

WIKSTROMOL, A NEW LIGNAN FROM *WIKSTROEMIA*  
*VIRIDIFLORA*\*

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*Wikstroemia viridiflora* (Meissn.) Hook. f. (= *W. indica* C. A. Mey) is reported to be effective in various ailments [1]. Chinese workers have reported diuretic acti-

vity in the bark and root cortex from which a flavonoid glycoside, wikstromin, was isolated [2]. Recently the crude plant extract was shown to exhibit potent anticancer activity [3].

\*CDRI Communication No. 2004. *Wikstroemia viridiflora* was collected and identified by Mr. B. N. Mehrotra from Gindi Park, Madras, and a voucher specimen No. 7018, has been preserved in the Herbarium of the Institute.

The EtOH extractive of the plant was macerated successively with hexane and EtOAc and the anticancer activity was found to reside mainly in the EtOAc fraction. This fraction on gross separation on Hyflosupercel gave

a  $C_6H_6$  eluate which showed 4 spots on TLC and its purification by various chromatographic procedures finally led to the isolation of substances A, B, C and D. One of these, D being a new substance, has been named as wikstromol.

Wikstromol,  $C_{20}H_{22}O_7$ , was phenolic in nature. IR absorption bands at 3513, 1768 and 1600, 1500, 1463  $cm^{-1}$  indicated the presence of a OH, a  $\gamma$ -lactone and an aromatic ring respectively in the molecule. It showed UV absorption at 215 ( $\log \epsilon$  4.014), 232 ( $\log \epsilon$  4.071) and 282 nm ( $\log \epsilon$  3.748) which was similar to that of matairesinol and other lignans which have been characterised from this plant (see *infra*). Its MS did not exhibit  $M^+$  but a base peak at  $m/e$  137 arising from the fragment ion  $(OMe \cdot OH) \cdot C_6H_5CH_2^+$  gave further evidence that wikstromol is a lignan. Its PMR spectrum displayed signals for six aromatic H (7.00–6.48), C-4 methylene as an unresolved broad doublet centered at 4.03,  $C_6$  methylene as a quartet centered at 3.02 and a complex multiplet due to the three protons on C-3, C-5 at 2.3–2.95 ppm. It yielded a diMe ether,  $C_{22}H_{26}O_7$ ,  $M^+$  402, and when PMR spectrum of this derivative was recorded with TAI, one proton singlet ( $-\text{CONHCO}-$ ) appeared at 9.1 ppm indicating the presence of an aliphatic OH group.

On acetylation wikstromol yielded two acetyl derivatives. The triacetate,  $M^+$  500, exhibited IR bands at 1786, 1770, and 1740  $cm^{-1}$  for a five membered lactone, phenolic and aliphatic acetates respectively. Its PMR showed acetoxy Me signals at 2.3 (for two) and 2.23 ppm (for one), but when the PMR was recorded in  $C_6D_6$ , these signals suffered a marked paramagnetic shift to 1.80 and 1.56 ppm respectively and the protons of the aliphatic region were better resolved for an unambiguous assignment. Thus, the  $C_{3,5}$  proton broad multiplet appeared at 1.88–2.52, the AB quartet of  $C_6$  methylene appeared at 2.93,  $J$  14 Hz, and the C-4 methylene appeared as two triplets at 3.7 and 4.12 ppm. The separation of a lactone methylene signal is markedly solvent dependent and its appearance as triplets indicated that  $J_{AB}$  of this methylene is *ca* equal to  $J_{3,4}$  (*cis*) and  $J_{3,4}$  (*trans*) and all the  $J$  values are 8 Hz. A similar observation has been reported in the case of helianthoidin [4].

The PMR of the corresponding diacetate showed signals for only two aromatic acetoxy groups (2.3 ppm). Under more vigorous conditions the diacetate could be converted to a triacetyl derivative. Thus, the presence of two phenolic and one aliphatic OH in wikstromol was confirmed and the nature of aliphatic OH was tertiary because of the absence of any diamagnetic shift of a carbinol proton on acetylation and the inertness of wikstromol to  $MnO_2$  oxidation.

