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IDENTIFICATION OF ALKALOID-CONTAINING DRUGS BY THIN-LAYER CHROMATOGRAPHY AFTER MICRO STEAM DISTILLATION

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SUMMARY

A method for the identification of some alkaloid-containing drugs, involving the micro steam distillate obtained using a TAS oven, utilising thin-layer chromatography in five solvent systems is described. Using Rhodamine B and Butter Yellow as reference substances the relative hR_x values of the two fastest moving Dragendorff-reacting compounds are plotted as a function of the solvent system used and the characteristic patterns obtained are included.

INTRODUCTION

A scheme for the identification of alkaloids involving consideration of their hR_F values in eight different thin-layer chromatographic (TLC) systems together with their characteristics in UV light (365 mμ) and after spraying with iodoplatinate solution was devised by WALDI *et al.*[1]. In order to utilise this scheme for the identification of alkaloid-containing drugs it is necessary to make an extract of the drug, but this is not easy on a small scale (5–25 mg powdered drug). However, the introduction by STAHL[2] of the TAS (thermomicro separation, transfer and application method according to STAHL) oven, which permits rapid steam distillation of the drug in micro quantities directly on to thin-layer plates, has made the identification of small amounts of alkaloid-containing drugs possible if the alkaloids present are volatile under the conditions prescribed.

This communication outlines a scheme whereby many alkaloid-containing drugs in common use may be identified by a TLC method without the need for standard reference alkaloids.

EXPERIMENTAL

Material

The powdered drugs investigated are the following:

Aconitum deinorhizum Stapf.	(Indian aconite)	*Atropa belladonna* Linn.	(Belladonna herb and root)
Aconitum napellus Linn.	(Aconite)		
Areca catechu Linn.	(Areca)	*Cephaëlis acuminata* Karsten	(Cartagena ipecacuanha)

Cephaëlis ipecacuanha Brot.	(Rio ipecacuanha)	*Nicotiana tabacum* Linn.	(Tobacco)
Cinchona sp.	(Cinchona)	*Papaver somniferum* Linn.	(Opium)
Claviceps purpurea Tulasne	(Ergot)	*Peumus boldus* Molina	(Boldo)
Colchicum autumnale Linn.	(Colchicum)	*Pilocarpus microphyllus* Stapf.	(Jaborandi)
Conium maculatum Linn.	(Hemlock)	*Piper nigrum* Linn.	(Black and white pepper)
Cytisus scoparius Link	(Broom tops)		
Datura innoxia Miller	(Datura herb)	*Physostigma venenosum* Balfour	(Calabar)
Datura stramonium Linn.	(Stramonium)		
Duboisia sp.	(Duboisia)	*Punica granatum* Linn.	(Pomegranate)
Ephedra sinica Stapf.	(Ephedra)	*Rauwolfia serpentina* Benth.	(Rauwolfia)
Erythroxylum truxillense Rusby	(Peruvian Coca)		
		Rauwolfia vomitoria	(African rauwolfia)
Galipea officinalis Hancock	(Angostura bark)		
Hydrastis canadensis Linn.	(Hydrastis)	*Strychnos nux-vomica* Linn.	(Nux Vomica)
Hyoscyamus niger Linn.	(Henbane)		
Lobelia inflata Linn.	(Lobelia)	*Veratrum album* Linn.	(White Hellebore)

Micro steam distillation

A Desaga TAS oven was used; oven temp., 275°; distillation time, 90 sec. The capillary end of the cartridge was packed with glass wool and the sample (10–20 mg) admixed with about 10 mg calcium hydroxide introduced. Silica gel of suitable moisture content (indicator pink) provided the moisture necessary for steam distillation.

