Mono-*O*-isopropylidine Derivatives of Digitoxin, Digoxin, and Ouabain

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The mono-*O*-isopropylidene derivatives of digitoxin, digoxin, and ouabain have been prepared. In the case of digoxin, this derivative was more lipid soluble than the parent compound and more water soluble than digitoxin or acetyldigitoxin. All derivatives were less active than the parent glycosides when they were assayed intravenously in cats. Mono-*O*-isopropylidene digoxin was 50 per cent absorbed when administered orally.

IT IS WELL KNOWN that digitoxin taken orally is completely absorbed (1), whereas this is not the case with digoxin. Gitoxin and ouabain are very poorly absorbed if at all. Acetyldigoxin is better absorbed than is digoxin when orally administered to cats (2). Evidence in the literature to date strongly supports the concept that the lipophilic properties of the cardiac glycosides (3) are an important factor in the degree to which they are absorbed orally.

Although digitoxin is well absorbed orally, its prolonged duration of action and cumulative properties sometimes are not desirable. Digoxin's duration of action and cumulative properties are more favorable; however, its oral absorption is less dependable as measured by its oral dose *versus* its LD_{50} in intravenous assays in cats. MLD is 230 ± 10 mcg./Kg. for digoxin in cat assays and an average oral dose for humans is 0.5 mg. *versus* MLD 330 ± 8 mcg./Kg. for digitoxin in cat assays and an average oral dose for humans is 0.1 mg. Therefore, it seemed of interest to explore the possibilities of preparing a derivative of digoxin whose lipophilic properties would be enhanced with the retention of effective hydrophilic properties. Dioxolanes and dioxanes enhance the lipophilic properties of the parent compound together with the retention of some hydrophilic properties *via* hydrogen bonding to the two ether oxygens.

DISCUSSION

The cardiac glycosides digoxin and digitoxin have two free adjacent *cis* hydroxyl groups on the terminal digitoxose residue that should yield monodioxolanes upon proper treatment. The isopropylidene derivatives are the most readily prepared dioxolanes under mild conditions that should preclude any undesirable changes in the structures essential for cardiac activity. The sugar residue of ouabain also should lend itself to the preparation of a mono-*O*-isopropylidene derivative. The method that yielded the best results utilized anhydrous copper sulfate, anhydrous acetone, room temperature, and continuous shaking. The course of the reaction was followed by paper chromatography. In the case of digoxin, the mono-*O*-isopropylidene derivative was mobile on formamide-impregnated paper, whereas digoxin remained at the starting line when formamide-saturated benzene was used as the mobile phase (Fig. 1). The use of this simple system led to erroneous results because no amount of time and anhydrous copper sulfate would eliminate some material at the starting line. The use of solvent system II (4) demonstrated that after the most effective reaction conditions, the substance at the starting line (with benzene as the mobile phase) moved faster than digoxin (Fig. 2). This product was very minor in amount and as yet has not been characterized. All attempts at fractional crystallization failed to yield a homogeneous mono-*O*-isopropylidene product even though in the case of digoxin and digitoxin this derivative was quite soluble in anhydrous ether. Separation of the minor by-product was effected by the use of a formamide-impregnated microcrystalline cellulose[1] column and benzene saturated with formamide as the eluant or by Whatman No. 31 paper and preparative paper chromatography methods.

All the mono-*O*-isopropylidene derivatives showed greater lipid solubility properties when compared to the parent glycosides as measured by their pronounced solubility in ether and their movement on formamide-impregnated paper with solvent system II (Figs. 2 and 3). This increase in lipid solubility was greatest with digoxin and was about midway between digitoxin and acetyldigitoxin. When examined by a reverse phase paper chromatographic system (5), digoxin showed greater water solubility than mono-*O*-isopropylidene digitoxin or acetyldigitoxin (Fig. 4). Mono-*O*-isopropylidene ouabain still exhibited the greatest water solubility (Fig. 5). All these derivatives gave a positive Raymond reaction which suggests that the α,β-unsaturated lactone ring was still intact. These changes in water and liposolubilities would suggest that mono-*O*-isopropylidene digoxin probably has the more favorable lipophilic–hydrophilic balance for good oral absorption.

