Cardiac Glycosides and Aglycones by Synthesis and Microbiological Conversion[1a]

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Pharmacological studies have been made with 20 cardiac glycosides and aglycones elaborated by partial synthesis or microbiological conversion. 20,22-Dihydrodigoxin is qualitatively active, but has $^1/_{18}$ the potency of digoxin (I). In contrast with 3-acetylstrophanthidin, the corresponding ester of digitoxigenin (II) is weaker than digitoxigenin. 14-Dehydroxylation and 14,15-epoxylation of cardenolides (III–VII) cause a loss of digitalis-like action in cats and frogs. 12β-Hydroxylation of resibufogenin (VIII) and marinobufagin (IX), both bufadienolides, confers and preserves a certain degree of activity in cats although resibufogenin itself is inactive. 3-Acetyl-17α-hydroxystrophanthidin (X) has a token cardiotoxic action, but an identical derivative of digitoxigenin (XI) is without effect with twice the dose. The slight decrease of potency of 19-dehydroouabain as compared with ouabain may indicate a favorable influence of the hydroxymethyl group in this most hydroxylated glycoside. Of the D-rhamnosides of digitoxigenin and strophanthidin, the β-glycosidic linkage is superior to the α-configuration. Deoxygenation of the carbohydrate ring of the monoside lowers the cardiotonic activity. The exceptionally high potency of strophanthidin α-L-mannoside can be explained on the same basis as that of convallotoxin.

It is common knowledge that cardiac glycosides can be hydrolyzed to aglycones and carbohydrates. Esterification of the secondary OH group at C-3 of an aglycone or reconstitution with a new sugar has been repeatedly accomplished. Nuclear changes of the aglycone have been affected in other positions by chemical means. Recently microbiological transformations have given rise to a variety of nuclear modifications.[2] During the last few years we had available 20 compounds for pharmacological studies. Eight partially synthetic glycosides listed in Table I were prepared by Zorbach and his co-workers.[3-6a] The chemical characterizations on digitoxigenin and strophanthidin β-D-rhamnosides and the 3-tetrahydropyranyl derivatives of digitoxigenin and strophanthidin have not been published. The two epoxides of digitoxigenin were prepared by partial synthesis,[6b] while that of digoxigenin and 12β-hydroxyresibufogenin and -marinobufagin were microbiological conversion products of *Fusarium lini* obtained by Schüpbach and Tamm.[7] The two isomers of 14-dehydroxy-15-ketodigitoxigenin were synthesized by K. Mayer (as yet unpublished), who gave us permission to mention our results on these two compounds. 3-Acetyl-17α-hydroxydigitoxigenin is an intermediate of totally synthetic digitoxigenin.[8] A corresponding intermediate of strophanthidin was also made available to us for assay by Dr. F. Sondheimer. Dihydrodigoxin was supplied by Dr. W. J. Bowen of the National Institute of Arthritis and Metabolic Diseases and 3-acetyldigitoxigenin by Aldrich Chemical Co., Milwaukee, Wis. 19-Dehydroouabain was first prepared by Mannich and Siewert[9]; our sample was supplied by Professor T. Reichstein.

Methods

The procedures of evaluating the cardiotoxicity of the 20 compounds were the same as those previously employed.[10,11] In brief, a stock solution of each substance in dilute ethanol was prepared, and a suitable dilution was injected intravenously into the femoral vein of the etherized cat. Electrocardiograms were recorded to demonstrate the digitalis-like action. The potency of each active compound was determined in groups of cats: 4 with 3-acetyl-17α-hydroxystrophanthidin, 6 with 3-acetyldigitoxigenin and dihydrodigoxin, and 10 with all others. Their geometric mean lethal doses and standard errors (LD $\pm$ S.E.) were computed and are listed in the middle column of Table I. They can be also converted to the reciprocals (number of mean LD values $\pm$ S.E./mg.) in order to arrive at a direct relationship of potency as shown in the last column. If a compound did not kill the etherized cats in large doses, its stock solution would be injected into the lymph sac of frogs in order to substantiate the absence of activity in another species of animals.

