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CHEMICAL INVESTIGATION OF INDIAN LICHENS—XXIV*

THE CHEMICAL COMPONENTS OF *ALECTORIA VIRENS* TAYL. CONSTITUTION OF A NEW DEPSIDONE, VIRENSIC ACID

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Abstract—The Himalayan lichen *Alectoria virens* Tayl. is found to contain besides D-arabitol, the unusual association of vulpinic acid and a new depsidone, virensic acid, whose structure is established as IIa.

ALECTORIA VIRENS TAYL. is a rare lichen, a sample of which was collected during a special expedition to the Himalayan lake Roopkund. The lichen resembles *Usnea pectinata* Tayl. but differs from it in possessing a yellowish green colour. The quantity of the lichen collected was only 90 g but it provided enough material for a detailed examination. Light petroleum extract yielded vulpinic acid.[1] A subsequent ether extraction gave a new depsidone 'virensic acid'. The residual lichen on acetone extraction yielded D-arabitol.

Asahina[2] examined by paper chromatography as well as by microchemical methods, a sample of *A. virens* Tayl. and identified the colouring matter as vulpinic acid. More recently Dhar *et al.*[3], reported that a sample obtained from Chakrata area contained atranorin and psoromic acid. In view of the present work the sample from Chakrata was re-examined and found to contain only vulpinic acid and virensic acid; hence the earlier report was an error. Based on the present work *Alectoria virens* seems to be so far the only species of *Alectoria* containing vulpinic acid and it contains this acid in association with a depsidone virensic acid.

Virensic acid is a colourless, optically inactive compound, m.p. 245–246°, and has the molecular formula $C_{18}H_{14}O_8$. It contained no methoxyl group, two hydroxyl groups, one aldehyde group and one free carboxyl group. It readily formed a monomethyl ester, a dimethyl ether methyl ester, a mono anil, a mono dinitrophenylhydrazone. It was unaffected by boiling with methanol during 18 hours which ruled out the possibility of its being a depside. It was hydrolysed by aqueous alkali to a dicarboxylic acid which has the molecular formula $C_{18}H_{16}O_9$. The dimethyl ether methyl ester on treatment with methanolic potash formed a new compound which contained one more methoxyl group and was soluble in aqueous alkali indicating that virensic acid is a depsidone. The ultra-violet spectrum [λ_{max}^{MeOH} 240, 308 mμ (log ϵ 4·47, 3·68) and λ_{min} 225, 285 mμ (log ϵ 4·34, 3·48)] and the infra-red spectrum (ester C=O band at 1725 cm^{-1}) also confirmed the presence of a depsidone system in virensic acid.

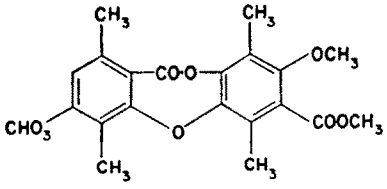
* Part XXIII: *J. Sci. Industr. Res. India* **18B**, 238 (1959).

[1] Y. Asahina and S. Shibata, *Chemistry of Lichen Substances* p. 43. Japan Society for the Promotion of Science, Tokyo (1954).

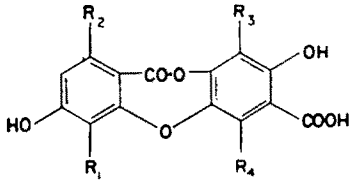
[2] Y. Asahina, *Fauna and Flora of Nepal Himalayas* (Edited by H. Kihara) p. 62. Fauna and Flora Research Society, Kyoto University, Japan (1955).

[3] M. L. Dhar, S. Neelakantan, S. Ramanujam and T. R. Seshadri, *J. Sci. Industr. Res. India* **18B**, 111 (1959).

Catalytic hydrogenation of virensic acid led to the up-take of 2 moles of hydrogen. That the absorption of hydrogen was solely due to the reduction of the —CHO group to the $—CH_3$ group was shown by the failure of the reduction product to exhibit carbonyl function and also by the infra-red spectrum. The reduction product produced a purple-red colour with alkali. This colour reaction is somewhat parallel to the behaviour of hypothamnolic acid[4] obtained by the reduction of thamnolic acid and may have significance. Complete methylation of the reduction product yielded a dimethyl ether methyl ester which was found to be identical with hypoprotocetraric acid methyl ether methyl ester (I) reported earlier.[5] Direct comparison was also effected with an authentic sample kindly furnished by Prof. Shibata, using mixed m.p., ultra-violet and infra-red spectra. Consequently the basic skeleton of virensic acid should have the structure II in which one of the substituents is a formyl group and the others are methyl groups. There are four possibilities (IIa, b, c, or d).



