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Communications to the Editor

17β-(3-Furyl)-5β-androstane-3β,14β,17α-triol (PST 2238). A Very Potent Antihypertensive Agent with a Novel Mechanism of Action

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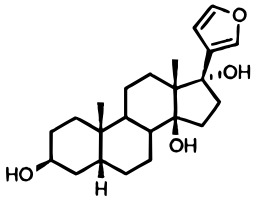
Recently the existence of an endogenous factor able to inhibit Na^+,K^+-ATPase and possibly involved in the pathogenesis of essential hypertension[1] has opened a new field in the study of compounds acting on this enzyme which is located in the cell membrane and promotes the outward transport of Na^+ and the inward transport of K^+.[2] The release of this factor is stimulated by the volume expansion secondary to a primary renal deficit in excreting sodium and water, and its pressor effect is ascribed to vascular constriction and a catecholaminergic nerve activation in these tissues through inhibition of the Na^+,K^+ pump.[3] Many substances, of widely different structures, called ouabain-like factor (OLF), have been claimed as the putative endogenous Na^+,K^+ pump inhibitor,[4] but today there is a rather converging opinion that the factor is a substance structurally correlated to ouabain, especially since it has been isolated from human plasma[5] and bovine hypothalamus[6] and characterized as a probable stereoisomer of ouabain. In fact, low doses of ouabain itself have a long-term pressor effect when chronically infused in rats.[7]

As a consequence of these findings, the endogenous OLF and/or its receptor may represent new, so far unexploited, pharmacological targets for the treatment of those forms of hypertension where an alteration in renal Na^+ handling is associated with increased OLF levels. A compound able to selectively counteract the pressor effect induced by the interaction of OLF with its receptor could have antihypertensive activity and should be devoid of adverse side effects caused by interference with other known mechanisms of blood pressure regulation.

As part of a program in this field, we synthesized and screened digitoxigenin derivatives for their selectivity for the Na^+,K^+-ATPase receptor and for their antihypertensive activity in rat models of genetic and experimental hypertension respectively sustained by elevated levels of endogenous OLF or by chronic infusion of low doses of exogenous ouabain. Among the many tested compounds, 17β-(3-furyl)-5β-androstane-3β,14β,17α-triol (PST 2238, **1**), was found particularly interesting and chosen for an in-depth evaluation.



PST 2238, **1**

In this communication we describe the synthesis and the preliminary *in vitro* and *in vivo* pharmacological characteristics of **1**.

The synthetic routes are shown in Schemes 1 and 2.

Compound **1** was prepared from the known compound **2**[8] by reduction with DIBAH[9] in THF at −65 °C (Scheme 1, 56% yield). A small percentage (about 5%) of the overreduction product **3** was also obtained. This highly polar compound was very difficult to remove by crystallization, and pure **1** could only be obtained by column chromatography.

Owing to the interest of compound **1**, a more practical pilot scale method that could avoid low reaction temperatures and chromatographic purification was necessary. The reduction at room temperature gave, as expected, a larger amount of the overreduction product

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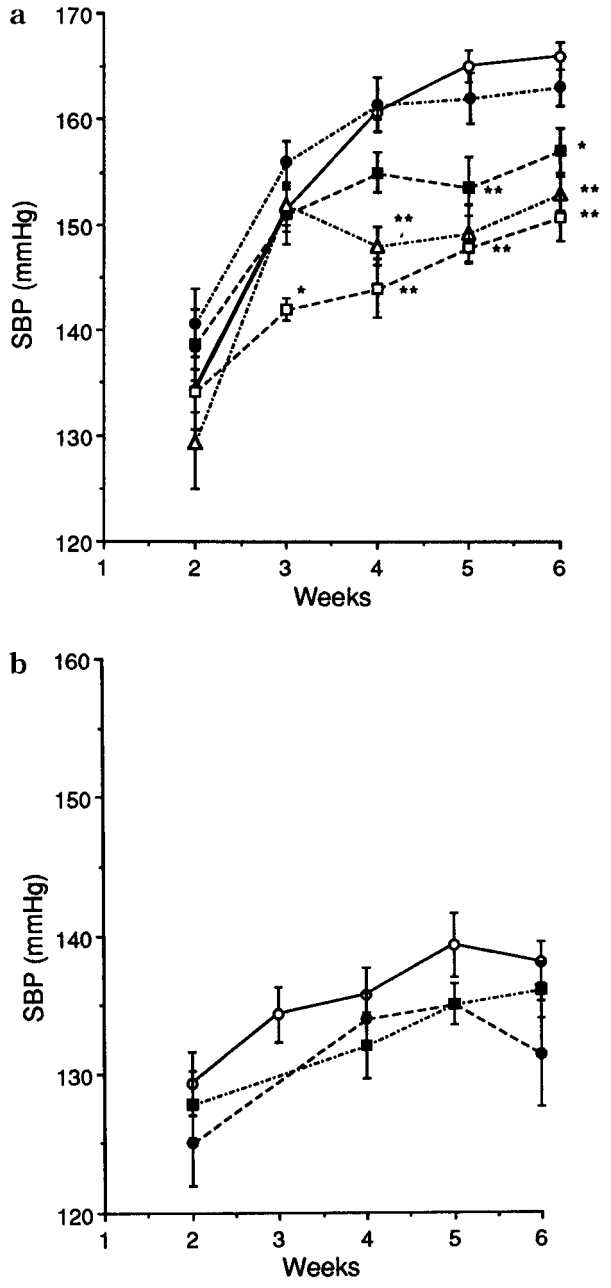


