Separation of the Alkaloidal Constituents of *Wrightia tomentosa* by Paper Partition Chromatography

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The alkaloids present in the bark of *Wrightia tomentosa*, which belongs to a closely allied genus and is widely used as a substitute of *Holarrhena antidysenterica*, have been separated using paper partition chromatography with a solvent system of chloroform–*n*-amyl-alcohol–alcohol–water (9:8:5:2) on paper strips buffered at pH 7.5 (KH_2PO_4 and $Na_2B_4O_7$ solution).

WRIGHTIA TOMENTOSA Roem and Schult (1), belonging to the natural order *Apocynaceae*, has been used by Indian ayurvedic physicians for many years for the treatment of menstrual disorders, renal complaints, and also as a remedy for amebic dysentery. The drug bark commonly is being utilized by drug dealers as an adulterant of *H. antidysenterica*, which it closely resembles, both in its morphological and anatomical characteristics (2). Preliminary examinations showed that the bark contained alkaloids; to isolate and identify the alkaloids, separation of the individual bases was attempted by paper chromatography. Several solvent and buffer systems were tried at different pH levels in order to obtain a system which gave a resolution of the constituents present in the alkaloidal mixture obtained from the plant material.

EXPERIMENTAL

Extraction and Separation of the Alkaloids.—The drug was obtained from local drug dealers, who sell it under the name of "Safed Kuraiya." The material was carefully compared to authentic samples[1] and was identified as *W. tomentosa*.

A weighed quantity of the dried drug powder (No. 60) was moistened with ammoniacal alcohol (1:40) and extracted in a Soxhlet extractor with alcohol (90%).

The hydroalcoholic extract thus obtained was concentrated under reduced pressure at 50° until a syrupy residue was left. It was then dissolved in 2 *N* HCl and shaken with chloroform in a separator. The coloring matter and other impurities were discarded along with the chloroform. The acid extract was made basic with ammonia, and the liberated alkaloidal bases were extracted with chloroform. The chloroformic extract, after being washed with water, was transferred to a distilling flask. The solvent was removed by distillation under reduced pressure. A portion of the dried residue thus obtained was weighed and dissolved in sufficient absolute alcohol to produce a 0.10% w/v solution of the total alkaloids. This solution was used for the separation of alkaloids on chromatographic papers in the subsequent experiments.

Apparatus and Reagents.—The apparatus employed was assembled in the laboratory. It consisted of a cylindrical glass jar (29 × 38 cm.) with a ground-glass upper rim. The jar was covered with a circular ground-glass lid having a small hole (1.5-cm. diameter) in the center. To hold the mobile phase, a half-cylindrical glass trough (27 × 3 cm.) was placed on a rack of glass rods inside the chamber at 34 cm. from the bottom. The stationary phase, equilibrated with the mobile phase, was kept at the bottom of the chamber in a 50-ml. beaker.

Strips of Whatman No. 1 chromatographic grade filter paper were cut 6 cm. or more wide and 40 cm. long, with fibers running lengthwise. A starting line was marked with a pencil 7.5 cm. from one end of the paper strip. Along this line circles were drawn 0.7 cm. in diameter and 1.5 cm. apart. The paper strips were stored in air tight containers. Several 0.1% w/v alcoholic solutions of the samples were applied to the paper with a calibrated glass capillary tube. A quantity between 30 to 50 mcg. per sample was used. The phase mixtures were prepared by shaking the solvents for about 1 hour in separators and allowing them to separate for 12 to 14 hours. The stationary phase was removed and used for saturating the chamber, whereas the mobile phase was used for the development of chromatograms.

When the chamber had been saturated with the stationary phase, the filter paper strips were transferred to the chamber. The end marked with the starting line was placed in the trough and held in position with a heavy glass rod. The papers were left for 18 to 24 hours for equilibration in the chamber. The mobile phase then was poured slowly into the trough through the center hole in the lid which was then immediately closed. The mobile phase was allowed to run to a distance of 28 to 30 cm. from the starting line, then the strips were removed from the jar and dried in the air. The solvent fronts were marked. The positions of the spots were detected by passing the strips quickly through modified Dragendorff's reagent (3). Orange-red spots from the alkaloids appeared on the yellow paper. The spots were marked with a copying pencil, since they usually disappeared after a few days. The R_f values were calculated.

Study of the Different Solvent Systems.—The different solvent systems listed in Table I first were studied for their ability to separate the maximum number of alkaloidal constituents. The number of spots separated in each case is recorded along with their R_f values. (Table I.) The R_f values recorded are representative of quadruplicate findings at 29°.

