

Some recent progress in the synthetic and medicinal chemistry of cardioactive steroid glycosides

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Abstract - Successful efforts to synthesize highly potent unnatural digitalis derivatives with a high margin of safety, which have been going on at the University of New Brunswick for some years, are reviewed.

INTRODUCTION

Digitalis preparations have been used in the treatment of heart disease for more than 200 years. The cardioactive glycosides contained in these preparations have the ability to slow the heart rate and, at the same time, to increase the contractility of the heart muscle (inotropic activity, connected with the release of Ca^{++}) and thus to improve, in general, the heart function. Because of this effect, the glycosides of digitalis belong to the top ten most prescribed drugs even today.

However, little progress has been made in efforts to decrease the dangerously high toxicity of these compounds. Most patients receive 60% of the toxic dose in order to obtain the desired therapeutic response and, as a consequence, digitalis glycosides are responsible for one-half of all the drug-induced deaths in hospitals.

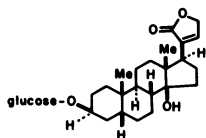
There seem to be two main reasons for the apparent lack of success of medicinal chemists to produce non-toxic cardenolide analogues.

- (1) The synthesis of digitalis cardenolides in the past was a difficult and inefficient process which did not lend itself to the rapid preparation of many derivatives in sufficient quantities for pharmacological testing.
- (2) There was, until recently, wide-spread belief that the inotropic effect and the toxicity of digitalis cardenolides cannot be separated as a matter of principle.

It was believed that both the toxicity and the inotropic effect are a direct result of the inhibition of membrane-bound $\text{Na}^+\text{K}^+\text{-ATPase}$, an enzyme involved in the Na^+K^+ transport across the cell membrane.

The compound "Actodigin" which we have first prepared from digitoxigenin (ref. 1) and later from testosterone (ref. 2) appeared to contradict these views.

"Actodigin" was studied extensively by R. Mendez, G. Pastelin and E. Kabela (ref. 3) in the failing heart of the dog heart lung preparation and in the intact anesthetized dog. It was found that the minimal therapeutic dose of "Actodigin" was a smaller percentage of both the irregularity dose and lethal dose than was the case with ouabaine and dihydroouabaine. Thus "Actodigin" displayed a greater margin of safety than the previously known natural glycosides and beside this, it showed a greater reversibility of the toxic effects. From these findings Dr. Mendez raised the question whether there might not be two separate receptors for the inotropic effect and the toxicity of digitalis glycosides in the heart muscle.



ACTODIGIN

Similar results were obtained somewhat later by investigators (ref. 4) at Columbia University who studied the contractility of isolated cat papillary muscle and isolated Purkynje fibers. Again, all effects were rapidly reversible and an equi-inotropic concentration of "Actodigin" caused significantly less electrophysiologic toxicity than ouabaine. Inhibition of $\text{Na}^+\text{K}^+\text{-ATPase}$ by "Actodigin" was studied by C. R. Ross and N. I. Pessah (ref. 5) who found that the inhibitory effect on this enzyme was completely

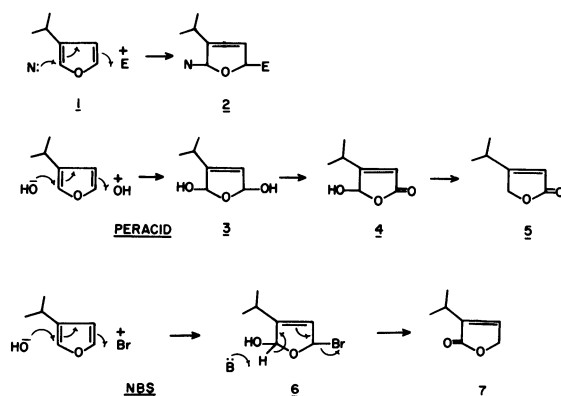
reversible, while the ouabaine inhibition can be only partly reversed. Finally, a few years ago George T. Okita (ref. 6) performed experiments which demonstrated that the inotropic effect and ATPase inhibition of natural digitalis glycosides, when measured independently, show a different time dependence and reversibility. He concluded that there must be two separate receptors in the heart muscle for the toxicity and the inotropic effect.

All this indicated that perhaps the situation was not as bleak as it seemed and that the therapeutic parameters of digitalis-like compounds might be improvable. As a result of this conclusion, we have embarked on the synthesis of a series of cardenolide analogues with the hope to prepare derivatives with an improved therapeutic index.

THE FURAN METHODOLOGY FOR THE SYNTHESIS OF CARDENOLIDES

The first task, of course, was the development of a new synthetic methodology which would allow us to prepare the various derivatives for pharmacological testing in gram quantities in a relatively short time. It occurred to one of us some time ago (cf. ref. 1) that one might use easily obtainable β -furyl derivatives of steroids as intermediates and oxidize them selectively to cardenolides.

Scheme 1



The oxidation mechanism of a substituted furan is shown in Scheme 1. It is initiated by an attack of an electrophile E^+ at the less-hindered α -position followed by a nucleophilic attack at the remaining α -site.

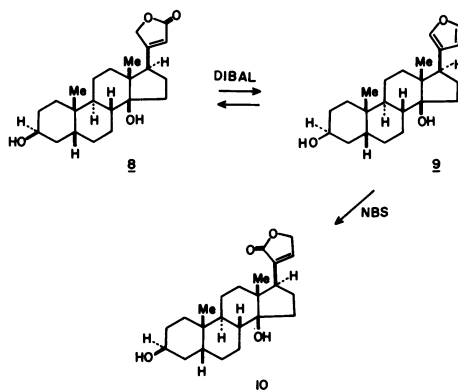
If the oxidizing agent is a peracid, the first formed intermediate (3) is oxidized further to the hydroxylactone (4). This compound may then be reduced by sodium borohydride to the unsaturated lactone (5).

In the case of *N*-bromosuccinimide, the intermediate bromohydrin (6) eliminates hydrobromic acid and yields the isomeric lactone (7).

When the model work with isopropyl furan was completed, the oxidation method was tried out with the furyl steroid (9) obtained by aromatization of digitoxigenin (8) with DIBAL (ref. 1). As expected, peracid followed by borohydride gave digitoxigenin in good yield [we improved this yield considerably later in Fredericton (cf. ref. 2)] and NBS gave the isomeric lactone (10). The glucoside of (10) is the already mentioned experimental drug "Actodigin".

