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LANCEOLARIN, A NEW ISOFLAVONE GLYCOSIDE OF *DALBERGIA LANCEOLARIA*[1]

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Abstract—The root-bark of *Dalbergia lanceolaria* contains a new isoflavone glycoside, now named lanceolarin. By the use of physical, chemical and degradative evidence its structure is deduced as the 7-apioglucoside of biochanin-A. It has also been shown that the position of linkage between apiose and glucose is 1 → 2 and that the glycosidic bonds are of β configuration.

Dalbergia, an important genus of trees (Leguminosae) has fifteen reported species in India,[2,3] many of which are of considerable commercial value and some provide folk medicines. The chemical study of these trees has revealed new isoflavones,[4–7] 4-phenyl coumarins[8] and dalbergenones[9–11] (dalberginoids, neoflavanoids). *Dalbergia lanceolaria* is cultivated in India as a host to the economically important lac insect and its leaves are used in the treatment of arthritic disabilities.[12] The present paper records the presence of 7-apioglucoside of biochanin-A, now named lanceolarin, in the root-bark of this tree. This is another example of the occurrence of the branched chain sugar apiose in glycosidic combination, the only instances known earlier being apiin[13] and graveobiosides A and B.[14] It may be pointed out that these compounds are flavones while lanceolarin is the first example of an isoflavone apioglucoside.

The root-bark was successively extracted with light petroleum, ether, acetone and alcohol and from the acetone concentrate lanceolarin was isolated as a colourless crystalline solid having a bitter taste. Its colour reactions and UV spectrum indicated that it belonged to the isoflavone group. A purple-violet ferric reaction suggested the

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existence of chelated phenolic hydroxyl group(s) and a positive Molisch's test, a band at 3400 cm^{-1} in the IR spectrum (alcoholic OH) and reduction of Tollens' reagent showed the presence of sugar groups. It was therefore inferred that the new compound is an isoflavone glycoside. Analytical data agreed with the formula $C_{27}H_{30}O_{14}$; one methoxyl group was present and acetylation gave a heptaacetate. Acid hydrolysis gave biochanin-A identified by m.m.p., colour reactions, cochromatography and preparation of derivatives. The sugars were found to be apiose and glucose by comparison with authentic specimens prepared by hydrolysis of apiin and graveobioside-A. Apiose was further confirmed by the characteristic yellow spot on the chromatograms showing an yellowish-white fluorescence in UV light when benzidine–trichloracetic acid reagent was used for developing.[15,16] Quantitative acid hydrolysis of lanceolarin showed that it contained biochanin-A, glucose and apiose in equimolar proportions.

Complete methylation of the glycoside followed by the removal of the sugar groups yielded 5,4'-dimethoxy-7-hydroxy isoflavone[17,18] identified by m.p., spectral data and preparation of the acetate and the ethyl ether. An authentic sample of the last substance has now been synthesized from genistein by partial ethylation of the 7-hydroxyl group followed by complete methylation. From this information it was concluded that in lanceolarin glucose and apiose are attached to the 7-hydroxyl group of biochanin-A as a biose unit. Partial hydrolysis of the glycoside under mild conditions removed apiose yielding biochanin-A 7-glucoside identical with sissotrin[7] and also a synthetic sample.[19] It, therefore, follows that the glucose residue is attached to the 7-hydroxyl group of biochanin-A and the apiose unit to one of the hydroxyl groups of glucose. The exact hydroxyl involved now remains to be established.

Earlier investigations[20] have established that, in apiin, 1-β-hydroxyl of the D-glucopyranose is condensed with the 7-hydroxyl of apigenin and the 1-β-hydroxyl of D-apiofuranose is condensed with the 2-hydroxyl group of the glucose residue; further the C_2 and the C_3 hydroxyl groups of apiose are *cis*-oriented. We have now used apiin as the reference compound to determine the positions and the nature of the glycosidic bonds in lanceolarin. For this purpose apiin and the new glycoside were exhaustively methylated,[20,21] the methylated glycosides were hydrolysed and the resulting methylated sugars compared by cochromatography on paper and thin-layers of silica gel. They were found to be the same viz. 3,4,6-trimethyl-D-glucopyranose and 2,3,4-trimethyl-D-apiofuranose, thus proving that in lanceolarin also the anomeric hydroxyl of apiose is condensed with the 2-hydroxyl of glucose. Finally it was found that the glycosidic bonds are susceptible to hydrolysis by almond emulsin showing that they are of β-configuration. These conclusions were also substantiated from optical rotation considerations.[20] The $[M]_D$ of lanceolarin is $-560{\cdot}1^\circ$ and that of biochanin-A 7-O-D-glucopyranoside is $-158{\cdot}3^\circ$. The difference is $-401{\cdot}8^\circ$ which is close to the $[M]_D$ of the β-methyl glycoside of D-apiofuranose, -405°. The complete structure of lanceolarin is therefore established as I.

