THE CHROMATOGRAPHIC AND SPECTRAL PROPERTIES OF STILBENE DERIVATIVES

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INTRODUCTION

A small number of groups of compounds are characterized by the intense blue fluorescence they show under ultraviolet light. The stilbenes constitute one of these groups. In recent years, the hydroxystilbenes of plants have attracted attention because of their fungicidal and fungistatic properties[1], their effect on pulping under acid conditions[2], the cause of coloration in pulps[3], etc. The study of stilbenes, however, is usually difficult on account of their instability and the small quantities present in plant tissues. In order to resolve some of the difficulties associated with their identification, this paper describes the spectrophotometric, paper chromatographic and thin-layer chromatographic properties of stilbenes from plant sources so as to assist in the prediction of their chemical structure. A chromatographic examination of substituted synthetic stilbenes was made recently[4].

EXPERIMENTAL

Chromatographic examination

R_F values of the stilbenes were obtained by spotting a few micrograms of each compound (in methanol) on Whatman No. 1 paper and all compounds were chromatographed by the descending technique under comparable conditions in a constant temperature room at 20°. The following solvents were used: (I) benzene–acetic acid–water (125:72:3); (II) *n*-butanol–acetic acid–water (6:1:2); (III) *m*-cresol–acetic acid–water (50:2:48); (IV) phenol–2 *N* acetic and hydrochloric acids (1:1); (V) *n*-butanol–ethanol–water (4:1:5); and (VI) 30 % acetic acid. The chromatograms were examined under U.V. light (254 nm and 365 nm) before and after exposure to ammonia vapour. Further visualisation of each spot was effected by spraying with diazotized *p*-nitroaniline in 20 % sodium acetate, diazotized *o*-dianisidine (0.2 g in the mixture of 10 ml of dioxane and 20 ml of water) with ammonia vapour, diazotized sulpanilic–sodium carbonate (Pauly's reagent), 0.5 % potassium permanganate followed by washing with water and 2 % aqueous solution of phosphomolybdic acid with ammonia vapour. The R_F values reported are averages of three or more determinations.

Chromatoplates of Silica Gel GF_{254} (E. Merck, A.G., Darmstadt) with a thickness of 0.25 mm were prepared in a constant temperature room at 20°. The following solvents were used: (A) methanol–chloroform–petroleum ether (b.p. 90–120°) (2:4:7);

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(B) methanol–chloroform–petroleum ether (b.p. 90–120°) (1:2:7); (C) benzene–methanol (9:1); (D) benzene–methanol–acetic acid (45:8:4); (E) benzene–dioxane–acetic acid (90:25:4); (F) chloroform–ethyl acetate–formic acid (5:4:1); and (G) toluene–ethyl formate–formic acid (5:4:1).

Ultraviolet spectra

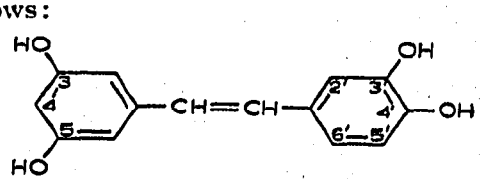
All spectra were determined in absolute ethanol solution with an Optica CF4 recording spectrophotometer using 1 cm silica cells. After determining the spectra of

