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Synthesis and evaluation of cardiotonic activity of simple butenolides

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Summary — Simple butenolide derivatives have been synthesized and their properties have been tested as inotropic agents. Although these compounds maintain the butenolide and the 14-hydroxyl group as compared with natural cardenolides, they lack inotropic activity.

Résumé — Synthèse et évaluation de l'activité cardiotonique des dérivés de buténolides. *Des dérivés simples de buténolides sont synthétisés et essayés pour leurs propriétés comme agents inotropiques. Bien que ces substances comportent le buténolide et le groupe hydroxyle-14 des cardénolides naturels, ils ne présentent pas d'activité inotropique.*

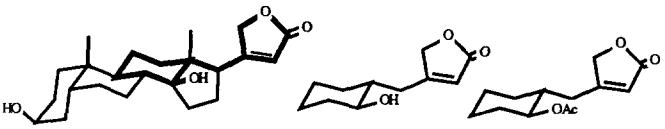
Introduction

The use of cardiotonic glycosides for the treatment of congestive cardiac insufficiency is limited by low therapeutic index of these compounds owing to their cummulative toxicity together with other undesirable effects such as arrhythmias, cephaleas, ventricular fibrillation, etc. [1, 2]. Accordingly, the search for simpler and less toxic compounds and a better understanding of the nature of the digitalis receptor, justifies the need for synthesizing simplified analogs of these substances.

Studies on the structure-activity relationship of cardiotonic glycosides and related substances [3–5] have shown that butenolide and other similar unsaturated systems are particularly important for biological activity, hence in the preparation of new compounds with possible cardiotonic activity it would seem appropriate to maintain this ring as a pharmacophore fragment. The literature contains references to several families of compounds in which there is a butenolide supported by more or less complex skeletons such as: simple aromatic and non-aromatic rings [6, 7, 8, 9], more complex aromatic systems [10, 11, 12, 13] stilbene derivatives [14–21] and systems close to the steroid skeleton [22, 23].

From the studies carried out on activity, it may be inferred that a greater similarity with the steroid skeleton, the molecular size and the substituents present in the compound, enhance the cardiotonic activity in these substances, although some of the simple models [10, 12] also show a certain degree of positive inotropic activity.

In the present work, we report on the synthesis of substances which, could show positive inotropic activity because they maintain the structural requirements considered to be essential to the cardenolides, the butenolide at C_{17} and the hydroxyl group at C_{14} [24] (fig 1). With this in mind, we synthesized 3-(*trans*-2-hydroxycyclohexylmethyl) but-2-enolide **1** on which the OH group and the lactone are kept in the same relative disposition on a cyclohexane ring equivalent to the C ring of the cardenolide (fig 1) in order to check whether these are in fact the minimal structural requirements for the activity of the cardenolides.



1 2

Fig 1. Fragment selected from the structure of cardenolide and the compounds synthesized, **1** and **2**.

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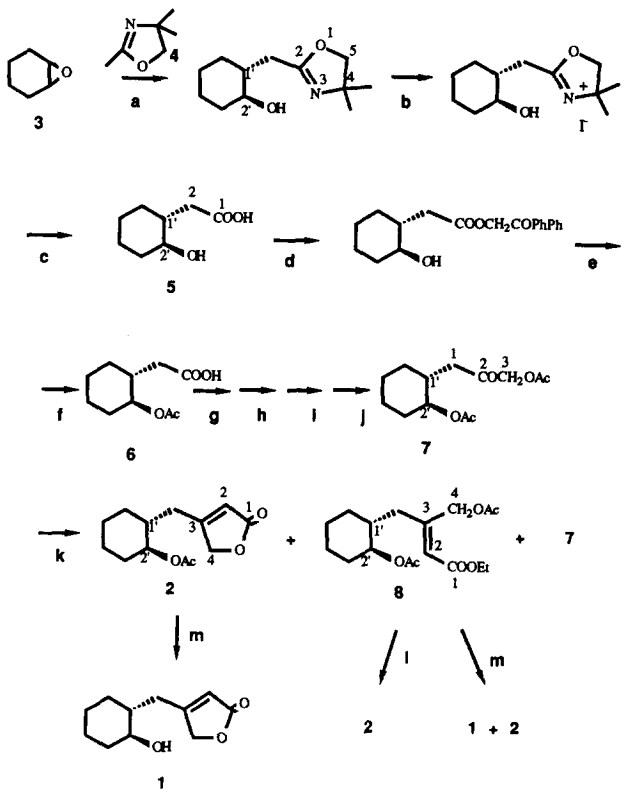
Chemistry

