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CARDIAC PROPERTIES OF THE $\Delta^{1,4}$-3-OXO-DERIVATIVE OF DIGITOXIGENIN

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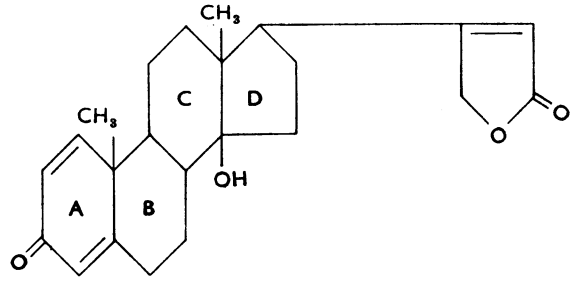
A new digitalis compound, the $\Delta^{1,4}$-3-oxo derivative of digitoxigenin ($\Delta^{1,4}$-3-oxo D), having the following structure has been obtained microbiologically from digitoxigenin by *Arthrobacter simplex*. It differs from digitoxin and digitoxigenin by having two double bonds in ring A and a cheto group in position 3 instead of a glycoside chain as digitoxin or an hydroxy group as digitoxigenin.



The compound was first prepared by Satoh & Wada (1960) by selenium dioxide oxidation of 3-dehydrodigitoxigenin. To our knowledge its biological properties have never been described. We thought the new compound to be worth testing as the results might help in determining the significance of the chair configuration of ring A for the biological activity of digitoxin. The $\Delta^{1,4}$ configuration makes ring A fully planar.

We tested the compound *in vivo* on guinea-pigs and *in vitro* on the guinea-pig isolated right ventricle preparation electrically driven. We also tested dehydroouabain (DH-ouabain), the ouabain derivative with the double bond in the lactone ring reduced, as representative of the semi-synthetic cardiac glycosides. Ouabain and DH-ouabain were also used as standard compounds in the initial stages of the research during the development of the *in vitro* method of assay.

$\Delta^{1,4}$-3-oxo D prepared microbiologically was found to have melting point (284–286° C) and infra-red and ultra-violet spectra identical with those of the compound prepared by Satoh & Wada (1960).

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METHODS

In vivo *preparations*

The activity of $\Delta^{1,4}$-3-oxo D was tested in male guinea-pigs anaesthetized with urethane, 2.5 g/kg subcutaneously, with the chest opened on the right side and artificially ventilated (22 strokes/min). A 1 mg/ml. solution in 70% alcohol in water was slowly infused at three different rates into an external jugular vein to determine the minimum dose inducing ventricular standstill. Immediately before entering the jugular vein the alcoholic solution of the drug was mixed with 0.9% saline in a constant ratio of 1:3. The polyethylene catheter in the jugular vein was linked through a three-ways connector to a 2 ml. syringe containing the alcoholic solution of the drug and to a 10 ml. syringe containing 0.9% saline. The barrels of both syringes were driven at the same speed by an infusion pump fitted with a multi-syringe attachment. This arrangement was devised to overcome the problems due to the poor solubility of the compound in water. In some animals the electrocardiogram (I, II and III leads simultaneously) was recorded at a paper speed of 50 mm/sec at 1 min intervals until the heart stopped beating.

Digitoxin and digitoxigenin (1 mg/ml. solution in 70% alcohol infused with the same procedure) and ouabain and DH-ouabain (0.1 and 1 mg/ml., respectively, in 0.9% saline infused at a constant rate of 0.116 ml./min) were used as reference compounds.

For each animal the log of the dose (in mg/kg) causing standstill was calculated and for each compound the mean lethal dose was obtained as the antilog of the mean log doses.

