RESEARCH PAPERS

PAPER CHROMATOGRAPHY OF SOME CARDIAC GLYCOSIDES AND THEIR DERIVATIVES

By Jeanette Harrison and S. E. Wright

From the Pharmacy Department, University of Sydney, Sydney, Australia

Received July 2, 1956

Several paper chromatographic systems have been used successfully for the separation of cardiac glycosides[1] and some information has been obtained about the relation between the chemical structure of several glycosides and their chromatographic behaviour. Schindler and Reichstein[2] chromatographed a number of aglycones and their glycosides on formamide impregnated paper and showed the importance of an increase in oxygen content of the aglycone on the rate of travel. Heftmann and Levant[3], also using formamide paper, demonstrated the influence of the degree of oxidation of the side chain at C(10) on the rate of travel of glycosides of the strophanthidin group. They also found that acetylation of the hydroxyl groups of the aglycone increased the rate of travel, presumably because the polarity of the molecule had been reduced.

Tschesche, Grimmer, and Seehofer[4] sought to overcome the adsorption effects of the paper and obtain a true partition effect by using a reversed phase system of chromatography in which the organic phase was applied to the paper, and the chromatogram developed with the aqueous phase. Using an amyl alcohol—water mixture, and also different mixtures of *n*-octanol, amyl alcohol, water, and formamide, very consistent R_F values were obtained and although adsorption effects were not eliminated, they appeared to be reduced to a minimum.

Owing to the apparent close approach to a true partition effect given by these systems, we have now used similar solvent mixtures to examine the effect on the chromatographic behaviour of structural differences in a number of digitalis glycosides and their derivatives. The movement of several of the substances on formamide paper has also been investigated.

Experimental

Materials

Ethylhexanol b.p., 183–184°; amyl alcohol (May and Baker) b.p., 128–132°; chloroform purified by passage through silica gel column; benzene b.p., 80°; formamide (Light and Company); filter paper, Whatman No. 4.

Chromatography Systems

[All solvents measured by volume.]

System I: ethyl hexanol–amyl alcohol–water–formamide (6:2:8:2).

System II: ethyl hexanol–amyl alcohol–water–formamide (6:2:1:4).

System III: amyl alcohol–water (1 : 1).

System IV: Chloroform–benzene (78 : 12) saturated with formamide; formamide impregnated paper.

The solvents for Systems I, II, and III were shaken together and allowed to separate. The organic phase was sprayed onto filter paper, 10 cm. × 30 cm., so that it was just saturated. Four substances (20–40 μg. in solution) were applied to the starting line 5 cm. from the end of the paper. The chromatograms were suspended in a glass tank and developed with the aqueous phase by the descending method at 18–24°, until the solvent front had advanced to 28 cm. from the starting line. The top of the chromatogram was raised out of the aqueous phase, allowed to drain for 10 minutes to prevent streaking, and then the chromatogram was dried at 100°. The substances were located by spraying lightly with a 33 per cent solution of trichloroacetic acid in chloroform containing one drop of 100 volume hydrogen peroxide in each 10 ml.[5] The chromatogram was heated in an oven at 110° for 10 minutes and examined under ultra-violet light. Substances which could not be detected readily by this reagent were revealed by treating the papers with 5 per cent *m*-dinitrobenzene in benzene, followed by 20 per cent aqueous sodium hydroxide.

For System IV, filter paper strips 1 cm. × 30 cm. were dipped in a freshly prepared solution of 25 per cent formamide in methanol, and excess solution removed by pressing lightly between filter paper. The substances in solution were applied to the strips along a starting line, 5 cm. from one end. The chromatograms were then developed in a horizontal tank for seven hours at 32°. The substances were detected on the paper with alkaline *m*-dinitrobenzene as described above.

At least two consistent R_F values were obtained for each substance when using the first three systems. To check the consistency of the results, each chromatogram included a standard glycoside. Digoxin was used in System I, digitoxin in System II, lanatoside C in System III, and digoxin in System IV.