Thin-layer chromatography

Silica Gel G layers, 250 μ thick, were activated at 110° for 30 min. The following solvent systems were used: (A) chloroform–acetone–diethylamine (50:40:10); (B) chloroform–diethylamine (90:10); (C) cyclohexane–chloroform–diethylamine (50:40:10); (D) cyclohexane–diethylamine (90:10); (E) benzene–ethyl acetate–diethylamine (70:20:10). Development temperature: 20–22°. Detection was carried out by means of the Dragendorff reagent[3].

RESULT AND DISCUSSION

The results are presented in Table I and Figs. 1–3. To allow for variation in adsorption, which affects the hR_F values, a dye mixture composed of Rhodamine B

TABLE I

$hR_{\text{Rhodamine}}$ AND $hR_{\text{Butter Yellow}}$ VALUES FOR THE TWO FASTEST MOVING DRAGENDORFF-REACTING COMPONENTS IN SOME ALKALOID-CONTAINING DRUGS

The figures quoted represent the mean of a minimum of five determinations.

Drug		*Solvent system* A		B		C		D		E	
		hR_R[a]	hR_B[b]	hR_R	hR_B	hR_R	hR_B	hR_R	hR_B	hR_R	hR_B
Aconite	(i)	118	99	156	97	300	100	1333	145	101	88
	(ii)	88	73	102	63	104	35	650	71	31	27
Indian aconite	(i)	117	98	153	94	300	100	1333	145	97	85
	(ii)	90	75	102	56	104	35	633	69	31	27
Angostura bark	(i)	126	95	162	99	308	94	744	137	100	87
	(ii)	85	64	109	67	164	50	611	112	75	65

TABLE I *(continued)*

Drug		Solvent system A		B		C		D		E	
		hR_R[a]	hR_B[b]	hR_R	hR_B	hR_R	hR_B	hR_R	hR_B	hR_R	hR_B
Areca	(i)	113	91	131	89	280	91	264	69	97	86
	(ii)	–	–	84	63	–	–	—	–	66	58
Belladonna herb	(i)	125	93	164	102	211	73	388	65	112	97
	(ii)	95	70	138	86	89	31	150	25	78	68
Belladonna root	(i)	125	93	160	100	278	96	675	113	110	96
	(ii)	95	70	134	84	207	72	475	90	78	68
Boldo	(i)	109	98	114	96	193	101	938	139	115	103
	(ii)	38	34	23	20	43	23	138	20	6	5
Broom	(i)	121	97	130	97	316	103	564	146	109	96
Calabar	(i)	104	86	136	79	179	52	83	10	95	75
Cinchona		Results not consistent									
