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C-GLYCOSIDES OF TAMARIND LEAVES

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Abstract—The leaves of *Tamarindus indica* L. have been found to contain two pairs of ring isomers vitexin and isovitexin (saponaretin) and orientin and iso-orientin (homo-orientin). They are best separated by preparative paper chromatography. The first two are the 8- and 6-*C*-glucopyranoside of apigenin and similarly the second two have been assigned the constitution of 8- and 6-*C*-glucopyranoside of luteolin.

THE TAMARIND tree is common all over India though more in the South, and a large number of uses have been listed for its various parts.[1,2] In the early stages of our work on these leaves Lewis and Neelakantan[3] reported the presence of four anthoxanthin glycosides in the aqueous extract. From spectral observations they considered two of them to be *O*-glycosides of luteolin and apigenin. However, they remarked on the poor hydrolysability of the glycosides which made positive identification difficult and considered that they were either stable stereoisomers of *O*-glycosides or *C*-glycosides. Later when our work was nearly over they reported in a brief note[4] that two of the compounds could be identified as vitexin and orientin. Since our study is more complete and the methods and observations are somewhat different, they are reported here; some of them have been given briefly earlier.[5]

We find that extraction with water is not efficient and complete extraction is possible only by using fresh leaves and as solvent successively hot ethanol, 80% and 60% ethanol. Circular paper chromatography of the concentrate indicated the presence of four pigments (*A* to *D*) together with large quantities of tartaric acid. Extraction of the concentrate with ethyl acetate removed all the pigments along with tartaric acid. Attempts to fractionate the pigments by concentrating the extracts in stages were only partially successful and only component *B* could be obtained pure. Similarly fractionation using lead acetate only gave component *B*. However all the pigments were readily separated by preparative paper chromatography.

The purified pigments were all soluble in water and insoluble in ether, and stable to acid hydrolysis being fully recovered even after prolonged boiling with mineral acid. Thus they all seemed to be *C*-glycosides.

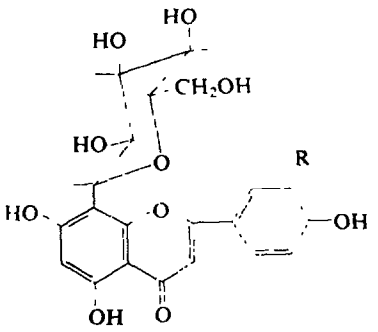
Component *A*, had the molecular formula $C_{21}H_{20}O_{11}$. Its colour reactions and spectra, i.r. and u.v. under different conditions, indicated close resemblance to luteolin with all the hydroxyl groups free. The melting point of the glycoside (265–267°) and of its acetate agreed with those of orientin. Identification was confirmed by comparison with an authentic sample using paper chromatography, spectroscopy and mixed melting point. The constitution of

[1] K. R. LAUMAS and T. R. SESHADRI, *J. Sci. Ind. Res. India* **17B**, 45 (1958).
[2] D. NARAYANAMURTI, P. RAMACHANDRA RAO and RULIA RAM, *J. Sci. Ind. Res. India* **16B**, 377 (1957).
[3] Y. S. LEWIS and S. NEELKANTAN, *Curr. Sci.* **31**, 508 (1962).
[4] Y. S. LEWIS and S. NEELAKANTAN, *Curr. Sci.* **33**, 460 (1964).
[5] V. K. BHATIA, S. R. GUPTA and T. R. SESHADRI, *Curr. Sci.* **33**, 581 (1964).

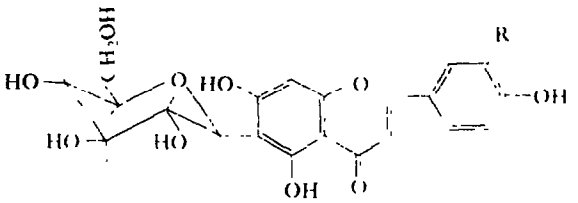
orientin is best represented as luteolin-8-*C*-glucopyranoside (I)[6] and not as furanoside.[7]

Component *B* had the molecular formula $C_{21}H_{20}O_{10}$. It resembled component *A* very closely but lacked the catechol grouping indicating that it was an apigenin derivative. Its identity with vitexin (II) was established by detailed comparison with an authentic sample. The partial methyl ether was prepared and subjected to oxidation with sodium metaperiodate. The product was reduced with sodium borohydride and subjected to acid hydrolysis yielding glycerol. This reaction has already been explained[5] as being given by flavone *C*-glycosides.

