Minor Phenolic Constituents of *Grevillea robusta* and *Hakea saligna*

Manju Varma, R. S. Varma, and
M. R. Parthasarathy [1]

Department of Chemistry, University of Delhi, Delhi-110007 (India)

Z. Naturforsch. **35 c,** 344–345 (1980);
received November 15, 1979

Proteaceae, *Grevillea robusta*, *Hakea saligna*, Lignans

Three hitherto unknown phenolic constituents epilyoniresinol, its glucoside and rhamnocitrin-3-O-rutinoside have been isolated from *G. robusta* wood and leaves besides kaempferol-3-O-rutinoside whereas 2,6-dimethoxybenzoquinone, lyoniresinol and its rhamnoside are obtained from *H. saligna* wood. Their structures were assigned on the basis of chemical and spectral evidence.

Previous work has shown the presence of several 5-*n*-alkyl resorcinol and macrocyclic phenols in the wood [2] and leaves [3] of *Grevillea robusta.* Parallel studies on Proteaceae members naturalised in India revealed the presence of other new phenolic components from wood [4, 5] and leaves [6] of *G. robusta* and *Hakea saligna.* We wish to report now the occurrence of few minor constituents from wood and leaves of these species, extraction procedures being described earlier [4–6].

G. robusta, wood: The combined acetone and methanol extract after fractionation on silica gel column followed by purification on preparative SiO_2-TLC in solvent EtOAc-H_2O-MeOH (40:5.4:6.6) afforded compounds (A) and (B).

Compound A, pinkish prisms from EtOAc/C_6H_6 (150 mg from 2 kg of wood) m. p. 198–200 °C, $[\alpha]_D^{MeOH} = -28.9°$ (Found: C, 63.0; H, 7.0; calculated for $C_{22}H_{28}O_8$ C, 62.8; H, 6.6). It gave colour reaction with $FeCl_3$ (changing green to dark blue), positive Mäule test and blue colour with *p*-$NO_2C_6H_4N_2^+ BF_4^-$ and 20% Na_2CO_3 overspray, a test for syringyl lignans. The IR spectrum displayed the aromatic and phenolic hydroxyl absorptions (ν_{max}^{KBr} 3472, 1600, 1493 and 1449 cm^{-1}) confirmed by the bathochromic shift in UV (from λ_{max}^{MeOH} 278 nm to 290 nm) on addition of alkali. It formed a dimethyl ether (**1 a**), m. p. 148–149 °C (Me_2SO_4–K_2CO_3-Me_2CO) and tetraacetate (**1 b**), m. p. 145 °C with Py/Ac_2O. PMR spectrum of acetate (60 MHz, $CDCl_3$, TMS, δ): 6.54 (s, 1H, Ar-H), 6.34 (s, 2H, Ar-H), 4.28 (m, 1H), 4.08 (m, 4H, $-CH_2O-$), 3.83 (s, 6H, ArO$-CH_3$), 3.76 (s, 3H ArO$-CH_3$), 3.23 (s, 3H, ArO$-CH_3$), 2.90–2.58 (m, 4H), 2.32 (s, 6H, ArO$-COCH_3$), 2.08 (s, 3H, $-CH_2O-COCH_3$) and 2.04 (s, 3H, $-CH_2O-COCH_3$). These data suggested it to be a disyringyl lignan, the physical properties being closely resembling to lyoniresinol [7]. However, its co-IR and co-TLC with authentic sample showed some minor differences. In view of these and its different $[\alpha]_D$, it was considered as an epimer of lyoniresinol with structure **1** or its enantiomer.