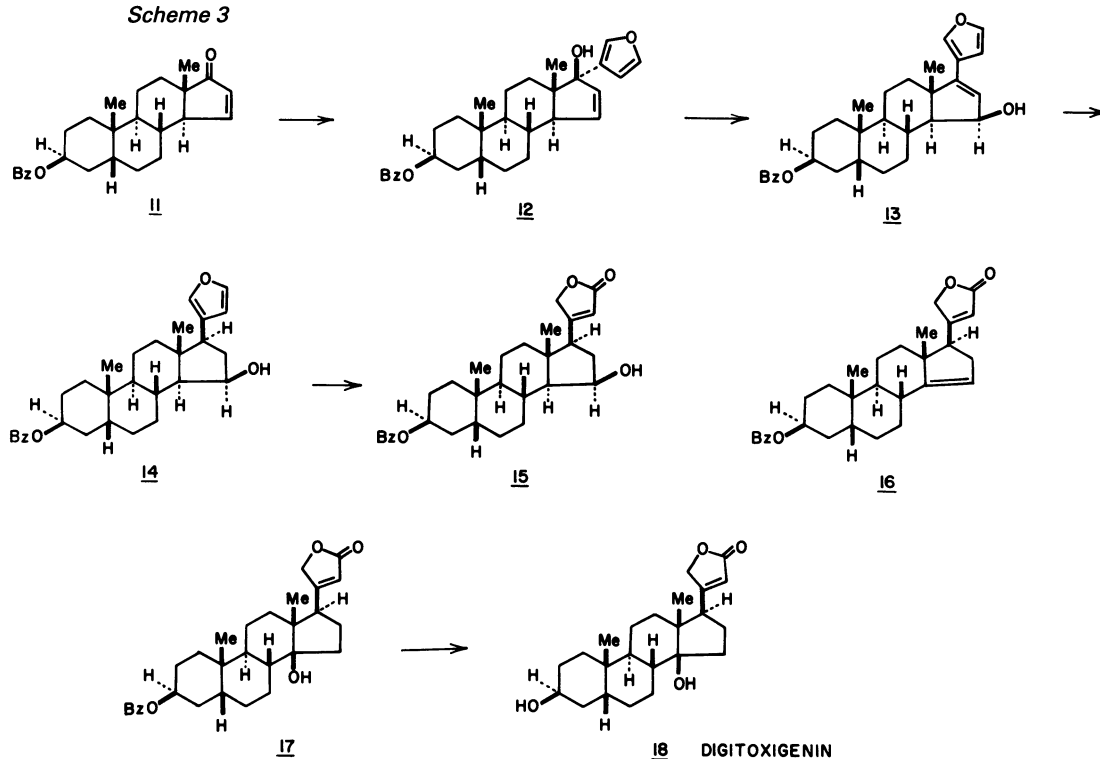
For the synthesis of digitoxigenin from common steroids we first prepared compound (11), obtainable in high yield by standard methods from testosterone (ref. 2, cf. also ref. 8). Treatment with β -furyl lithium yielded the tertiary alcohol (12), which by acetylation and allylic rearrangement gave stereospecifically the 15- β -hydroxy derivative (13). Stereospecific hydrogenation gave the furyl derivative (14) and this was treated with *m*-chloroperbenzoic acid followed by sodium borohydride to yield the cardenolide (15). Mesylation, elimination, and standard functionalization of the 14-15 double bond in the intermediate (16) gave finally benzyl-digitoxigenin (17) in excellent yield. Debonylation by hydrogenolysis was quantitative and yielded crystalline digitoxigenin (18).

Scheme 2



The synthesis just described is a good illustration of the most advantageous stereochemical strategy for the construction of cardenolides. (It is surprising that this strategy does not seem to be clear to many investigators.) Due to the preferential reactivity of steroids at C_{17} from the α side in C/D trans systems and from the β side in C/D cis systems, it is advantageous to use C/D trans starting materials. This way it is easy to set up the lactone group in the required 17- β configuration, but this alone does not solve the problem. It is necessary to create simultaneously a "handle" for the subsequent inversion of C_{14} and the introduction of

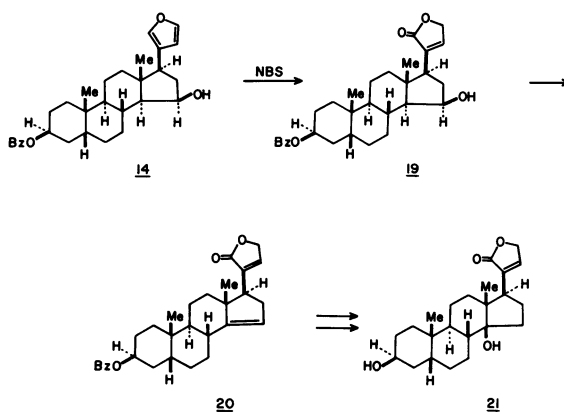
Scheme 3



the substituent in this position. We see that our system can accomplish both these tasks very simply.

The synthesis of the isolactone (21) was accomplished as follows (ref. 2) (Scheme 4). The already mentioned intermediate (14) was converted by NBS followed by zinc-acetic acid to the isolactone (19). Mesylation, elimination, functionalization of the 14-15 double bond and debenzoylation yielded the desired compound (21). The β -glucoside of (21) is the already mentioned experimental drug "Actodigin".

Scheme 4



The next step in the development of the "furan approach" to the cardenolides was the use of substituted furans. Alkoxy or silyloxy furans can be hydrolyzed quantitatively to unsaturated lactones under very mild conditions and thus they can serve as masked lactone synthons.