TABLE I

STILBENES EXAMINED

Stilbene	*Substituents**								*Common name*
	3	*4*	*5*	*2'*	*3'*	*4'*	*5'*	*6'*	
1	—	—	—	—	—	—	—	—	*cis*-Stilbene
2	—	—	—	—	—	—	—	—	*trans*-Stilbene
3	—	OH	—	—	—	—	—	—	4-Hydroxystilbene
4	OH	—	OH	—	—	—	—	—	Pinosylvin
5	—	OH	—	—	—	OH	—	—	4,4'-Dihydroxystilbene
6	OH	—	OH	—	—	OH	—	—	Resveratrol
7	OH	—	OH	—	OH	OH	—	—	3,5,3',4'-Tetrahydroxystilbene
8	OH	—	OH	OH	—	OH	—	—	Oxyresveratrol
9	OH	R	OH	OH	—	OH	—	—	Chlorophorin
10	OH	—	OMe	—	—	—	—	—	Pinosylvin monomethyl ether
11	—	—	—	—	OMe	OH	—	—	3'-Methoxy-4'-hydroxystilbene
12	OH	—	OH	—	OH	OMe	—	—	Rhapontigenin
13	—	OMe	—	—	OMe	OH	—	—	4,3'-Dimethoxy-4'-hydroxystilbene
14	OMe	—	OMe	—	—	OH	—	—	Pterostilbene
14A	OMe	—	OMe	—	—	OA	—	—	Acetate of pterostilbene
15	OMe	OH	—	—	OMe	OH	—	—	3,3'-Dimethoxy-4,4'-dihydroxystilbene
16	OMe	OMe	—	OMe	—	OMe	—	OMe	3,4,2',4',6'-Pentamethoxystilbene
6G	OG	—	OH	—	—	OH	—	—	Resveratrol glucoside
7G	OG	—	OH	—	OH	OH	—	—	Astringin
10G	OG	—	OMe	—	—	—	—	—	Pinosylvin monomethyl ether glucoside
11G	—	—	—	—	OMe	OG	—	—	3'-Methoxy-4'-hydroxystilbene glucoside
11GA	—	—	—	—	OMe	OGA	—	—	Acetate of 3'-methoxy-4'-hydroxystilbene glucoside
12G	OG	—	OH	—	OH	OMe	—	—	Rhapontin
12GA	OGA	—	OA	—	OA	OMe	—	—	Acetate of rhapontin
13G	—	OMe	—	—	OMe	OG	—	—	4,3'-Dimethoxy-4'-hydroxystilbene glucoside
13GA	—	OMe	—	—	OMe	OGA	—	—	Acetate of 4,3'-dimethoxy-4'-hydroxystilbene glucoside
8A	OA	—	OA	OA	—	OA	—	—	Acetate of oxyresveratrol
7GA	OGA	—	OA	—	OA	OA	—	—	Acetate of astringin

* OMe = OCH_3; OG = O-D-glucoside; OGA = acetate of glucoside; OA = acetate; R = CH_2—CH=C(CH_3)—CH_2—CH_2—CH=C(CH_3)—CH_3; numbering as follows:



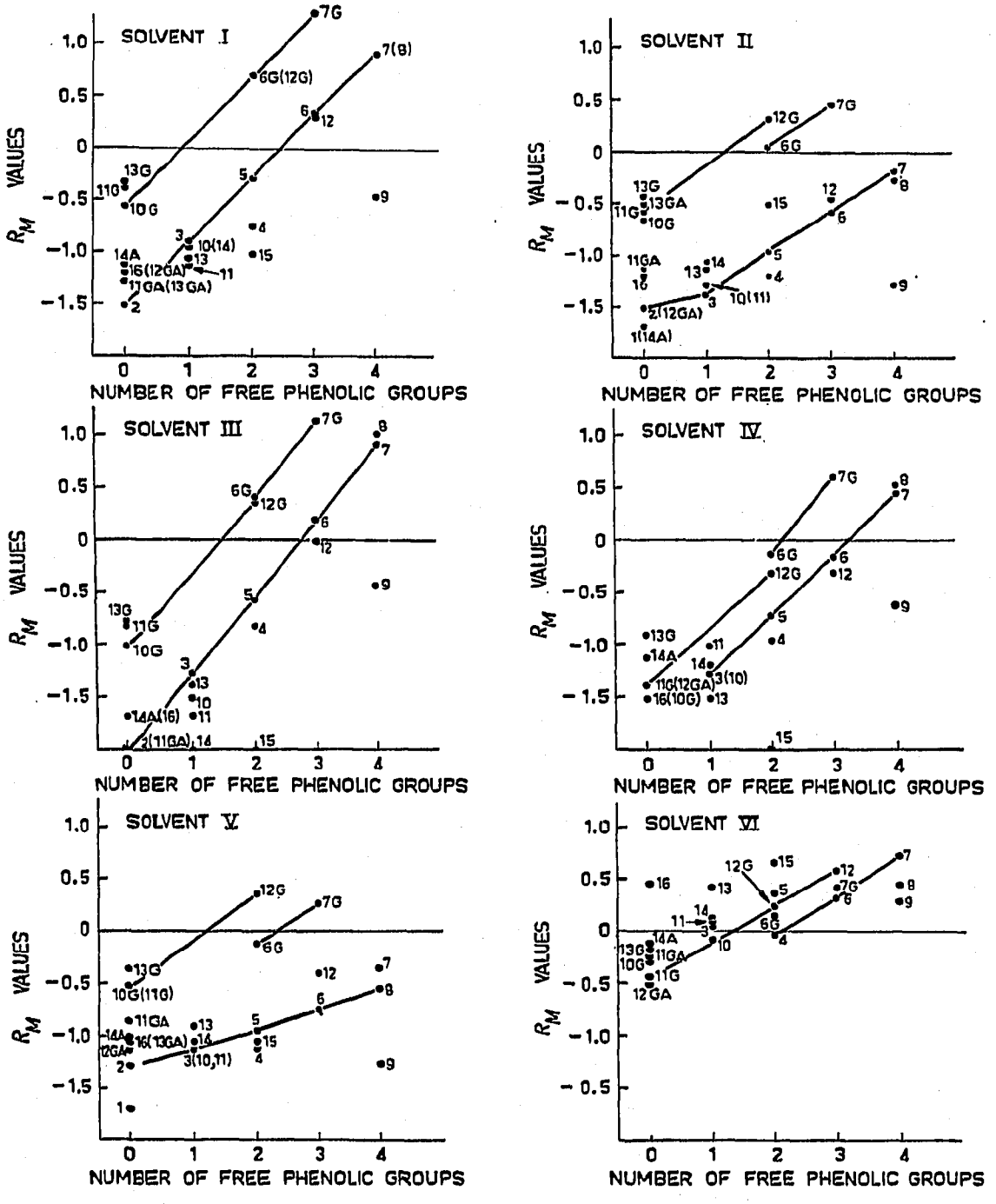


Fig. 1. The relation of R_M values determined with different solvents to the number of free phenolic groups in different stilbenes. The composition of the solvents is given in the text and the numbers on the graphs correspond to the stilbenes listed in Table I.

ethanol solutions of suitable concentration, an excess of powdered anhydrous sodium acetate[5] was added and allowed to settle in the cuvette before measurements were made. For the determination of the spectra in ethanolic sodium ethylate solutions[6], 0.2 ml of 0.1 *M* sodium ethylate was added to 3.8 ml of the solution of each compound and the spectra were determined after 5 min. In order to determine the spectra in

TABLE II

PAPER CHROMATOGRAPHY OF STILBENES

Stilbene*	Solvent**											
	I		II		III		IV		V		VI	
	$R_F \times 100$	R_M	$R_F \times 100$	R_M	$R_F \times 100$	R_M	$R_F \times 100$	R_M	$R_F \times 100$	R_M	$R_F \times 100$	R_M
1	99	—1.999	98	—1.690	—	—	—	—	98	—1.690	00	0.0
2	97	—1.509	97	—1.509	99	—1.999	—	—	95	—1.279	00	0.0
3	89	—0.908	96	—1.380	95	—1.279	95	—1.279	93	—1.124	47	0 052
4	77	—0.525	94	—1.195	87	—0.826	90	—0.954	93	—1.124	52	—0.035
5	66	—0.288	90	—0.954	79	—0.575	84	—0.720	90	—0.954	31	0.348
6	32	0.327	79	—0.575	39	0.194	59	—0.158	85	—0.753	33	0.308
7	11	0.908	60	—0.176	11	0.908	26	0.454	69	—0.348	16	0.720
8	11	0.908	65	—0.269	08	1.061	23	0.525	78	—0.550	27	0.432
9	74	—0.454	95	—1.279	73	—0.432	81	—0.630	95	—1.279	34	0.288
10	90	—0.954	95	—1.279	97	—1.509	95	—1.279	93	—1.124	55	—0.088
11	93	—1.124	95	—1.279	98	—1.690	91	—1.005	93	—1.124	46	0.070
12	33	0.308	74	—0.454	51	—0.017	67	—0.308	72	—0.410	21	0.575
13	92	—1.061	93	—1.124	96	—1.380	97	—1.509	89	—0.908	28	0.410
14	90	—0.954	92	—1.061	99	—1.999	94	—1.195	92	—1.061	44	0.105
14A	93	—1.124	98	—1.690	98	—1.690	93	—1.124	91	—1.005	58	—0.140
15	96	—1.005	76	—0.501	99	—1.999	99	—1.999	92	—1.061	18	0.659
16	94	—1.195	94	—1.195	98	—1.690	97	—1.509	92	—1.061	26	0.454
6G	17	0.689	47	0.052	28	0.410	57	—0.122	57	—0.122	42	0.140
7G	05	1.279	24	0.454	07	1.124	20	0.602	35	0.269	28	0.410
10G	78	—0.550	82	—0.659	91	—1.005	97	—1.509	77	—0.525	66	—0.288
11G	71	—0.389	79	—0.575	87	—0.826	96	—1.380	77	—0.525	73	—0.432
11GA	95	—1.279	93	—1.124	99	—1.999	—	—	88	—0.865	63	—0.232
12G	17	0.689	33	0.308	30	0.368	67	—0.308	31	0.348	37	0.232
12GA	94	—1.195	97	—1.509	97	—1.509	96	—1.380	93	—1.124	76	—0.501
13G	69	—0.348	73	—0.432	86	—0.788	89	—0.908	70	—0.368	60	—0.176
13GA	95	—1.279	91	—1.005	—	—	—	—	92	—1.061	—	—

* See Table I for constitution.