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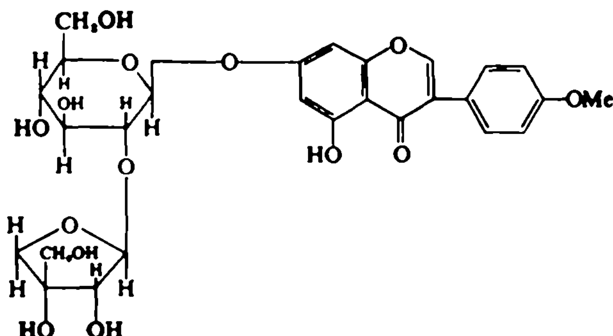

I

EXPERIMENTAL

[Paper chromatography was carried out on Whatman No. 1 filter paper and TLC on silica gel. For the glycosides and the aglycones the following solvents were used: (a) 5% AcOH (b) 30% AcOH (c) n-BuOH–AcOH–water, 6:1:2 (d) benzene–AcOH–water, 125:72:3 (e) n-BuOH–ammonia, 85:15 (f) MeOH–chf, 11:89 (g) MeOH–chf, 30:70; for the sugars: (h) moist n-BuOH (i) moist phenol (j) n-BuOH–AcOH–water, 4:1:5, upper layer (k) n-BuOH–water–pyridine–benzene, 5:3:3:1, upper layer (l) n-BuOH or n-propanol–ammonia–water, 6:2:1; for the methylated sugars: (m) benzene–EtOH, 6:1 (n) n-BuOH–water–CCl_4, 4:4:3, lower layer. Ethanolic $FeCl_3$ was used as the developer for the isoflavone glycosides and the isoflavones and aniline hydrogen phthalate and benzidine–trichloracetic acid for the sugars.

Isolation of lanceolarin. Air-dried and coarsely powdered root-bark of *Dalbergia lanceolaria* (1 kg) was successively extracted with light petroleum (60–80°), ether, acetone and alcohol (4 × 3 l., 4 hr each time). The acetone concentrate on standing for about a week deposited a colourless, crystalline solid which was filtered and washed with cold acetone. It was further purified by 2 recrystallizations from MeOH colourless needles, m.p. 165–170° sintering earlier and collecting at 190–193°, yield, 4 g. (Found: C, 54·7; H, 5·1; OMe, 4·8. $C_{27}H_{30}O_{14}$, H_2O requires: C, 54·4; H, 5·4; OMe, 5·2%.) $[\alpha]_D^{25}$ −96·9° (*c*, 1·032; 80% MeOH). UV absorption (mμ): λ_{max}^{EtOH} 262 (4·55), 335 (4·06); $\lambda_{max}^{EtOH-NaOAc}$ 262; $\lambda_{max}^{EtOH-AlCl_3}$ 271. IR spectrum (nujol, cm^{-1}): 3400 (broad), 1660, 1615, 1580, 1300, 1255, 1180, 1020, 830. R_f values: 0·5 (paper, descending, solvent c, 25°); 0·7 (paper, circular, solvent a, 23°); 0·72 (thin-layer, silica gel, solvent g, 30°).

Lanceolarin is sparingly soluble in water, acetone, MeOH and AcOEt in the cold; it readily dissolves in warm water from which it is not reprecipitated on cooling. It has a bitter, astringent taste. With alcoholic $FeCl_3$ it gives a violet-brown colour becoming greenish-brown with excess of the reagent. A light yellow colour is produced when the substance is reduced with Mg and HCl in ethanolic soln; a red colour is obtained when a strong soln is treated with NaHg, left overnight and then acidified with conc. HCl. Durham test for isoflavanones is negative. It dissolves to a pale yellow soln in conc H_2SO_4 without any fluorescence. Tollens' reagent is rapidly reduced but the reduction of Fehling's reagent is slow. Molisch's test is answered.

