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Synthesis and evaluation of cardiotonic activity of diterpenic butenolides

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Summary — Four diterpenic cardenolide analogs have been prepared from sandaracopimaric acid and tested for inotropic activity. Three of them displayed an unexpected negative inotropic activity.

Résumé — **Synthèse et évaluation de l'activité cardiotonique de buténolides diterpéniques.** *À partir de l'acide sandaracopimarique, on réalise la synthèse de quatre analogues diterpéniques des cardénolides et puis on a essayé leurs propriétés inotropiques. Trois d'entre eux présentent une activité inotropique négative inattendue.*

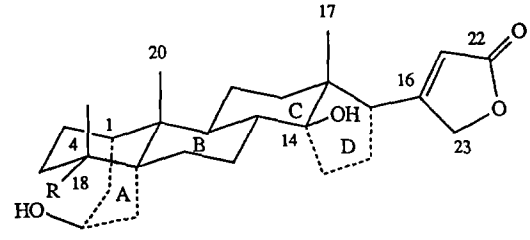
cardenolide analog / sandaracopimaric acid / inotropic activity / diterpenic butenolide

Introduction

The cardiac glycosides are drugs used in the treatment of congestive heart failure, a cardiovascular disease with a high mortality rate [1]. The use of these compounds is limited by their toxicity and low therapeutic index, which, together with the debate concerning their mechanism of action [2–5], have led to growing interest in the search for simpler and less toxic analogues that could help in defining the nature of digitalis receptors.

In earlier studies [6] we described the synthesis of cyclohexane analogues of cardenolides, in which the butenolide and the OH group were maintained in an arrangement similar to that of the C-ring of natural cardenolides. Their lack of activity as positive inotropic agents made it clear that these simple models did not contain the minimum structural requirements for activity.

Continuing along this same line of research, the present work describes the results obtained with cardenolide diterpene analogues **1**, **2**, **3** and **4**. Like the cardiac glycosides, these synthesized compounds maintain the butenolide and the OH group at 14β. The most significant differences are the absence of the D ring, the *trans* junction of A and B rings and a different substitution pattern of the A ring (fig 1). Ester **1** and acid **2** have a carboxyl function at C_{18}, while compounds **3** and **4** have a hydroxyl or a glucoside group, respectively.



1 R = COOMe **3** R = CH_2OH
2 R = COOH **4** R = CH_2C glucose

Fig 1. Superposition of the digitoxigenin molecule (dotted lines) with the proposed analogues **1–4**.

As can be observed in figure 1, in spite of the differences there is great structural analogy between compounds **1–4** and the genins of natural cardiac glycosides.

The *trans* junction of rings A and B should not produce a loss of cardiotonic activity since there are some compounds such as uzarigenine and gomphoside [7] with high cardioactivity even though the junction of those rings is *trans*. The genins 5α-H and 5β-H are equipotent although their glycosides are quite different; according to Brown *et al* [8, 9] this means that the stereochemistry of the A/B junction mainly affects the spatial arrangement allowing the sugar to bind to the receptor.

Regarding ring A, the main differences are the lack of functionalization at position 3 and the presence of substituents such as: a methyl group on C_4, as well as an oxygenated group at C_{18}. The latter occupies a spatial area similar to that occupied by the OH group at C_3 in the cardiac glycosides, and thus it could maintain the drug–receptor interaction in its own area. Nor should it be expected that the lack of a D ring implies a lack of activity, since the butenolide could adopt an adequate arrangement in order to bind to the receptor.

The great structural similarity of these compounds with the cardiac glycosides, on the one hand, and the absence of a complete steroid skeleton, on the other, lend interest to their synthesis within the search for new cardiotonic agents and could contribute to acquire a better definition of the nature of the digitalic receptor.

Chemistry

Synthesis of compounds **1**, **2**, **3** and **4** was carried out from sandaracopimaric acid according to the reaction sequence shown in scheme 1 [10, 11].

In a first step, positions 14 and 16 were functionalized by sequential and selective hydroboration-oxidation of the Δ^{15} and $\Delta^{8(14)}$ double bonds, in which, after acetylation, the diacetate ester **5** and triacetate **6** were obtained with a 14β-OAc stereochemistry [12].

Partial saponification of these compounds yielded as main products the 16-hydroxy-derivatives **7** and **8** as a consequence of the greater accessibility of acetate on C_{16}. Oxidation of the alcohols yielded the acids, which were transformed into the acetoxyketones **9** and **10**, substrates that were used for the construction of butenolide.

The Horner–Emmons reaction [13] of **10** with triethyl phosphonoacetate mainly yielded the product with *E* stereochemistry of the double bond, whose lactonization is not produced under either acid or base treatments. As a consequence, the Reformatsky reaction was chosen as a means of access to the butenolides.

The reaction of **9** and **10** with ethyl bromoacetate and zinc [14] yielded mixtures of compounds, mostly the unsaturated lactones **11** and **14** and the saturated **12** and **15**. Acid hydrolysis [15] of **11** and **14** yielded lactones **1** and **3**, respectively.

Hydrolysis of the methyl ester at C_{18} was carried out from lactones **12a + 12b** by reaction with KOH-saturated/*t*-BuOH. Dehydration of the obtained epimeric lactone mixture (the lactones were previously acetylated) followed by later hydrolysis of the acetate group finally yielded the γ-lactone **2**.

