Phytochemistry, 1976, Vol. 15, pp. 1025–1027. Pergamon Press. Printed in England.

NEW ISOFLAVONOID GLYCOSIDES FROM *DALBERGIA PANICULATA*

MADHANAM R. PARTHASARATHY, TIRUVENKATA R. SESHADRI✝ and RAJENDER S. VARMA
Department of Chemistry, University of Delhi, Delhi-1101 007, India

(*Revised received* 20 *December* 1975)

Key Word Index—*Dalbergia paniculata*; Leguminosae; isoflavonoids; 8-*C*-glucopyranosylprunetin; biochanin-A7-*O*-β-(6-*O*-α-L-rhamnopyranosido)-D-glucopyranoside; formononetin 7-*O*-β-(6-*O*-α-L-rhamnopyranosido)-D-glucopyranoside.

Abstract—The methanolic extract of the bark of *Dalbergia paniculata* has yielded three new isoflavonoid glycosides whose structures have been determined. They are 8-*C*-glucosylprunetin and biochanin-A and formononetin 7-rutinosides.

INTRODUCTION

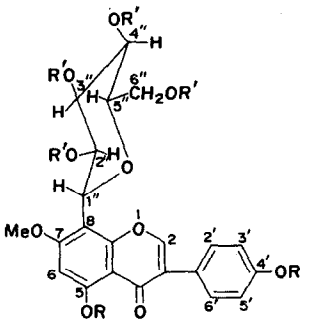
In earlier communications [1–3] on the components of the bark of *Dalbergia paniculata*, were reported the isolation from benzene and acetone extracts of biochanin-A and formononetin and from subsequent methanol extract, of a new genistein-di-*C*-glucoside, paniculatin. In the present reinvestigation of the same bark using larger quantities we have now isolated besides the known isoflavone glucosides sissotrin and ononin, three new glycosides in minor yields from the methanolic extract by column chromatography followed by preparative TLC. The structure elucidation of these three compounds is now discussed.

RESULTS AND DISCUSSION

The first new glycoside was resistant to acid and enzymic hydrolysis. Vigorous treatment with hydroiodic acid gave genistein. These observations coupled with other degradative experiments suggested it was 8-*C*-glucosylprunetin (1a). This was confirmed by the NMR spectrum of its acetate in which the signal position for 2″-acetoxyl (δ1.72) is characteristic of 8-*C*-glucosylflavones [4–6] as in the acetates of volubilin (1.70) [7], vitexin (1.75) [8] and orientin (1.72) [9] and by mass spectral data [10].

The other two compounds were found to be *O*-glycosides and by acid hydrolysis and permethylation studies, they were identified as biochanin-A and formononetin 7-*O*-rutinosides respectively. These assignments were also supported by the NMR spectra of their acetates [11] and configurations at anomeric centres by optical rotatory considerations. In the case of biochanin-A 7-*O*-rutinoside, further confirmation was provided by comparison with a synthetic sample prepared by methylation with diazomethane of genistein 7-*O*-rutinoside (sphaerobioside) [12]. A 7-rhamnosylglucoside of biochanin-A has been previously reported from *Baptisia* by Markham *et al.* [13], but the nature of the sugar linkage was not determined by these workers.

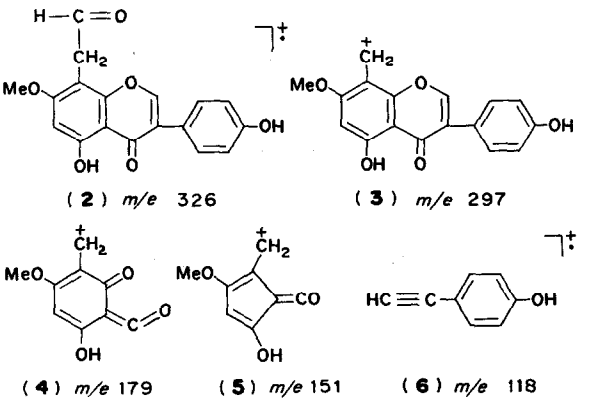
All the components of the bark of *D. paniculata* are based on the parent isoflavones, daidzein and genistein.



