

CHEMICAL INVESTIGATION OF INDIAN LICHENS

Part I. Chemical Components of *Roccella montagnei*

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Received October 5, 1940

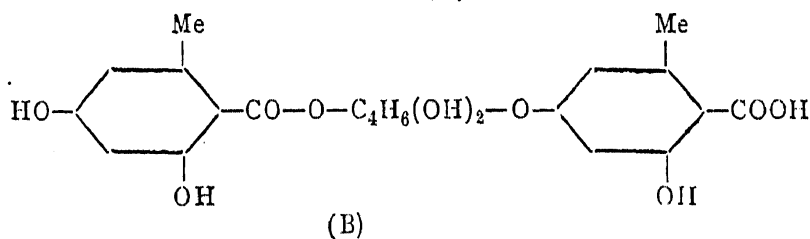
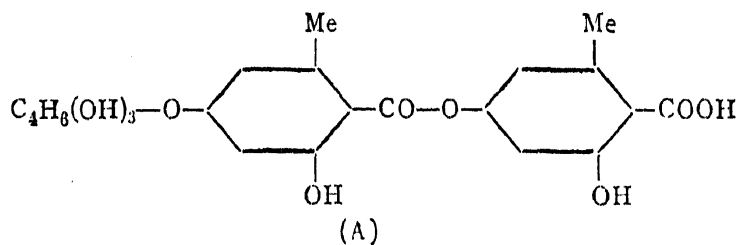
THE lichens, known as *Sailaja* in Sanskrit, *Charila* in Hindi, *Kalpasi* in Tamil and *Rathipuchi* or *Rathipavvu* in Telugu, have excited a good deal of interest as regards their biological nature, botanical classification and chemical composition. They are chiefly found on the barks of trees and on rocks, and are very slow growing plants representing a symbiosis between fungi and algæ. Their growth is dependent upon the humidity of the atmosphere and in general they are widely distributed over the surface of the globe. Being exceedingly diversified in their form, appearance and texture, more than three thousand species of them have been recorded by the botanists. They have found use in various ways as dye-stuffs (orchil, cudbear and litmus), food-stuffs and as material for making drugs. Excepting the work of Chopra¹ on the lichens of the Sikkim Himalayas, no detailed information is available about the distribution in India of the numerous species of lichens of economic importance. Watt² has recorded that the true Orchil (*Roccella tinctoria*) was to be found in Ceylon on the stems of certain palms, and that samples of this material were sent to the colonial and Indian exhibition (1886) as a drug from Madras. However, no knowledge appeared to exist of its value as a dye. An allied species (*Roccella fuciformis*) was recorded by Lindsay³ as growing on the mango trees at Pondicherry.

The lichen, *Roccella montagnei*, occurs in abundance in Waltair and the neighbourhood and is chiefly found on the cashewnut, mango, pongamia, tamarind and banyan trees. The identification was made by sending a sample to Kew and we are thankful to the Director of that herbarium for it.

Hesse⁴ was the first to examine a sample of this lichen obtained from Angola and he isolated from it erythrin and oxyroccellic acid. Subsequently Ronceray⁵ detected the presence of orcinol also. Erythrin was, however, found to be the main component. The methods that they employed could not yield complete information regarding the composition of the lichen. A thorough examination of the Indian sample has now been made using a series of solvents in succession for purposes of extraction. Further, a number of samples obtained during different parts of the year and from different trees have also been analysed.

As a result of this investigation it has been found that the important components of this lichen are: Erythrin, Erythritol, Lecanoric acid, Orcinol and Roccellic acid. Of these, previous investigators did not obtain erythritol, lecanoric acid and roccellic acid. Oxyroccellic acid, which Hesse claimed to have isolated from the Angola sample, could not be found in the course of our work. Its place has possibly been taken up by roccellic acid. Samples collected from various trees were found to be very similar, but considerable variation in composition was noticed in samples obtained in different parts of the year.

