days. Removal of the HCl below 37° and digestion of the residue with papain[10] gave a solution in which protocatechuic acid was the only ether-soluble phenol.

Fusion of powdered, fully hardened oothecae (2 g) with KOH (20 g) and water (10 ml) at 270–280° for 2 h in an atmosphere of nitrogen with stirring gave *p*-hydroxybenzoic acid (approx. yield 3.6 %). This acid was identified in the ethereal extract of the acidified aqueous solution of the melt by the use of paper chromatography[11]. Several other phenols were present in extremely small amounts. The *p*-hydroxybenzoic acid was probably formed from tyrosine.

Amino phenols could not be detected in hydrolysates of the oothecae. Protocatechuic acid and tyrosine were the only phenolic compounds identified. Of these protocatechuic acid is present uncombined in the original material. Exhaustive extraction of the powdered, fully hardened oothecae with hot water yielded 1 % of protocatechuic acid (identified as above) which is more than sufficient to account for the amounts of this acid identified in the hydrolysates. For these experiments the oothecae were cleaned by washing in water[7] and no doubt some protocatechuic acid was lost in the process. The lysine content of the white ootheca (9.7 %) was considerably higher than that of the fully hardened ootheca (3.6 %) suggesting that lysine may be involved in the hardening process.

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The use of specific amino acid decarboxylases for the identification of C-terminal groups

Although methods for N-terminal grouping have been adequately developed, there is no completely satisfactory method, as yet, for the characterisation of C-terminal groups. A widely used method, in which the C-terminal amino acid is released into the suspension medium by carboxypeptidase, provides results which may be difficult