SYNTHETIC CARDENOLIDES AND RELATED PRODUCTS

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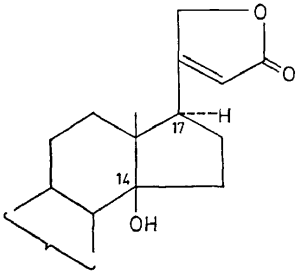
ABSTRACT

Since the publication in 1785 of one of the great classics of clinical medicine, 'An Account of the Foxglove and Some of its Medical Uses' by William Withering[1], the steroidal glycosides of digitalis have maintained a unique position among drugs. Their usefulness as life saving products is unchallenged and yet very few drugs are known to possess a narrower margin of safety. Their toxicity has been described as an extension of the pharmacologic activity which in turn has been related to structure in a 'none or all' fashion. Chemical modifications to their structure had not produced results of practical significance: digitalis materials continue to be essentially products of extraction, qualitatively all similar, and still a challenge to the synthetic medicinal chemist.

HISTORICAL

The isolation, purification and identification of digitalis materials was a formidable task and many illustrious names such as Schmiedeberg, Kiliani, Cloetta, Windaus, Stoll, Jacobs, Elderfield and Reichstein are associated with it.

A considerable amount of synthetic work in the cardenolide field was reported in the nineteen forties by Ruzicka, Plattner, and coworkers in Zurich. Efficient methods for the synthesis of the butenolide ring and the introduction of the 14β-hydroxyl group were developed on model substances, but failed when applied to steroids with the desired C/D *cis* junction (I).



(I)

Stereochemical control at the 17-position was not achieved (*Figure 1*) or, alternatively the reaction conditions were too drastic for the survival of the tertiary alcoholic function in 14β[2].

The breakthrough came in 1962 when Sondheimer and coworkers announced the synthesis of digitoxigenin, the first synthesis of a cardenolide[3]. Isomerization of the 17β-side chain to the thermodynamically more stable 17α-configuration was largely avoided, as well as the dehydration of the labile 14β-hydroxyl function during the elaboration of the butenolide ring

