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CONSTITUTION OF LEPRAPINIC ACID

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Abstract—Leprapinic acid isolated from a *Lepraria* lichen, possibly *L. citrina* is shown to have the structure methyl 2-methoxypulvinate (I) assigned to it earlier.[1] Methanolysis of 2-methoxypulvinic dilactone yields two isomeric products.

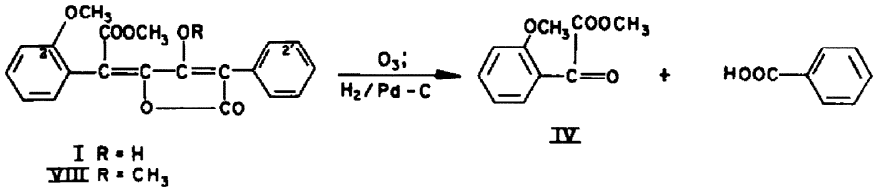
A LEPRARIA lichen which occurs as yellow powder on rocks was collected from Rahla range of forests (Kula Dist., Western Himalayas) in September, 1964. On extraction with benzene it yielded a golden yellow compound identified as leprapinic acid and unaccompanied by any other compound of this group. This acid has previously been found alone in *Lepraria citrina*,[1] accompanied by calycin and leprapinic acid methyl ether in *L. chlorina* and along with calycin in *Biatora lucida*.[2] The lichen may be therefore *L. citrina*.

The structure of leprapinic acid as 2-methoxyvulpinic acid was based on degradation experiments and confirmed by its synthesis.[3] Based on its reaction with *o*-phenylenediamine, Mittal and Seshadri[1] suggested that the carbomethoxyl group in leprapinic acid was situated adjacent to the phenyl ring carrying the methoxyl group as given in I.

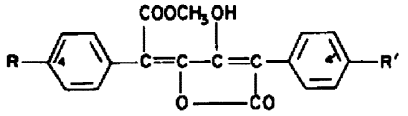
In view of new observations made during our reinvestigation of the constitution of pinastric acid (II) and isopinastric acid (III)[4–6] it was desirable to study the constitution of leprapinic acid in more detail. The method of ozonolysis[4] which is found to be the most suitable for the study of the constitution of unsymmetrical derivatives of vulpinic acid has now been employed. In this reaction leprapinic acid (I) furnished methyl *o*-methoxybenzoylformate (IV) as the neutral fraction and benzoic acid as the acid fraction. The identity of the ester (IV) was proved by its degradation to *o*-methoxybenzoic acid (V). These products of ozonolysis are in agreement with expectation from the structure proposed by Mittal and Seshadri[1] and hence confirms structure I.

It has now been established[6] that during the methanolysis of unsymmetrical *p*-methoxy derivative of pulvinic dilactone, both the lactone rings open to yield the two possible position isomers. The 4-methoxy compound (isopinastric acid III) is the major product and the 4′-methoxy compound (pinastric acid II) the minor product. This is influenced by the preferential deactivation of the far lactone group by the *p*-methoxyl. In the case of *o*-methoxypulvinic dilactone (VI) the same effect as indicated by arrows brings about a larger fission of the lactone ring near the substituted nucleus and yields a higher proportion of a less soluble higher melting fraction which

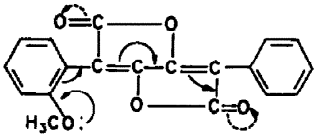
[1] O. P. Mittal and T. R. Seshadri, *J. Chem. Soc.* 3053 (1955).
[2] P. K. Grover and T. R. Seshadri, *J. Sci. Ind. Res.* **18B**, 238 (1959).
[3] O. P. Mittal and T. R. Seshadri, *J. Chem. Soc.* 1734 (1956).
[4] S. C. Agarwal and T. R. Seshadri, *Tetrahedron* **19**, 1965 (1963).
[5] S. C. Agarwal and T. R. Seshadri, *Tetrahedron* **20**, 17 (1964).
[6] S. C. Agarwal and T. R. Seshadri, *Indian J. Chem.* **2**, 17 (1964).