Synthesis of the glucosylated derivative was performed by reaction of dihydroxylacetone **3** with acetobromoglucose and Fetizon's reagent [16]. Hydrolysis of the acetate groups under mild conditions led to 18-*O*-β-D-glucopyranoside **4**.

Pharmacology

The activity of compounds **1–4** has been evaluated in guinea pig left auricle. The preparation was electrically stimulated to obtain a contractile response. The inotropic effects observed must be those of the compounds being tested and not the result of a possible compensatory reaction of the variations in the cardiac frequency. Parallel to this, tests were made on the vehicles used to dissolve the products and eliminate their possible effects.

The results obtained with compounds **1–4** and their corresponding vehicles on the inotropic response in isolated and stimulated guinea pig left auricle are expressed graphically through the construction of dose–response curves that are shown in figure 2. The results obtained with digoxin as reference are included (fig 3).

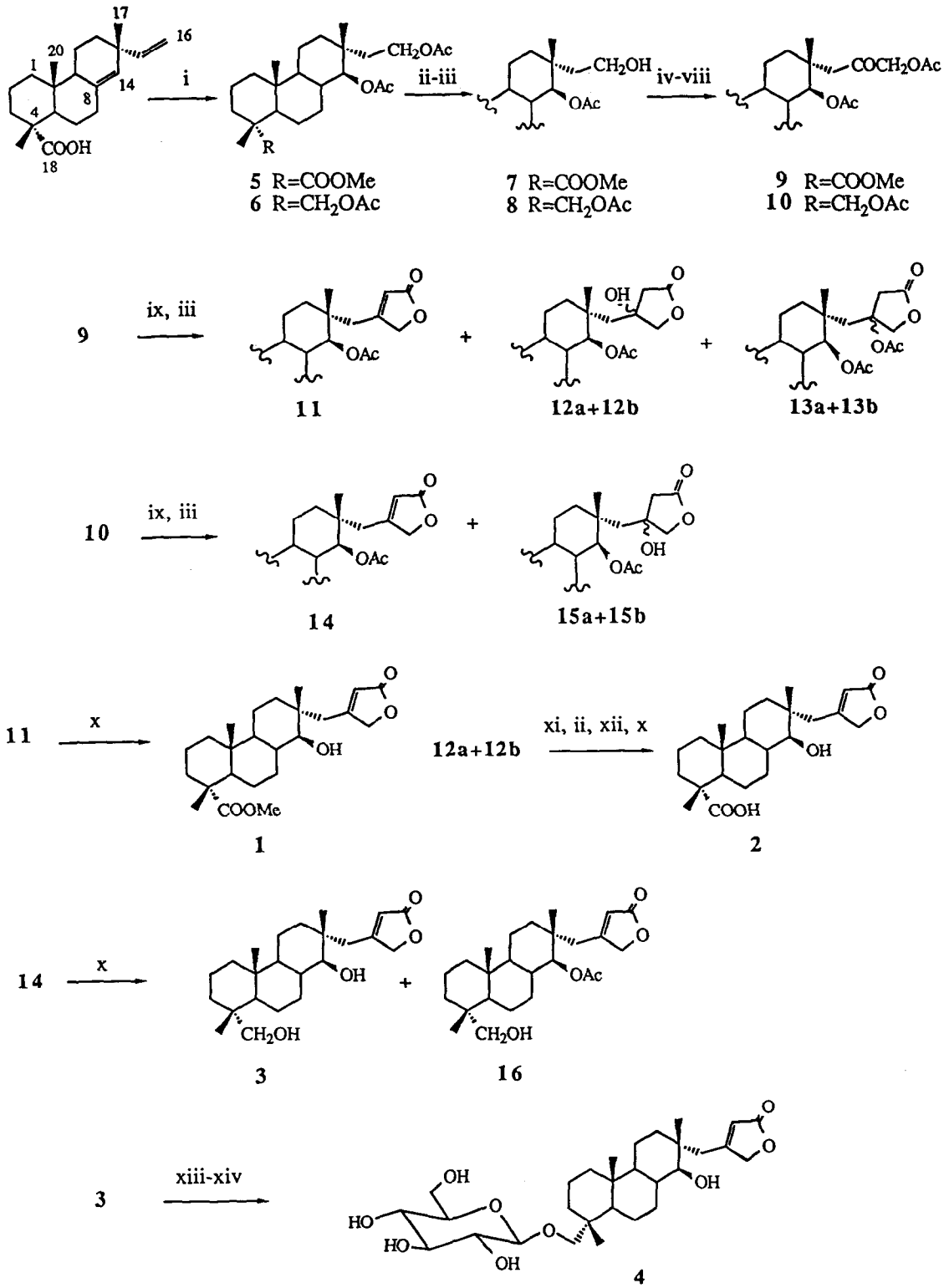
Compound **1** did not significantly modify the inotropic response as compared to the control preparations. Products **2**, **3** and **4** did not produce the expected inotropic effect either; on the contrary, they produced a negative inotropic effect as compared to the control preparations. The ED_{50} calculated from the dose–response curves were 1.51 E-4, 1.17 E-4, 1.76 E-4 for **2**, **3** and **4**, respectively.