** See text for composition.

TABLE III

THIN-LAYER CHROMATOGRAPHY OF STILBENES ($R_F \times 100$)

*Stilbene**	*Solvent***						
	A	*B*	*C*	*D*	*E*	*F*	*G*
1	94	90	97	—	95	99	98
2	99	98	97	—	99	99	99
3	87	68	52	—	84	94	74
4	71	51	25	—	72	83	61
5	67	45	23	—	73	85	61
6	36	24	13	—	51	71	59
7	18	17	07	—	30	57	49
8	21	16	06	—	29	58	47
9	39	14	26	—	49	86	64
10	52	31	54	—	75	96	72
11	58	43	75	—	87	97	82
12	28	07	19	—	40	71	56
13	56	38	76	—	86	96	79
14	52	31	50	—	74	96	71
15	60	42	82	—	88	97	72
6G	07	10	02	11	03	08	07
7G	04	06	00	08	00	06	04
10G	28	12	17	44	06	19	23
11G	29	14	15	44	04	10	16
12G	08	01	02	15	03	08	07
13G	30	12	15	45	03	12	16

* See Table I for constitution.
** See text for composition.

ethanolic boric acid–sodium acetate[7], 0.8 ml of a saturated solution of boric acid in absolute ethanol and an excess of powdered anhydrous sodium acetate was added to 3.2 ml of the solution of each compound. After shaking and allowing to stand for 20 min, the spectrum of each solution was determined.

RESULTS AND DISCUSSION

All the stilbenes examined are tabulated in Table I together with their structural details.

R_F values for the stilbenes examined are given in Table II. When using solvent system I, containing benzene, special care was taken to ensure a stable temperature and a preliminary equilibration of the papers for one hour or longer was made. Nevertheless the R_F values determined with this solvent fluctuated sometimes in the range of $\pm$ 0.02.

Among the irrigating solvents examined, no solvent was suitable for unhydroxylated stilbenes, *i.e. cis*- and *trans*-stilbenes, as the R_F values were either too high or too low. In all the solvent systems examined, the mobility of stilbenes depends to a large measure on the number of free phenolic groups present in the aglycone. The more highly hydroxylated stilbenes had, as a rule, lower R_F values than the less hydroxylated. Glucosidation of a hydroxyl group causes a large decrease in R_F values in these solvents, except phenol–2 *N* acetic acid–hydrochloric acid and 30 % acetic acid. In the case of the former solvent, glucosidation of a hydroxyl group in the molecule has an irregular effect on the R_F value, which may be either increased or decreased. In

TABLE IV

COLOUR REACTIONS AND FLUORESCENCE OF STILBENES

Stilbene	*U.V. light*	*U.V. light + NH_3 vapour*	*Diazotized p-nitro-aniline*	*Diazotized o-dianisidine (+ NH_3)*	*Diazotized sulphanilic acid*	*0.5% $KMnO_4$**	*Phospho-molybdic acid*
1	weak dull purple	weak dull purple	—	—	—	1	—
2	weak purple	weak purple	—	—	—	2	—
3	purple	blue	yellow-brown	(brown)	purple → yellow	3	violet
4	violet	violet	yellow brown	purple	orange → yellow-brown	7	strong violet
5	purple	blue	yellow-brown	yellow (colourless)	orange → black-brown	3	violet
6	violet	bright blue	brown	purple	yellow-brown	6	strong violet
7	purple	bright white-blue	yellow-brown	ashen purple	—	2	purple
8	bright blue	bright white-blue	brown	purple	purple → black-brown	5	purple
9	violet	blue	yellow-brown	ashen purple	black-brown	4	violet
10	purple	pale white-blue	yellow-brown	purple (red-purple)	red → yellow-brown	6	faint violet
11	violet	sky blue	black-brown	(brown)	purple → yellow-brown	3	violet
12	purple	white-purple	brown	faint brown	orange → yellow-brown	2	ashen violet
13	violet	blue	black-brown	(brown)	—	3	violet
14	weak purple	bright blue	yellow-brown	(faint purple)	—	4	faint violet
14A	weak violet	weak violet	—	—	—	1	—
15	pale purple	purple	—	—	—	0	—
16	purple	purple	—	—	—	1	—
6G	violet	bright blue	yellow-brown	ashen purple	yellow-brown	4	faint violet
7G	bright blue	whitish yellow-green	brown	faint black-brown	orange	5	ashen violet
10G	purple	purple	—	—	—	1	—
11G	weak purple	weak purple	faint yellow-brown	—	—	2	—
11GA	pale blue	pale blue	—	—	—	0	—
12G	violet	white-pink	red-brown	ashen purple	yellow-brown → brown	5	violet
12GA	weak violet	pale white	—	—	—	0	—
13G	purple	purple	faint yellow-brown	—	—	2	—
13GA	pale purple	pale purple	—	—	—	0	—

* The figure shows the relative sensitivity of each compound with 0.5% potassium permanganate reagent.

