

THE ISOLATION AND IDENTIFICATION OF THREE ALKALOIDS FROM *ACACIA BERLANDIERI**

HERMAN R. ADAMS† and BENNIE J. CAMP
Department of Biochemistry and Nutrition, Texas A and M University,
College Station, Texas, U.S.A.

(Accepted for publication 5 May 1966)

Abstract—A method is described for the isolation and identification of three β -phenylethylamine alkaloids from *Acacia berlandieri* (guajillo). The isolated amines were identified by means of paper, thin-layer, and gas-chromatography as tyramine, N-methyltyramine and hordenine.

INTRODUCTION

Acacia berlandieri (guajillo) is a member of the family *Leguminosae*. It is a deciduous shrub 3 to 14 ft in height with pinnate leaves and few or no prickles. This plant grows in its greatest density in the Edwards Plateau of Texas and Northern Mexico.

During periods of extended drought, sheep and goats may ingest the plant exclusively for periods of 6 to 12 months before developing a locomotor ataxia of the hindquarters. The condition is colloquially called "guajillo wobbles" or "limberleg".

CAMP and LYMAN [1] isolated the sympathomimetic amine, N-methyl- β -phenylethylamine, from guajillo. Further investigation revealed the presence of alkaloids other than N-methyl- β -phenylethylamine. The present study was initiated to elucidate the chemical nature of these unidentified alkaloids.

MATERIALS AND METHODS

Extraction procedure

Thirty-five pounds of ground leaves from guajillo were extracted with 70% ethanol adjusted to a pH of 2 with concentrated HCl. The extraction procedure was repeated 3 times, and a final extraction was made using 33% ethanol.

The combined liquid extracts were concentrated under reduced pressure to a volume of 5 l., then diluted to 10 l. with water. A plant pigment precipitated upon the addition of water and was removed by filtration. The filtrate was concentrated under reduced pressure to 4 l. A saturated solution of sodium hydroxide was added to the filtrate and a yellow precipitate formed which was removed by centrifugation.

The basic supernatant solution was further clarified by the addition of lead acetate and lead subacetate [2], and the heavy precipitate was removed by centrifugation. Concentrated HCl was added to the solution to precipitate the excess lead as lead chloride, and

*Supported in part by a grant from the Herman Frasch Foundation, New York, N.Y. Contribution of the Texas Agricultural Experiment Station.

†Based in part upon a thesis presented to the Graduate School of Texas A and M University by Herman R. Adams in partial fulfillment of the requirements for the degree of Master of Science.

the precipitate was removed by filtration. The acidic filtrate was adjusted to pH 8 with sodium carbonate, and the phenols were extracted into chloroform. The chloroform extract was extracted repeatedly with an aqueous sodium hydroxide solution to separate the phenolic amines from the non-phenolic amines.

The alkaline aqueous phase was made acidic with concentrated HCl and then alkaline once again with sodium carbonate. Then the phenolic amines were extracted into chloroform or ether.

Chromatographic techniques

Paper chromatography. A sample of the crude plant extract was chromatographed two-dimensionally on Whatman No. 1 paper using the following solvent systems: (1) *n*-butanol saturated with water : acetic acid (10:1); (2) *n*-butanol : ethanol : ammonium hydroxide (90:10:1). Ascending chromatography was used to develop the paper strips suspended by glass rods in sealed chromatography chambers.

The hydrochloride salts of the isolated amines and reference amines were dissolved in chloroform or methanol and spotted on Whatman No. 1 paper. The paper strips were developed ascendingly in the solvent systems: (1) *sec*-butanol : water : formic acid : acetic acid (30:4:3:1); (2) *iso*-propanol : acetic acid : water (15:3:2).

Thin layer chromatography. Silica Gel G (E. Merck) plates of 0.25 mm thickness were used to chromatograph the reference compounds and the plant amines. The plates were activated and stored at 100° in an electric oven. Top and side boundaries were cut in the plate material with a pencil to facilitate smoother solvent fronts and to stop the solvent fronts at a desired height.

The plant amines and reference amines were applied as the hydrochloride salts on thin layer plates. The solvent systems that gave satisfactory results were: (1) *sec*-butanol : water : glacial acetic acid (15:3:2), (2) *n*-butanol : glacial acetic acid : water (12:3:5), (3) *iso*-propanol : glacial acetic acid : water (15:3:2), and (4) *n*-butanol saturated with water.