When the ED_{50} of **2** and **3** are compared, **3** is shown to be more potent; however, these values cannot be directly compared with the ED_{50} of product **4**, since different vehicles were used. Hence, we have calculated the relative potency of all the compounds, taking into account the effect of the vehicle used. The values of relative potency of **4** *versus* **3**, **2** and **1** were 1.36, 1.91 and 6.58, respectively (fig 4).

Discussion

The lack of inotropic activity of compound **1**, and the increase in the negative inotropic activity that compounds **2**, **3** and especially **4** exhibit, clearly indicate the importance of hydrosolubility in the access of the substance to the receptor.

If the receptor model proposed by Thomas *et al* [17], which considers 3 binding sites (A, B, C), is taken into consideration, these compounds (**1–4**) have structural characteristics that are appropriate for binding to the receptor. Thus, at site A binding could



Scheme 1. i) [10–12]; ii) Ac_2O/Pyr; iii) $NaHCO_3$-H_2O/MeOH; iv) Jones (ox); v) $SOCl_2$; vi) CH_2N_2; vii) HCl (g); viii) NaOAc/Ac_2O; ix) Reformatsky; x) conc HCl/Et_2O/EtOH; xi) KOH/t-BuOH; xii) $SOCl_2$/Pyr; xiii) Fetizon, acetobromoglucose; xiv) Et_3N/H_2O/MeOH.

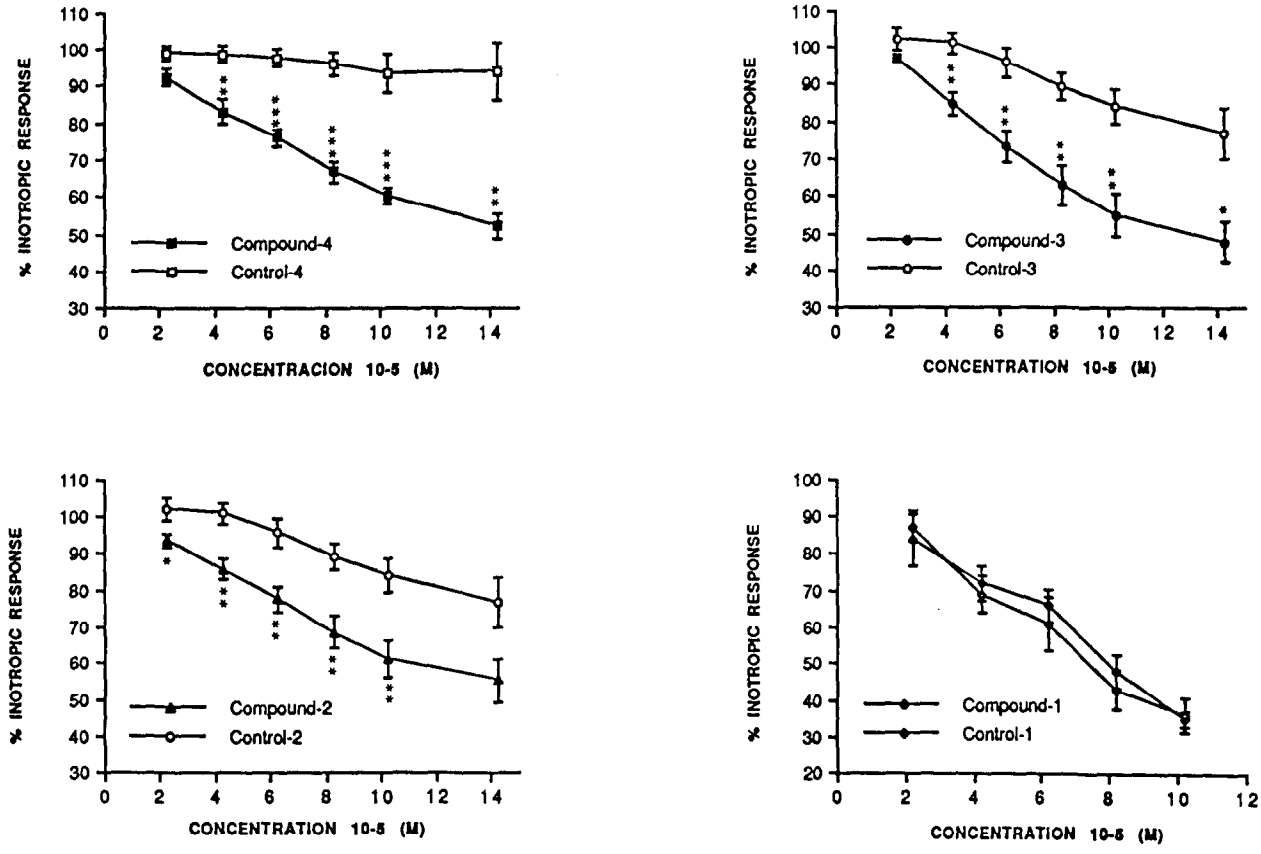
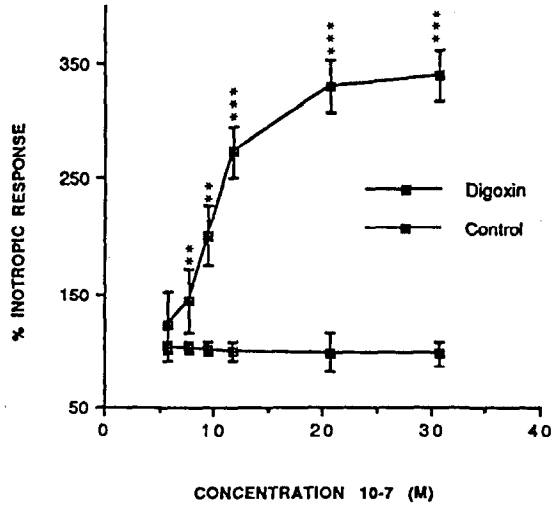