Wikstromol diMe ether on  $LiAlH_4$  reduction yielded a compound (1)  $M^+$  406, which was identified as carinol diMe ether by direct comparison (mp, IR, UV, PMR, MS) with an authentic sample. A similar treatment of wikstromol led to the production of carinol [5] which confirmed the bisguaiacyl moiety in the molecule. The stereochemistry at centres C-2 and C-3 is identical with helianthoidin, because of the similarity of the benzylic and lactone methylene protons signal in the PMR spectra of the two substances in  $C_6D_6$ , as previously mentioned. The structure of wikstromol is, therefore, elucidated as (2).

The substances A, B and C were identified as arctigenin, matairesinol and pinoresinol [6] respectively.

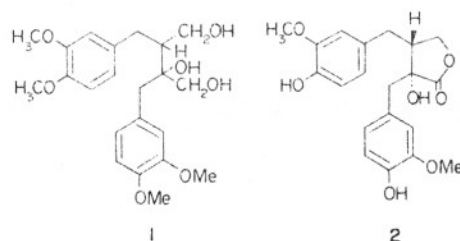


Fig. 1.

## EXPERIMENTAL

Mp's are uncorrected. PMR spectra were recorded in  $CDCl_3$  with TMS as internal standard.  $R_f$  values refer to Si gel plates using  $FeCl_3-K_3Fe(CN)_6$  as spray reagent. The EtOH extract of the powdered plant (3 kg) was macerated successively with hexane and EtOAc to yield hexane-soluble (23 g) and EtOAc-soluble (57 g) fractions. The latter fraction was chromatographed on Hyflosuperpel (300 g) and  $C_6H_6$  (17 g), EtOAc (34 g) and MeOH (3 g) fractions were collected. The  $C_6H_6$  eluate was re-chromatographed on Si gel (500 g) in  $C_6H_6$ -MeOH (99:1) and 62 fractions (150 ml each) were collected and screened for various components by TLC in  $C_6H_6$ -MeOH (24:1). Substance A was obtained from eluate 3 (0.713 g) by re-chromatography on Si gel ( $C_6H_6$ -MeOH, 99:1). From eluates 4–9 (3.16 g), substance B was isolated by dry Column Si gel chromatography ( $CHCl_3$ -MeOH, 99.5:0.5). Substance C was isolated via its acetyl derivative from chromatographic fractions 10–21 (1.87 g). The purification of the substance D was achieved by chromatography of fractions 37–62 (3.73 g) on Si gel in  $CHCl_3$ .

**Compound A (arctigenin).**  $C_{21}H_{24}O_6$ , 372 ( $M^+$ ) mp, 99–100°.  $R_f$  0.4 ( $C_6H_6$ -MeOH, 24:1).  $\lambda_{max}^{EtOH}$  (nm): 230, 281 ( $\log \epsilon$  4.16, 3.76).  $\nu_{max}^{CHCl_3}$  ( $cm^{-1}$ ): 3400 (OH), 1768 ( $\gamma$  lactone) 1605, 1520, 1470 (aromatic). PMR (ppm): 2.58 (4H, *br* s, C-5, 6), 2.9 (2H, *br*, C-2, 3), 3.81 (9H, s, OMe), 3.9–4.40 (2H, *m*, C-4), 4.8 (1H, *br*, OH quenched by  $D_2O$ ), 6.5–6.92 (6H, *m*, aromatic H). **Monoacetate.** amorphous,  $\nu_{max}^{CHCl_3}$ : 1786 (phenolic OAc) PMR (ppm): 2.28 (3H, s, OCOMe). MS ( $m/e$ ) ( $M^+$  absent) 372, 352, 324, 315, 287, 278, 234, 223, 191, 149. **Monomethylether.** mp 125–26°.

**Compound B (matairesinol).**  $C_{20}H_{22}O_6$ , 358 ( $M^+$ ), mp, 115–116°.  $R_f$  0.2 ( $C_6H_6$ -MeOH, 24:1).  $\lambda_{max}^{EtOH}$  (nm): 232, 283 ( $\log \epsilon$  4.06, 3.74).  $\nu_{max}^{CHCl_3}$  ( $cm^{-1}$ ): 3560 (OH), 1765 ( $\gamma$ -lactone) 1600, 1505, 1463 (aromatic). PMR (ppm): 2.53 (4H, s, C-5, 6), 2.95 (2H, *br*, C-2, 3), 3.86 (6H, s, OMe), 3.95–4.4 (2H, *m*, C 4), 4.65 (2H quenched by  $D_2O$ ), 6.4–7.0 (6H, *m*, aromatic). **Diacetate.** mp 110°. **DiMe ether.** mp 125–26°.