30 % acetic acid, R_F values of the glucosides were higher than those of their aglucones. Solvent system III was not very suitable for stilbenes as the R_F values of some compounds were too high (0.95), although fairly suitable R_F values were obtained with the glucosides.

The correlation between chromatographic behaviour and the molecular structure is shown distinctly when the function $R_M = \log (1/R_F - 1)$ (ref. 8) was substituted for the R_F value. The R_M values of the stilbenes examined are given in Table II and these values in each solvent have been plotted against the number of hydroxyl substituents in Fig. 1. Straight-line relationships exist between the R_M values of stilbenes and the number of their free phenolic hydroxyl substituents. However the straight-line relationship is disturbed, to a greater or lesser extent, by the introduction of methoxyl and acetoxyl groups and a di-isoprenoid side-chain into the stilbene molecule.

The R_F values obtained by means of thin-layer chromatography are given in Table III. An increase in the number of phenolic hydroxyl groups in the stilbene molecule lowered the R_F values in all of the six solvent systems used. This was the same in the case of paper chromatography. However, methylation of the hydroxyl groups had an irregular effect on the R_F value and a large fall in R_F value is caused by the glucosidation of a hydroxyl group. Increase in the mobility of the stilbene glucosides was effected by using the benzene–methanol–acetic acid (45:8:4) solvent system, but this solvent, unfortunately, separates into solvent phases. This solvent system (D) may be useful for the separation of the stilbene glucosides, but not for the aglucones.

The colour reactions and the fluorescent colours of the stilbenes are noted in Table IV. The stilbenes have, in general, a blue to purple fluorescence under U.V. light before and after exposure to ammonia vapour. The exceptions—rhapontin (12 G) and astringin (7 G)—emit different fluorescent colours, rhapontin has a white-pink fluorescence when exposed to ammonia vapour, and similarly astringin emits a white yellow-green fluorescence. *trans*-Stilbene emits stronger purple fluorescence than the

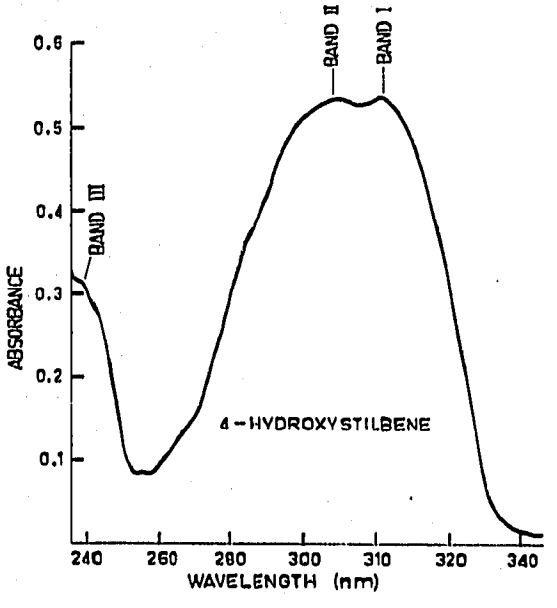


Fig. 2. U.V. absorption spectrum of 4-hydroxystilbene.