Gas-liquid chromatography. A Warner-Chilcott Company Model 604 gas chromatograph, equipped with a flame ionization detector, and a 10/1 output splitter for preparative work, was used to chromatograph the plant amines and reference samples. The column was prepared according to the method of BROCHMANN *et al.* [3]. The column consisted of chromosorb W (Johns-Manville) 60-80 mesh, which was deactivated with hexamethyldisilazane [4] and coated with 30 per cent SE-30 [5]. The column material was packed in stainless steel tubing (3/8 in. × 6 ft). The column was operated isothermally at 202° and the output splitter temperature was maintained at 268°. The carrier gas (nitrogen) flow rate was 296 ml per min. All of the samples were chromatographed as the free base in methanol. Collections were made in U shaped glass tubes at room temperature.

Chromogenic reagents

A 0.1% solution of ninhydrin in 95 % ethanol was utilized to test for primary and secondary amines. Ehrlich's reagent [6] was used to test for the presence of the indole group. Dragendorff's reagent [7] was used because it was found to be specific for certain of the alkaloids present. Phenolic compounds were detected with Pauly's [8] reagent.

A spray reagent for secondary aliphatic amines [9] consisted of a 1% nitroprusside solution to which 10% by volume of acetaldehyde was added. The chromatogram was

sprayed with this reagent, and then it was sprayed with a 2 % solution of sodium carbonate and heated at 100° for 5 min. A blue color was positive for secondary aliphatic amines.

The sodium picrate reagent [10] for hydrocyanic acid and cyanides was prepared by combining 1 ml of a saturated solution of picric acid with 3 ml of 10% sodium hydroxide.

Reference samples

Tyramine, 3-hydroxytyramine, and hordenine were obtained commercially. N-methyl- β -phenylethylamine was prepared by the method of CAMP and MOORE [11].

N-methyltyramine was synthesized by the procedure of KIRKWOOD and MARION [12], starting with the intermediate β -(4-methoxyphenyl)-ethylamine. The method was altered in that the N-methyltyramine was precipitated from anhydrous ether as the hydrochloride salt by using alcoholic hydrogen chloride.

RESULTS AND DISCUSSION

A sample of the crude acidic plant extract was chromatographed two-dimensionally on a series of paper chromatograms. A concentrated spot that was positive with Dragendorff's reagent and ninhydrin was identified by comparative chromatography as N-methyl- β -phenylethylamine, as reported by CAMP and LYMAN [1]. Two unidentified spots of lower concentration trailed behind the N-methyl- β -phenylethylamine and gave positive ninhydrin and Pauly's tests but negative Dragendorff's, Ehrlich's and sodium picrate tests. The positive ninhydrin and Pauly's test indicated that the compounds were phenolic amines.

When the chloroform extract containing the phenolic compounds was chromatographed on thin layer plates, four phenolic compounds were detected with Pauly's reagent. Two of the compounds gave a positive ninhydrin test, a third gave negative ninhydrin test but a positive Dragendorff's test. The fourth phenol was negative with ninhydrin and Dragendorff's tests.

The alkaloids were extracted from the plant material in minute quantities, and comparative chromatography was employed in an attempt to identify some or all of the compounds. The reference compounds were selected from the β -phenylethylamine series of alkaloids [13], because N-methyl- β -phenylethylamine had previously been isolated from guajillo [1]. The phenolic compounds in the β -phenylethylamine series that could have been isolated by the extraction procedures and also given positive Pauly's tests were tyramine, N-methyltyramine and hordenine. 3-Hydroxytyramine gave a negative Pauly's test and was eliminated as one of the phenolic amines.

Reference samples of tyramine and N-methyltyramine were chromatographed on paper along with the chloroform extract containing the phenolic alkaloids of guajillo. When sprayed with Pauly's reagent, two of the plant amines had R_f values corresponding to tyramine and N-methyltyramine. Tyramine gave a characteristic purple color and N-methyltyramine gave a grey color when tested with ninhydrin. The plant amines of comparable R_f values gave identical colors. One of the phenolic compounds corresponding to N-methyltyramine gave a positive test for a secondary amine when treated with the nitroprusside reagent (Table 1).

