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Note

Thin-layer chromatography of steroidal alkaloids

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For our work on the biosynthesis and metabolism of steroidal alkaloids[1] and for the qualitative analysis of fractions obtained by high-pressure liquid chromatography[2] a rapid method of identification by thin-layer chromatography (TLC) was required. Although several satisfactory TLC methods have been reported[3], they were not applicable to all the steroidal alkaloids in which we were interested. We have therefore devised a new method, based on the previously described sulfuric acid test[4].

EXPERIMENTAL

The 26 alkaloids listed in Tables I and II were applied in solutions of 1 mg/ml of acetone to precoated silica gel G plates, 250-μm layer (Uniplates; Analtech, Newark, Del., U.S.A.)*. For the determination of the response to sulfuric acid (Table I) 5-μg samples of each steroid were applied, the plates were lightly sprayed with 50% aqueous H_2SO_4, the spots were observed in long-wave (366 nm) UV light and in daylight while they were lying on a hot-plate at a surface temperature of 80°, and the time of appearance of the response was noted. The limit of detection was similarly determined, but with decreasing quantities of steroids and at both 80° and 300°.

The R_F values listed in Table II were obtained by spotting 10 μg of each steroid 2 cm from the bottom edge of TLC plates, 20 × 20 cm, which had been scribed to give 1-cm wide bands. The solvents, which were of Spectroquality (Burdick & Jackson, Muskegon, Mich., U.S.A.), were mixed in the proportions (by volume) shown in Table II, and 50 ml of each mixture were placed in a square tank, $21\frac{1}{2} \times 21\frac{1}{2} \times 6$ cm. The plates were introduced in the tanks and the time required for the solvent mixture to ascend to the prescored finish line, 15 cm above the starting points, was noted. After development and air-drying, the plates were heated at 300° until charring occurred.

RESULTS AND DISCUSSION

The responses of 26 steroidal alkaloids to the sulfuric acid test are listed in

* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

TABLE I

RESPONSE OF STEROIDAL ALKALOIDS TO SULFURIC ACID

Abbreviations: BE = blue; BT = bright; DK = dark; GN = green; GY = gray; LC = lilac; LT = light; MDQ = minimum detectable quantity; NIL = no response observed under experimental conditions; OE = olive; PE = pale; PK = pink; PU = purple; RE = rose; UV = ultraviolet radiation of 366 nm; VY = very; YW = yellow.

No.	*Alkaloid*	*Daylight*					*UV light*				
		Starting		*Final color*	*MDQ (μg)*		*Starting*		*Final color*	*MDQ (μg)*	
		Time (sec)	*Color*		*80°*	*300°*	*Time (sec)*	*Color*		*80°*	*300°*
1	Tomatidine	130	PK	BE-GN	0.20	0.10	0	BT-BE	GN	0.04	0.04
2	Tomatidenol	86	PK	RE	0.25	0.10	76	LT-BE	BT-BE	0.05	0.05
3	Soladulcidine	300	LT-GN	BE-GN	1.00	0.25	210	BE-GN	GN	0.05	0.05
4	Solasodine	110	PK	PU	0.25	0.10	111	LT-BE	BT-BE	0.03	0.03
5	Veramine	100	GY	GY	0.85	0.10	23	PK	TAN	0.07	0.05
6	9α-Hydroxytomatidene	250	PK	GY	0.90	0.25	131	GY	TAN	0.08	0.08
7	7α-Hydroxytomatidene	80	BE	BE-GY	1.00	0.25	96	PK-BE	GY-GN	0.05	0.05
8	7α,11α-Dihydroxytomatidine	270	LT-BE	BE	8.00	0.50	322	PK-BE	PK	0.30	0.30
9	9α,11α-Dihydroxytomatidine	NIL	NIL	NIL	1.00	0.20	322	PK-BE	PK	0.20	0.10
10	Demissidine	132	PK	PE-LC	40.0	1.00	118	BE	BE	0.25	0 25
11	Solanidine	74	RE	DK-RE	0.25	0.10	0	LT-BE	LT-BE	0.10	0.10
12	5β-Solanidan-3-one	NIL	NIL	NIL	14.0	0.25	NIL	NIL	NIL	2.00	0.05
13	Solanid-4-en-3-one	437	LT-PK	NIL	80.0	0.25	300	BE-GY	GY-GN	10.0	0.07
14	Rubijervine	60	TAN	LC	0.50	0.25	119	DK-BE	PK	0.06	0.04
15	Isorubijervine	53	RE	LC	0.90	0.35	89	LT-BE	LT-BE	0.08	0.06
16	Veralobine	NIL	NIL	NIL	80 0	0.25	360	VY-LT-BE	VY-PE-BE	2.00	0.07
17	Verarine	166	YW	OE	0.15	0.10	106	YW	BT-YW	0.02	0.02
18	Cyclopamine	NIL	GY	GY	0.25	0.10	0	PK-BE	TAN	0.05	0.10
19	Jervine	330	LT-YW	GY	0.50	0.30	151	BE	BT-GN	0.05	0.05
20	Veratramine	139	YW	LT-OE	0.40	0.01	59	LT-BE	BT-YW	0.02	0.02
21	Veramarine	70	PK	OE	1.20	0.07	64	YW	BT-BE	0.02	0.02
22	Solanocapsine	NIL	NIL	NIL	4.00	0.01	NIL	NIL	NIL	0.10	0.01
23	Verazine	120	PK	LC	2.00	0.90	86	TAN	LT-LC	0.40	0.07
24	Tomatillidine	80	PK	LC	0.20	0.10	92	TAN	LT-LC	0.10	0.05
25	Etioline	60	PK	LC	0.30	0.20	45	LT-BE	BT-BE	0.05	0.05
26	Veralkamine	118	LC	PU-GY	0.35	0.25	64	TAN	TAN	0.04	0.04

