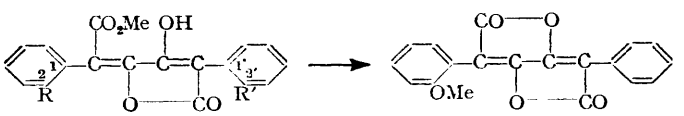
*Chemical Investigation of Indian Lichens. Part XIX.**
Lepraria : *Constitution of Leprapinic Acid.*

By O. P. MITTAL and T. R. SESHADRI.

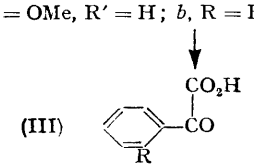
[Reprint Order No. 6370.]

Two Indian lichens of the genus *Lepraria* have been examined. *L. flava* (Schreb) Ach. yielded pinastric acid, and *L. citrina* Schaer yielded a new lichen acid, leprapinic acid. Its constitution has been established as methyl 2-methoxypulvinate, the methoxyl group being located in the phenyl nucleus next to the ester group.

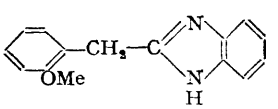
INDIAN lichens belonging to the genus *Lepraria* have not been studied before. They occur as deep yellow or orange powders on trees. Two of them have now been collected and examined. The first was obtained from the bark of pine trees on Kodaikanal hills in South India and was identified as *L. flava* (Schreb) Ach. by Mr. D. D. Awasthi of Lucknow University. Extraction with light petroleum yielded orange yellow crystals, identified as pinastric acid [*L. flava* Quercina was examined by Zopf (*Annalen*, 1905, **338**, 35) who reported the presence of calycin and calyciarin along with pinastric acid]. The second sample was collected from the bark of deodar trees in Simla and was identified by Dr. G. A. Llano of Washington University as *Lepraria citrina* Schaer. This yielded a light petroleum-soluble, golden-yellow compound, which is a new member of the pulvinic acid series and is named leprapinic acid [the simpler names, leprarin and lepraric acid, have been used for other substances (Zopf, *Annalen*, 1897, **295**, 257; Hesse, *J. prakt. Chem.*, 1898, **58**, 465)]. It has the formula $C_{18}H_{10}O_4(OMe)_2$. One methoxyl group is hydrolysed by alkali and hence is present in an ester group. The other is affected only by hydriodic acid and hence constitutes an ether group. The product of hydrolysis by alkali has a free carboxyl group and when refluxed with acetic anhydride yields a dilactone in the same way as does pulvinic acid. This dilactone was different from 4-methoxypulvinic dilactone (Asano and Kameda,



(I; *a*, R = OMe, R′ = H; *b*, R = H, R′ = OMe) (II)



(III)



(IV)

Ber., 1935, **68**, 1565). Though its melting point was very close to that of 3-methoxypulvinic dilactone (Asano and Huziwara, *J. Pharm. Soc. Japan*, 1939, **59**, 83), it gave a depression with an authentic sample. It should therefore be 2-methoxypulvinic dilactone

* Part XVIII, *J. Sci. Ind. Res., India*, 1954, **13**, *B*, 403.

(II). This has been confirmed by degradation by alkaline potassium permanganate to *o*-methoxybenzoic and benzoic acid.

Leprapinic acid is therefore a methyl *o*-methoxypulvinate (I*a* or *b*). After oxidation with cold alkaline permanganate, evidence for the formation of two glyoxylic acids (III; R = H and OMe respectively, besides oxalic acid, was obtained, but the acids were not isolated and characterised owing to lack of material. Condensation with *o*-phenylenediamine and subsequent alkaline fission (Schönberg and Sina, *J.*, 1946, 601) gave 2-2'-methoxybenzylbenziminazole (IV), also obtained from *o*-methoxyphenylacetic acid. This reaction establishes that the methoxyl group is attached to the benzene ring which is close to the ester group.

