Phytochemistry, 1980, Vol. 19, pp. 2801 2802. © Pergamon Press Ltd. Printed in England 0031-9422/80/1201-2801 $02.00/0

WAIROL, A NEW COUMESTAN FROM *MEDICAGO SATIVA*

DAVID R. BIGGS and G. JOHN SHAW

Applied Biochemistry Division, DSIR, Palmerston North, New Zealand

(*Received* 3 *April* 1980)

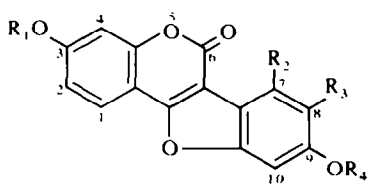
Key Word Index—*Medicago sativa*; Leguminosae; lucerne; 3-hydroxy-7,9-dimethoxycoumestan; wairol.

Abstract—A new coumestan isolated from *Medicago sativa* has been characterized as 3-hydroxy-7,9-dimethoxycoumestan and named wairol.

Previous chemical investigation of fungal- and viral-infected lucerne foliage has resulted in the isolation of eight structurally related coumestans [1, 2]. The present communication describes the isolation and structural characterization of a new coumestan, wairol (**1**) from the same source.

In the isolation procedure, **1** behaved very similarly to the known coumestan 3-hydroxy-8,9-dimethoxycoumestan (**3**), but was finally separated from it by repeated chromatography on silicic acid and Sephadex LH-20. Identification of **3** was confirmed by chromatographic, UV and MS comparisons with a sample of authentic material. The characterization of wairol as **1** was suggested by its UV spectral properties. Two noteworthy features were the presence of an absorption band at longer wavelength than 250 nm (265 nm) and of greater relative intensity than the absorption band at 345 nm. These spectral features are characteristic of the 7,9-disubstituted coumestans trifoliol (**4**) and repensol (**6**) [1, 3, 4]. In contrast, other coumestans isolated from lucerne have revealed a corresponding absorption band below 250 nm, which in all cases was of lower relative intensity than the longer wavelength band at 340–350 nm. Bathochromic shifts in the UV maxima of wairol were observed on addition of NaOAc, consistent with a free-OH group at C-3 [1]. In support of this, methylation of wairol gave only the monomethyl ether derivative (**2**).

High resolution MS of wairol gave a strong molecular ion peak at *m/e* 312 ($C_{17}H_{12}O_6$), with prominent fragment ions at *m/e* 297 (M^+-CH_3), 283 (M^+-CHO) and 269 (M^+-CH_3-CO). The MS of **3** gave a very similar fragmentation pattern however the ion at *m/e* 283 was relatively weak. Similarly in **4** the corresponding ion at *m/e* 269 was minor. The enhanced fragmentation pathway leading to *m/e* 283 in wairol can be rationalized in terms of a proximity effect between the lactone carbonyl and a C-10 methoxyl group. This is supported by the finding that the MS of **5**, prepared by reaction of authentic trifoliol (**4**) with diazomethane, gave only a negligible ion at *m/e* 283. Exhaustive methylation of **4** afforded a product which was chromatographically and mass spectrometrically indistinguishable from **2**.



	R_1	R_2	R_3	R_4
1	H	OMe	H	Me
2	Me	OMe	H	Me
3	H	H	OMe	Me
4	H	OH	H	Me
5	Me	OH	H	Me
6	H	OH	H	H

Wairol was detected in extracts of lucerne field-infected with *Stemphylium botryosum*, as well as in *Aschochyta*-infected plants, but in much smaller quantity. Neither wairol nor other coumestans were detected in fresh, apparently healthy foliage. Wairol, therefore, like other coumestans, appears to be a post-infectional product of lucerne.

EXPERIMENTAL

High and low resolution MS were recorded on AEI MS9 and AEI MS30 mass spectrometers respectively, at 70 eV.