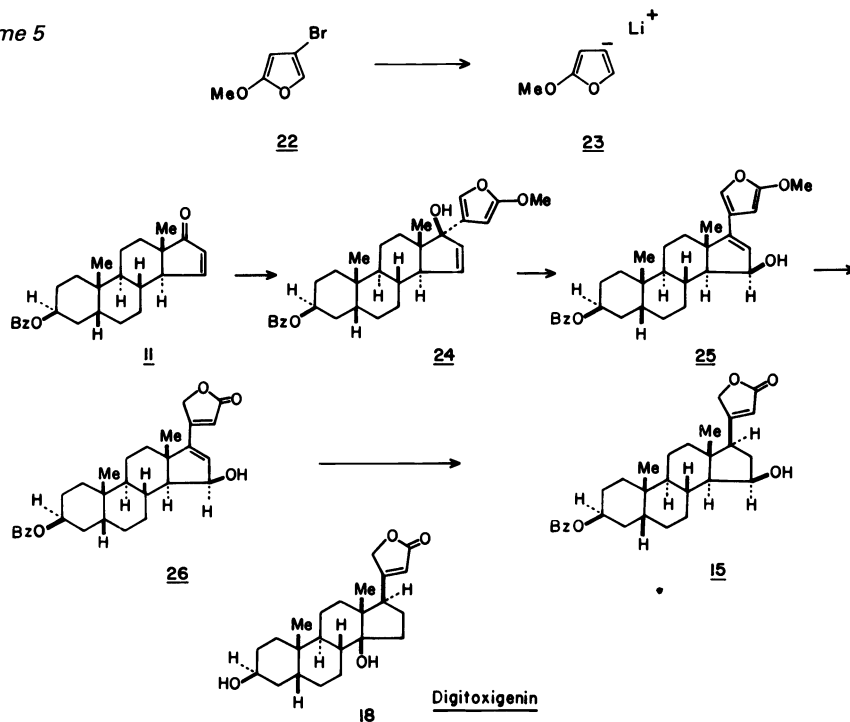
We have used successfully both silyloxy (ref. 7) and methoxy (ref. 8) furyl derivatives to synthesize cardenolides; however, only the methoxy derivatives are stable enough to lend themselves to the allylic rearrangement, which is an obligatory step in our synthesis of digitoxigenin. The new improved synthesis of this compound (Scheme 5) involved first the preparation of the methoxy bromofuran (22) which was transformed by *n*-butyllithium to the organometallic reagent (23).

Treatment of our starting ketone (11) with (23) gave the tertiary alcohol (24). Allylic rearrangement yielded (25) and a very mild acidic hydrolysis gave the cardenolide (26) in excellent overall yield. Selective hydrogenation finally provided our known intermediate (15) which we had previously converted to digitoxigenin (18).

In spite of this success of the methoxyfuran method, we still prefer to use our earlier methodology involving a regioselective furan oxidation. The regioselectivity and yield of this last process has been raised to such a level that there is practically no room for

improvement (Note a).

Scheme 5



Since we wished to synthesize also digoxigenin analogues, we have prepared (ref. 10) in high yield by an adaptation of known methods the ketone (28) from desoxycholic acid (27). The synthesis of digoxigenin (29) and isodigoxigenin (30) followed uneventfully by the already described process.

CARDENOLIDES WITH A CHIRAL CENTER IN THE LACTONE RING AND THE TOPOLOGY OF THE DIGITALIS RECEPTOR(S)

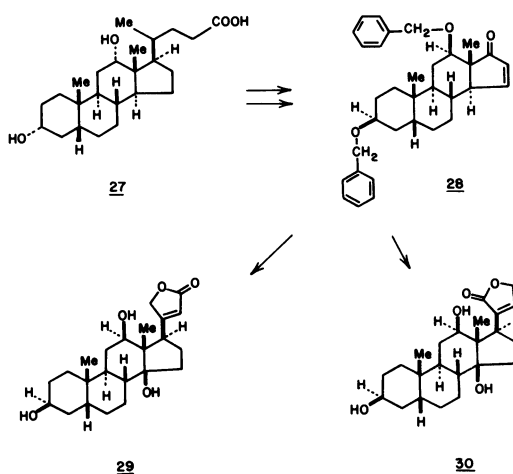
With the new efficient synthetic methodology established, we have naturally started on a vigorous programme of molecular manipulation in the hope of finding less toxic digitalis analogues by trial and error. However, there exists also the possibility to approach the problem in a more rational manner, by applying the principles of chemical topology (ref. 11) to the presumed digitalis receptors in the heart muscle.

Our reasoning went as follows: If the two receptor hypothesis is correct, then it would seem that the topology of both receptors must be quite similar since, prior to our work a linear relationship was observed between the positive inotropic effect and the toxicity of the various natural cardenolide derivatives examined.

However, this need not be necessarily true.

It is, for example, conceivable that both receptors (for toxicity and inotropy) would require contact with the same face of the steroid molecule but with a different face of the two non-identical faces of the unsaturated

Scheme 6

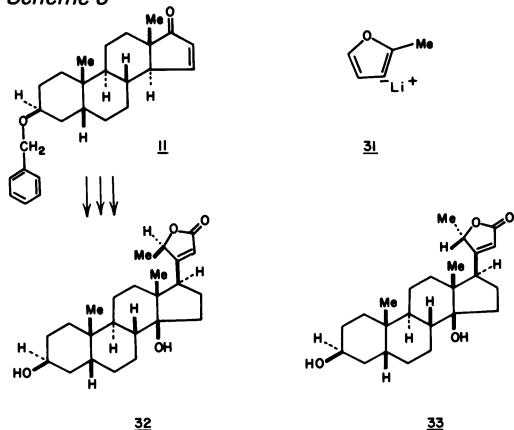


Note a: S. P. Tanis and D. B. Head have much later (ref. 9) also used substituted furans as butenolide equivalents. However, their claim that furan oxidation methods are questionable because of regiochemical ambiguities can be written off as the usual disparagement of earlier work.

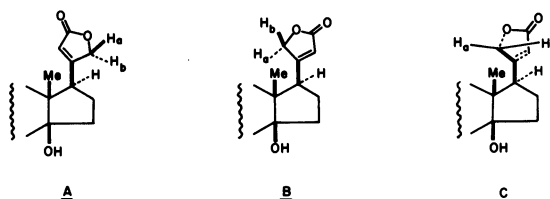
lactone. The geometry of one receptor might thus require rotamer A and the geometry of the other receptor rotamer B. A natural cardenolide would fit both receptors, since the lactone may rotate and present H_a to one and H_b to the other receptor. If we, however, replace one of the two diastereotopic H-atoms H_a and H_b , respectively, by a larger group, a cardenolide would result which is indistinguishable from the natural compound by one receptor and presents the larger group instead of a H-atom to the other one. Thus, if this simple idealized situation in fact exists in the heart muscle, epimeric 21-alkyl-cardenolides could have remarkable pharmacological properties. One of the epimers could show a positive inotropic effect without being toxic, the other epimer could be cardiotoxic without being inotropic.

To test whether at least a partial dissociation of inotropy and toxicity on the basis of the above considerations is possible, we have decided to prepare first the two epimeric 21-methyl digitoxigenins (ref. 12).

