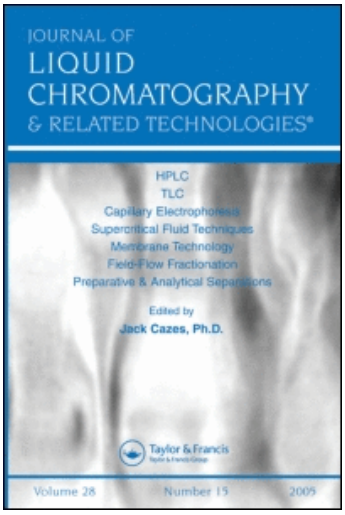

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Application of Thin Layer, Ion Exchange and High Performance Liquid Chromatography to Separate Pharmacologically Active Components of an African Arrow Poison of Plant Origin

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APPLICATION OF THIN LAYER, ION EXCHANGE AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO SEPARATE PHARMACOLOGICALLY ACTIVE COMPONENTS OF AN AFRICAN ARROW POISON OF PLANT ORIGIN

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ABSTRACT

We have applied thin layer chromatography (TLC), ion exchange chromatography (IEC) and, high performance liquid chromatography (HPLC) to separate and identify the pharmacologically active components of an african arrow poison of plant origin. On the basis of Rf values obtained from TLC, the active components of the toxin are unlike d-tubocurarine, atropine and, scopolamine. Dowex 1 x 2 IEC of 630 mg of crude toxin on a 2.5" x 33" column with step gradient elution (NaCl, 0.1 - 1.0M and NaOH, 0.1M) led to the identification of three distinct peaks. When the components of each of the three peaks were subjected to HPLC, the results confirmed the homogeneity of each of the isolated peaks except for the third peak which was a doublet.

INTRODUCTION

The people of Africa who prepared this Arrow Poison are primarily agricultural people. However, hunting and fishing are secondary occupations which have provided

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ways and means of getting animal protein to enrich their diets. These people use bows and poisoned arrows to hunt antelopes, deer, gazelle and many game which abound in the African Savanna. The poison is heat extracted from plants and the identification and taxonomic classification of the plants is in progress and will be reported elsewhere.

The use of arrow poison in Africa is widespread although there is very little information in the published literature about african arrow poisons. Results of a casual survey indicate that right across Africa, the arrow poisons used are prepared from different sources. The lethality of the poison we have investigated appears to be due to muscle paralysis and cardio-respiratory collapse (1, 2).

The potential of identifying and characterizing the active components of the arrow poison is great. For example, the muscle relaxing component would be useful in surgical operations, electroconvulsive therapy, and also in other therapeutic modalities requiring muscle relaxation. Therefore, the purpose of this investigation was to separate toxicological and pharmacological active components of an african arrow poison of plant origin using TLC, IEC and HPLC.

Our data from thin layer chromatography indicated that the toxin, on the basis of R_f values, is different

from d-tubocurarine, atropine, scopolamine and other common alkaloids of medicinal values. The results of IEC led to the resolution of crude toxin into three distinct peaks. Subjecting each of the peaks to HPLC confirmed each of the peaks to be homogeneous, except for the third peak which was a doublet.

MATERIALS AND METHODS

The arrow poison was obtained from Kenya where the natives heat extract the poison from plants. The taxonomic identification of the plants from which the poison is extracted and the methods of preparation of the toxin will be published elsewhere. A 630 mg portion of the poison was weighed and dissolved in deionized water and the resulting solution had a pH of 7.0 with a conductivity of 200 megaohms. Other chemicals used in this investigation were obtained from the following sources: d-tubocurarine, scopolamine, atropine, sodium chloride and sodium hydroxide (Sigma Chemicals, St. Louis, Mo.); Dowex 1 x 2 ion exchange resins (Biorad Labs Richmond, CA), all the solvents for TLC (Curtin Matheson, Dallas, TX), the reagents for HPLC (Burdick and Jackson Laboratories, Inc. Muskegon, Michigan) and, TLC plates coated with silica gel 60F-254 (EM Laboratories, Inc. Elmsford, N.Y. 10523).

Thin Layer Chromatography (TLC)

Portions of the solution of the arrow poison and similar solutions of atropine, scopolamine and d-tubocurarine were spotted on silica gel plates were then set in different solvent systems to facilitate movement. The solvent systems used were marked 1, 2, 3, 4, and 5 and were of the following composition: ethyl acetate, 2-propanol, 25% ammonia (45:35:5); chloroform, cyclohexane, diethylamine (3:6:1); butanol, water, acetic acid (12:3:1); ethylacetate, 2-propanol, 25% ammonia (9:7:2) and; methanol ammonia (2.0M) and ammonia nitrate (1.0M) respectively. The solvent front was allowed to migrate approximately 95% of the distance possible before removing each plate. The migration of each other components was identified with the use of UV light. The results of thin layer chromatography was expressed as R_f values.

Ion Exchange Chromatography (IEC)

A 170 ml. solution of the arrow poison containing 630 mg. of the original poison was charged on to a 1200 ml. Dowex 1 x 2 column in the formate form. The column was then eluted with the following eluants in sequence: 0.1M NaOH - 0.1 M NaCl for 10 column volumes; 0.2 MNaCl 0.1 MNaOH for 7.2 column volumes; 0.4M NaCl - 0.1 MNaOH for 5.3 column volumes and; 1.0M NaOH for 14 column volumes. Ten ml. fractions were collected and the absorb-

ance at 260 nm was determined on a spectrophotometer (Beckman DB). The results were plotted as absorbancy against fraction number.

