

# CHEMICAL INVESTIGATION OF INDIAN LICHENS

## Part IX. Some Lichens on Sandal Trees—*Parmelia tinctorum* and *Usnea japonica*

BY T. R. SESHADRI AND S. SANKARA SUBRAMANIAN

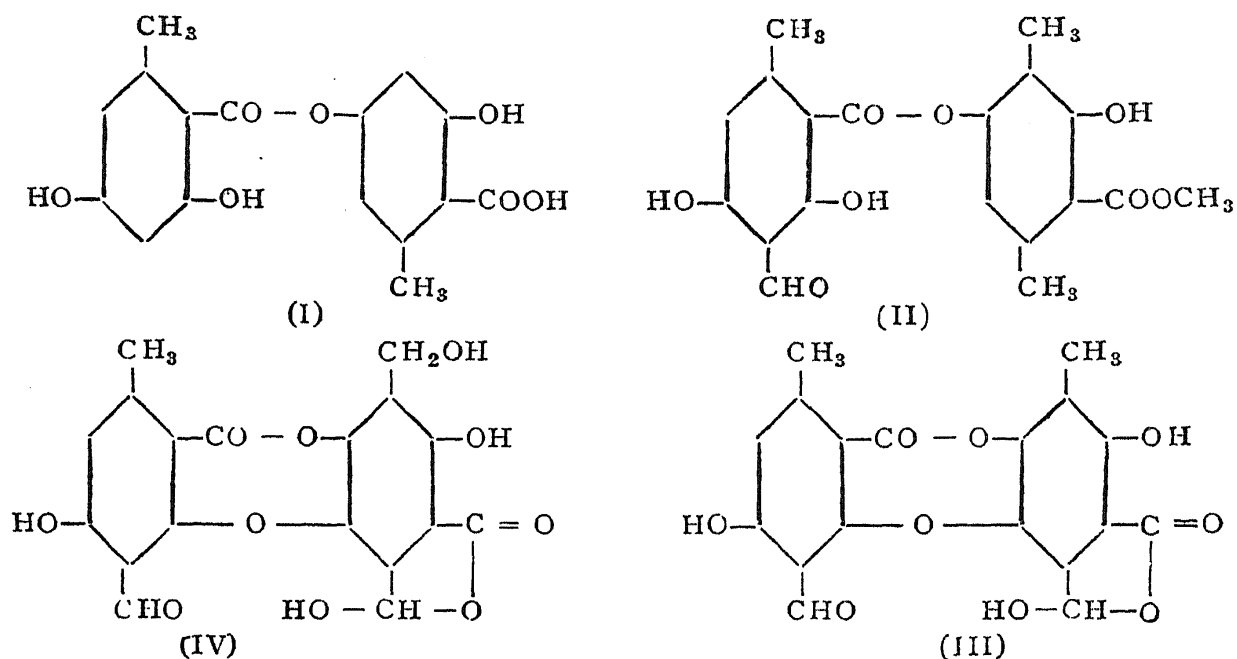
(From the Departments of Chemistry and Chemical Technology, Andhra University, Waltair)