During the years 1938 and 1939 about ten samples in all were extracted. Two typical cases are described in the experimental part. It was noticed that whenever erythrin was available in large quantity lecanoric and roccellic acids were absent and only small quantities of erythritol were obtained, and when lecanoric acid was found to be present it was accompanied by considerable quantities of erythritol and very little of erythrin. For obtaining good yields of erythrin samples collected during the wet months were found to be the best. These statements may not hold good always. But the details of the occurrence of erythrin and lecanoric acid along with erythritol in *Rocella montagnei* are highly interesting. They indicate that these two compounds are closely related. Of the two alternative formulæ (A)⁶ and (B)⁷ for erythrin both satisfy all known reactions and properties of the compound and there has been no unequivocal evidence in support of any one to the exclusion of the other. But the facts mentioned above lend support to formula (A) in which it is represented as the erythrityl ether of lecanoric acid. Work is in progress with a view to establish the constitution definitely.



Experimental

Two representative extractions of the lichen are described below. At the beginning the following series of solvents were employed in succession: light petroleum, benzene, ether, acetone, alcohol and water. Since it was

found that benzene and ethyl alcohol did not extract any appreciable quantity they were left out.

Extraction I: Erythrin, Erythritol and Orcinol.—The fresh lichen obtained from the trees was dried in the sun and ground into coarse powder in a coffee-mill. The extraction was carried out in a Soxhlet extractor designed to take up about 1 kilogram of the material, and the process was carried out first with light petroleum (60–80°) for about 24 hours. After removing the solvent from this extract, a residue amounting to 1.3% of the weight of the lichen was obtained. This consisted mostly of fatty and waxy matter along with carotene and xanthophyll. This was not further investigated. The fat-free lichen powder was then extracted with ether for 24 hours and subsequently with acetone for 48 hours. These extracts were separately examined as described below:

The ether extract amounted to 1.4% and consisted of a semi-solid mass. Part of it dissolved in water and after filtration a pale yellow crystalline solid was left behind. It could be readily recrystallised from acetone and was obtained in the form of bundles of pale yellow needles melting at 156–57°. (Found: C, 54.6; H, 5.6; $C_{20}H_{22}O_{10}$, H_2O requires C, 54.5; H, 5.5%.) This substance gave an orange-red colour with bleaching powder and an alcoholic solution of it turned violet on the addition of a drop of ferric chloride solution while with an excess of the reagent it turned reddish brown. From all these reactions it was identified as erythrin. The water-soluble portion was recovered by the concentration of the aqueous extract. It dissolved easily in ether, alcohol and water and gave all the reactions of orcinol. It gave a deep violet colour with ferric chloride and the characteristic homofluorescein reaction with chloroform and alkali. But the sample was contaminated with a tenacious impurity which rendered purification extremely difficult and wasteful. The yield of the crude orcinol was about 0.3%.

The acetone extract, amounting to about 7.5% of the lichen, was also a sticky mass. It could be divided into two portions by treatment with water. The water-insoluble portion was separated by filtration and washing with small quantities of water. It was readily recrystallised from acetone and was found to be erythrin by comparison with the sample obtained from the ether extract. The aqueous extract yielded a semi-solid mass on concentration and drying in a desiccator. When treated with a little ether a white crystalline solid separated out. This was filtered and washed with small quantities of ether. When recrystallised from alcohol, it came out as large colourless hexahedral crystals melting at 122°. It possessed a sweet taste. (Found: C, 39.6; H, 8.0; $C_4H_{10}O_4$ requires C, 39.3; H, 8.2%.)

It was therefore concluded to be erythritol and the identification was confirmed by the preparation of the benzoyl derivative melting at 185–86°, the acetyl derivative melting at 86°, and the formate melting at 150–51°. The ether extract gave rise to a little orcinol on evaporation.

The residual lichen powder was finally boiled with water for 48 hours and the aqueous extract concentrated. This gave no colouration with ferric chloride and a blue colour with iodine. It reduced twice the amount of Fehling's solution after hydrolysis with hydrochloric acid than before hydrolysis, was insoluble in ammonio-copper sulphate solution and gave no acetyl derivative. It was therefore identified as isolichenin.

The yields of the pure crystalline compounds obtained from this sample of lichen were as below: erythritol 2.0 % and erythrin 5.3%.

Extraction II: Roccellic acid, Erythritol, Lecanoric acid and Orcinol.—The light petroleum extract was just the same in quantity and composition as in the case of sample (1). The ether extract (yield 1.0%) was treated with water just as before and the water-insoluble solid recrystallised from acetone. The substance thus obtained was in the form of colourless rectangular rods and melted at 132–33°. (Found: C, 67.5; H, 10.7; $C_{17}H_{32}O_4$ requires C, 68.0; H, 10.6%.) It was soluble in benzene, acetone, ether and alcohol and very sparingly soluble in water. $[\alpha]_D$ in ethyl alcohol = +17.6°. It was an acid by nature dissolving readily in sodium bicarbonate solution and gave no colour with bleaching powder or with ferric chloride in alcoholic solution. Similar to succinic acid it gave the fluorescein reaction when fused with resorcinol. In view of all these properties the compound was identified as roccellic acid.