Fig 2. Cumulative concentration response curves to **4**, **2**, **3** and **1** in the left atrium isolated from guinea pigs. Each value is the mean from 5 preparations; vertical lines show the SEM. *, ** and *** indicate $P < 0.1$, $P < 0.01$ and $P < 0.001$, respectively, when compared to the corresponding control values.



take place by the butenolide ring, at point B by the hydrocarbon skeleton and at point C by the oxygenated function at C_{18} or by a hydroxyl group of glucose in compound **4**. Taking all this into consideration, the lack of positive inotropic activity of these compounds could be either because they interact with the receptor itself, as antagonists, or because they bind to a different receptor, since the molecule does not adopt the appropriate conformation for interacting with the digitalis receptor as it does not have a D ring. This could be due to the fact that the arrangement of the heteroatoms of the butenolide is

← **Fig 3.** Cumulative concentration response curve to digoxin in the left atrium isolated from guinea pigs. Each value is the mean from 5 preparations; vertical lines show the SEM. *, ** and *** indicate $P < 0.1$, $P < 0.01$ and $P < 0.001$, respectively, when compared to the corresponding control values.

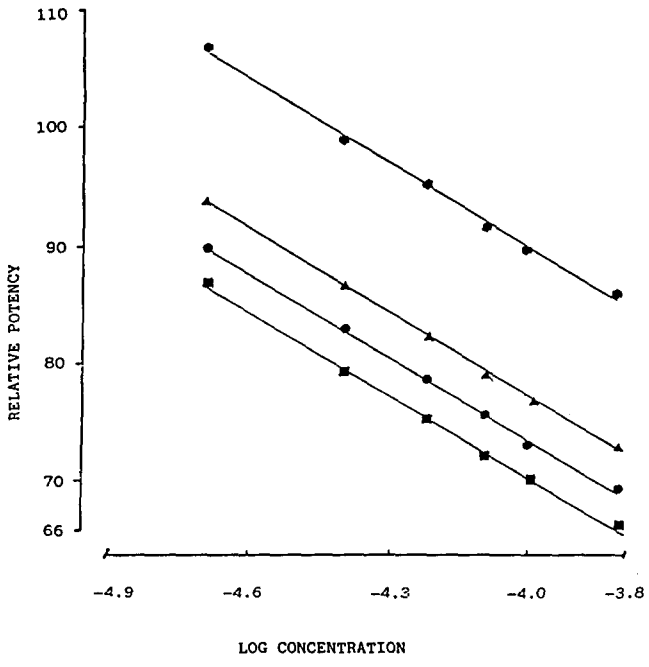


Fig 4. Relative inotropic potency to compounds **1** ( ), **2** (▲), **3** (●) and **4** (■) in the left atrium isolated from guinea pigs.

modified with respect to the ring system, a determining factor [4] in the positive inotropic activity of the cardenolides.

Another possible explanation of the inotropic effect observed would be a blockage of the calcium slow channels. Cardiac glycosides are known to exert an important effect on the amount of calcium present in the cell surface and on the flow of calcium into the cell. Hence, in various studies it has been demonstrated that digitalis compounds increase the activity of the slow stream of calcium input and there is also evidence that digitalis compounds increase the amount of calcium stored in an extracellular deposit located in the glucocalix, and thus the calcium is available for transfer to the interior of the cell to activate the contractile mechanism [18, 19]. These compounds, given their great structural similarity, could act on the deposits and the flow of calcium, but producing the opposite effect.

Experimental protocols

Chemistry

The solvents and reagents were purified and dried by standard techniques. Mps are uncorrected. IR spectra were taken in film and NMR spectra (200 MHz for ^1H, 50.3 MHz for ^{13}C) were obtained in $CDCl_3$ solution, unless otherwise stated. Chemical shifts are reported in ppm (δ) downfield from internal TMS. Optical rotations were measured at 20°C on a digital polarimeter. Mass spectra were obtained under electron impact (70 eV) and ultraviolet spectra were obtained in ethanol.

Methyl 14β-acetoxy-16-hydroxy-13-epi-pimaran-18-oate (7) and 14β,18-diacetoxy-13-epi-pimaran-16-ol (8)

70 ml of saturated $NaHCO_3$ (aq) were added to a stirred solution of **5** (7.4 g, 17 mmol) in 210 ml of MeOH and maintained with vigorous stirring for 30 h at 25°C. The mixture was then diluted with water, extracted with EtOAc and washed with brine. After the usual work up the crude product (7.1 g) was chromatographed over silica gel to yield: unreacted **5** (1.7 g, 22.7%; hexane/EtOAc 6:4); methyl 16-acetoxy-14β-hydroxy-13-*epi*-pimaran-18-oate (75 mg, 1.3%; hexane/EtOAc 6:4); **7** (3.7 g, 65.9%; hexane/EtOAc 6:4) and methyl 14β,16-dihydroxy-13-*epi*-pimaran-18-oate (170 mg, 2.8%; EtOAc).