Figure 1. (A) Effect of compound **1** on the development of hypertension in MHS during long-term oral treatment at different doses once a day, for 6 weeks. Systolic blood pressure (SBP) was recorded weekly at the tail, 6 h after the treatment, starting from the second week of treatment: MHS controls (○); **1**, 0.1 μg/kg (●); **1**, 3 μg/kg (■); **1**, 90 μg/kg (△) and **1**, 3 mg/kg (□). (B) Long-term oral treatment of MNS rats with **1**. SBP was recorded according to same schedule as for MHS rats: MNS controls (○); **1**, 90 μg/kg (●); and **1**, 3 mg/kg (■). Data are a mean ± SEM; $n = 7$ for each group. Statistical differences from the control group was calculated by two-way ANOVA followed by Dunnett's *t*-test: $*p < 0.05$; $**p < 0.01$.

3 (40−45%), together with **1** (45−50%) and **4** (5−10%), but the crude mixture, i.e. compounds **3** and **4**, proved oxidizable with activated MnO_2 in boiling dioxane/acetic acid/water (10:2:1) to crude **1** (85−90% purity) without any trace of **3** (Scheme 2). After two crystallizations from acetone/water and methanol, pure **1** (≥99%) was obtained in 41% yield from **2**.

Compound **1** displaced [^{3}H]ouabain from the dog kidney Na^+,K^+-ATPase receptor[10,11] ($IC_{50} = 1.5 \times 10^{-6}$ M), was devoid of cardiac inotropic activity in isolated guinea pig atria, and showed no affinity up to 10^{-4} M with general (α_1, α_2, β_1, β_2, A_1, A_2, M_1, M_2, H_1, H_2, 5-HT_1, 5-HT_2, Ca^{2+} channels, TXA_2/PGH_2, PAF, $GABA_A$, $GABA_B$, DA-NE-5-HT uptake, glutammate, glycine, benzodiazepine) and hormonal (estrogenic, progestinic, androgenic, mineralcorticoid) receptors.

The antihypertensive activity of **1** was tested both in genetic hypertensive rats of the Milan strain (MHS) and in Harlan Sprague−Dawley (SD) rats made hypertensive by chronic infusion of low doses of ouabain (OS rats).

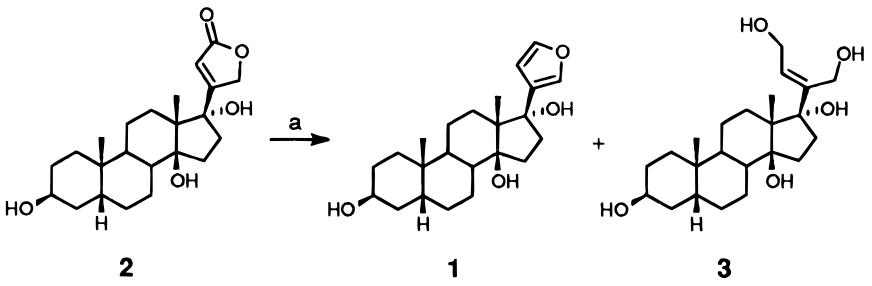
MHS rats develop hypertension due to a primary renal abnormality in tubular Na^+ reabsorption[12] which is sustained by a faster cell Na^+ transport[13,14] driven by an up-regulation of the activity and expression of the basolateral Na^+,K^+ pump.[15] The development of hypertension is paralleled by an increase of the tissue and plasma levels of OLF in MHS, compared with their normotensive controls, Milan normotensive rats (MNS).[16] Therefore, MHS rats represent a good model of spontaneous hypertension in which a potential antihypertensive compound able to interfere with the Na^+,K^+ pump and OLF may be effective.