Table I. Although sulfuric acid is a universal reagent for all but the volatile organic compounds, it shows a great deal of specificity. Compounds differing in only minor structural details may give differential responses to sulfuric acid. Thus, the C-22 isomers tomatidine and soladulcidine can be distinguished by this test and both compounds can be differentiated from their 5-dehydro analogs, tomatidenol and solasodine. Even the various hydroxy derivatives of tomatidine differ in their response to the sulfuric acid test. The sensitivity of the detection in daylight is greatly increased by carrying out the reaction at higher temperature, but the detection in UV is not much improved thereby.

TABLE II

$R_F \times 100$ VALUES FOR STEROIDAL ALKALOIDS

Solvent systems [developing time, in minutes]: 1 = *n*-hexane–ethyl acetate (1:1) [58]; 2 = *n*-hexane–ethanol (1:1) [102]; 3 = dichloromethane–methanol (23:2) [45]; 4 = dichloromethane–methanol (9:1) [42]; 5 = dichloromethane–acetone (4:1) [42]; 6 = *n*-hexane–acetone (1:1) [48]; 7 = dichloromethane–methanol–acetic acid (85:13:2) [50]; 8 = dichloromethane–methanol–ammonia (100:100:1) [62].

No.	*Alkaloid*	*Solvent system*							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1	Tomatidine	47	90	49	66	37	87	73	94
2	Tomatidenol	47	92	46	66	40	89	73	96
3	Soladulcidine	26	87	40	53	23	85	71	97
4	Solasodine	27	85	41	59	23	82	70	96
5	Veramine	10	75	27	35	9	60	68	93
6	9α-Hydroxytomatidene	8	86	27	37	7	60	45	91
7	7α-Hydroxytomatidene	4	88	20	35	5	53	37	93
8	7α,11α-Dihydroxytomatidine	0	69	5	6	0	10	10	93
9	9α,11α-Dihydroxytomatidine	0	69	5	6	0	10	10	93
10	Demissidine	58	85	21	33	19	87	57	97
11	Solanidine	59	83	42	61	15	95	81	89
12	5β-Solanidan-3-one	75	85	61	50	43	0	91	100
13	Solanid-4-en-3-one	63	83	48	50	39	0	71	89
14	Rubijervine	15	73	17	13	7	56	55	82
15	Isorubijervine	19	81	27	33	11	60	43	91
16	Veralobine	11	75	43	43	17	64	55	100
17	Verarine	3	60	22	17	3	19	64	73
18	Cyclopamine	3	46	25	21	5	17	57	81
19	Jervine	0	35	26	19	3	14	53	83
20	Veratramine	5	76	28	17	11	36	37	91
21	Veramarine	3	29	12	8	5	17	27	60
22	Solanocapsine	0	2	0	0	0	0	5	12
23	Verazine	69	92	59	64	83	84	92	97
24	Tomatillidine	69	91	61	69	82	84	92	97
25	Etioline	1	47	12	14	3	9	47	77
26	Veralkamine	3	52	19	14	5	22	60	73

Table II shows the $R_F \times 100$ values of 26 steroidal alkaloids in eight solvent systems. Although the C-22 isomers can be separated by TLC, tomatidine and soladulcidine are not separable from their 5-dehydro analogs in any of the solvent systems used. However, demissidine is separated from its dehydro analog, solanidine,

in several solvent systems. Interesting differences in the order of mobilities are observed in some cases. For instance, in *n*-hexane–acetone (1:1) the mobilities are in the order solanidine > tomatidine > solasodine, but in dichloromethane–methanol (23:2) they are tomatidine > solasodine = solanidine, and in dichloromethane–acetone (4:1) they are tomatidine > solasodine > solanidine. Solanidine moves ahead of demissidine in most solvent systems, but the order is reversed in dichloromethane–methanol–ammonia (100:100:1). Isorubijervine is ahead of its position isomer rubijervine in all solvent systems, except in dichloromethane–methanol–ethyl acetate (85:13:2), where the mobilities are in the reverse order. Thus, most of the steroidal alkaloids can be identified by judicious selection of solvent systems in combination with the sulfuric acid test.

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