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# A NOTE ON THE COLOURING MATTER OF LAC LARVAE

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The results of a preliminary investigation on the components of the larvae was reported earlier (1). It was found that the colouring matter is not extracted by the usual organic solvents whereas all other products like resin and wax are easily removed by them. The colouring matter thus left behind could then be isolated in a pure condition. Since it happens to be an acid and different from what has been earlier called laccaic acid (2), for purposes of distinction we propose to call it "Lacciferic Acid". Its investigation using the small amount available seems to provide valuable insight into the nature of lac dye.

Extraction of the pigment:

The kusumi larvae were collected alive at the time of "swarming" in July 1961, stored in 70% alcohol and extracted within a week from the date of collection. The cold alcohol extract was filtered and the residual larvae successively extracted with hot acetone, ether, petroleum ether and chloroform. This sequence of solvents was found to be most suitable in view of the moisture content of the original

larvae. Cold alcohol does not extract any pigment but removes part of the resin. The remaining part of the resin and some wax were dissolved by hot acetone. The other solvents removed rest of the wax esters. The residual larval matter, free from wax and resin was extracted with M/15 phosphate buffer adjusted to pH 6.8 at 60° for 5 hr. The extract which was deep violet in colour gave a red-violet powder on bringing the pH to 2 with hydrochloric acid. This product was found to be a mixture of two pigments by paper chromatography; the major component was violet and the minor red which agreed with an acid degradation product of the former and hence was not further studied. The violet component was isolated as a single entity by preparative paper chromatography on Whatman paper (3 mm) impregnated with a phosphate buffer pH 6.8 and developed with the solvent system, methanol:ethyl acetate:light petroleum (60-80°):water (1:2:2:2, lower layer). The purified product was a violet powder (Found: C, 35.6; H, 4.6; N, 8.7; S, 0.7; ash, 21.1%). This compound which appears to be the parent has been given the above mentioned name 'laccíferic acid'. It decomposes with charring above 300°. It is practically insoluble in all organic solvents but readily soluble in water at and above pH 6.8, forming a pink solution and in concentrated hydrochloric and sulphuric acids giving a deep red solution. It is insoluble in dilute mineral acids. It is apparently a salt as indicated by the ash content. Its infrared spectrum showed a broad band at 1667-1639 $cm^{-1}$ possibly due to amide

groups and quinonoid carbonyls and another at 1562-1538 $cm^{-1}$ in the aromatic region. Its U.V. and visible spectra at pH 6.8 showed $\lambda_{max}$ 260, 515 and 556 mμ.

Acid hydrolysis studies:

The acid hydrolysis of laceiferic acid has been studied under various conditions of acid concentration, temperature and time. A number of closely related degradation products were formed, as indicated by paper chromatography. None of them could be isolated pure.

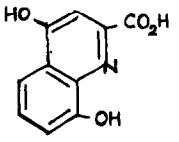
By boiling with 6N-hydrochloric acid for 5 hr under reflux peptide chains are knocked off from the chromophore and at least 6 amino acids are split off as shown by paper chromatography using butanol:acetic acid:water (4:1:5, upper layer). The remainder is a mixture of red compounds readily extracted into n-butanol and having the same characteristic spectra in the visible region as the starting material. This product has an aliphatic acid carbonyl (1724 $cm^{-1}$) and an aromatic acid carbonyl (1681 $cm^{-1}$). The mixture is soluble in organic solvents such as n-butanol and methanol and also in water. Boiling with 5N hydrochloric acid under reflux for 70 hr gave a black polymeric insoluble amorphous mass. The clear aqueous filtrate on dilution with water and cooling yielded a red crystalline product (Found: C, 54.0; H, 4.1; N, 1.4%). This showed two rings on paper chromatography with the solvent system already described. The visible spectrum of this product was identical at pH 6.8 with that of the original pigment, showing the retention

of the chromophore ($\lambda_{max}$ 240, 290, 515 and 555 mμ). In the infrared spectra it showed four absorption maxima in the carbonyl and aromatic regions (1724, 1685-1667, 1613, 1563 $cm^{-1}$). These results suggest that lacciferic acid has a peptide chain which is removed by acid hydrolysis, leaving in part the main chromophore intact, though other side reactions do take place as shown by the presence of the polymeric mass.