Extraction procedure. Field-grown lucerne foliage (2.5 kg dry wt, cv Wairau, infected with *Aschochyta imperfecta*) were extracted with EtOH, and the extracts treated as described previously [5]. The phenolic extract (EtOAc) was chromatographed on Al_2O_3 (500 g, Woelm, neutral, activity grade 1), with $CHCl_3$, EtOH and EtOH–H_2O (1:1). The EtOH fractions were further chromatographed by PC (HOAc–H_2O, 3:7) and the fluorescent material at R_f 0.1–0.3 on Si gel with $CHCl_3$. The least polar fluorescent fractions were further chromatographed by PLC (Si gel, Et_2O–hexane, 3:1).

Wairol (**1**) was detected as a violet fluorescent band, R_f *ca* 0.4 on prep. TLC, only partially separated from bright blue fluorescent **2**. Repeated chromatography (4×) on Sephadex LH-20 with $CHCl_3$–HOAc (19:1) yielded *ca* 100 μg (based on ε for trifoliol [4]) of chromatographically homogenous **1**. λ_{max}^{EtOH} (nm) 218, 265, 303, 345 (rel. int.: 100, 39, 11, 37); $\lambda_{max}^{EtOH+NaOAc}$ (nm) 218, 268, 298, 310, 369; $\lambda_{max}^{EtOH+KOH}$ (nm) 218, 270, 298, 310, 379. MS (probe) *m/e* (rel. int.) 312.0633 (M^+; 100) (Calc. for $C_{17}H_{12}O_6$ 312.0665), 297 ($C_{16}H_9O_6$, 40), 283 ($C_{16}H_{11}O_5$, 43), 269 ($C_{15}H_9O_5$, 12).

3-Hydroxy-8,9-dimethoxycoumestan (**3**) was isolated by repeated chromatography of a bright blue fluorescent band ex prep. TLC on Sephadex LH-20 as for **1**. UV. as lit. [1]. MS (probe) *m/e* (rel. int.): 312 (M^+: $C_{17}H_{12}O_6$, 100), 297 (80), 283 (3), 269 (10). R_f: PC 0.41, 0.60 (HOAc H_2O, 6:4, C_6H_6-HOAc-H_2O 125:72:3); TLC Si gel 0.33, 0.32 ($CHCl_3$-EtOH 40:1, Et_2O hexanes 3:1).

Wairol monomethyl ether (**2**). To a solution of wairol (*ca* 100 μg) in EtOH was added an excess of CH_2N_2. After 5 min the soln was taken to dryness. The product co-chromatographed with and gave identical MS characteristics to an exhaustively methylated sample of trifoliol (**4**), prepared by reaction with Me_2SO_4 [3]. MS (probe) *m/e* (rel. int.): 326 (M^+; 100), 311 (52), 297 (46), 283 (36). R_f: PC 0.58, 0.93 (HOAc-H_2O 6:4, C_6H_6-HOAc-H_2O 125:72:3); TLC Si gel 0.70, 0.16 ($CHCl_3$-EtOH 40:1, Et_2O-hexanes 1:1).

3,9-Dimethoxy-7-hydroxycoumestan (**5**). Methylation of **4** with CH_2N_2 afforded **5**, crystals from MeOH (mp 208–212 uncorr.) (lit. [3] 209–212). MS (probe) *m/e* (rel. int.). 312 (M^+: 100), 297 (72), 283 (3), 269 (12).

Acknowledgements The authors are grateful to Miss P. A. Dittmer for technical assistance, to Dr. E. M. Bickoff of the U.S. Dept. of Agriculture, Albany, California for samples of trifoliol and 3-hydroxy-8,9-dimethoxycoumestan, to Dr G. Lane of this Division for helpful discussions and to Dr J. Lancashire of the Grasslands Division, DSIR for samples of field-infected lucerne plants.

REFERENCES

1. Bickoff, E. M., Spencer, R. R., Witt, S. C. and Knuckles, B. E. (1969) *Studies on the Chemical and Biological Properties of Coumestrol and Related Compounds*, Technical Bulletin 1408, U.S. Dept. of Agriculture.
2. Wong, E. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.) p. 780. Chapman and Hall, London.
3. Livingston, A. L., Bickoff, E. M., Lundin, R. E. and Jurd, L. (1964) *Tetrahedron* **20**, 1963.
4. Wong, E. and Latch, G. C. M. (1971) *Phytochemistry* **10**, 466.
5. Wong, E. (1962) *J. Sci. Food Agric.* **13**, 304.