7: Oil. $[\alpha]^{20}$ (λ) = + 8.7° (589), + 9.3° (578), + 10.5° (546), + 19.3° (436), + 32.8° (365), c = 1.14% ($CHCl_3$). IR: 3520, 1740, 1270, 1160, 1040 cm^{-1}. ^1H NMR: 0.88 (s, 3H; Me-20); 0.93 (s, 3H; Me-17); 1.15 (s, 3H; Me-19); 2.06 (s, 3H; -OAc); 3.62 (s, 3H; -OMe); 3.67 (m, 2H; H-16); 4.50 (d, 1H, *J* = 10.1 Hz; H-14).

Under the same conditions **6** (1.1 g, 2.7 mmol) yielded: unreacted **6** (331.7 mg, 27.3%; hexane/EtOAc 7:3); 16,18-diacetoxy-13-*epi*-pimaran-14β-ol (12.1 mg, 1.1%; hexane/EtOAc 7:3); 14β,16-diacetoxy-13-*epi*-pimaran-18-ol (19.8 mg, 1.8%; hexane/EtOAc 1:1); **8** (656 mg, 59.6%; hexane/EtOAc 1:1); 18-acetoxy-13-*epi*-pimaran-14β,16-diol (11.9 mg, 1.2%; hexane/EtOAc 1:1) and 14β-acetoxi-13-*epi*-pimaran-16,18-diol (27.7 mg, 2.8%; hexane/EtOAc 3:7).

8: mp = 98–101°C; [α] = + 0.2° (589), + 0.2° (578), + 0.7° (546), + 1.2° (436); c = 0.59% ($CHCl_3$). IR: (4% $CHCl_3$): 3600, 3400, 1730, 1250, 1040, 980 cm^{-1} NMR ^1H: 0.82 (s, 3H; Me-19); 0.89 (s, 3H; Me-20); 0.93 (s, 3H; Me-17); 2.05 (s, 3H; -OAc); 2.07 (s, 3H; -OAc); 3.59 (d, 1H, *J* = 10.9 Hz; H-18); 3.69 (t, 2H, *J* = 7.4 Hz; H-16); 3.82 (d, 1H; *J* = 10.9 Hz; H-18); 4.50 (d, 1H, *J* = 10.1 Hz; H-14).

By oxidation of 16-hydroxy group with Jones reagent (ox), followed by standard treatments [6] with $SOCl_2$, CH_2N_2, HCl (g) and $NaOAc/Ac_2O$ acetoxyketone **9** was obtained from **7** and acetoxyketone **10** from **8**.

9: mp = 82–86°C; [α] = + 10.2° (589), + 10.9° (578), + 12.5° (546), + 23.1° (436), + 43.3° (365); c = 0.98% ($CHCl_3$). IR: (4% $CHCl_3$): 1745, 1720, 1250, 1170, 1130, 1030 cm^{-1} NMR ^1H: 0.88 (s, 3H; Me-20); 1.07 (s, 3H; Me-17); 1.16 (s, 3H; Me-19); 2.06 (s, 3H; -OAc); 2.14 (s, 3H; -OAc); 2.23 (s, 2H; H-15); 3.62 (s, 3H; -OMe); 4.53 (d, 1H, *J* = 9.7 Hz; H-14); 4.55 (s, 2H; -CH_2OAc).

10: mp = 120–124°C; [α] = + 22.3° (589), + 23.1° (578), + 27.3° (546), + 51.7° (436), + 99.3° (365); c = 0.98% ($CHCl_3$). IR: (4% $CHCl_3$): 1750, 1730, 1250, 1160, 1120, 1040, 960 cm^{-1} NMR ^1H: 0.82 (s, 3H; Me-19); 0.89 (s, 3H; Me-20); 1.07 (s, 3H; Me-17); 2.05 (s, 3H; OAc); 2.08 (s, 3H; -OAc); 2.15 (s, 3H; -OAc); 2.23 (s, 2H; H-15) 3.58 (d, 1H, *J* = 10.9 Hz; H-18); 3.83 (d, 1H, *J* = 10.9 Hz; H-18); 4.54 (d, 1H, *J* = 10.1 Hz; H-14); 4.55 (s, 2H; -CH_2OAc).

Reformatsky reaction

A solution of ethyl bromoacetate (35 ml, 314 mmol) and α-acetoxyketone **9** (2.6 g, 39.7 mmol) in dry benzene was added dropwise to a stirred mixture of active granulated zinc (2.6 g, 39.7 mmol) in dry benzene. The mixture was refluxed for 6 h, treated with dilute HCl (aq) and extracted with EtOAc to yield 3 g of reaction product. After column chromatography 18-methoxy-18-oxo-13-*epi*-pimaran-14β,16-olide (100 mg, 5.2%)

and another complex mixture (2.2 g) were obtained. This latter mixture was stirred in $NaHCO_3$–H_2O/MeOH (100/120 ml) for 72 h at 25°C and was extracted with EtOAc to give 1.25 g of reaction product. The aqueous layer was acidified and after extraction yielded 960 mg.

