alginate were 0.9982, 0.9987, and 0.9983, respectively. The processes giving rise to the second-order elimination from the matrix are not apparent immediately since a shrinking core phenomenon with decreasing core surface area is occurring.

This technique of controlling the diffusion rate from silicone polymers was recently applied by the authors to salts of amphetamine and the barbiturates, and preliminary *in vitro* and *in vivo* results are encouraging. This simple method of drug delivery may find use also in chronic toxicity studies in small animals.

## REFERENCES


(1) T. J. Roseman and S. H. Yalkowsky, *J. Pharm. Sci.*, **63**, 1639 (1974).

(2) P. J. Dzuik and B. Cook, *Endocrinology*, **78**, 208 (1966).

(3) C. C. Chang and F. A. Kincl, *Fertil. Steril.*, **21**, 134 (1970).

(4) T. J. Roseman and W. I. Higuchi, *J. Pharm. Sci.*, **59**, 353 (1970).

(5) T. J. Roseman, *ibid.*, **61**, 46 (1972).

(6) J. Haleblian, R. Runkel, N. Mueller, J. Christopherson, and K. Ng, *ibid.*, **60**, 541 (1971).

(7) P. Kratochvil, G. Benagiano, and F. A. Kincl, *Steroids*, **13**, 505 (1970).

(8) Y. W. Chien and H. J. Lambert, *J. Pharm. Sci.*, **63**, 515 (1974).

(9) Y. W. Chien, H. J. Lambert, and D. E. Grant, *ibid.*, **63**, 365 (1974).

(10) J. W. McGinity, C. S. Mehta, and A. B. Combs, "Proceedings of First International Conference on Pharmaceutical Technology," Paris, France, 1976, p. 235.

(11) J. W. McGinity and C. S. Mehta, *Pharmacol. Biochem. Behav.*, **9**, 705 (1978).


## ACKNOWLEDGMENTS


Supported in part by a grant from the University Research Institute, University of Texas at Austin.


# Wikstromol, Antitumor Lignan from *Wikstroemia foetida* var. *oahuensis* Gray and *Wikstroemia uva-ursi* Gray (Thymelaeaceae)


**STERLING J. TORRANCE, JOSEPH J. HOFFMANN, and JACK R. COLE ×**



Received September 11, 1978, from the *Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, AZ 85721.* Accepted for publication November 1, 1978.



**Abstract** □ The ethanol extracts of *Wikstroemia foetida* var. *oahuensis* and *Wikstroemia uva-ursi* showed antitumor activity against the P-388 lymphocytic leukemia (3PS) test system. One PS-active constituent of both plants was the lignan wikstromol. Several inactive compounds were identified as daphnoretin, pinoresinol, and syringaresinol.



**Keyphrases** □ Wikstromol—isolation from plant material, antineoplastic activity □ Antineoplastic agents—wikstromol, isolated from plant material


In the continuing search for plants having antitumor constituents, it was found that the chloroform fractions of *Wikstroemia foetida* var. *oahuensis* Gray and *Wikstroemia uva-ursi* Gray[1] (Thymelaeaceae) ethanol extracts were active against the P-388 lymphocytic leukemia (3PS) test system[2].

## DISCUSSION

The lignan wikstromol (I) was isolated from both *Wikstroemia* species. This compound was isolated recently from *Wikstroemia viridiflora* (1) and *Daphne odora* (Thymelaeaceae) (2). Wikstromol is the enantiomer of nortrachelogenin (II, also called pinopalustrin), isolated from *Trachelospermum asiaticum* var. *intermedium* (Apocynaceae) (3) and *Pinus palustris* (Pinaceae) (4).

Comparison of the sample spectra (IR, PMR, and mass) with literature values (1, 3) for wikstromol and nortrachelogenin and comparison of the sample's physical constants with those of these two lignans (Table I) suggested that it was wikstromol. Confirmation was by direct comparison of authentic spectra[3] (IR and PMR) and of the sample[3] of the nortrachelogenin dimethyl ether with the study sample dimethyl ether. The spectra were identical, and TLC behaviors were indistinguishable. Unfortunately, the study sample could not be made to crystallize, and an authentic sample of wikstromol, or its dimethyl ether, was unobtainable.