TABLE V

SPECTRA OF STILBENES

No.	Stilbene	No. of band	λ_{max} (nm) in EtOH	+ NaOEt	$\Delta\lambda_{max}$ (band I)	+ satd. NaOAc	$\Delta\lambda_{max}$ (band I)	+ NaOAc–H_3BO_3	$\Delta\lambda_{max}$ (band I)
1	*cis*-Stilbene	II	281	281	0*	281	0*	281	0*
2	*trans*-Stilbene	I	310	310	0	310	0	310	0
		II	298	298		298		298	
3	4-Hydroxystilbene	I	322	355	33	322	0	322	0
		II	309	323S		308		307	
		III	235S	245		—		—	
4	Pinosylvin	I	312	316	4	312	0	311S	−1
		II	303	—		303		301	
		III	—	256		—		—	
5	4,4′-Dihydroxystilbene	I	330	350	30	329	−1	330	0
		II	307	328		304		303	
6	Resveratrol	I	323	338S	15	323	0	323	0
		II	310	324		310		311	
		III	—	251		—		—	
7	3,5,3′,4′-Tetrahydroxystilbene	I	330	350	20	334	4	343	13
		II	309S	—		307S		310	
		III	253	256		229		264	
8	Oxyresveratrol	I	332	353	21	331	−1	326	−6
		II	305	317		306		307	
		III	296S	308S		295S		294	
9	Chlorophorin	I	335	358	23	330	−5	327	−8
		II	307	347		309		308S	
		III	296S	307S		296		293	
10	Pinosylvin monomethyl ether	I	309	314	5	310S	1	309S	0
		II	302	306S		303		301	
11	3′-Methoxy-4′-hydroxy-stilbene	I	327	365	38	328	1	326	−1
		II	305	317S		305		305	
		III	234	253		235		—	

12	Rhapontigenin	I	327	325	−2	326	−1	316S	−11
		II	310S	310S		308S		307	
		III	—	—		241		296S	
13	4,3′-Dimethoxy-4′-hydroxy-stilbene	I	332	360	28	332	0	332	0
		II	308	317		307		306	
		III	—	257		—		—	
14	Pterostilbene	I	324	358	34	317	−7	303	−21
		II	313	324S		—		—	
14A	Acetate of pterostilbene	I	314	315	1	310	−4	309	−5
15	3,3′-Dimethoxy-4,4′-dihydroxystilbene	I	336	359	23	337	1		
		II	310S	308S		309S			
		III	298S	—		296S			
16	3,4,2′,4′,6′-Pentamethoxy-stilbene	I	335	335	0	335	0	335	0
		II	327S	327		326S		327	
		III	243	246		243S		243	
6G	Resveratrol glucoside	I	324	345	21	324	0	324	0
		II	310	328		311		311	
7G	Astringin	I	331	346	15	330	−1	344	13
		II	297	307		308S		309	
10G	Pinosylvin monomethyl glucoside	I	310	311	1	310	0	310	0
		II	302	302		302		302	
11G	3′-Methoxy-4′-hydroxy-stilbene glucoside	I	322	322	0	322	0	322	0
		II	304	303		303		303	
11GA	Acetate of 3′-methoxy-4′-hydroxystilbene glucoside	I	321	321	0	320	−1		
		II	303	305		304			
12G	Rhapontin	I	329	327	−2	330	1	323 (316S)	−6
		II	308S	308		309S		309	
		III	—	240		—		—	

(continued on p. 332)

TABLE V (*continued*)

No.	*Stilbene*	*No. of band*	λ_{max} *(nm) in EtOH*	*+NaOEt*	$\Delta\lambda_{max}$ *(band I)*	*+satd. NaOAc*	$\Delta\lambda_{max}$ *(band I)*	*+NaOAc–H_3BO_3*	$\Delta\lambda_{max}$ *(band I)*
12GA	Acetate of rhapontin	I	323	323	0	323	0	322 (315S)	—1
		II	305S	305S		308S		306	
13G	4,3′-Dimethoxy-4′-hydroxy-stilbene glucoside	I	330	330	0	330	0	329	—1
		II	307S	306S		306S		307S	
		III	234S	—		278		—	
13GA	Acetate of 4,3′-dimethoxy-4′-hydroxystilbene glucoside	I	328	329	1	328	0		
		II	308S	307S		308S			
		III	—	—		235			
8A	Acetate of oxyresveratrol	II	302	302	0*	301	—1*	302	0*
7GA	Acetate of astringin	I	312	312	0	312	0	312	0
		II	301	301		301		301	
		III	237	235		232		233	

S = inflection.
* = $\Delta\lambda_{max}$ of band II.

