

Synthesis and Biological Evaluation of 14 β -Methoxy Digitalis Derivatives*

M. Gobbini,** N. Almirante, A. Cerri, G. Padoani and P. Melloni

Prassis Istituto di Ricerche Sigma-Tau, Via Forlanini, 3, 20019 Settimo Milanese (MI), Italy. Tel +39 2 33500388, Fax +39 2 33500408, Email: MC3405@mclink.it

Received: 11 November 1997 / Accepted: 15 January 1998 / Published: 25 January 1998

Abstract: The synthesis and biological evaluation of 14 -methoxy derivatives of digitoxigenin and of other digitalis-like compounds are reported. These compounds have a 14 -oxygen, which can be a hydrogen bonding acceptor, as is the case of 14 ,15 -epoxide derivatives, but not a hydrogen bonding donor as is the case of 14 -hydroxy derivatives. All the new 14 -methoxy derivatives show a considerable reduced binding affinity on Na⁺,K⁺-ATPase when compared with the 14 -hydroxy analogues and also with the 14 ,15 -epoxy derivatives. These results could mean that the digitalis receptor does not permit the presence of a bulky substituent in the 14 region, even of relatively small volume like the methyl group.

Keywords: 14 -Methoxy digitalis derivatives; binding affinity; Na⁺,K⁺-ATPase.

Introduction

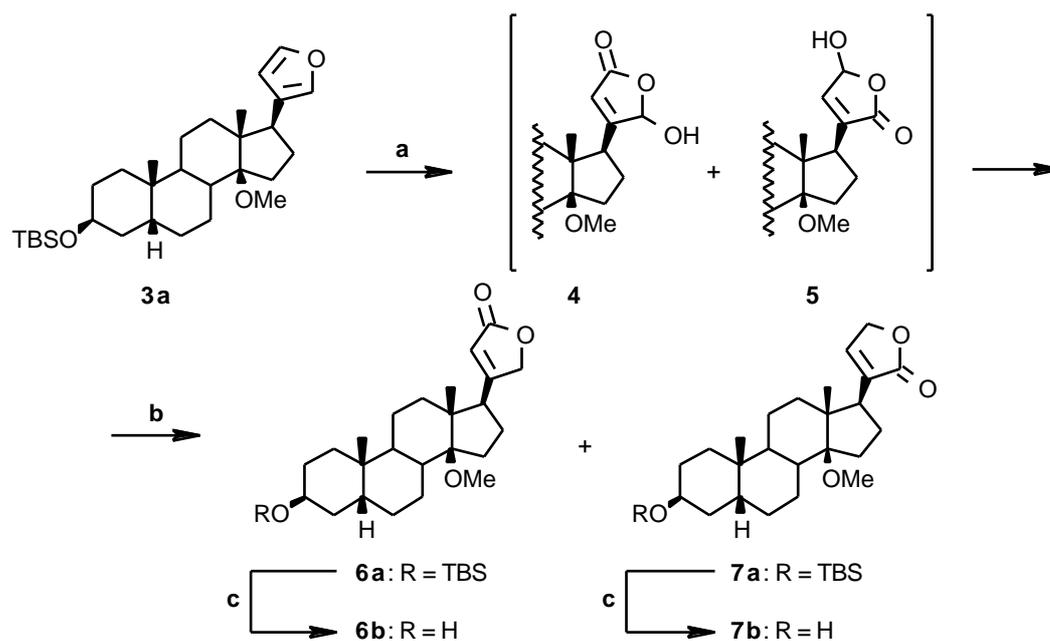
Digitalis cardiac glycosides are well known drugs clinically used for treatment of congestive heart failure [1]. Their action is mainly due to inhibition of Na⁺,K⁺-ATPase, an enzyme located in the cell membrane and promoting the outward transport of Na⁺ and the inward transport of K⁺ [2]. Recently the existence of endogenous digitalis-like factors that may be responsible for essential hypertension [3] has opened a new field in the study of compounds acting on the Na⁺,K⁺-ATPase. The most potent inhibitors of Na⁺,K⁺-ATPase are cardenolides such as digitoxigenin (Figure 1) with the following structural characteristics: 17 -unsaturated lactone, 3 - and 14 -hydroxy substituents and A/B and C/D *cis* ring junctions. The 14 -hydroxy

group is involved in hydrogen bonding with the receptor and plays an important role in binding digitalis compounds to Na⁺,K⁺-ATPase receptor; in fact compounds in which this group is absent show very low binding affinity or no affinity at all [4]. However, the known derivatives with a 14 ,15 -epoxy group (Figure 1) show high binding affinities although not as high as the 14 -hydroxy analogues (Table 1).

