Phytochemistry, 1976, Vol. 15, p. 1789. Pergamon Press. Printed in England.

LACOUMARIN FROM *LAWSONIA INERMIS*

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(*Received* 27 *March* 1976)

Key Word Index—*Lawsonia inermis* (*Syn*: *L. alba*); Lythraceae; coumarins; 5-alloxy-7-hydroxycoumarin.

Plant. Lawsonia inermis, whole plant. *Uses.* [1]. *Previous work.* [2–8].

Present work. This communication decribes the isolation and structural elucidation of a new coumarin, named lacoumarin. The air-dried whole plant (2 kg) was extracted exhaustively with hot EtOH and the solvent-free residue chromatographed on a Si gel column. Elution with petrol gave lawsone [9], and with petrol–C_6H_6 (1:3) gave laxanthones I and II [2]. The fraction obtained from the petrol–C_6H_6 (9:1) eluate, was purified by preparative-TLC using C_6H_6, and named lacoumarin (80 mg), mp 162–164°. It analysed for $C_{12}H_{10}O_4$ (M^+ 218), gave a green colour with EtOH–$FeCl_3$, blue UV fluorescence and negative flavonoid colour reactions. ν_{max}^{KBr} 3250 (–OH), 1720, 1640 (conj. C=O) cm^{-1}; λ_{max}^{MeOH} 250, 330 nm. *MS.* 218 (M^+ 100%), 203 (57%), 190 (100%), 178 (50%), 175 (76%), 163 (100%), 149 (62%). On acetylation with $(Ac)_2O$ and Py it gave an acetate which crystallised from C_6H_6–petrol, mp 136–7°. PMR of the acetate ($\delta CDCl_3$, TMS as internal standard), 2.30 (3H, *s*, –O–CO–Me), 4.65 (2H, *d*, –O–CH_2–), 5.22–5.65 (2H, *m*, >C=CH_2), 5.80–6.12 (1H, *m*, >C=CH–C<), 6.32 (1H, *d*, *J* 9 Hz, H–C_3), 6.55 and 6.75 (2H, *dd*, *J* 2 Hz, H–C_6 and C_8), 8.15 (1H, *d*, *J* 9 Hz, H–C_4). IR and UV spectra indicated the possibility of the compound being a coumarin with a free OH group. Signals at δ6.32 (*d*, *J* 9 Hz) and 8.15 (*d*, *J* 9 Hz) in the PMR spectrum of the acetate showed the presence of unsubstituted C-3 and C-4 in the coumarin ring. The presence of an –OCOMe group in lacoumarin acetate was shown by the singlet at δ2.30 whereas the presence of an allyloxy group was supported by the signals at δ4.65 (2-H, –OCH_2), 5.22–5.65 (2H, *m*, >C=CH_2), 5.80–6.12 (1H, *m*, –CH=C<). The aromatic protons at δ6.55 and 6.75 were doublets (*J* 2 Hz) which could be attributed to *m*-coupling between the C-6 and C-8 protons. Hence, the compound could be either 5-allyloxy-7-acetoxycoumarin or 5-acetoxy-7-allyloxycoumarin. However, it was found to be indentical (mp, mmp, co-TLC and superimposable IR) with 5-allyloxy 7-acetoxycoumarin obtained by the selective allylation [10] of 5,7-diacetoxycoumarin, Lacoumarin itself is 5-allyloxy-7-hydroxycoumarin and this was confirmed by comparison with a synthetic sample [10] (mp, mmp, co-TLC and superimposable IR).

REFERENCES

1. Dastur, J. F., (Edition, 1962) *Medicinal Plants of India and Pakistan*, p. 101. Taraporevala, Bombay.
2. Bhardwaj, D. K., Murari, R., Seshadri, T. R., Radhika, S. *Phytochemistry* (communicated).
3. Agarwal, S. R., Ghatak, S. N., and Dhingra, D. R. (1959) *J. Indian Oil and Soap* **25**, 145.
4. Latif, A. (1959) *Indian J. agric. Sci.* **29**, (2–3) 147.
5. Vishnevetskaya, S. G. (1962). *Maslob-Zhir. Prom.* **28**, (2) 30–32, [*Chem. Abstr.*, **57**, (1962), 952 f].
6. Kedlaya, K. J., Selvarangan, R. and Nayudamma, Y. (1963) *Leather Sci.* (*Madras, India*) **10**, 305.
7. Machatizke, H., Vaughan, W. R. Charlotte, L. W. and White, G. R. (1967) *J. Pharm. Sci.* **56**, 86.
8. Prasad, V. and Gupta, S. C. (1967) *Indian J. Exp. Biol.* **5**, 193.
9. Lal, J. B. and Dutt, S. B. (1933) *J. Indian Chem. Soc.* 577.
10. Ahluwalia, V. K., Seshadri, T. R. and Venkateswarlu, P. (1969) *Indian J. Chem.* **7**, 115.