(1a) R = R′ = H
(1b) R = R′ = COMe
(1c) R = Me; R′ = H
(1d) R = Et ; R′ = H

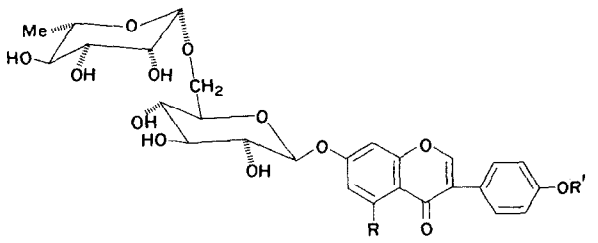
Fig. 1.

In the *O*-glycosides methylation appears to be exclusively in the side phenyl, which presumably has taken place after glycoside formation involving the more reactive 7-position. Further, the two *C*-glucosides are genistein derivatives and have phloroglucinol-A ring which possesses the necessary reactivity for the entry of glucose



(2) *m/e* 326 (3) *m/e* 297
(4) *m/e* 179 (5) *m/e* 151 (6) *m/e* 118

Fig. 2.



(7a) R = OH; R′ = Me
(7b) R = OH; R′ = H
(7c) R = H; R′ = Me

Fig. 3.

units in the nuclear position. It would appear that 8-*C*-glucosylgenistein is the first product and it undergoes (1) methylation of reactive 7-hydroxyl giving 8-*C*-glucosylprunetin which probably prevents further *C*-glucosylation and (2) *C*-glucosylation in the 6-position yielding the genistein di-*C*-glucoside, paniculatin.

EXPERIMENTAL

UV spectra were recorded in MeOH and IR spectra as KBr discs. NMR spectra were determined using $CDCl_3$ and TMS as internal standard. The MS were recorded by direct inlet method at 75 eV ionisation potential.

Extraction of the bark of Dalbergia paniculata. Air dried and coarsely powdered bark (5 kg) was successively extracted with light petrol (60–80°), C_6H_6, $(Me)_2CO$ and MeOH in the cold. The light petrol extract yielded sitosterol. The C_6H_6 and $(Me)_2$ CO extracts found similar (TLC) were mixed, concentrated and chromatographed over Si gel yielding biochanin-A, (1.8 g), mp 212–213° in C_6H_6–EtOAc (7:3) eluates, formononetin (800 mg), mp 257–258° in C_6H_6–EtOAc (13:7) eluates, sissotrin (80 mg), mp 205–206° in C_6H_6–EtOAc (1:1) eluates followed by mixture of sissotrin and ononin (140 mg) in later fractions. This mixture was subjected to preparative TLC in solvent (e) to give ononin (50 mg), mp 242–243°. Identities in all cases were confirmed by direct comparison (mmp, co-TLC and co-IR) with authentic specimens. The MeOH extract was subjected to detailed examination. The cold MeOH extracts (5 × 61) were concentrated under red. pres. and the resulting semi-solid (23 mg), was successively extracted with Et_2O, EtOAc and MeOH. Et_2O and EtOAc extracts were worked up together [1] to give sissotrin an ononin. The MeOH soluble fraction was conc under red. pres. and paniculatin (7 g) was obtained and purified by repeated crystallisations (MeOH). A colourless solid (8 g) could be precipitated from mother liquor by adding dry Et_2O and it was chromatographed over Si gel (350 gm). The C_6H_6–EtOAc (1:1) eluates (2 l.) contained compound biochanin-A, formononetin, sissotrin and ononin in trace amounts (TLC). The EtOAc eluates (2.5 l.) yielded 8-glucosylprunetin (200 mg) followed by a mixture (250 mg) of the two new rutinosides in EtOAc–MeOH (99:1) eluates. This mixture was separated by preparative chromatography over Si gel thin layers in solvent (e). Final elution of the column with EtOAc–MeOH (4:1) afforded paniculatin (1.5 g).