In vitro *preparations*

The activity of $\Delta^{1,4}$-3-oxo D was tested on the guinea-pig isolated right ventricle preparation driven by rectangular wave pulses (1/sec, 10 msec duration, 1 or occasionally 2 or 3 V) through the electrodes shown in Fig. 1. The method is basically that described by Sanyal & Saunders

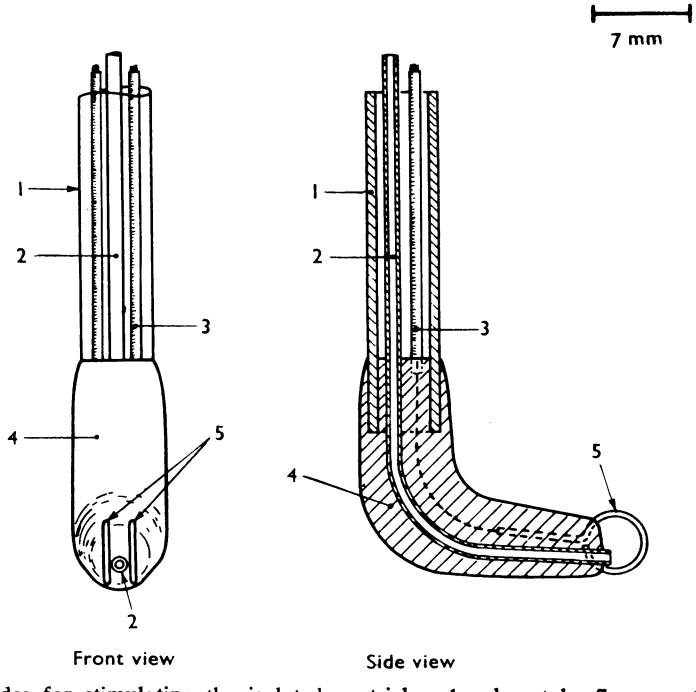

Fig. 1. Electrodes for stimulating the isolated ventricle. 1=glass tube 7 mm external diameter; 2=polyethylene tube connected with the O_2+CO_2 supply; 3=wires connecting stimulator to electrodes; 4=silicone rubber adhesive (Silastic RTV 731—Dow Corning Co.) covered by a film of flexible collodion; 5=platinum electrodes.

(1957). Contractions were recorded on lightly smoked kymograph paper using an isotonic Gimbal type lever with 7.5× magnification and a constant tension of 1 g. The stylus of the lever was made by a balsa stick 2×2 mm in section. Before the beginning of the stimulation the preparations were left to equilibrate for 15–30 min in modified Ringer solution of the following composition (g/100 ml.): NaCl 0.9 ; KCl 0.042 ; $CaCl_2$ 0.0615 ; NaOH 0.042 ; and glucose 0.1. This is basically the solution first described by Sanyal & Saunders (1957), but with the calcium concentration doubled ; it was found that lower calcium concentrations reduced the contractions and the responsiveness to ouabain and higher concentrations caused contracture and insensitivity to electrical stimulation. The medium was bubbled continuously with pure oxygen or with a mixture of 95% oxygen and 5% carbon dioxide ; after equilibration with the gas it had pH 8.2 and pH 6.9 respectively. All experiments were carried out at 32° C in an isolated organ-bath whose volume was kept constant at 60 ml. by an overflow.

An 0.8 mg/ml. solution of the compound in 70% alcohol was slowly infused into the bottom of the bath at a constant rate of 0.04 ml./min. The infusion of the drug was initiated only when the amplitude of contractions had been constant for at least 5 min. An 0.2 mg/ml. digitoxin and an 0.4 mg/ml. digitoxigenin solution in 70% alcohol infused at a constant rate of 0.04 ml./min, and an 0.1 mg/ml. ouabain and a 1 mg/ml. DH-ouabain solution in Ringer, infused at the constant rate of 0.07 ml./min, were used as reference compounds. A number of experiments were done infusing 70% alcohol or Ringer solution.