Young prehypertensive MHS rats were orally treated with **1** (0.1−3−90 μg/kg and 3 mg/kg once a day) for 6 weeks, and starting from the second week of treatment, systolic blood pressure (SBP) and heart rate (HR) were recorded weekly at the tail 6 h after treatment. In parallel, age-matched normotensive MNS controls were similarly treated with **1** at 90 μg/kg and 3 mg/kg, and SBP and HR were recorded according to the same schedule as for MHS rats. Compound **1** reduced the development of hypertension in MHS at doses from 3 μg/kg to 3 mg/kg, the ineffective dose of **1** in this rat strain being 0.1 μg/kg (Figure 1A). The calculated ED_{50} at the end of the treatment was 4 μg/kg. In MNS rats, compound **1** did not affect SBP (Figure 1B). HR was not affected at any tested dose in both strains (data not shown).

The hypotensive effect of compound **1** was also tested in already hypertensive male adult MHS rats. SBP was recorded daily at the tail 6 h after treatment with oral doses of 0.1−1−3 and 10 mg/kg for a period of 10 days, followed by 6 days of washout. SBP was gradually lowered by **1**, and the hypotensive effect, which was seen until the 10th day of treatment, started to be statistically significant at the 3rd day with the doses of 10 and 0.1 mg/kg (−19 and −11 mmHg respectively vs controls, $p < 0.01$) and at the 5th day with the doses of 3 and 1 mg/kg (−21 and −27 mmHg respectively vs controls, $p < 0.01$). The hypotensive effect of compound **1** was seen to be long lasting since SBP recorded 24 h after treatment in rats receiving the dose of 10 mg/kg was significantly lower than in MHS controls, starting from the 3rd day of treatment. During the 6 days of washout, the SBP of treated rats returned gradually to the levels of the MHS controls (data not shown). HR was not affected at any tested dose (data not shown).

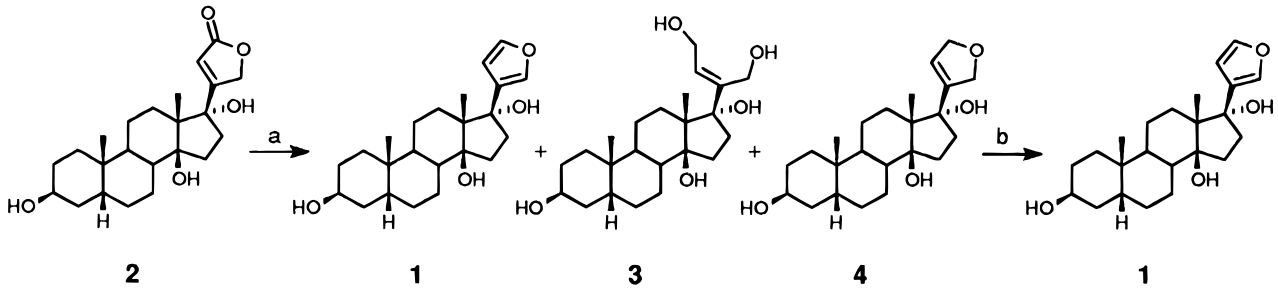
The antihypertensive activity of **1** was also tested in a model of experimental hypertension sustained by chronic infusion of low doses of ouabain.[7] Normotensive SD rats of 150−180 g of body weight were made hypertensive (OS rats) by subcutaneous implantation of osmotic Alzet minipumps which released ouabain at the dose of 50 μg/kg/day for 8−10 consecutive weeks (first experiment). In parallel, a second group of SD rats received, through the minipumps, only saline and was

Scheme 1[a]



[a] Reagents and conditions: (a) DIBAH, 1 M in *n*-hexane, THF, −65 °C, 5 h.