Acetate. The glycoside (0·8 g) was acetylated by heating with Ac_2O (5 ml) and dry pyridine (5 ml) at 100° for 2 hr. The acetate had m.p. 181–182° from (benzene–pet. ether). [Found: C, 56·3; H, 5·1; acetyl, 40·8. $C_{41}H_{44}O_{21}$ requires: C, 56·4; H, 5·1; 7 CH_3CO + 1 HCO (from the 2-carbon of isoflavone), 39·5%.] $[\alpha]_D^{30}$ −47·9° (*c*. 1·252, DMF).

Acid hydrolysis of lanceolarin. The glycoside (1 g) was refluxed with 4% H_2SO_4aq (100 ml). It first dissolved and when the soln began to boil the aglycone started to separate. After refluxing for 4 hr, the mixture was kept in the refrigerator overnight, the colourless solid was filtered off and crystallized from MeOH, m.p. 211–213°. It was identified as biochanin-A by colour reactions, m.m.p. determination, co-chromatography (paper and thin-layer of silica gel), preparation of the acetate and the methyl ether and comparison with authentic samples.[7]

The aqueous filtrate from the above was neutralized with $BaCO_3$, the Ba salts were filtered off and the filtrate evaporated to dryness on a water-bath. The brownish syrup was dissolved in warm MeOH, filtered and concentrated and was examined by chromatography. R_f values: 0·26 and 0·58

(paper, ascending, solvent h, 25°) and 0·46 and 0·77 (TLC, solvent i, 25°). The sugars were identified as glucose and apiose by comparison with authentic sugars prepared by the hydrolysis of apiin and graveobioside-A.

Methylation and acid hydrolysis. The glycoside (1 g) was methylated by refluxing with Me_2SO_4 (5 ml) and K_2CO_3 (10 g) in dry acetone (150 ml) soln for 70 hr. The brown oily product was hydrolysed with 4% H_2SO_4aq (100 ml) for 3 hr and the colourless solid that separated was crystallized from acetone to yield 7-hydroxy-5,4′-dimethoxy isoflavone, m.p. 294–296° (lit.[17,18] 294°). It did not give any ferric reaction. UV absorption (mμ): λ_{max}^{MeOH} 255 (4·57), 311 (inflexion); $\lambda_{max}^{MeOH-NaOAc}$ 265; $\lambda_{max}^{MeOH-AlCl_3}$ 255, 315 (inflexion).

The acetate (Ac_2O–AcONa) had m.p. 84–86° (lit.[21] 83–84°). (Found: C, 67·0; H, 5·2. $C_{19}H_{16}O_6$ requires: C, 67·1 and H, 4·7%.) The ethyl ether (Et_2SO_4–K_2CO_3–acetone method) melted at 155–156° and did not depress the m.p. of an authentic sample (see later).

Quantitative acid hydrolysis of lanceolarin. Lanceolarin (190·14 mg) was refluxed with 4% H_2SO_4aq (25 ml) for 3 hr and the mixture was kept in the refrigerator overnight. The aglycone was filtered through a sintered glass crucible and washed with a little distilled water. The filtrate was extracted with ether, the extract dried and the solvent removed. The combined aglycone samples were dried at 110° for 2 hr. Total yield of biochanin-A, 89·5 mg; 47·1%. $C_{27}H_{30}O_{14}$, H_2O requires for the aglycone $C_{16}H_{12}O_5$, 47·7%.

Partial hydrolysis. The glycoside (0·5 g) was refluxed with H_2SO_4aq (0·5%; 50 ml) for 30 min. The compound went into soln and after a few min a colourless solid separated. It was filtered off, washed with a little cold water and crystallized from MeOH, m.p. 220–222°; $[\alpha]_D^{30}$ −35·5° (*c*, 1·644, DMF). Its acetate, prepared by the Ac_2O–pyridine method melted at 204–205° (AcOEt); $[\alpha]_D^{30}$ −16·97° (*c*, 1·885, DMF). The compound was identified as biochanin-A 7-glucoside by comparison with sissotrin[7] and a synthetic specimen[19] and cochromatography (paper and TLC).

