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Note

Thin-layer chromatographic study of acetyldigitoxin isomers

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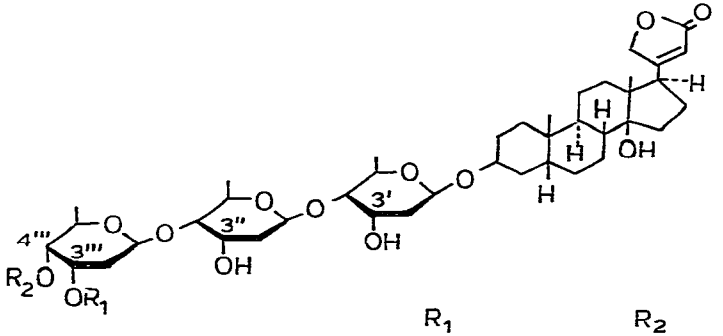
In the preparation of monoacetyl derivatives of digitoxin[1] the 3‴-acetyldigitoxin (α-monoacetyldigitoxin) (I) formed may contain varying amounts of the 4‴-acetyl isomer (β-monoacetyldigitoxin) (II) or, *vice versa*, product II may be contaminated with I. These two compounds may be interconverted in ion-containing solvents or on prolonged contact with adsorbents (*i.e.*, alumina or silica gel)[2]. Highly diverse results were obtained during thin-layer chromatographic (TLC) assay on silica gel of the isomer ratio in preparations containing these substances, and isomerization occurring in the course of the TLC procedure was suggested. Subsequent experiments confirmed that practically no interconversion occurs either in solution or in the course of development, but that 3‴ → 4‴ or 4‴ → 3‴ isomerization proceeds at a detectable rate in the absence of solvents after the application of the spots to the thin-layer plates.



	R_1	R_2
I	CH_3CO	H
II	H	CH_3CO

This note reports a study of the reaction rate, and the development of a procedure to suppress the undesirable isomerization.

EXPERIMENTAL

α-Acetyldigitoxin and β-acetyldigitoxin were prepared according to Stoll and Kreis[1].

Adsorbents

Homogeneous coatings. Glass TLC plates (20 × 20 cm) were prepared with the help of a Camag automatic TLC Coater. The 0.3 mm layers were formed by use of one of the following commercial adsorbents: neutral aluminium oxide (Type T; E. Merck, Darmstadt, G.F.R.) (1); silica gel G (Type 60; E. Merck) (2); silica gel HR (E. Merck) (3); Kieselguhr N (Macherey, Nagel & Co., Düren, G.F.R.) (4); calcium carbonate (Chinoin, Nagytétény, Hungary) (5).

Combined coating. A 4 cm wide calcium carbonate layer, followed by a 16 cm wide layer of silica gel G (both on the same 20 × 20 cm plate and 0.3 mm thick), were formed using the above apparatus modified in our Laboratory to ensure correct partitioning.

Determination of R_F values on different adsorbents

Samples of 10 μg of alcoholic solutions of α-acetyldigitoxin and β-acetyldigitoxin (each containing 200 μg/ml) were applied to the above plates, and developed in one series with benzene–acetone (60:40) and in a second series with benzene–acetone (80:20). The method of identification was as described later.

Determination of the rate of isomerization on different adsorbent coatings

An ethanolic solution (5000 μg/ml) of α-acetyldigitoxin (95%) was applied to coatings 1–5 to give a total coverage of 200 μl on *ca.* 18 cm^2 of surface, and the solvent was evaporated in a stream of air. The plates were left in the dark (relative humidity 48–51%; temperature, 18–23°) for 30 min, 3 h or 22 h. The adsorbent bands were then scraped off with a suitable tool[3] and washed with ethanol (5 ml) on a frit. 10 μl of the filtrate obtained were spotted onto a plate covered with the combined coating, and assayed as below.

Simultaneous determination of the rate of isomerization homogeneous silica gel and combined silica gel–calcium carbonate coatings

Ethanolic α-acetyldigitoxin or β-acetyldigitoxin solution (10 μl each) was applied, at different times prior to development, to the silica gel G coated plate and to the combined silica gel–calcium carbonate plate and developed in a glass chamber with benzene–acetone (60:40). The solvent was evaporated, and the plates sprayed with 10% aqueous sulphuric acid, then kept for 5 min in an oven at 120°. Under UV irradiations bright, light blue, fluorescent spots appeared when the cool plates were sprayed with paraffin oil.