The component *C* agreed with component *A* in all its chemical reactions and spectral properties but its melting point (235°) was different from *A* (265°) and agreed with that of



(I) R = OH, orientin
(II) R = H, vitexin



(III) R = OH, homoorientin
(IV) R = H, isovitexin (saponaretin)

homo-orientin (III).[7] This was supported by the melting point of the tetra-methyl ether which agreed with that of homo-orientin methyl ether. Finally the identity of *C* with homo-orientin was established by comparison with an authentic sample. Its constitution is probably 6-*C*-glucosyl luteolin (III) from the observations of Koeppen[8] that it contains only four aliphatic hydroxyl groups and yields, on periodic acid oxidation under mild conditions 1 mole of formic acid, and further that in acid solution it is in equilibrium with orientin (I). Analogous cases earlier studied in detail are vitexin (II) and isovitexin (IV)[9] which have been shown to exhibit

[6] B. H. KOEPPEN and D. G. ROUX, *S. African Med. J.* **38**, 154 (1964).
[7] L. HORHAMMER and H. WAGNER, In *Recent Developments in the Chemistry of Natural Phenolic Compounds*, p. 187. Pergamon Press, Oxford (1961).
[8] B. H. KOEPPEN, *Chem. & Ind. (London)* 2145 (1962).
[9] R. M. HOROWITZ and B. GENTILI, *Chem. & Ind. (London)* 498 (1964).

such an isomeric change. The unexpected facility of this change in these *C*-glycosides as compared with *C*-methyl-flavones could be attributed to the high solubility in water of these *C*-glycosyl compounds. In view of this isomeric relationship homo-orientin (III) should more appropriately be called iso-orientin.

A detailed study of the component *D*, (m.p., 238–39°), by the above mentioned methods showed that it was isovitexin (IV). Confirmation was provided by comparison with an authentic sample.

Thus tamarind leaves contain two pairs of ring isomers orientin and iso-orientin, and vitexin and isovitexin. Vitexin (II) is present as the major component with orientin (I) next, the iso compounds (III and IV) being found in smaller quantities. The presence of a sugar substituent in the nucleus may perhaps protect the polyphenols from oxidative change.

EXPERIMENTAL

R_f values were determined by circular paper chromatography on Whatman No. 1 filter paper. The solvent systems were: (*a*) water saturated with phenol; (*b*) phenol saturated with water; (*c*) *n*-butanol:acetic acid:water (4:1:5), top layer; and (*d*) aqueous layer; (*e*), 30% acetic acid; and (*f*) 15% acetic acid. The flavonoids were revealed by exposure to ammonia vapour. The u.v. spectra determined in ethanol are marked (i), with added sodium acetate (ii), with added aluminium chloride (iii), with boric acid and sodium acetate (iv) and with sodium ethoxide (v); $\log \epsilon$ values are given in brackets.

Extraction

Fresh leaves (2 kg) were extracted with boiling alcohol (4 × 4 l.; 10 hr each time). The residual leaves were successively extracted with 80% and 60% boiling alcohol (10 hr in each case). The solvent was removed from the combined extracts under reduced pressure. The aqueous extract (1·8 l.) was then extracted with petroleum ether (60–80°; 12 × 1 l.) and ether (5 × 1 l.) to remove chlorophyll and waxy matter. During these extractions some inorganic matter (4 g and 20 g respectively) was thrown down. This consisted mainly of potassium hydrogen tartrate. In the second case the solid also brought down a small amount of a pigment (R_f 0·68, solvent system *a*). The pigment was extracted from the inorganic matter with hot water and combined with the main aqueous solution.

The aqueous solution which gave a pink colour with magnesium and hydrochloric acid on chromatography in (*b*) showed the presence of four rings, R_f 0·54 (*A*), 0·70 (*B*), 0·78 (*C*), and 0·87 (*D*). The concentrated aqueous solution (250 ml) was continuously extracted with ethyl acetate changing the solvent after every 24 hr. The first two such extracts contained all the four pigments, and on concentration deposited a dark brown solid containing the pigments *A*, *B* and *C* along with a large quantity of tartaric acid, the mother liquor still showing the presence of all the four pigments. The next six fractions contained only *A*, *B* and *C* and on concentration deposited them as a mixture with a large amount of tartaric acid. The last four fractions contained only component *B* and on concentration gave it almost free of tartaric acid. The above-mentioned mixtures were freed from tartaric acid by dissolving them in water, neutralizing the acid with $KHCO_3$ and extracting the pigments with ethyl acetate.

Isolation of the pigment B

To the mixture of the pigments *A*, *B* and *C* (200 mg) in water (100 ml) was added a solution of neutral lead acetate (5 g, 20 ml water). The precipitate was filtered off and the filtrate

treated with a solution of basic lead acetate (2 g, 100 ml water). On working up the precipitates a mixture of all the three pigments was obtained but on concentration to about 10 ml *B* was obtained pure. Yield 20 mg.

