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Synthesis and Biological Activity of the Ketals of Digitoxigenone and Digoxigenone and Some Acetals of Digitoxigenin and Digoxigenin

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Abstract □ The preparation of the 3,3-ethylenedioxy derivatives of digitoxigenone and 3-dehydrodigoxigenin; and the 3β-1′-(6′-*O*-acetyl-2′,3′,4′-trideoxyaldohexopyranosyl) derivatives of digitoxigenin and digoxigenin have been described. Their pharmacological activities have been determined.

Keyphrases □ Digitoxigenone, digoxigenone ketals—synthesis □ Digitoxigenin, digoxigenin acetals—synthesis □ Pharmacological screening—cardiac aglycone derivatives □ IR spectrophotometry—structure

The minimal essential structural requirements for maximum cardiotonic activity of the cardiac glycosides is present in the genin digitoxigenin. Although the sugar residues at C-3 play a secondary nonessential role in the biological activities of the cardiac glycosides, their contribution to physical, as well as other properties, may be quite significant. For maximum activity, the hydroxyl group at C-3 should have the β-(axial) configuration that is present in many naturally occurring cardiac glycosides (1). An appreciable loss in activity is found in the α-(equatorial) epimers, especially 3-epidigoxigenin, which is almost devoid of activity (2). An intact glycosidic linkage at the C-3 hydroxyl group is no bar to the biological activity of the cardiac glycosides. This is because they exert a positive and powerful effect upon certain sodium- and potassium-activated ATPases in *in vitro* studies (3). One might thus postulate that the unshared pairs of electrons on the oxygen function at C-3 are one of the essential parameters for activity. It thus would be of interest, from the cardiotonic activity point of view, to test the activity of structurally related derivatives of the cardiac aglycones possessing an oxygen function at both the β- and the α-configurations at C-3. A cyclic ketal of the C-3 keto genins would provide the desired test compound. To the authors' knowledge, such a semisynthetic aglycone derivative has not been prepared. The authors therefore have prepared, for biological testing, the ethylene ketals of digitoxigenone and digoxigenone.

Recently it has been reported (4) that the completely deoxygenated tetrahydropyranyl derivatives of some cardiac aglycones are less active than the parent aglycones, and that deoxygenation in the sugar component also leads to decreased potency. The partially deoxygenated tetrahydropyranyl derivatives, preparation of which is described in this communication, are of considerable interest because they represent a stage between the significantly active monoglucoside (5) derivatives and the less active completely deoxygenated ones. These derivatives of digitoxigenin and digoxigenin were prepared from 2-hydroxymethyl-2,3-dihydro-4*H*-pyran (V), a racemic dideoxyglucal.