Phytochemistry, 1976, Vol. 15, pp. 1789–1791. Pergamon Press. Printed in England.

WIKSTROMOL, A NEW LIGNAN FROM *WIKSTROEMIA VIRIDIFLORA**

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(*Revised received* 15 *April* 1976)

Key Word Index—*Wikstroemia viridiflora*; Thymelaeaceae; lignan; arctigenin; matairesinol; pinoresinol; wikstromol.

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

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

*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.

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.

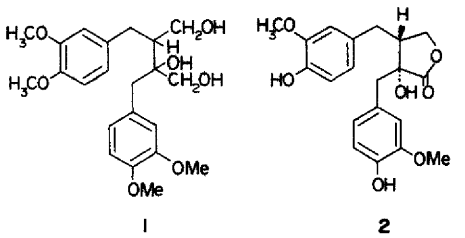
Wikstromol, $C_{20}H_{22}O_7$, was phenolic in nature. IR absorption bands at 3513, 1768 and 1600, 1500, 1463 cm^{-1} indicated the presence of a OH, a γ-lactone and an aromatic ring respectively in the molecule. It showed UV absorption at 215 (log ϵ 4.014), 232 (log ϵ 4.071) and 282 nm (log ϵ 3.748) 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 $(OMe \cdot OH)$-$C_6H_5CH_2^+$ 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 (–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 (mmp, IR, UV, PMR, MS) with an authentic sample. A similar treatment of wikstromol led to the production of carinol [5] which confirmed the bisguiacyl 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.



1 2

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 Hyflosupercel (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). λ_{max}^{EtOH} (nm): 230, 281 (log ϵ 4.16, 3.76). ν_{max}^{KBr} (cm^{-1}): 3400 (OH), 1768 (γ 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). λ_{max}^{EtOH} (nm): 232, 283 (log ϵ 4.06, 3.74). ν_{max}^{KBr} (cm^{-1}): 3560 (OH), 1765 (γ-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°. $[\alpha]_D$ + 84.0° (*c*, 2.0 Me_2CO). λ_{max}^{EtOH} (nm): 208, 230, 283 (log ϵ 4.26, 4.24, 3.657). ν_{max}^{KBr} (cm^{-1}): 3420 (OH), 1600, 1510, 1460, (aromatic). PMR (ppm): 3.05 (2H, *m*, C-β), 3.9 (2H, *m*, C-γ-ax.) 4.25 (2H, *m*, C-γ -eq.) 4.73 (2H, *d*, C-α 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°. ν_{max}^{KBr} (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 $[\alpha]_D$ +72° (*c*, 0.37, $CHCl_3$). R_f, 0.29 (C_6H_6–MeOH, 24 : 1). $\lambda_{max}^{(EtOH)}$ (nm): 215, 232, 282.5 (log ϵ 4.014, 4.077, 3.748). $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3513 (OH). 1768 (γ lactone), 1600, 1513, 1463 (aromatic). PMR (ppm): 2.3–2.95 (3H, *m*, C-3, 5). 3.02, (2H, *q*, J_{AB} 14 Hz, C6 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 Al_2O_3 and crystallized from MeOH, mp 96–97°, $[\alpha]_D$ +35° (*c* 1.47, $CHCl_3$). λ_{max}^{EtOH} (nm): 231, 261 (log ε 4.510, 3.730). $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3560 (OH), 1770 (γ 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 D_2O), 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 (M^+), 387, 373, 345, 327, 306, 294, 278, 263, 250, 233, 219, 195, 151, 135. Found: C, 63.00; H, 6.1 $C_{22}H_{26}O_7$ requires C, 63.1: H, 6.2%.