From the kymograph tracings were obtained:

1. The minimum inotropic dose (MInD), the dose provoking the earliest increase in the amplitude of contractions ;

2. The minimum irregularity dose (MIrD), the dose provoking volleys of irregular contractions lasting more than 60 sec (" permanent " irregularities). By irregular contractions is meant contractions not following the rate of electrical stimulation, faster and more vigorous than driven contractions. Extrasystoles appearing at irregular intervals were not considered " permanent " irregularities ;

3. The standstill dose (SD), the dose provoking standstill of the muscle and total insensitivity to electrical stimulation.

MIrD was estimated as shown diagramatically in Fig. 2. As the amplitude of contractions following the slow infusion of the drugs increases steadily the line B in Fig. 2 may be easily drawn.

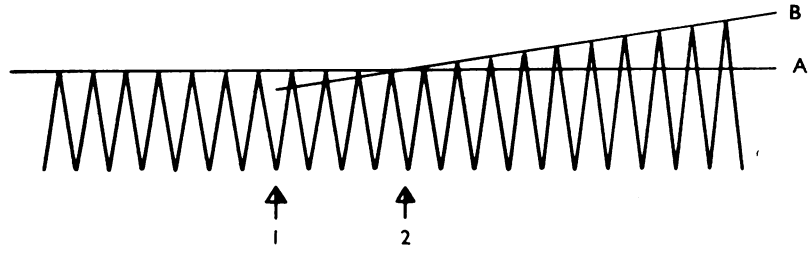

Fig. 2. Calculation of minimum inotropic dose (MInD) on the isolated electrically driven right ventricle of guinea-pigs following slow infusion of cardioactive drugs. Vertical lines represent contractions of the ventricle (contractions upwards). The tops of the contractions before infusion of the drug are joined by a straight line (line A). As the infusion starts (at arrow 1) only when the amplitude of contractions was steady for at least 5 min, line A is parallel to the base line. The tops of the contractions after drug are joined by a second straight line (line B). As the amplitude of contractions after drug increases steadily until the effect of the drug is maximum, line B forms a constant angle with the base line. The intersection between lines A and B (at arrow 2) represents the earliest increase in the amplitude of contractions and it represents the MInD.

For each experiment the logs of MInD, MIrD and SD, expressed in μg as total amounts of drug infused, were calculated; the mean values of many experiments were computed as the antilogs of the mean log doses. For each experiment the following ratios were calculated:

log (MIrD/MInD), equal to log MIrD − log MInD;
log (SD/MInD), equal to log SD − log MInD;
and log (SD/MIrD), equal to log SD − log MIrD.

The mean values of the ratios obtained in a number of similar experiments were computed as the antilogs of the mean logs of the ratios. The fiducial limits ($P=0.05$) of the mean values of the doses and ratios were computed as the antilogs of the log limits. The statistical significance of the differences were assayed with the t test at the 5% level.

MInD, MIrD and SD may be related to the actual concentrations of the drug in the bath by interpolation on a nomogram relating duration of infusion to total amount infused and concentration in bath for each drug. The nomogram was based on the equation:

$$\ln (1-C/Co) = -Qt/V$$

where C is the concentration of the drug at the time t in the bath of constant volume V (60 ml.), Q the rate of infusion in ml./min, t the duration of the infusion and Co the concentration of the drug in the solution infused. As in our experimental conditions C/Co is small and the equation approximates to $C/Co = Qt/V$, C is now linearly related to t since Co, Q and V are constants. This linear relationship is assumed in the nomogram used to calculate MInD, MIrD and SD as actual concentrations.