High Performance Liquid Chromatography

Each of the fractions under the peaks separated by IEC was evaporated and redissolved in minimum amount of double deionized water and filtered through a millipore filter (pore size, 0.45 um) before subjecting the solution to HPLC. A 20 ul aliquot of each solution was separated on a Perkin-Elmer Series 3 liquid chromatograph (Perkin - Elmer, Norwalk, Connecticut) equipped with a deuterium power supply, a digital programmer, an optical unit for detection of UV absorption at 260 nm, a stainless steel column (25 x 0.26 cm) packed with Silica - A (HC-ODS-SIL-S, Lot 34) and model 023 recorder. Adsorbed compounds were eluted using 95% (v/v) acetonitrile as the solvent flow rate was maintained at 1.0 ml/min. With a pressure drop of 1800 psig at ambient temperature.

RESULTS AND DISCUSSION

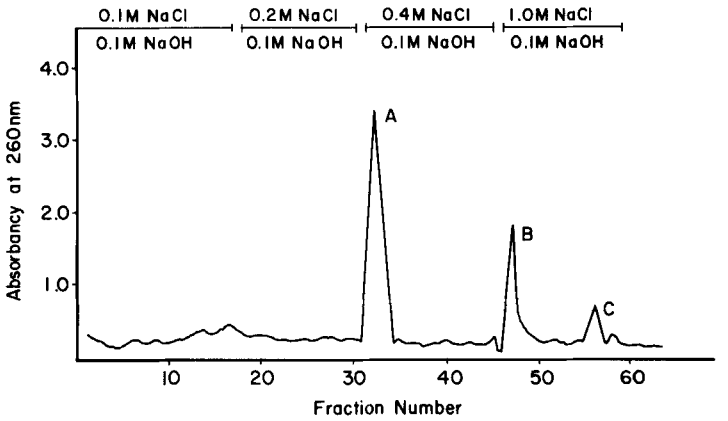
The results of TLC on silica gel are presented in Table 1. Of the different solvent systems used, the components of the arrow poison appear to either have not moved or only moved in solvent system 4 which has composed of ethylacetate, 2-propanol, and 25% ammonia (9:7:2).

TABLE 1

Results of Thin Layer Chromatography on Silica Gel. The spots were one application per spot.

Solvent System*	Agents spotted on silica gel plates and their respective R_f values.			
	Atropine	Arrow Poison	d-Tubocurarine	Scopolamine
1	0.368	0	0	0
2	0.330	0	0.71	0.60
3	0.720	0	0	0
4	0	0.90	0.57	0.60
5	0.06	0	0.06	0.06

*The solvent systems marked 1,2,3,4 and 5 are of the following composition Ethyl acetate, 2-proponol, 25% ammonia (45:35:5); chloroform, cyclohexane diethylamine (30:60:10); butanol, water, acetic acid (60:15:5); ethylacetate, 2-proponol, 25% ammonia (45:35:10); methanol ammonia (2M) and ammonium nitrate (1M), respectively.



Dowex 1x2 ion exchange chromatography of 630 mg of crude arrow poison on a 2.5" x 33" column with step gradient elution.

FIGURE 1

Separation of 630 mg. of arrow poison by ion exchange chromatography. For the details of the procedure refer to Materials and Methods section. Each of the peaks A, B and C were further subjected to HPLC to test their homogeneity.

The application of IEC of the arrow poison resulted in the successful separation of the active components of the poison. The results are presented in Fig. 1. The results of the HPLC are presented in Fig. 2. Peak A and B appear to be homogenous while peak C is resolved further into two peaks.

The lack of similarity between the components of arrow poison with d-tubocurarine was surprising. Of the arrow poisons of plant origin d-tubocurarine is normally considered to be the prototype component (3). The sepa-

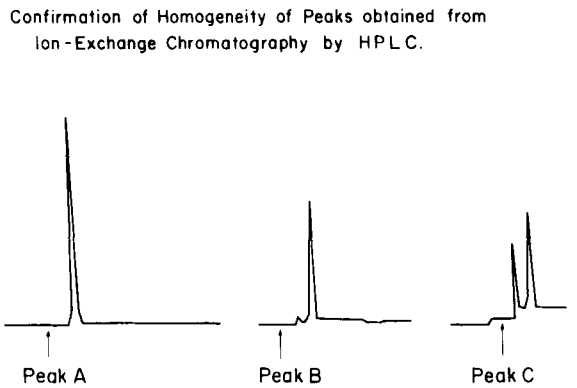


FIGURE 2

HPLC chromatograms of the fractions isolated by IEC. The conditions for the separation are specified in the test.

ration of the arrow poison on TLC was not adequate and therefore we resorted to IEC. The IEC separation was very satisfactory as shown in Fig. 1. The peaks were distinct and therefore easily identifiable. However, the method is tedious and time consuming. The results of HPLC provided additional support to the validity of IEC and also provided additional resolution of peak C into doublets. Relative to IEC, we found HPLC to be rapid and efficient. The major drawback we ran into with HPLC was that the fractions of the arrow poison separated were too small to be used for toxicological and pharmacological studies as had been done previously with the unseparated poison (1).

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