Treatment of the cyclohexane oxide **3** with the anion of 2,4,4-trimethyl-2-oxazoline generated by treatment of **4** with BuLi at –78°C [25, 26] resulted in the opening of the oxiranic ring. Alkylation of the hydroxyoxazoline formed and later hydrolysis afforded the hydroxyacid **5**, with a global yield of 33.9% from **3**. The *trans* stereochemistry of the substituents in compound **5** was checked in the ^1H NMR spectrum by the multiplicity of the hydrogen atom geminal to the hydroxyl group, resonating at 300 ppm (1H, ddd, $J_{2'-3'e}$ = 4.4 Hz, $J_{2'-3'a}$ = 9.8 Hz, $J_{2'-1}$ = 9.8 Hz), indicating the existence of two *trans*–diaxal couplings and one equatorial-axial coupling.

Conversion of the hydroxyacid **5** into the acetoxyacid **6** was carried out by acetylation of the phenylphenacyl ester and hydrogenolysis, since direct acetylation of **5** leads to lactonization in the presence of different acetylating agents.



Scheme 1. a = BuLi: –78°C; b = MeI; c = NaOH; d = $BrCH_2COPhPh$; e = Ac_2O/Et_3N/benzene; f = Zn/acetic acid; g = Cl_2SO/benzene; h = diazomethane/ether; i = HCl (g); j = Ac_2O/NaOAc; k = Horner-Emmons; l = $NaHCO_3$/MeOH; m = HCl/EtOH/ether.

Conversion of **6** into the acyl chloride allowed us to lengthen the chain by reaction with diazomethane. Treatment of the diazoketone obtained by HCl (g) and later substitution of the chloride by acetate in the presence of $NaOAc/Ac_2O$ afforded compound **7**, in which the *trans*-diequatorial stereochemistry of the substituents of the cyclohexane ring is conserved.

The acetoxyketone **7** reacted with triethylphosphonoacetate under the conditions of a Horner-Emmons reaction [27], affording a mixture of substances comprising the following: the acetoxybutenolide **2** (11.4%), the expected condensation product **8** (40.6%) and the starting compound **7** (21.4%), together with small non-isolated amounts of the condensation product with the *E* configuration. These compounds were characterized by their spectroscopic properties. In its IR spectrum, substance **2** exhibited absorptions of the acetoxyl group (1 740 cm^{-1}) and its NMR showed the hydrogen geminal to the acetoxyl 4.53 (1H, ddd, $J_{2'-3'e}$ = 4.4 Hz, $J_{2'-3'a}$ = 9.8 Hz, $J_{2'-1'}$ = 9.8 Hz) and the butenolide 4.70 (2H, brs) and 5.89 (1H, brs) ppm.

Compound **8** was transformed quantitatively into **2** by treatment with a saturated solution of sodium bicarbonate in methanol water, while treatment under acid conditions (HCl in $EtOH/Et_2O$) afforded a mixture of acetoxylactone **2** (17.7%) and the hydroxylactone **1** as the major product (68.7%). Likewise, acid treatment of **2** yielded the lactone **1** (82.1%).

Results and Discussion

In the assays carried out with compounds **1** and **2** on the rate and force of beating of isolated Guinea pig atria, no positive inotropic activity was observed at the concentrations employed compared with digoxin, as shown in the D/R curves (fig 2) of these substances. Neither were any modifications in cardiac rate noted.

In view of the lack of positive inotropic activity in the compounds assayed, it may be inferred that such a simple model does not correspond to the essential minimum responsible for the action, and it would be necessary to construct other molecules to extend the model. Taking into account that the model proposed for the digitalis receptor by Thomas, Schwartz and Pitts [28] has 3 anchorage sites (A, B, C) and that in substances **1** and **2** there are groupings that are able to bind to site A, and partially so to site B, the lack of response could be due to the fact that the interaction with the receptor is very fast. Accordingly, it would be necessary to include some other structural requirement able to produce stronger hydrophobic interactions at site B and a group able to establish favourable interactions with site C.