RESULTS

In vivo *preparations*

Table 1 records the doses of digitoxin, digitoxigenin and $\Delta^{1,4}$-3-oxo D, infused at three different rates, which caused ventricular standstill in anaesthetized guinea-pigs. With digitoxin it was possible to find the optimum rate of infusion giving the true lowest toxic

TABLE 1

LETHAL DOSES OF DIGITOXIN, DIGITOXIGENIN AND $\Delta^{1,4}$-3-OXO D DETERMINED ON MALE ANAESTHETIZED GUINEA-PIGS

Values are means with fiducial limits ($P=0.05$) in mg/kg. The allotment of animals to the treatment was random. n = Number of animals

Compound	Rate of infusion (mg/min)			
	0·157	0·078	0·039	0·019
Digitoxin		1·27 1·56–1·04 n=7	1·03 1·27–0·82 n=7	1·04 2·19–0·49 n=5
Digitoxigenin	1·83 2·01–1·21 n=8	2·11 2·47–1·67 n=8	2·58 3·18–2·10 n=7	
$\Delta^{1,4}$-3-oxo D	3·24 4·53–2·31 n=7	5·08 6·89–3·75 n=7	5·13 7·45–3·54 n=6	

dose, while with digitoxigenin and $\Delta^{1,4}$-3-oxo D the optimum rate of infusion was not determined. As it was not feasible to increase the rates of infusion because of the alcoholic content of the solutions, the toxic doses in Table 1 may not represent, particularly for $\Delta^{1,4}$-3-oxo D, the true lowest toxic doses. The activity ratios cannot therefore be precisely defined. However if we take for each compound the lowest value

recorded, $\Delta^{1,4}$-3-oxo D is 1.8-times less active than digitoxigenin and 3.1-times less than digitoxin, and digitoxigenin is 1.8-times less active than digitoxin.

The electrocardiogram proves that $\Delta^{1,4}$-3-oxo D has an activity similar to that of other compounds: the drug prolonged the P-R interval, depressed the S-T segment, inverted the T wave and provoked atrioventricular block, ventricular fibrillation and standstill of the heart. It is worth mentioning that in some experiments the heart, brought to standstill by the compound, regained spontaneously its normal activity after a few minutes of electrical and mechanical inactivity.

The infusion of 70% alcohol influenced the heart activity at doses much higher than those infused with the compound and brought about different changes in the electrocardiogram.

DH-ouabain was less toxic than ouabain. Their respective mean toxic doses and fiducial limits were 3.6 (3.5–3.9) mg/kg and 0.29 (0.28–0.32) mg/kg, 10 guinea-pigs being used for each compound.

The oral activity of the compound was tested by intraduodenal administration of single doses and monitoring the heart activity by the electrocardiogram for 2 hr after dosing. $\Delta^{1,4}$-3-oxo D was two times less active than digitoxin as judged by the appearance of arrhythmias.

In vitro *preparations*

Ouabain and DH-ouabain. Both compounds influenced the ventricle activity in the same way. In low doses they increased the amplitude of the contractions and in higher