Received May 30, 1949

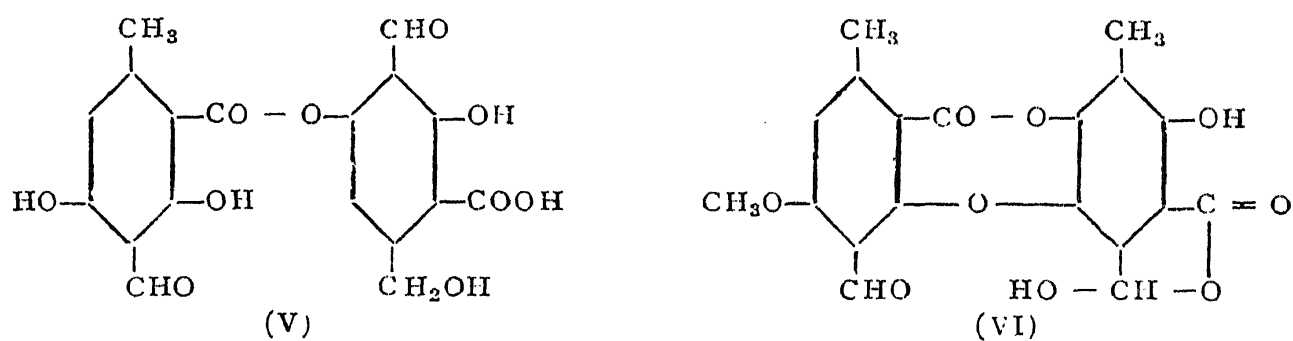
A LARGE number of species of the *Parmelia* from different parts of the world have been studied by different workers and they seem to differ much in chemical composition. Of the various chemical components of these lichens atranorin, lecanoric, usnic and salazinic acids appear to be the most frequently occurring and a few other acids like stictic, nor-stictic, protocetraric and caperatic are present in certain cases. An Indian lichen, *Parmelia abessinica* growing in the western Andhra districts, was examined by Sastri and Seshadri<sup>1</sup> and found to contain atranorin and lecanoric acid as major components and salazinic acid as a minor component.

The lichen examined now grows on the sandal trees of the Coorg area on the western highlands of South India. The trees do not suffer any adverse effects on account of its growth as contrasted with *Ramalina tayloriana* previously reported.<sup>2</sup> A sample of the lichen was sent to Prof. Y. Asahina of Tokyo and kindly identified by him as *Parmelia tinctorum* Despr. This species is found in all the warmer regions of the world including Japan. Hesse<sup>3</sup> originally examined a sample of this lichen found on the Cinchona bark of the Madras Province and isolated from it atranorin and lecanoric acid. This finding has been confirmed with a Japanese sample (Private communication from Prof. Asahina).

In our study of this lichen a cold ether extract contained, along with some colouring matter of the carotenoid type, lecanoric acid (I) (5.0%) and atranorin (II) (0.8%). A subsequent extraction with hot acetone yielded nor-stictic acid (III) (1.0%). Of these components nor-stictic acid is of rather rare occurrence. It was first isolated by Asahina from *Lobaria pulmonaria*<sup>4</sup> and later from *Parmelia acetabulum*<sup>5</sup> which according to Hesse<sup>6</sup> contained only atranorin and salazinic acid. In our earlier publication on *Parmelia abessinica*<sup>1</sup> attention was drawn to the biogenetic relationship among the three components: lecanoric acid, atranorin and salazinic acid (IV). Nor-stictic acid occurring in *P. tinctorum* is more closely related to atranorin than salazinic acid is. Of these compounds atranorin is found to be definitely toxic to fish whereas lecanoric and nor-stictic acids are nontoxic.



Along with the predominant growth of *P. tinctorum* a small quantity of a lichen kindly identified by Prof. Y. Asahina as *Usnea japonica* Wain. is also found. This lichen has been hitherto known to occur only in Japan and Formosa. Using the small sample sent for identification, Prof. Asahina could detect the presence of usnic and stictic acids by his micro-chemical method.<sup>7</sup> In his opinion *U. articulata* v. *asperula*<sup>8</sup> which he had found earlier to contain usnic and stictic acids is synonymous with *U. japonica*. In our detailed examination of the Indian sample of *U. japonica* usnic and barbatolic acids (V) could be obtained from the ether extract and stictic acid (VI) from the subsequent acetone extract. The isolation of barbatolic acid from this source is significant since its occurrence along with stictic acid is again of interest from the point of view of biogenesis.<sup>9</sup>