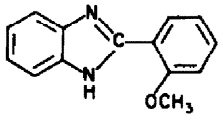
I R = H
VIII R = CH_3



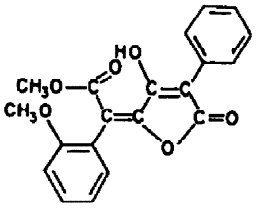
II R = H ; R' = OCH_3
III R = OCH_3; R' = H



VI



VII



IX

when crystallized from methanol agreed in all its properties with the naturally occurring leprapinic acid.[3] However, a small amount of a lower melting and more soluble fraction was also obtained. A repetition of the experiments of Mittal and Seshadri on the action of *o*-phenylenediamine on leprapinic acid yielded the same result i.e., 2-(*o*-methoxybenzyl)-benziminazole (VII) was formed.[1] Unlike the case of pinastric acid this did not lead to any discrepancy regarding the constitution[5] because the dilactone (VI) produced initially in the reaction opens preferentially to yield the 2-methoxy compound. In the absorption spectrum leprapinic acid has the maxima at 270, 316 mμ and its (2'-methoxy) isomer at 227, 288, 355 mμ and this would agree with their being position isomers (cf. absorption spectra of pinastric and isopinastric acid).[6]

In the IR spectrum the low hydroxyl and low carbonyl stretching frequencies of leprapinic acid and its isomer in the solid state and in solution reveal strong intramolecular hydrogen bonding which is absent in the case of leprapinic acid methyl ether (VIII; Table 1). Hence a *trans- trans* configuration (IX) may be assigned to both leprapinic acid and its isomer.

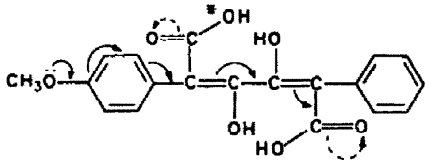
The natural occurrence of pinastric acid (II) and of leprapinic acid (I) and non-occurrence of their respective position isomers which have not so far been isolated from natural sources may have some biogenetic significance. In regard to pinastric acid, it does not appear that the *p*-methoxypulvinic dilactone is an intermediate stage. If it were so, subsequent methanolysis would have yielded predominantly isopinastric acid as it has been found in the laboratory. A more likely route is monolactone formation from dihydroxy dicarboxylic acid (X). This would take place

TABLE 1. INFRA-RED ESTER C=O ABSORPTION OF LEPRAPINIC ACID DERIVATIVES

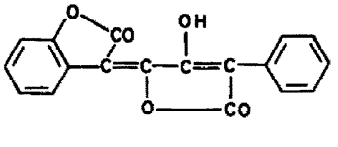
Compound	Cm^{-1} (in KBr)	Cm^{-1} (in Dioxane)	Cm^{-1} Shift
1. Leprapinic acid	1686	1684	-2
2. Isomer of leprapinic acid.	1689	1689	—
3. Leprapinic acid methyl ether.	1736	—	—
4. Vulpinic acid methyl ether.	1730	—	—

preferentially to yield pinastric acid skeleton which could be later easily esterified. Here also the methoxyl group will be playing an important part in bringing about preferential lactone ring closure involving the stronger acid*.

In the formation of leprapinic acid the mechanism seems to be different because of the presence of an *ortho* substituent. It seems probable that esterification takes place between the carboxyl and nuclear *o*-phenolic hydroxyl followed by the bridge lactonization. This would produce calycin (XI) as an intermediate and methanolysis of it could be expected to give leprapinic acid. The fact that calycin and leprapinic acid and its methyl ether have been isolated from *Lepraria chlorina* and *Biatora lucida*[2] supports this view.



