Gas chromatography of digitoxigenin and digoxigenin*

Determination of the urinary excretion of digitalis compounds can improve dose control of digitalis in difficult clinical situations. FRIEDMAN, BINE, BYERS AND BLAND[1] have shown by bioassay that satisfactory complete digitalization is associated with a urinary digitoxin excretion of 40 μg per day, and that a urinary excretion of 80 μg per day is associated with imminent danger of toxicity. Unfortunately, their bioassay method is too laborious for routine clinical use.

Gas–liquid chromatography (GLC) has proven to be useful in the micro-analysis of steroids[2], sterols[3], bile acids[3,4], and sapogenins[5]. Since the aglycone moieties of digitalis compounds are steroids (Fig. 1), it was decided to investigate their behaviour when subjected to GLC.

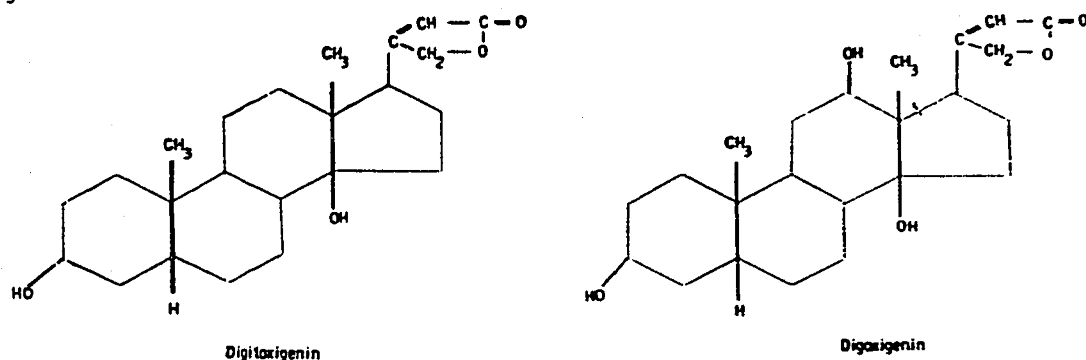

Fig. 1. Structures of digitoxigenin and digoxigenin.

Methods

A Barber Colman Model 10 gas chromatograph was used, employing 12 ft., 4 mm I.D. glass columns packed with 0.75 % SE-30 coated onto 100–140 mesh siliconized Gas Chrom P. A tritium argon ionization detector was used.

Trimethylsiloxy (TMS) derivatives of digitoxin, digoxin, digitoxigenin, and digoxigenin were made by the following reaction[6–8]:

$$2\ ROH + (CH_3)_3SiNHSi(CH_3)_3 \rightarrow 2\ ROSi(CH_3)_3 + NH_3.$$

1 mg of each reference compound was dissolved in 0.2 ml dry tetrahydrofuran and 0.4 ml hexamethyldisilazane was added. 0.1 ml trimethylchlorosilane was added to catalyze the reaction. The reaction mixture stood overnight at room temperature under a drying tube containing silica gel. The next morning any remaining liquid was evaporated with nitrogen. 2.0 ml of 30–60° petroleum ether was added, the residue triturated and centrifuged. The petroleum ether supernate was decanted, evaporated with nitrogen, and dissolved in 0.1 ml chloroform or light petroleum ether for GLC. The resulting structures of digitoxigenin-TMS and digoxigenin-TMS may be as shown in Fig. 2.

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1 mg of each of the other compounds studied was dissolved in 0.1 ml chloroform or tetrahydrofuran for GLC*.

Results

Digitoxigenin-TMS and digoxigenin-TMS each gave single, separate, reproducible gaussian peaks on a column having 400 theoretical plates for cholestane (Fig. 3). Chromatographic conditions are given in the figure. Retention times were: cholestane 6.2 min; digitoxigenin-TMS 37.5 min; digoxigenin-TMS 47.5 min. The separation factor of digitoxigenin-TMS and digoxigenin-TMS was 1.26.

GLC of digitoxin, digoxin, and digoxigenin gave no useful peaks at all. GLC of

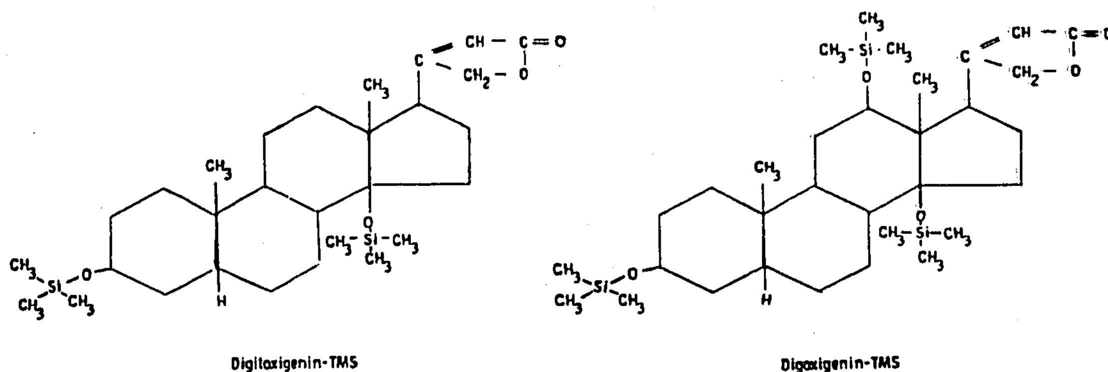

Fig. 2. Probable structures of digitoxigenin-TMS and digoxigenin-TMS.