Scheme 8



Scheme 7



Starting with the steroid (11) and the furyllithium derivative (31), we have prepared by our already described synthetic route (cf. Scheme 3) the epimeric cardenolides (32) and (33) in good yield and in a ratio 3.5:1. The (21R)-21-methyl digitoxigenin formula (32) was assigned to the major epimer on the basis of an X-ray crystallographic analysis (ref. 13). As all our cardioactive steroid analogues, compounds (32) and (33) were first tested as β -glucosides (Note b). The comparison of the data for the glucosides of (32) and (33) with similar data for digitoxin can be found in Table 1. The glucoside of the (R)-epimer (32) is only slightly less potent than digi-

toxin, but its toxicity is dramatically reduced as measured by both ratios ID/MTD and LD/MTD. Parallel with this reduction of toxicity, we note a reduction of Na^+K^+ -ATPase inhibition by almost 2 orders of magnitude. It seems that the two receptor hypothesis is the best explanation of these data and that the inotropy receptor does have the topological properties conjectured above.

On the other hand the toxicity receptor does not show an analogous geometry. Blockade of either of the two faces of the lactone ring reduces toxicity very strongly. This may mean that the topology of the toxicity receptor is such, that both diastereotopic H-atoms, H_a and H_b , of a natural cardenolide come into contact with the receptor and that the conformation required for the toxicity receptor corresponds to the rotamer C (Scheme 7).

TABLE 1

Potency, margin of safety and inhibition of human and guinea pig Na^+K^+ -ATPase of cardenolide derivatives

Compound	ED	ID/MTD	LD/MTD	Human ATPase*	Guinea Pig ATPase*
β -Glucoside of (32)	0.7	45	128	0.55	25
β -Glucoside of (33)	3.0	33	65	2.10	100
Digitoxin	0.45	2.92	5.07	0.009	0.24
β -Glucoside of (35)	0.25	49	88	0.34	50
β -Glucoside of (36)	0.70	45	84	1.4	120
Actodigin	0.65	20	88	1.9	160

*ATPase inhibition given by concentration (in μ M) required for 50% inhibition. Effective dose (ED): single injection effective dose. MTD = Minimal Therapeutic Dose. ID = Irregularity Dose, and LD = Lethal Dose.

Note b: The pharmacological tests of the majority of our compounds have been carried out under the direction of Professor Rafael Mendez at the Instituto Nacional de Cardiologia Ignacio Chavez, Mexico City. The inhibitions of human and guinea pig Na^+K^+ -ATPase were determined by Professor Kurt R. H. Repke, Berlin, DDR.

The synthesis of the two epimeric methyl isodigitoxigenins (35) and (36) was carried out next, starting with the steroid (11) and the furyl lithium derivative (34). By this time the process became routine and proceeded uneventfully (ref. 14). Neither of the two epimers yielded to X-ray analysis, but the configurations were assigned without any difficulty by Barton's method of molecular rotation differences with reference to the known configurations of (32) and (33) (ref. 15). Finally, the epimers (35) and (36) were converted to β -glucosides and submitted to Professor Mendez for study. The results of this study were quite interesting (ref. 16).

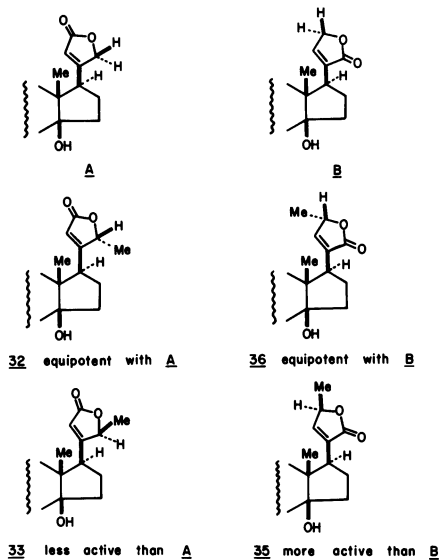
Again, the R-epimer (35) was more potent than the S-epimer (36). However, in this case it was the less active S-epimer (36) which was equipotent with the parent substance actodigin (see Table 1).

A tentative, but fully consistent explanation of this seemingly strange situation is based on the following assumptions:

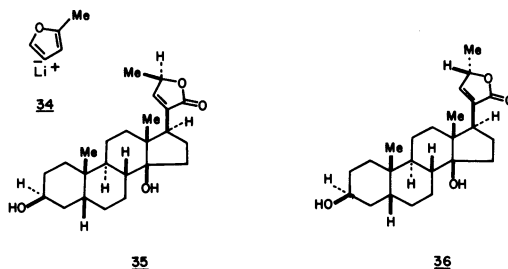
- 1) The methyl epimer [(32), (33) or (35), (36)] which presents a hydrogen to the inotropy receptor cannot be distinguished by this receptor from the corresponding unsubstituted parent compound (digitoxin or actodigin).
- 2) The epimer which presents to the inotropy receptor a methyl group may be either less active or more active than the corresponding unsubstituted parent compound, since there is a priori no reason to believe that the parent compound represents the best possible fit to the receptor.

If we assume that the steroid skeleton makes contact with the inotropy receptor on its β -face, the consistent picture portrayed in Scheme 10 emerges.

Scheme 10



Scheme 9



The inotropy receptor requires the rotamer A of digitoxin and B of actodigin. Consequently, the glucoside of the R-epimer (32) is equipotent with digitoxin and the S-epimer (33) is less active. In the same manner, the S-epimer (36) is equipotent with actodigin B and the R-epimer (35) is more active. There is no controversy in the opposite effects of the methyl group in compounds (33) and (35) since these methyl groups come into contact with the inotropy receptor at topologically different sites.

Regardless of theory, the β -glucoside of (35), i.e., "(23R)-23-methylactodigin", is a remarkable compound. If we consider the ID/MTD is the more important definition of the margin of safety (Note c) and that besides the margin of safety the second most important of the parameters in Table 1 is potency, then (23R)-23-methylactodigin is by far the best derivative listed.

