CARDIOTONIC ACTION OF RING A-MODIFIED CARDENOLIDES, WITH SPECIAL REFERENCE TO CLEAVAGE OF THE RING

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Abstract—Cardiotonic activities of six newly synthesized ring A-modified cardenolides, four 3,5-seco-4-nor-cardenolides and two 4-oxa-cardenolides, were studied in isolated frog hearts and guinea pig atria. These compounds were 14-hydroxy-3,5-seco-4-nor-5-oxo-14β-card-20(22)-enolid-3-oic acid (IIa), and its methyl ester (IIb); 5β, 14- and 5α, 14-dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ols (IIIa and IIIb); 3-dehydro-4-oxa-azarigenin (IVA) and 3-dehydro-4-oxa-digitoxigenin (IVb). In the frog heart (Straub's preparation), positive inotropic effects were demonstrated with all compounds except IIa. The pattern of response was the same as that to digitoxigenin (I). The relative potencies obtained on the basis of the concentration of each compound in which a contracture was brought about, the potency of I being taken as standard, were as follows: I (1.0), IIa (--), IIb (0.03-0.1), IIIa (0.01), IIIb (<0.01), IVa (<0.01), and IVb (0.03-0.1). The positive inotropic effect of IIb was also confirmed in guinea pig atria. IIb and IVb potentiated dose-dependently the K-contracture of the frog ventricular muscle just as was seen with I, indicating that the cardiotonic activity of the compounds is the same in nature as that of digitoxigenin. It was clearly demonstrated that the perhydrocyclopentanophenanthrene nucleus is not an indispensable requirement for cardiotonic activity.

In the course of our studies on the structure-activity relationship of the cardenolide, several 3,5-seco-4-nor-cardenolides, in which the ring A of the steroid nucleus is cloven, were prepared from digitoxigenin (I). Furthermore, two 4-oxa-cardenolides were derived from the 3,5-seco-4-nor-cardenolide (I). In this paper, we describe the cardiotonic activities of six new compounds in comparison with that of digitoxigenin (I) as tested in isolated frog hearts and guinea pig atria.

A part of the present work has appeared as preliminary communications (2, 3).  

MATERIALS AND METHODS

The six new compounds used in this study are as follows, the synthesis of which will be reported elsewhere (1): 14-hydroxy-3,5-seco-4-nor-5-oxo-14β-card-20(22)-enolid-3-oic acid (IIa), its methyl ester (IIb), 5β, 14-dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ol (IIIa), 5α, 14-dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ol (IIIB), 3-dehydro-4-oxa-azarigenin (IVA), and 3-dehydro-4-oxa-digitoxigenin (IVB). Their structural formulae are...
indicated in Fig. 1.

Stock solutions of the compounds were prepared with 95% ethanol in a concentration of 1.0 mg/ml. Before experiments, these stock solutions were diluted to the desired concentrations with 0.6% saline for frog hearts and 0.9% for guinea pig atria.

**Isolated frog hearts (Straub's preparation):** Male frogs (Rana nigromaculata) of 25-40 g body weight were used. The method of assay is the same as described in the previous papers (2, 4, 5). The contraction was recorded isotonically on a smoked drum. The Straub's cannula contained 2 ml of Ringer's solution, the composition of which (in mM) was: NaCl 111, KCl 2.7, CaCl₂ 1.8, NaHCO₃ 15, and glucose 2.7 and such was aerated with 95% O₂ + 5% CO₂.

The heart was first made hypodynamic by reducing the concentration of calcium to 0.6 mM, 1/3 of the normal, and was allowed to equilibrate for about 20 min. The effect of the compound was then tested in the following way. Starting from a subthreshold dose, a small amount (10-70 μl) of a test solution was added to the cannula every 15 min so that a stepwise increase in the cumulative concentration of the test compound was achieved until the heart went into contracture. The way of increasing the cumulative concentration was: 10⁻ⁿ, 3 x 10⁻ⁿ, 10⁻ⁿ⁻¹ g/ml, ... The height of contraction always reached a plateau before the next addition was made.

The relative potencies were obtained on the basis of the final concentration of each compound at which contracture of the heart (a marked rise in the diastolic tension, usually accompanied by arrest) was brought about. The potency of digitoxigenin in the same lot of animals was taken as 1.0. The experiments were carried out at room temperature of 18-24°C.

**Potassium-induced contracture in the isolated frog ventricular muscle:** The procedure is basically the same as described by Takeda et al. (6). A circular strip was dissected out from the middle portion of the frog ventricle, and cut open to give a muscle preparation about 1 mm thick and 5-8 mm long. This tissue was suspended in an organ bath containing 10 ml of Ringer's solution aerated with 95% O₂ + 5% CO₂. The resting tension, ranging 0.3-0.5 g, was determined in each preparation so that the twitch tension became maximum. The muscle was stimulated electrically by square wave pulses, 5 msec duration, at the rate of 0.2 Hz throughout the experiment, except when K-contracture was induced and recorded. Stimulation was made via a hook holding the lower end of the preparation. The pulses
were provided by an electronic stimulator (Nihon Kohden MSE-3). The contractile tension was recorded isometrically on an ink-writing oscillograph with the aid of a force-displacement transducer (Nihon Kohden SB-1T) and a carrier amplifier (Nihon Kohden RP-5). The upper end of the muscle was connected to the transducer by string.