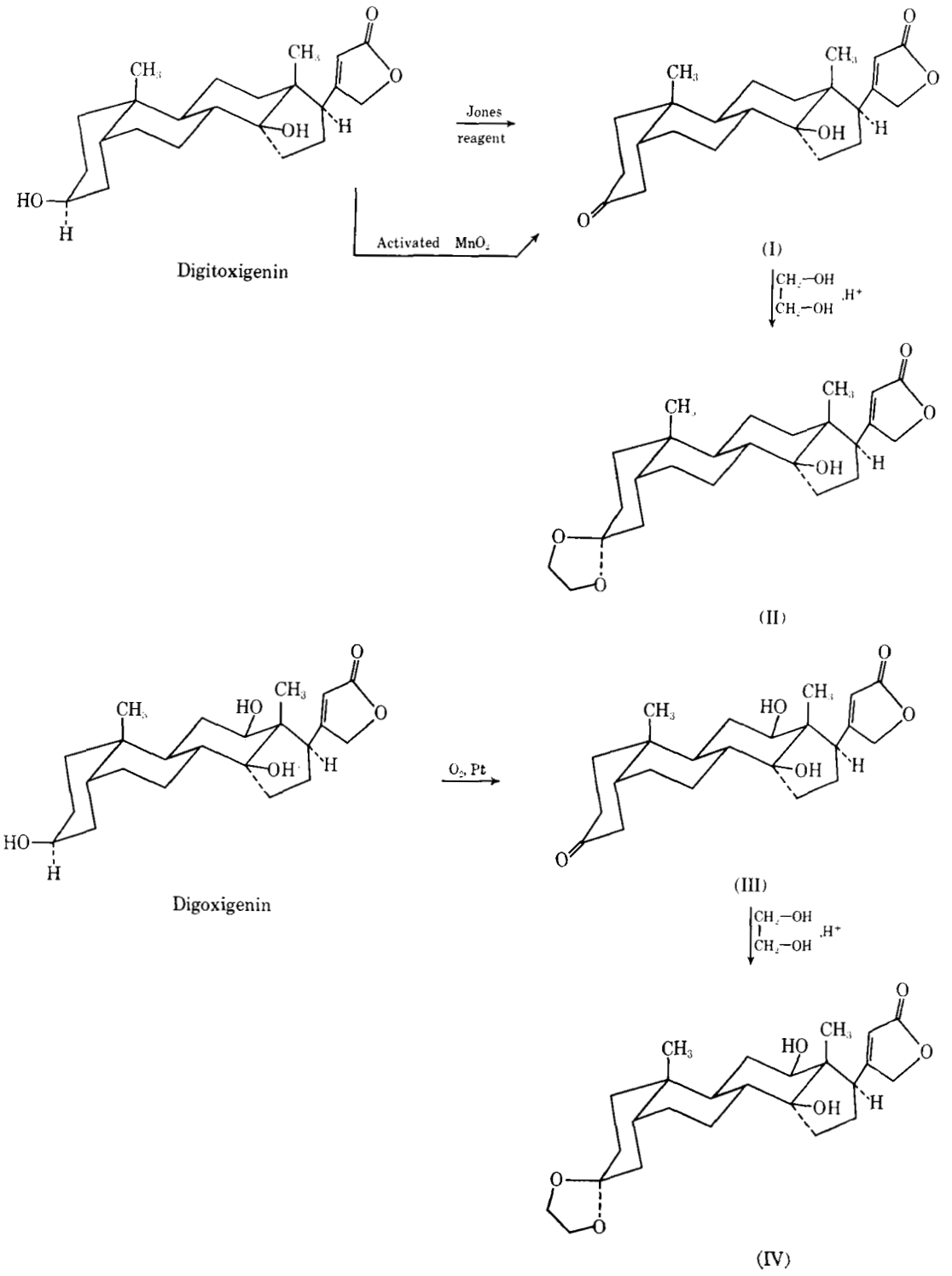
The ethylene ketals were prepared primarily by the method described by Dean and Christiansen (6) (Scheme I).

To gain some experience with the Peterson and Gisvold method for preparing tetrahydropyranyl derivatives (7), cholesterol was used as a model compound (Scheme II). All attempts to condense compound V with cholesterol were unsuccessful. Intramolecular reaction of the primary alcohol function with the enol ether double bond (8), or intermolecular condensation to produce the polymer (VII), are possible explanations. These condensations would compete with the acid-catalyzed addition of cholesterol to the enol ether double bond. This view is supported by the authors' finding that conversion of V to the acetate (VIII) circumvents the difficulty.

EXPERIMENTAL

Digitoxigenin—Two grams of digitoxin was hydrolyzed by the method of Yamada (2). The digitoxigenin thus obtained was recrystallized twice from ethyl acetate yielding 740 mg. (73%) of digitoxigenin, m.p. 251–253°, reported (9) 253–255°.

Digitoxigenone (I)—*Method a: Oxidation Using Jones Reagent* (2)—Digitoxigenin 280 mg., dissolved in 38 ml. of acetone (previously distilled with potassium permanganate), was cooled to 10°. Twenty-five hundredths milliliter of Jones reagent (10) was added rapidly while the mixture was stirred vigorously. After 2 min. the reaction mixture was diluted with 40 ml. of water. The product was extracted with ethyl acetate, the extract was washed successively with 5%