The amount of ethanol to effect solution of the 20 substances varied. Attempts were always made to prepare a 0.1% solution in 47.5–50% alcohol. This was successful with 16 substances. 3-Acetyl-17α-hydroxydigitoxigenin required 71% ethanol, 3-acetyldigitoxigenin 95%, and digitoxigenin β-D-rhamnoside 100%. A stock solution of 0.05% in 55% ethanol was necessary for 14α-dehydroxy-15-ketodigitoxigenin. The final dilutions for the cat work needed much adjustment in order to titrate the end point within 30–60 min./kg. of cat weight. Thus, strophanthidin β-D-rhamnoside and strophanthidin α-L-mannoside were used in a ratio of 1:200,000; digitoxigenin 2-deoxy-β-D-alloside, strophanthidin α-D-mannoside, and 3-tetrahydropyranylstrophanthidin, 1:50,000; dihydrodigoxin, 1:5000; and 14α,15α-epoxy-14-anhydrodigitoxigenin, 14β,15β-epoxy-14-anhydrodigitoxigenin and -digoxigenin, and 3-acetyl-17α-hydroxystrophanthidin, 1:-10,000. The following substances were so insoluble in saline that their stock solutions were intermittently injected into the cats' vein through a three-way microburet[12]: 3-acetyldigitoxigenin, 3-acetyl-17α-hydroxydigitoxigenin, 3-tetrahydropyranyldigitoxigenin, and digitoxigenin β-D-rhamnoside.

Results

It is obvious that all the partially synthetic glycosides are active to various degrees (Table I). 19-Dehydroouabain, 3-acetyldigitoxigenin, 3-acetyl-17α-hydroxystrophanthidin, 12β-hydroxyresibufogenin and -marinobufagin, and dihydrodigoxin are also active, al-

(1) (a) The work at Indiana University was supported in part by U. S. Public Health Service Grant HE-07714 and Indiana Heart Association. (b) To whom all inquiries regarding this paper should be addressed at Indiana University School of Medicine.

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TABLE I

ACTIVITY AS MEASURED BY LD VALUES IN CATS

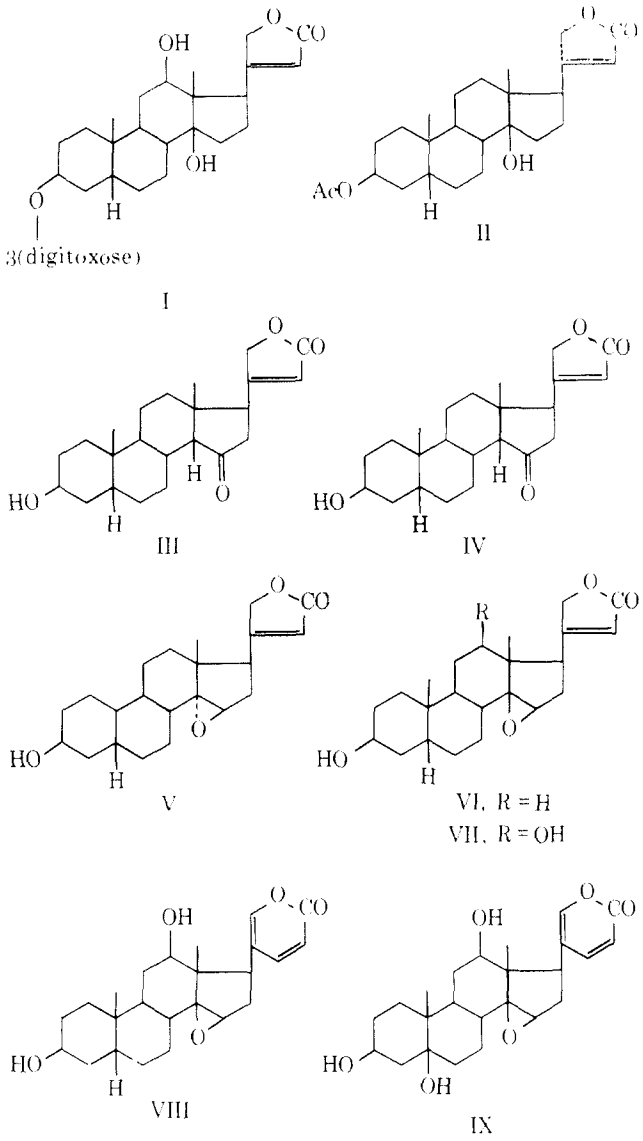
Compd.	Mean LD ± S.E., mg./kg.	No. of mean LD values ± S.E./mg.
Digitoxigenin β-D-rhamnoside	0.3563 ± 0.0387	2.807 ± 0.305
Digitoxigenin 2-deoxy-β-D-alloside	0.1860 ± 0.0102	5.376 ± 0.295
3-Tetrahydropyranyl-digitoxigenin	2.429 ± 0.244	0.412 ± 0.041
14α-Dehydroxy-15-ketodigitoxigenin[a]	1 cat survived 3.666, another 4.695	
14β-Dehydroxy-15-ketodigitoxigenin[a]	1 cat survived 4.845, another 5.875	
14β,15β-Epoxy-14-anhydrodigitoxigenin	1 cat survived 5.88; ECG suggestive	
14α,15α-Epoxy-14-anhydrodigitoxigenin	1 cat survived 5.52	
14β,15β-Epoxy-14-anhydrodigitoxigenin	1 cat survived 11.27	
Dihydrodigoxin	4.1920 ± 0.3680	0.239 ± 0.021
3-Acetyldigitoxigenin	0.8910 ± 0.0670	1.121 ± 0.084
3-Acetyl-17α-hydroxydigitoxigenin	1 cat survived 3.983, another 4.037	
3-Acetyl-17α-hydroxystrophanthidin	2.081 ± 0.3481	0.481 ± 0.080
3-Tetrahydropyranyl-strophanthidin	0.5552 ± 0.0572	1.801 ± 0.185
Strophanthidin β-D-rhamnoside	0.0989 ± 0.0046	10.111 ± 0.470
Strophanthidin α-D-rhamnoside	0.1387 ± 0.0073	7.210 ± 0.379
Strophanthidin α-D-mannoside	0.2538 ± 0.0207	3.940 ± 0.321
Strophanthidin α-L-mannoside	0.0693 ± 0.0057	14.430 ± 1.187
12β-Hydroxyresibufogenin	1 cat died with 4.58, and another with 3.78	
12β-Hydroxymarinobufagin	1 cat died with 3.35, and another with 2.66	
19-Dehydroouabain	0.1649 ± 0.0055	6.064 ± 0.202