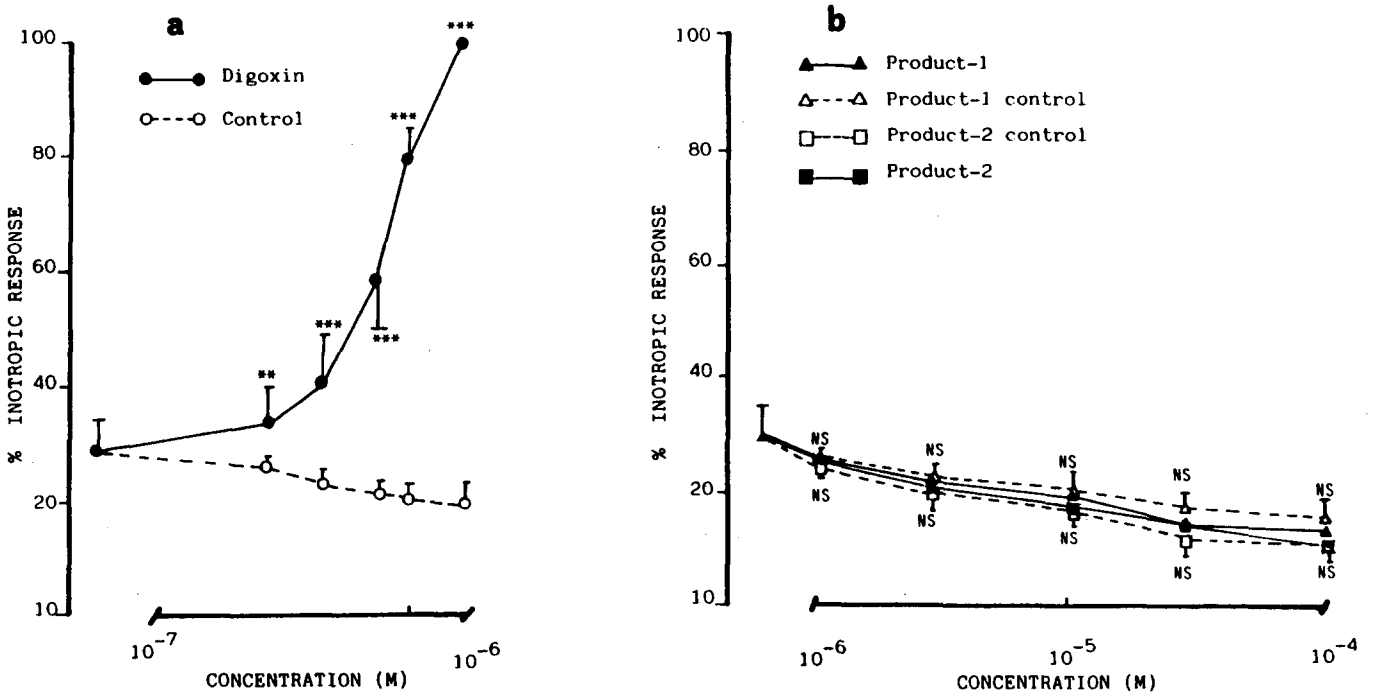


Fig 2. Cumulative concentration-response curves to digoxin (a), **1** and **2** (b) in left atrium isolated from guinea pigs. Each value is the mean from 5 preparations; vertical lines show the SEM. ** and *** indicate $P < 0.01$ and $P < 0.001$, respectively, when compared to the corresponding control values.

Experimental protocols

Chemistry

General: the solvents and reagents were purified and dried by standard techniques. Mps are uncorrected. The IR spectra were taken on film and NMR spectra were obtained in $CDCl_3$ solution (200 MHz for ^1H, 50.3 MHz for ^{13}C) unless otherwise stated. Chemical shifts are reported in ppm *(∂)* downfield from internal TMS. Mass spectra were obtained under electron impact (70 eV).