Isolation of the Individual Pigments

The mixture of *A*, *B*, *C* and *D* (100 mg) was separated on Whatman No. 3 MM using solvent (*e*). The individual pigments were separately extracted with methanol (3 × 500 ml, 4 hr each time), and finally concentrated to 3–4 ml when the pigment separated out. Component *A* recrystallized from ethanol as pale yellow globular clusters m.p., 265 67° (decomp.), 25 mg (0·04 per cent of air-dry leaves); *B* from pyridine–ether mixture or 80% ethanol as yellow plates, m.p. 252° (decomp.), 37 mg (0·06 per cent) and *C* and *D* from methanol as yellow microcrystalline solids, m.p. 234–5°, 20 mg (0·03 per cent) and m.p. 238–39°, 10 mg (0·02 per cent) respectively.

Component A. It was readily soluble in aqueous NaOH and Na_2CO_3 and gave an olive-green colour with alcoholic $FeCl_3$ and a pink colour with Mg and HCl, m.p. 265–67° (decomp.), undepressed on admixture with authentic orientin. Ultraviolet data: (λ max) (i) 258, 270 and 350 mμ (4·1, 4·1 and 4·1); (ii) 279 and 380 mμ (4·1 and 4·1); (iii) 269, 350 and 380 mμ (4·1, 4·1 and 4·0); (iv) 262 and 371 mμ (4·4 and 4·3); (v) 272 and 402 mμ (4·2 and 4·3). I.r. (cm^{-1}) (KBr disc) 3571 (s), 1653 (s), 1613 (s), 1562 (s), 1504 (s), 1429 (s), 1361 (s), 1348 (s), 1274 (s), 1227 (s), 1190 (s), 1105 (s), 1087 (s), 1031 (m), 1010 (m), 980 (m), 930 (m), 848 (m), 817 (m) and 788 (m). (Found: C, 55·5; H, 4·5. Calc. for $C_{21}H_{20}O_{11}$: C, 56·2; H, 4·5%). The pigment and an authentic sample of orientin had the same chromatographic behaviour in solvent systems *a*, *b*, *c*, *d* and *f* having R_f values 0·53, 0·66, 0·51, 0·57 and 0·41 (19°).

Acetylation. Component *A* (50 mg) was acetylated using acetic anhydride (2 ml) and pyridine (1 ml) at 100° for 4 hr. Repeated crystallization of the product from aqueous acetic acid gave colourless prisms, m.p. 196–97°; 40 mg. It gave no colour with alcoholic $FeCl_3$. (Found: C, 56·4; H, 4·8. Calc. for $C_{37}H_{36}O_{19}$: C, 56·6; H, 4·6%).

Component B. It was soluble in aqueous alcohol and aqueous Na_2CO_3 and NaOH. It gave a brown colour, with $FeCl_3$, and with Mg and HCl developed a pink colour, and gave a positive Molisch's test; m.p. and mixed m.p. with an authentic sample of vitexin, 252°. Ultraviolet data: (λ max) (i) 270 and 334 mμ (4·3 and 4·3); (ii) 279 and 388 mμ (4·4 and 4·2); (iii) 274, 303, 340 and 380 mμ (4·3, 4·2, 4·3 and 4·1); (iv) 270 and 335 mμ (4·1 and 4·1); (v) 280 and 400 mμ (4·2 and 4·3). I.r. (cm^{-1}) (KBr disc) 3448 (s), 1653 (s), 1550 (s), 1538 (s), 1504 (m), 1418 (m), 1355 (m), 1282 (m), 1105 (m), 1036 (w) and 830 (m). (Found: C, 58·4; H, 4·7 Calc. for $C_{21}H_{20}O_{10}$: C, 58·3; H, 4·7%). Component *B* and an authentic sample of vitexin had the same chromatographic behaviour in solvent systems *a*, *b*, *c* and *d* and had R_f values 0·70, 0·80, 0·66 and 0·76 (34°).

Acetylation. Component *B* (100 mg) was acetylated as described earlier. Repeated crystallization from ethyl acetate–petroleum ether gave the acetate as rectangular plates, m.p., 257–59°; 80 mg. It gave no colour with alcoholic $FeCl_3$ (Found: C, 57·2; H, 5·0. Calc. for $C_{35}H_{34}O_{17}$: C, 57·9; H, 4·7%).

Methylation. Component *B* (70 mg), in a mixture of dioxan (10 ml), ethanol (15 ml) and water (0·5 ml) was methylated using diazomethane (from 2 g of nitrosomethyl urea) in ether (50 ml). At the end of 24 hr more of diazomethane in ether was added. After 72 hr the pale yellow semisolid residue left after evaporating the solvents crystallized from dry acetone as light yellow needles, m.p. 264° (45 mg). It gave a purple-red colour with alcoholic $FeCl_3$. (Found: C, 56·1; H, 5·6. Calc. for $C_{23}H_{24}O_{10}\cdot 2H_2O$: C, 56·7; H, 4·8%).