Wikstromol triacetate. Crystallized from EtOH mp 162°, $[\alpha]_D$ +118.8° (*c* 1.56, $CHCl_3$), λ_{max}^{EtOH} (nm): 226, 274, 280 (log ε 4.04, 3.72, 3.69). ν_{max}^{KBr} (cm^{-1}), 1786 (phenolic OAc), 1770 (γ 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 (M^+), 458, 416, 374, 398, 356, 220 and 137. Found: C, 62.95; H, 5.75 $C_{26}H_{28}O_{10}$ requires C, 62.4; H, 5.6%.

Wikstromol diacetate. Colourless powder, $\nu_{max}^{CHCl_3}$ (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 (M^+), 416, 374, 352, 323, 280, 265, 233, 210, 205, 192, 155, 149, 137 (base peak).

$LiAlH_4$ reduction of wikstromol dimethyl ether. To a soln of wikstromol diMe ether (206 mg) in THF, a suspension of $LiAlH_4$ (400 mg) in Et_2O 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, C_6H_6–MeOH, 23 : 2), was purified by chromatography over Si gel. The $CHCl_3$ eluate yielded a residue (131 mg) which crystallized from hexane –C_6H_6 (1 : 1) as colourless needles (50 mg), mp 130–131°, $[\alpha]_D$, +9° (*c*, 1.0, EtOH). λ_{max}^{EtOH} (nm): 230, 280, 290, ν_{max}^{KBr} (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 D_2O), 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 M^+, 388, 357, 254, 237, 219, 189, 160, 151. Found: C, 64.8: H, 7.42 $C_{22}H_{30}O_7$ requires C, 65.20; H, 7.35%.

Acknowledgements—We thank Mr. Edward Samson for technical assistance during this work. We also gratefully acknowledge that the plant material was collected under a NIH (USA) scheme (Grant No. PL-480 134304) and anticancer screening data was obtained from the cancer chemotherapy. National Service Centre, Bethesda, U.S.A.

REFERENCES

1. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956), *Glossory of Indian Medical Plants*, p. 258. Council of Scientific & Industrial Research, New Delhi.
2. Tseng, K. F. and Chao, C. Y. (1963) *Yao Hsuch Hsuch Pao* **10**, 286; (1963) *Chem. Abstr.* **59**, 15372.
3. Dhar, M. L., Dhawan, B. N., Prasad, C. R. Rastogi, R. P., Singh, K. K. and Tandon, J. S. (1974) *Indian J. Exp. Biol.* **12**, 512.
4. Burden, R. S., Crombie, L. and Whiting, D. A. (1969) *J. Chem. Soc.* 693.
5. Pal, R., Kulshreshtha, D. K. and Rastogi, R. P. (1975) *Phytochemistry* **14**, 2302.
6. Paech, K. and Tracey, M. Y. (1955) *Moderne Methoden der Pflanzenanalyse*, p. 428. Springer-Verlag, Berlin.

Phytochemistry, 1976, Vol. 15, pp. 1791–1793. Pergamon Press. Printed in England.

3,5,4'-TRIHYDROXYSTILBENE AS A PHYTOALEXIN FROM GROUNDNUTS (*ARACHIS HYPOGAEA*)

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(*Received* 16 *March* 1976)

Key Word Index—*Arachis hypogaea*; Leguminosae; groundnut; *Helminthosporium carbonum*; stilbene; phytoalexin; antifungal compound; resveratrol.

Abstract—*Cis* and *trans*-resveratrol (3,5,4'-trihydroxystilbene) have been isolated from the infected hypocotyls of *Arachis hypogaea* and implicated as phytoalexins.

INTRODUCTION

Although phytoalexin biosynthesis has been associated with infected stems [1], seeds [1] and immature pods [2] of the groundnut (*Arachis hypogaea* L.), the chemical nature of the compound or compounds involved has not been reported. As yet, no experimental data has been provided to substantiate the claim that (like many leguminous species), roots and leaves of *A. hypogaea* produce pterocarpanoid phytoalexins [3]. Other work [4] suggests that a pre-infectional antifungal compound occurs in the tissues of groundnut pods. This paper presents evidence to show that groundnut hypocotyls accumulate a mixture of *cis* and *trans*-resveratrol following infection by the non-pathogenic fungus, *Helminthosporium carbonum*.

RESULTS AND DISCUSSION

Antifungal material in hypocotyl diffusates (see Experimental) was detected by TLC ($CHCl_3$–MeOH, 100:4, Merck Si gel, F 254) bioassay [5] using *Cladosporium herbarum* as the test organism. Only one area (extending from the origin to R_f 0.11) highly inhibitory to the spore