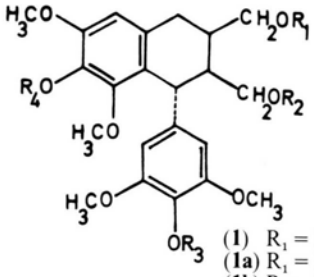
Compound B, brownish gum (90 mg) gave all tests for compound (A) and a positive Molisch's test. IR spectrum (ν_{max}^{KBr} 3448, 1608, 1493 and 1449 cm^{-1}) and UV (λ_{max}^{MeOH} 275 and $\lambda_{max}^{MeOH\text{-}NaOH}$ 280 nm) were almost identical with compound (A). The acid hydrolysis afforded glucose as sugar and compound (A) as aglycone m. p. 197–198 °C. The PMR spectrum of heptaacetate (Found: C, 57.2; H, 6.1; $C_{42}H_{52}O_{20}$ requires C, 57.7; H, 5.9) showed in addition to the signals for compound (A), a complex multiplet at δ 5.16–4.19 ascribable to sugar protons. The major difference, however, was in the methoxyl region where the highest field methoxyl of compound (A) (δ 3.23) was now appearing as a pair of peaks (δ 3.32 and 3.22). This behaviour, ascribed to the presence of rotamers, has been reported in the case of lyoniresinol-2α-O-rhamnoside acetate [7]. For similar reasons, it was identified as epi-lyoniresinol-2-O-glucoside (**1 c**).

G. robusta leaves: The column chromatography of acetone extract afforded a glycosidic fraction from which two closely moving compounds (C) and (D) were obtained by preparative SiO_2-TLC in solvent $CHCl_3$–MeOH–H_2O (33:13.5:1.8). Both of them gave greenish brown colour with $FeCl_3$, colour tests for flavonoids and were found to be glycosidic in nature.

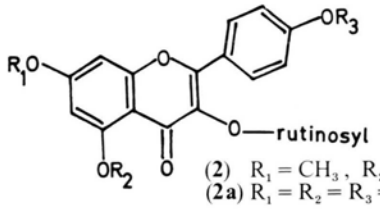
Compound C, yellow plates from MeOH, m. p. 167–168 °C (200 mg from 500 g of leaves). UV λ_{max}^{MeOH} 270, 343 nm with no shift on addition of NaOAc but a bathochromic shift of long wavelength maximum to 405 nm on addition of $AlCl_3$. On total acid hydrolysis with H_2SO_4 it gave glucose and rhamnose as sugars and rhamnocitrin as aglycone identified by comparison with authentic sample (co-TLC, co-IR and m. m. p.). Permethylation and hydrolysis by Hakomori's method [8] yielded two meth-

Reprint requests to Dr. M. Varma, School of Physical and Mol. Sciences, University College of North Wales, Bangor, Gwynedd LL57 2DG, U. K.

0341-0382/80/0300-0344 $ 01.00/0



(**1**) $R_1 = R_2 = R_3 = R_4 = H$.
(**1a**) $R_1 = R_2 = H$, $R_3 = R_4 = CH_3$.
(**1b**) $R_1 = R_2 = R_3 = R_4 = COCH_3$.
(**1c**) $R_1 = R_3 = R_4 = H$, R_2 = glucose.



(**2**) $R_1 = CH_3$, $R_2 = R_3 = H$.
(**2a**) $R_1 = R_2 = R_3 = H$.

ylated sugars identified as 2,3,4-trimethyl glucopyranose (R_{TMG} 0.84) and 2,3,4-trimethyl rhamnopyranose (R_{TMG} 0.98) confirmed by direct comparison with the samples obtained under similar conditions from rutin. That the compound (C) is rhamnocitrin-3-O-rutinoside (**2**) was inferred from PMR of its octaacetate m. p. 124–125 °C (Found: C, 55.7; H, 5.6; $C_{44}H_{48}O_{23}$ requires C, 55.9; H, 5.1): δ 8.13 (d, 2H, $J = 9$ Hz, 2′,6′-H), 7.02 (d, 2H, $J = 9$ Hz, 3′,5′-H), 6.82 (d, 1H, $J = 2$ Hz, 8-H), 6.61 (d, 1H, $J = 2$ Hz, 6-H), 5.16–4.89 (m, sugar protons), 3.85 (s, 3H, ArO–CH_3), 2.43 (s, 3H, ArO–$COCH_3$), 2.38 (s, 3H, ArO–$COCH_3$), 2.09, 2.03, 1.95 (m, 18H, –$OCOCH_3$) and 1.07 (br, 3H, C–CH_3).