Scheme 2[a]



[a] Reagents and conditions: (a) DIBAH neat, THF, room temperature, 2 h; (b) MnO_2, dioxane, AcOH, H_2O, reflux, 2.5 h.

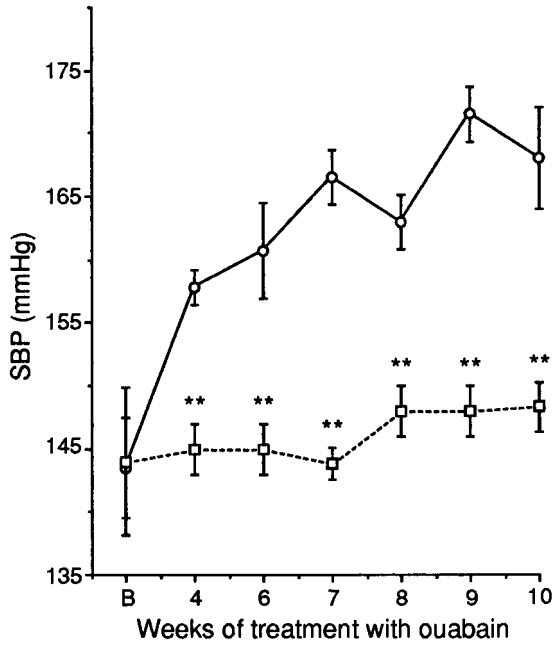


Figure 2. Pressor effect of Ouabain in SD rats. Normotensive SD rats of 150−180 g of body weight were made hypertensive (OS rats, ○) by subcutaneous implantation of osmotic Alzet minipumps which released ouabain at the dose of 50 μg/kg/day for 10 consecutive weeks. In parallel, a second group of SD rats received through the minipumps only a saline solution and were taken as normotensive controls (CS rats, □). B: basal before starting with ouabain treatment. Data are a mean ± SEM; $n = 8$ for each group. Statistical differences from the OS control group was calculated by Student's *t*-test: $^{**}p < 0.001$.

taken as normotensive controls (CS rats). After 4 weeks of ouabain treatment, SBP significantly rose from the 145−147 mmHg of CS rats up to 160−165 mmHg in OS rats (Figure 2).

In a second experiment, the antihypertensive activity of **1** was tested in OS and CS rats. Four groups of OS rats were orally treated with **1** at doses of 0.1−1−10 and 100 μg/kg once a day for 4 weeks, while one group of OS and one of CS rats received only vehicle (methocel, 0.5% v/v) (Figure 3A). In parallel a group of CS rats was orally treated with **1** at 100 μg/kg or vehicle, according to the same schedule as for OS rats (Figure 3B). SBP and HR were recorded weekly at the tail, 6 h after treatment. While compound **1** did not affect SBP in CS rats, Figure 3B, it gradually lowered SBP in OS rats at all the tested doses, and this reached the same level as that of CS rats after 4 weeks of treatment (Figure 3A). Moreover, the subsequent interruption of treatment for 1 week (washout period) of OS rats on **1** brought their SBP back to the level of OS controls (data not shown). HR was never affected at any dose in either OS or CS rats.

Compound **1** showed antihypertensive activity also in other rat models of volume-dependent hypertension, such as the deoxycorticosterone acetate (DOCA) + salt, DOCA + salt + ouabain, and reduced renal mass models (data not shown).

The discrepancy between the concentration active *in vitro* on isolated Na^+,K^+-ATPase and the very low doses of **1** active *in vivo* in MHS and OS rats may be explained by recent and still unpublished results obtained in cultured rat renal epithelial cells where it has been observed that, after 5 days of incubation with **1**, the compound is able to antagonize the effect of ouabain on the Na^+,K^+ pump at 10^{-9} M.

In conclusion, compound **1**, which is able to antagonize *in vitro* the binding of ouabain to Na^+,K^+-ATPase, is also able to reduce SBP *in vivo* when chronically given at oral doses of μg/kg both in genetic and experimental animal models of hypertension sustained respectively by elevated levels of OLF or ouabain. The hypotensive effect of **1**, when administered in adult MHS rats, takes some days to develop and it is long-lasting, being present also 24 h after treatment. This suggests that **1** does not act on mechanisms that acutely modulate blood pressure, such as the adrenergic system, the Ca^{2+} channels, or the vascular contractility, but probably acts on long-term pressor mechanism. Moreover, a possible