Coca	(i)	130	96	157	143	289	100	663	110	107	94
	(ii)	45	33	140	128	241	83	513	85	87	75
Colchicum	(i)	117	98	102	63	296	99	1367	149	119	104
	(ii)	77	64	–	–	36	12	567	62	36	32
Datura herb	(i)	123	91	149	93	263	91	763	127	100	87
	(ii)	92	68	123	76	207	72	550	91	67	58
Duboisia	(i)	118	88	147	92	259	90	765	128	101	88
	(ii)	88	65	123	76	207	72	563	94	70	61
Ephedra		S		ND		ND		S		BS	
Ergot		S;ND		ND		ND		S		S	
Hellebore, white	(i)	111	92	134	96	277	96	571	148	107	95
	(ii)	92	76	81	58	77	27	86	22	51	45
Hemlock	(i)	121	99	152	98	260	103	1183	158	123	100
	(ii)	111	91	141	91	237	93	1017	136	112	91
Henbane	(i)	120	89	151	91	267	92	350	58	106	92
	(ii)	90	67	123	76	200	69	150	25	75	65
Hydrastis	(i)	103	86	169	92	259	89	763	142	115	98
	(ii)	23	19	–	–	222	76	525	98	100	85
Ipecacuanha, Rio	(i)	116	98	184	100	304	104	988	184	112	95
	(ii)	84	70	139	76	244	84	713	130	96	82
Ipecacuanha,	(i)	116	98	184	100	304	104	988	184	115	98
Cartagena	(ii)	77	65	137	74	240	82	700	130	99	84
Jaborandi	(i)	76	62	168	97	83	24	50	6	114	89
	(ii)	49	40	115	66	46	13	–	–	41	32
Lobelia	(i)	119	96	132	98	245	100	347	86	119	91
	(ii)	Second component not consistent in consecutive determinations									
Nux Vomica		Results not consistent									
Opium	(i)	118	99	163	89	248	85	538	100	99	84
	(ii)	107	90	110	60	200	68	238	44	87	74
Pepper, black	(i)	117	93	133	99	292	95	200	52	101	90
Pepper, white	(i)	123	98	131	100	288	94	200	52	101	90
Pomegranate	(i)	121	97	131	98	288	94	564	146	112	99
	(ii)	31	25	86	64	36	12	436	113	56	49
Rauwolfia	(i)	108	94	163	97	400	89	167	37	127	86
	(ii)	75	66	116	69	152	34	75	17	104	70
Rauwolfia, African	(i)	113	99	159	94	394	88	183	41	139	93
	(ii)	74	65	120	71	147	33	67	15	104	70
Stramonium	(i)	120	89	147	92	263	91	750	125	100	87
	(ii)	90	67	117	73	196	68	525	88	67	58
Tobacco	(i)	119	94	131	98	288	94	607	157	109	96
	(ii)	101	81	116	86	248	81	357	93	90	79