A preliminary screening for cardiac activity was carried out in pigeons and activity still was present although of a lower order. Intravenous assays

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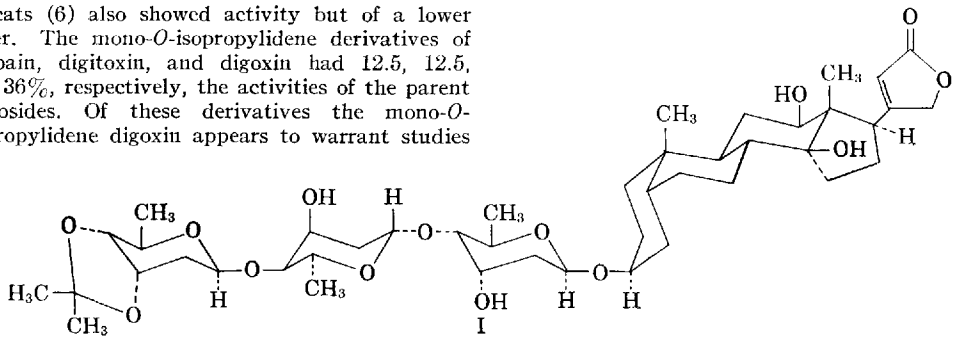
[1] Marketed as Avicel by American Viscose Co., Marcus Hook, Pa.

in cats (6) also showed activity but of a lower order. The mono-*O*-isopropylidene derivatives of ouabain, digitoxin, and digoxin had 12.5, 12.5, and 36%, respectively, the activities of the parent glycosides. Of these derivatives the mono-*O*-isopropylidene digoxin appears to warrant studies



3‴,4‴-*O*-Isopropylidene Digoxin

on its oral absorption because of its physical properties. Although it has 36% of the activity of digoxin, its degree and reliability of absorption might more than offset the reduction in activity. Its toxicity and duration of action also would have to be considered. These might be modified in view of the fact that some cyclic ketals are stable *in vivo* and also might be true of this compound. Thus, the rate of hydrolysis *in vivo* of the sugar residues and other *in vivo* transformations might be altered to provide favorable or unfavorable biological effects.

EXPERIMENTAL

The details of some of the paper chromatographic techniques used in these studies have been described previously (4). Solvent system II was used to very good advantage for the development of some of the paper chromatograms. The original glycosides as well as the reaction products separated effectively by this mobile system. Benzene saturated with formamide provided a simple and rapid mobile system to detect the extent of isopropylidene formation on Whatman No. 1 paper treated with 30% formamide in acetone. It also was used as a very fast moving system for preparative paper and powdered cellulose column studies. A descending reverse system described by Tschesche *et al.* (5) was used to compare the relative hydrophobic properties of the parent glycosides and their

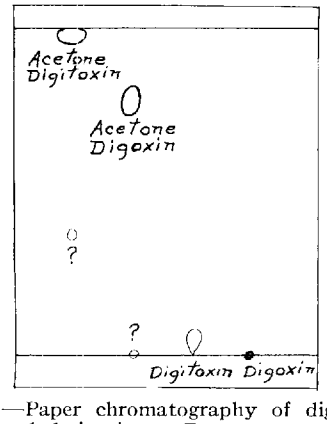


Fig. 1.—Paper chromatography of digoxin, digitoxin, and derivatives. Benzene as mobile phase.

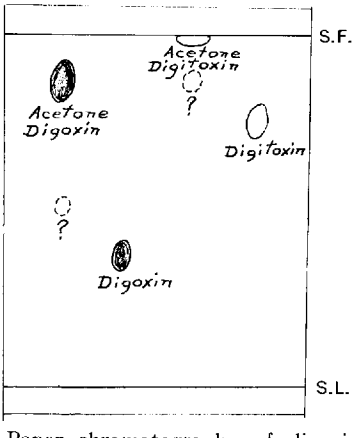


Fig. 2.—Paper chromatography of digoxin, digitoxin, and derivatives. System II as mobile phase.

isopropylidene derivatives. The Raymond reagent was used to detect the glycosides and their reaction products on paper.

Preparation of Isopropylidene Derivatives.—Reagent grade acetone was dried overnight with anhydrous calcium sulfate. It then was carefully decanted or filtered into a flask that was dried in the oven at 110°. (*Caution:* no powdered calcium sulfate should be in the distilling flask.) The acetone was distilled into a 250-ml. conical flask equipped with a drying tube. A forerun of 20% was discarded and subsequently 200 ml. of acetone was collected. The dry glycoside, 100 mg. of digoxin or ouabain or 400 mg. of digitoxin, was added to the flask and the flask carefully heated and shaken to dissolve as much as possible of the glycoside. After cooling to room temperature, 5 Gm. of anhydrous copper sulfate (Allied Chemical) was added. The flask was stoppered with a close fitting stopper and the mixture shaken continuously. The course of the reaction was followed by examining a sample by paper chromatography using formamide-impregnated paper and solvent system II. A period of 22 to 24 hr. of shaking was necessary to completely eliminate the presence of the parent glycoside, although in all cases a small amount of some other slower moving product also could be detected. This slower moving product gave an initial blue color with the Raymond reagent

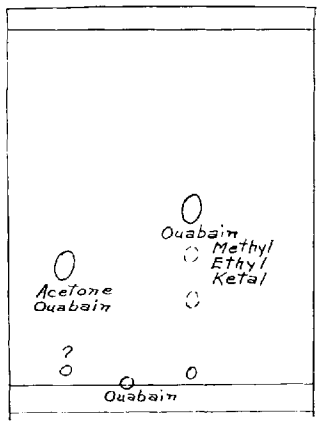


Fig. 3.—Paper chromatography of ouabain and derivatives. System II as mobile phase.