Herein, we report the synthesis and biological evaluation of novel 14 -methoxy derivatives of digitoxigenin and of other digitalis-like compounds. These compounds have a 14 -oxygen, which can be a hydrogen bonding acceptor, as is the case of 14 ,15 -epoxide derivatives, but not a hydrogen bonding donor as is the case of 14 -hydroxy derivatives. Comparison of the

* Part of this paper was presented at The First International Electronic Conference on Synthetic Organic Chemistry (ECSOC-1), <http://www.mdpi.org/ecsoc-1.htm>, 1-30 September 1997.

** To whom correspondence should be addressed.

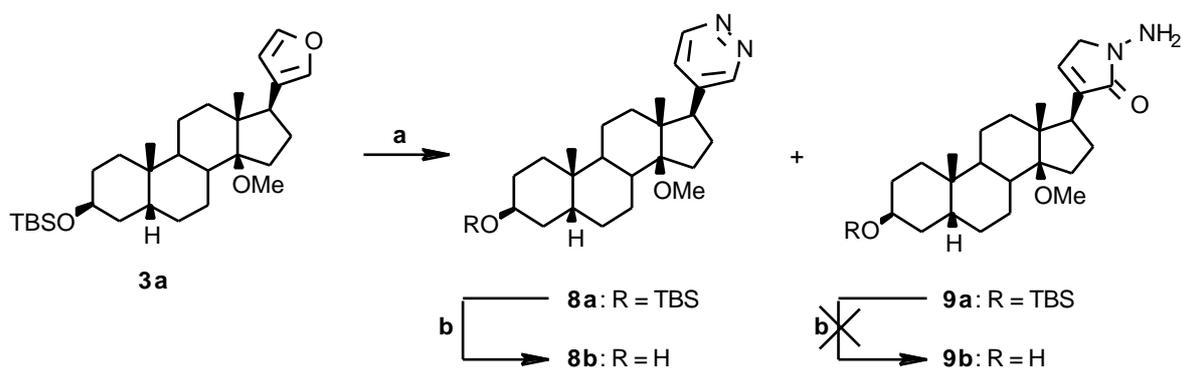


Reagents and conditions: **a**: *m*-chloroperbenzoic acid, AcOH, AcONa, CHCl₃, rt; **b**: NaBH₄, CH₂Cl₂/water, rt, (**6a** 49%; **7a** 13%); **c**: conc. HCl, CHCl₃/MeOH, rt (**6b** 81%; **7b** 58%).

Scheme 2. Synthesis of 14 -methoxy-digitoxigenin and 14 -methoxy-isodigitoxigenin.

The 17 -(4-pyridazinyl) derivative **8a** (Scheme 3) was prepared by reacting the crude 17 -(3-furyl) derivative **3a** with NBS in dioxane/water in the presence of AcONa and then with hydrazine [7] to give, after chromatographic purification, the desired **8a** (24% from **2**) and the N-amino

lactam derivative **9a** as a side product (20% from **2**); **8a** was deprotected with *n*-Bu₄NF in THF at reflux temperature (81% yield), while **9a** degraded to a complex mixture under the same conditions.



Reagents and conditions: **a**: NBS, AcONa, dioxane/water, 5 °C; then hydrazine, water, rt, (**8a** 24%; **9a** 20%); **b**: *n*-Bu₄NF, THF, reflux (**8b** 81%; **9b** degradation).

Scheme 3. Synthesis of 17 -(4-pyridazinyl)-14 -methoxy derivative.

The synthesized compounds were evaluated, in comparison with 14,15-epoxy and/or 14-hydroxy analogues, for displacement of the specific [³H]-ouabain binding from the Na⁺,K⁺-ATPase receptor [13a] isolated

from dog kidney and purified according to Jørgensen [13b]. The biological data are showed in Table 1.