[a]R = Rhodamine
[b]B = Butter Yellow
S = streaked; ND = not detected using Dragendorff's reagent; BS = base line spot.

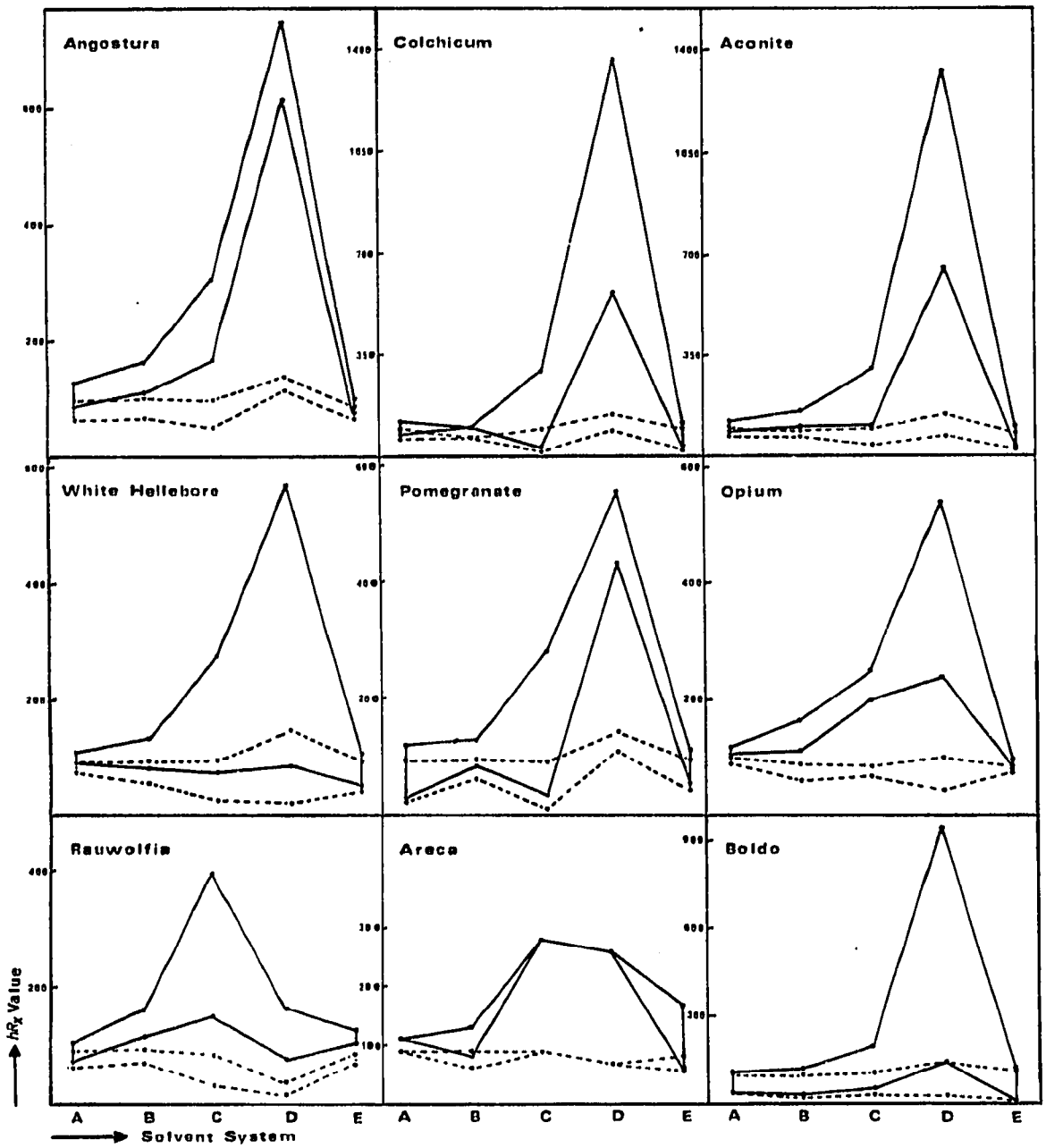

Fig. 1. Patterns obtained by plotting hR_x values against solvent system. ———, $hR_{\text{Rhodamine}}$; - - - - -, $hR_{\text{Butter Yellow}}$.

and Butter Yellow was chromatographed on each plate at the same time as the volatilised compounds. The rates of movement of the two fastest moving Dragendorff-reacting (DR) components were correlated with those of the two reference dyes ($hR_{\text{Rhodamine}}$ and $hR_{\text{Butter Yellow}}$).

Dragendorff's reagent was chosen for detection in preference to iodoplatinate used by WALDI *et al.*[1] because, after development in the five solvent systems, the micro steam distillate from a single drug in many cases yielded a complex array of

