PAPER CHROMATOGRAPHY OF METHYL RESERPATE DERIVATIVES

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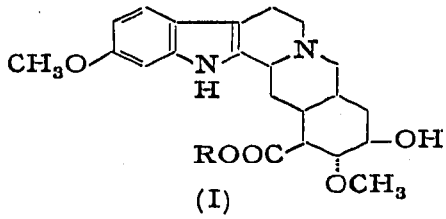
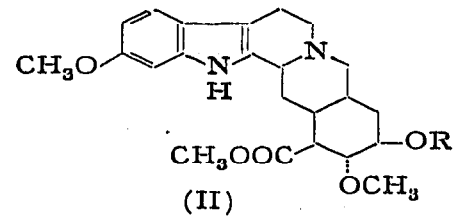
INTRODUCTION

Interest in reserpine and the unique activity of some reserpine analogs has led to the preparation of other derivatives[1-3]. A series of reserpic acid esters of type I, ethers of methyl reserpate type II and methyl 18-*epi*-reserpate and ethers thereof (III) were prepared[3]. Paper chromatography was an invaluable aid in the preparation and isolation of many of these derivatives. Crude reaction mixtures were chromatographed to determine relative amounts of starting material and reaction products. In addition, paper chromatography was used to evaluate the efficiency of the isolation procedure and to estimate the purity of the final products. The usual ZAFFARONI type systems used for the Rauwolfia alkaloids were modified to obtain the desired separation[4-6].



(I)



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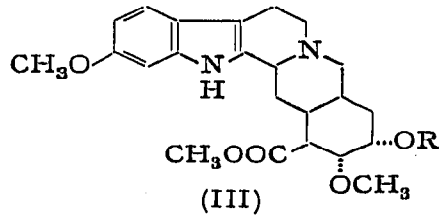


(III)

EXPERIMENTAL

Whatman No. 1 paper was used throughout in standard chromatographic jars set up for descending method of development.

Solvent systems

The immobile phase was prepared by adjusting the pH of formamide to 5.6 by adding approximately 5 % benzoic acid. It was observed that this helped to prevent the

elongation and tailing of spots. Before impregnating the chromatograms, the adjusted formamide was diluted to 50 % and 30 % with methanol. The formamide diluted to 50 % was used for systems A, B, C, and D. The 30 % solution was used for systems E and F. Formamide, without benzoic acid, was diluted to 50 % with methanol and used as the immobile phase for systems G, H, and I.

A = Chloroform
B = Chloroform containing 10 % pyridine
C = Benzene–chloroform (1:1) containing 2 % pyridine
D = Xylene–methyl ethyl ketone (1:1)
E = Xylene–methyl ethyl ketone (3:1)
F = Toluene–ethyl acetate (1:2) containing 2 % pyridine
G = Benzene
H = Benzene–cyclohexane (1:1)
I = Benzene–chloroform (1:1)

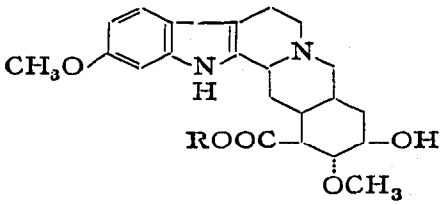
Procedure

Points for the application of samples were marked on the strips, usually six per strip. The strips were impregnated with the formamide–methanol solutions. The excess solvent was removed by blotting between two sheets of clean filter paper. The samples were dissolved in chloroform or chloroform–methanol (1:1) and spotted in concentrations of 5–10 μg. The chromatograms were developed by the descending method and the solvent allowed to run to about 1 or 2 inches from the end of the chromatogram. The time for development was 4 to 6 h, depending on the system used. After developing, the chromatograms were air dried and then sprayed lightly with glacial acetic acid and heated for 5–10 min in an oven at 90° to enhance the greenish-yellow fluorescent color produced by the alkaloids. The chromatograms were viewed under a long wave (3660Å) ultra-violet lamp. To produce visible colored spots the chromatograms were dried at 90° for 2 to 3 h to completely remove the formamide and then sprayed with Dragendorff's reagent[7]. On drying, an orange color developed for the alkaloids. The chromatograms were then washed with $N/20$ H_2SO_4[8]. This method increases the sensitivity approximately ten times.

RESULTS AND DISCUSSION

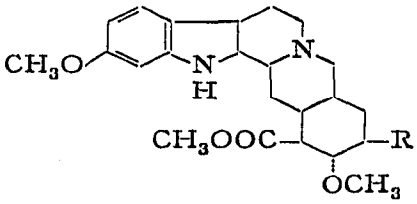
Tables I, II, and III list the R_F values of 23 alkaloid derivatives in nine solvent systems. These values were an average of two or more runs. The degree of separation and R_F values may vary considerably with the formamide systems. This can be attributed to the concentration of sample, variations in room temperature, amount of formamide remaining on the paper and the purity of the formamide. However, the components will appear in the same order even if the R_F values do vary. It was necessary to run standards and not depend entirely on R_F values for the identity of components. The solvent systems containing benzoic acid in the immobile phase gave more compact spots and less variation of R_F values. Systems without benzoic acid showed some elongation of spots and distinct separations of closely running compounds were difficult.