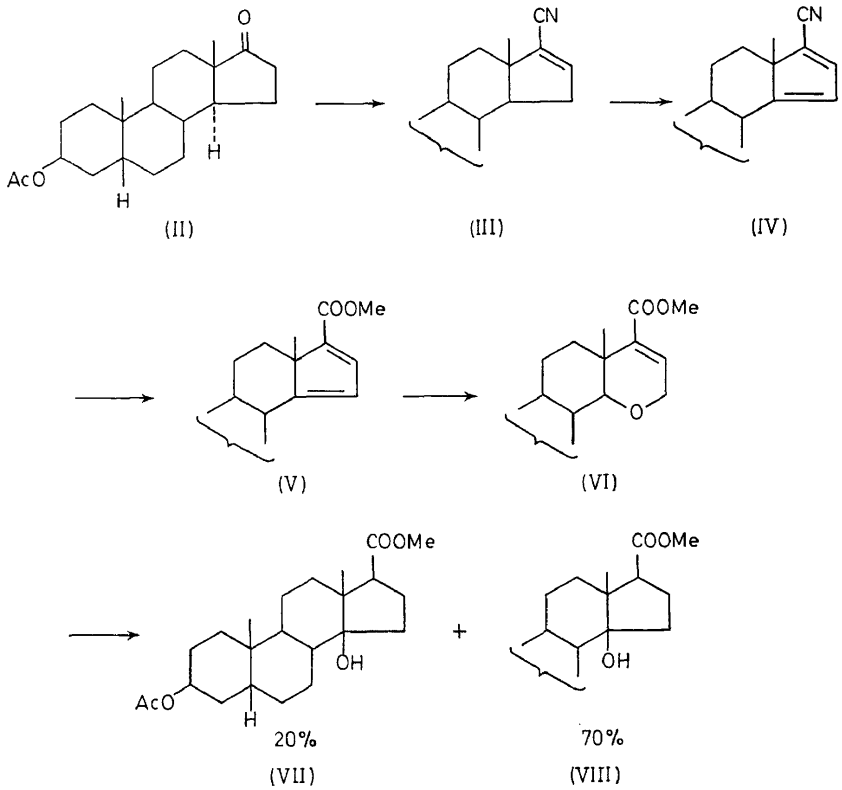


Figure 1

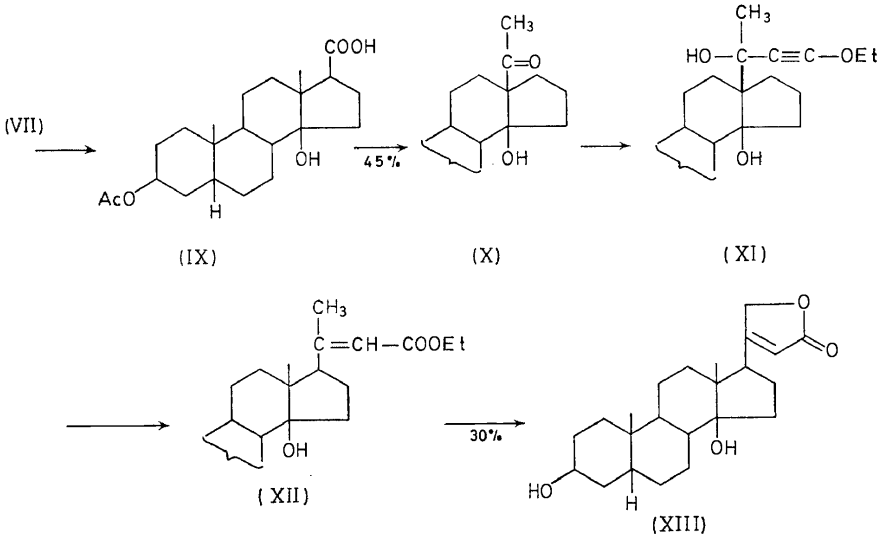
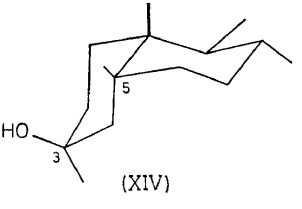


Figure 2

(*Figure 2*). This very elegant and important work, however, suffers from the difficulty of securing the starting material (VII) in attractive yields.

An added complication to the chemistry of cardenolides is the coprostanol-like configuration of the A/B ring junction (3β-hydroxyl group) (XIV).



Certain steroid sapogenins such as smilagenin contain this feature. Commercially available steroid intermediates suitable for large scale synthesis are, however, mainly from Mexican origin, and these (derived from diosgenin) possess double bonds in either position 5 or 4 and their transformation to the desired structure (XIV), albeit recently improved[4] is still not satisfactory.

A somewhat better utilization of steroid intermediates available from Mexico was reported from our laboratory in 1963 with the synthesis of the aglycone periplogenin (XX) from desoxycorticosterone acetate[5] (*Figure 3*).

The appropriate Δ^{14} double bond was obtained by eliminating a 14α-OH group introduced microbiologically. We had previously found[6] that a 14,15 unsaturation could conveniently be introduced by a novel 14,21 dehydrohalogenation of a 17,21-dihalosteroid in refluxing dimethylformamide as depicted in *Figure 4*.