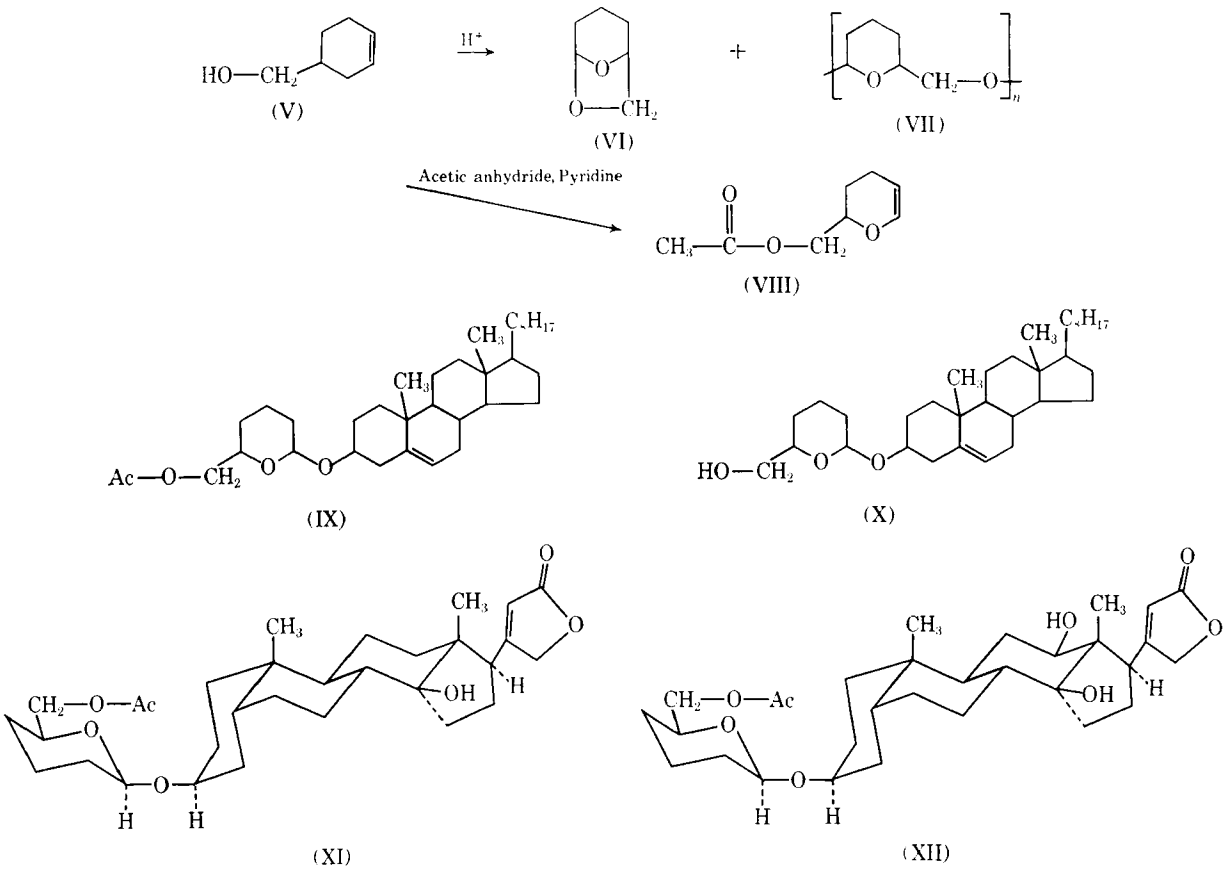
Scheme I

sodium bicarbonate solution and water, and was dried over sodium sulfate. Removal of the solvent yielded a residue that was crystallized from acetone. Yield 235 mg. (85%) of rod-shaped crystals, m.p. 201–203°. Reported (2) 203–204°. IR spectrum[1] (KBr pellet) shows characteristic peaks at 3,470 cm.$^{-1}$ (OH); 1,785, 1,765–1,740 cm.$^{-1}$ (C=O of the lactone ring); 1,715 cm.$^{-1}$ (C=O of C-3); 1,625 cm.$^{-1}$ (C=C).

[1] All IR spectra were recorded on a 237B Perkin-Elmer grating spectrophotometer.