TABLE I

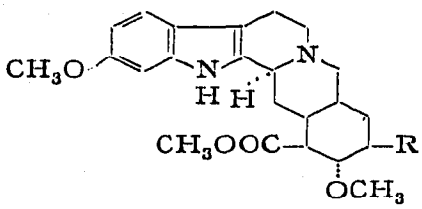


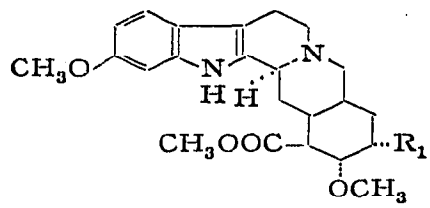
R	R_F values								
	A	B	C	D	E	F	G	H	I
CH_3	0.15	0.55	0.08	0.03	0.05	0.23	0.00	0.00	0.10
CH_3CH_2	0.24	0.65	0.15	0.05	0.10	0.35	0.10	0.00	0.30
$CH_3CH_2CH_2$	0.30	0.83	0.21	0.05	0.10	0.40	0.30	0.00	0.40
$(CH_3)_2CH$	0.30	0.83	0.21	0.05	0.10	0.40	0.30	0.00	0.40
$CH_3(CH_2)_3$	0.35	0.90	0.30	0.12	0.15	0.50	0.43	0.00	0.50
$(CH_3)_2CHCH_2$	0.35	0.90	0.30	0.12	0.15	0.50	0.43	0.00	0.50
$(CH_3)_2CHCH_2CH_2$	0.40	0.90	0.35	0.25	0.15	0.50	0.45	0.10	0.75
$CH_3(CH_2)_4CH_2$	0.50	0.90	0.40	0.35	0.25	0.50	0.50	0.15	0.85

TABLE II



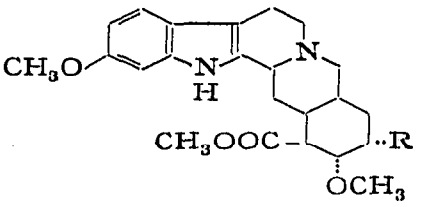
R	R_F values								
	A	B	C	D	E	F	G	H	I
OCH_3	0.45	0.65	0.40	0.25	0.25	0.50	0.50	0.15	0.75
OCH_2CH_3	0.45	0.85	0.50	0.30	0.25	0.55	0.70	0.30	0.95
$OCH_2(CH_2)_2CH_3$	0.90	0.95	0.85	0.50	0.60	0.75	0.90	0.65	0.85
$OSO_2C_6H_4Br$	0.90	0.90	0.90	0.55	0.75	0.75	0.70	0.50	0.95
$OSO_2C_6H_5$	0.83	0.95	0.80	0.40	0.65	0.60	0.90	0.35	0.95
OOCH	0.14	0.55	0.10	0.00	0.03	0.15	0.00	0.00	0.05





R	A	B	C	D	E	F	G	H	I
OH	0.18	0.70	0.25	0.18	0.34	0.40	0.20	0.01	0.40
OCH_3	0.65	0.85	0.70	0.45	0.55	0.70	0.95	0.55	0.95
R_1									
OCH_3	0.45	0.85	0.50	0.35	0.45	0.55	0.85	0.75	0.85

TABLE III



R	R_F values								
	A	B	C	D	E	F	G	H	I
OCH_3	0.40	0.60	0.35	0.03	0.20	0.34	0.05	0.00	0.20
OCH_2CH_3	0.45	0.85	0.50	0.25	0.25	0.55	0.55	0.13	0.80
$OCH(CH_3)_2$	0.75	0.90	0.60	0.30	0.30	0.55	0.75	0.40	0.90
$OCH_2CH_2CH_3$	0.75	0.90	0.60	0.40	0.50	0.65	0.75	0.40	0.90
$OSO_2C_6H_4Br$	0.85	0.85	0.75	0.45	0.55	0.85	0.85	0.15	0.85
OH	0.10	0.55	0.10	0.00	0.00	0.25	0.01	0.00	0.35

ACKNOWLEDGEMENT

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SUMMARY

A paper chromatographic procedure for the separation of methyl reserpate, its derivatives and isomers has been described. The method has proven to be a valuable tool in following the synthesis, isolation and purification of closely related alkaloids.

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