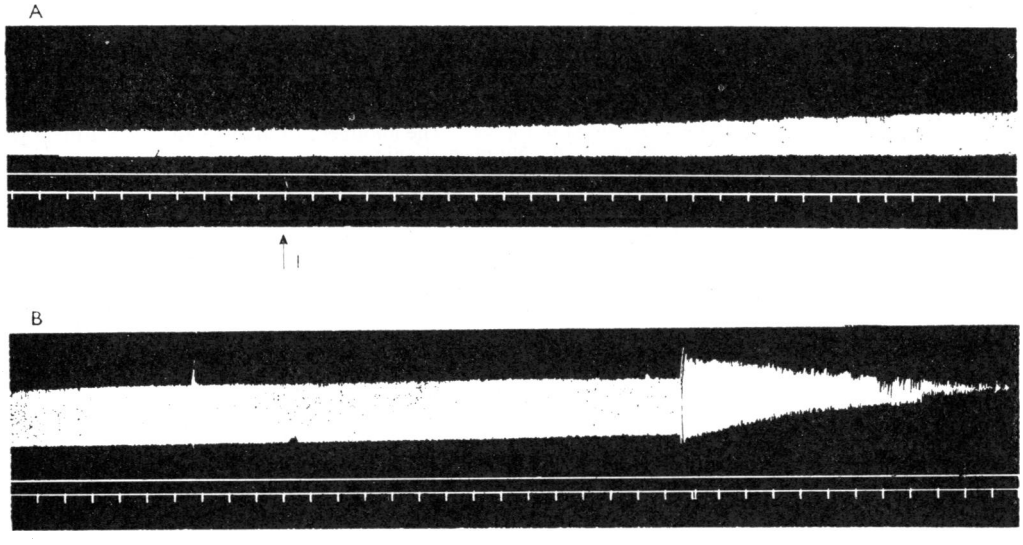

Fig. 3. Effect of continuous infusion of DH-ouabain on the contraction of guinea-pig isolated ventricle electrically driven (1 V; 10 msec; 1/sec). From top to bottom: isotonic contractions of the ventricle (contraction upwards); events marker; and time interval, 30 sec. Interval between A and B = 17 min. At the arrow DH-ouabain (1 mg/ml.) was infused at the rate of 0.07 ml./min.

doses they provoked permanent irregularities, increase in the diastolic tension on the recording lever and standstill (Fig. 3). The activity of the preparation did not change during control trials lasting at least 1 hr.

Table 2 records the mean values of MInD, MIrD and SD and their fiducial limits ($P=0.05$). Fifteen experiments were done with ouabain and fifteen with DH-ouabain.

TABLE 2

MInD, MIrD AND SD OF OUABAIN AND DH-OUABAIN DETERMINED ON THE GUINEA-PIG ISOLATED RIGHT VENTRICLE PREPARATION ELECTRICALLY DRIVEN

Values are means with fiducial limits ($P=0.05$) in μg. Ouabain (15 experiments) was infused into the bath at the constant rate of 0·116 μg/ml./min; DH-ouabain (13 experiments) at 1·16 μg/ml./min. The Ringer solution was bubbled with a mixture of 95% oxygen and 5% carbon dioxide; after equilibration with the gas it had pH 6·9

	Ouabain	DH-ouabain
MInD	49·3 64·2– 37·8	297·2 435·8– 202·7
MIrD	150·4 183·7–123·1	2,112·8 2,943·4–1,516·5
SD	212·2 246·3–182·8	2,688·9 3,693·1–1,957·7
MIrD/MInD	3·0 4·1–2·3	7·1 9·6–5·3
SD/MInD	4·3 5·7–3·2	9·0 12·4–6·6
SD/MIrD	1·4 1·5–1·3	1·3 1·4–1·1

In two experiments it was, however, impossible to bring the muscle to standstill with DH-ouabain and therefore the statistical evaluation of the results is based on a different number of experiments. DH-ouabain is about six times less active than ouabain as far as MInD is concerned and about thirteen to fourteen times less active as far as MIrD and SD are concerned. It follows that the ratios MIrD/MInD and SD/MInD are about two times higher for DH-ouabain than for ouabain while the ratio SD/MIrD is similar for both drugs. The differences in the ratios are statistically highly significant. The mean maximum increase in the amplitude of contraction, measured when MIrD was reached, was similar for both drugs: 216% for ouabain and 203% for DH-ouabain.

To test differences in the binding force of the drugs to myocardial fibres, in a second set of experiments MInD and MIrD were determined three times on the same preparation. The experiment was performed as described above but 30 sec after the beginning of permanent irregularities the preparations were thoroughly rinsed with 350 ml. Ringer solution without exposure of the muscle to the air. As each preparation regained its control level of activity a second and a third infusion were performed. Table 3 records the MInD and MIrD for each one of the three infusions as mean values of 14 experiments for each compound. In these experiments DH-ouabain was less active than ouabain but once more the ratio MIrD/MInD was higher for DH-ouabain than for ouabain. Pooling the results of the three infusions and revaluating the mean values of MInD and MIrD, MInD of DH-ouabain was about seven times and MIrD about 15 times that of ouabain. The ratio MIrD/MInD was two times higher for DH-ouabain than for ouabain, consistent with the values of Table 2. The values of Table 3 related to the first infusion differ sometimes considerably from the corresponding values of Table 2. A plausible explana-