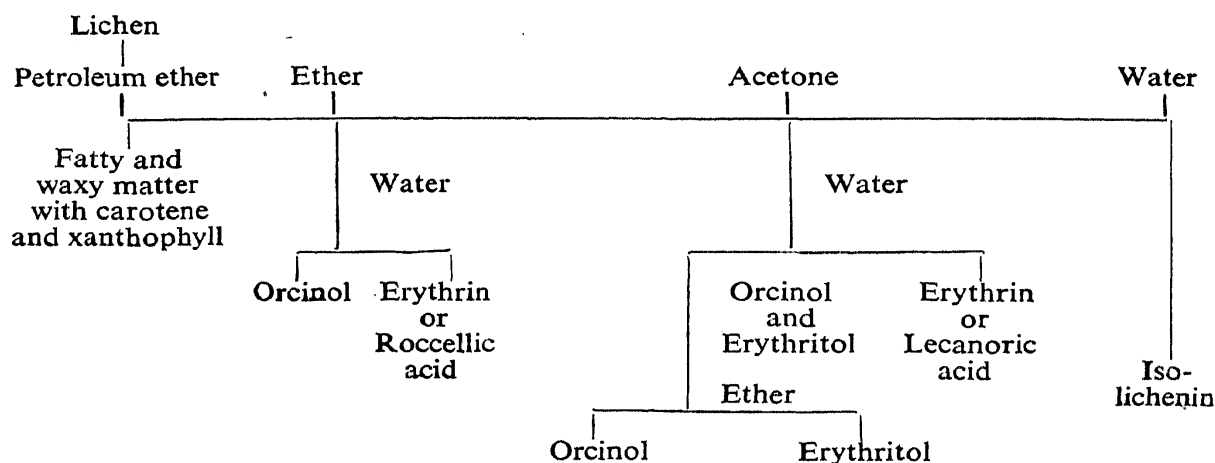
During the course of the extraction of the lichen with acetone erythritol began to separate out in the form of large crystals in the receiver. After 48 hours when the extraction was found to be over it was removed by filtration and subsequently washed with small quantities of acetone. The acetone solution was then distilled in order to recover the solvent and the residual amorphous solid treated with water. A highly coloured solid was left undissolved. The aqueous solution was found to contain some more erythritol and orcinol and these were recovered by evaporating the solution and treating the residue with ether as has already been described. The purification of the coloured water-insoluble solid was effected as follows: It was dissolved in the minimum quantity of acetone and diluted with an equal volume of water when a precipitate consisting mostly of colouring matter separated out immediately. It was filtered off, the filtrate diluted with a large quantity of water and repeatedly extracted with ether. On evaporating the ether

solution a yellow solid was obtained which crystallised from acetone as pale yellow elongated needles melting at 166° with decomposition. Further purification was effected by dissolving the substance in the minimum quantity of acetone-ether mixture (1:1) and adding to it benzene or chloroform. An amorphous solid that first separated out was removed and subsequently colourless needles of lecanoric acid were formed. The melting point now went up to $173-74^{\circ}$ (decomp.). The substance gave a blood-red colour with bleaching powder and a stable violet colour with ferric chloride and agreed closely in all respects with an authentic sample of the acid and the mixed melting point was undepressed.

Final extraction of the lichen with boiling water yielded isolichenin as before.

The yields of the different crystalline compounds were (1) Roccellic acid 0.8%, (2) Erythritol 3.5% and (3) Lecanoric acid 1.0%.

The following table summarises the processes adopted for the separation of the various components of the lichen:



Summary

The lichen *Rocella montagnei* collected in Waltair has been found to differ markedly in composition, depending upon the season. Erythrin, Erythritol, Lecanoric acid, Roccellic acid and Orcinol are the main components besides isolichenin. The present findings differ from previous work in that erythritol and roccellic acid are now found in this lichen whereas oxyroccllic acid is absent. The constitution of erythrin as the erythrityl ether of lecanoric acid is supported.

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