Phytochemistry, 1971, Vol 10, pp. 1335 to 1337 Pergamon Press. Printed in England.

SHORT COMMUNICATION

(−)-HYOSCYAMINE IN *DUBOISIA HOPWOODII*

G. S. KENNEDY*

Department of Botany, University of Queensland, Brisbane, Australia

(*Received* 1 *September* 1970)

Abstract—Tropane alkaloids were found in *Duboisia hopwoodii* F. Muell. Their location was restricted to the roots of the plant. The pyridine alkaloids were concentrated in its leaves. All *Duboisia* species therefore, have unexpectedly similar alkaloids but locate them differently within the plant.

INTRODUCTION

Duboisia, a small genus within the Solanaceae, consists of three species which have either pyridine alkaloids, tropane alkaloids, or a mixture of both alkaloid types. *D. hopwoodii* F. Muell. has only the pyridine alkaloids, nicotine and nornicotine.[1] It is an infrequently occurring desert shrub with leaves chewed by Australian Aborigines or used by them to poison water holes. *D. leichardtii* F. Muell. has only tropane alkaloids,[2,3] while *D. myoporoides* R. Br. has 14 alkaloids possessing pyridine, piperidine or tropane ring structure.[4,5] The latter two species are rainforest trees, important commercially as sources of hyoscine or hyoscyamine.

The present study was originally intended to investigate the possibility that *D. hopwoodii* fails to produce tropane alkaloids because of its inability to cyclize or esterify tropane alkaloids precursors. *D. hopwoodii* roots were therefore examined for alkaloids like hygrine or *cis*-tropine. Roots in related genera have been shown largely responsible for tropane and pyridine alkaloids synthesis[6] and to contain a more complex alkaloids pattern than the leaf.[7,8] The root alkaloids of *D. leichhardtii*[9] and *D. myoporoides*[10] have been recently studied.

RESULTS AND DISCUSSION

Three samples of *D. Hopwoodii* were obtained from locations more than 600 miles apart. All root samples contained substantial amounts of hyoscyamine and hyoscine; these

* Present address: Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48823, U.S.A

[1] W. BOTTOMLEY and D E. WHITE, *Australian J Sci. Res* A **4**, 107 (1951)
[2] K. LOFTUS HILLS, W. BOTTOMLEY and P I. MORTIMER, *Australian J. Appl Sci.* **5**, 276 (1954)
[3] E. I. ROSEMBLUM and J. TAYLOR, *J. Pharm Pharmacol* **6**, 410 (1954).
[4] K. LOFTUS HILLS, W BOTTOMLEY and P I. MORTIMER, *Australian J. Appl Sci.* **5**, 258 (1954).
[5] P. I. MORTIMER and S. WILKINSON, *J. Chem. Soc.* 2967 (1957)
[6] K. MOTHES, in *The Alkaloids* (edited by R. H. F MANSKE and H. L. HOLMES), p 7, Academic Press, New York (1960).
[7] E. WADA, R. KISAKI and I. IHIDA, *Arch Biochem. Biophys.* **80**, 258 (1959).
[8] W. C. EVANS and M. WELLENDORF, *J. Chem. Soc.* 1406 (1959).
[9] W. J. GRIFFIN, *Australian J. Pharm.* **51**, 519 (1967).
[10] J. F COULSON and W J GRIFFIN, *Planta Med.* **16**, 174 (1968)

TABLE 1. ALKALOID CONTENTS OF *D. hopwoodii* FROM VARIOUS ORIGINS

	Alkaloid (% dry wt)*								
	1	2	3	4	5	6	7	8	9
Alice Springs									
Medium root	0 3	0 5	0	0	0	0 2	0 2	0 8	0 8
Coarse root	0 2	0 5	0	0	0	0 1	0	0 1	0·8
Stem wood	0	0 3	0	0	0	0 1	0	0 1	0·4
Stem bark	0	0 5	0	0	0 2	0 1	0	0 6	0 4
Old leaf	0	2 2	0	0	0 4	0 1	0 1	0	0
Middle leaf	0	2 4	0	0	0 4	0 1	0	0	0
Young leaf	0	1 6	0	0 2	0 1	0 2	0 1	0	0
Kalgoorlie									
Coarse root	0·1	0 6	0	0	0	0	0	0·2	0 4
Middle leaf	0	1 8	0	0 3	0	0 2	0 2	0 1	0
Kimba									
Medium root	0 1	0 3	0 1	0 1	0	0 1	0·1	0 1	0 5
Coarse root	0 2	0·4	0 1	0 1	0	0	0·1	0 2	0 3
Middle leaf	0	3 2	0	0 2	0	0	0	0 1	0

* Key: 1 cuscohygrine, 2 normicotine, 3 anabasine, 4 nicotine, 5 unknown pyridine alkaloid as nicotine, 6 hygrine, 7 isopelletierine, 8 hyoscine, 9 hyoscyamine The samples were divided into medium roots (approximately 3–5 mm in diameter), coarse roots (> 1 cm in diameter), yellow, senescing leaves, leaf bulk and young leaves (< 3 cm in length).