**Compound C (pinoresinol).**  $C_{20}H_{22}O_6$ , mp 118–120°.  $[\alpha]_D^{25} + 84.0^\circ$  (c, 2.0 Me $_2$ CO).  $\lambda_{max}^{EtOH}$  (nm): 208, 230, 283 ( $\log \epsilon$  4.26, 4.24, 3.657).  $\nu_{max}^{CHCl_3}$  ( $cm^{-1}$ ): 3420 (OH), 1600, 1510, 1460, (aromatic). PMR (ppm): 3.05 (2H, *m*, C- $\beta$ ), 3.9 (2H, *m*, C- $\gamma$ -ax.), 4.25 (2H, *m*, C- $\gamma$ -eq.), 4.73 (2H, *d*, C- $\alpha$  H), 6.82–6.86 (6H, *m*, aromatic), 4.15 (2H, OH quenched by  $D_2O$ ). MS: ( $m/e$ ): 358  $M^+$ , 344, 327, 291, 272, 259, 234, 221, 205, 196, 191, 151, 137. **Diacetate.** mp 165–166°.  $\nu_{max}^{CHCl_3}$  ( $cm^{-1}$ ): 1754 (phenolic OAc). MS: ( $m/e$ , 442 ( $M^+$ ), 400, 358, 328, 234, 221, 205, 179, 163, 152, 137. **DiMe ether.** mp 107°.

**Compound D (wikstromol).** Colourless powder  $[\alpha]_D^{25} + 72^\circ$  (c, 0.37,  $CHCl_3$ ).  $R_f$  0.29 ( $C_6H_6$ -MeOH, 24:1).  $\lambda_{max}^{EtOH}$  (nm): 215, 232, 282.5 ( $\log \epsilon$  4.014, 4.077, 3.748).  $\nu_{max}^{CHCl_3}$  ( $cm^{-1}$ ): 3513 (OH), 1768 ( $\gamma$  lactone), 1600, 1513, 1463 (aromatic). PMR (ppm): 2.3–2.95 (3H, *m*, C-3, 5), 3.02, (2H, *q*,  $J_{AB}$  14 Hz,  $C_6$  methylene), 2.53 (1H, *br*, OH quenched by  $D_2O$ ), 3.85 (6H, s, OMe), 4.03 (2H *br* *d*, C-4 methylene), 5.65 (2H, *br*, OH quenched by  $D_2O$ ) 6.48–7.00 (6H, *m*, aromatic H).

**Wikstromol diMe ether.** Wikstromol (100 mg) was reacted with ethereal  $CH_2N_2$  at 0° and the progress of the reaction was followed by TLC. After 5 days a single spot was obtained and the reaction mixture was evaporated to yield a viscous residue (114 mg) which was purified by filtration through neu-

tral  $\text{Al}_2\text{O}_3$  and crystallized from MeOH, mp 96–97°.  $[\alpha]_D^{25} + 35^\circ$  (c 1.47,  $\text{CHCl}_3$ ),  $\lambda_{\text{max}}^{\text{EtOH}}$  (nm): 231, 261 (log  $\epsilon$  4.510, 3.730),  $\nu_{\text{max}}^{\text{CHCl}_3}$  ( $\text{cm}^{-1}$ ): 3560 (OH), 1770 ( $\gamma$  lactone), 1600, 1510, 1460 (aromatic). PMR (ppm): 2.0–2.9 (3H, m, C-3, 5) 2.98 (2H, q,  $J_{AB}$  14 Hz, C-6, methylene), 3.03 (1H, OH quenched by  $\text{D}_2\text{O}$ ), 3.85 (12H, s, OMe), 4.08 (2H, br d, C-4 methylene), 6.5–6.9 (6H, m, aromatic H). MS ( $m/e$ ): 402 ( $\text{M}^+$ ), 387, 373, 345, 327, 306, 294, 278, 263, 250, 233, 219, 195, 151, 135. Found: C, 63.00; H, 6.1  $\text{C}_{22}\text{H}_{26}\text{O}_7$  requires C, 63.1; H, 6.2%.