X



XI

EXPERIMENTAL

The powdery lichen (30·00 g) separated from rocks by brushing was extracted with cold benzene (3 × 24 hr). The combined extracts were filtered and the filtrate concentrated to a small volume and light petroleum (60–80°) added. On standing golden yellow long rectangular plates separated together with some waxy matter. The product was washed with cold MeOH and crystallized from hot MeOH as golden yellow plates (1·5 g), m.p. 164–65°. It dissolved in $NaHCO_3$ aq, Na_2CO_3 aq and NaOH aq and was recovered unchanged on acidification. It did not give any colour with alcoholic $FeCl_3$ or bleaching powder. It agreed in all its properties with leprapinic acid;[1] mixed m.p. with an authentic sample[3] was undepressed. Its identity was further confirmed by UV and IR spectra and by circular paper chromatography.[3] λ_{max}^{MeOH} 270, 316 (inf.) mμ (log ε 4·18, 3·92); IR (KBr): 3030 (w), 1776 (s), 1686 (s) 1621 (s), 1603 (s), 1499 (m), 1458 (m), 1441 (s) cm^{-1}. (Found: C, 67·9; H, 4·5 Calc. for $C_{20}H_{16}O_6$: C, 68·2; H, 4·5.)

Leprapinic acid methyl ether (*methyl O-methyl-2-methoxypulvinate*) (VIII)

Leprapinic acid (0·1 g) was treated with an ethereal solution of diazomethane containing traces of MeOH for 24 hr. The methyl ether crystallized from MeOH as colourless needles, m.p. 150–52° (the earlier reported[3] low m.p. 118–20° is due to impurity); λ_{max}^{MeOH} 229 (inf.), 261, 336 mμ (log ε 4·27, 4·14, 4·44); IR ($CHCl_3$): 2994 (m), 2899 (w), 1773 (s), 1736 (s), 1634 (s), 1603 (m), 1493 (m), 1453 (m), 1435 (m) cm^{-1}. (Found: C, 68·8; H, 5·3. Calc. for $C_{21}H_{18}O_6$: C, 68·8, H, 4·9%.)

Ozonolysis of leprapinic acid (I)

A solution of leprapinic acid (0·5 g) in dry ethyl acetate was subjected to ozonolysis for ½ hr as described.[4] Fractionation of the solution gave the following:

Acid fraction. The $NaHCO_3$ aq extract was acidified, extracted with ether (4 × 50 ml) and ether recovered from the extract. The residue crystallized from water (animal charcoal) as colourless long needles, m.p. 120–121° alone or mixed with benzoic acid.

Neutral fraction (IV). Evaporation of the residual ethyl acetate solution (red. press.) gave a sweet smelling yellow oil. It was identified as methyl *o*-methoxybenzoylformate (i) by the formation of a 2,4-dinitrophenylhydrazone which crystallized from EtOH as yellow needles, m.p. 209–211° (Found: C, 51·8; H, 4·3. $C_{16}H_{14}N_4O_7$ requires: C, 51·3; H, 3·7%) and (ii) by conversion into *o*-methoxybenzoic acid as follows: A portion of the oil was heated under reflux with 10% KOH aq at 100° for 20 min. The solution was cooled, acidified and extracted with ether. The residue obtained on removal of ether was warmed with a few drops of conc. H_2SO_4 when it turned brown with the evolution of CO. The mixture was diluted with water, extracted with ether and ether recovered. The residue crystallized from water as colourless flakes, m.p. 98–99° alone or mixed with *o*-methoxybenzoic acid.

Methanolysis of 2-methoxypulvinic dilactone (VI)

The dilactone, m.p. 172–173° (0·4 g) obtained by hydrolysis and lactonization of natural leprapinic acid,[1] was subjected to methanolysis as described earlier.[3] The product was boiled with MeOH and filtered. The insoluble residue crystallized from benzene as golden yellow rectangular plates (0·2 g), m.p. 162–164°; mixed m.p. with a sample of leprapinic acid was undepressed; UV and IR spectra were identical.

The methanolic filtrate on concentration gave a yellow solid which on repeated crystallization from MeOH furnished yellow prisms (0·07 g), m.p. 132–34°, sintering at 126°; λ_{max}^{MeOH} 227 (inf.), 288, 355 mμ (log ε 4·21, 4·25, 3·82); IR (KBr): 2994 (w), 1783 (s), 1689 (s), 1618 (s), 1600 (s), 1497 (m), 1441 (s) cm^{-1}. (Found: C, 68·1; H, 4·8. $C_{20}H_{16}O_6$ requires: C, 68·2; H, 4·5%.)

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