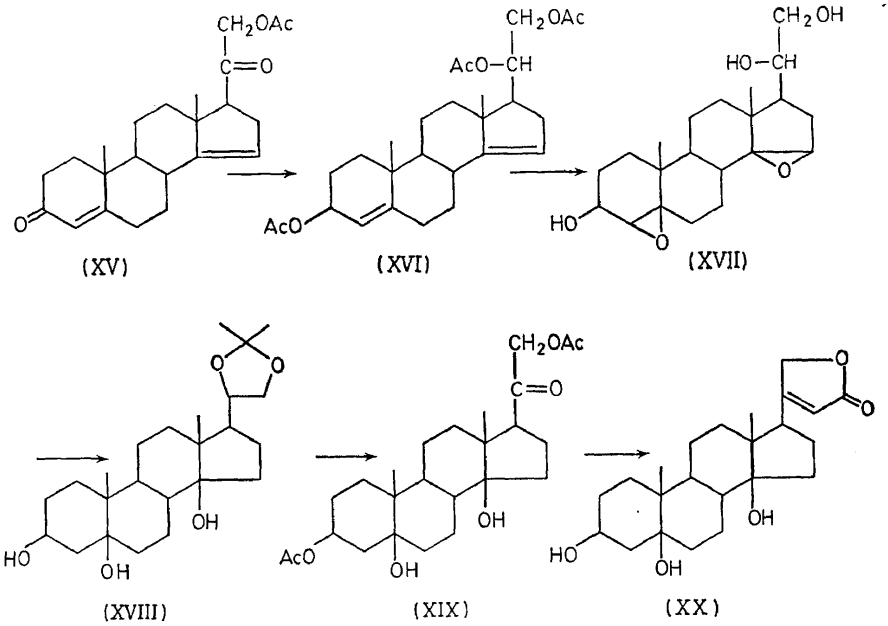


Figure 3

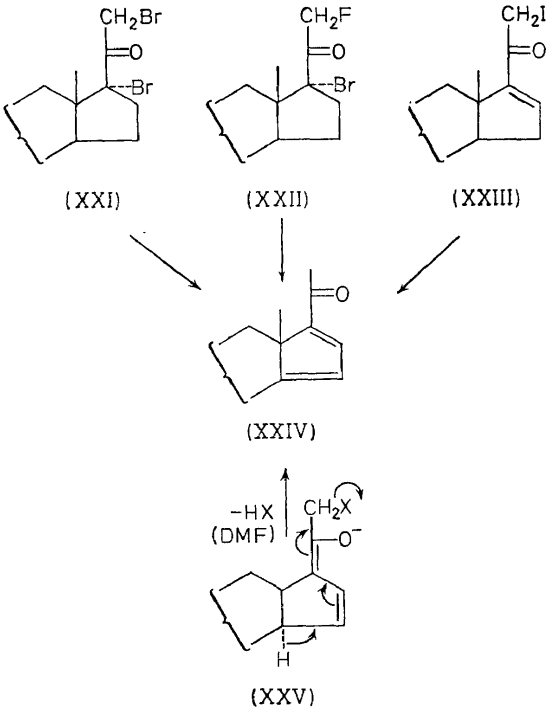
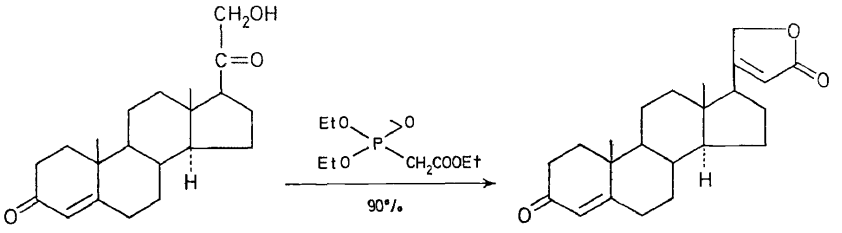


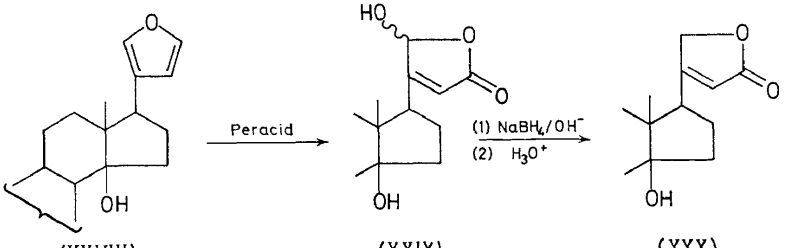
Figure 4

Two novel and efficient syntheses of the butenolide ring were reported in 1966. One by Ruschig and collaborators[7] utilizing a Wittig reagent;



(XXVI) (XXVII)

and the second, from our laboratories[8] by an oxidation reaction of a furan intermediate, followed by reduction:



(XXVIII) (XXIX) (XXX)

Other syntheses of the aglycone digitoxigenin have been reported since the first one of Sondheimer[9–11].

MODIFICATIONS OF THE CARDENOLIDE STRUCTURE

Lactams

We were interested in modifying those structural features common to most digitalis materials, such as the unsaturated lactone ring, on the assumption that common features account for the qualitative resemblance of all active (but toxic) products. One of the modifications reported from our laboratories[12] is the synthesis of novel 'aza' cardenolides, in which the lactone side chain has been replaced by lactams. Naturally occurring cardenolides such as digitoxin and digoxin (or their aglycones) were treated with ammonia or methylamine at room temperature or in autoclave at 110°C.

