CHROM. 6051

Thin-layer chromatographic and spectral properties of certain lactones and related compounds

During recent years there has been an increasing interest in lactones because of their contribution to certain food flavors[1] and their known carcinogenic properties[2–4]. In spite of this wide interest, there has been little effort to investigate the thin-layer chromatographic (TLC) properties of the toxic members of this group.

In connection with our studies on the possible occurrence of mycotoxins in tobacco, we have found it of value to examine the TLC behavior of a number of lactones and related compounds, many of which are derived from molds and are considered either toxic or antibiotic in biological systems. With the possible exception of the chromatography of some carbohydrate lactones[5,6], the only solvent systems used previously for the TLC separation of lactones have included diisopropyl ether[7,8]. In order to avoid the inherent hazardous character of this solvent (due to the formation and subsequent explosion of peroxides[8,9]) we have tried some of the solvent systems commonly used for the TLC of aflatoxins, namely mixtures of chloroform and methanol. Our results are presented below.

For purposes of identification of unknown spots we have also found it useful to have data on the UV and IR spectra of standard samples of these lactones and related compounds. This information is included herewith.

Experimental

The thin-layer plates used were Silica Gel (5763), EM-Reagents, precoated analytical glass plates (20 × 20 cm, 250 μ), obtained from Brinkmann Instruments, Inc., New York*. The solvents were highest purity analytical grade obtained commercially and were used without further purification. The lactones and related compounds were obtained from a variety of sources, as indicated in Table I. A number of them were gifts of Dr. F. H. STODOLA, Northern Marketing and Nutrition Research Division, ARS, USDA, Peoria, Ill. Solid samples for chromatography were dissolved (1–15 mg/ml) in chloroform or methanol and spotted (1–20 μl) at 2 cm from the bottom of the plate. Liquid samples were handled similarly but without solvent. Plates were placed in previously equilibrated, lined tanks and allowed to develop until the solvent front had traveled *ca.* 16–17 cm. This generally required 60–70 min. Plates were then removed and air-dried at room temperature. Spots were detected by spraying with a 2% solution of iodine in methanol[8] and/or by the hydroxamic acid method of KORTE AND VOGEL[7]. Since some compounds were detectable only with the first and others with the second, both methods were used on the same plate. The iodine spray was used first, detectable spots marked, and the iodine allowed to evaporate off; then the hydroxamic acid method was applied and the remaining spots marked.

UV absorbance spectra were obtained on a Beckman DBG using ethanol or methanol solutions of the standard compounds. IR spectra were obtained on a

* Mention of company or trade names does not imply endorsement by the Department over others not named.

TABLE I

CHROMATOGRAPHED SUBSTANCES

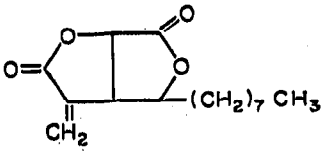
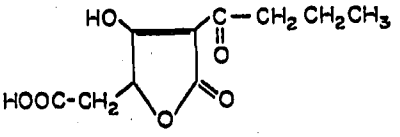
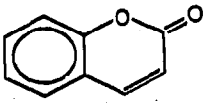
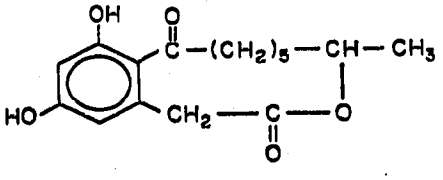
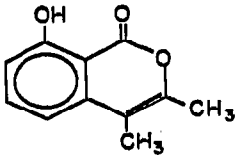
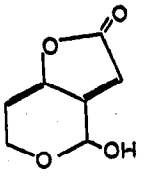
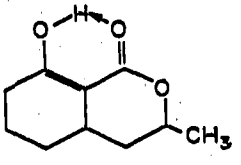
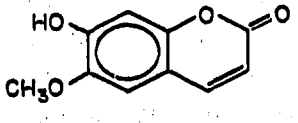
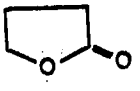
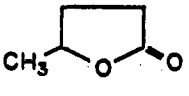
No.	*Lactone*	*Structure*	*Source*
1	Avenaciolide		F. H. STODOLA
2	Carlosic acid		F. H. STODOLA
3	Coumarin		Aldrich Chemical
4	Curvularin		F. H. STODOLA
5	Oospolactone		F. H. STODOLA
6	Patulin		F. H. STODOLA
7	Ramulosin		F. H. STODOLA
8	Scopoletin		Mann Laboratories
9	Butyrolactone		Chem. Service
10	4-Valerolactone		Eastman Chemicals