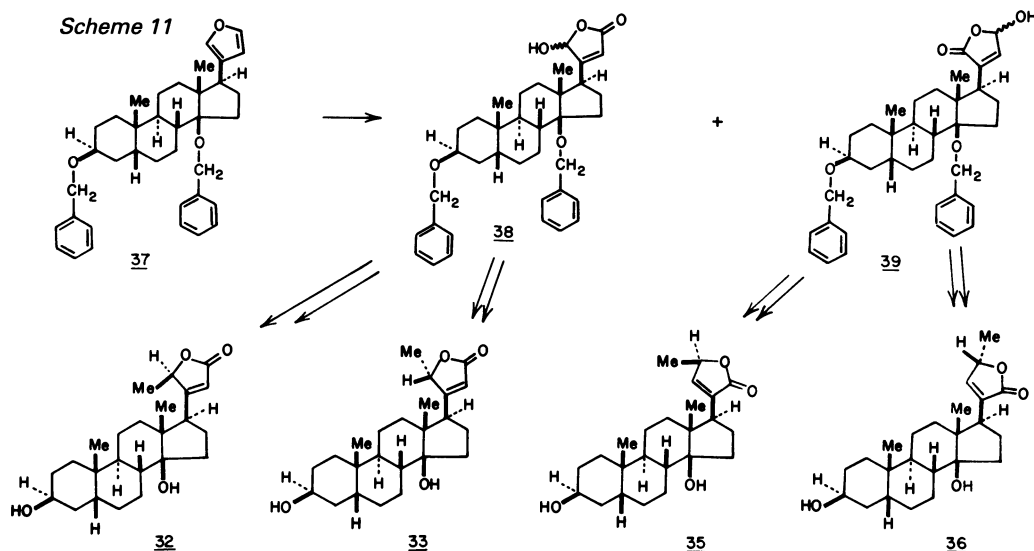
In summary it seems that the two receptor hypothesis and the assumption that toxicity, but not potency, is a function of ATPase inhibition is the best explanation of the data in Table 1.

Since the glucosides of compounds (32) and (35) turned out to have good potency and a high margin of safety, the development of a short high yield process for the preparation of these materials from digitoxigenin was desirable, regardless of the high cost of the starting material.

Such a process is portrayed in Scheme 11 (ref. 12, 15). The starting material was the derivative (37) easily prepared from digitoxigenin by reduction with DIBAL, followed by benzylation. Oxidation of compound (37) with *m*-chloroperbenzoic acid yielded the two easily separable lactols (38) and (39). Due to the influence of the 14-benzyloxy group (unlike in our synthesis of digitoxigenin--vide supra), the oxidation was not regioselective and the

.....
 Note c: Private communication of Drs. R. Mendez and E. Kabela. (Patients would be expected to receive sometimes the irregularity dose but hardly ever the lethal dose.)

two isomers were obtained in a ratio 2:1. Since compounds (38) and (39) are masked aldehydes, treatment of these materials with methyl lithium, acidification, and catalytic debenzoylation yielded the expected epimeric methyl-digitoxigenins (32) and (33) and methyl-isodigitoxigenins (35) and (36), respectively. By good fortune, the more active R-isomers (32) and (35) were obtained as major products. (The compounds (32) and (33) were obtained in a ratio 8:1.)



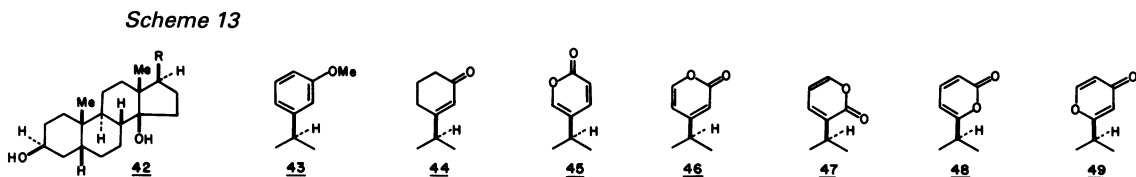
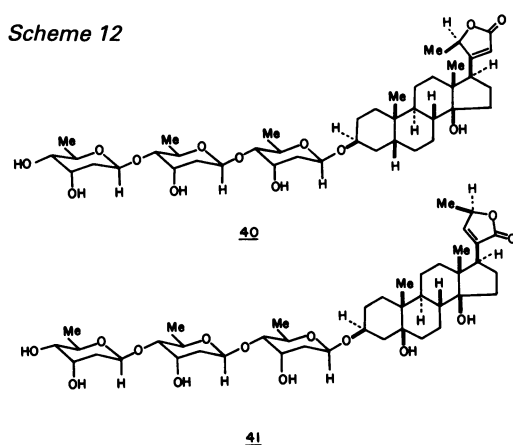
However, the greatest stroke of luck was the finding that this method of preparation of the interesting steroids (32) and (35) could be applied without change to digitoxin. This enabled us to prepare (21R)-21-methyldigitoxin (40) (ref. 17) and (23R)-23-methylisodigitoxin (41) (ref. 15) in good yields.

These compounds contain the steroids (32) and (35) in combination with the natural digitoxin glycosidic sidechain and they are being studied at the time of writing.

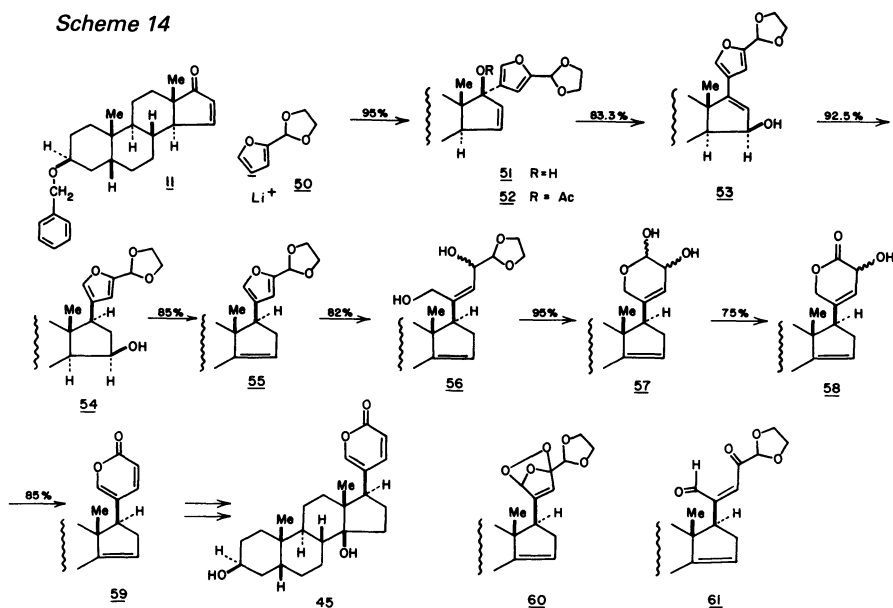
SYNTHESIS OF BUFALIN AND ITS ISOMERS

Besides the rational approach described above, we have naturally also used our efficient synthetic methodology for random molecular manipulation in the hope of discovering active and non-toxic derivatives by chance. Thus we have prepared a large number of compounds of the general formula (42) and sent them to Professor Mendez's Institute in the form of the corresponding β -glucosides. The R groups in these derivatives were mostly aromatic systems like furans, thiophenes, pyridines and pyridones. Several of them turned out to be interesting, but at this point we shall mention only the anisole derivative (43) and its Birch reduction product (44) (ref. 18). The glucoside of (44), in spite of the absence of a lactone group, showed in pharmacological tests both a potency and a margin of safety superior to those of natural cardioactive steroid glycosides.