One lactol-amide (XXXII) and/or two isomeric γ-lactams ('A' and 'B' in 60% and 3% yield respectively) were obtained, consistent with structures (XXXIV) (*Figure 5*). In no instance was a γ-crotonolactam obtained, not surprisingly in view of the great tendency of cardenolides to cyclize to the 'iso'-form (XXXIII) in basic medium.

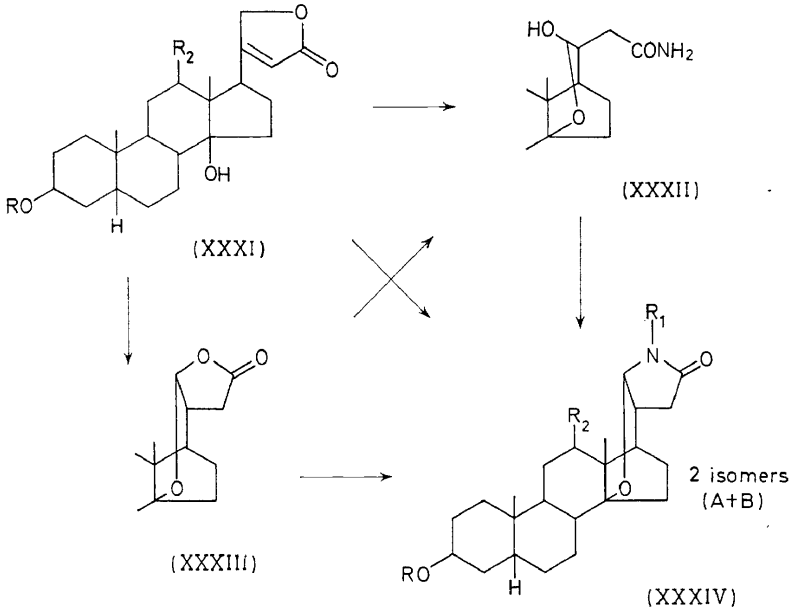
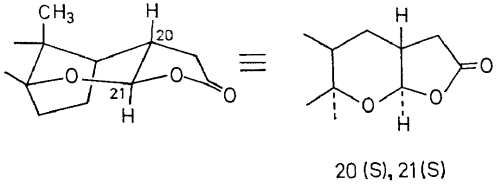


Figure 5

We assume in fact that the 'iso' structure (XXXIII) is an intermediate since the same products were obtained by reacting the latter with ammonia or amines. The stereochemistry of the predominant isodigitoxigenin (XXXIII; $R = R_2 = H$) has been postulated on conformational ground by Reichstein[13], as having the 20(S), 21(S) *trans* fusion of the E/F rings (XXXV):

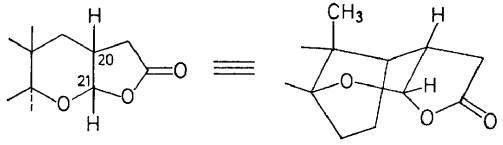


(XXXV)

Our n.m.r. data, however, do not support the *trans* configuration. The coupling constant J for the 21-proton is 5 Hz and consistent with an axial-equatorial dihedral angle $\phi \approx 40°$ indicating a *cis* junction †, which, for

† The *trans* junction of the lactone ring in α-santonin, as one classical example, obtains a coupling constant $J = 11$ Hz for the C_1-proton.

reasons given below, we describe as 20(S), 21(R) (XXXVI):



20 (S),21 (R)

(XXXVI)

Isodigitoxigenin (XXXIII) was converted into the known[13] lactolester (XXXVII) by mild alkaline hydrolysis, followed by acidification with acetic acid and diazomethane treatment (*Figure 6*).