TABLE 3

MInD AND MIrD OF OUABAIN AND DH-OUABAIN DETERMINED ON THE GUINEA-PIG ISOLATED RIGHT VENTRICLE PREPARATION ELECTRICALLY DRIVEN

Values are means with fiducial limits ($P=0.05$) in μg. Ouabain (7 experiments) was infused into the bath at the constant rate of 0·116 μg/ml./min; DH-ouabain (7 experiments) at 1·16 μg/ml./min. MInD and MIrD were determined three times on the same preparation. The Ringer solution was bubbled with oxygen; after equilibration with the gas it had pH 8·2

		Ouabain	DH-ouabain
1st infusion	MInD	37·1 46·1–29·9	207·3 292·1–147·2
	MIrD	83·2 98·9–66·9	1,221·9 1,845·7–808·6
	MIrD/MInD	2·2 3·0–1·6	5·9 9·2–3·7
2nd infusion	MInD	29·4 44·6–19·3	281·2 442·6–178·7
	MIrD	97·1 136·2–69·1	1,520·0 2,149·5–1,074·5
	MIrD/MInD	3·3 5·2–2·1	5·4 9·3–3·1
3rd infusion	MInD	40·2 78·7–20·6	280·6 426·2–184·7
	MIrD	111·6 161·0–77·4	1,883·8 3,065·1–1,157·7
	MIrD/MInD	2·8 5·0–1·5	6·7 10·8–4·2
Means of the three infusions	MInD	35·3 44·4–28·0	253·9 310·8–207·4
	MIrD	95·9 112·2–81·9	1,518·0 1,878·4–1,226·7
	MIrD/MInD	2·7 3·4–2·1	6·0 7·6–4·7

tion may be the different pH of the medium, 6.9 for the experiments summarized in Table 2 and 8.2 for those in Table 3. The differences between the values of MInD and MIrD recorded in the two sets of experiments do not detract from the validity of the fact that their ratios are really different for ouabain and DH-ouabain.

To overcome the objection that invalid conclusions might have been drawn from the infusion method because differences in latent periods before the onsets of the inotropic and toxic effects had biased the determination of MInD and MIrD, a third set of experiments was performed. The drugs instead of being slowly infused into the bath were added in stepwise increasing doses, without rinsing the preparation, at 15-min intervals until the heart stopped beating; 0.125, 0.125, 0.25, 0.5, 1 and 2 ml. of a 1 mg/ml. solution of DH-ouabain or an 0.1 mg/ml. solution of ouabain were added in succession. The dose that within 15 min increased the amplitude of contractions is referred to as MInD and that which provoked permanent irregularities as MIrD. For each compound seven experiments were performed. With ouabain in five out of seven experiments MIrD was twice MInD and in two experiments it was four times. With DH-ouabain the ratio of the two doses equalled 4 in only one experiment out of seven, equalled 8 in five experiments and 32 in one. The values of the ratio, higher for DH-ouabain than for ouabain, were similar to those obtained in the first two sets of experiments.

Digitoxin, digitoxigenin and $\Delta^{1,4}$-3-oxo D. The activity of $\Delta^{1,4}$-3-oxo D on the isolated ventricle was in all aspects similar to that of digitoxin and digitoxigenin. In low doses it increased the amplitude of contractions, and in higher doses it provoked irregular contractions, increase of the diastolic tension on the recording lever and standstill. The influence of the solvent (70% alcohol) was tested in analogous experimental conditions. It never increased the amplitude of contractions or provoked irregularities, increase of diastolic tension or standstill. Seventy per cent alcohol does not modify the reactivity of the ventricle to digitalis compounds. As shown before the effect of ouabain may be reproduced several times on the same preparation. Ouabain (50 μg) was therefore tested on the same preparation in the absence and in the presence of alcohol (2.4 ml. corresponding to the quantity infused in 1 hr). The inotropic effect of ouabain in the presence of alcohol was only slightly less than in absence of alcohol. In one experiment the maximum increase in the amplitude of contractions provoked by ouabain in presence of alcohol was 200%, while the corresponding value in absence of alcohol was 220%. The decrease observed was similar to that experienced with repeated administration of ouabain alone.

