Journal of Chromatography, 189 (1980) 79–85

CHROM. 12,461

CACTUS ALKALOIDS

XL. IDENTIFICATION OF MESCALINE AND OTHER β-PHENETHYLAMINES IN *PERESKIA*, *PERESKIOPSIS* AND *ISLAYA* BY USE OF FLUORESCAMINE CONJUGATES

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(First received May 15th, 1979; revised manuscript received October 11th, 1979)

SUMMARY

The combined methods of analytical thin-layer chromatography (TLC), high-performance TLC, and gas-liquid chromatography (GLC) have been used to identify specific alkaloids in twelve cactus species. TLC of fluorescamine conjugates, prepared from primary amines in the alkaloid extracts, served to substantiate results from the other chromatographic identifications. Traces of the hallucinogen, mescaline, were identified and quantitated by GLC in *Islaya minor* Backbg., *Pereskia corrugata* Cutak, *Pereskia tampicana* Weber, and *Pereskiopsis scandens* Br. and R., demonstrating that this alkaloid is distributed through all three cactus tribes.

INTRODUCTION

Fluorescamine, 4-phenylspiro[furan-2(3H),1′-phthalan]-3,3′dione (Fluram; Roche, Nutley, N.J., U.S.A.), was first synthesized and utilized by Weigele and coworkers[1–3] for the fluorimetric assay of amino acids and primary amines. It has been used as a chromatographic spray reagent to detect amino acids[4,5], peptides[6] and cactus alkaloids (both primary and secondary amines)[7]. Imai *et al.*[8] have detected amino acids, peptides and other primary amines by first conjugating these compounds with fluorescamine and then performing analytical thin-layer chromatography (TLC). Farid[9] has recently used high-performance liquid chromatography to assay the fluorescamine conjugate of aminocaproic acid.

Our previous isolation and identification of many alkaloids in the cactus tribes *Opuntieae* and *Cereeae* served as an impetus for phytochemical examination of the previously uninvestigated third cactus tribe, the *Pereskieae*. The problem of obtaining suitable quantities of these rare, leafy, tropical species for alkaloid isolations prompted our development of a simple microanalytical technique, employing TLC separation of fluorescamine conjugates, to substantiate results obtained by conventional TLC and gas–liquid chromatography (GLC) of the non-derivatized alkaloids.

EXPERIMENTAL

Plant material

Cuttings of the cacti (Table I) were obtained from authenticated plants growing at Grigsby Cactus Gardens, Vista, Calif., U.S.A.; Abbey Gardens, Carpinteria, Calif., U.S.A.; Fairchild Tropical Gardens, Miami, Fla., U.S.A.; Botanical Gardens, The University of Michigan, Ann Arbor, Mich., U.S.A.; and the Desert Botanical Gardens, Phoenix, Ariz., U.S.A. Reference photographs are on file. Specimens were frozen, freeze-dried and passed through a 2-mm screen in a Wiley mill. Samples weighing 20 g, or proportionally less, were extracted, as previously described[10], to yield extracts A (alkaloids), B (non-alkaloidal material) and C (water soluble alkaloids). Extracts A and C were pooled for each species and subjected to anion-exchange chromatography[11], to separate the phenolic and non-phenolic alkaloids.

Analytical TLC

Analytical TLC and, in some cases, radial high-performance TLC (HPTLC)[12] were utilized to determine the presence of alkaloids in the phenolic and non-phenolic alkaloid extracts. For analytical TLC, silica gel plates (Merck 60, F-254, 5 × 20 cm) were spotted with 5–15 μl of the extracts (in 1.0 ml of ethanol) or with reference alkaloids (1–5 μg) and chromatographed in solvent systems A, B, E, F and G as previously described[13]. A Camag 28600 Series U-chamber system with a digital solvent delivery system (flow-rate set at 1 μl/sec) was utilized for HPTLC[12], employing the same five solvent systems on special silica gel plates (Merck 60, F-254, for nano-TLC, 5 × 5 cm). Evidence for the presence of a specific alkaloid in the extracts was based on co-chromatography with the reference alkaloid in the five different solvent systems. Visualization was accomplished by spraying sequentially with fluorescamine, dansyl chloride and tetrazotized benzidine or iodoplatinate[7,11]. Fluorescamine conjugates were separated by TLC on the silica gel plates using ethyl ether–glacial acetic acid (19:1) and visualized under long wave UV light; for HPTLC of the conjugates ethyl acetate–methanol (17:2) and ethyl ether–ethanol (2:1) were also found to be useful.