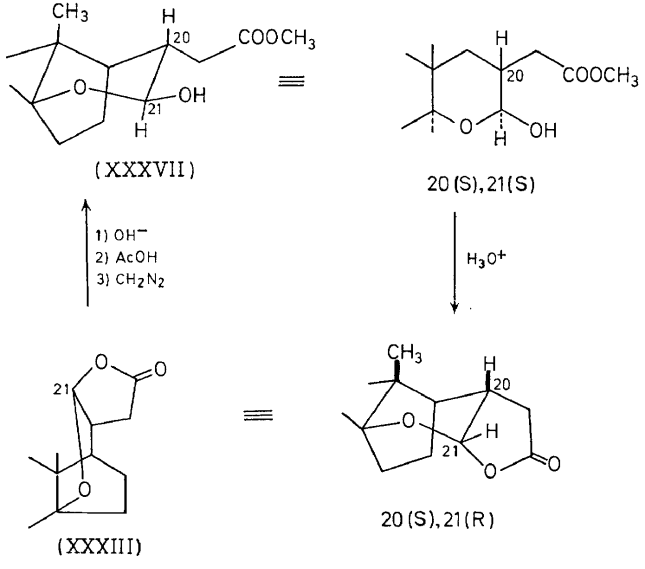


Figure 6

The C_{21} proton in (XXXVII) has now a coupling constant (W/2) of 14 Hz indicating a *trans*-diaxial configuration of the 20–21 protons. A mutarotation of lactol (XXXVII) was observed in tetrahydrofuran-water ($[\alpha]_D$ + 5·7° to + 10·4°)[14]. Reacidification of the lactol-ester regenerates isodigitoxigenin (XXXIII) in excellent yield. Only one *trans*-diaxial configuration of the 20–21 protons is sterically possible for isodigitoxigenin [the 20(S), 21(S) (XXXV)] and therefore the (anomeric) centre at C-21, (but not both 20 *and* 21), is epimerized to give lactol (XXXVII) (*Figure 6*).

The major lactam 'A' (XXXIV, R = H) which is isolated in 60 per cent yield has a coupling constant J = 6 Hz for the 21 proton (axial-equatorial)

and we assign the same *cis* structure 20(S), 21(R) (XXXVIII) present in isodigitoxigenin. The minor lactam 'B' has coupling constant W/2 = 6 Hz for the 21 proton, and therefore must possess the alternative *cis* configuration 20(R), 21(S) (XXXIX) as indicated in the *Figure 7*.

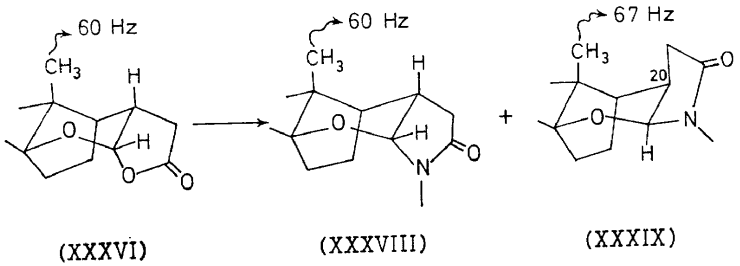


Figure 7

Additional evidence for the structure of the minor lactam (XXXIX) is provided by a deshielding of the C_{18} protons (67 Hz) due to proximity to the lactam function. Epimerization at C_{20} of an intermediate amide-aldehyde followed by lactamization is the probable mechanism of formation of this minor isomer.

An analogous situation was encountered when gitoxigenin (XL), which possesses a 16β-hydroxyl function, was similarly treated with ammonia or amines (*Figure 8*).

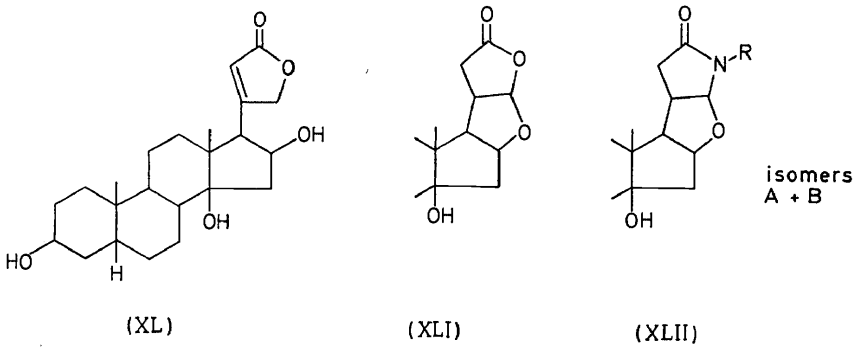
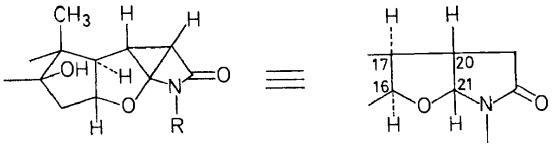


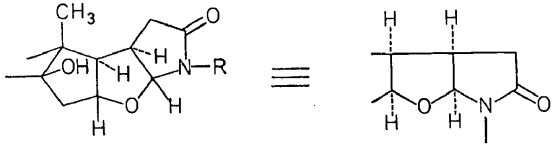
Figure 8

A major and a minor isomer (A and B) were isolated in a 15:1 ratio. Similar lactamizations were reported by Barton *et al.*[15] in their paper on the diterpenoid bitter principle Clerodin, and more recently by German workers[16] who describe the bicyclic system hexahydrofuro [2,3*b*]pyrrol-2-one. Only the two *cis* structures are clearly possible (*Figure 9*).