TABLE VI

RELATIONSHIP BETWEEN SPECTRA (BAND I) AND STRUCTURE OF STILBENES

Stilbene	λ_{max} (nm) *in EtOH (band I)*	*Stilbene*	λ_{max} (nm) *in EtOH (band I)*	$\Delta\lambda$*
Introduction of one hydroxyl group				
trans-Stilbene	310	4-Hydroxystilbene	322	12
Pinosylvin	312	Resveratrol	323	11
Pterostilbene	324	3,3′-Dimethoxy-4,4′-dihydroxystilbene	336	12
4-Hydroxystilbene	322	4,4′-Dihydroxystilbene	330	8
Resveratrol	323	Oxyresveratrol	332	9
Resveratrol	323	3,5,3′,4′-Tetrahydroxystilbene	330	7
Introduction of one methoxyl group				
4-Hydroxy stilbene	322	3′-Methoxy-4′-hydroxystilbene	327	5
3′-Methoxy-4′-hydroxystilbene	327	4,3′-Dimethoxy-4′-hydroxystilbene	332	5
Modification of a hydroxyl group to methoxyl group				
Pinosylvin	312	Pinosylvin monomethyl ether	309	—3
3,5,3′,4′-Tetrahydroxystilbene	330	Rhapontigenin	327	—3
Other types				
4,4′-Dihydroxystilbene	330	3,3′-Dimethoxy-4,4′-dihydroxystilbene	336	6
4-Hydroxystilbene	322	Pterostilbene	324	2
4-Hydroxystilbene	322	Resveratrol	323	1
trans-Stilbene	310	Pinosylvin	312	2

* $\Delta\lambda$ = Difference of λ_{max} (band I) between stilbenes.

cis-isomer under U.V. light. The detecting reagent, 0.5 % potassium permanganate, was decolourized by all the stilbenes examined, except the acetylated compounds. Of the different spray reagents used, diazotized *p*-nitroaniline reagent was found to be the most suitable.

The spectral data of the stilbenes examined are tabulated in Table V and a typical spectrum of a stilbene, 4-hydroxystilbene, is shown in Fig. 2. Stilbenes exhibit, as a rule, high intensity absorption in the 308–336 nm region (band I) and the 281–313 nm region (band II). However, some stilbenes do not show band II, but have instead an inflection in this region. These stilbenes are 3,5,3′,4′-tetrahydroxystilbene, 4,3′-dimethoxy-4′-hydroxystilbene glucoside and its acetate, 3,3′-dimethoxy-4,4′-dihydroxystilbene, rhapontin and its acetate, rhapontigenin and 3,4,2′,4′,6′-pentamethoxystilbene. The spectra of both *cis*-stilbene and the acetate of oxyresveratrol show only one absorption peak and this corresponds to band II. On the other hand, the acetate of pterostilbene shows in its spectrum an absorption maximum appropriate to band I. Our results in neutral solution confirm those of ERDTMAN[9] for stilbenes 2, 4 and 10 (see Table V), KING *et al.*[10] for stilbenes 7, BRAUDE[11] for stilbenes 1, 2, 3 and 5, and WALCZAK AND WIENIAWSKI[12] for stilbenes 11G and 13GA.

Compared with *trans*-stilbene, which is the more elongated isomer, the *cis*-isomer shows an absorption maximum (band II) of lower intensity and of shorter

TABLE VII

INFLUENCE OF SODIUM ETHYLATE ON THE SPECTRA (BAND I) OF STILBENES

No. of group	*Stilbene*	λ_{max} *(nm)*		$\Delta\lambda$*
		EtOH	*NaOEt–EtOH*	
a	3′-Methoxy-4′-hydroxystilbene	327	365	38
	Pterostilbene	324	358	34
	4-Hydroxystilbene	322	355	33
	4,3′-Dimethoxy-4′-hydroxystilbene	332	360	28
b	3,3′-Dimethoxy-4,4′-dihydroxystilbene	336	359	23
	Chlorophorin	335	358	23
	Oxyresveratrol	332	353	21
	4,4′-Dihydroxystilbene	330	350	20
	3,5,3′,4′-Tetrahydroxystilbene	330	350	20
	Resveratrol glucoside	324	345	21
c	Pinosylvin monomethyl ether	309	314	5
	Pinosylvin	312	316	4
d	*cis*-Stilbene	218**	281**	0***
	Acetate of oxyresveratrol	302**	302**	0***
	trans-Stilbene	310	310	0
	Pinosylvin monomethyl glucoside	310	311	1
	Acetate of astringin	312	312	0
	Acetate of pterostilbene	314	315	1
	Acetate of 3′-methoxy-4′-hydroxystilbene glucoside	321	321	0
	3′-Methoxy-4′-hydroxystilbene glucoside	322	322	0
	Acetate of rhapontin	323	323	0
	Acetate of 4,3′-dimethoxy-4′-hydroxystilbene glucoside	328	329	1
	4,3′-Dimethoxy-4′-hydroxystilbene glucoside	330	330	0
	3,4,2′,4′,6′-Pentamethoxystilbene	335	335	0
e	Resveratrol	323	338	15
	Astringin	331	346	15
	Rhapontin	329	327	—2
	Rhapontigenin	327	325	—2

* $\Delta\lambda = \lambda_{max}$ (NaOEt)—λ_{max} (EtOH) of the long wavelength, band I.
** λ_{max} of band II.
*** $\Delta\lambda$ of band II.

wave-length. BRAUDE[11], SCOTT[13], GILLAM AND STERN[14] and RIEZEBOS AND HAVINGA[15] have obtained similar results for *cis*- and *trans*-stilbenes. Free phenolic hydroxyl substitution in stilbene molecules raises the wavelength maxima, especially for the *para*-derivative. The introduction of one hydroxyl group into the benzene rings of stilbene produces a bathochromic shift of band I in the range of 7–12 nm (see Table VI) and also the introduction of one methoxyl group into the molecule causes a shift of 5 nm of band I to the longer wavelength. However, the conversion of a hydroxyl group into a methoxyl group in the stilbenes produces a small hypsochromic shift (3 nm).