The aqueous filtrate was worked up for the sugars as earlier and apiose was identified. A weak spot corresponding to glucose was also found indicating that slight hydrolysis of the biochanin-A 7-glucoside also had occurred. The apiose spot was strong and agreed with an authentic sample in chromatographic behaviour and fluorescence in UV light when benzidine-trichloracetic acid reagent was employed as developer.

Further hydrolysis of the partial hydrolysis product by refluxing with 6% H_2SO_4aq gave biochanin-A and glucose.

Permethylation[20,21] of lanceolarin and acid hydrolysis of the product. Lanceolarin (0·5 g) was dissolved in a mixture of dimethyl formamide (15 ml) and DMSO (15 ml) and to the soln were added BaO (5 g) and $Ba(OH)_2$ octahydrate (3 g). The mixture was kept at 5° and to the well-stirred mixture was added Me_2SO_4 (10 ml) during 45 min. An inert atmosphere was maintained by bubbling purified H_2 through the reaction mixture. After stirring for 24 hr, water (20 ml) was added and the mixture extracted with chf (100 ml) and AcOEt (40 ml). Removal of the solvents from the combined extracts under red. press. left the fully methylated glycoside as a syrup. This was hydrolysed by refluxing with 5% H_2SO_4aq for 3 hr, the mixture neutralized with $BaCO_3$, filtered and the salts washed with distilled water. The filtrate was evaporated to dryness on a steam-bath, the residue taken up in warm MeOH, the soln filtered and concentrated to about a ml. The methylated sugars were identified as 3,4,6-trimethyl-D-glucopyranose and 2,3,4-trimethyl-D-apiofuranose by cochromatography with the methylated sugars obtained from apiin by exhaustive methylation and subsequent hydrolysis.

Enzymic hydrolysis of lanceolarin. Lanceolarin (0·2 g) was dissolved in citrate buffer (pH 5·0; 100 ml), almond emulsin soln[22] (50 ml) was added and the mixture kept at 37° with occasional mixing. Biochanin-A began to separate after a few hr. After allowing to stand for 7 days the mixture was extracted with ether; the ether soln on removal of the solvent gave biochanin-A whose identity was confirmed by comparison with an authentic sample. The filtrate was worked up for the sugars and glucose and apiose were identified. A small amount of unhydrolysed lanceolarin was also detected by cochromatography.

Enzymic hydrolysis of sissotrin. Hydrolysis of sissotrin[7] (0·2 g) was also carried out as in the above case and biochanin-A, glucose and a small amount of unchanged sissotrin were identified in the products.

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Synthesis of 7-ethoxy-5,4'-dimethoxyisoflavone

(i) *7-Ethoxy-5,4'-dihydroxyisoflavone.* To a soln of genistein (1 g) in dry acetone (100 ml) were added anhyd $NaHCO_3$ (4 g) and Et_2SO_4 (0·65 ml) and the mixture was refluxed for 18 hr. The product was stirred with 5% Na_2CO_3aq to remove unchanged genistein, the insoluble solid was filtered off and washed with water. This was further purified by dissolution in 2% NaOH and reprecipitation. The 7-ethoxy-5,4'-dihydroxyisoflavone was crystallized from MeOH (charcoal), m.p. 175–176°; 0·4 g. It gave a purple ferric reaction. (Found: C, 68·5; H, 5·0. $C_{17}H_{14}O_5$ requires: C, 68·5; H, 4·7%.) UV absorption (mμ): λ_{max}^{EtOH} 260 (4·59), 290 (inflexion), 330 (inflexion); $\lambda_{max}^{EtOH-NaOAc}$ 260; $\lambda_{max}^{EtOH-AlCl_3}$ 270.

(ii) *7-Ethoxy-5,4'-dimethoxyisoflavone.* The above monoethyl ether (0·5 g) was refluxed with redistilled Me_2SO_4 (2·5 ml) and anhyd K_2CO_3 (10 g) in dry acetone (100 ml) soln for 20 hr and the product crystallized from EtOH, m.p. 156–157°; 0·3 g. (Found: C, 69·6; H, 5·9. $C_{19}H_{18}O_5$ requires: C, 69·9; H, 5·5%.)

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