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Note

High-pressure liquid chromatography of digitoxigenin and its glycosides

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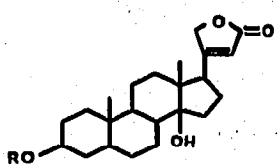
The cardiac glycosides are a therapeutically important group of plant constituents and the necessity for micro-methods of identification is evident in such fields as therapeutics[1], pharmacology[2], analytical chemistry[3], and phytochemistry[4]. Techniques available include paper chromatography[5], thin-layer chromatography[6], gel filtration[7], and gas–liquid chromatography[8]. Recent developments in the field of liquid–liquid chromatography indicated that the speed of analysis with high-pressure systems, sensitivity and ease of recovery of samples were advantages which would be beneficial in the identification of digitoxigenin and its glycosides.

EXPERIMENTAL

A DuPont 820 high-pressure liquid chromatograph fitted with an ultraviolet detector was used for analysis. An SCX ion-exchange column (3 ft. × ¼ in. O.D.) was eluted with 4% amyl alcohol in distilled water at 45°. 1% solutions in 50% alcohol of digitoxigenin, digitoxigenin monodigitoxoside, digitoxigenin bisdigitoxoside, digitoxin and lanataside A (for structures, see Fig. 1) were prepared and 1-μl samples used for injection through a rubber septum on to the column.



Compound		*R*
Digitoxigenin	I	H
Digitoxigenin monodigitoxoside	II	digitoxose
Digitoxigenin bisdigitoxoside	III	$(digitoxose)_2$
Digitoxin	IV	$(digitoxose)_3$
Lanataside A	V	$(digitoxose)_2$-acetyldigitoxose-glucose

Fig. 1. Digitoxigenin and its glycosides.

RESULTS AND DISCUSSION

Several columns and solvent mixtures were tried but resolution occurred only with the ion-exchange columns SAX and SCX, the latter producing better separations. The elution of digitoxigenin decreased with the series of alcohols methyl, ethyl, isopropyl and amyl, the latter solvent at a concentration of 4% in distilled water giving the shortest elution time (Fig. 2). With alcoholic systems operating slightly

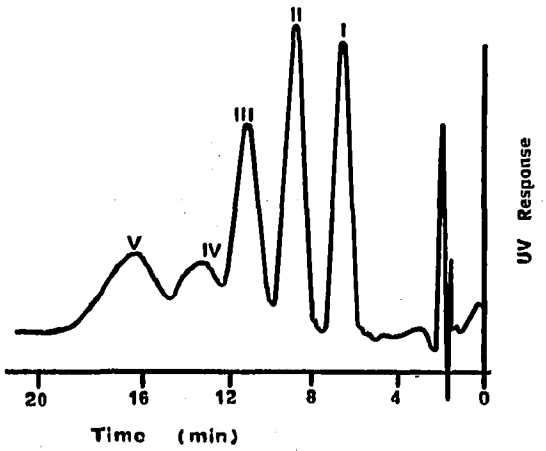


Fig. 2. High-pressure liquid chromatogram of digitoxigenin and its glycosides. SCX ion-exchange column; eluent, 4% amyl alcohol in distilled water; temperature, 45°. Meaning of Roman numbers as indicated in Fig. 1.

above ambient temperature the peak shapes were greatly improved, but because of solvent frothing prolonged degassing of solvent mixtures was required. Elution times at 45° were: digitoxigenin, 6.7 min; digitoxigenin monodigitoxoside, 8.7 min; digitoxigenin bisdigitoxoside, 10.9 min; digitoxin, 13.4 min; lanataside A, 16.7 min. The identity of the peaks was confirmed by collecting samples from several injections and running thin-layer plates (silica gel G, 250 μm) eluted with chloroform–methanol–water (80:19:1), together with standard references.

An increase in the molecular weight of the series digitoxigenin to lanataside A produced increases in the time of residence on the column and it is likely that in this instance the ion-exchange resin matrix is merely acting as an adsorbent. The technique has a similar resolving power and sensitivity to thin-layer chromatography, but has the advantages over gas–liquid chromatography that derivatives such as the trimethylsilyl ethers do not have to be synthesized before analysis and low temperatures are used, preventing thermal elimination of the C_{14} tertiary hydroxyl group.

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