8-Glucosylprunetin crystallised as plates from MeOH and was found to be pure by TLC, mp 286–287°, yield (200 mg) (Found: C, 56.5; H, 5.0. $C_{22}H_{22}O_{10}$, H_2O requires C, 56.89; H, 5.2%). λ_{max}^{MeOH} 261, 287 (infl.) nm; $+AlCl_3$ 270, 299 (infl.) nm; $+AlCl_3 + HCl$ 270, 278 (infl.) nm; +NaOAc 260, 285 (infl.) nm; $+NaOAc + H_3BO_3$, 260, 295 (infl.) nm; +NaOMe, 268, 294 (infl.) nm. ν_{max}^{KBr} 3450 br. (OH), 1650 (conjugated CO), 1600, 1580, 1500 (aromatic), 1425, 1360, 1300, 1260, 1170, 1145, 1080, 1034, 1010 (*C*-glucosyl), 875, 827, 764 cm^{-1}; MS: M^+ 446 (11%), 326 ($M^+ - C_4H_8O_4$, 41%), 313 (40%), 297 [$M^+ - (C_4H_8O_4 + CHO)$, 100%], 283 (9%), 267 (13%) 206 (33%), 191 (12%), 179 (5%), 151 (46%), 149 (20%), 118 (18%) and 55 (15%). The hexa-acetate crystallised from C_6H_6–light petrol as plates, mp 128–130° (Found: C, 58.6; H, 5.2. $C_{34}H_{34}O_{16}$ requires C, 58.4; H, 4.9%); λ_{max}^{MeOH} 259, 294 (infl.) nm; ν_{max}^{KBr} 1736, 1626, 1595, 1493, 1361, 1190 br, 1087, 1031, 1010, 905, 846, 760 and 730 cm^{-1}. NMR: δ 7.90 (*s*, 2-H), 7.50 (*d*, *J* Hz, 2′ and 6′-H), 7.15 (*d*, *J* 9 Hz, 3′ and 5′-H), 6.65 (*s*, 6-H), 4.00–5.50 (*m*, sugar protons), 3.95 (*s*, OMe), 2.40 (*s*, s-O*Ac*); 2.30 (*s*, 4′-O*Ac*), 2.03 (*br s*, 3″, 4″, 6″-O*Ac*) and 1.72 (*s*, 2″-O*Ac*). The dimethyl ether (prepd by methylation with $Me_2SO_4/K_2CO_3/Me_2CO$) crystallised from $CHCl_3$ mp 120–122° (Found: C, 60.9; H, 5.9; $C_{24}H_{26}O_{10}$ requires C, 60.8; H, 5.5%). λ_{max}^{MeOH} 262 nm. Its one mole consumed 1.88 moles of periodate.

Ethylation of 8-glucosyl-prunetin by Et_2SO_4–K_2CO_3–$(Me)_2$ CO afforded a gummy mass. A soln of this product in $(Me)_2$ CO was oxidizing with powdered $KMnO_4$ by refluxing for 5 hr. The product was identified as *p*-ethoxybenzoic acid by mmp. and Co-TLC with an authentic sample. Oxidation of 8-glucosyl-prunetin with $FeCl_3$ gave glucose, identified by PC, and treatment with HI/phenol gave genistein, identified by mmp and Co-TLC.

Biochanin A 7-rutinoside. Crystallised from MeOH as tiny plates yield 120 mg, mp 153–154°. $[\alpha]_D^{20}$ −51.86 (*c*, 1.0003 in MeOH) (Found: C, 52.4; H, 6.3; $C_{28}H_{32}O_{14}$, $3H_2O$ requires C, 52.0; H, 5.9%). λ_{max}^{MeOH} 262 nm (log ϵ 4.44); $+AlCl_3$ 271 nm; $+AlCl_3 + HCl$ 271 nm; +NaOAc 262 nm; $+NaOAc + H_3BO_3$ 262 nm. ν_{max}^{KBr} 3400 br (OH) 1650 (conjugated CO), 1620, 1580, 1510 (aromatic), 1438, 1360, 1290, 1242, 1180, 1060, 830 and 780 cm^{-1}. MS: m/e M^+ 284 (100%), 132 (48%) and 152 (32%). It underwent acid hydrolysis when (11.50 mg) was refluxed with 4% H_2SO_4 aq. (1 ml) for 3 hr. The aglycone after drying at 100°, weighed (4.42 mg), 38.43% $C_{28}H_{32}O_{14}$ requires for the aglycone $C_{16}H_{12}O_5$, 40.65%. It formed needles from MeOH, mp 211–213°. It was identified as biochanin-A (mmp, co-TLC, superimposable IR spectrum and preparation of acetate). The aq. mother liquor revealed the presence of glucose and rhamnose by PC.