Table 4 records the mean values with their fiducial limits (20 experiments) of MInD, MIrD and SD which decrease in the order digitoxin, digitoxigenin and $\Delta^{1,4}$-3-oxo D. Table 4 also shows that the ratios MIrD/MInD and SD/MInD decrease in the order $\Delta^{1,4}$-3-oxo D, digitoxigenin and digitoxin. The ratio SD/MIrD decreases in the order digitoxin, digitoxigenin and $\Delta^{1,4}$-3-oxo D.

TABLE 4

MInD, MIrD AND SD OF DIGITOXIN, DIGITOXIGENIN AND $\Delta^{1,4}$-3-OXO D DETERMINED ON THE GUINEA-PIG ISOLATED RIGHT VENTRICLE PREPARATION ELECTRICALLY DRIVEN

Values are means with fiducial limits ($P=0{\cdot}05$) in μg. Digitoxin was infused into the bath at the constant rate of 0·133 μg/ml./min; digitoxigenin at 0·266 μg/ml./min; $\Delta^{1,4}$-3-oxo D at 0·533 μg/ml./min. There were 20 experiments with each drug. The Ringer solution was bubbled with a mixture of 95% oxygen and 5% carbon dioxide; after equilibration with the gas it had pH 6·9

	Digitoxin	Digitoxigenin	$\Delta^{1,4}$-3-oxo D
MInD	67·7 56·8–80·7	75·7 61·1–93·8	169·9 138·4–207·9
MIrD	132·3 119·4–146·5	225·8 181·2–281·3	883·9 807·6–967·5
SD	286·1 246·4–332·1	454·3 382·4–539·9	1,324·5 1,235·7–1,420·3
MIrD/MInD	1·9 1·6–2·4	3·0 2·3–3·9	5·2 4·4–6·2
SD/MInD	4·2 3·6–5·0	6·0 4·6–7·7	7·8 6·4–9·5
SD/MIrD	2·1 1·9–2·5	2·0 1·7–2·4	1·5 1·4–1·6

DISCUSSION

The experiments reported in this paper show that the activity of $\Delta^{1,4}$-3-oxo D, a digitalis compound obtained microbiologically with *Arthrobacter simplex* from digitoxigenin, is qualitatively similar to that of digitoxin and digitoxigenin. *In vivo* it provokes ventricular standstill and brings about the electrocardiographic alterations peculiar to digitalis; *in vitro* low doses increase the amplitude of contractions while higher doses

provoke irregular contractions, increase of diastolic tension and standstill. It closely resembles DH-ouabain. Both are less active, *in vivo* as well as *in vitro*, than their parent compounds and both have *in vitro* MIrD/MInD and SD/MInD ratios higher than the parent compounds, that is both exert inotropic effects at much lower fractions of the toxic doses than their parent compounds.

The differences in the ratios considered may find an explanation in differences in latent periods necessary for the full development of the response to digitalis. It is known that this response is of slow onset and that *in vitro* it depends on the concentration and on the duration of exposure. Taeschler, Schalch & Cerletti (1963) have shown that, in contrast to that of natural glycosides, the DH-ouabain cardiac effect measured on isolated right auricles of the guinea-pig was immediate and scarcely depended upon the duration of exposure. It may therefore be that an immediate onset of the cardiac action of $\Delta^{1,4}$-3-oxo D and of DH-ouabain is the main reason for the larger ratios where MInD is involved. However the results of some of our experiments with ouabain and DH-ouabain are not in full agreement with this explanation. The ratio MIrD/MInD calculated from the slow infusion method is not dissimilar from that calculated from the single dose method that avoids the influence of potential differences in latent periods.