(Trans-2-hydroxycyclohexyl)acetic acid **5**

A solution containing 2,4,4-trimethyl-2-oxazoline (23 g, 0.2 mol) in anhydrous THF (120 ml) was cooled to –78°C under N_2. A solution of *n*-butyllithium (1.6 M in hexane, 126 ml, 0.2 mol) was added dropwise and the lithiooxazoline slowly precipitated from solution. The suspension was stirred for 30 min at –78°C. The epoxide **3** (20 g, 0.2 mol) was added at –45°C, stirred for 4–5 h and then allowed to warm to room temperature. The solution was acidified to pH 2 and was extracted with EtOAc to remove unreacted epoxide and then neutralized with 4% NaOH. The oxazoline was removed by extraction with EtOAc and the extracts were dried (Na_2SO_4) and concentrated to give 4,4-dimethyl-2-(1-hydroxycyclohex-2-yl)methyl-2-oxazoline (28 g, 65.1%).

The oxazoline (20 g, 94.8 mmol) was converted to the methiodide salt by stirring in excess methyl iodide at room temperature for 12 h. Once the excess MeI had been evaporated, the methiodide salt (32 g) was recrystallized from acetonitrile/ether (mp = 142–145°C) (23.8 g, 71.2%). The oxazoline methiodide (23.8 g) was added to 90 ml of 1 N NaOH and the mixture was stirred for 15 h at room temperature. The solution was acidified and extracted to give **5** (5.9 g, 73.2%). Overall yield 33.9% from **3**.

Mp = 88–90°C. IR (KBr): 3260, 2550, 1685, 1450, 1110, 1070 cm^{-1}.

^1H NMR (DMSO): 1.81 (dd, 1H, $J_{2a-1'} = 9.5$ Hz, $J_{2a-b} = 15.2$ Hz; H_{2a}); 2.70 (dd, 1H, $J_{2b-1'} = 3.7$ Hz, $J_{2a-b} = 15.2$ Hz; H_{2b}); 3.00 (ddd, 1H, $J_{2'-3'e} = 4.4$ Hz, $J_{2'-3'a} = 9.8$ Hz, $J_{2'-1'} = 9.8$ Hz; $H_{2'}$).

(Trans-2-acetoxycyclohexyl)acetic acid **6**

Potassium fluoride (2.93 g, 51.6 mmol) and phenylphenacyl bromide (6.32 g, 23.4 mmol) were stirred in dry DMF (25 ml) at room temperature for 1 min. The hydroxyacid **5** (3.7 g, 23.4 mmol) was then added and the reaction mixture was stirred at room temperature for 48 h. The product was extracted with EtOAc, the organic layer was washed with water, dried and evaporated to give, after chromatography over SiO_2, the phenylphenacyl ester (3.5 g, 42.5%). From the same chromatography and from the mother liquors 1.03 g of **5** were recovered (27.8%).

Acetylation of 3.2 g of the phenyl phenacyl ester with Ac_2O in Et_3N/benzene afforded the acetoxy derivative (3.1 g, 86%). 2.7 g of 4-phenylphenacyl (*trans*-2-acetoxycyclohexyl) acetate in acetic acid (68 ml) and Zn (5.4 g) were stirred for 4 h at room temperature. The solution was filtered, extracted with EtOAc and fractionated into acidic and neutral fractions. From the acidic fraction the acetoxyacid **6** (1.15 g, 83.9%) was isolated.

IR: 3 500–2 500, 1 745, 1 710, 1 240 cm^{-1}.

^{1}H NMR: 2.02 (s, 3H; –OAc); 2.15 (dd, 1H, $J_{2a-1'}$ = 7.6 Hz, J_{2a-b} = 14.6 Hz; H_{2a}); 2.46 (dd, 1H, $J_{2b-1'}$ = 5.9 Hz, J_{2a-b} = 14.6 Hz; H_{2b}); 4.50 (ddd, 1H, $J_{2'-3'e}$ = 4.4 Hz, $J_{2'-3'a}$ = 9.8 Hz; $J_{2'-1'}$ = 9.8 Hz; $H_{2'}$).