In order to follow up this lead, we have decided to synthesize bufalin (45) (ref. 19) and its isomers (46), (47), (48) (ref. 20), and (49) (ref. 21). The synthesis of the derivatives (45) - (48) involved our furan strategy, but two different furan ring openings had to be developed to make all of the systems readily available. We shall illustrate these two variants by briefly describing the synthesis of natural bufalin (45) and of α -isobufalin (46).



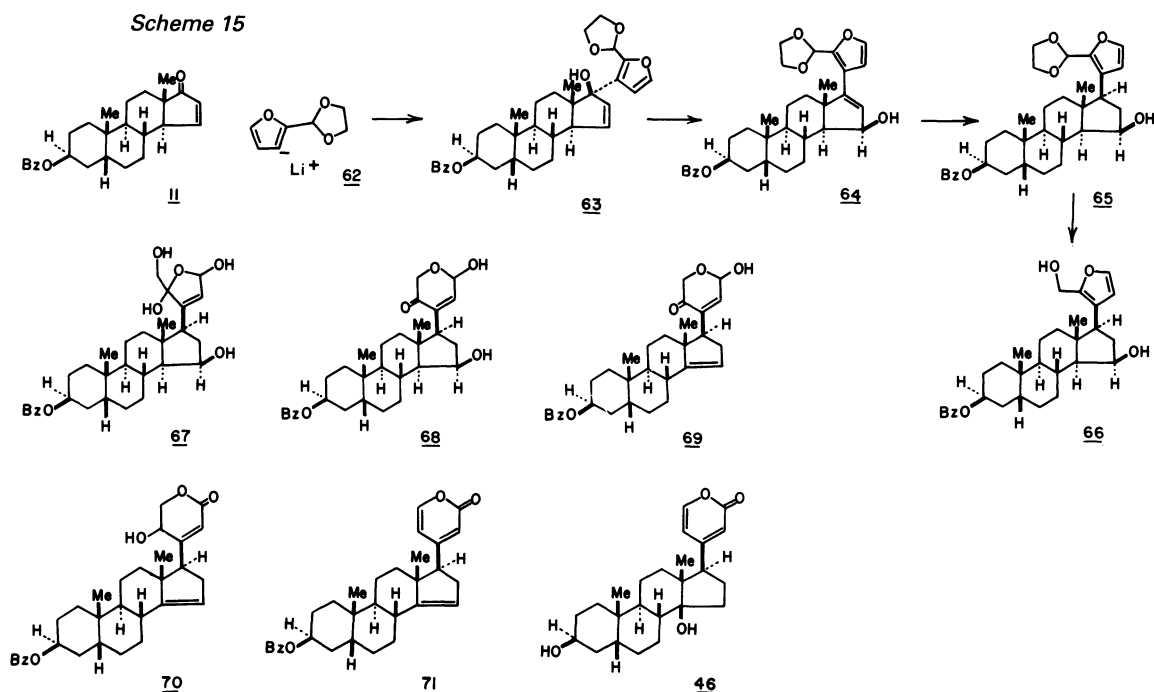
Scheme 14



We might add that our preparation of (45) which is portrayed in Scheme 14 constitutes the first truly simple and efficient synthesis of natural bufalin recorded in the literature.

The starting materials were our usual steroid (11) and the organometallic reagent (50) prepared by metallation of the corresponding bromoderivative with *n*-butyllithium. The first steps proceeded uneventfully exactly as in our digitoxigenin synthesis (*vide supra*) and compound (54) was reached in a high overall yield. The 15- β -OH group was now eliminated with mesyl chloride in pyridine and the beautifully crystalline Δ -14,15 derivative (55) was ready for the furan ring opening. This was achieved by a sensitized oxygen addition followed by treatment with an excess of dimethylsulfide and finally reduction with NaBH_4 . This one pot procedure yielded the mixture of the epimeric diols (56), presumably via the endoperoxide (60) and keto aldehyde (61) which were not isolated. Mild acidic hydrolysis of (56) gave the pyranose derivative (57) which was selectively oxidized ($\text{Ag}_2\text{CO}_3/\text{Celite}$) to the lactone (58). The assembly of the α -pyrone system was completed by elimination of the remaining hydroxyl with mesyl chloride and triethylamine and the resulting bufadienolide (59) was converted to bufalin (45) by a standard functionalization of the 14-15 double bond, followed by hydrogenolysis of the benzyl protecting group.

Scheme 15



The second variant of furan ring opening may be best illustrated by the preparation of α -isobufalin (46). In this case the furan system was opened by the action of *m*-chloroperbenzoic acid which we discovered in connection with our original studies on actodigin (*vide supra*).

The preparation of (46) is portrayed in Scheme 15. Our standard starting material (11) and the organometallic reagent (62) were converted in high yield, exactly as in the bufalin synthesis, to compound (65) which by deacetalization and NaBH_4 reduction gave the primary alcohol (66). When (66) was oxidized with *m*-chloroperbenzoic acid, the first formed intermediate (67) isomerized immediately to the more stable pyranose derivative (68) which was obtained in a 90% yield. Compound (68) now required only some functional group adjustment to yield the desired α -isobufalin (46).

Protection of the hemiacetal hydroxyl by monoacetylation, elimination of the C_{15} hydroxyl by thionyl chloride and pyridine and saponification yielded compound (69).

Oxidation of the hemiacetal group to a lactone with chromic acid and reduction of the ketone with zinc borohydride gave the hydroxy lactone (70) and finally mesylation and elimination yielded the α -pyrone (71). Functionalization of the 14-15 double bond followed by hydrogenolysis of the benzyl group gave finally the beautifully crystalline α -isobufalin (46).

We shall conclude this section by presenting a table of selected β -glucosides, together with their potencies (ED) and margins of safety (ID/MTD, LD/MTD).

SOME SELECTED ANALOGUES AND THEIR ACTIVITIES

In the interest of brevity, all the chemistry in this section has been deleted and we present only some of the results in Table 3.