Experimental

Lepraria flava (*Schreb*) *Ach.* (*Pinastric Acid*).—Since complete separation of the lichen from the bark was not feasible whereas the bark contained no extractable matter except wax, the mixture of the two was extracted 4 times with cold light petroleum (b. p. 40—60°), 24 hr. each time. The combined extracts were filtered and concentrated to 50 c.c. An orange-yellow solid separated and next morning was filtered off and washed with small amounts of light petroleum to remove adhering wax. It crystallised from ether–light petroleum as golden-yellow stout rectangular prisms, m. p. 204—205°, was readily soluble in ether and hot ethyl alcohol and sparingly so in cold alcohol, dissolved in aqueous sodium hydrogen carbonate, carbonate, and hydroxide and was precipitated unchanged on acidification, and gave no colour with ferric chloride or bleaching powder, and an orange colour with concentrated sulphuric acid (Found : C, 67·9; H, 4·4. Calc. for $C_{20}H_{16}O_6$: C, 68·2; H, 4·5%). It did not depress the m. p. of pinastric acid. The acetate, prepared by boiling acetic anhydride, crystallised from ethyl acetate as pale yellow needles, m. p. 172—173° (cf. Zopf, *loc. cit.*).

Lepraria citrina *Schaer : Isolation of Leprapinic Acid.*—The lichen along with the bark was extracted with cold light petroleum as in the previous case. The solid obtained on concentration of the extract crystallised from ether–light petroleum as golden-yellow long rectangular tablets, m. p. 159—160°. It was soluble in aqueous sodium hydrogen carbonate from which it was reprecipitated by acid, and in ethyl alcohol or ether, but sparingly so in light petroleum. It gave no colour with ferric chloride or bleaching powder, and a deep yellow colour with sulphuric acid (Found : C, 68·2; H, 4·7; OMe, 18·2. $C_{20}H_{16}O_6$ requires C, 68·2; H, 4·5; 2OMe, 17·6%).

Hydrolysis to 2-methoxypulvinic acid. Leprapinic acid (0·25 g.), barium hydroxide (0·5 g.), and water (15 c.c.) were refluxed for 15 min. On cooling and acidification, a copious yellow precipitate of *2-methoxypulvinic acid* was obtained. This was dried and crystallised from dry benzene, yielding golden-yellow thick rhombohedral plates and prisms, m. p. 213—214° (Found : C, 67·6; H, 4·6. $C_{19}H_{14}O_6$ requires C, 67·5; H, 4·1%).

2-Methoxypulvinic dilactone. The above acid (100 mg.) was refluxed with acetic anhydride (2·5 c.c.) for ½ hr. and the clear yellow solution cooled in ice; the yellow crystals that separated were filtered off, washed with a small quantity of ether, and crystallised from benzene, yielding lemon-yellow elongated rectangular prisms of the *dilactone*, m. p. 172—173° (Found : C, 71·2; H, 4·1; OMe, 9·8. $C_{19}H_{12}O_5$ requires C, 71·2; H, 3·8; 1OMe, 9·7%).

Oxidation of leprapinic acid. (*a*) Leprapinic acid (150 mg.), water (15 c.c.), potassium permanganate (300 mg.) and anhydrous sodium carbonate (150 mg.) were refluxed for 2 hr. The mixture was then cooled and acidified (sulphuric acid), and sulphur dioxide passed in till the mixture was colourless. It was then extracted with ether and the extract evaporated. A colourless residue (A) was obtained which melted indefinitely between 68° and 96° and gave no colour with ferric chloride.

The mixture (A) (75 mg.), acetic anhydride (1 c.c.), and hydriodic acid (*d* 1·7, 1 c.c.) were refluxed for 1½ hr., poured into an ice-cold saturated solution of sulphur dioxide, and extracted with ether. By evaporation of the ether, a colourless solid (B) was obtained, giving a violet colour with alcoholic ferric chloride and with methyl alcohol and sulphuric acid the smell of methyl salicylate.