TABLE I *(continued)*

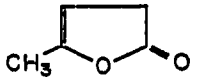
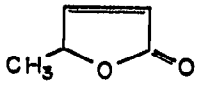
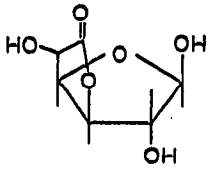
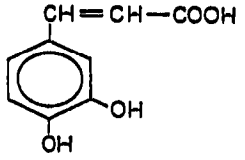
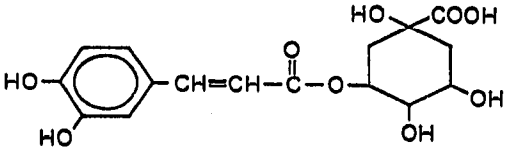
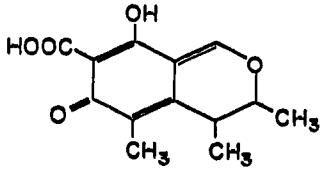
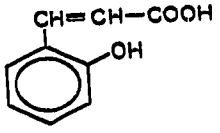
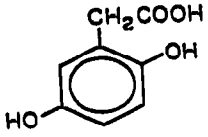
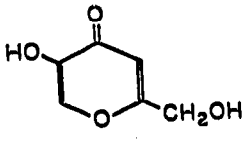
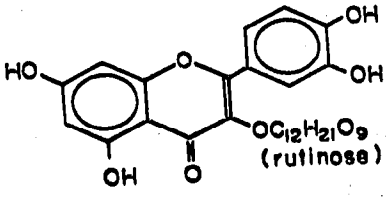
No.	*Lactone*	*Structure*	*Source*
11	α-Angelica lactone		Aldrich Chemical
12	β-Angelica lactone		K & K Laboratories
13	D-Glucuronolactone		Mann Laboratories
Related compound			
1	Caffeic acid		Calbiochem
2	Chlorogenic acid		Sigma Chemical
3	Citrinin		F. H. Stodola
4	o-Coumaric acid		Bios Laboratories
5	Homogentisic acid		Sigma Chemical
6	Kojic acid		Sigma Chemical
7	Rutin		Eastern Regional Res. Lab., USDA

TABLE II

R_F VALUES OF LACTONES AND RELATED COMPOUNDS IN VARIOUS SOLVENT SYSTEMS

Solvent systems: A = chloroform–methanol (3:1); B = chloroform–methanol (85:15); C = chloroform–methanol (95:5); D = chloroform–methanol (95:5) satd. with water; E = methanol–chloroform (3:1).