Compound D, yellow needles from MeOH, m. p. 180–182 °C. It was identified as kaempferol-3-O-rutinoside (**2 a**) by UV spectral analysis with usual shift reagents [9] and other experiments as described for compound (C). A direct comparison with authentic sample (co-TLC, co-IR and m. m. p.) confirmed the identity.

Hakea saligna wood: The combined acetone and methanol extract on SiO_2 column chromatography gave compounds (E), (F) and (G).

Compound E, from C_6H_6-EtOAc (1:4) eluates as pinkish prisms (125 mg from 2.8 kg of wood), m. p. 192–193 °C, $[\alpha]_D^{MeOH} = 0$ °. It answered tests for syringyl lignans and showed resemblance to compound (A) (TLC, IR). It was identified as (±) lyoniresinol [7] by comparison with an authentic sample (co-TLC, co-IR and m. m. p.).

Compound F, from EtOAc eluates, yellow needles (140 mg), m. p. 258–260 °C. It gave typical tests for quinones [10] IR (ν_{max}^{KBr} 1698 and 1650 cm^{-1}). It was inferred as 2,6-dimethoxy benzoquinone (UV and PMR) and confirmed by comparison with authentic sample (co-TLC, co-IR and m. m. p.).

Compound G, from EtOAc-MeOH (19:1) eluates, purified by preparative SiO_2-TLC in solvent EtOAc-H_2O-MeOH (40:5.4:6.6), amorphous solid m. p. 108–109 °C. It answered tests for syringyl lignans and also the Molisch's test. On hydrolysis it gave rhamnose as sugar and lyoniresinol as aglycone. Its identitiy as lyoniresinol-2α-O-rhamnoside [7] was confirmed by direct comparison with an authentic specimen (co-TLC, co-IR).

It is the first report of the natural occurrence of the epimer of lyoniresinol and its glucoside as well as rhamnocitrin-3-O-rutinoside. The other compounds has been reported from different sources; this, however, is the first report of their occurrence in Proteaceae. The minor stereochemical differences in the lyoniresinol based lignan constituents of both the woods and the occurrence of glucoside in *G. robusta* and rhamnoside in *H. saligna* are the main distinctive features. However, a definite conclusion on the role of any of these compounds as chemotaxonomic markers requires investigation of more representative samples from different regions.

[1] We are grateful to Dr. John W. Rowe, U. S. D. A., Forest Products Laboratory; Madison, Wis., USA, for the Samples of Lyoniresinol and Its Rhamnoside.
[2] E. Ritchie, W. C. Taylor, and T. K. Vautin, Australian J. Chem. **18,** 2015 (1965); M. Rasmussen, D. D. Ridley, E. Ritchie, and W. C. Taylor, Australian J. Chem. **21,** 2989 (1968).
[3] J. R. Cannon, P. W. Chow, M. W. Fuller, B. H. Hamilton, B. W. Metcalf, and A. J. Power, Australian J. Chem. **28,** 2257 (1973).
[4] R. S. Varma, Manju, and M. R. Parthasarathy, Phytochemistry **15,** 1418 (1976).
[5] Manju and M. R. Parthasarathy, Ind. J. Chem. Sec. B, **15,** 1090 (1977).
[6] Manju, R. S. Varma, and M. R. Parthasarathy, Phytochemistry **16,** 793 (1977).
[7] F. D. Hostettler and M. K. Seikel, Tetrahedron **25,** 2325 (1969).
[8] S. Hakomori, J. Biochem. **55,** 205 (1964).
[9] J. B. Harborne, T. J. Mabry, and H. Mabry, The Flavonoids, Chapman and Hall, London 1975.
[10] R. H. Thomson, Naturally Occurring Quinones, Academic Press, London 1971.