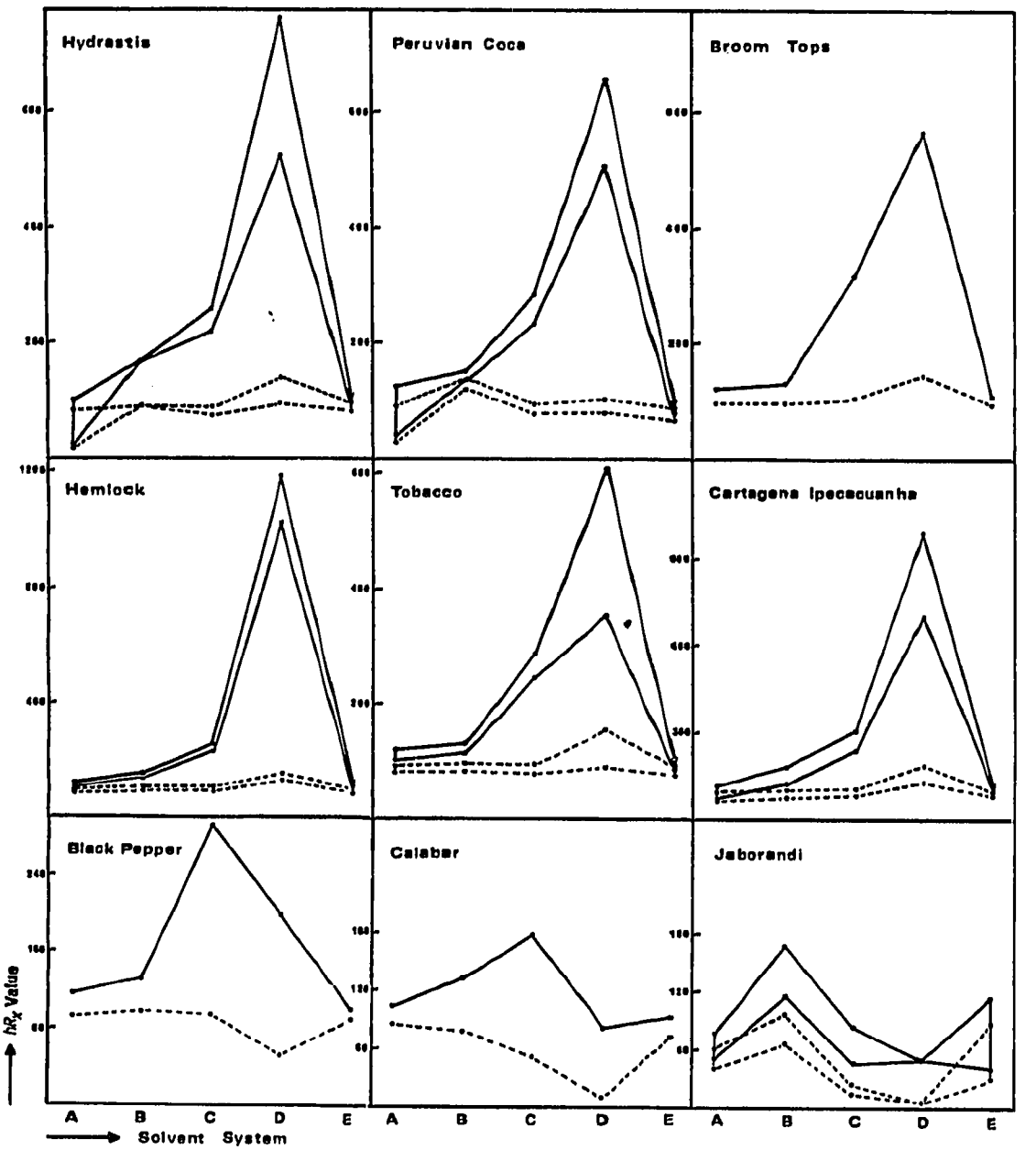

Fig. 2. Patterns obtained by plotting hR_x values against solvent system. ———, $hR_{\text{Rhodamine}}$; - - - - -, $hR_{\text{Butter Yellow}}$

coloured zones in the range white—grey-brown—yellow—orange—blue—pink—purple with the latter reagent whereas a much more simple picture resulted when the former reagent was used.

On plotting the $hR_{\text{Rhodamine}}$ and $hR_{\text{Butter Yellow}}$ values as a function of the solvent system used, some interesting patterns emerged. After spraying with Dragendorff's reagent in some cases (Broom tops, Calabar, Pepper) only one DR component was obtained, and these corresponded in position and reaction with Dragendorff's