I



II

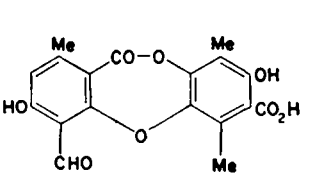
a, R_1 = CHO, $R_2 = R_3 = R_4 = CH_3$
b, R_2 = CHO, $R_1 = R_3 = R_4 = CH_3$
c, R_3 = CHO, $R_1 = R_2 = R_4 = CH_3$
d, R_4 = CHO, $R_1 = R_2 = R_3 = CH_3$

The correct structure (III) was deduced for virensic acid based on the following considerations. The structure IId is eliminated because a formyl group in the position shown would not exist free and would be present as a lactol (cf. salazinic acid,[6] and stictic acid[7]) and this would show a band in the infra-red spectrum at 1754 cm^{-1} not found in the spectrum of virensic acid. Further, the lactol grouping would not readily be reduced catalytically. The structure IIb is eliminated for similar reasons since the dicarboxylic acid obtained by the opening of the lactone ring does not show the lactol absorption, and undergoes catalytic reduction easily as does the original compound. The structure IIc is ruled out since the dimethyl ether methyl ester of virensic acid after lactone ring opening does not show any colour with alcoholic ferric chloride. If R_3 were a CHO group, then under the above conditions a new *ortho* hydroxy carbonyl system would be formed which would give a characteristic colour with alcoholic ferric chloride. Further, the spectral data of the ether ester after lactone ring opening does not support the presence of an *ortho* hydroxy aldehyde group. Hence III provides the correct structural formula for virensic acid.

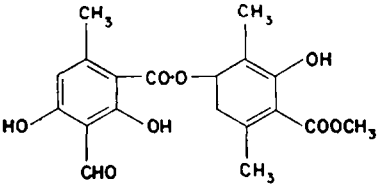
Virensic acid, as a depsidone has therefore a structure closely analogous to the depside atranorin (IV) which is common in the lichens. It is found in *Alectoria sulcata*

[4] Y. Asahina, F. Fuzikawa and M. Aoki, *Ber. Dtsch. Chem. Ges.* **74**, 928 (1941); Y. Asahina and T. Kusaka, *Ibid.* **69**, 450 and 1896 (1936).
[5] Y. Asahina and Y. Tanase, *Ber. Dtsch. Chem. Ges.* **67**, 414 and 716 (1934).
[6] Y. Asahina and S. Shibata, *Chemistry of Lichen Substances* p. 130. Japan Society for Promotion of Science, Tokyo (1954).
[7] Y. Asahina and S. Shibata, *Chemistry of Lichen Substances* p. 134. Japan Society for Promotion of Science Tokyo (1954).

Nyl.[8] but is absent in *A. virens*. Possibly virensic acid has taken its place owing to the presence in this species of the required oxidizing agent.



III



IV

EXPERIMENTAL*

Extraction of Alectoria virens *Tayl.*

(a) *With light petroleum* (*vulpinic acid*): The cut lichen (90 g) was extracted in a Soxhlet with light petroleum (b.p. 60–80°) for 24 hr. Evaporation of the extract left a solid residue (3·47 g) which on repeated crystallizations from benzene afforded yellow needles, m.p. 148–149°. The substance was identified as vulpinic acid; mixed m.p. with an authentic sample was not depressed and the ultra-violet and infra-red spectra of both the specimens were identical.

(b) *With ether* (*virensic acid*): The residual lichen was extracted with ether for 24 hr. A crystalline solid (4·3 g) separated out from the extract. Repeated crystallizations from dioxane–acetone gave *virensic acid* as colourless long needles, m.p. 245–247° (Found: C, 60·4; H, 4·4; OMe, nil; eq. wt. by lactone titration, 166·3 and 167·7; $C_{18}H_{14}O_8$ requires: C, 60·3; H, 3·9%; M.W. 358·3). Light absorption in methanol: λ_{max}: 240, 308 mμ (log ϵ 4·47, 3·68), λ_{min}: 225, 289 mμ (log ϵ 4·34, 3·48). Main I.R. bands: 3509–3333 (w), 2985 (m), 1724 (s), 1639 (s), 1613 (s), 1543 (m), 1422 (m), 1389 (m), 1348 (m), 1305 (w), 1266 (s), 1242 (m), 1198 (m), 1149 (s), 1121 (m), 1015 (w), 881 (w), 843–833 (w), 794 (w), 781 (w), 743 (m), 704 (w) cm^{-1}. Virensic acid gave a wine-red colour with alcoholic ferric chloride, a faint yellow colour with sodium hydroxide, violet colour changing to brown-red with conc sulphuric acid, and no colour with bleaching powder or conc nitric acid. It did not give the homofluorescein reaction. It formed a mono *dinitrophenylhydrazone*, which crystallized from acetone as orange rectangular plates, m.p. 256–258° (with sintering at 249°) (Found: C, 54·1; H, 3·1; $C_{24}H_{18}O_{11}N_4$ requires: C, 53·5; H, 3·4%). Virensic acid formed a mono *anil* which crystallized from acetone as golden yellow polygonal plates, m.p. 225–227° (Found: C, 66·3; H, 4·6; $C_{24}H_{19}O_7N$ requires: C, 66·5; H, 4·4%).