Partial hydrolysis: The glycoside (20 mg) was dissolved in Killiani reagent (AcOH–HCl–H_2O, 3.5:1:5.5) (0.65 ml) and heated at 100° for 15 min, when a colourless solid separated. It crystallised from MeOH (needles), mp 218–220°; λ_{max}^{MeOH} 262 nm (log ϵ 4.34), 330 (infl.) nm; +NaOAc 262 nm. It was identified as biochanin-A 7-*O*-glucoside by comparison with sissotrin (mmp, co-TLC and co-IR). In the aq. filtrate, rhamnose was identified by PC.

Permethylation and hydrolysis. NaH dispersion in oil (50%, 10 mg) was added to a solution of the 7-rutinoside (7 mg) in Me_2SO_4 (2 ml) and the mixture was kept at 80° for 1 hr. After cooling, MeI (1 ml) was added and the mixture was left overnight. The product was poured into iced H_2O and extracted with $CHCl_3$. It was permethylated once more until it was chromatographically homogenous. It was hydrolysed with Killiani's mixture (3 ml). The hydrolysate was examined for methylated sugars when 2,3,4-tri-*O*-methyl-D-glucopyranose (R_G 0.84) and 2,3,4-tri-*O*-methyl-L-rhamnopyranose (R_G 1.02) were identified by direct comparison with authentic samples (from permethylation and hydrolysis of rutin) by PC in *n*-BuOH–EtOH–H_2O (5:1:4).

Biochanin-A rutinoside acetate crystallized from C_6H_6–light petrol as flakes, mp 170–172°. λ_{max}^{MeOH} 260 nm; ν_{max}^{KBr} 1745, 1629, 1568, 1508, 1440, 1364, 1282, 1127, 1060, 904, 835 and 800 cm^{-1}. NMR: δ3.83 (*s*, OMe), 2.05 (*br s*, 6 alc. O*Ac*), 2.42 (*s*, 1 phenolic O*Ac*), 1.28 (*br s*, rhamnose Me) [4, 11], 3.60–5.50 (*m*, sugar protons), 6.65 (*d*, *J*3 Hz, 6-H), 7.40 (*d*, *J*9 Hz, 2′ and 6′-H); the highfield doublet overlapping with signal of proton at 8-position, 6.93 (unresolved, 3′,5′ and 8-H) and 7.95 (*s*, 2-H).

Methylation of sphaerobioside. To the soln of sphaerobioside (4 mg) in MeOH (2.0 ml), ethereal CH_2N_2 was added till the yellow colour persisted. After 22 hr the mixture was worked up; the product crystallised from MeOH as colourless cubes, mp 155–157° found to be identical with biochanin A 7-rutinoside (mp, mmp and co-TLC).

Formononetin 7-rutinoside crystallised from MeOH as thin rectangular rods, yield 115 mg, mp 171–172°, $[\alpha]_D^{20}$ −80.74 (*c*, 0.99 in MeOH). (Found; C, 53.2; H, 6.4. $C_{28}H_{32}O_{13}$, $3H_2O$ requires C, 53.3; H, 6.0%). λ_{max}^{MeOH} 261 nm (log ε 4.32): $+AlCl_3$ 262 nm; $+AlCl_3 + HCl$ 262 nm; +NaOAc 261 nm; $+NaOAc-H_3BO_3$ 261 nm. ν_{max}^{KBr} 3400 *br* (OH), 1630 (carbonyl), 1580, 1510, 1440 (aromatic C=C), 1378, 1282, 1244, 1200, 1178, 1070, 890, 830, 785 and 690 cm^{-1}. MS: M^+ 268 (100%) and *m/e* 132 (50%). It underwent acid hydrolysis when (10.32 mg) was refluxed with aq. H_2SO_4 (4%, 1 ml) for 3 hr on a H_2O-bath affording aglycone (3.96 mg), 38.37%. $C_{28}H_{32}O_{13} \cdot H_2O$ requires for the aglycone $C_{16}H_{20}O_4$, 40.15%. The product crystallised as needles from MeOH, mp 257–259°; λ_{max}^{MeOH} 257, 300 (infl.) nm; +NaOAc 265, 300 (infl.) nm and it was indistinguishable from an authentic sample of formononetin (mmp, co-TLC and co-IR). The sugars from the aq. mother liquor were identified as glucose and rhamnose by PC.

