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Note

High-performance liquid chromatography of thalicarpine, hernandaline, hernandalinol and dehydrothalicarpine

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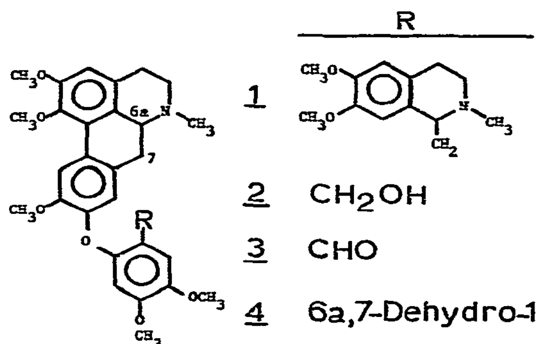
Thalicarpine (1) is a benzyltetrahydroisoquinoline–aporphine alkaloid which was first isolated from *Thalictrum*[1,2] and *Hernandia*[3] species, and its structure was proven by total synthesis[4]. Thalicarpine is of interest as an antitumor compound[5,6], and hernandalinol (2) was obtained as a microbial metabolite of thalicarpine, presumably by reduction of the intermediate hernandaline (3)[7]. Dehydrothalicarpine (4) is commonly formed by air oxidation when (1) is incubated in aqueous medium.



Further microbial and mammalian metabolism studies with thalicarpine would be greatly facilitated by the availability of a sensitive, simple and rapid analytical technique. This report describes the development of a high-performance liquid chromatographic (HPLC) system useful in the detection of compounds 1–4.

EXPERIMENTAL

Thalicarpine was obtained from the National Cancer Institute (Bethesda, Md., U.S.A.)[7]. Hernandalinol was produced by $NaBH_4$ reduction of hernandaline, a product of the $KMnO_4$ oxidation of thalicarpine[7]. Dehydrothalicarpine was prepared

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by palladium on carbon treatment of 1 according to the procedure of Cava *et al.*[8]. All compounds were fully characterized and gave single spots on thin-layer chromatograms.

HPLC experiments were performed using a Waters Assoc. (Milford, Mass., U.S.A.) ALC/GPC 202 instrument equipped with an M6000 solvent delivery system, a U6K universal injector, and a 254-nm differential UV detector. Alkaloids were best separated from one another on a Waters Assoc. μPorasil column (0.4 × 30 cm) with a solvent system of cyclohexane–chloroform–diethylamine (25:150:0.3). Nominal operating conditions employed were 1900 p.s.i. at a flow-rate of 2.4 ml/min. Samples of alkaloids and extracts were dissolved in chloroform.

Thin-layer chromatography (TLC) was performed on 0.25-mm thick layers of silica gel GF_{254} (Merck, Darmstadt, G.F.R.), and all plates were activated for 30 min at 120° before use. Solvent systems used in TLC analysis were (a) benzene–methanol–58% NH_4OH (80:30:0.1), and (b) acetone–100% ethanol (50:1). Visualization of developed TLC plates was accomplished with 254-nm UV light, and by spraying plates with Dragendorff's reagent, or with 2,4-dinitrophenylhydrazine reagent[7].

Urine specimens were collected from patients being treated with thalicarpine at an average dose of 1100 mg/m^2 of body area administered intravenously. The times of collecting of urines varied from 4 to 24 h following administration of the drug. Urines were pooled, and stored under toluene in the cold until required for analysis. All urine specimens were divided into portions which received one of the following treatments.

(A) *Untreated.* Samples of 50 ml of urine were adjusted to pH 8.0 with 1.0 *N* NaOH and were exhaustively extracted with four equal volumes of chloroform. The chloroform extracts were dried over anhydrous Na_2SO_4 before being concentrated to dryness. The dried extracts were redissolved in 1.0 ml of chloroform for use in HPLC and TLC analyses.

(B) *Glusulase treated.* Sufficient 2 *M* sodium acetate buffer was added to a volume of urine resulting in a final buffer concentration of 0.1 *M*. The urine specimens were adjusted to pH 5.0 exactly and were reacted with a combination β-glucuoronidase and sulfatase enzyme preparation (1000 Fishman units and 500 sulfatase units/ml of urine; Endo Labs., Richmond Hill, N.Y., U.S.A.) at 37° for 24 h. Incubation mixtures were then adjusted to pH 8.0 and extracted as described before. The efficiency of the enzyme preparation was determined by measuring the rate of hydrolysis of phenolphthalein-β-glucuronidase added to urine samples.

RESULTS AND DISCUSSION

Several types of columns and solvent systems were initially examined for their abilities to separate the alkaloids. Phenyl-Bondapak (Waters Assoc.) and μPorasil columns with acetonitrile–ethanol or chloroform–methanol mixtures were tried without success. Hernandaline and hernandalinol were well resolved with acetonitrile–0.1% ammonium carbonate (1:1) on a Phenyl-Bondapak column, while a 7:1 mixture of these solvents could be used to separate 1 and 2 from one another. None of these chromatographic systems could be used to resolve all four of the alkaloids.