TABLE 2

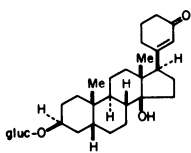
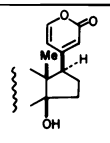
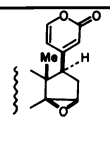
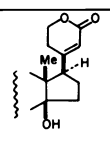
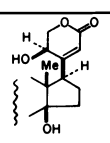
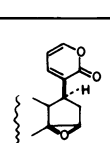
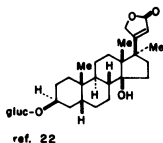
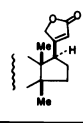
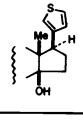
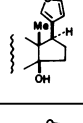
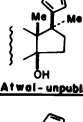
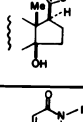
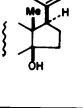
	ED	ID MTD	LD MTD
	0.30	10.25	27.09
	0.15	9.20	21.50
	0.65	11.50	22.40
	0.20	15.33	42.96
	0.25	24.57	43.19
	Inactive		

TABLE 3

	ED	ID MTD	LD MTD
 ref. 22	3.5	could not be determined	could not be determined
 ref. 24	inactive	cardiotoxic ATPase inhibitor	
 ref. 18	inactive	anti-inotropic	
	0.36	12.87	22.44
 S. K. Atwal - unpublished	inactive		
 ref. 25	4.0-4.5	37.21	90.10
 ref. 26	5.20	45.76	90.00

STERESELECTIVE β -GLYCOSYLATION OF DIGITOXOSE AND THE SYNTHESIS OF DIGITOXIN

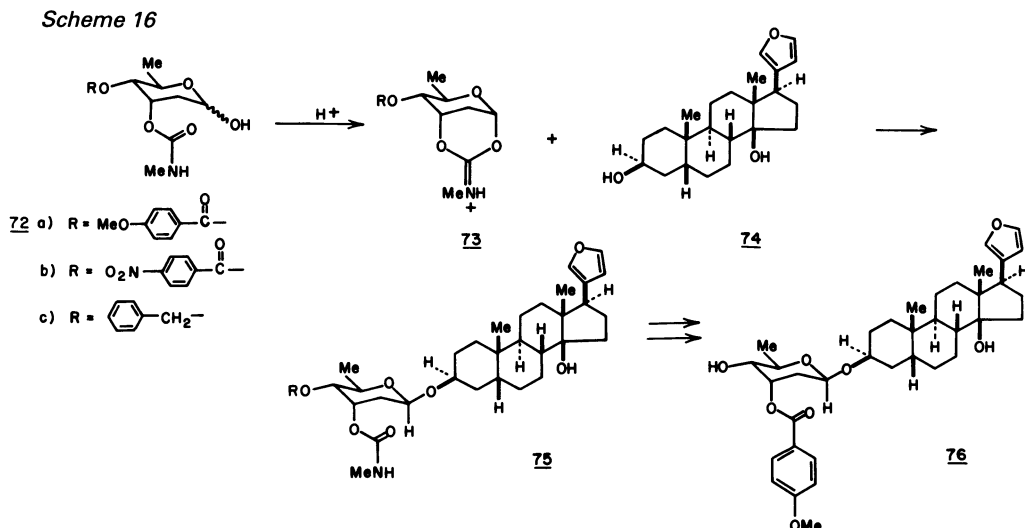
The steroidal derivatives which we have discussed so far and many more (a total of some 50 compounds) have been submitted for pharmacological testing in the form of their β -glucosides. As we have seen, some of them not only showed a high level of inotropic activity, but also a margin of safety many times wider than the precariously narrow margin displayed by the natural digitalis glycosides used in therapy.

However, as our studies progressed, it became gradually clear that glucosides have a duration of action too short to be (according to the opinion of Dr. Mendez) of practical use. Besides duration of action, there are other important therapeutic parameters to be considered. The compounds must be water soluble, orally acceptable, and must be unable to cross the blood-brain barrier and thus be free of central effects. Also, in all these respects glucosides cause difficulties. Thus we came to the conclusion that it would be desirable to attach the natural digitoxin glycosidic chain instead of glucose to our best synthetic steroids.

However, since digitoxin itself, in spite of its importance, has never been synthesized, it was clear that a fundamental improvement in the stereoselective β -glycosylation of digitoxose had to be achieved, before this ambition could be realized.

We have developed two novel stereoselective β -glycosylation techniques for digitoxose and achieved an efficient digitoxin synthesis by a combination of these methods (ref. 27).

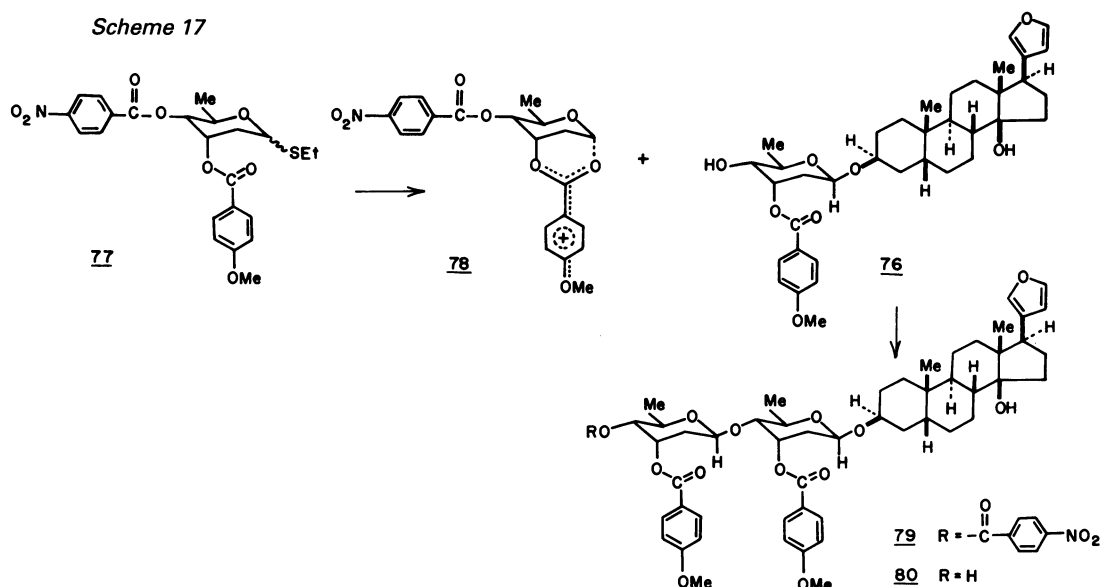
The first method is portrayed in Scheme 16. The digitoxose derivative (72) and the furyl steroid (74) were treated with *p*-toluenesulfonic acid in CH_2Cl_2 -benzene and the product (75) was obtained in good yield on the basis of starting material not recovered. The β -stereoselectivity of this method was presumably due to the intermediacy of the bridged species (73). This stereoselectivity seemed to depend considerably on the nature of the group R. Thus with the three derivatives tried (72a, b, and c) the ratios β : α were 7:1, 8:1, and 3:1. These ratios were quite reproducible and we believe that the size of the group R is important for keeping the relatively large urethane group axial. In spite of the least favourable β : α ratio, we have selected for reasons of blocking group manipulation the series c for the digitoxin synthesis. (We hope to improve the process later by using a heavily substituted benzyl group as R.) The urethane group in the product (75) was removed by LiAlH_4 reduction, the liberated hydroxyl was *p*-methoxybenzoylated and finally the benzyl group was deblocked by hydrogenolysis. The intermediate (76) was obtained in high yield and it was ready for the second stage glycosylation.