Action of hydriodic acid. To the suspension of the pigment *B* (80 mg) in phenol (0·5 ml) hydriodic acid (0·8 ml; d 1·7) was added gradually, the mixture gently refluxed (135–37°) for 7 hr, cooled and poured into an aqueous solution of sodium bisulphite. Repeated crystallization from ethyl acetate–petroleum ether (60–80°) gave a yellow product, m.p. >300° (18 mg). It gave a brown colour with alcoholic ferric chloride and a pink-red colour with magnesium and hydrochloric acid. Ultraviolet data: (λ max) (i) 270 and 338 mμ (4·2 and 4·1); (ii) 275 and 355 mμ (4·1 and 3·9); (iii) 278 and 354 mμ (4·3 and 4·2); (iv) 270 and 338 mμ (4·2 and 4·0); (v) 280, 320 and 397 mμ (4·0, 3·8 and 4·0). Infra red (cm^{-1}) (KBr disc) 3333 (OH); 1653 (conjugated CO) and 1613, 1562, 1493, 1460 (aromatic C—C). The aglycone and an authentic sample of apigenin had the same chromatographic behaviour in solvent systems *a*, *b*, *c* and *d* having R_f values 0·46, 1·0, 0·58 and 1·0 (34°).

Periodic acid oxidation of the methyl ether of component B followed by borohydride reduction. To a solution of the methyl ether (2 mg) in water (0·2 ml) was added $NaIO_4$ (2 mg), the mixture kept at room temperature for 4 hr, $NaBH_4$ (2 mg) in water (0·1 ml) added, the solution kept at room temperature overnight, and then heated with HCl (1 N, 0·2 ml) at 100° for 15 min. Ascending chromatography of this solution with ethyl acetate:pyridine:water (10:4:3) system as the irrigating solvent and as spray a mixture of aqueous $NaIO_4$ (2%, 4 parts) and $KMnO_4$ (1% $KMnO_4$ in 2% aqueous Na_2CO_3) gave a yellow spot on a pink background with an R_f 0·37 (32°). Glucose treated in the same manner gave R_f 0·37 and so also its mixture with that from component *B*.

Component C. It was highly soluble in aqueous Na_2CO_3 and NaOH. With alcoholic $FeCl_3$ it gave brownish-green colour, with Mg and HCl acid a pink colour and a positive Molisch's test. Ultraviolet data (λ max): (i) 257, 270 and 350 mμ (4·2, 4·2 and 4·2); (ii) 279, and 390 mμ (4·3 and 4·2); (iii) 271 and 290 mμ (4·2) and 4·1); (iv) 262 and 372 mμ (4·4 and 4·4); (v) 270 and 401 mμ (4·3 and 4·3). I.r. (cm^{-1}) (nujol mull) 3509 (m), 1639 (m), 1613 (m), 1562 (m), 1493 (m), 1287 (m), 1258 (w), 1247 (w), 1217 (w), 1172 (m), 1099 (m), 1085 (m), 1031 (m), 1010 (m), 964 (w), 848 (w), 830 (w) and 716 (w). The pigment and an authentic sample of homo-orientin in solvent systems *a*, *b*, *c*, *d* and *f* gave R_f values 0·54, 0·78, 0·55, 0·70 and 0·44 (19°).

Methylation. Component *C* (30 mg) was methylated using diazomethane in ether. The methyl ether crystallized from dry acetone as light yellow granules m.p. 265–67°; 15 mg. (Found: C, 57·2; H, 5·0. Calc. for $C_{25}H_{30}O_{12}$: C, 57·5; H, 5·7%).

Component D. It was soluble in aqueous Na_2CO_3 and NaOH. With alcoholic $FeCl_3$ it gave a brown colour, on reduction with Mg and HCl a pink colour and a positive Molisch's test. The compound had m.p. 239° undepressed by an authentic sample of isovitexin. Ultraviolet data (λ max): (i) 272 and 337 mμ (4·5 and 4·4); (ii) 279, and 372 mμ (3·6 and 4·4); (iii) 274, 302, 340 and 379 mμ (4·4, 4·3, 4·4 and 4·4); (iv) 270 and 335 mμ (4·4 and 4·4); (v) 279, 332 and 400 mμ (4·3, 4·0 and 3·7). I.r. (cm^{-1}) (nujol mull) 3472 (s), 1653 (s), 1615 (s), 1587 (s), 1565 (m), 1502 (m), 1285 (m), 1248 (m), 1220 (m), 1193 (m), 1170 (m), 1107 (w), 1081 (m), 1036 (m), 1010 (m), 969 (m), 833 (m) and 718 (m). The pigment and an authentic sample of isovitexin had the same chromatographic behaviour in solvent systems *a*, *b*, *c*, *d* and *f* giving R_f values 0·68, 0·87, 0·72, 0·78 and 0·60 (19°).

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