

# CHEMICAL EXAMINATION OF INDIAN LICHENS

## Part VII. Chemical Components of *Parmelia abessinica* (Rathipuvvu)

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RATHIPUVVU (Telugu, meaning rock flower) is a lichen occurring abundantly in the rocky areas of the Western Andhra Districts of Bellary, Anantapur and Cuddapah in South India. It is used largely as a food material and some times is considered a delicacy. It also finds use in indigenous medicine. A sample of this was submitted to Kew for identification and was pronounced to be almost identical with *Parmelia abessinica* (Kremp).

A large number of species of *Parmelia* from different parts of the world have been studied by other workers. They seem to differ widely in chemical composition and even varietal differences are considerable. Since *Parmelia abessinica* has not so far been examined and since it is one of the most important of the Indian lichens, it has now been subjected to a detailed investigation using various solvents for extraction.

Careful cold percolation with petroleum ether removed most of the plastid pigments consisting of chlorophyll, carotene and xanthophyll. Subsequent extraction in a Soxhlet with the same solvent enabled the isolation of a crystalline compound which was identified as atranorin. In further succession ether, chloroform, acetone, alcohol and water were employed. The ether extract contained lecanoric acid; chloroform and alcohol extracted very small amount of amorphous material from which nothing definite could be isolated. Salazinic acid was the main component of the acetone extract and isolichenin was present in the aqueous extract. The same substances could be obtained by employing only ether, acetone and water in succession. Then the ether extract contained along with colouring matter, atranorin and lecanoric acid and they had to be separated carefully by using benzene and chloroform in which the former was soluble and the latter not. The other two extracts yielded salazinic acid and isolichenin. The yields of the various components were: Atranorin 1.1%, Lecanoric acid 3.3%, Salazinic acid 0.1 to 0.5%, Isolichenin 3.4%. Employing samples obtained in different

years it was noticed that though the other components did not show marked change in yield, salazinic acid content varied appreciably.

In identifying the substances and studying their reactions certain new observations were made. (1) Methylation of lecanoric acid which was originally done by Fischer<sup>1</sup> using diazomethane can be equally well carried out using methyl iodide and potassium carbonate in acetone medium. Obviously in this and similar cases such as atranorin, chelation is not strong enough to interfere with the methylation of hydroxyl groups ortho to the carbonyl groups. (2) A pure sample of anhydrous salazinic acid is obtained by boiling the impure sample with pure acetone commercially available. Owing to the presence of impurities it dissolves fairly easily. From this solution the anhydrous acid crystallises out slowly. On the other hand if 80% acetone is employed for crystallisation the mono-hydrate is obtained.

### *Experimental*

Samples of the lichen were obtained from Bellary, cleaned from adhering impurities, dried in the sun and stored. The results of proximate analysis expressed as percentage were as follows: moisture 13.9, reducing sugar 0.25, sucrose expressed as invert sugar 2.34, starch (lichenin) 27.39, nitrogen 0.98, ash 9.75. The ash contained phosphates, carbonates and silicates of iron, calcium and magnesium. The plastid pigments consisted of chlorophyll, carotene and xanthophyll.

### *Extraction with solvents*

*Petroleum ether: Atranorin.*—The dry lichen powder (500 g.) was extracted in a modified Soxhlet apparatus with petroleum ether (b.p. 60–80°). The first few siphonings yielded dark green solutions due to the removal of the plastid pigments. This portion was discarded and the extraction continued. The colour of the extracts was only pale yellow. After about 20 hours the process was complete and no more was extracted. The yellow residue obtained on distilling the solvent was washed with small quantities of petroleum ether, ether and acetone in order to remove all colour. It was then crystallised once from xylene and then from benzene. Colourless prismatic crystals melting at 194–95° were obtained. The yield of the pure product was 5.5 g. 1.1%. [Found: C, 60.8; H, 5.2; OCH<sub>3</sub>, 7.8; C<sub>19</sub>H<sub>18</sub>O<sub>8</sub> requires C, 60.9; H, 4.8; OCH<sub>3</sub> (ester) 8.3%.] The substance was easily soluble in hot chloroform and benzene and less soluble in other solvents. It was insoluble in aqueous sodium bicarbonate and only slowly in sodium carbonate to produce a yellow solution. It was readily soluble in potash with a deep yellow colour. With alcoholic ferric chloride it formed a wine red colour

and bleaching powder produced no change. The substance was therefore identified as atranorin. This was confirmed by the production of the characteristic chloroform containing compound by long boiling with this solvent and the preparation of the methyl ether by the action of methyl iodide and potassium carbonate, melting at  $123^{\circ}$  (Curd and Robertson<sup>2</sup>). [Found: C, 63.2; H, 6.0;  $\text{OCH}_3$ , 30.0;  $\text{C}_{22}\text{H}_{24}\text{O}_8$  requires C, 63.4; H, 5.8;  $\text{OCH}_3$ , 29.8%.]