Partial hydrolysis. The glycoside (20 mg) was treated with the Killiani's reagent (0.70 ml) on a boiling H_2O-bath for 15 min. The solid that separated crystallised as needles from MeOH, mp 240–241°, λ_{max}^{MeOH} 259 nm, 300 (infl.) nm; +NaOAc 260 nm, 305 (infl.) nm. Mmp on admixture with an authentic specimen of ononin was undepressed. Further confirmation was provided by co-TLC and identical UV and IR spectra. The aq. filtrate revealed the presence of rhamnose by PC. Further hydrolysis with Killiani reagent for 3 hr gave formononetin and glucose.

Permethylation and hydrolysis. The 7-rutinoside (7 mg) was subjected to permethylation as described above and the methylated sugars after Killiani hydrolysis of the product were identified as 2,3,4-tri-*O*-methylglucopyranose and 2,3,4-tri-*O*-methyl-L-rhamnopyranose.

Formononetin 7-rutinoside acetate crystallized from C_6H_6–light petrol as needles (22 mg), mp 120–122°. (Found: C, 57.8; H, 5.5; $C_{40}H_{44}O_{19}$ requires C, 58.0; H, 5.3%). λ_{max}^{MeOH} 259 nm; ν_{max}^{KBr} 1736, 1626, 1608, 1550, 1493, 1429, 1359, 1227, 1058, 1031, 882, 828, 806 and 782 cm^{-1}. NMR: (δ, 100 MHz) 1.30 (*br s*, rhamnose $C\underline{H}_3$) [4, 11], 2.06, 2.08, 2.12 and 2.14 (*s*, 6 alc. O*Ac*), 3.85 (*s*, $OC\underline{H}_3$), 3.50–5.50 (*m*, sugar protons), 6.95 (*d*, *J* 9 Hz, 3′, 5′–H), 7.50 (*d*, *J* 9 Hz), 2′, 6′–H), 7.10 (unresolved 8–H), 7.30 (*m*, 6H), 8.04 (*s*, 2–H) and 8.25 (*d*, 9 Hz, 5–H).

Acknowledgements—Our thanks are due to Prof. T. J. Mabry, University of Texas, Austin and Prof. H. Wagner, University of Munich, Germany for a specimen of Sphaerobioside. One of the authors (R.S.V.) is grateful to the CSIR, New Delhi, for a Research Fellowship.

REFERENCES

1. Parthasaraty, M. R., Seshadri, T. R. and Varma, R. S. (1974) *Curr. Sci.* **43,** 74.
2. Parthasarathy, M. R., Seshadri, T. R. and Varma, R. S. (1974) *Indian J. Chem.* **12,** 518.
3. Narayanan, V. and Seshadri, T. R. (1971) *Indian J. Chem.* **9,** 14.
4. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, p. 267, Springer-Verlag, New York.
5. Hillis, W. E. and Horn, D. H. S. (1965) *Australian J. Chem.* **18,** 531.
6. Gentili, B. and Horowitz, R. M. (1968) *J. Org. Chem.* **33,** 1571.
7. Chawla, H. M., Chibber, S. S. and Seshadri, T. R. (1974) *Phytochemistry* **13,** 2301.
8. Batterham, T. J. and Highet, R. H. (1964) *Australian J. Chem.* **17,** 428.
9. Koeppen, B. H. and Roux, D. G. (1965) *Biochem. J.* **97,** 444.
10. Prox, A. (1968) *Tetrahedron* **24,** 3697.
11. Mabry, T. J., Kagan, J. and Rosler, H. (1964) *Nuclear Magnetic Resonance Analysis of Flavonoids*, The University of Texas, Publication No. 6418, Austin.
12. Wagner, H., Horhammer, L., Bohringer, W. and Farkas, L. (1967) *Chem. Ber.* **100,** 101.
13. Markham, K. R., Mabry, T. J. and Swift, Jr. W. T. (1970) *Phytochemistry* **9,** 2359.