K-contractures were elicited by replacing the Ringer’s solution in the bath with oxygenated K-rich solution. Potassium concentrations of the K-rich solutions were changed by replacing NaCl with equimolar amounts of KCl, except the solution containing 120 mM potassium. In the latter, 111 mM NaCl was replaced by 117.3 mM KCl. Application of K-rich solution lasted for 1–2 min, during which the tension development reached a plateau. Then the preparation was washed with fresh Ringer’s solution, and more than 20 min elapsed before the next administration. The intervals of 20 min were sufficient to obtain a reproducible size of contractures. The experiments were also performed at room temperature.

Isolated guinea pig atria: The right and left atrial preparations were prepared from freshly excised hearts of stunned and bled male guinea pigs (470–540 g). The left atrial preparation, stimulated at a constant rate (4 Hz, duration 1 msec, voltage twice threshold) by a square-wave electronic stimulator, was used for the study of the inotropic effect. The right atrial preparation, which retained a spontaneous beat, was used to assess the chronotropic effect and its contractile force was also recorded. The preparations were suspended separately in 10 ml organ baths containing Krebs-bicarbonate solution aerated with 95% O₂ : 5% CO₂ and kept at 37°C. The composition of the solution (in mM) was: NaCl 118, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.18, MgSO₄ 1.18, NaHCO₃ 24.9 and glucose 11.1.

To obtain optimum responses, the initial tensions of the preparations were set at 0.2–0.3 g. The contractile tension was recorded isometrically on an ink-writing oscillograph with a force-displacement transducer coupled with a carrier amplifier. The rate of spontaneous beat was recorded with a cardiotachometer (Nihon Kohden RT-2) into which the output of the carrier amplifier was fed. Before the drugs were added to the bath, the preparation was washed several times and allowed to equilibrate for 1 hr after mounting. The administration of the drugs was made in a cumulative manner.

RESULTS

Assay of the cardiotonic activity with Straub’s preparation

The six compounds were assayed with two lots of frogs. Four animals were allocated to each compound. In each lot, digitoxigenin was taken as the standard. A typical experiment with IIb is shown in Fig. 2, and the results of assay are summarized in Table 1. With digitoxigenin, the hearts of all eight frogs went into contracture at the concentration of 3·10⁻³ g/ml. Compounds IIb, IIIa and IVb brought about a contracture at the concentrations indicated in the Table. The whole pattern of response of the Straub’s preparation to IIb, IIIa and IVb was not distinguishable from that to digitoxigenin. While slight increases in the height of contraction were induced by IIIb and IVa, these compounds did not cause a contracture even at 3·10⁻² g/ml, the highest concentration tested. With
FIG. 2. Typical response of the isolated frog heart (Straub’s preparation) to the compound IIb. At the arrow, the smoked drum was stopped for 10 min, and the normal Ringer’s solution was replaced with the solution containing 0.6 mM Ca, 1/3 of the normal. The concentration of IIb was increased cumulatively at the dots.

**TABLE 1.** Final concentrations and the relative potencies of the compounds used.

| Compound | Final concentration | Relative potency |
|----------|---------------------|-----------------|
| I        | $3 \times 10^{-7}$  | 1.0             |
| IIa      | $>3 \times 10^{-3}$ | (inactive)      |
| IIb      | $3 \times 10^{-8}$ $\sim 10^{-5}$ | 0.03–0.1       |
| IIIa     | $3 \times 10^{-5}$  | 0.01            |
| IIIb     | $>3 \times 10^{-5}$ | $<0.01^*$       |
| IVa      | $>3 \times 10^{-5}$ | $<0.01^*$       |
| IVb      | $3 \times 10^{-6}$ $\sim 10^{-5}$ | 0.03–0.1       |

Final concentration: concentration of the compound at which a contracture was brought about. Relative potency: Digitoxigenin (I) was taken as standard. $^*$: no tendency to contracture at $3 \times 10^{-5}$ g/ml. Number of frogs is given in parentheses.

IIa, however, any sign of increased contractility was not observed even at the highest concentration.

The heart rates ranged between 40 to 70/min when the preparations were set up. No consistent change was caused in heart rate either by reduction of calcium in Ringer’s solution, or by the cumulative addition of the compounds tested, until the concentration reached one or two steps prior to the final. Then the heart rate dropped quickly, and arrest followed. In this regard, there was no distinction between digitoxigenin and IIb, IIIa and IVb.

**Increase in K-contracture**

In order to ascertain that the mode of cardiotonic action of the active compounds is of the same nature as that of digitoxigenin, the effects of IIb and IVb on K-contracture of the frog ventricular muscle were examined. Four experiments were performed, respectively.