By acetylation and chromatography of both fractions: **11** (430 mg, hexane/EtOAc 65:35) and methyl 14β-acetoxy-16-carboxymethyl-16-hydroxy-16-hydroxymethyl-13-*epi*-pimaran-18-oate-γ-lactone (**12a** + **12b**) (280 mg, hexane/EtOAc 1:1) were isolated from the neutral fraction and methyl 14β, 16-diacetoxy-16-carboxymethyl-16-hydroxymethyl-13-*epi*-pimaran-18-oate γ-lactone (**13a** + **13b**) (38 mg, hexane/ EtOAc 6:4) and **12a** + **12b** (590 mg, hexane/EtOAc 1:1) were obtained from the aqueous layer.

Methyl 14β-acetoxy-16-carboxymethylidene-16-hydroxymethyl-13-epi-pimaran-18-oate-γ-lactone ***11***. Foam $[\alpha]^{20}$ (λ) = + 24.9° (589), + 26.3° (578), + 29.9° (546), + 52.4° (436), c = 1.22% ($CHCl_3$). IR: 1790, 1755, 1725, 1640, 1250, 1180, 1040 cm^{-1}. UV λ_{max} nm (ε): 212 (20300). ^{1}H-NMR: 0.89 (s, 3H; Me-20); 1.00 (s, 3H; Me-17); 1.16 (s, 3H; Me-19); 2.08 (s, 3H; -OAc); 2.23 (d, 1H, *J* = 13.8; H-15); 2.30 (d, 1H, *J* = 13.8 Hz; H-15); 3.63 (s, 3H; -OMe); 4.52 (d, 1H, *J* = 10.0 Hz; H-14); 4.72 (m, 2H; H-21); 5.90 (brs, 1H; H-22).

Under the same conditions α-acetoxyketone **10** (1.3 g, 2.7 mmol) yielded: 18-acetoxy-13-*epi*-pimaran-14β,16-olide (50 mg, 5.1%), diacetate of 16-carboxymethyliden-16-hydroxymethyl-13-*epi*-pimaran-14β,16-diol γ-lactone (**14**) (344.5 mg, 27.2%), diacetate of 16-carboxymethyl-16-hydroxymethyl-13-*epi*-pimaran-14β, 16,18-triol γ-lactone (**15a** + **15b**) (457.5 mg, 33.6%) and acetate of 16-acetoxymethyl-16-carboxymethyl-13-*epi*-pimaran-16,18-diol ε-lactone (35 mg, 4.4%).

14: Foam. [α] = + 22.4° (589), + 23.6° (578), + 26.9° (546), + 47.3° (436); c = 1.07% ($CHCl_3$). UV: λ_{max} 213 (ε = 7574) nm. IR (4% $CHCl_3$): 1790, 1750, 1730, 1635, 1250, 1040, 980, 950, 860 cm^{-1} NMR ^{1}H: 0.83 (s, 3H; Me-19); 0.89 (s, 3H; Me-20); 1.01 (s, 3H; Me-17); 2.06 (s, 3H; -OAc); 2.09 (s, 3H; -OAc); 2.27 (s, 2H; H-15); 3.58 (d, 1H, *J* = 10.9 Hz; H-18); 3.69 (t, 2H, *J* = 7.4 Hz; H-16); 3.83 (d, 1H, *J* = 10.9 Hz; H-18); 4.52 (d, 1H, *J* = 10.2 Hz; H-14); 4.73 (m, 2H; H-21); 5.90 (brs, 1H; H-22).

*Methyl 16-carboxymethylidene-14β-hydroxy-16-hydroxymethyl-13-*epi*-pimaran-18-oate γ-lactone (**1**) and 16-carboxymethylidene-16-hydroxymethyl-13-epi-pimaran-14β, 18-diol γ-lactone (**3**)*

11 (85 mg, 0.19 mmol) in Et_2O/EtOH/concentrated HCl (1:1:0.6) was maintained for 4 days at room temperature and then extracted with EtOAc. By chromatography (hexane/EtOAc 6:4) of the reaction product, unreacted **11** (37.2 mg, 43.4%) and **1** (31.8 mg, 40.8%) were obtained.

1: mp = 115–117°C; $[\alpha]^{20}$ (λ) = + 8.6° (589), + 10.9 (578), + 19.1 (546), c = 0.70% ($CHCl_3$). IR: 3600, 1790, 1720, 1640, 1260, 1110, 1040, 990, 900, 870 cm^{-1}. UV λ_{max} nm (ε): 217 (5050). MS: 404 (M^+, 21), 345 (61), 307 (61), 247 (99), 229 (90), 173 (17). Anal Calcd for $C_{24}H_{36}O_5$: C, 71.29; H: 8.91. Found: C, 70.24; H, 9.52. ^{1}H-NMR: 0.88 (s, 3H; Me-20); 0.94 (s, 3H; Me-17); 1.17 (s, 3H; Me-19); 2.41 (d, 1H, *J* = 13.5; H-15); 2.53 (d, 1H, *J* = 13.5; H-15); 2.88 (d, 1H, *J* = 9.7 Hz; H-14); 3.65 (s, 3H; -OMe); 4.77 (d, 2H, *J* = 1.9 Hz; H-21); 5.86 (brs, 1H; H-22). ^{13}C-NMR: 38.2 (1), 18.1 (2), 36.9 (3), 47.6 (4), 49.5 (5), 23.8 (6), 30.8 (7), 38.8 (8), 54.2 (9), 36.3 (10), 19.5 (11), 35.8 (12), 39.4 (13), 81.1 (14), 40.4 (15), 167.8 (16), 17.5 (17), 179.2 (18), 16.7 (19), 14.6 (20), 75.1 (21), 118.4 (22), 174.0 (23), 51.8 (OMe).