A second explanation of the differences of the ratios considered may be based on the assumption of the existence of two separate mechanisms responsible for the inotropic and for the irregularity standstill effects. $\Delta^{1,4}$-3-oxo D and DH-ouabain may have influenced them differently from digitoxin, digitoxigenin and ouabain; the greater ratios may therefore reflect an actual wider gap between cardiotonic and cardiotoxic doses.

The possible existence of separate mechanisms for inotropic and toxic effects of digitalis is suggested by differences in cation (sodium, potassium and calcium) concentrations found in rabbit hearts, in rabbit auricles, in cat papillary muscle and in guinea-pig auricles treated with therapeutic and toxic doses of ouabain and digitoxigenin. Contrary to the hypothesis that the inotropic effect of cardiac glycosides is caused by a decrease in intracellular potassium and an increase in intracellular sodium, Farah & Witt (1963); Klauss, Kuschinsky & Lüllmann (1963) and Lee (1963) have found that the cation concentrations changed only when toxic doses of digitalis were used. Furthermore, Vick (1959) found that in guinea-pig hypodynamic isolated auricles nonsteroid lactones produced toxic effects (contracture) without provoking cardiotonic effects (positive inotropic effects) and that steroid lactones (cardiac glycosides) could produce positive inotropic effects without provoking contracture. It has been suggested that the two effects may be brought about by two different mechanisms.

The correct explanation of our results cannot yet be stated. For the time being it is enough to point at differences in the activity of different digitalis compounds and to leave open the question whether the toxic manifestations of digitalis overdosage are based on the same mechanisms responsible for the inotropic effects.

The results reported in this paper indicate that some parts of the digitalis molecule, hitherto thought to be essential for its activity, may be altered without destroying the fundamental properties of the molecule but provoking a dissociation between some of the properties thought to be closely linked. The double bond in the lactone ring may be reduced, as in DH-ouabain, and ring A, that in digitoxin is bent with a chair

configuration, may be made fully planar as in $\Delta^{1,4}$-3-oxo D. The resulting compounds still retain the main properties of a digitalis compound but *in vitro* provoke an inotropic effect with a smaller fraction of the lethal dose than do digitoxin and ouabain. The alterations in the structure do not instead modify the progression of the glycoside effect from a cardiotonic phase to a cardiotoxic phase, as shown by the fact that the ratio SD/MIrD remained almost the same for all compounds. The lack of the glycoside chain in $\Delta^{1,4}$-3-oxo D is not the only cause of these results. The new compound has an MIrD/MInD statistically different from that of digitoxigenin.

The reliability of the method used for testing *in vitro* digitalis activity is shown by the agreement between the results reported in this paper and those reported in the literature and obtained with other techniques (Jacobs & Hoffman, 1927; Vick, Kahn & Acheson, 1957; Lipicky, 1959; Vick, 1959; Taeschler *et al.*, 1963; Kahn, Van Atta & Johnson, 1963; Acheson, Kahn & Lipicky, 1964; Ehmer, Jahr, Kuschinsky, Lüllmann, Reuter & Wollert, 1964).

SUMMARY

1. The dose provoking ventricular standstill in anaesthetized guinea-pigs has been determined for digitoxin, digitoxigenin, ouabain, DH-ouabain and for a new derivative of digitoxigenin, obtained microbiologically, $\Delta^{1,4}$-3-oxo D. The latter compound was less active than the parent compounds but provoked similar electrocardiographic alterations.

2. The minimum doses provoking a positive inotropic effect, irregular contractions and cardiac standstill have been determined for the same compounds on isolated right ventricles of guinea-pigs electrically driven. $\Delta^{1,4}$-3-oxo D was less active than digitoxin and digitoxigenin but it exerted an inotropic effect at a smaller fraction of the lethal dose than did the parent compounds.

3. The *in vivo* and the *in vitro* effects of $\Delta^{1,4}$-3-oxo D closely resemble that of DH-ouabain.

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