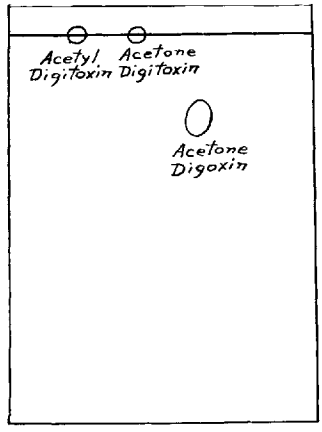


Fig. 4.—Reversed phase paper chromatography of derivatives of digoxin and digitoxin.

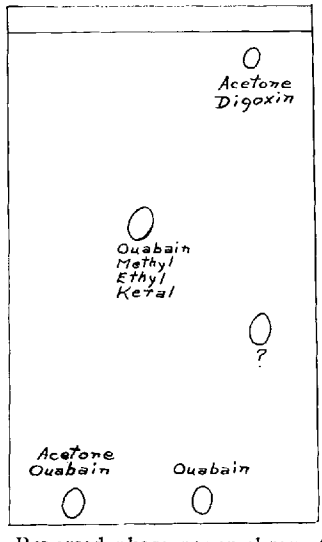


Fig. 5.—Reversed phase paper chromatography of ouabain and derivatives of ouabain and digoxin.

but very shortly turned a yellow green. This was not true with the faster moving isopropylidene derivative that retained its blue color as do the parent glycosides. The reaction mixture then was allowed to stand to permit most of the copper sulfate to settle out, after which time it was filtered through a dense filter paper to remove all the very finely divided suspended copper sulfate. The acetone was removed by distillation with the aid of a water pump and in the absence of air. The amorphous residue readily dissolved in 35 ml. of anhydrous ether in the case of digoxin and digitoxin; somewhat more ether was necessary in the case of ouabain. The ether was concentrated until turbidity appeared. The flask was stoppered, and upon standing a crystalline product was obtained. Upon further concentration, and in the case of digitoxin and digoxin the addition of a judicious amount of isopropyl ether, additional amounts of the crystalline product were obtained. In the case of ouabain the first and second crops of crystals exhibited homogeneity as manifested by their behavior on paper chromatograms using solvent system II and formamide-impregnated paper. Mono-*O*-isopropylidene ouabain melted at 174°.

Anal.—Calcd. for mono-*O*-isopropylidene ouabain, $C_{32}H_{48}O_{12}$: C, 61.54; H, 7.70. Found: C, 61.64; H, 7.42.

In the case of digitoxin and digoxin all attempts to fractionally crystallize their isopropylidene derivatives yielded products that still showed the presence of a small amount of a second product.

An attempt was made to improve upon the above described general method by adding drying agents anhydrous calcium sulfate or a molecular sieve[2] type 3A, to the reaction mixture. In the first case no advantage was gained in the yield of the acetonide. Furthermore, diacetone alcohol was obtained upon removal of the acetone. This substance moved with the solvent front on a paper chromatogram and gave a violet color. A combination of anhydrous calcium sulfate and a small crystal of *p*-toluenesulfonic acid was not satisfactory. In the case of the use of a molecular sieve, quite unsatisfactory results were obtained.

Prolonged shaking beyond 24 hr. did not lead to complete conversion of the glycosides to the acetonides. As the time of shaking was increased, the isopropylidene derivative slowly was converted to a secondary product. This was demonstrated by paper chromatography when the single spot of the original product appeared as two adjacent spots. About equal intensity of these two spots (Raymond reaction) was obtained after 120 hr. of shaking. No further investigations have been completed in this area at the time of this writing.

The general procedure used for the preparation of the isopropylidene derivatives was used with methyl ethyl ketone with analogous results. The derivatives were more lipid soluble and less water soluble than the acetonides. At the present time these derivatives have not been prepared in a homogeneous state. These studies have been suspended until more data have been obtained on the biological activities of the acetonides.