## EXPERIMENTAL

*Parmelia tinctorum*

*Ether extract (Atranorin and lecanoric acid).*—500 g. of the powdered lichen was extracted with ether twice by cold maceration (each time lasting for 24 hrs.). The combined ether extract was concentrated to a low bulk when a colourless solid was obtained (Fraction A). This was filtered off,

washed with a small quantity of cold acetone and crystallised twice from benzene when it was obtained in the form of colourless prismatic rods melting at 196-97°; yield, 4.0 g. (Found: C, 60.7; H, 5.2;  $C_{19}H_{18}O_8$  requires C, 60.9; H, 4.8%.) The substance was easily soluble in hot benzene and chloroform, moderately in ether and petroleum ether and sparingly in cold alcohol and acetone. It was insoluble in aqueous sodium bicarbonate and only slowly it gave a yellow solution with aqueous sodium carbonate. It was readily soluble in potassium hydroxide solution with a deep yellow colour and with conc. sulphuric acid it gave a deep yellow solution. With alcoholic ferric chloride it gave a wine-red colour and with bleaching powder no colour. The substance was identified as atranorin and this was confirmed by preparing its methyl ether melting at 123-24° by the action of methyl iodide and potassium carbonate in dry acetone medium. Direct comparisons with authentic samples were also made.

The ether mother-liquor left after removing fraction A was evaporated to dryness and the residue treated with hot benzene to remove the small quantities of atranorin still present. The benzene-insoluble solid (fraction B) was taken in cold acetone in which it quickly dissolved and to this solution was added excess of chloroform when a colourless solid separated. This was filtered off and crystallised from acetone-chloroform mixture (1:3) when colourless needles melting at 175-76° were obtained; yield, 25.0 g. (Found: C, 60.8; H, 4.3;  $C_{16}H_{14}O_7$  requires C, 60.4; H, 4.4%.) The substance was soluble in sodium bicarbonate solution and it gave a red-violet colour with alcoholic ferric chloride and bluish-red with bleaching powder. It was identified as lecanoric acid and the identity was confirmed by fission of the depside link by means of (1) aqueous alkali (5.0%) when orsellinic acid, m.p. 176°, and orcinol were obtained, (2) methyl alcoholic alkali (2.0%) when methyl orsellinate, m.p. 142-43°, and orsellinic acid were got and (3) absolute alcohol when ethyl orsellinate, m.p. 132°, and orcinol were the products. On methylation with methyl iodide and anhydrous potassium carbonate in dry acetone medium, trimethyl ether of lecanoric acid methyl ester melting at 145-46° was obtained as colourless needles. (Found: C, 64.0; H, 6.2;  $C_{20}H_{22}O_7$  requires C, 64.1; H, 5.9%.)

*Acetone extract (Nor-stictic acid).*—The lichen residue left after ether extraction was extracted three times with hot acetone (about 6 hrs. each time). The brown solution was distilled to remove the solvent. The residue was boiled with excess of pure chloroform and filtered when a colourless solid was left behind. This was crystallised from hot 80% acetone when colourless needles turning red brown at 260° and melting with decomposition at 285° were obtained; yield, 5 g. (Found: C, 57.6; H, 3.5;  $C_{18}H_{12}O_9$  requires C, 58.0; H, 3.2%.) It was very bitter to the taste, insoluble in

chloroform, benzene and water and sparingly soluble in ether, cold acetone and alcohol. In alcoholic solution it gave a wine-red colour with ferric chloride. It dissolved slowly in aqueous sodium bicarbonate giving a yellow colour. When boiled with a concentrated solution of potassium carbonate it formed a red solution from which separated red crystals (in the form of aggregates of long needles) of the potassium salt of nor-stictic acid which could be easily identified by the characteristic crystal appearance under the microscope.<sup>5</sup> Its identity was confirmed by preparing the acetyl derivative melting at 212–13° and the dianil melting at 265° (decomp.).

*Toxicity tests.*—Using fresh-water fish (*Haplochilus panchax*) as the test animals and following the usual procedure employed in these laboratories,<sup>10</sup> atranorin was found to be toxic in a concentration of 25 mg. per litre in 20 minutes; gelatin in a concentration of 1 g. per litre was used in this experiment to facilitate the solution of the compound in water. Lecanoric and nor-stictic acids were non-toxic even in a concentration of 100 mg. per litre during 24 hours.