Gas–liquid chromatography

Traces of mescaline (3,4,5-trimethoxy-β-phenethylamine) and 3,4-dimethoxy-β-phenethylamine were detected by TLC and HPTLC in extracts of several of the cactus species screened (Table I). GLC on a Varian Series 2700 gas chromatograph equipped with a flame-ionization detector and employing a 1.5 m × 0.32 cm 1.5% OV-101 (stainless steel) column (chromosorb G, 100–200 mesh) was performed on the non-phenolic alkaloid fractions to quantitate the amount of mescaline (Table II). The oven temperature was set at 150°, and the nitrogen carrier gas flow was 30 ml/min. Concentrations of alkaloids were estimated by comparing peak areas of reference standards to those from the non-phenolic fractions. Nearly identical retention times and peak height enhancement with references served to substantiate the alkaloid identities in the extracts.

Preparation of fluorescamine conjugates

Fluorescamine conjugates of eleven β-phenethylamine (β-PEA) alkaloid reference standards (mescaline; 3,4-dimethoxy-β-PEA; 2,5-dimethoxy-β-PEA; β-hydroxy-

TABLE I

ALKALOID SCREENING RESULTS AND METHODS OF ANALYSIS

*Species screened and source**	*Alkaloid identified*	*Method of analysis*			
		*TLC***	*TLC of fluorescamine conjugate*	*HPTLC****	*GLC****
Islaya minor Backbg. (T.) from Grigsby	3,4-Dimethoxy-β-phenethylamine	×	×	×	×
	Mescaline	×	×	×	×
	β-Phenethylamine	×	×		
	Corypalline	×			
	Hordenine	×			
	3-Methoxytyramine	×	×		
	N-Methyltyramine	×			
	Tyramine	×	×		
	Pellotine	×			
Melocactus delessertianus Lem. from Abbey	Tyramine	×		×	
M. maxonii (Rose) Gürke from Abbey	Tyramine	×		×	
Pereskia aculeata Miller from Fairchild	Tyramine	×	×		
P. autumnalis (Eichlam) Rose from Univ. of Michigan	β-Phenethylamine	×	×		
	Tyramine	×	×		
P. corrugata Cutak from Fairchild	3,4-Dimethoxy-β-phenethylamine	×	×	×	×
	Mescaline	×	×	×	×
	3-Methoxytyramine	×	×		
	Tyramine	×	×		
P. cubensis Br. and R. from Univ. of Michigan	Tyramine	×	×		
P. godseffiana (Sand.) Knuth from Univ. of Michigan	Tyramine	×	×		
P. grandifolia Haw. from Univ. of Michigan	p-Methoxy-β-hydroxy-β-phenethylamine	×	×		
	3-Methoxytyramine	×	×		
	Tyramine	×	×		
P. grandiflora Hort. from Univ. of Michigan and Desert Bot. Gard.	β-Hydroxymescaline	×	×		
	Tyramine	×	×		
P. pittiache (Karwinsky) Br. and R. from Abbey	β-Phenethylamine	×	×		
	Tyramine	×	×		
P. tampicana Web. from Univ. of Michigan	3,4-Dimethoxy-β-phenethylamine	×	×	×	×
	Mescaline	×	×	×	×
	p-Methoxy-β-hydroxy-β-phenethylamine	×	×	×	
	β-Phenethylamine	×	×		
	Tyramine	×	×		

(Continued on p. 82)

TABLE I *(continued)*

*Species screened and source**	*Alkaloid identified*	*Method of analysis*			
		*TLC***	*TLC of fluorescamine conjugate*	*HPTLC****	*GLC****
Pereskiopsis chapistle (Web.) Br. and R. from Abbey	*p*-Methoxy-β-hydroxy-β-phenethylamine	×	×		
	β-Phenethylamine	×	×		
	3-Methoxytyramine	×	×		
	Tyramine	×	×		
P. scandens Br. and R. from Abbey	3,4-Dimethoxy-β-phenethylamine	×	×	×	×
	Mescaline	×	×	×	×
	Tyramine	×	×		

* For source explanation see *Plant material* section.

** Co-chromatography with reference materials in five systems using solvents and visualization aids as previously described[7,13].

*** HPTLC and GLC were used primarily to provide additional evidence for the identifications of 3,4-dimethoxy-β-phenethylamine and mescaline.

TABLE II

GLC DATA AND MESCALINE QUANTITATION

3,4 dm PEA = 3,4-Dimethoxy-β-phenethylamine. All injection volumes 10 μl; ref = reference alkaloid; np = non-phenolic alkaloid extract.