(c) *Extraction with acetone* (D-*arabitol*): The remaining lichen was extracted with acetone for 24 hr and the dark brown residue left on evaporation of the solvent was macerated with water and the insoluble portion filtered off. The aqueous extract on evaporation in a dessicator left a syrup which on trituration with methanol afforded a solid. It was recrystallized from methanol–ether mixture to yield D-arabitol, m.p. 99–102° (0·07 g); $[\alpha]_D^{28°}$ +7·35 (*c*, 0·07 in saturated aqueous borax). A mixed m.p. with an authentic sample showed no depression.

Methyl virensate

A solution of virensic acid (0·1 g) in dry acetone (25 ml) was heated with sodium hydrogen carbonate (0·15 g) and dimethyl sulphate (0·2 ml) for 8 hr. The sodium salts were filtered off and the filtrate on evaporation yielded a residue (0·08 g) of *methyl virensate*, which crystallized from ethyl acetate as colourless long flat needles, m.p. 215–216·5° (Found: C, 61·3; H 4·4; OMe 8·3; $C_{19}H_{16}O_8$ requires: C, 61·3; H, 4·3; 1 OMe, 8·3%). Light absorption in methanol: λ_{max} 243, 300, 316 mμ (log ϵ 4·49, 3·85, 3·78), λ_{min} 228, 294, 310 mμ (log ϵ 4·38, 3·70, 3·75). Main I.R. bands: 3390–3279 (w), 1724 (s), 1639 (s), 1600 (shoulder), 1563 (m), 1429 (m), 1408 (shoulder), 1372 (m), 1361 (shoulder), 1342 (shoulder), 1316 (s), 1282 (s), 1267 (s), 1250 (s), 1198 (s), 1172 (m), 1149 (s), 1124 (m), 1074 (m), 1022 (m), 971 (w), 855 (w), 796 (m), 763 (m) cm^{-1}. The ester gave a wine-red colour with alcoholic ferric chloride.

* All the I.R. spectra were taken in KBr disk unless otherwise stated.

[8] Y. Asahina and H. Hayashi, *J. Pharm. Soc, Japan* **48**, 1094 (1928).

Methyl virensate dimethyl ether

A solution of virensic acid (0·1 g) in dry acetone (25 ml) was heated with anhydrous potassium carbonate (2 g) and dimethyl sulphate (0·5 ml) for 18 hr. The product was crystallized from benzene–light petroleum and the *ether ester* was obtained as colourless fine needles m.p. 160–162° (Found: C, 62·6; H, 4·5; OMe, 23·4; $C_{21}H_{20}O_8$ requires: C, 63·0; H, 5·0; 3 OMe, 23·2%). Light absorption in methanol: λ_{max} 301, 316 mμ (log ε 3·57, 3·42), λ_{min} 294, 308 mμ (log ε 3·36, 3·26). Main I.R. bands: 2874 (w), 1709 (s), 1681 (m), 1587 (m), 1527 (m), 1451 (m), 1414 (m), 1399 (m), 1379 (m), 1368 (m), 1333 (m), 1274 (s), 1258 (shoulder), 1220 (shoulder), 1208 (m), 1190 (m), 1166 (w), 1140 (s), 1124 (m), 1087 (w), 1048 (w), 1029 (w), 990 (w), 962 (w), 909 (w), 855 (w), 793 (w), 769 (w) $cm.^{-1}$

Opening of the lactone ring of virensic acid

A solution of virensic acid (0·1 g) in 10% aqueous potassium hydroxide (2 ml) was kept at room temp for 1 hr. The solution was acidified and extracted with ether. The solid obtained from ether solution was crystallized from alcohol. The *diacid* was obtained as colourless rectangular prisms and needles, m.p. 216–217° (Found: C, 56·1; H, 4·4; $C_{18}H_{16}O_9.\frac{1}{2}H_2O$ requires: C, 56·1; H, 4·4%). It gave a pinkish-brown colour with ferric chloride and a deep yellow colour with bleaching powder and with sodium hydroxide. Light absorption in methanol: λ_{max} 227, 270, 305 and inf. 333 mμ (log ε 4·50, 4·23, 3·72, and 3·54), λ_{min} 252, 300 mμ (log ε 4·09, 3·64). Main I.R. bands: 3448 (w), 2899 (w), 1681 (m), 1621 (s), 1431 (m), 1393 (shoulder), 1379 (m), 1342 (w), 1266 (s), 1220 (m), 1198 (m), 1176 (w), 1105 (m), 901 (w), 820 (w), 797 (w) cm^{-1}.