20(S), 21(R)

(XLIII)



20(R), 21(S)

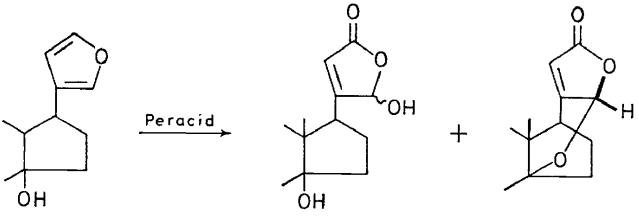
(XLIV)

Figure 9

The major isomer is assigned the 20(S), 21(R) one (XLIII) on steric ground. The 21-H coupling constant is J = 5 and 6 Hz respectively as expected for *cis* junctions.

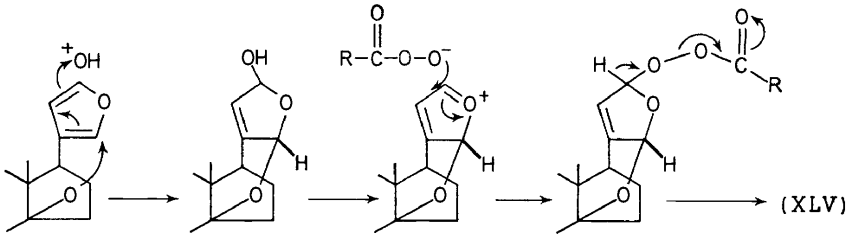
14,21 Bridged Butenolides

In the course of the peracid oxidation of the furan intermediate (XXVIII) to give the hydroxy butenolide (XXIX), we isolated an unsaturated γ-lactone consistent with structure (XLV). It was possible to increase the

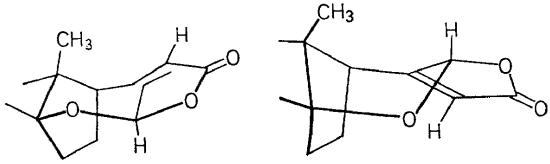


(XXVIII) (XXIX) (XLV)

proportion of (XLV) by changing the conditions of the reaction: with excess *m*-chloroperbenzoic acid in acetic acid containing sodium acetate at room temperature the yield of (XLV) was 33 per cent. The probable mechanism involves an internal nucleophilic attack of the 14β-hydroxyl function concerted with the elecrophilic addition of the OH^+ species on the less hindered double bond, as in (XLVI), followed by exchange with the peroxide anion as in (XLVIII) and oxidation (XLIX) to lactone (XLV).

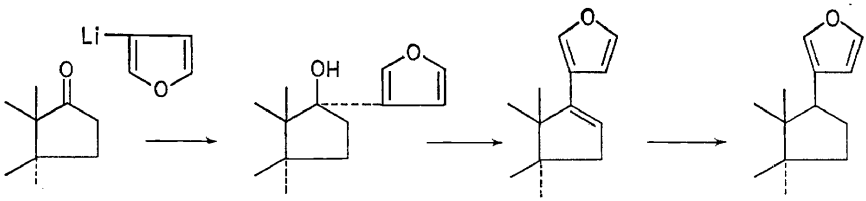


Only two isomeric structures (L) and (LI) are possible for (XLV) and on conformational ground we assign (L) to the compound isolated.