We should point out that the masking of the cardenolide as a furan derivative is an essential feature of the synthesis since it enabled us to remove blocking groups by vigorous alkaline hydrolysis or hydride reduction.

At this stage it became rapidly clear that we could not use our acid catalysed method for the second and third coupling. With glycosidic bonds present, both in starting materials and products, degradation prevailed on synthesis and the higher glycosides were isolated only in traces. It was obviously necessary to perform the second and third glycosylation in a neutral medium and we decided to use mercury catalysed cleavage of ethyl thioglycosides as the coupling reaction. Glycosylation via phenyl thioglycosides was first described by Ferrier and several subsequent uses of thioglycosides in glycosylation reactions may be found in the literature (ref. 28).

Scheme 17



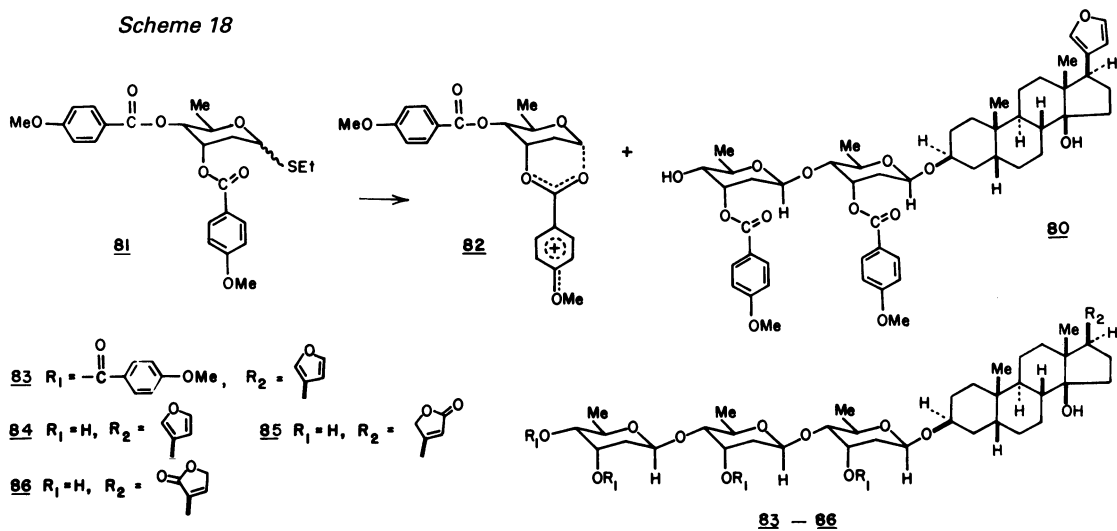
Our aim was to trigger by the mercury catalysed cleavage of the thioglycoside bond the formation of a 1,3-bridged species which would react stereoselectively and yield a β -glycoside. After the methyl urethane group somewhat predictably failed to perform in neutral medium, it turned out that a *p*-methoxybenzoyl group was equal to the task. We have found that both anomers of the thioglycoside (77) (Scheme 17) gave with the protected monodigitoxoside (76) in CH_2Cl_2 in the presence of HgCl_2 and CdCO_3 60% of the β -glycoside (79) besides 27% of unreacted starting material. In some runs small amounts (cca. 2%) of the corresponding α -glycoside were easily separated. This was an unexpectedly good result and it indicated that the bridged species (78) probably played a role in the glycosylation reaction.

Ammonolysis of the product (79) gave the deprotected derivative (80) ready for the final coupling. It must be mentioned that this method of glycosylation was only mildly stereoselective when applied to the first glycosylation step and consequently other factors than 1,3-bridging must play a role in the practically complete stereospecificity of the reaction (76) - (79).

The final glycosylation of the protected bisdigitoxoside (80) (Scheme 18) was performed under the same conditions. Since it was no longer necessary to differentiate the individual ester groups in the triglycoside sidechain of the product (83), the easily prepared α or β thioglycosides (81) were used for the coupling. The crystalline product (83) was obtained in a yield of 58% besides 25% of the starting material (80). No product with an α -glycosidic bond was found and thus the coupling was fully stereospecific.

The final steps of the synthesis were simple. Deblocking with LiAlH_4 gave the beautifully crystalline "furyldigitoxin" (84). Oxidation of this material with *m*-chloroperbenzoic acid followed by NaBH_4 reduction yielded 74.6% of crystalline digitoxin (85). Finally oxidation of furyldigitoxin (84) with NBS gave 65% of crystalline isodigitoxin (86).

Scheme 18



OUTLOOK

The synthesis of digitoxin, just described, is clearly not ideal for attaching the digitoxin sidechain to artificial cardenolide and bufadienolide analogues. However, the experience gained enabled us to design the construction of a tridigitoxose derivative which will be suitable for a one-step attachment to artificial steroids. Work in this direction is in progress. In spite of the fact that this last process is not yet ready at the time of writing, we have in our hands several derivatives which are combinations of artificial steroids with a wide margin of safety and the digitoxin glycosidic sidechain (cf. compounds (40), (41), and (86)). Pharmacological studies with these, at the present time, expensive materials are in progress.

Another possibility of altering the duration of action and other pharmacologic parameters of some of our relatively non-toxic steroids is to convert them into aminoglycoside derivatives (ref. 29). Several such derivatives have been prepared and are being tested.

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