Method b: Oxidation Using Activated Manganese Dioxide—Activated manganese dioxide (11) (5 g.) was added to 250 mg. of digitoxigenin dissolved in 50 ml. of acetonitrile. The mixture was shaken for 21 hr. at room temperature, filtered, and the acetonitrile removed *in vacuo*. The residue was crystallized from acetone and 200 mg. of white rods, m.p. 185–187°, were obtained. Two recrystallizations from acetone gave 150 mg. yield (62%), m.p., 199–201°. Mixed melting point with the ketone of the previous oxidation showed no depression.

3,3-Ethylenedioxy-14β-hydroxy-20(22)-cardenolide (II)—Digitoxigenone (I, 180 mg.) was ketalized by azeotropic distillation for 5



Scheme II

hr. in 85 ml. of benzene with 15 mg. of *p*-toluenesulfonic acid monohydrate and 7 ml. of ethylene glycol. Crystallization of the crude solid product from a mixture of benzene and excess ether afforded 160 mg. of white fine needles. Yield 88%, m.p. 249–252°. A sample for analysis was recrystallized twice from ether and dried *in vacuo* at 80° for 24 hr. and had a m.p. of 253–256.5°. Infrared spectrum (KBr pellet) showed characteristic peaks at 3,460 cm.$^{-1}$ (OH); 1,780, 1,765–1,740 cm.$^{-1}$ (C=O of the lactone ring); 1,626 cm.$^{-1}$ (C=O); 1,115, 1,105, 1,090, 1,085 cm.$^{-1}$ (C—O—C); absence of the 1,715 cm.$^{-1}$ peak of the C-3 keto group.

Anal.—Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 72.30; H, 8.40.

Digoxigenin—Two grams of digoxin was hydrolyzed by the same method used for digitoxin. The digoxigenin thus obtained was recrystallized from ethyl acetate containing a trace of methanol. Yield 850 mg. (85%) of digoxigenin, m.p. 209–211°. Two recrystallizations afforded 780 mg., m.p. 219–221°.

3-Dehydrodigoxigenin (III)—Digoxigenin 760 mg. was oxidized by the method of Tamm and Gubbler (12) using oxygen and platinum. The product was crystallized from acetone. Yield (84%), m.p. 253–255°, reported (12) 248–253°. IR spectrum (mineral oil) shows characteristic peaks at 3510, 3,485 cm.$^{-1}$ (OH); 1,770, 1,745–1,725 cm.$^{-1}$ (C=O of the lactone ring); 1,700 cm.$^{-1}$ (C=O of C-3); 1,622 cm.$^{-1}$ (C=C).

3,3-Ethylenedioxy-12β,14β-dihydroxy-20(22)-cardenolide(IV)—3-Dehydrodigoxigenin (III, 200 mg.) was ketalized by azeotropic distillation for 7 hr. in 85 ml. of benzene with 18 mg. of *p*-toluenesulfonic acid monohydrate and 7 ml. of ethylene glycol. Crystallization of the crude solid product from methanol afforded 135 mg. (67%) of white prismatic crystals, m.p. 243–247°. A sample for analysis was recrystallized from a trace of methyl alcohol in ether. When dried *in vacuo* at 85° for 18 hr. it gave a m.p. of 249–252°. Mixed m.p. with the ketone 3-dehydrodigoxigenone gave a m.p. of 225–237°. IR spectrum (mineral oil) shows characteristic peaks at 3,505, 3,480 cm.$^{-1}$ (OH); 1,775, 1,732 cm.$^{-1}$ (C=O of lactone ring); 1,612 cm.$^{-1}$ (C=C); 1,140, 1,095, 1,082, 1,075 cm.$^{-1}$ (C—O—C); absence of the 1,700 cm.$^{-1}$ peak of the C-3 keto group.

Anal.—Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.00; H, 8.65.

2-Acetoxymethyl-2,3-dihydro-4H-pyran (VIII)—2-Hydroxymethyl-2,3-dihydro-4*H*-pyran(V) was acetylated by the method of Zelinsky *et al.* (8) and the product was obtained in 54% yield, b.p. 55° at 1 mm.; n_D^{20} 1.4578.