In addition to the PS-active wikstromol, three inactive compounds were isolated from both *W. foetida* var. *oahuensis* and *W. uva-ursi*. These compounds were daphnoretin (III), pinoresinol (IV), and syringaresinol (V). In each case, identity was confirmed by comparison with authentic specimens[4].

Wikstromol (I) demonstrated activities of 154, 146, 137, 141, and 130% test/control (T/C) at does of 16, 10, 4, 2, and 1 mg/kg, respectively. Activity in the PS test system is defined as an increase in the survival of treated animals over that of control animals resulting in a T/C ≥ 130%[5].

## EXPERIMENTAL[6]

Wikstromol was isolated similarly from *W. foetida* var. *oahuensis* and *W. uva-ursi*. The procedure for isolation from the former follows.

Whole *W. foetida* var. *oahuensis* plants were ground in a Wiley mill and stored at −10° prior to extraction. The ground material (9 kg) was exhaustively extracted in a Lloyd-type extractor with petroleum ether

[1] *W. foetida* var. *oahuensis* and *W. uva-ursi* were collected in Hawaii in September 1975 and March 1973, respectively. Identification was confirmed by Dr. Robert E. Perdue, Jr., Medicinal Plant Resources Laboratory, U.S. Department of Agriculture, Bethesda, Md. Reference specimens are maintained by the Department of Agriculture.

[2] Of the Drug Evaluation Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md.

[3] The authors are indebted to Prof. Dr. Sansei Nishibe, Department of Pharmacognosy, Higashi Nippon Gakuen University, Ishikari-Tobetsu, Hokkaido, Japan, for spectra and sample of nortrachelogenin dimethyl ether.

[4] The authors thank Prof. Dr. G. Legler, Institute für Biochemie der Universität Köln, Germany, for sample of daphnoretin. They are grateful also to Dr. Cornelius Steelink, Department of Chemistry, University of Arizona, Tucson, Ariz., for specimens of pinoresinol and syringaresinol.

[5] John D. Douros, Natural Products Branch, National Cancer Institute, Bethesda, Md., personal communication, Sept. 1977.

[6] Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR and mass spectra were recorded on a Beckman IR-33 and Hewlett-Packard quadrupole spectrometer (model 4930), respectively. PMR spectra were recorded on Varian T-60 and EM-360L spectrometers, and optical rotations were taken on a Perkin-Elmer model 241 MC polarimeter.

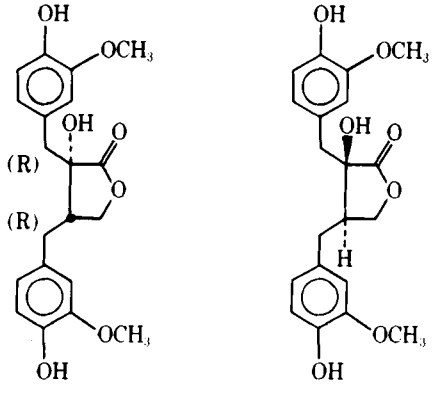



*0022-3549/79/0500-0664$01.00/0*

**Table I—Comparison of Physical Constants**

| Constant | Wikstromol | Study Sample | Nortrachelogenin | Daphne Lignan | Pinopalustrin |
|---|---|---|---|---|---|
| Melting point | Amorphous | Amorphous | Amorphous | Amorphous | Amorphous |
| $[\alpha]_D$ | +72° (chloroform) | +41° (chloroform) | −17° (ethanol) | +48° | — |
| | | Dimethyl Ethers | | | |
| Melting point | 96–97° | Amorphous | 97–98.5° | — | 96–97°, 127–128° |
| $[\alpha]_D$ | +35° (chloroform) | +26° (chloroform) | −46° (ethanol) | — | −39° (chloroform) |

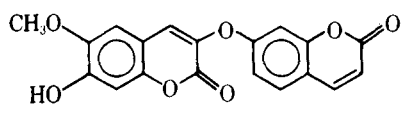
(bp 30–60°) followed by 95% ethanol. The air-dried ethanol extract was partitioned between chloroform and water (1:1). Removal of the solvent from the chloroform phase provided a sticky, green residue (227 g). The latter was extracted with hexane (5 × 0.5 liter) and ether (4 × 0.5 liter), and the hexane-insoluble, ether-soluble material (84 g after solvent removal) was chromatographed over silica gel 60[7] (2 kg, 9.5 × 80 cm). Elution was begun with chloroform–methanol (98:2 v/v), and wikstromol (I) was eluted with chloroform–methanol (97:3 v/v). Final purification of wikstromol was by preparative TLC [chloroform–methanol–water (188:12:1 v/v/v)].