#### *Usnea japonica*

*Ether extract (d-usnic acid and barbatolic acid).*—The lichen (50 g.) in coarse powder form was extracted in a Soxhlet extractor with ether and the extract concentrated to a small bulk and allowed to stand overnight when a crystalline solid (about 1.4 g.) separated. This was found to be a mixture when examined under the microscope since two different crystalline forms could be seen, yellow prisms and colourless tiny plates. It was digested with petroleum ether (400 c.c.) and filtered. From the petroleum ether filtrate on concentration crystallised yellow prismatic rods melting at 202–03°; yield, 0.8 g.  $[\alpha]^{30}$ , +472°. It gave a deep yellow solution with aqueous potassium hydroxide and in alcoholic solution it gave a reddish brown colour with ferric chloride. Mixed melting point with an authentic sample of *d*-usnic acid from *R. tayloriana* was undepressed. The petroleum-ether insoluble residue was washed with hot benzene (20 c.c.). The benzene-insoluble solid was dissolved in sufficient quantity of ether and the ether solution concentrated when a colourless crystalline solid separated in the form of flat needles melting at 204–05° (decomp.) (sintering at 195°); yield, 0.4 g. (Found: C, 55.1; H, 3.7;  $C_{18}H_{14}O_{10}$  requires C, 55.4; H, 3.6%.) It was easily soluble in aqueous sodium bicarbonate giving a yellow colour and with aqueous potash gave an intense yellow solution. With alcoholic ferric chloride it gave a deep brown-red colour and no colour with bleaching powder. It was therefore identified as barbatolic acid and this was confirmed by preparing its acetate, m.p. 160–62° (decomp.), by means of acetic anhydride and fused sodium acetate.

*Acetone extract (Stictic acid).*—The residue left after ether extraction was extracted with hot acetone and this extract, on concentration to a low bulk, deposited some colourless crystalline powder. This was filtered off and crystallised twice from anhydrous acetone when a colourless crystalline solid was obtained in the form of aggregates of fine needles. It melted at 265° (decomp.) with sintering and change of colour at 255°; yield, 0.8 g. (Found, C, 59.4; H, 3.4; C<sub>19</sub>H<sub>14</sub>O<sub>9</sub> requires C, 59.1; H, 3.6%.) It was sparingly soluble in alcohol, acetone and ether and insoluble in benzene and chloroform. It was slowly soluble in aqueous sodium bicarbonate giving a yellow colour and in aqueous potash it gave a bright yellow solution which changed in colour to brown-red on standing without depositing any crystalline salt like salazinic and nor-stictic acids. With alcoholic ferric chloride it gave a violet colour which turned brown with addition of an excess of the reagent. With concentrated sulphuric acid it gave an intense yellow solution which immediately changed to deep red and from this solution separated a reddish brown flocculent precipitate on the addition of a small quantity of water. From these reactions it was identified as stictic acid and this was confirmed by preparing the dianil melting at 232°.

Our thanks are due to Prof. Y. Asahina for kindly identifying the lichens and for his interest in this work.

#### SUMMARY

Two lichens are found on the sandal trees of Coorg which do not suffer adverse effects on this account. *Parmelia tinctorum* was earlier reported to contain atranorin and lecanoric acid; considerable amounts of nor-stictic acid are now isolated from it. *Usnea japonica* is reported for the first time in India. Besides usnic and stictic acids already known in this source, barbatolic acid also has now been found to be present in it.

#### REFERENCES

1. Sastri and Seshadri .. *Proc. Ind. Acad. Sci.*, A, 1942, **16**, 137.
2. Seshadri and Subramanian .. *Ibid.*, 1949, **30**, 15.
3. Hesse .. *J. Pract. Chem.*, 1904, **70**, 449.
4. Asahina and Yanagita .. *Ber.*, 1934, **67**, 799.
5. Asahina and Fuzikawa .. *Ber.*, 1935, **68**, 946.
6. Hesse .. *J. Pract. Chem.*, 1901, **63**, 537.
7. Asahina .. *Acta Phytochim.*, 1934, **8**, 47.
8. Asahina and Tukamoto .. *Ber.*, 1934, **67**, 965.
9. Seshadri .. *Proc. Ind. Acad. Sci.*, A, 1944, **20**, 1.
10. Krishnaswami and Seshadri .. *Ibid.*, 1942, **16**, 231.