Quantitative assay

A Camag Z-Scanner connected to an Opton PMQ II spectrophotometer was used. Apparatus: mercury lamp; filter (366 nm); diaphragm; chromatogram; monochromator (490 nm); photomultiplier; amplifier; Radelkis OH-814 recorder. Quantitation was based on the fluorescence intensity of the spots.

RESULTS AND DISCUSSION

Starting from α-acetyldigitoxin, the percentage of β-acetyldigitoxin in the isomer mixture as a function of time on different adsorbents, as well as the R_F values

TABLE I

ISOMERIZATION OF α-ACETYLDIGITOXIN AS A FUNCTION OF TIME ON DIFFERENT ADSORBENTS

Adsorbent	*Percentage of β-acetyldigitoxin*			R_F			
	0.5 h	*3 h*	*22 h*	*Benzene–acetone (60:40)*		*Benzene–acetone (80:20)*	
				α	*β*	*α*	*β*
Aluminium oxide	30.4	40.3	40.5	0.24	0.28	*	*
Silica gel G	8.2	18.6	34.0	0.46	0.52	0.06	0.07
Silica gel HR	5.1	14.8	26.2	0.56	0.61	0.07	0.08
Kieselguhr N	1.8	5.4	10.8	**	**	0.78	0.78
Calcium carbonate	0.2	0.3	0.5	**	**	**	**

* At the start.
** At solvent front.

obtained on them in different developing solvents, are given in Table I. It is apparent that isomerization proceeds most rapidly on aluminium oxide. The reaction rate gradually decreases in the order silica gel G > silica gel HR > Kieselguhr, and is hardly perceptible in the case of calcium carbonate. On the other hand, the trend in the R_F values is the reverse of that of the rate of reaction. Since all of the parameters remain constant in these experiments, except for the adsorbents, and in such cases it is generally assumed[4] that adsorbent activity and R_F values vary in opposite directions, it may be concluded that the isomerization rate increases with increasing adsorbent activity.

Fig. 1 shows the difference between homogeneous silica gel coated and combined silica gel–calcium carbonate coated plates as a function of sample–adsorbent contact period. While on the traditional, homogeneous, silica gel coated plates the 22 h contact period led to an equilibrium approaching an isomer ratio of *ca.* 1:1, on

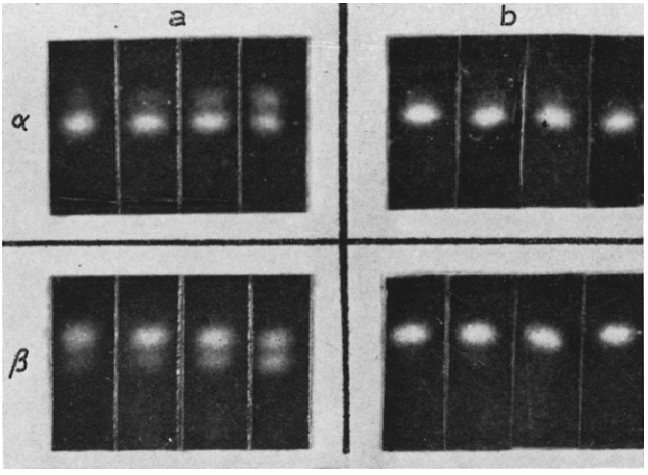


Fig. 1. Chromatogram of α-acetyldigitoxin and β-acetyldigitoxin on thin-layer plates coated with homogeneous (a), and combined (b) adsorbent, starting development immediately or 0.5 h, 3 h or 22 h following application.

the combined silica gel–calcium carbonate coated plates the isomerization is practically negligible.

The scanograms (Fig. 2) prepared from the chromatograms may form the basis of qualitative and quantitative assays, using surface integrals (peak height and width at half height).

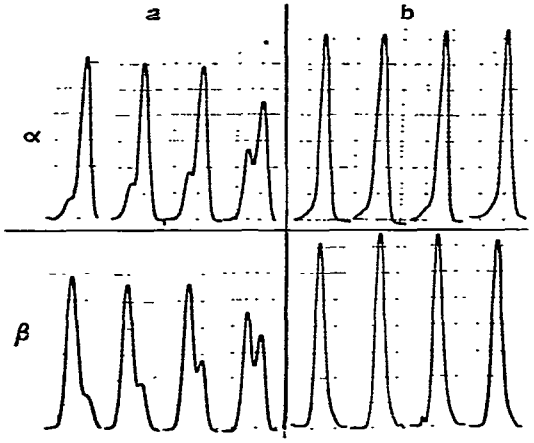


Fig. 2. "Scanogram" of chromatograms demonstrated in Fig. 1.

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