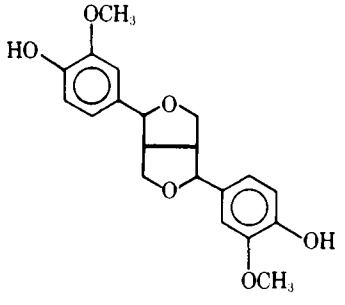

I



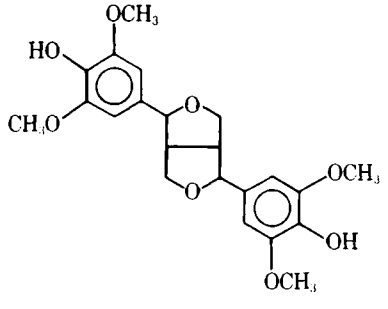

II




III




IV




V

Wikstromol was obtained as an amorphous powder, $[\alpha]_D^{25}$ +41° (c 0.93, $CHCl_3$) whose IR, PMR, and mass spectra were identical to those reported for wikstromol (1) and nortrachelogenin (3).

Wikstromol dimethyl ether, prepared in the usual way with diazomethane, was obtained as an amorphous semisolid, $[\alpha]_D^{25}$ +26.3° (c 1.09, $CHCl_3$). Its spectra (IR, PMR, and mass) were in accord with those in the literature (1, 3), and its IR and PMR spectra were identical in all respects with authentic nortrachelogenin dimethyl ether spectra[3]. In several solvent systems, TLC behaviors of our material and authentic nortrachelogenin dimethyl ether[3] were indistinguishable.

Daphnoretin (III) and syringaresinol (V) were isolated from other fractions of the preparative TLC used to obtain wikstromol. Daphnoretin was obtained as yellow needles, mp 246–248°. Recrystallization from methanol–chloroform provided colorless needles, mp 261–262°. Comparison with an authentic sample (identical IR spectra and undepressed mixed melting point) confirmed its identity. The PMR and mass spectra were in accord with Structure III. Syringaresinol was obtained as colorless cubelets from methanol, mp 178–180°. Comparison with an authentic specimen[4] (identical IR and undepressed mixed melting point) confirmed its identity.

Pinoresinol (IV) was obtained from the wikstromol-containing column fraction by preparative TLC using benzene–ethyl acetate–methanol (30:10:2 v/v/v). Recrystallization from ether gave colorless cubelets, mp 120–121°, identical (superimposable IR spectra and undepressed mixed melting point) with an authentic sample[4].

## REFERENCES


(1) S. Tandon, and R. P. Rastogi, *Phytochemistry*, **1976**, 1789.

(2) S. Kogiso, K. Wada, and K. Munakata, "Report of the 26th International Congress of Pure and Applied Chemistry," Tokyo, Japan, 1977, p. 291.

(3) S. Nishibe, S. Hisada, and I. Inagaki, *Phytochemistry*, **1971**, 2231. S. Nishibe, S. Hisada, and I. Inagaki, *Chem. Pharm. Bull.*, **19** (4), 866 (1971). *Ibid.*, **20** (9), 2075 (1972). *Ibid.*, **20** (12), 2710 (1972). *Ibid.*, **21** (5), 1108 (1973).

(4) B. Carnmalm, H. G. H. Erdtman, G. C. Harris, and T. F. Sanderson, *Acta Chem. Scand.*, **B31**, 433 (1977).


## ACKNOWLEDGMENTS


Supported in part by Contract NOI-CM-33750 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014; The Elsa U. Pardee Foundation, Midland, MI 48640; and Grant 1-R01-CA22336-01A1 from the National Cancer Institute.


[7] Merck.