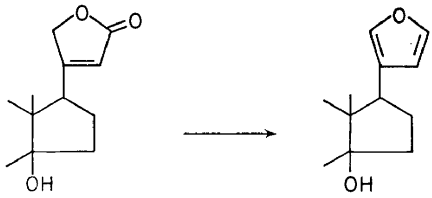


Isomeric Butenolides

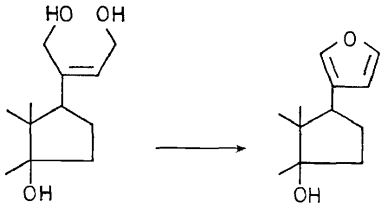
The already discussed transformation of a furan function (XXVIII) into hydroxy-butenolide (XXIX) and into the natural lactone (XXX) of digitoxigenin, appealed to us since furan intermediates are readily accessible by several routes. Examples are the reaction of a 17-carbonyl function with the appropriate furyl lithium derivative:



or by hydride reduction of a butenolide[17]:

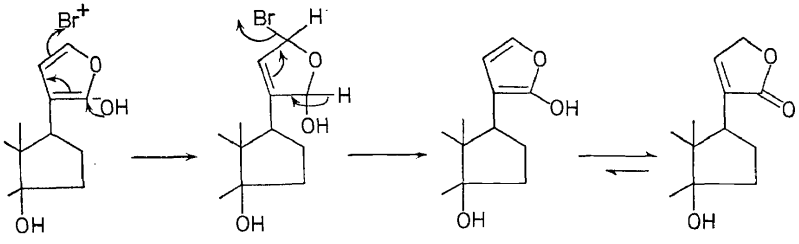


or by ring closure of an appropriate 1,4-diol:



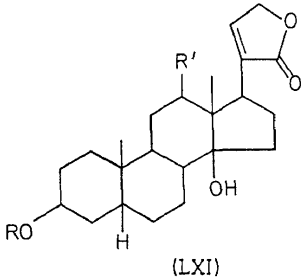
(LVI) (XXVIII)

When these furans were however treated with *N*-bromosuccinimide[8], the isomeric butenolides (LX) were the products of the reaction:



(LVII) (LVIII) (LIX) (LX)

Of particular interest are the substituted 3β,14-dihydroxy-21-oxo-23-desoxo-5β-card-20(22)-enolides (LXI)[18] which formally differ from the



(LXI)

natural cardenolides only in the position of attachment of the lactone ring to the steroid nucleus†.

Biological activity

It has long been known that the therapeutic dose for digitalis materials is a fairly constant fraction of the lethal dose, and uncomfortably close to the amount causing toxic effects such as arrhythmias. Officially adopted testing procedures are based on this seemingly indissoluble association of

† But have different chemical behaviour: notably they do not form isocardenolides under alkaline conditions.

toxic and therapeutic properties. New compounds have been considered of potential medicinal use only if they elicited sufficient toxicity *in vivo*.

In vitro the activity has been correlated to the ability of the drug to inhibit the membrane-bound (Na + K)-activated adenosine-triphosphatase, (Na—K) ATPase[19, 20].

Repke and coworkers[19] identify the 'active group' (Wirkgruppierung) as the unsaturated carbonyl of the lactone side chain which presumably binds the hydroxyl of the phosphoric acid residue in the phosphorylated enzyme, resulting in an overall inhibition of the ATPase system.

That, however, the ATPase inhibition of cardiotonic agents would not necessarily reflect their activity was advanced by Kupchan *et al.*[21], who found hellebrigenin (a bufadienolide) to have 30 times more affinity for the enzyme than strophanthidin (a cardenolide), and yet to possess the same cartiotonic activity of the latter on the guinea pig atrium. That the ATP-ase inhibition might actually be related to the arrhythmia-provoking properties of digitalis because of loss of intracellular potassium was suggested by Mason and Braunwald[22].

Our studies seem to provide experimental evidence that, indeed, the useful cardiotonic activity may not be related to inhibition of adenosine-triphosphatase. When our synthetic derivatives (LXI) were tested *in vitro*, exceptionally low activity was found (or less than one hundredth the ATP-ase inhibitory activity of the naturally occurring isomers (digitoxigenin). *In vivo*, compounds (LXI) were compared with naturally occurring cardenolides for their ability to increase the force of myocardial contraction in dogs with pentobarbital induced heart failure. Following intravenous injection, the isomeric cardenolides (LXI), in contrast to the standards, elicited inotropic activity at a dose considerably smaller than the toxic one.

More experiments and (as Withering wrote about foxglove) 'Time will fix the real value upon this discovery.'

Acknowledgements

I have the fortune of being associated with a group of enthusiastic colleagues whose ability and persistence are responsible for the success of this work. Drs Y. Lefebvre and J. M. Ferland have admirably developed the novel syntheses of butenolides and Dr G. Beaulieu has skilfully performed the *in vivo* assays.

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