Plant/dry weight of sample	*Peak**	*Retention time (sec)*	*Calculated amount alkaloid injected (μg)*	*Total mescaline in sample (μg)*	*Mescaline in plant (%)*
I. minor (20 g)	Mescaline (ref)	337	20	—	—
	3,4 dm PEA (ref)	200	20	—	—
	Mescaline (np)	337	17	340	0.0017
	3,4 dm PEA (np)	200	38	760	0.0038
P. corrugata (20 g)	Mescaline (ref)	400	30	—	—
	3,4 dm PEA (ref)	200	30	—	—
	Mescaline (np)	395	5	100	0.0005
	3,4 dm PEA (np)	197	9	180	0.0009
P. tamaulipana (12 g)	Mescaline (ref)	337	30	—	—
	3,4 dm PEA (ref)	195	30	—	—
	Mescaline (np)	330	8	160	0.0013
	3,4 dm PEA (np)	190	15	300	0.0025
P. scandens (20 g)	Mescaline (ref)	4[illegible]3	10	—	—
	3,4 dm PEA (ref)	[illegible]16	10	—	—
	Mescaline (np)	395	2.2	44	0.0022
	3,4 dm PEA (np)	211	2.9	58	0.0029

mescaline; 3,5-dihydroxy-4-methoxyamphetamine; 3-methoxy-4-hydroxy-β-PEA; normetanephrine; 4-methoxy-β-PEA; β-PEA; 4-methoxy-β-hydroxy-β-PEA; and tyramine) were prepared by dissolving 1 mg of primary amine hydrochloride in 0.4 ml of distilled water and 2.5 mg of sodium carbonate; 3 mg of fluorescamine was dissolved in 0.2 ml of acetone and added to the primary amine alkaloid solution.

The reaction mixture was immediately shaken on a Vortex mixer for 15 sec, and 1.0 ml of distilled water followed by 1.0 ml of ethyl acetate was added. The aqueous layer was extracted, and two additional 1.0 ml portions of ethyl acetate were used for subsequent extractions. The combined ethyl acetate extracts were reduced to a small volume under rotary vacuum and analyzed immediately by TLC and HPTLC. Although secondary amines produce UV quenching when sprayed with fluorescamine[7], no conjugates were detected by using the above procedure with five different secondary amines (such as N-methylmescaline and N-methyltyramine). Samples of several alkaloid–fluorescamine conjugates were prepared in the above way and submitted for mass spectrometry (MS) analyses to confirm the expected molecular weights (Table III).

TABLE III

MASS SPECTRUM AND TLC RESULTS OF ALKALOID–FLUORESCAMINE CONJUGATES

Fluorescamine conjugate	R_F* *in ethyl ether–glacial acetic acid (19:1)*	*Spectrum type*	*Observed parent ion (m/e)*	*Calculated MW*	
				Acid conjugate	*Lactone*
2,5-Dimethoxy-β-phenethylamine	0.68	—	—	—	—
3,4-Dimethoxy-β-phenethylamine	0.44	CI–MS	442	459	441
β-Hydroxymescaline	0.35	—	—	—	—
3-Methoxy-4-hydroxy-β-phenethylamine	0.51	—	—	—	—
4-Methoxy-β-phenethylamine	0.69	CI–MS EI–MS	412 411	429	411
Mescaline	0.41	CI–MS EI–MS	472 471	489	471
β-Phenethylamine	0.73	CI–MS EI–MS	382 381	399	381
Tyramine	0.60	—	—	—	—

* Average of four determinations.

A larger 270-μmol portion of fluorescamine was reacted with an equivalent amount of mescaline hydrochloride to prepare enough mescaline conjugate for closer characterization (Fig. 1). Upon volume reduction of the ethyl acetate, yellow crystals of the conjugate were obtained; however, these required reversed-phase low-pressure chromatography on a 0.6 × 30 cm column of C_{18}-silica gel (methanol–water–acetonitrile, 5:3:1) for purification: m.p. 120–124° (from ethyl acetate); 54% yield; UV $\lambda_{max.}^{methanol}$ (ε) 275 nm (18,200) and 390 nm (6450); IR $\nu_{max.}^{KBr}$ 3300–2500 cm^{-1} (broad),

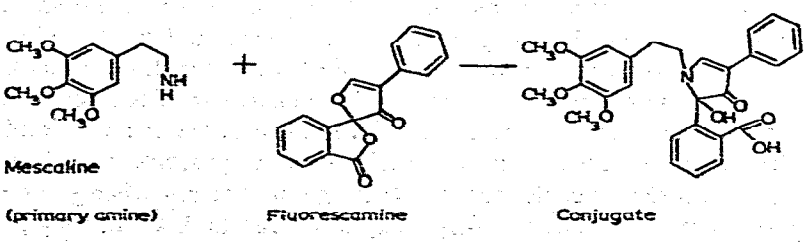

Fig. 1. The fluorescamine conjugate of mescaline.

