Aromatic Tanning in the Myriapod Cuticle

BROWN[1] has found that the precursor of the quinone responsible for the tanning of certain structures in *Mytilus* and of the egg capsule in *Fasciola* is probably an amino-acid or protein, in contrast to the alcohol-soluble phenol operative in the cockroach ootheca and the insect cuticle[2,3,4]. He suggests that the tanning mechanism may have evolved from a type where the phenolic hydroxyl groups of tyrosine become oxidized to the quinone without removal of the side-chains. It is therefore interesting to find, in myriapods, evidence which appears to indicate the retention of this suggested original mechanism of tanning.

A comparative study of the cuticle of various myriapods has clearly shown that the endocuticle subjacent to regions of extensive sclerotization differs considerably from the endocuticle of the arthrodial membranes, where sclerotization is slight. In the sclerites of *Lithobius forficatus*, where an appreciable outer thickness is tanned, the inner layer, although histologically similar to that of the arthrodial membrane, shows unexpected chemical properties. It is intensely positive to Millon's reagent and reduces ammoniacal silver nitrate, thus appearing to be rich in phenolic substances. A protein is probably involved, since the sclerite endocuticle has an iso-electric point in the region of *p*H 5, whereas that of the arthrodial membrane endocuticle is much lower (*p*H 2·8–3·0). The substance responsible for this higher iso-electric point does not dissolve in water or alcohol, and treatment of the cuticle with diaphanol does not remove the difference between the two regions of endocuticle as would be expected if due to a free phenol. In *Haplophilus subterraneus* the inner part of the exocuticle is colourless and yet is different from the endocuticle in being optically homogeneous and refractile. This is an optical appearance of the substance, which, in the endocuticle of *Lithobius*, betrays its presence only on certain chemical tests being applied. The foregoing observations suggest that there is a precursor of sclerotin which is not water- or alcohol-soluble. This substance has accordingly been termed pro-sclerotin.

These facts also suggest that, in a myriapod, the protein precursor of sclerotin and the phenolic substance responsible for its becoming tanned are possibly the same substance, pro-sclerotin. It seems possible that tyrosine is the unit of this self-tanning protein, and, indeed, it has an iso-electric point similar to that of pro-sclerotin. Observations of other workers are not inconsistent with these views. Browning[5] records an iso-electric point of the endocuticle of *Tegenaria* of *p*H 5·0–5·5, similar to that of *Lithobius*. These figures are very different from those for the endocuticle of *Sarcophaga* larvæ[6] and the chitin (endocuticle) of the foregut of *Homarus*[7], both of which have unhardened cuticles; but I have determined the iso-electric point of the endocuticle of *Calliphora* after formation of the puparium and found it to be in the region of *p*H 5 and not *p*H 3 as might have been expected.

The epicuticle of *Sarcophaga* larvæ[6] and the 'cuticle' (epicuticle) of the foregut of *Homarus*[7] have iso-electric points higher than those of the inner layers, again in the region of *p*H 5. The outer layers in these two cases appear to be related to pro-sclerotin, and their chemistry and staining reaction confirm this view. Dennell[6] records the fact that the junction of the endocuticle and exocuticle of *Sarcophaga* puparia stains red with Mallory, like the unhardened epicuticle of the larva. The sclerite endocuticle of *Lithobius* stains red with Mallory. It appears that red-staining with Mallory and elevation of iso-electric point go hand-in-hand—that it is, in fact, a property of pro-sclerotin.

The outer layers of *Sarcophaga* and *Homarus* are, however, described as being free from chitin, whereas there is no evidence in myriapods of a similar chitin-free layer. All regions respond to the chitosan test. The two histological layers of a myriapod have thus been termed endocuticle and exocuticle. They are optically distinct, but merge one into the other by way of pro-sclerotin. Most differences in the myriapod cuticle can apparently be explained by regarding it as a chitinous matrix impregnated to varying extents by pro-sclerotin which may or may not be tanned.

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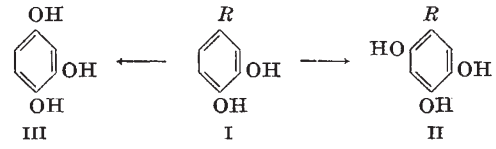
Quinone Tanning in the Animal Kingdom

THE communication under the above title by Brown[1] prompts us to record that we have been engaged on the same problem from the chemical point of view during the past few years. Our approach was based on the assumption that *combined* tyrosine (in a protein) could be oxidized to *combined* 3 : 4-dihydroxyphenylalanine (I) [$R = CH_2.CH(NH.COR').CO.NHR'$, where R' is part of the protein residue], which might then undergo oxidation in either of two ways, namely: (i) direct hydroxylation of the benzene nucleus in the 6-position to give (II), (ii) oxidative elimination of R to produce hydroxyquinol (1 : 2 : 4-trihydroxybenzene) (III). In either case, a 1 : 2 : 4-trihydroxybenzene derivative would be produced.



From our point of view, the combined amino-acid side-chain of (II) is of relatively little importance, since ring closure of such a compound to an indole derivative[2] can no longer occur. The simplest and most readily accessible prototype of (II) is clearly 2 : 4 : 5-trihydroxytoluene, which is readily prepared, as its triacetate, by the Thiele acetylation[3] of toluquinone. Hydroxyquinol is similarly available, starting from benzoquinone. When dilute aqueous solutions of either of these trihydroxy-compounds are applied to human skin, there develops during eight to twenty-four hours a brownish-black stain. We are of the opinion that the trihydroxy-compounds, functioning as reducing agents, convert some of the S.S bonds of the keratinous protein into .SH groups, which then react with the hydroxyquinone produced