[a] Perhaps a better nomenclature should be 3β-hydroxy-15-oxo-5β,14α-card(20:22)enolide and 3β-hydroxy-15-oxo-5β,14β-card-(20:22)enolide, respectively

though the last substance has a very low potency. All the cats showed typical electrocardiographic changes and died with ventricular fibrillation. The two isomers of ketodigitoxigenin, two isomers of the epoxide of 14-anhydrodigitoxigenin, 14β,15β-epoxy-14-anhydrodigoxigenin, and 3-acetyl-17α-hydroxydigitoxigenin showed no cardiotoxicity in the doses indicated in Table I. The results in frogs were also negative with doses varying from 23–54 mg./kg.

Discussion

The results of this series of cardiac steroids reveal certain interesting points. The first is the preservation of activity of dihydrodigoxin (I) in cats, in the ratio of 1:18 to digoxin.[11] Windaus, *et al.*,[13] and



Cloetta[14] reported that hydrogenation of digitalinum verum to saturate the double bond of the lactone ring destroyed the activity on the frog's heart. Jacobs and Hoffmann[15] on the other hand found that in frogs, cymarin was 23 times as effective as dihydrocymarin, and ouabain 16 times as effective as dihydroouabain. Acheson and his associates[16–18] demonstrated in the dog heart-lung preparation the positive inotropic action of dihydroouabain, dihydrodigoxin, and dihydrodigitoxin. Taeschler, *et al.*,[19] confirmed the above observations with dihydroouabain on the isolated auricle of the guinea pig. Bach and Reiter[20] estimated that in the guinea pig the LD of ouabain by continuous infusion was 80 times that of dihydroouabain, and the LD of digoxin 60 times that of di-

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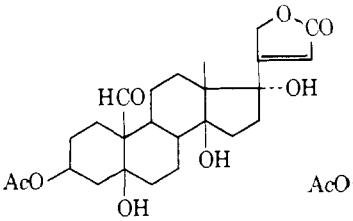
hydrodigoxin. Wright and co-workers[21] reported vastly reduced activity of dihydrodigoxin but found dihydrodigitoxigenin and -digoxigenin to be virtually inactive. In our previous papers[22,23] we showed that doses of 36–66 mg./kg. of dihydrostrophanthidin were unable to kill etherized cats, and doses of 5–6 mg./kg. of the hydrogenated products of cinobufagin and marinobufagin were also not fatal to cats. It appears that saturation of the double bond of the lactone ring greatly reduces the cardiotoxicity of glycosides or aglycones, and in some instances it may completely abolish the digitalis-like effect as in the case of dihydrostrophanthidin.