Opening of the lactone ring of methyl virensate dimethyl ether

Methyl virensate dimethyl ether (0·08 g) was mixed with methanolic potassium hydroxide (5 ml; 10%) and kept at room temp for 2½ hr. Acidification and extraction with ether followed by evaporation of ether from the extract gave a product which crystallized from ethyl acetate–light petroleum as colourless stout rectangular tablets, m.p. 134–135° (Found: C, 61·2; H, 5·8; OMe, 28·1; $C_{22}H_{24}O_9$ requires: C, 61·1; H, 5·6; 4 OMe, 28·7%). It did not give any colour with alcoholic ferric chloride. Light absorption in methanol: λ_{max} 226, 280, 305, 325 mμ (log ε 4·45, 3·84, 3·26, 3·19), λ_{min} 274, 298, 317 mμ (log ε 3·78, 3·18, 3·08). Main I.R. bands: 3448 (s), 2959 (m), 1718 (vs), 1667 (s), 1600 (s), 1553 (m), 1460 (s), 1399 (s), 1370 (shoulder), 1340 (m), 1316 (shoulder), 1282–1266 (s), 1242 (shoulder), 1205–1190 (s), 1170 (s), 1149 (s), 1111 (s), 1075 (m), 1053 (m), 1020 (shoulder), 1010 (m), 990 (m), 978 (m), 901 (m), 890 (m), 848 (m), 833 (m), 800 (w), 781 (w), 769 (w), 763 (w), 746 (w), 716 (w), 671 (w) cm^{-1}.

Catalytic reduction of virensic acid

A solution of virensic acid (0·1 g) in methanol (15 ml) was shaken with hydrogen in the presence of palladized charcoal (0·1 g, 10%) until no further absorption took place. During 2 hr hydrogen absorbed was 13·5 ml at 32·1° and 736·2 mm. The catalyst was removed by filtration and methanol evaporated *in vacuo*. The residue (0·06 g) crystallized from ethyl acetate as clusters of stout rectangular prisms, m.p. 240° (Found: C, 62·7; H, 5·0; $C_{18}H_{16}O_7$ requires: C, 62·8; H, 4·7%). It gave a violet colour with alkalis and alkali carbonates and a pale red colour with bleaching powder. Light absorption in methanol: λ_{max} 225, 265, 280 mμ (log ε 4·42, 4·08, 4·05), λ_{min} 245, 277 mμ (log ε 4·05, 3·98). Main I.R. bands: 3559 (m), 3226 (m), 3125 (m), 2899 (m), 2564–2326 (w), 1689 (s), 1626 (m), 1592 (s), 1550 (m), 1481 (m), 1439 (m), 1401 (m), 1370 (m), 1304 (s), 1258 (s), 1209 (m), 1149 (s), 1075 (m), 1042 (w), 1020 (w), 1009 (m), 893 (w), 855–840 (w), 803 (m), 784 (w), 752–735 (w), 714–704 (w) cm^{-1}.

Complete methylation of reduced virensic acid

A solution of the above compound (0·1 g) in methanol was treated with excess of diazomethane in ether and kept overnight in the refrigerator the unchanged diazomethane decomposed by acetic acid and the solvent removed *in vacuo*. The product crystallized from ethyl acetate as colourless stout rectangular tablets, m.p. 169–170°. It did not give any colour with alcoholic ferric chloride. A mixed m.p. with an authentic specimen of hypoprotocetraric acid methyl ether methyl ester was undepressed. The ultra-violet and the infra-red spectra of both the specimens were identical. Light absorption in

methanol: λ_{max} 269 mμ (log ϵ 3·99), λ_{min} 247 mμ (log ϵ 3·85), [authentic specimen had the absorption: λ_{max} 269 mμ (log ϵ 3·92), λ_{min} 245 mμ (log ϵ 3·68)]. Main I.R. bands: ($CHCl_3$) 2959 (m), 2890 (m), 1712 (s), 1592 (s), 1553 (m), 1447 (s), 1429 (shoulder), 1399 (s), 1333 (s), 1307 (m), 1274 (s), 1176 (m), 1130 (s), 1111 (m), 1083 (m), 1052 (m), 1030 (m), 1005 (m), 996 (m), 972 (m), 899 (w). 841 (w) cm^{-1}.

The same product was also obtained by the reduction of virensic acid dimethyl ether methyl ester (0·1 g) in methanol with hydrogen in the presence of palladized charcoal (0·1 g, 10%) and working up the product as usual.

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