*3-Acetoxy-1-(*trans*-2-acetoxycyclohexyl)acetone 7*

Acetoxyacid **6** (1.1 g) was converted into the acylchloride, with thionylchloride (25 ml) in dry benzene. An ethereal solution (50 ml) of the acylchloride was added dropwise to an excess of ethereal diazomethane maintained at –5°C to 0°C. After 3 h the solution was treated with dry hydrogen chloride for 30 min. After extraction 1-(*trans*–2-acetoxycyclohexyl)-3-chloroacetone was obtained (1.1 g, 95%).

990 mg of this compound, acetic anhydride (40 ml) and anhydrous sodium acetate (5.7 g) were refluxed under N_2 for 4 h. After extraction, the organic layer was evaporated and the residue was chromatographed over SiO_2 to give **7** (750 mg, 70%).

IR: 1760, 1740, 1715, 1380, 1240, 1040 cm^{-1}.

^{1}H NMR: 2.00 (s, 3H; 2'–OAc); 2.13 (s, 3H; 3–OAc); 2.28 (dd, 1H, $J_{1a-1'}$ = 7.7 Hz, J_{1a-b} = 15.8 Hz; H_{1a}); 2.43 (dd, 1H, $J_{1b-1'}$ = 4.7 Hz, $J_{1b-1'}$ = 15.8 Hz; H_{1b}); 4.45 (ddd, 1H, $J_{2'-3'e}$ = 4.4 Hz, $J_{2'-3'a}$ = 9.8 Hz; $J_{2'-1'}$ = 9.8 Hz; $H_{2'}$); 4.64 (s, 2H; H_3).

Horner–Emmons reaction of 7

Triethyl phosphonoacetate (0.48 ml, 2.4 mmol) was added dropwise under N_2 at 5°C to a slurry of NaH (62 mg, 80% parafine, 2.1 mmol) in dry benzene (5 ml). After the addition, the solution was stirred at room temperature until gas evolution had ceased. Acetoxymethylacetone **7** (350 mg, 1.4 mmol) was added to the mixture and the reaction was allowed to stand for 30 min at room temperature; for 20 min at 40°C and for 21 h at 25°C. The solution was cooled and extracted with ether. The product (480 mg) was chromatographed over SiO_2 to give:

Eluted with hex/EtOAc (8:2): 180 mg (40.6%) of ethyl 4-acetoxy-3-(*trans*-2-acetoxycyclohexylmethyl)-(*Z*)-2-butenoate **8**.

IR: 1740, 1710, 1650, 1450, 1380, 1240 cm^{-1}.

^{1}H NMR: 1.27 (t, 3H, *J* = 7.2 Hz; -O-CH_2CH_3-); 2.01 (s, 3H, 3'–OAc); 2.10 (s, 3H; 4-OAc), 4.15 (c, 2H, *J* = 7.2 Hz; -O-CH_2CH_3-); 4.48 (ddd, 1H, $J_{2'-3'e}$ = 4.4 Hz, $J_{2'-3'a}$ = 9.8 Hz, $J_{2'-1'}$ = 9.8 Hz; $H_{2'}$); 5.18 (d, 1H, J_{4a-b} = 14.8 Hz; H_{4a}); 5.28 (d, 1H, J_{4b-a} = 14.8 Hz; H_{4b}); 5.74 (s, 1H; H_2).

Eluted with hex/EtOAc (7:3): unreacted **7** (75 mg, 21.4%) and 36 mg (11.5%) of 3-(*trans*-2-acetoxycyclohexylmethyl)but-2-enolide **2**.

Mp = 80–84°C. IR: 1780, 1750, 1740, 1640, 1240 cm^{-1}.

^{1}H NMR: 2.00 (s, 3H; -OAc); 2.21 (dd, 1H, J_1 = 8.0 Hz, J_2 = 15.6 Hz; –CH_2–); 2.62 (dd, 1H, J_1 = 4.9 Hz, J_2 = 15.6 Hz; –CH_2–); 4.53 (ddd, $J_{2'-3'e}$ = 4.4 Hz, $J_{2'-3'a}$ = 9.8 Hz, $J_{2'-1'}$ = 9.8 Hz; $H_{2'}$); 4.70 (brs, 2H; H_4); 5.84 (brs, 1H; H_2).