TABLE I

R_F VALUES OF COMPOUNDS OF A, B, AND C SERIES OF DIGITALIS GLYCOSIDES

Solvent system	R_F Values		
	I	II	III
Digitoxigenin	0·10	0·60	—
Gitoxigenin	0·27	—	—
Digoxigenin	0·46	—	—
Digitoxin	0·12	0·65	—
Gitoxin	0·29	—	—
Digoxin	0·47	—	—
Lanatoside A	0·61	—	—
Lanatoside B	0·80	—	0·14
Lanatoside C	0·89	—	0·34
Deacetyl lanatoside A	0·69	—	0·08
Deacetyl lanatoside B	—	—	0·24
Deacetyl lanatoside C	—	—	0·44

RESULTS

Solvent System I was found to be suitable for most of the substances and was evidently of fairly wide application, whereas System II was only useful for those compounds of low water solubility, and System III was used for the more water soluble lanatosides. In Table I, a comparison is made of the results obtained for analagous compounds belonging to the

TABLE II

R_F VALUES OF GLYCOSIDES OF THE A SERIES AND DERIVATIVES

Solvent system	R_F Values		
	I	II	III
Acetyldigitoxigenin	0·05	0·35	—
β-Anhydrodigitoxigenin	0·08	0·37	—
Digitoxigenin	0·10	0·60	—
Dihydrodigitoxigenin	0·14	0·61	—
3-*epi*-Digitoxigenin	—	0·62	—
Digitoxin	0·12	0·65	—
Digitoxigenone	0·22	0·75	—
Lanatoside A	0·61	—	—
Deacetyl lanatoside A	0·69	—	0·08

TABLE III

R_F VALUES OF GLYCOSIDES OF THE B SERIES AND DERIVATIVES

Solvent system	R_F Values		
	I	II	III
Gitoxigenin	0·27	—	—
Gitoxin	0·29	—	—
Lanatoside B	0·80	—	0·14
Deacetyl lanatoside B	—	—	0·24

TABLE IV

R_F VALUES OF GLYCOSIDES OF THE C SERIES AND DERIVATIVES

Solvent system	R_F Values		
	I	II	III
$\Delta^{14:15}$ Anhydrodigoxigenin	0·07	0·51	—
14-Deoxydigoxigenin*	0·11	0·53	—
Diacetyldigoxigenin	0·12	0·57	—
12-Acetyldigoxigenin	0·23	0·76	—
Acetyldigoxin	0·34	—	—
Digoxigenin	0·46	—	—
Digoxin	0·47	—	—
Dihydrodigoxigenin	0·48	—	—
Dihydrodigoxin*	0·55	—	—
Digoxigenin-3-one*	0·58	—	—
Lanatoside C	0·89	—	0·34
Deacetyl lanatoside C	—	—	0·44

* Description to be published.

three digitalis glycoside series; A (derivatives of digitoxigenin); B (derivatives of gitoxigenin); C (derivatives of digoxigenin). R_F values of different members of the three series and their derivatives are compared in Tables II, III, and IV.

Owing to the nature of the formamide system, consistent R_F values were not readily obtained, but it was possible to observe the order in

TABLE V

COMPARISON OF DIGITALIS GLYCOSIDES AND DERIVATIVES OF THE A, B, AND C SERIES ON SYSTEM IV

Sequence from slow to fast moving substances.

(i) *A Series*	(ii) *B Series*	(iii) *C Series*
Deacetyl lanatoside A	Deacetyl lanatoside B	Deacetyl lanatoside C
Lanatoside A	Lanatoside B	Lanatoside C
Digitoxigenin	Gitoxigenin	Digoxigenin
Anhydrodigitoxigenin and digitoxigenin at solvent front	Gitoxin	Digoxigenin-3-one
		Digoxin
		β-Anhydrodigoxigenin
		Acetyldigoxin
		14-Deoxydigoxigenin, 12-acetyldigoxigenin, and diacetyldigoxigenin at solvent front

which the compounds travelled. This has been tabulated for each of the three series in Table V.

DISCUSSION

Tables I–IV illustrate the effect of the presence and positions of hydroxyl groups in the genins on the R_F values of these substances. A gradual increase in R_F values can be observed for both glycosides and genins passing from the A series through the B series to the C series (Table I). This transition has been noted on other chromatographic systems[6,7] and would appear to be due to an increase in the polarity of the molecules. The presence of a second hydroxyl group in the genin would be expected to produce an increase in R_F values in the reversed phase systems used in this study, but a hydroxyl group at position C(12) (digoxigenin and its glycosides) appears to contribute more to this effect than does a C(16) hydroxyl group (gitoxigenin and its glycosides). The β orientated lactone ring at C(17) would appear to make the C(16) hydroxyl group which is also β orientated[7] less available for hydrogen bonding.

From a comparison of the R_F values of digoxigenin, digitoxigenin and the corresponding $\Delta^{14:15}$ anhydro compounds (Tables II and IV), it appears that the loss of the C(14) β hydroxyl group in the formation of the anhydrogenin produces a definite decrease in mobility, despite the fact that this hydroxyl group is hindered by the neighbouring β-methyl group at position C(13). The effect of the loss of this C(14) β hydroxyl group is also shown in 14-deoxydigoxigenin which has a much lower R_F value than digoxigenin. The C(14) deoxy compound, which still retains hydroxyl groups at C(3) and C(12), has an R_F value very close to that of digitoxigenin (hydroxyl groups at C(3) and C(14)). This would indicate that the hydroxyl groups at C(12) and C(14) contribute equally to the rate of movement and possibly to the polarity of the compounds.