*Ether: Lecanoric acid.*—The lichen residue left after extraction with petroleum ether was dried and then extracted with ether for 24 hours. The first few extracts were coloured and were discarded. After distilling off the ether from the final extract a greenish solid was left. It was tested for any water-soluble portion and found to contain none. It was then dissolved in excess of cold acetone and diluted with an equal volume of water when most of the colouring matter separated out. It was quickly filtered and the filtrate diluted with a large excess of water and repeatedly extracted with ether. On distilling the ether a colourless solid was obtained which was easily soluble in alcohol, ether and acetone and insoluble in benzene and chloroform. It was finally purified by crystallisation from dilute acetone when colourless needles were obtained in an yield of 16.5 g. It melted with decomposition at  $175^{\circ}$ . (Found: C, 57.4; H, 4.9; loss on drying at  $110^{\circ}$ , 5.4.  $\text{C}_{16}\text{H}_{14}\text{O}_7$ ,  $\text{H}_2\text{O}$  requires C, 57.2; H, 4.8;  $\text{H}_2\text{O}$ , 5.4%.) The substance was soluble in sodium bicarbonate, gave a violet red colour with alcoholic ferric chloride and bluish red with bleaching powder and reduced ammoniacal silver nitrate solution. It was identified as lecanoric acid and the identity was confirmed by fission of the depside link and identifying the products and by methylation using excess of methyl iodide and potassium carbonate, in anhydrous acetone medium. The acid in acetone solution was treated with excess of methyl iodide and potassium carbonate and the mixture was boiled for 60 hours. The solvent was then distilled off and water added in order to dissolve the potassium salts. The product was filtered and crystallised from aqueous acetone when it came out as colourless silky needles melting at  $145\text{--}46^{\circ}$ . It did not give any colour with alcoholic ferric chloride. [Found: C, 64.2; H, 6.1;  $\text{OCH}_3$ , 31.6;  $\text{C}_{20}\text{H}_{22}\text{O}_7$  requires C, 64.1; H, 5.9;  $\text{OCH}_3$ , 33.2%.] Cf. Fischer.<sup>1</sup>

#### *Extraction with acetone*

*Salazinic acid.*—The lichen residue left after ether extraction was again extracted with acetone in the same extractor for about 32 hours. The brown solution was distilled in order to remove the solvent and the residue boiled with a large excess of acetone. At this stage most of the solid dissolved

leaving behind a little coloured amorphous matter. The solution was quickly filtered, concentrated to some extent and allowed to stand. Colourless crystals (short needles) slowly separated out in good quantity. When heated, the substance was found to turn brown at about  $260^{\circ}$  to sinter at about  $270^{\circ}$  and swell and char at about  $280^{\circ}$ . [Found: C, 55.5; H, 3.0;  $C_{18}H_{12}O_{10}$  requires C, 55.7; H, 3.1%.] It did not undergo any loss on drying at  $120^{\circ}$  *in vacuo* for 5 hours. It was insoluble in ether and chloroform and very sparingly soluble even in acetone. It could however be recrystallised from aqueous acetone (80%) when it came out as the monohydrate. The crystalline appearance was almost the same as the anhydrous sample except that the needles of the hydrate were longer and thinner and on heating it turned brown at  $240^{\circ}$ , sintered at  $255^{\circ}$  and swelled markedly and charred at about  $260^{\circ}$ . [Found: C, 53.2; H, 3.5; and loss on drying at  $120^{\circ}$ , 4.2;  $C_{18}H_{12}O_{10}, H_2O$  requires C, 53.2; H, 3.4; loss on drying 4.4%.] The substance was bitter to the taste, gave a brown red colour with alcoholic ferric chloride and formed a red solution with hot potassium carbonate yielding crystals of potassium salt having the characteristic crystal appearance of potassium salazinate (Asahina<sup>3</sup>). Its identity as salazinic acid was established by the preparation of the dianil by boiling the acid and aniline in 80% acetone solution. It was obtained as golden yellow prisms which were very sparingly soluble in most solvents and on heating turned orange yellow at about  $240^{\circ}$  and finally decomposed (charred) at about  $280^{\circ}$ . [Found: N, 5.4;  $C_{30}H_{22}O_8N_2$  requires N, 5.2%.]

#### *Extraction with water*

*Isolichenin*.—The final lichen residue was heated with water in a boiling water-bath for 30 hours and filtered hot. On concentration over a water-bath an amorphous solid was obtained which gave the reactions of isolichenin. Its acetyl derivative crystallised from alcohol as needles melting at  $115^{\circ}$ . [Found: C, 49.7; H, 5.8;  $C_6H_7O_5(COCH_3)_3$  requires C, 50.0; H, 5.6%.]

#### *Summary*

A complete examination of the lichen *Parmelia abessinica* is reported. Atranorin and lecanoric acid form the major lichen acids and salazinic acid the minor component. The presence of these three important lichen acids together is highly significant in regard to the biogenesis of these compounds.

#### REFERENCES

1. Fischer .. *Ber.*, 1913, 46, 3253.
2. Curd and Robertson .. *J.C.S.*, 1933, 130.
3. Asahina .. *Acta Phytochimica*, 1934, 8, 55.