^{13}C NMR: 21.0 (-OAc), 24.3 (4'), 24.9 (5'), 31.0 (3'), 31.8 (6'), 32.4 (-CH_2-), 40.6 (1'), 73.7 (4), 76.5 (2'), 118.4 (2), 170.0 (3), 170.0 (1), 170.4 (-OAc).

MS: *m/e* 239 [(M+1)$^+$, 2], 196 (8), 178 (60), 111 (63), 81 (100).

Lactonization of 8

85 mg of **8** in Et_2O/EtOH/HCl (1.5:1.5:0.3 ml), were maintained for 3 d at room temperature and were then extracted with EtOAc. The crude product was chromatographed over SiO_2 to give **2** (17.7%) and 3-(*trans*-2-hydroxycyclohexylmethyl)but-2-enolide **1** (68.7%).

Mp = 60–64°C. IR (4% $CHCl_3$): 3620, 1785, 1750, 1650, 1450, 1060, 1040 cm^{-1}.

^{1}H NMR: 2.26 (dd, 1H, J_1 = 9.0 Hz, J_2 = 15.1 Hz; –CH_2–); 2.94 (dd, 1H, J_1 = 4.0 Hz, J_2 = 15.1 Hz; –CH_2–); 3.24 (ddd, 1H, $J_{2'-3'e}$ = 4.4 Hz, $J_{2'-3'a}$ = 9.8 Hz, $J_{2'-1'}$ = 9.8 Hz; $H_{2'}$); 4.80 (brs, 2H; H_4); 5.85 (brs, 1H; H_2).

^{13}C NMR: 24.8 (4'), 25.4 (5'), 30.9 (6'), 32.2 (-CH_2-), 36.1 (3'), 44.9 (1'), 73.2 (4), 74.13 (2'), 116.4 (2), 168.9 (3), 173.6 (1).

MS: *m/e* 196 (M$^+$, 5), 178 (18), 151 (36), 111 (88), 98 (100).

50 mg of **8** in 20 ml of methanol/$NaHCO_3$ aq salt (1:1.5) were stirred for 30 h at room temperature. By chromatography of the reaction product: unreacted **8** (5.8 mg, 11.6%) and **2** (23 mg, 65.2%) were isolated.

Saponification of 2

Reaction of **2** under the same condition employed in the acidic lactonization of **8**, yielded 82% of the hydroxylactone **1**.

Pharmacology

Guinea pigs of both sexes weighing between 600 and 800 g were killed by cervical dislocation. The hearts were excited quickly and both atria were disected free. The spontaneously beating right atrium was suspended in a bath containing 5 ml of Krebs-Henseleit solution of the following composition (g/l): NaCl, 6.9; KCl, 0.35; $CaCl_2$, 0.28; KH_2PO_4, 0.16; $MgSO_4$ x 7 H_2O, 0.29; $NaHCO_3$, 2.1 and glucose, 1.8; oxigenated with a mixture of 5% CO_2 in O_2 and maintained at 37°C. The left atrium was dissected and set up in a 10 ml bath and platinum electrodes were placed on either side for direct electrical stimulation at a frequency of 3 Hz, with 1 msec square pulses, at 10 V.

The tissues were attached to a force displacement traducer connected to a Letica polygraph to record the contractions of the preparations. Spontaneous heart rate was measured using a cardiotachometer triggered by the amplified signal from the right atrium.

Following an equilibratium period of 30 min, cumulative dose response curves to digoxine, **1** and **2** were constructed, increasing concentrations of the drug at 10 min intervals. The drug concentrations used were in the 10^{-7}–10^{-4} range. Dose response curves with the vehicle employed for disolution of drugs were used as controls. Inotropic responses were expressed as the percentage of maximal response to digoxin.

The results obtained from 5 experiments were expressed as means ± SE of the means. Statistical analysis of results performed by the unpaired 2-tailed Student's *t*-test. Probability levels of less than 0.05 were taken to indicate statistical significance.

The drug used in this study were solubilized in:
— Tween 80: H_2O (10:90) for digoxin;
— Tween 80: H_2O (5:95) for **1**;
— Tween 80: alcohol 50: H_2O (5:95:100) for **2**.

Acknowledgments

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