3β-1′-(6′-O-Acetyl-2′,3′,4′-trideoxyaldohexopyranosyl)-Δ^5-cholestene (IX)—Cholesterol 1 g. was dissolved in 10 ml. of chloroform and 0.4 ml. of 2-acetoxymethyl-2,3-dihydro-4*H*-pyran (VIII), one capillary drop of phosphorus oxychloride was added and the solution warmed to 40° for 0.5 hr. The solution was allowed to cool and then diluted to about 100 ml. with ether. The mixture was washed with a cold dilute solution of sodium bicarbonate followed by a washing with water. After drying over sodium sulfate, the solvents were removed *in vacuo* and the residue crystallized from ethyl alcohol containing few drops of acetone. Yield 880 mg. (86%) of white feathery crystals, m.p. 79–81°. A sample for analysis was prepared by recrystallization from a mixture of acetone and ethanol. When dried at 50° for 18 hr. it melted at 86–88°.

Anal.—Calcd. for $C_{35}H_{58}O_4$: C, 77.44; H, 10.77. Found: C, 77.23; H, 10.70.

3β-1′-(2′,3′,4′-Trideoxyaldohexopyranosyl)-Δ^5-cholestene (X)—Compound IX (0.267 g.) was dissolved in a mixture of 7 ml. of methanol and 7 ml. of ethanol and cooled in an ice bath. Six milliliters of 0.1 *N* sodium hydroxide solution was added. The solution was kept for 15 hr. at refrigerator temperature, diluted with ether, and washed with water until the washings were neutral. The ethereal layer was dried over sodium sulfate, solvent removed *in vacuo* and the residue crystallized from ether containing a trace of methanol. Two hundred and ten milligrams of white crystalline compound, m.p. 130–132° was obtained. A sample for analysis was recrystallized from ether and dried *in vacuo* at 40° for 24 hr.; m.p. 131–132.5°; yield 75%.

Anal.—Calcd. for $C_{33}H_{56}O_3$: C, 79.14; H, 11.27. Found: C, 79.25; H, 11.85.

3β-1′-(6′-O-Acetyl-2′,3′,4′-trideoxyaldohexopyranosyl)-14β-hydroxy-20(22)-cardenolide(XI)—Digitoxigenin 300 mg. was

dissolved in a mixture of 10 ml. chloroform and 1 ml. of 2-acetoxymethyl-2,3-dihydro-4*H*-pyran(VIII), one drop of a solution of 2 drops of phosphorus oxychloride in 10 ml. ethyl acetate was added and the solution warmed to 50° for 0.5 hr. After the solution had cooled to room temperature, the chloroform was removed *in vacuo*, 20 ml. of aliphatic naphtha[2] was added and solution cooled in the freezer. The semisolid material that separated was collected and dried in a desiccator over sulfuric acid for 2 hr. The derivative then was crystallized from a few drops of ether and 10 ml. of isopropyl ether; m.p. 189–194°; yield 239 mg. (62%). A sample for analysis was crystallized twice from isopropyl ether and dried *in vacuo* at 50° for 24 hr., m.p. 200–202°.

Anal.—Calcd. for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74. Found: C, 70.07; H, 9.38.

3β - 1′ - (6′ - O - Acetyl - 2′,3′,4′ - trideoxyaldohexopyranosyl)-12β,14β-dihydroxy-20(22)-cardenolide(XII)—Digoxigenin 500 mg. was dissolved in 10 ml. of ethyl acetate and 10 ml. of chloroform. The solution was cooled and 1.5 ml. of 2-acetoxymethyl-2,3-dihydro-4*H*-pyran(VIII) was added, followed by the addition of six drops of a solution of two drops of phosphorus oxychloride in 10 ml. ethyl acetate. The solution was warmed to 50° for 10 min. After cooling the solution to room temperature, the solvents were removed *in vacuo* and the residue was dissolved in 30 ml. of aliphatic naphtha and cooled in the freezer. A semisolid material that separated was collected by decanting off the naphtha. This process was repeated twice. The semisolid product was dissolved in 3 ml. of benzene and, when an excess of ether was added, 350 mg. of white fine powder, m.p. 132–135°, was obtained. A sample for analysis was recrystallized from ether and dried *in vacuo* at 40° for 24 hr.; yield (55%); m.p. 136–138°.