1680 cm^{-1} (sh), and 1230 (sh); chemical ionization mass spectrometry (CI-MS) *m/e* 472 ($M+H^+$ of the lactone); electron impact (EI)-MS exact mass 471.167 (calc. 471.168 for the lactone) and low resolution *m/e* 181 (M-190 from β-cleavage of mescaline); ^1H nuclear magnetic resonance (NMR) (80 MHz, $CDCl_3$) δ 2.78 (m, 2H, β-CH_2 of mescaline), δ 3.21–3.41 (m, 2H, α-CH_2 of mescaline), δ 3.52, 3.70 and 3.75 (bs, s, s, 3 mescaline ring CH_3O's), δ 6.23 (bs, 2H, *o*-protons of mescaline), the remainder of the spectrum consisted of overlapping and ill-defined peaks integrating to account for the remaining ten conjugated and aromatic protons.

The conjugate preparation procedure was extended to the phenolic and non-phenolic cactus alkaloid fractions. The alkaloid extract was condensed to dryness and 0.5–1.0 ml of 1 *N* sodium carbonate was added; the remainder of the preparation was identical to that described above for the reference alkaloids with the exception that five, rather than three, ethyl acetate extractions were made. The conjugated extracts were then subjected to TLC (Table I).

RESULTS AND DISCUSSION

Chromatographic results of the alkaloid screenings for many of the cactus species investigated indicated the presence of a number of phenolic and non-phenolic alkaloids. The primary amines identified by conventional TLC and HPTLC had these identifications confirmed by TLC of their fluorescamine conjugates. Table I lists the alkaloids identified in each species and the methods of analysis used. In each case the TLC identification of primary amines in the extracts was confirmed by TLC of their fluorescamine conjugates; HPTLC and GLC were used primarily to provide additional evidence for the identifications of 3,4-dimethoxy-β-phenethylamine and mescaline. Table II summarizes the GLC data and includes estimates of mescaline concentrations.

Mass spectral data for the fluorescamine conjugates of representative alkaloids confirmed that the conjugates are converted to their respective lactones when heated[1], *e.g.*, in the MS probe. Table III shows the MS results and R_F values of the alkaloid–fluorescamine conjugates.

These results demonstrate a new method for identifying small quantities (100–500 pg, refs. 5, 7) of primary β-phenethylamines. The specificity of fluorescamine for primary amines makes it superior to dansyl chloride which will also form conjugates with secondary amines, imidazoles and phenols. The method should be useful for alkaloid identifications in cases where large quantities of plant material cannot be obtained for actual alkaloid isolation and where an additional method is needed to substantiate TLC and GLC results that employ the underivatized alkaloids. A disadvantage of the method is the relative instability of the fluorescent conjugates which are both heat and acid labile[1,14]. Conjugates chromatographed in the ethyl ether–acetic acid (19:1) system lost fluorescence within one hour after development.

Tyramine was found in the phenolic fraction of all the cactus species screened; thus, the presence of this alkaloid seems to be a common feature of the *Pereskia* and their relatives. Since the other β-phenethylamines likely involve tyramine as a biogenetic precursor[15], the widespread occurrence of tyramine is not surprising. The detections of β-phenethylamine, corypalline, β-hydroxymescaline and 4-methoxy-β-hydroxy-β-phenethylamine are either novel or unusual in the cactus family, and

isolation studies are needed to confirm these chromatographic identifications.

Traces of mescaline and 3,4-dimethoxy-β-phenethylamine were identified in *I. minor*, *Pereskia corrugata*, *P. tampicana* and *Pereskiopsis scandens*. An individual would have to ingest kilograms of these plant materials to receive a hallucinogenic dose of mescaline, so these species pose no drug abuse problems. However, the discovery of this alkaloid in these cacti is still interesting. Its presence in *Pereskiopsis* (tribe *Opuntieae*), *Islaya* (tribe *Cereeae*) and *Pereskia* (tribe *Pereskieae*) indicates that the genetic ability to produce mescaline is common to all three tribes of the cactus family. The capacity to accumulate large quantities of mescaline, however, has yet to be demonstrated among the *Opuntieae* and the *Pereskieae*[16,17].

ACKNOWLEDGEMENTS

We thank Dr. I. Jardine and Ms. L. Sellers for help with the MS data, Mr. J. Kozlowski for providing the NMR spectra, Dr. M. Cushman and Mr. H. Burliss for the use of the GLC equipment, and Dr. J. Hembree for discussions on the C_{18} chromatography. Support was provided, in part, by a grant from the Cactus and Succulent Society of America and Biomedical Research Support Grant, RR-05586, from the National Institutes of Health. Our gratitude for supplying plant material is extended to Mr. D. Grigsby, Ms. M. Collins, Dr. G. Hatfield and Mr. R. Engard.

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