Under the same conditions **14** (500 mg, 1.1 mmol) gave 14β-acetoxy-16-carboxymethyliden-16-hydroxymethyl-13-*epi*-pimaran-18-ol γ-lactone (**16**) (267 mg, 58.7%) and 16-carboxymethyliden-16-hydroxymethyl-13-*epi*-pimaran-14β,18-diol γ-lactone (**3**) (129.1 mg, 31.6%).

3: mp = 225–228°C; [α] = + 3.0° (589), 3.3° (578), + 3.7° (546), + 6.6° (436); c = 0.67% (MeOH). IR (1% KBr): 3400, 1780, 1720, 1630, 1185, 1160, 1050, 1025, 995, 860 cm^{-1}. UV: λ_{max} 216 (ε = 18 700) nm. MS: 361 (M^+-Me, 1), 345 (100), 257 (5), 247 (7), 229 (13), 147 (9). Anal Calcd for $C_{23}H_{36}O_4$: C, 73.40; H, 9.57. Found: C, 73.69; H; 9.17. NMR ^{1}H (C_5D_5N): 0.83 (s, 3H; Me-19); 0.90 (s, 3H; Me-20); 1.07 (s, 3H; Me-17); 2.49 (d, 1H, *J* = 13.2 Hz; H-15); 2.66 (d, 1H, *J* = 13.2 Hz; H-15); 2.96 (d, 1H, *J* = 9.7 Hz; H-14); 3.29 (d, 1H, *J* = 10.8 Hz; H-18); 3.64 (d, 1H, *J* = 10.8 Hz; H-18); 4.73 (s, 2H; H-21); 5.91 (s, 1H; H-22). ^{13}C-NMR (C_5D_5N): 39.2 (1), 19.0 (2), 36.2 (3), 38.2 (4), 48.3 (5), 21.7 (6), 31.9 (7), 39.2 (8), 54.7 (9), 36.9 (10), 20.2 (11), 36.1 (12), 40.0 (13), 80.6 (14), 40.9 (15), 169.6 (16), 18.3 (17), 71.6 (18), 18.1 (19), 15.2 (20), 75.5 (21), 118.3 (22), 174.4 (23).

*16-Carboxymethylidene-14β-hydroxy-16-hydroxymethyl-13-*epi*-pimaran-18-oic acid γ-lactone (**2**)*

Saponification of methylester group in C_{18}: **12a** + **12b** (250 mg, 0.56 mmol) in saturated KOH(*t*-BuOH) (20 ml) was stirred under N_2 at 100°C for 1 h and at 40°C for 12 h. The reaction mixture was acidified and extracted with EtOAc to give 16-carboxymethyl-16-hydroxymethyl-14β,16-dihydroxy-13-*epi*-pimaran-18-oic acid γ-lactone (184 mg, 83.7%).

This compound was acetylated and dehydrated with $SOCl_2$ to give 14β-acetoxy-16-carboxymethylidene-16-hydroxymethyl-13-*epi*-pimaran-18-oic acid γ-lactone. The hydrolysis of this latter compound (65 mg, 0.15 mmol) under acidic hydrolysis conditions previously stated, yielded unreacted compound (32 mg, 50%) and **2** (22 mg, 38.1%).

2: Foam. $[\alpha]^{20}$ (λ) = – 3.4° (589), 3.7° (578) – 4.1° (546), c = 1.00% (Pyr). IR (1% KBr): 3600–2700, 3500, 1725, 1695, 1630, 1200, 1100, 1030, 990 cm^{-1}. MS: 390 (M^+, 2), 345 (5), 293 (10), 275 (10), 247 (31), 229 (21), 173 (8). Anal calcd for $C_{23}H_{34}O_5$: C, 70.77; H, 8.72. Found: C, 70.97; H, 8.92. ^{1}H-NMR (C_5D_5N): 0.90 (s, 3H; Me-20); 1.06 (s, 3H; Me-17); 1.39 (s, 3H; Me-19); 2.49 (d, 1H, *J* = 13.2; H-15); 2.69 (d, 1H, *J* = 13.2; H-15); 2.97 (d, 1H, *J* = 9.5 Hz; H-14); 4.89 (s, 2H, H-21); 6.06 (s, 1H; H-22). ^{13}C-NMR (C_5D_5N): 37.8 (1), 18.8 (2), 35.9 (3), 47.6 (4), 49.9 (5), 24.5 (6), 31.8 (7), 39.3 (8), 54.7 (9), 36.5 (10), 19.9 (11), 38.7 (12), 40.0 (13), 80.4 (14), 40.6 (15), 169.3 (16), 17.9 (17), 181.4 (18), 17.5 (19), 14.8 (20), 75.4 (21), 116.3 (22), 174.3 (23).