Anal.—Calcd. for $C_{31}H_{46}O_8 \cdot H_2O$: C, 65.93; H, 8.57. Found: C, 65.73; H, 8.31.

BIOLOGICAL RESULTS

These compounds were assayed for cardiac toxicity by the cat method as described in the "USP XIII" (13). The following minimum lethal doses $\pm SE$ mg./kg. values were obtained: Ethylene ketal of digitoxigenone[3] (II), 3.380 ± 0.03; ethylene ketal of digoxigenone (IV), 1.2 ± 0.2; acetal of digitoxigenin (XI), 0.46 ± 0.01; acetal of digoxigenin (XII), 1.33 ± 0.33; and ouabain, 0.11 ± 0.02.

The compounds were tested for cardiotonic activity by the cat papillary muscle (14). According to the method, the muscle was mounted in a 100 ml. bath containing Krebs-Henseleit solution at 38° and aerated with 5% carbon dioxide in oxygen. The muscle was stimulated by a rectangular pulse at a rate of two pulses per second, voltage and pulse duration were adjusted to obtain maximal contractions which were recorded using a transducer.[4] After recording the control contractions, the muscle was made hypodynamic by changing to a bicarbonate-free low calcium solution and aerating with pure oxygen. When the contractions were down to 30% of the control values, the drug was introduced in a volume less than 1 ml. The doses of the drugs that brought contractions back to control values were found to be: ethylene ketal of digitoxigenone (II), 2.0 mg.; ethylene ketal of digoxigenone (IV), 0.09 mg.; acetal of digitoxigenin (XI), 0.04 mg.; acetal of digoxigenin (XII), 0.03 mg.; and ouabain, 0.08 mg.

No direct correlation could be observed between cardiac toxicity (*in vivo*) and cardiotonic activity (*in vitro*), except that the ethylene ketal of digitoxigenone (II) was the least toxic *in vivo* and the least active *in vitro*. The acetal of digitoxigenin (XI) was much more toxic than the acetal of digoxigenin (XII), *in vivo*, yet their cardiotonic activities were not much different *in vitro*.

Based on *in vivo* studies, the ethylene ketal of digitoxigenone (II) was about three times as toxic as 3-epidigitoxigenin but only one-seventh as toxic as digitoxigenin. Although 3-epidigoxigenin is almost inactive (2), the ethylene ketal of digoxigenone (IV) is one-third as toxic as digoxigenin. It can be concluded that, when an oxygen function is present in the α- and the β-configuration at C-3, in a simple ketal structure of the cardiac aglycones, the compounds retain significant activity. Therefore, the presence of an oxygen function in the α-configuration at C-3 does not inhibit all the activity contributed by the presence of oxygen in the β-configuration at the same position.

The acetals of digitoxigenin and digoxigenin (XI and XII) were significantly active in both *in vivo* and *in vitro* tests. Both are more potent than the completely deoxygenated tetrahydropyranyl derivatives (4) but they are less active than the corresponding, naturally occurring glycosides, digitoxin and digoxin (1). They are much less active than the monoglucoside derivatives (5). It can be concluded that the order of activity of the tetrahydropyranyl ethers of the cardiac aglycones follows the number of oxygen functions, mainly hydroxyl groups, present in the sugar moiety. The fully oxygenated monoglucosides show maximum activity, which decreases gradually as the number of oxygens in the sugar moiety decreases. One finally reaches the tetrahydropyranyl derivatives, which are the least active in the series.

Using ouabain as a reference standard in both instances, *in vitro* activity (cat papillary muscle) was higher than *in vivo* activity (cat method). Direct application of drug to its site of action in *in vitro* tests, rather than administering it to the intact animal, as was done in *in vivo* tests, could account for the difference in activities. Distribution and metabolic factors may play a significant role in the *in vivo* tests.

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[3] H. B. Haag and J. D. Woodley, *J. Pharmacol. Exptl. Therap.*, **51**, 360(1934).
[4] LVDT.

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