*Wikstromol triacetate*. Crystallized from EtOH mp 162°.  $[\alpha]_D^{25} + 118.8^\circ$  (c 1.56,  $\text{CHCl}_3$ ),  $\lambda_{\text{max}}^{\text{EtOH}}$  (nm): 226, 274, 280 (log  $\epsilon$  4.04, 3.72, 3.69),  $\nu_{\text{max}}^{\text{KBr}}$  ( $\text{cm}^{-1}$ ): 1786 (phenolic OAc), 1770 ( $\gamma$  lactone), 1740 (OAc), 1600, 1510, 1460 (aromatic). PMR (ppm): 2.3 (6H, s, OCOMe), 2.23 (3H, s, OCOMe), 2.34–3.06 (3H, m, C-3, 5), 3.16 (2H, q,  $J_{AB}$  14 Hz, C-6 methylene), 3.85 (6H, s, OMe) 4.25 (2H, br d, C-4 methylene), 6.44–7.2 (6H, m, aromatic H). Ms ( $m/e$ ): 500 ( $\text{M}^+$ ), 458, 416, 374, 398, 356, 220 and 137. Found: C, 62.95; H, 5.75  $\text{C}_{26}\text{H}_{28}\text{O}_{10}$  requires C, 62.4; H, 5.6%.

*Wikstromol diacetate*. Colourless powder,  $\nu_{\text{max}}^{\text{CHCl}_3}$  ( $\text{cm}^{-1}$ ): 3450 (OH), 1760 (phenolic OAc). PMR (ppm): 2.3 (6H, s, OCOMe) 2.4–3.16 (3H, m, C-3, 5), 3.05 (2H, q,  $J_{AB}$  14 Hz, C-6 methylene), 3.80 (6H, s, OMe), 4.09 (2H, br d, C-4 methylene), 6.6–7.1 (6H, m, aromatic). MS ( $m/e$ ): 458 ( $\text{M}^+$ ), 416, 374, 352, 323, 280, 265, 233, 210, 205, 192, 155, 149, 137 (base peak).

*LiAlH<sub>4</sub> reduction of wikstromol dimethyl ether*. To a soln of wikstromol diMe ether (206 mg) in THF, a suspension of  $\text{LiAlH}_4$  (400 mg) in Et<sub>2</sub>O was added and refluxed for 3 hr. The mixture was worked up and the residue (185 mg), showing one major spot on TLC ( $R_f$  0.25,  $\text{C}_6\text{H}_6$ -MeOH, 2:3:2), was purified by chromatography over Si gel. The  $\text{CHCl}_3$  eluate yielded a residue (131 mg) which crystallized from hexane- $\text{C}_6\text{H}_6$  (1:1) as colourless needles (50 mg), mp 130–131°.  $[\alpha]_D^{25}$

+ 9° (c. 1.0, EtOH).  $\lambda_{\text{max}}^{\text{EtOH}}$  (nm): 230, 280, 290,  $\nu_{\text{max}}^{\text{KBr}}$  ( $\text{cm}^{-1}$ ), 3401, (OH), 1600, 1517 and 800 (aromatic). PMR (ppm): 2.05–2.68 (1H, m, C-3), 2.7–3.12 (2H, m C-5), 2.92 (2H, s, C-6), 3.0–3.3 (2H, br OH quenched by  $\text{D}_2\text{O}$ ), 3.52 (2H, s, C-1), 3.7 (2H, br d, C-4), 3.85 (12H, s, OMe), 6.68–7.00 (6H, m, aromatic H). MS ( $m/e$ ): 406 ( $\text{M}^+$ ), 388, 357, 254, 237, 219, 189, 160, 151. Found: C, 64.8; H, 7.42  $\text{C}_{22}\text{H}_{30}\text{O}_7$  requires C, 65.20; H, 7.35%.

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