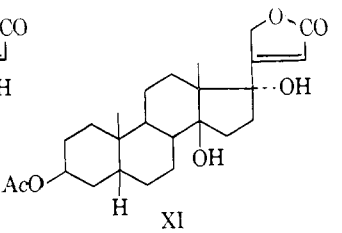
Acetylation of the secondary OH at C-3 (II) decreased the activity of digitoxigenin almost by 50%.[11] It follows the example of 3-acetylscillirosidin,[24] but is contrary to 3-acetylstrophanthidin[25] and several acetylbufadienolides.[10] 3-Acetylation therefore produces unpredictable changes of cardiac potency.

A free tertiary OH at C-14 is consistently present in natural cardenolides and their glycosides. Only recently Meyer and his co-workers[26,27] demonstrated a 14β,15β-epoxide ring in cinobufagin and marinobufagin, both of which are cardiotonically active. In the present series of Table I, 14α,15α-epoxy-14-anhydrodigitoxigenin (V) and 14β,15β-epoxy-14-anhydrodigitoxigenin (VI) and -digoxigenin (VII) are all inactive in the doses studied. The results confirm our previous observations with samples of another source.[28] Wright, *et al.*,[21] stated that β-anhydro- and 14-deoxydigoxigenin had no demonstrable toxicity. 12β-Hydroxyresibufogenin (VIII) and -marinobufagin (IX) are active but of low potency. The electrocardiographic changes are characteristic. It should be pointed out that unaltered resibufogenin[29] has no effect upon the heart. Ragab, *et al.*,[30] prepared 14α- and 14β-artebufogenin in which H replaced OH at C-14 with a keto group at C-15, and found that the β-isomer was active but the α-isomer was inert. They postulated that the *cis* C/D linkage was more important than 14β-OH group for the preservation of cardiac action. Nonetheless, the most potent compounds having 10 LD values/mg. or more in cats invariably have a free 14β-OH group without an exception.

Another pair of interesting aglycones are 3-acetyl-17α-hydroxystrophanthidin (X) and -digitoxigenin (XI). The latter has no cardiac effect in the 4 mg./kg. dose in cats, but the former produces digitalis-like action electrocardiographically and causes death in one-half the dose. The activity, although low, may



X



XI

be due to the presence of 5-hydroxy and 10-aldehyde groups.

19-Dihydroouabain differs from ouabain in that it has an aldehyde group at C-10 instead of a hydroxymethyl group. It has a lower potency than ouabain which registers 8.62 LD values/mg.[27] Such depreciation of action only occasionally occurs as in case of cymarol *vs.* cymarin.[31]

Of the 8 synthetic monosides of digitoxigenin and strophanthidin, several observations can be made. First, the potency of a glycoside depends on the optical rotation of the sugar and the glycosidic linkage. In nature the D-sugars are usually conjugated by β-configuration, and the L-sugars by α-isomerism.[32] If an enantiomorphic form of a natural sugar, such as D-rhamnose, is coupled with a cardenolide, the β-anomer has a higher activity. Thus in our series digitoxigenin β-D-rhamnoside surpasses the potency of the α-D-isomer[11]; and similarly, strophanthidin β-D-rhamnoside is superior to its α-D-isomer (Table I). Espeally significant is the pair of isomers synthesized by Zorbach, *et al.*,[6] namely the mannosides of strophanthidin. Apparently the α-L-configuration contributes far more pharmacological effect than does the α-D.

There is another change in the carbohydrate portion of the molecule that affects the cardiac activity, that is, deoxygenation lowers the potency of monosides, as exemplified by the 3-tetrahydropyranyl derivatives of digitoxigenin and strophanthidin. In fact the superiority of strophanthidin α-L-mannoside over convallotoxin (6-deoxy-α-L-mannoside of strophanthidin) can be explained by the same reasoning.[6] The number of LD values/mg. of digitoxigenin 2-deoxy-β-D-alloside is practically the same as that of its 2-deoxy-β-D-glucoside.[11] These two deoxy glycosides differ from each other only by the configuration at C-3 of the pyranoside which is apparently unimportant biologically.[4]

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