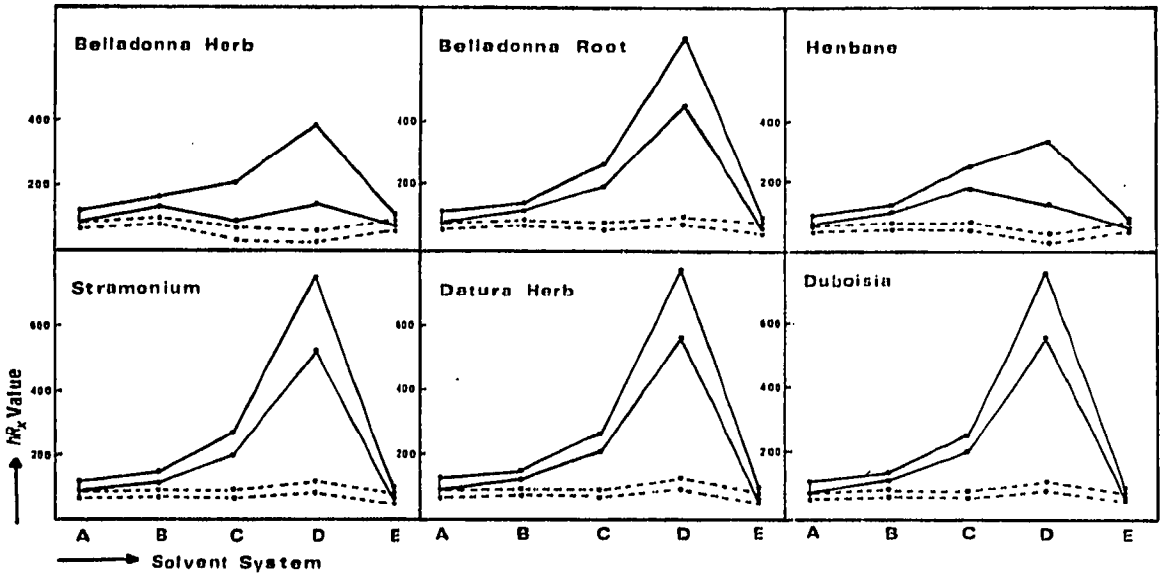

Fig. 3. Patterns obtained by plotting hR_x values against solvent system. ———, $hR_{\text{Rhodamine}}$; - - - - -, $hR_{\text{Butter Yellow}}$.

reagent with the alkaloids sparteine, physostigmine and piperine, respectively, when applied on the same plates. In most cases, however, two or more DR components resulted and for the purpose of identification of the drug the two fastest moving DR components in each of the five solvent systems were considered. It is of interest to note that where more than two DR components are present the same alkaloid (or DR compound) is not necessarily considered in each of the five solvent systems. Reference to Figs. 1–3 shows that the patterns produced when the $hR_{\text{Rhodamine}}$ and $hR_{\text{Butter Yellow}}$ values for the two fastest moving DR components in the respective drugs are plotted as a function of the solvent system, characteristically different pictures are obtained thus making it possible to identify the powdered drug. Although the majority of alkaloids-containing drugs can be identified by this method it can be seen from Table I that the experimental conditions described are not entirely satisfactory for all the drugs.

Lobelia

The fastest moving DR component was constant in repetitive determinations in the five solvent systems. However, the position of the second component varied and for this reason the pattern for this drug has been omitted.

Ephedra

It is recognised that ephedrine is not successfully chromatographed in a basic solvent system and, therefore, the steam distillate from Ephedra was developed using chloroform–methanol–glacial acetic acid. In addition to Dragendorff's reagent iodoplatinate and 2′,7′-difluorescein were used as detection reagents. While the reference ephedrine was detected with each spray reagent the products of the steam distillate resulted in a streak. Since the reference ephedrine could be detected after steam distil-

lation it would appear that with Ephedra other products of the steam distillate interfere with the separation in the solvent systems tested.

Cinchona, Ergot and Nux Vomica

Although the alkaloids in Nux Vomica (strychnine, brucine), Ergot (ergotonine) and Cinchona (quinine, quinidine, cinchonine) could be detected in the steam distillate, the pattern of some other DR compounds in some solvent systems was not consistent.

Solanaceous drugs

It is interesting to note that the fastest moving DR component present in Belladonna root, Datura, Duboisia and Stramonium is absent from Belladonna herb and Henbane under the experimental conditions described (Fig. 3).

This procedure offers a novel, chemical method for the identification of many alkaloid-containing drugs (in mg quantities) in the powdered condition. Conventional identification of these drugs would normally require a detailed knowledge of their microscopy whereas the method outlined requires a simple technique which could be handled in any chemical laboratory. The method is also advantageous because it does not depend on the availability of standard reference alkaloids.

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