alkaloids were absent or barely detected in corresponding leaf samples (Table 1). (—)-Hyoscyamine was isolated by preparative TLC; the other identities rest on chromatographic data. It was observed that young roots about 0·5 cm in diameter contained the widest range of alkaloids, but at a comparatively low total alkaloids content. The leaves, although of high alkaloid content, contained mostly normicotine. These leaf data are consistent with previous results, although nicotine predominating *D. hopwoodii* plants are also quite common.[1]

Alkaloids corresponding chromatographically with cuscohygrine, anabasine, isopelletierine and hygrine were also present in the roots (Table 1). Anabasine and isopelletierine have previously been found in *D. myoporoides*;[5] cuscohygrine[11] and hygrine[12] have been found in related genera which contain tropane alkaloids. Tetramethylputrescine, a major component in the roots of *D. leichhardtii*[9] and *D. myoporoides*,[10] may have cochromatographed with cuscohygrine.

It is apparent that the characteristically simple leaf alkaloid pattern in *D. hopwoodii* is not due to inability to synthesize tropane alkaloids. Instead, the *D. hopwoodii* leaf preferentially accumulates pyridine alkaloid. The converse situation has been observed in *D. leichardtii* where pyridine alkaloids are abundant in the fine roots but occur only in trace amounts in the leaves.[13] Little attention has been paid to the mechanisms by which this type of localization is achieved. In the present case, alkaloids presumably produced in the

[11] R P. VAN HAGA, *Nature* **174**, 833 (1954)
[12] A. ROMEIKE, *Naturwissenschaften* **53**, 82 (1966)
[13] G S KENNEDY, Ph D Thesis, Univ of Queensland (1963).

root, move in the xylem sap to the leaf where accumulation is subject to the influence of selective uptake, permeability or metabolism.

The primary contribution of this investigation has been to demonstrate that tropane alkaloids are in fact synthesized by *D. hopwoodii*. In the samples examined, their occurrence is almost entirely restricted to the root—they do not accumulate in the leaf. Since entirely different sets of data may be obtained by investigating roots or leaves, this finding has some general relevance to biochemical taxonomy.

EXPERIMENTAL

Materials

Air-dried *D hopwoodii* leaf, stem and root samples were obtained from near Kalgoorlie, Western Australia, Kimba, South Australia and Alice Springs, Northern Territory. Reference alkaloids were very kindly given by Dr W. Bottomley and Dr P I Mortimer. Hygrine picrate and cuscohygrine were obtained from Koch–Light

Alkaloid Analysis

Milled plant material, extracted with 80% ethanol, was applied to a Duolite C-10 resin H^+ form. The resin was washed successively with water, 80% ethanol and the alkaloids displaced with 95% ethanol containing 2N NH_4OH, then concentrated to a small volume and acidified. The extracts were chromatographed on treated papers with two solvent systems[14,15] which adequately separated individual tropane alkaloids, isopelletierine, hygrine and cuscohygrine (the latter alkaloids were also detected with 0 4% 2,4-dinitrophenylhydrazine in 2N HCl) The pyridine alkaloids were separated by chromatography on buffered paper and TLC.[16,17] Semiquantitative measurement was made using a matching spot method similar to that already described.[18]

Isolation of (−)-Hyoscyamine

Kimba (South Australia) *D hopwoodii* root (19 g) was extracted in methanol, the extract evaporated, dissolved in 0 2 N H_2SO_4, made alkaline and extracted into $CHCl_3$ The $CHCl_3$ was concentrated and separated by TLC on a preparative scale [17] On elution, the hyoscyamine zone yielded a solid residue (3 2 mg, $[\alpha]_D^{20}$-19 (ethanol), i r and u v spectra identical with (−)-hyoscyamine and a reineckate m p 156 (uncor)

Hydrolysis with barium hydroxide yielded products chromatographically identical with tropine and tropic acid

Acknowledgements—I thank Dr. J R Cannon, University of Western Australia, the Forestry Officers, Kalgoorlie and Mr G Chippendale, Alice Springs for collecting *D. hopwoodii* samples. The studies were partially financed by a grant from the Rural Credits Development Fund, Commonwealth Reserve Bank of Australia.

[14] R E A Drey, *J. Pharm Pharmacol* **10**, 241 T (1958).
[15] R E A Drey, *J. Pharm. Pharmacol* **11**, 64 (1959)
[16] L Leiserson and T. B. Walker, *Anal Chem* **27**, 1129 (1955).
[17] K Teichert, E Mutschler and H Rochelmeyer, *Dtsch Apotheker-Ztg.* **100**, 477 (1960)
[18] R N Jeffrey and W H. Eoff *Anal. Chem.* **27**, 1903 (1955)