Gas-liquid chromatography was employed both as an analytical tool and as a preparative method of purification. A comparison of retention values between standards (Fig. 1) and a chloroform extract containing both phenolic and non-phenolic amines (Fig. 2) demonstrated the plant amines to be N-methyl- β -phenylethylamine, tyramine and N-methyltyramine. It was impossible to detect hordenine in the presence of N-methyltyramine on

TABLE 1. PAPER CHROMATOGRAPHY OF AMINES ISOLATED FROM GUAJILLO

Sample*	Solvent system†		Spray reagent		
	(1)	(2)	Pauly's	Nitroprusside and acetaldehyde	Ninhydrin
	<i>R_f</i>				
Tyramine	0.56	0.73	Red	Negative	Purple
N-methyl-tyramine	0.64	0.76	Red	Blue	Grey
Plant amines	0.58	0.70	Red	Negative	Purple
	0.64	0.76	Red	Blue	Grey

*Sample was spotted as hydrochloride salt.

†Solvent systems: (1) *sec.*-BuOH: H₂O: HCOOH: HOAc (75:10:7.5:2.5)

(2) *iso*-PrOH: HOAc: H₂O (75:15:10).

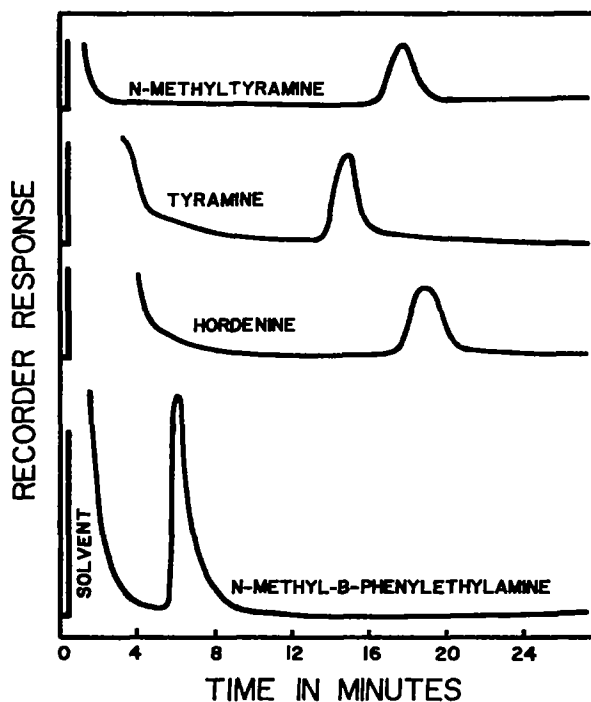


FIG. 1. GAS-LIQUID CHROMATOGRAPHY OF REFERENCE SAMPLES ON A CHROMOSORB W COLUMN COATED WITH 30% SE-30. Vaporizer temperature was 268° and column temperature was 202°.

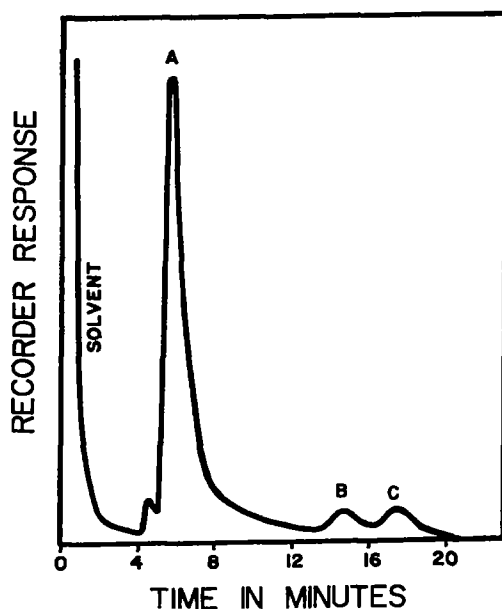


FIG. 2. GAS-LIQUID CHROMATOGRAPHY OF PLANT AMINES ON A CHROMOSORB W COLUMN COATED WITH 30% SE-30.

Vaporizer temperature was 268° and column temperature was 202°. The major peaks are (A) *N*-methyl- β -phenylethylamine, (B) tyramine, (C) *N*-methyltyramine and hordenine.

the analytical column because of the minute quantity of hordenine present in the plant extract.

A chloroform extract containing the phenolic alkaloids of guajillo was subjected to preparative gas-liquid chromatography and the peaks corresponding to tyramine, *N*-methyltyramine and hordenine were collected. The phenolic plant amines collected from gas-liquid chromatography were spotted with standards on thin layer plates. The chromatograms were developed in 4 solvent systems and the phenolic compounds were detected with Pauly's reagent. In each solvent system, the R_f values for the three phenolic amines isolated from guajillo were comparable to the R_f values obtained with reference samples of tyramine, *N*-methyltyramine and hordenine (Table 2).