After equilibration, K-contractures were induced by three concentrations of K-rich solutions containing 60, 80 and 120 mM K$^+$. The responses were regarded as control. Thereafter, K-contractures in the presence of the test compound were studied. The K-contractures were induced about 1 hr after administration of the agents. The result of a typical experiment is shown in Fig. 3. K-concentration-tension curves were shifted by both
digitoxigenin and IIb, to the left, in a parallel manner. Similar results were confirmed in three other experiments with IIb, and in the four experiments with IVb.

Responses of guinea pig atria

Four experiments with compound IIb, and three experiments with digitoxigenin were carried out. Fig. 4 depicts a typical example of the responses to IIb.

In the left atrial preparations, the contractile force began to increase progressively at $3 \times 10^{-7} - 10^{-5}$ g/ml of IIb. It increased with concentration and reached a peak at $3 \times 10^{-6} - 10^{-5}$ g/ml. Arrhythmic contractions which did not follow the electrical stimulation appeared abruptly and the contractile force decreased gradually. In this course it was observed that the resting tension tended to increase markedly, and all four atria reached the contracture at $10^{-5}$ g/ml in half an hour or more (Fig. 4, B). Though the initiation of the response of the right atrium delayed, in general, from that of the left atria, the marked increases in the

![Figure 3](image)

**Fig. 3.** Effects of digitoxigenin and the compound IIb on the K-concentration-tension curves of the frog ventricular muscle. The concentrations (g/ml) of the compounds are indicated under the curves.

![Figure 4](image)

**Fig. 4.** Typical responses of the right atrium (A) and the left atrium (B) of guinea pig to the compound IIb. The concentration of the compound was increased cumulatively at the arrows. The intervals of drug administration were 15-20 min.
resting tension were constantly elicited by $10^{-5}$ g/ml of IIb. The spontaneous beating rates of the right atria ranged 160 to 220 beats/min and did not change appreciably until the concentration of IIb reached $10^{-5}$ g/ml. It was then consistently observed that a sudden increase in the beating rate (sudden quickening) preceded the contracture (Fig. 4, A).

The course of events was the same as that caused by digitoxigenin, which induced the contracture and sudden quickening at the concentration of $10^{-6}$ g/ml in all three experiments, indicating that digitoxigenin is about ten times more potent than IIb.

**DISCUSSION**

Against the long-standing belief (7, 8) on the structure-activity relationship of the cardenolide, it was demonstrated earlier that the oxygen function at C-3 of the steroid nucleus is not an indispensable requirement for cardiotonic activity, because 3-deoxydigitoxigenin (9) proved to be nearly equipotent with digitoxigenin when tested for its effect on the isolated hearts (4) or for its inhibitory action on Na⁺, K⁺-ATPase (10, 11). To determine whether or not the steroidal skeleton of the cardenolide is an essential structural requirement for cardiotonic activity, 3,5-seco-4-nor-cardenolides were synthesized by cleaving the ring A of digitoxigenin (1). Moreover, 4-oxa-cardenolides which are derivable from the 3,5-seco-4-nor-cardenolide were prepared (1). To our knowledge, the synthesis and pharmacological examination of these types of the cardenolide have never been reported.

It has been demonstrated in the present study that the steroid nucleus is not an indispensable requirement for the cardiotonic activity, since of the four 3,5-seco-4-nor-cardenolides (IIa, IIb, IIIa and IIIb) IIb and IIIa produced definite cardiotonic activities. The reason why IIa is inactive but its methyl ester IIb is active is not aptly explainable. The finding that IIIa (5β-hydroxy compound) has a weak but definite cardiotonic activity while its 5α-hydroxy isomer IIIb is much less active, on the other hand, is suggestive of the previous observations that digitoxigenin having 3β-hydroxy group is much more potent than its 3-epimer (3-epidigitoxigenin) in various bioassay methods (5, 11, 12).

As for the cardiotonic activity of the 4-oxa-cardenolides, IVa and IVb, which are isomeric only at C-5 position, it has been found that IVb (5β) is much more potent than IVa (5α). This finding indicates the similar structure-activity relationship observed with the cardenolide aglycones in regard to the configuration at C-5 that the A/B cis-cardenolides (e.g., digitoxigenin, digoxigenin) are much more potent than the A/B trans-cardenolides. (e.g., uzarigenin, syriogenin) (5).

The whole pattern of responses of the Straub’s preparation to the compounds IIb, IIIa and IVb were not distinguishable from that to digitoxigenin. The same was true of the response of the guinea pig atria to IIb. Furthermore, the K-contracture of frog ventricular muscle was potentiated by IIb and IVb. These observations indicate that the cardiotonic action of these compounds is of the same nature as the characteristic action of cardiotonic steroids.

The results in the present study concerning the cardiotonic activity of the 3,5-seco-4-nor-cardenolides and 4-oxa-cardenolides have clearly demonstrated that the perhydrocy-
clopentanophenanthrene nucleus is not an indispensable requirement for the cardiotonic activity. The heterocyclic cardenolide analogs other than the 4-oxa-cardenolide may be expected to possess cardiotonic activity.

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