Excellent separations were obtained with a μPorasil column using a cyclohexane–chloroform–diethyl amine (25:150:0.3) solvent system. All of the alkaloids

TABLE I

HPLC AND TLC PROPERTIES OF THALICARPINE, DEHYDROTHALICARPINE, HERNANDALINE AND HERNANDALINOL

Compound	*HPLC**		*TLC R_F values*	
	Retention volume (ml)	*Limits of detection (ng)*	*Benzene–methanol–NH_4OH (80:30:0.1)*	*Acetone–ethanol (50:1)*
Dehydrothalicarpine	5.4	40	0.7	0.33
Hernandaline	6.7	40	0.75	0.45
Thalicarpine	17.7	400	0.65	0.05
Hernandalinol	33.1	400	0.65	0.32

* Analyses were performed with a μPorasil column at 1900 p.s.i., 2.4 ml/min using cyclohexane–chloroform–diethylamine (25:150:0.3).

** Silica gel GF_{254} TLC plates, 0.25 mm, visualized with 254 nm light and with Dragendorff's reagent.

were well separated from one another within 15 min. Average retention volumes, and limits of detectability (signal to noise ratio of 2:1) of the alkaloids are presented in Table I, and a typical HPLC chromatogram is shown in Fig. 1. Although peak sharpness and resolution could be improved by the addition of more diethylamine to the solvent system, this would also require the use of a detector at higher wavelength (280 nm).

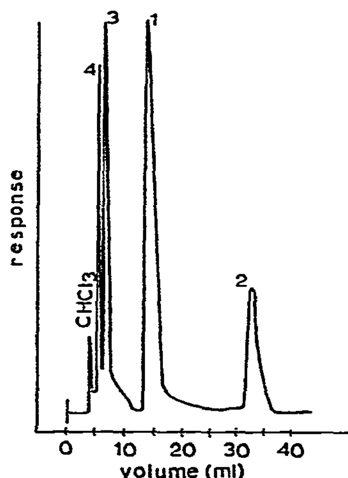

Fig. 1. HPLC Separation of thalicarpine (1), hernandalinol (2), hernandaline (3) and dehydrothalicarpine (4) achieved at 25° under the following conditions: cyclohexane–chloroform–diethyl amine (25:150:0.3) at a flow-rate of 2.3 ml/min, 1900 p.s.i. using a 254-nm UV detector.

The HPLC and TLC systems were used to detect thalicarpine and potential thalicarpine metabolites 2–4 in urine specimens collected from patients receiving 1 as a drug. The presence or absence of each compound was confirmed by spiking extracts with the known alkaloids, and by examining the extracts by TLC. A typical HPLC chromatogram of a chloroform extract of a urine specimen is shown in Fig.

2. All of the urine extracts examined contained thalicarpine and dehydrothalicarpine. Dehydrothalicarpine was probably present as an artifact formed during urine sample workup. The amounts of 4 ranged from 1.4–4% of the amounts of thalicarpine also found in samples. The total amount of thalicarpine recovered ranged from 0.25%–0.64% of the doses administered to the patients. These results are consistent with those of Palm *et al.*[9], who reported that little urinary or biliary excretion of thalicarpine occurs in test animals. Neither 3 nor 2 could be conclusively demonstrated to occur in any of the urine extracts.

Chromatographic systems described in this report will be useful in performing further microbial and mammalian metabolism studies with thalicarpine.

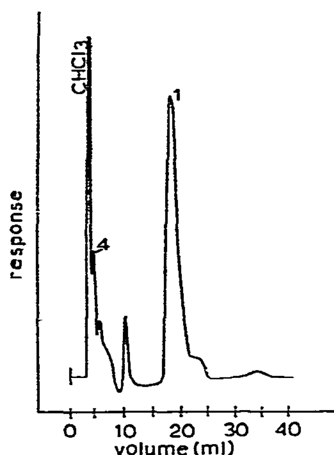

Fig. 2. HPLC analysis of a chloroform extract of urine performed on μPorasil using cyclohexane–chloroform–diethyl amine (25:150:0.3) at a flow-rate of 2.4 ml/min at 1900 p.s.i. using a 254-nm UV detector.

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REFERENCES

1 S. M. Kupchan, K. K. Chakravarti and N. Yodoyama, *J. Pharm. Sci.*, 52 (1963) 985.
2 P. L. Schiff, Jr. and R. W. Doskotch, *Lloydia* 33 (1970) 403.
3 M. Tomita, H. Furukawa, S. T. Lu and S. M. Kupchan, *Tetrahedron Lett.*, (1965) 4309.
4 S. M. Kupchan, A. J. Liepa, V. Kameswaran and K. Sempuka, *J. Amer. Chem. Soc.*, 95 (1973) 2995.
5 J. L. Hartwell and B. J. Abbott, *Advan. Pharm. Chemother.*, 7 (1968) 117.
6 N. M. Mollov, K. B. Duchevska, K. Silyanovska and S. Stoicev, *C. R. Acad. Sci.*, 21 (1968) 605; *C.A.*, 69 (1968) 58087.
7 T. Nabih, P. J. Davis, J. F. Caputo and J. P. Rosazza, *J. Med. Chem.*, 20 (1977) 914.
8 M. P. Cava, D. L. Edie and J. J. Saa, *J. Org. Chem.*, 40 (1975) 3602.
9 P. E. Palm, M. S. Mick, E. P. Arnold, D. W. Yesair and M. M. Callahan, *U.S. Nat. Techn. Inform. Serv., PB Report No. 201914*, 1971, 164 pp.; *C.A.* 76 (1972) 68093g.