*18-O-β-D-glucopyranoside of 16-carboxymethylidene-16-hydroxymethyl-13-*epi*-pimaran-14β,18-diol γ-lactone (**4**)*

To a solution of **3** (130 mg, 0.33 mmol) in dry benzene, freshly prepared Fetizón's reagent [20] (3.2 g, 5.8 mmol), mercuric cyanide (182 mg, 0.68 mmol) and mercuric bromide (182 mg; 0.51 mmol) were added. Acetobromoglucose (461 mg; 1.12 mmol) dissolved in dry benzene was added dropwise with stirring over 5 min. The mixture was stirred at room temperature for 3 h, then filtered through celite and the organic layer was washed with aqueous sodium bicarbonate solution and water. After the usual workup, the crude product (420 mg) was chromatographied over silica gel yielding: 18-*O*-β-D-tetraacetylglucopyranoside of 16-carboxymethylen-16-hydroxymethyl-13-*epi*-pimaran-14β,18-diol γ-lactone (133.9 mg, 54.5%; Hexane/EtOAc 4:6) and unreacted product **3** (52 mg, 40%; hexane/EtOAc 2:8).

3 (130 mg, 0.24 mmol) was dissolved in MeOH (7 ml) and Et_3N (7 ml) and water (1 ml) were added. The solution was kept at room temperature for 84 h. The mixture was evaporated

to dryness and applied to a silica gel column (CH_2Cl_2/MeOH 95/5) to give **4** (42 mg, 43.1%).

4: mp = 232–234°C; [α] = – 1.8°(589), – 2.0° (578), – 2.3° (546); – 2.7°(436); c = 1% (MeOH). IR (1% KBr): 3420, 1785, 1745, 1635, 1100, 1080, 1040 cm^{-1}. UV: λ_{max} 209 (ε = 9680) nm. MS: 502 (M^+-2·H_2O, 1), 375 (10), 281 (89), 219 (100), 147 (1), 131 (47). Anal calcd for $C_{29}H_{46}O_9$: C, 64.68; H, 8.55. Found: C, 65.02; H, 8.77. NMR ^1H (CD_3OD): 0.84 (s, 3H; Me-19); 0.91 (s, 3H; Me-20); 0.94 (s, 3H; Me-17); 2.44 (d, 1H, *J* = 13.5 Hz; H-15); 2.54 (d, 1H, *J* = 13.5 Hz; H-15); 2.82 (d, 1H, *J* = 9.8 Hz; H-14); 3.03 (d, 1H, *J* = 9.4 Hz; H-18); 3.31 (d, 1H, *J* = 9.4 Hz; H-18); 4.21 (d, 1H, *J* = 7.6 Hz; H-1'); 4.85 (d, 2H, *J* = 1.6 Hz; H-21); 5.88 (brs, 1H; H-22). ^{13}C-NMR (CD_3OD): 39.1 (1), 18.7 (2), 36.3 (3), 37.8 (4), 49.0 (5), 21.7 (6), 31.5 (7), 9.0 (8), 55.0 (9), 37.1 (10), 20.2 (11), 36.5 (12), 40.0 (13), 81.2 (14), 40.8 (15), 170.4 (16), 18.0 (17), 80.0 (18), 18.0 (19), 15.1 (20), 76.1 (21), 118.2 (22), 175.9 (23), 104.4 (1'), 74.4 (2'), 77.4 (3'), 71.2 (4'), 71.7 (5'), 62.5 (6').

Pharmacology

Guinea pigs of both sexes, weighing between 550 and 750 g, were killed by cervical dislocation. The hearts were excited quickly and the atria were dissected free. The left atrium was dissected and set up in a bath containing 10 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; $MgSO_4 \cdot 7H_2O$, 0.29; $NaHCO_3$, 2.1 and glucose, 1.8; oxygenated with a mixture of 5% CO_2 in O_2 and maintained at 37°C. Two platinum electrodes were placed on either side for electrical stimulation at a frequency of 3 Hz, with 1 ms square pulses, at 10 V (Harvard stimulator, double pulse, variable delay).

The tissues were attached to a force displacement transducter connected to a Letica poligraph to record contractions of the preparations.

Following an equilibration period of 30 min, cumulative dose–response curves for **1**, **2**, **3** and **4** drugs were constructed, increasing concentrations of the drug at 10 min intervals. The digoxin was employed as reference. Dose–response curves with the vehicle employed for dissolution of drugs were used as control. The inotropic responses were expressed as the percentage of the vasal response.

The results were obtained from a minimum of 5 experiments for each one of the products and were expressed as the mean ± SE mean. Statistical analysis of results were performed by unparied 2-tailed Student's *t*-test. Probability levels of < 0.05 were taken to indicate statistical significance.

Drugs used in this study were solubilized in: water:Tween 80:alcohol (100:5:95) for **1**; water:Tween 80 (99:1) for **2** and **3**; water for **4**; water:Tween 80 (90:10) for digoxin.

The relative potency of all compounds was determined (using a computer program: Tallarida and Hurray, 1984) as the difference between the effect of the solvent and the compound at each point in the concentration response curves.

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