A change in the orientation of the C(3) hydroxyl group from β to α (*epi*-digitoxigenin, Table II), has little effect upon the R_F value, and *epi*-digitoxigenin could not be separated from digitoxigenin by the use of these solvent systems.

Acetylation of hydroxyl groups in the genin produces a consistent lowering of R_F values which is in accord with a decrease in polarity. Thus, digoxigenin, 12-acetyldigoxigenin and 3 : 12-diacetyldigoxigenin follow one

another in that order. A similar effect was noted by Heftmann and Levant for acetates of glycosides and genins on formamide impregnated paper[3].

A comparison of the R_F values of the deacetyl lanatosides A, B, and C, with the corresponding desgluco-derivatives—digitoxin, gitoxin, and digoxin—shows that the glucose residue has an important effect upon the rate of travel of the molecule. It would appear that the four free hydroxyl groups of the glucose molecule contribute a good deal more to the R_F value than the one free hydroxyl group of each digitoxose residue. The digitoxose hydroxyl group appears to be hindered as there is very little difference in R_F values between the desglucoglycosides—digitoxin, gitoxin, and digoxin—and their respective genins. Evidently, for these systems at least, the effect of a hydroxyl group at position C(3) in the genin is about equal to the total effect of the three digitoxose residues. This does not occur in paper chromatographic systems in which water is the stationary phase, as the genins tend to follow the solvent front in systems designed to separate the desglucoglycosides. Acetylation of hydroxyl groups on the sugars of the glycosides has a similar effect on the R_F value to acetylation of hydroxyl groups on the genin. Thus acetyldigoxin follows digoxin, and lanatosides A, B, and C, follow the corresponding deacetyl lanatosides.

As System IV is the reverse of the previous ones used, the organic phase being the mobile phase, substances having a high R_F value on Systems I, II, and III would be expected to be correspondingly slow moving on System IV. Thus, a decrease in rate of travel is observed for analogous compounds passing from the A series through the B series to the C series. Acetylation of a compound increased its rate of travel in all instances, and addition of a glucose molecule to a desglucoglycoside to produce a deacetyl lanatoside decreased the rate of movement. The main deviation from this rule was found in the behaviour of the genins and the desglucoglycosides. Contrary to expectation the genin, in every case, travelled more slowly than its desglucoglycoside. This indicates that for this system, the hydroxyl group at position C(3) appears to be of greater significance than the hydroxyl groups of the three digitoxose molecules.

The effect of structural differences upon the R_F values so far discussed appears to conform with changes in the polarity of the molecules. However, it is not possible to attribute all changes in R_F values to an increase or decrease in polarity. Oxidation of hydroxyl groups to ketones would be expected to produce a definite lowering of R_F values in the first three systems. Instead it is found that digitoxigenone and digoxigenin-3-one travel ahead of the corresponding genins. Reduction of the unsaturated linkage in the lactone group to give dihydrodigitoxigenin, dihydrodigoxin, and dihydrodigoxigenin, which should result in a decrease in polarity, does not give the expected decrease in R_F values. In these examples it would appear that either partition effects do not follow polarity changes, or that adsorption effects of the paper have an influence on R_F values.

Summary

1. The behaviour of a number of digitalis glycosides and their derivatives has been studied on three chromatographic systems composed of a stationary organic phase and a mobile aqueous phase, and on one system with reversed order of polarities.

2. On the first three systems, members of the C series of digitalis glycosides always travelled ahead, followed by corresponding members of the B and A series in that order. The differences in rate of travel between members of these three series are ascribed to differences in the number and positions of hydroxyl groups on the steroid nucleus.

3. The presence of glucose increases the rate of travel of the glycosides, whereas the presence of digitoxose has little effect. Acetylation of an hydroxyl group retards the movement, whereas conversion to a ketone increases the rate of travel. Formation of the $\Delta^{14:15}$ anhydro compound greatly decreases the rate of travel. Hydrogenation of the lactone ring has little effect.

4. Chromatography of these compounds on the fourth system, as expected, generally reversed the results obtained with the previous systems.

The authors wish to thank Dr. H. Silberman of the Pharmacology Department, University of Sydney, for helpful advice and assistance, Sandoz A.G., Basel, for supplies of lanatosides A, B, and C, deacetyl lanatosides A, B, and C, and acetyldigoxin, and Professor T. Reichstein for *epi*-digitoxigenin.

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