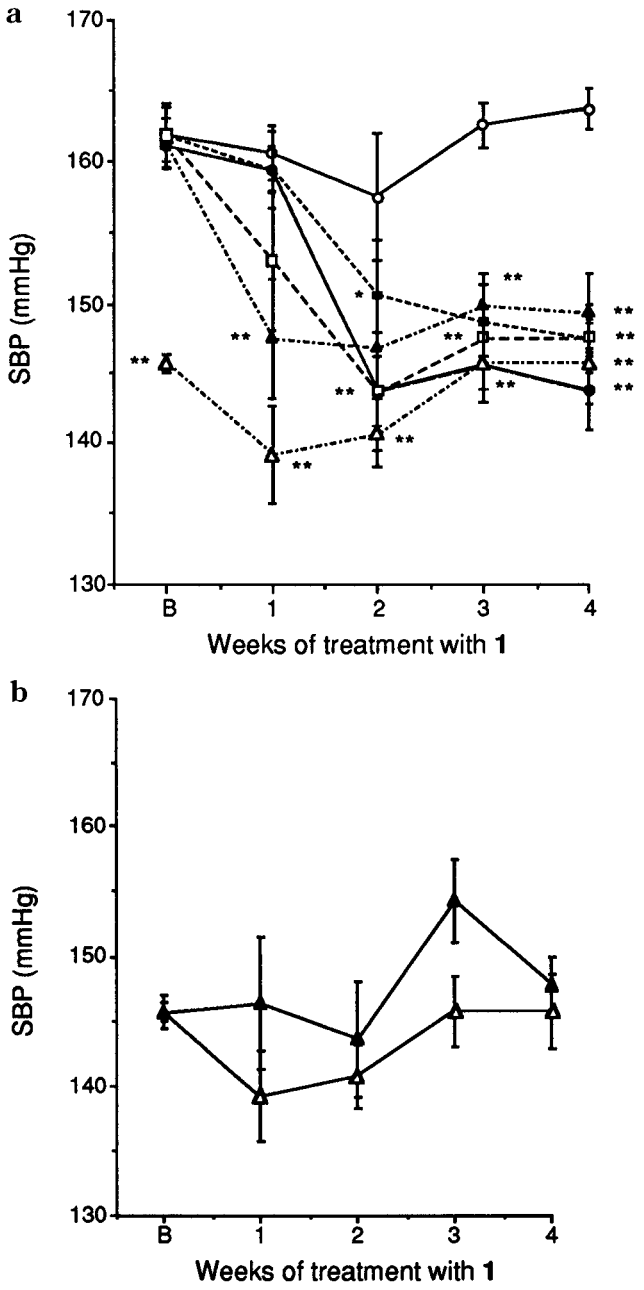


Figure 3. (A) Long-term oral treatment of OS rats with **1**. Four groups of OS rats were orally treated with **1** at different doses once a day, for 4 weeks, while one group of OS and one of CS rats (controls) received only vehicle (methocel, 0.5% v/v). SBP was recorded weekly at the tail, 6 h after the treatment: OS controls (○); CS controls (△); **1**, 0.1 μg/kg (●); **1**, 1 μg/kg (■); **1**, 10 μg/kg (▲); and **1**, 100 mg/kg (□). Data are a mean ± SEM; $n = 7$ for each group. Statistical differences from the OS control group were calculated by two-way ANOVA followed by Dunnett's *t*-test: $*p < 0.05$; $**p < 0.01$. (B) Long-term oral treatment of CS rats with **1**. SBP was recorded according to same schedule as for OS rats: CS controls (△); **1**, 100 μg/kg (▲). Data are a mean ± SEM; $n = 7$ for each group.

β-blocking or vasodilating activity may be excluded for **1** since it does not affect HR at any tested doses and at any time. The product seems not to interact with general and hormonal receptors and to be devoid of cardiac effects typical of digitalis compounds. Acute and chronic toxicological studies, both in rat and monkey, indicate that the ratio between the antihypertensive dose and that which induces the first observable toxic effects is higher than 1:10000. Therefore, this product is under study as a prototype of a new class of potential antihypertensive drugs able to selectively antagonize the pressor effect of ouabain or OLF. More detailed pharmacological and toxicological results will be described in a future paper.

Supporting Information Available: Experimental procedures and analytical data for compounds **1**, **3**, and **4** (5 pages). Ordering information is given on any current masthead page.

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