Just as in the case of the flavonoid compounds[16], the spectra of the stilbenes also undergo considerable shift with the use of reagents such as sodium ethylate, powdered sodium acetate and boric acid–sodium acetate. Bands I and II in the spectra of polyhydroxystilbenes undergo, as a rule, bathochromic shifts with sodium ethylate.

The bathochromic shifts of band I with sodium ethylate were 1–38 nm (see Table V). Sodium ethylate seems to ionize phenolic groups located at any position of the stilbene molecule, because it is difficult to correlate the location and number of hydroxyl groups with the spectral shifts. However, the stilbenes can be divided into five groups depending on the changes which take place in their spectra with sodium ethylate (see Table VII):

(a) Compounds which contain only one of the hydroxyl groups in the *para*-position of the benzene rings of the stilbenes, have shifts of 28–38 nm.

(b) Stilbenes which contain an equal number of hydroxyl groups in both benzene rings in the molecule, have shifts of 20–23 nm.

(c) Compounds containing one or more hydroxyl groups in one of either benzene rings, *e.g.*, pinosylvin monomethyl ether or pinosylvin, show bathochromic shifts of 4–5 nm.

(d) The spectra of those compounds in which free phenolic hydroxyl groups are either absent or substituted by glucosyl and acetyl groups do not shift with sodium ethylate. In addition, acetylation and glucosidation of a phenolic hydroxyl group nullify their effects on the absorption.

(e) The shift in absorption maximum after the addition of sodium ethylate to some stilbenes is accompanied by a decrease in the absorption of band I. This band in the spectrum of resveratrol, in particular, becomes merely an inflection of very low intensity.

On the other hand, the shifts, after the addition of sodium ethylate to the stilbenes in groups a–d, are accompanied by a decrease in the absorption-intensity of band II. *o*-Dihydroxy compounds, *e.g.*, 3,5,3′,4′-tetrahydroxystilbene and astringin, rhapontin and rhapontigenin apparently decompose rapidly in strongly alkaline solutions, because the spectrum of these compounds in sodium ethylate tends to decrease the intensity of absorption to a considerable extent. The spectra of chlorophorin, pterostilbene and its acetate also showed a considerable decrease in the absorption-intensity with alkaline solution. Stilbenes exhibit no significant change in the positions of bands I and II of their spectra on the addition of sodium acetate, but, as exceptions, chlorophorin, pterostilbene or the acetate of pterostilbene have hypsochromic shifts of 5, 7 and 4 nm, respectively (see Table V). In the case of both, 3,5,3′,4′-tetrahydroxystilbene and astringin, band I in their spectra shifted (3 nm towards the longer wavelength) on the addition of both boric acid and sodium acetate. This is due, evidently, to the presence of *ortho*-dihydroxyl groups in these stilbene molecules. 3,5,3′,4′-Tetrahydroxystilbene can be, in consequence, distinguished from the 3,5,2′,4′-tetrahydroxyl derivative, which shows a hypsochromic shift (6 nm) in response to the addition of boric acid–sodium acetate. Chlorophorin, pterostilbene and its acetate, rhapontin and rhapontigenin, on the other hand, showed hypsochromic shifts (5–21 nm) of band I in the presence of boric acid and sodium acetate.

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SUMMARY

The R_F values of 26 stilbene aglycones and glucosides have been determined in several solvent systems by means of paper chromatography and thin-layer chromatography. Most stilbenes in paper chromatograms showed a straight-line relationship between R_M value and the number of free phenolic hydroxyl groups.

The absorption spectra of 28 stilbenes in alcohol with or without added sodium acetate, sodium ethylate and boric acid–sodium acetate have been measured. Interpretations of the spectral changes have been made in terms of the structure of stilbenes which also can be divided into several groups due to spectral changes with added sodium ethylate. The presence of *ortho*-dihydroxyl groups in stilbene molecules can be detected by the spectral changes with boric acid–sodium acetate.

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