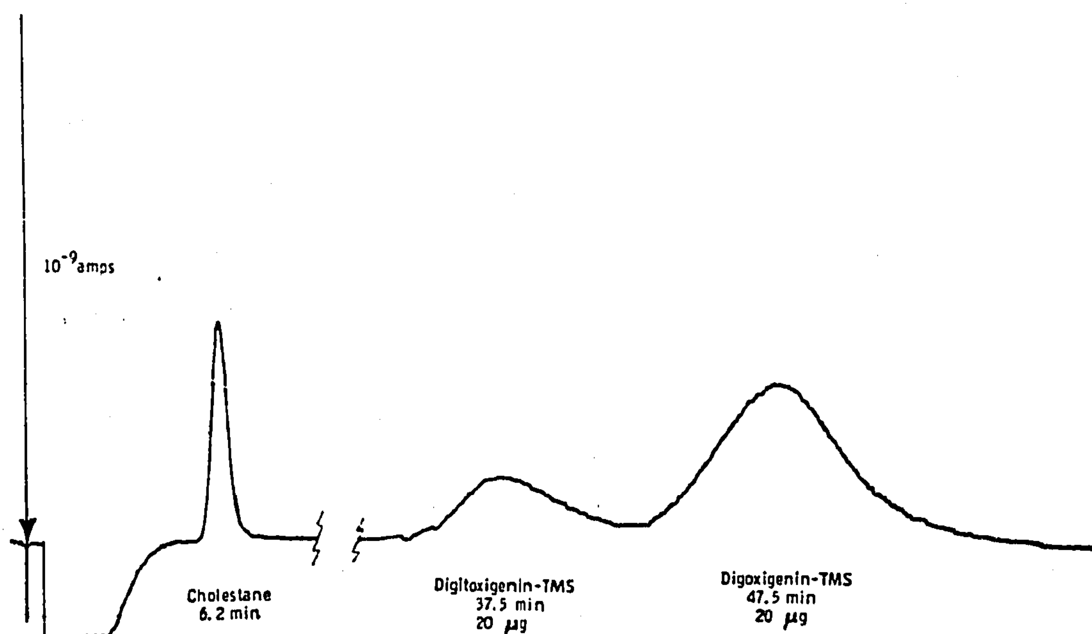

Fig. 3. Separation of digitoxigenin-TMS from digoxigenin-TMS by gas–liquid chromatography. A cholestane reference marker has been included in the mixture for chromatography. The chromatogram has been interrupted between cholestane and digitoxigenin-TMS to save space in the illustration. Column: 12 ft. 4 mm I.D. 0.75 % SE-30, on 100–140 mesh Gas Chrom P. Column temp.: 228°. Detector temp.: 220°. Flash heater temp.: 348°. Detector volts: 1000. Inlet pressure: 40 lbs. Gas flow: 104 ml/min. Electrometer gain: 10^{-9} A.

* Digitoxigenin, digoxigenin, and digoxin were generously supplied by Burroughs-Wellcome and Company.

digitoxigenin gave a characteristic series of peaks which might have some use for identification purposes. These peaks were felt to represent pyrolysis products. For these studies, the column temperature was 249°, detector temperature 212°, flash heater temperature 307°, detector volts 1000, inlet pressure 60 p.s.i., and argon flow 130 ml/min.

Discussion

To our knowledge this represents the first separation of digitalis compounds by GLC. Amounts of TMS derivatives at present detectable permit the potential application of this method to urinary digitalis excretion studies. Hydrolysis of urinary glycosides to their aglycones may be required.

Potential determination of blood digitalis levels in patients on maintenance digitalis therapy will probably require at least a one-thousand fold increase in sensitivity. The bioassay method of FRIEDMAN AND BINE[9], which is sensitive to 0.05 μg/ml of serum, permits detection of digitalis in blood for 2 to 3 h following an intravenous full digitalizing dose of digitoxin, and cannot detect digitalis in the blood of patients on oral maintenance therapy. It seems desirable that a method for blood analysis be at least capable of detection of 0.01 μg in 10 to 20 ml of blood. An attempt to obtain this increased sensitivity by production of halogenated aglycones for GLC, using electron capture detection, is at present in progress.

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