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Separation of the hydroxylation products of digitoxigenin by thin-layer chromatography

During a study on the metabolism of cardenolides it was necessary to separate and identify some of the hydroxylation products of digitoxigenin. Thin-layer chromatography (TLC) provided a most useful device in the solution of this problem. Existing systems were concerned primarily with the separation of cardiac glycosides or the separation of cardiac genins from parent glycosides[1–6]. This laboratory had previously reported three TLC systems that separated digitoxigenin from two of its metabolites (3-epidigitoxigenin and 3-dehydrodigitoxigenin), and from gitoxigenin and digoxigenin[7]. NOVER *et al.*[8] have recently reported on a TLC study of the relationship of chromatographic behavior and structure of a number of cardiac glycosides, their genins, and the peracetylated derivatives. In a subsequent paper, NOVER *et al.*[9] developed the mathematics of this relationship and discussed the problems encountered. These authors used four aqueous solvent systems[8]. None of the four systems provides a single system which yields good resolution of all of the cardenolides that we were interested in. In this communication a single TLC system for the separation of a wide variety of hydroxylation products of digitoxigenin is presented.

Experimental

Reagents. All reagents were analytical grade. The coating material was Silica Gel H (EM Reagents, Art. No. 7736). The developing solvent was a mixture of cyclohexane–acetone–acetic acid (65:33:2). The chromogenic agents employed were an aqueous solution of sulfuric acid (20%) and anisaldehyde reagent which was composed of 0.5 ml anisaldehyde, 1.0 ml sulfuric acid, and 50 ml acetic acid[10]. The latter solution was prepared immediately prior to use.

Preparation of the thin-layer plates. The Silica Gel H (30 g) was shaken vigorously with 75 ml of water for 60–90 sec and the slurry was spread over five glass plates (20 × 20 cm) to a thickness of 250 μ with a Kensco (Kensington Scientific Corp.) apparatus. The coated plates were air-dried at room temperature, then activated for 2 h in a 110° oven, and stored in a desiccator.

Procedure. Two chromatography tanks, each containing 100 ml of developing solvent, were prepared. The tanks were allowed to equilibrate for 24 h at room temperature (23–25°) before use. Equilibration was facilitated by covering one wall of each tank with a sheet of Whatman No. 1 filter paper. The materials to be chromatographed (10–20 μg in 10 μl) were routinely spotted 1 cm apart and 1.5 cm from the bottom of the plates. The plates were developed four times by alternating between the two tanks. During each development the developing solvent was allowed to reach the top of the TLC plate (about 45 min) before the chromatogram was removed. A stream of warm air was employed to remove the developing solvent from each plate before subjecting it to the next development. After the final development, each chromatogram was sprayed with either the sulfuric acid solution or the anisaldehyde reagent and placed in a 110° oven for 5–10 min for plates sprayed with sulfuric acid solution and 4–5 min for plates sprayed with the anisaldehyde reagent. Each spot, at the time of its appearance on the chromatogram, was marked and the color noted.

After cooling, the chromatograms were xeroxed to provide a permanent record and to facilitate the determination of R_F values.

Results

The average R_F values and colors of the hydroxylation products of digitoxigenin are given in Table I. The results for other related cardenolides, glycosides, and several steroids are presented for comparison. Each value is the average of two or more chromatograms. As indicated in Table I, satisfactory separation of a wide variety of related compounds can be achieved. In all cases the colors were intense, and the spots extremely small in size, indicating little diffusion during the process of development.

TABLE I

R_F VALUES OF CARDENOLIDES AND SOME RELATED GLYCOSIDES AND STEROIDS

Name	R_F	*Color with sulfuric acid*	*Color with anisaldehyde*
Digitoxigenin	0.53	Blue-green	Blue-green
3-Epidigitoxigenin	0.49	Blue-gray	Blue
3-Dehydrodigitoxigenin	0.60	Tan	Red
1β-Hydroxydigitoxigenin (Acovenosigenin)	0.35	Yellow-green	Light blue
5β-Hydroxydigitoxigenin (Periplogenin)	0.33	Light green	Dark green
7β-Hydroxydigitoxigenin	0.41	Gray	Gray
11α-Hydroxydigitoxigenin (Sarmentogenin)	0.23	Green	Light green
1β,7β-Dihydroxydigitoxigenin	0.24	Tan	Green
5β,7β-Dihydroxydigitoxigenin	0.27	Light blue	Blue
Digoxigenin	0.24	Yellow	Dark blue
3-Epidigoxigenin	0.21	Light yellow	Blue
3-Dehydrodigoxigenin	0.29	Light yellow	Blue-violet
Gitoxigenin	0.30	Purple	Green
Diginatigenin	0.12	Gold	Blue-green
Digitoxin	0.26	Blue-gray	Light green
Digitoxigenin bisdigitoxoside	0.35	Blue-gray	Light green
Digitoxigenin monodigitoxoside	0.44	Blue-gray	Light green
Digoxin	0.09	Gray-brown	Blue
Gitoxin	0.12	Dark purple	Light blue
Strophanthidin	0.22	Yellow green	Pink-violet
Strophanthidol	0.15	Gold	Violet
Strophanthin (*k*-strophanthin)	0.00	Brown	Blue
Ouabain (*G*-strophanthin)	0.00	Olive green	Gold
β-Sitosterol	0.87	Purple	Red-pink
Cholesterol	0.87	Red-pink	Red-pink

Compounds with relatively close R_F values could be separated and identified. It was observed that each developing solvent could be used for approximately 10 days without a significant change in R_F values.

In conclusion, the TLC system presented in this communication has made an important contribution to our study of the metabolism of cardenolides.

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1 M. L. DOW, R. D. KIRCHHOEFER AND J. F. BROWER, *J. Pharm. Sci.*, 60 (1971) 298.
2 L. FAUCONNET AND M. WALDESBÜHL, *Pharm. Acta Helv.*, 38 (1963) 423.
3 G. RABITZSCH AND S. JÜNGLING, *J. Chromatogr.*, 42 (1969) 146.
4 L. TAN, *J. Chromatogr.*, 45 (1969) 68.
5 E. WATSON AND S. M. KALMAN, *J. Chromatogr.*, 56 (1971) 209.
6 W. E. WILSON, S. A. JOHNSON, W. H. PERKINS AND J. E. RIPLEY, *Anal. Chem.*, 39 (1967) 40.
7 S. J. STOHS, L. A. REINKE AND M. M. EL-OLEMY, *Biochem. Pharmacol.*, 20 (1971) 437.
8 L. NOVER, G. JÜTTNER, S. NOACK, G. BAUMGARTEN AND M. LUCKNER, *J. Chromatogr.*, 39 (1969) 419.
9 L. NOVER, G. BAUMGARTEN AND M. LUCKNER, *J. Chromatogr.*, 39 (1969) 450.
10 D. KRITCHEVSKY, D. S. MARTAK AND G. H. ROTHBLAT, *Anal. Biochem.*, 5 (1963) 388.

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