Purification of Mono-O-isopropylidene Digitoxin.—Microcrystalline cellulose,[1] 15 Gm., was

[2] Linde Molecular Sieves, Union Carbide Corp., Linde Division, New York, N. Y.

mixed with 25 ml. of 30% formamide and carefully dried on a large watch crystal with constant stirring. The microcrystalline cellulose then was intimately mixed with an equal volume of finely powdered quartz. A column 8 × 2.2 cm. was packed in a tube 10 × 2.2 cm. Mono-*O*-isopropylidene digitoxin, 100 mg., was powdered and intimately mixed with a small amount of the cellulose–quartz mixture and placed at the top of the above column. The column then was developed with benzene saturated with formamide. One-milliliter fractions were collected and those from 1 to 8 contained only mono-*O*-isopropylidene digitoxin (detected by paper chromatography). Fraction 16 contained only digitoxin and some of the intermediate fractions contained mixtures. This experiment was repeated three more times, and the rate of flow of the benzene held to 15 drops/min. Similar results were obtained as with the first trial. The mono-*O*-isopropylidene digitoxin fractions from all the runs were dissolved in a small amount of methylene chloride, isopropyl ether was added, and the methylene chloride was removed by distillation. The mono-*O*-isopropylidene digitoxin readily crystallized and appeared to be homogeneous by paper chromatographic analysis. It melted at 213–215°.

Anal.—Calcd. for mono-*O*-isopropylidene digitoxin, $C_{44}H_{68}O_{13}$: C, 65.60; H, 8.44. Found: C, 66.11; H, 8.95.

Purification of Mono-O-isopropylidene Digoxin.—When the same column technique as applied to mono-*O*-isopropylidene digitoxin was applied in an attempt to purify this derivative, very unsatisfactory results were obtained. This was not expected because of the similar results that were obtained on paper under the same conditions where only the desired derivative moved on paper impregnated with formamide and benzene saturated with formamide as the mobile phase. As an expediency measure, purification *via* Whatman No. 31 paper was carried out using the descending technique. The crude acetone digoxin product was heavily spotted all along the starting line which was 10 cm. from the end of the paper. This fast running paper provided complete elution of the mono-*O*-isopropylidene digoxin within 1 hr. with the retention of the unwanted products at the starting line. Two and even three starting lines, 2 cm. apart, equally impregnated with the same amounts of crude acetonated digoxin on the same length of paper gave results equally as good, with the retention of the unwanted products at each of the starting lines. Concentration of the benzene eluates left an amorphous residue that was stirred with water several times to remove the small amount of formamide that was present. This resulted in a solid product that gave a single spot on a paper chromatogram. Crystallization of this product was most difficult. A crystalline product readily can be obtained directly from the acetonation reaction; however, it will contain a small amount of the secondary reaction product. It melted at 155°.

Anal.—Calcd. for mono-*O*-isopropylidene digoxin, $C_{44}H_{68}O_{14}$: C, 64.31; H, 8.28. Found: C, 63.70; H, 8.14.

Establishment of Ketal Structure.—The periodate-benzidine test (7) on paper can be used very effectively to detect the presence of a *cis* glycol structure. The substance is spotted on paper and then sprayed with a saturated aqueous solution of potassium metaperiodate. After a suitable time it then is sprayed with a benzidine reagent (10 vol. of 0.1 *M* benzidine in 50% aqueous ethanol is mixed with 2 vol. of acetone and 1 vol. of 0.2 *N* HCl). The presence of a *cis* glycol structure is indicated by a colorless area surrounded by a blue background. Four milligrams each of digoxin, mono-*O*-isopropylidene digoxin, digitoxin, mono-*O*-isopropylidene digitoxin, ouabain, and mono-*O*-isopropylidene ouabain were dissolved in 1 ml. each of methanol. Equal quantities of each of these solutions were spotted on Whatman No. 1 paper and sprayed with the periodate solution. In the case of digoxin and digitoxin and their mono-*O*-isopropylidene derivatives a 5-min. waiting period was necessary. The parent glycosides all gave a positive test for the presence of a glycol structure, whereas the mono-*O*-isopropylidene derivatives gave a negative test.

It should be pointed out here that much of the benzidine in this reagent will crystallize out upon standing a few hours. If the mixture is warmed the benzidine will redissolve, and this reagent can be used directly with satisfactory results.

Bioassay of the Glycoside Acetonides.—These derivatives were assayed by Chen (6), who obtained the following mean $LD_{50} \pm$ S. E. mg./Kg. values. Ouabain acetonide, 0.8837 ± 0.0714 (10 cats); digitoxin acetonide, 2.520 ± 0.2147 (6 cats); and digoxin acetonide, 0.6392 ± 0.0356 (10 cats).

Subsequent to the review of this manuscript, oral studies (6) in cats indicated that mono-*O*-isopropylidene digoxin had 50% absorption from the jejunum which is the same as digoxin.

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