The acid (B) (50 mg.) was refluxed with anhydrous acetone (10 c.c.), methyl sulphate (excess), and sodium hydrogen carbonate (250 mg.) for 4 hr. (Saraiya and Shah, *Current Sci.*, 1949, **18**, 218). The acetone solution was filtered, evaporated, treated with cold water, and left overnight in the refrigerator. The esters were extracted with ether, and the ether extract

was washed with aqueous 5% sodium hydroxide (2 × 25 c.c.) (aqueous solution D; ether solution C). The solution (C) was evaporated, and the residue refluxed with aqueous 5% sodium hydroxide (20 c.c.) for 1 hr., cooled, acidified (hydrochloric acid), and extracted with ether which removed a colourless material soluble in aqueous sodium hydrogen carbonate and giving no ferric chloride colour. After sublimation in vacuum, this formed colourless needles m. p. 122° alone or mixed with benzoic acid. Circular paper chromatography at 35° (Whatman No. 1) with ammonia saturated with butan-1-ol as the solvent and a buffered solution of *p*-bromophenol-blue as indicator (Fewster and Hall, *Nature*, 1951, **168**, 78) gave only one ring with R_F 0·59, which agreed with the value given by benzoic acid.

The solution (D) was acidified and extracted with ether. The ether extract on being subjected to the treatment given to (C) left a brownish residue, soluble in aqueous sodium hydrogen carbonate and giving a violet ferric reaction. On sublimation in vacuum it deposited colourless needles, m. p. 158° alone or mixed with salicylic acid. In circular paper chromatography at 30° with 0·1% alcoholic ferric chloride as the indicator it gave a ring with R_F 0·68 agreeing with salicylic acid.

(*b*) Leprapinic acid (100 mg.) was oxidised by Spiegel's method (*Ber.*, 1881, **14**, 1689) (2N-potassium permanganate, 50 c.c.; aqueous 5% sodium hydroxide, 25 c.c.) in the cold for 4 hr. The solution was acidified and sulphur dioxide passed in. Extraction with ether and evaporation of the extract gave an oil. Its solution in acetone was examined by paper chromatography at 35° with *p*-bromophenol-blue as indicator. Two rings were visible, one sharp with R_F 0·30 (oxalic acid), the other diffuse having R_F 0·57—0·62. Phenylglyoxylic acid gave a sharp ring with R_F 0·59. It seemed therefore that this was present in the degradation products along with its methoxy-analogue having a similar R_F value.

Condensation of leprapinic acid with o-phenylenediamine. Leprapinic acid (0·2 g.), *o*-phenylenediamine (0·15 g.), and *NN*-dimethylaniline (10 c.c.) were refluxed at 200—210° for 4 hr. and, after cooling, poured into dilute acid (50 c.c.). The brown solid was filtered off, washed with dilute acid, then with water, and dried. The *product* crystallised from ethyl acetate as small orange-red prisms, m. p. 271—272° (decomp.) (Found: C, 73·3; H, 5·1. $C_{25}H_{18}O_4N_2$ requires C, 73·2; H, 4·4%). It was soluble in dilute aqueous potassium hydroxide, giving a yellow solution from which it was reprecipitated by acid. It was difficultly soluble in benzene and ethyl alcohol, but very soluble in ethyl acetate.

This product was refluxed with alcoholic 10% potassium hydroxide (5 c.c.) for 5 hr. Colourless potassium salts were then filtered off. The filtrate was concentrated under reduced pressure. Colourless crystals separated. When washed with water and dried, they had m. p. 186—187° alone or mixed with 2-2′-methoxybenzylbenziminazole (below). A micro-Zeisel test was positive. The mixed m. p. with 2-benzylbenziminazole (Walther and Pulawski, *J. prakt. Chem.*, 1899, **59**, 253), m. p. 189°, was considerably depressed.

2-2′-*Methoxybenzylbenziminazole.*—Equal quantities of *o*-methoxyphenylacetic acid and *o*-phenylenediamine were heated at 200—210° in the presence of *NN*-dimethylaniline for 4 hr., and dimethylaniline then distilled off at 68—70°/8 mm. The brownish residue was washed with a small quantity of dilute hydrochloric acid and crystallised from dilute alcohol, yielding the *product* as colourless thick rectangular plates, m. p. 186—187° (Found: C, 75·3; H, 6·2. $C_{15}H_{14}ON_2$ requires C, 75·6; H, 5·9%), sparingly soluble in ether but highly soluble in alcohol and acetic acid.

We express our gratitude to Professor S. Shibata of Tokyo University for samples of pinastric acid and 3-methoxypulvinic dilactone, and to Mr. D. D. Awasthi and Dr. G. A. Llano for the botanical identification of the lichen samples.

UNIVERSITY OF DELHI, DELHI. [*Received, April 28th*, 1955.]