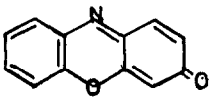
Alkaline hydrolysis:

The pigment decomposes rapidly at room temperature in alkaline solutions, particularly in presence of air; in hydrogen atmosphere the decomposition is slow. The pink alkaline solution becomes almost dull wine red after some time in the presence of air and turns yellow on acidification. In a typical experiment the pigment was treated with aqueous sodium hydroxide (2.5 N) for 24 hr at room temperature followed by refluxing for 3 hr. The yellow solution obtained on acidification when extracted with n-butanol gave a yellow-brown solid containing nitrogen. Paper chromatography of this product with butanol:acetic acid:water (4:1:5, upper layer) gave the following results. The primary product was a compound with an intense yellowish green fluorescence in U.V. characteristic of phenolic amines. The second main component gave a bluish violet fluorescence in U.V. and this has been identified as xanthurenic acid (I) by direct comparison with an authentic sample using chromatography and co-chromatography. Other

fluorescent rings were also present and the nature of the components is unknown.




(I)

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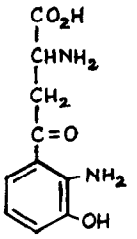


(III)

The retention of nitrogen and the chromophore in the acid hydrolysed product and the nature of fission in alkaline solution suggest a phenoxazone (II) chromophore in lacciferic acid. Further, the formation of fluorescent products during alkaline fission, along with their solubility, U.V. and visible spectra suggest that lacciferic acid may belong to ommochrome group of pigments (3).

As with ommochromes the deep red solution of the pigment in hydrochloric acid, on the addition of sodium nitrite, changed to yellow. The original red colour was regenerated on warming the yellow solution with sulphur dioxide. Further the aqueous solution obtained from the acid hydrolysis, showed the presence of 3-hydroxy kynurenine (III) identified by chromatography and co-chromatography with an authentic sample.

Methylation studies:

Methylation of the crude acid degraded pigment with either diazomethane or dimethyl sulphate-potassium carbonate in acetone medium gave a mixture of methyl ether esters. The separation of these products was found to be difficult and at this stage only gross features could be inferred from the infrared spectrum. It showed a distinct absorption pattern in the carbonyl and aromatic regions (1741, 1724, 1712, 1682, 1655, 1625, 1573 and 1555 $cm^{-1}$). More peaks are found after methylation than in the parent acid given earlier.

Musso and co-workers (4) have described three characteristic absorptions in the infrared for a number of phenoxazone derivatives: 1661-1647, 1626-1595 and 1590-1570 $cm^{-1}$. Absorptions in these regions are also found in the spectrum of the methyl ether esters of the degradation products of laccíferic acid. In the parent laccíferic acid however wide unresolved bands are present. The U.V. and visible spectra of the degradation products show very characteristic absorption in methanol (288, 490 and 518 mμ) which shift in alkaline solution to longer wave lengths (260, 515 and 555-65 mμ). These are shown by model phenoxazones also.

Relation of laccíferic acid with laccaic acid:

It has been suggested that the laccaic acid obtained from stick lac (2), has a purpurin chromophore. In order to compare it with laccíferic acid, powdered kusum stick lac was extracted with phosphate buffer at pH 6.8 and acidified to pH 2; a red-violet powder was obtained similar to

lacciferic acid, and it had similar qualitative analysis, but quantitatively it was different and it gave a different spot in paper chromatography with the same solvent as used for lacciferic acid earlier (Found: C, 53.5; H, 6.0; N, 4.6; S, 0.9%). However, on acid hydrolysis it gave the same series of compounds, indicating that it is a partially decomposed stage of lacciferic acid. The earlier workers generally employed a stage of acid treatment in the extraction of stick lac and it would appear that laccaic acid reported in the literature is actually a mixture of the acid degradation products of lacciferic acid. Even prolonged extraction with hot water decomposes lacciferic acid to a large extent giving a mixture of red compounds.

The action of alkali on purpurin showed considerable difference compared to lacciferic acid. Upon alkaline degradation under similar conditions, the pink alkaline solution of purpurin deposited a brown precipitate and the mixture on acidification gave an orange solid. No fluorescent compound was obtained from the reaction mixture and this is in marked contrast to the behaviour of lacciferic acid from the larvae and laccaic acid extracted from stick lac as given above and hence they may not have a purpurin structure.

Summarising the results, lacciferic acid is best extracted from the larvae with aqueous buffer. It appears to belong to the ommochrome group of pigments and resembles

ommins in complexity and in spectral properties. Laccaic acid seems to be a degradation product and belongs to the same group.

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