Of 16 solvent systems studied, only five—*viz.*, *8*, *9*, *11*, *12*, and *13*—produced a separation resulting in a maximum number of spots (*i.e.*, three) in each case.

Separation on Buffered Papers.—To obtain more effective separation of the alkaloids, the decision was made to use the partition chromatography technique on suitably buffered papers at various pH values. Buffer solutions of pH 4, 5, 6, 7, 7.5, and 8 were prepared by mixing the quantities of the two desired solutions mentioned in Table II.

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[1] From the Department of Pharmaceutics and the ayurvedic herbarium, Banaras Hindu University, Varanasi, India.

TABLE I.—SOLVENT SYSTEMS STUDIED

Solvent System	Compn.	Spots, No.	R_f
1	Petroleum ether–water (1:1)	1	0.00
2	Ether–water (1:1)	1	0.15
3	Benzene–water (1:1)	1	0.00
4	Chloroform–water (1:1)	Tailing	...
5	Carbon tetrachloride–water (1:1)	1	0.00
6	Ethyl acetate–water (1:1)	2	0.12, 0.08
7	*n*-Propyl alcohol–water (1:1)	1	0.84
8	*n*-Butyl alcohol–water (1:1)	3	0.81, 0.60, 0.35
9	*n*-Amyl alcohol–water (1:1)	3	0.68, 0.14, 0.07
10	Dioxan–water (7:3)	1	0.88
11	Ethyl alcohol–water (7:4)	3	0.93, 0.84, 0.82
12	*n*-Butyl alcohol–ammonia–water (4:1:5)	3	0.75, 0.70, 0.65
13	*n*-Butyl alcohol–HCl–water (10:3:7)	3	0.67, 0.66, 0.63
14	Ether–ammonia–water (7:3:4)	1	1.00
15	Ether–AcOH–water (7:3:4)	1	0.90
16	*n*-Propyl alcohol–AcOH–water (10:1:10)	1	0.87

TABLE II.—BUFFER SOLUTION

pH	ml. of 0.05 *M* Succinic Acid	ml. of 0.1 *M* Pot. Dihydrogen Phosphate	ml. of 0.05 *M* Borax
4	82.2	...	17.8
5	63.2	...	36.8
6	...	87.7	12.3
7	...	62.3	37.7
7.5	...	54.4	45.6
8	...	46.5	53.5

TABLE III.—*n*-BUTANOL–WATER (1:1) SYSTEM STUDIED ON BUFFERED PAPERS (pH 4 TO 8)

pH	Spots, No.	R_f
4	4	0.78, 0.32, 0.28, 0.14
5	4	0.85, 0.40, 0.37, 0.28
6	4	0.87, 0.61, 0.21, 0.14
7	4	0.77, 0.45, 0.35, 0.29
7.5	5	0.90, 0.71, 0.60, 0.45, 0.32
8	3	0.94, 0.90, 0.86

TABLE IV.—*n*-AMYL ALCOHOL–WATER (1:1) SYSTEM STUDIED ON BUFFERED PAPERS (pH 4 TO 8)

pH	Spots, No.	R_f
4	4	0.61, 0.42, 0.08, 0.06
5	4	0.52, 0.46, 0.35, 0.33
6	4	0.51, 0.40, 0.05, 0.02
7	4	0.88, 0.54, 0.40, 0.11
7.5	5	0.90, 0.60, 0.50, 0.34, 0.15
8	3	0.95, 0.93, 0.80

The paper strips were dipped in the buffer solutions and dried—first at room temperature for 8 hours, then at 48° in a hot-air oven for 12 hours. Finally, they were stored in air-tight containers.

The alkaloid spots produced using solvent systems *8* and *9* were further apart than the spots produced by any other system; therefore, only these systems were utilized for the separation of alkaloids on buffered papers. The number of spots with their R_f values are recorded in Tables III and IV.

From the two systems studied at various pH levels, the best resolution is effected at pH 7.5. A critical observation of these chromatograms reveals that the spots separated in the latter system (amyl alcohol–water, 1:1) are distributed nearly uniformly, starting right from the starting line to the solvent front; whereas in the former system, they lie mostly in the latter half.

An additional observation in the latter system was that one of the spots (R_f 0.15) had a tendency to resolve into two spots. Therefore, an attempt was made to find a still better solvent system which would cause further separation and would resolve the alkaloids located around the spot. A number of different combinations of *n*-amyl alcohol with chloroform, alcohol, and water were studied at pH 7.5; the separation of six spots was achieved with the solvent system chloroform–*n*-amyl alcohol–alcohol–water (9:8:5:2). The six spots thus separated exhibited R_f values of 0.83, 0.60, 0.40, 0.20, 0.15, and 0.10, respectively.

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