No.	*Lactone*	*Solvent system*				
		A	*B*	*C*	*D*	*E*
1	Avenaciolide	0.69	0.66	0.59	0.16	0.71
2	Carlosic acid	0.06	0.05	0.02	0.00	0.54
3	Coumarin	0.70	0.65	0.59	0.29	0.69
4	Curvularin	0.66	0.48	0.21	0.01	0.70
5	Oospolactone	0.72	0.68	0.63	0.43	0.70
6	Patulin	0.65	0.51	0.28	0.02	0.67
7	Ramulosin	0.71	0.67	0.61	0.32	0.70
8	Scopoletin	0.66	0.56	0.40	0.05	0.66
9	Butyrolactone	0.65	0.59	0.52	0.32	0.63
10	4-Valerolactone	0.66	0.60	0.55	0.36	0.65
11	α-Angelica lactone	0.63	0.64	0.56	0.43	0.65
12	β-Angelica lactone	0.62	0.59	0.51	0.32	0.68
13	D-Glucuronolactone	0.25	0.10	0.01	0.00	0.55
	Related compound					
1	Caffeic acid	0.26	0.13	0.02	0.00	0.64
2	Chlorogenic acid	0.05	0.01	0.02	0.00	0.58
3	Citrinin	0.24	0.08	0.01	0.01	0.71
4	o-Coumaric acid	0.44	0.31	0.08	0.00	0.61
5	Homogentisic acid	0.18	0.08	0.02	0.00	0.66
6	Kojic acid	0.47	0.34	0.09	0.00	0.60
7	Rutin	0.07	0.02	0.00	0.00	0.39

TABLE III

ULTRAVIOLET AND INFRARED ABSORBANCE MAXIMA OF LACTONES AND RELATED COMPOUNDS

No.	*Lactone*	*$UV_{max.}$ (nm)*	*Principal $IR_{max.}$ (cm^{-1})*
1	Avenaciolide	203[a]	1100, 1200, 1220, 1290, 1765
2	Carlosic acid	203, 247[a]	1000, 1075, 1275, 1690, 1740
3	Coumarin	209, 272, 310[b]	1110, 1170, 1700
4	Curvularin	203, 219, 268, 298[a]	1150, 1700
5	Oospolactone	207, 227, 234, 258, 340[a]	1160, 1215, 1670
6	Patulin	270[a]	1030, 1160, 1200, 1765
7	Ramulosin	262[a]	1225, 1295, 1640
8	Scopoletin	204, 227, 345[b]	1250, 1280, 1695
9	Butyrolactone	210[b]	990, 1035, 1160, 1780[c]
10	4-Valerolactone	223[b]	1160, 1780[c]
11	α-Angelica lactone	217[b]	1090, 1170, 1255, 1800[c]
12	β-Angelica lactone	226[b]	1100, 1155, 1760, 1780[c]
13	Glucuronolactone	234[a]	1035, 1125, 1190, 1350, 1750
	Related compound		
1	Caffeic acid	216, 241, 325[b]	1265, 1435, 1590, 1635
2	Chlorogenic acid	218, 233, 243, 331[b]	960, 1280, 1440, 1510, 1600, 1635, 1685
3	Citrinin	207, 249, 315[a]	1255, 1270, 1500, 1620
4	o-Coumaric acid	215, 272, 325[b]	1200, 1310, 1590, 1660
5	Homogentisic acid	207, 295[a]	1145, 1300, 1500, 1685
6	Kojic acid	215, 267[a]	1125, 1215, 1340, 1600
7	Rutin	205, 255, 360[b]	1195, 1290, 1355, 1495, 1595, 1650

[a] In absolute methanol.
[b] In 95% ethanol.
[c] In carbon tetrachloride, all other IR spectra obtained on KBr micropellets.

Beckman IR-8 using KBr micropellets of the solid compounds. Liquids were dissolved in carbon tetrachloride.

Results

TLC data are presented in Table II. Each R_F value shown is the average of at least two runs. Spots were well formed and movement was highly reproducible. No one solvent system appeared to be ideal for every compound, but all ran well in at least one system.

These data indicate that it is possible to apply TLC methods to separation problems involving any of the tested compounds using only very simple solvent systems. Obtaining adequate movement on the plate is merely dependent upon the system chosen. It is also conceivable that mixtures of several of these lactones could be resolved by selection of the proper system in one or two dimensions.

UV and IR absorbance maxima are presented in Table III for the benefit of those who may find such information valuable for identification purposes. These data apparently have not been reported previously for a number of the compounds listed.

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