The extraction procedure and chromogenic reagents classified the isolated compounds as phenolic amines. The retention values of gas-liquid chromatography for reference samples and the isolated plant amines were comparable. From these findings it was concluded that the isolated plant alkaloids were tyramine, *N*-methyltyramine and hordenine.

SUMMARY

(1) A procedure is described for the isolation of three phenolic amines from *Acacia berlandieri* by means of solvent extraction and gas chromatography.

(2) The chromogenic reagents and chromatography techniques used to identify and isolate the amines are presented.

(3) The plant amines were found to be in the β -phenylethylamine series of alkaloids. Comparative paper, thin layer, and gas-liquid chromatography identified the isolated phenolic amines as tyramine, *N*-methyltyramine, and hordenine.

TABLE 2. THIN LAYER CHROMATOGRAPHY OF GLC PURIFIED PLANT AMINES*

Sample†	Solvent systems‡				Spray reagent
	(1)	(2)	<i>R_f</i> (3)	(4)	Pauly's
GLC purified plant amines	0.10	0.42	0.14	0.06	Red
	0.39	0.56	0.51	0.27	Red
	0.28	0.51	0.31	0.17	Red
Hordenine	0.10	0.42	0.14	0.06	Red
Tyramine	0.39	0.56	0.51	0.27	Red
N-methyltyramine	0.28	0.51	0.31	0.17	Red

*0.25 mm Silica Gel G Plates.

†Samples were spotted as hydrochloride salt.

‡Solvent systems: (1) *sec.*-BuOH: HOAc: H₂O (75:15:10).

(2) *n*-BuOH: HOAc: H₂O (60:15:25)

(3) *iso*-PrOH: HOAc: H₂O (75:15:10)

(4) *n*-BuOH saturated with H₂O.

REFERENCES

- [1] CAMP, B. J. and LYMAN, C. M., The isolation of N-methyl- β -phenylethylamine from *Acacia berlandieri*. *J. Am. pharm. Ass., Sci. Ed.*, **25**, 719, 1956.
- [2] WHITE, E. P., Alkaloids of the *leguminosae*. *N.Z. J. Sci. Technol.* (B), **25**, 137, 1944.
- [3] BROCHMANN, E., HANSEN, A. and SVENDSEN, B., Separation and identification of sympathomimetic amines by gas-liquid chromatography. *J. pharm. Sci.*, **51**, 938, 1962.
- [4] BOHEMEN, J., LANGER, S. H., FERRUT, R. H. and PURNELL, J. J., A study of the adsorptive properties of firebrick in relation to its properties as a solid support in gas-liquid chromatography. *J. chem. Soc.*, 2440, 1960.
- [5] HORNING, E. C., MOSCATELLY, E. A. and SWEELY, C. C., Polyester liquid phases in gas-liquid chromatography. *Chem. Ind.*, 751, 1959.
- [6] BLOCK, R. J., DURRUM, E. L. and GUNTER, Z., *A Manual of Paper Chromatography and Paper Electrophoresis*. 2nd ed., Academic Press, New York, p. 137, 1958.
- [7] BLOCK, R. J., DURRUM, E. L. and GUNTER, Z., *A Manual of Paper Chromatography and Paper Electrophoresis*. 2nd ed., Academic Press, New York, p. 361, 1958.
- [8] BLOCK, R. J., DURRUM, E. L. and GUNTER, Z., *A Manual of Paper Chromatography and Paper Electrophoresis*. 2nd ed., Academic Press, New York, p. 132, 1958.
- [9] FEIGL, F., *Spot Tests*, Vol. II, Elsevier, New York, p. 189, 1954.
- [10] SIMMONS, J. S. and GENTZKOW, C. J., *Medical and Public Health Laboratory Methods*. Lea and Febiger, Philadelphia, p. 532, 1955.
- [11] CAMP, B. J. and MOORE, J. M., A quantitative method for the alkaloid of *Acacia berlandieri*. *J. Am. pharm. Ass., Sci. Ed.*, **49**, 158, 1960.
- [12] KIRKWOOD, S. and MARION, L., The biogenesis of alkaloids, I.—The isolation of N-methyltyramine from barley. *J. Am. chem. Soc.*, **72**, 2522, 1950.
- [13] MANSKE, R. H. F. and HOLMES, H. L., *The Alkaloids*, Vol. III, Academic Press, New York, p. 313, 1953.