Phytochemistry, 1971, Vol. 10, pp. 465 to 466. Pergamon Press. Printed in England.

LAURACEAE

OCCURRENCE OF ISOBOLDINE IN *NECTANDRA PICHURIM*

G. FERRARI and O. FERVIDI

Simes S.p.A., Lab. Ricerche Chimiche, Milano, Italy

and

M. FERRARI

Istituto di Chimica Organica dell'Università di Milano, Italy

(*Received* 20 *April* 1970)

Plant. Nectandra pichurim (H.B.K.) Mez (*Syn. Ocotea pichurim* H.B.K.).
Source. State of São Paulo, Brasil.
Uses. As a substitute for sassafras.[1]
Previous work. A tincture of leaves showed growth control of *Entamoeba histolytica.*[2]
Leaves and stems. Extracted with EtOH until exhaustion and the solvent removed. The residue was extracted with 10% HOAc alkalinized with aqueous ammonia, extracted with $CHCl_3$ (this procedure was repeated twice) and evaporated to dryness. Chromatography on a neutral Al_2O_3 column afforded from MeOH an alkaloid (0·635 per cent of the weight of dry plant material), which was crystallized from EtOAc and stored under N_2.

Isoboldine, $C_{19}H_{21}NO_4$. Found: m.p. (*) 115–20° and 172–5°; $[\alpha]_D$ + 43° ($CHCl_3$), $[\alpha]_D$ + 50·5° (EtOH); HCl-ide m.p. 255–60°; picrate m.p. 182–4° dec. (Required: m.p. 121–6° and 180°[3]; $[\alpha]_D$ + 43° ($CHCl_3$)[4]; $[\alpha]_D$ + 41·2° (EtOH);[3] HCl-ide m.p. 270° dec.;[4] picrate m.p. 163–5° dec.[5] CH_3J-ide m.p. 175–80° dec. (from MeOH–ether).

U.v.,[3] m.s.[6] and NMR[7] of the base and u.v. of the HCl-ide[4] were in full accordance with those described for authentic material. i.r. spectrum and R_fs on buffered paper according to Jurd[8] were identical to those of authentic isoboldine. Further evidence of identity was furnished by its diacetate and by its correlation with glaucine. Diacetate $C_{23}H_{25}NO_6$ was prepared via Ac_2O in pyridine and was recrystallized from isopropyl ether; m.p. 151–3°. Found: C, 67·62; H, 6·20; N, 3·35%. (Required, C, 67·14; H, 6·12; N, 3·40%.) Glaucine was obtained with CH_2N_2 in ether[9] and was identical with authentic material isolated from *Glaucium flavum* Crantz[10] by mixed m.p., $[\alpha]_D$, co-chromatography and i.r. analysis.

* M.ps are uncorrected.

[1] F. C. HOEHNE, *Plantas e Substancias Vegetais, tòxicas e medicinais*, Departamento de Botanica do Estado, São Paulo, 1939.
[2] F. PRADO JR., *Brasil Med.* **63**, 43 (1949).
[3] T. TSCHESCHE, P. WELZEL, R. MOLL and G. LEGLER, *Tetrahedron* **20**, 1435 (1964).
[4] K. BERNAUER, *Helv. Chim. Acta* **50**, 1583 (1967).
[5] A. H. JACKSON and J. A. MARTIN, *Chem. Commun.* **18**, 420 (1965).
[6] A. H. JACKSON and J. A. MARTIN, *J. Chem. Soc.* (c) 2181 (1966).
[7] A. H. JACKSON and J. A. MARTIN, *J. Chem. Soc.* (c) 2222 (1966).
[8] L. JURD, *J. Chromatog.* **4**, 374 (1960).
[9] A. H. JACKSON and J. A. MARTIN, *J. Chem. Soc.* (c) 2061 (1966).
[10] J. SLAVÍK and L. SLAVÍKOVÁ, *Coll. Czech. Chem. Commun.* **24**, 3141 (1959).

Acknowledgements—The authors are indebted to Prof. Dr. R. Tschesche, Bonn, for a sample of isoboldine, and to Dr. Moysés Kuhlmann, São Paulo, for the identification of plant material. They also thank Dr. G. Severini Ricca for NMR spectra and Dr. T. Salvatori for mass spectra.

Phytochemistry, 1971, Vol. 10, pp. 466 to 468. Pergamon Press. Printed in England.

LEGUMINOSAE

COUMESTANS IN DISEASED WHITE CLOVER

E. WONG and G. C. M. LATCH

Department of Scientific and Industrial Research, Palmerston North, New Zealand

(*Received* 19 *May* 1970)

Abstract—Four coumestans have been detected in white clover (*Trifolium repens*) infected with various foliar pathogens, and have been identified as coumestrol, 12-*O*-methylcoumestrol, trifoliol and 7,10,12-trihydroxycoumestan (*repensol*) by chromatographic and spectroscopic methods. Repensol has not previously been reported as a natural product.

INTRODUCTION

EIGHT coumestans, including coumestrol[1,2] (I), 12-*O*-methylcoumestrol[3] (II) and trifoliol[2] (IV) have been reported as minor constituents in alfalfa[4] (*Medicago sativa*), and later work has shown that the concentration level of these compounds in the plant is markedly increased by the presence of fungal infection.[4,5] Coumestrol and trifoliol have also been isolated by Bickoff and co-workers from large scale extractions of white clover (variety Ladino).[6,7] Their concentration levels however were normally very low, and they were usually not readily discernible on chromatograms of clover extracts. We have now found that when white clover is infected with the foliar pathogens *Pseudopeziza trifolii* and *Uromyces* sp. (rust), coumestrol and trifoliol increase markedly in concentration and are readily detectable

[1] E. M. BICKOFF, A. N. BOOTH, R. L. LYMAN, A. L. LIVINGSTON, C. R. THOMPSON and F. DE EDS, *Science* **126**, 969 (1957).
[2] E. M. BICKOFF, A. L. LIVINGSTON, S. C. WITT, B. E. KNUNCKLES, J. GUGGOLZ and R. R. SPENCER, *J. Pharm. Sci.* **53**, 1496 (1964).
[3] E. M. BICKOFF, A. L. LIVINGSTON, S. C. WITT, R. E. LUNDIN and R. R. SPENCER, *J. Agri. Food Chem.* **13**, 597 (1965).
[4] For a review, see E. M. BICKOFF, R. R. SPENCER, S. C. WITT and B. E. KNUCKLES, *Studies on the Chemical and Biological Properties of Coumestrol and Related Compounds*, Technical Bulletin 1408, U.S. Dept of Agriculture (1969).
[5] E. M. BICKOFF, G. M. LOPER, C. H. HANSON, *Crop Sci.* **7**, 259 (1967).
[6] E. M. BICKOFF, A. L. LIVINGSTON and J. GUGGOLZ, *J. Agri. Food Chem.* **13**, 151 (1965).
[7] A. L. LIVINGSTON, E. M. BICKOFF, R. E. LUNDIN and L. JURD, *Tetrahedron* **20**, 1963 (1964).