Table 1. Binding affinity on Na⁺,K⁺-ATPase

Compound	Binding ^a	Compound	Binding ^a
Digitoxigenin	7.2	17-(3-furyl) derivative 1	6.6
Digitoxigenin-14,15-epoxy	6.6	17-(3-furyl)-14,15-epoxy	5.2
Digitoxigenin-14-methoxy 6b	5.4	17-(3-furyl)-14-methoxy 3b	4.3
Isodigitoxigenin	5.4	17-(4-pyridazinyl) derivative	7.0
Isodigitoxigenin 14-methoxy 7b	<4.0	17-(4-pyridazinyl)-14-methoxy 8b	4.9

^aAverage of three values (-log IC₅₀). The affinity for the receptor site of Na⁺,K⁺-ATPase was evaluated by the displacement of the specific [³H]-ouabain binding from Na⁺,K⁺-ATPase receptor [13a] isolated from dog kidney and purified according to Jørgensen [13b].

Conclusion

All the new 14-methoxy derivatives show a considerable reduced binding affinity when compared with the 14-hydroxy analogues and also with the 14,15-epoxy derivatives; the reduction in the affinity varies from 65 times for **6b**, the most potent 14-methoxy derivative, to 200 times for **3b**; the 14-methoxy derivative of isodigitoxigenin **7b** was almost devoid of any affinity.

These results could mean that the digitalis receptor does not permit the presence of a bulky substituent in the 14 region, even of relatively small volume like the methyl group. In fact the reduced binding affinities of the 14-methoxy derivatives do not seem to depend on the impossibility of being hydrogen donors since the two epoxy derivatives reported in Table 1 show high binding affinity although lower than that of the 14-hydroxy analogues.

Experimental

General

Melting points were measured on a capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Redox, Cologno Monzese, Italy. ¹H-NMR spectra were recorded on a Bruker AC-300 spectrometer at 300.13 MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as internal standard. Chromatography was carried out on silica gel

(Baker 7024-02). Solvents and reagents (Aldrich) were used as purchased.

3β-Hydroxy-14β-methoxy-17β-(3-furyl)-5β-androstane **3b**

To a solution of 17-(3-furyl)-5-androstane-3,14-diol **1** [11] (5.4 g, 15.08 mmol) in 47 mL of DMF and 18.6 mL of triethylamine, *tert*-butyldimethylsilyl chloride (10.0 g, 66.34 mmol) were added at 0 °C and the mixture was allowed to warm to room temperature. After 4 hrs the mixture was poured into water and extracted with CH₂Cl₂. The organic layer was dried over Na₂SO₄ and evaporated to dryness under reduced pressure and the crude residue was filtered through a short pad of silica gel (cyclohexane) to give 3-*tert*-butyldimethylsilyloxy-17-(3-furyl)-5-androstan-14-ol **2** (6.4 g, 90%) as an amorphous solid that was used for the next step without further purification.

A suspension of **2** (6.4 g, 13.56 mmol) and KH (2.7 g, 67.88 mmol) in 90 mL of dry THF was heated at 70 °C for 1 hr; then MeI (2.53 mL, 40.6 mmol) were added. After 30 min the mixture was poured into water and extracted with CH₂Cl₂. The organic layer was dried over Na₂SO₄ and evaporated to dryness under reduced pressure to give 3-*tert*-butyldimethylsilyloxy-14-methoxy-17-(3-furyl)-5-androstane **3a** (6.59 g) as an amorphous solid; ¹H-NMR (300 MHz, CDCl₃): 0.04 (6H, s, OSit-BuMe₂), 0.78 (3H, s, CH₃), 0.91 (9H, s, OSit-BuMe₂), 0.98 (3H, s, CH₃), 2.69 (1H, m, 17-H), 3.39 (3H, s, OCH₃), 4.07 (1H, brs, 3-H), 6.39 (1H, brs, 22-H), 7.18 (1H, brs, 21-H), 7.32 (1H, brs, 23-H).

A solution of **3a** (3.0 g, 6.35 mmol) in 57 mL of a 1.1 M solution of *n*-Bu₄NF (63.5 mmol) in THF was heated at 70 °C under nitrogen for 1 hr and then poured into a saturated aq. solution of NaCl. The mixture was extracted with AcOEt and the organic layer was dried over Na₂SO₄, evaporated to dryness under reduced pressure and purified by flash-chromatography (n-hexane to n-hexane/AcOEt 80:20 v/v) to give **3b** (2.3 g, quantitative from **2**) as a white solid, m.p. 88-91 °C (dec); ¹H-NMR (300 MHz, CDCl₃): 0.78 (3H, s, CH₃), 1.01 (3H, s, CH₃), 2.69 (1H, m, 17 -H), 3.39 (3H, s, OCH₃), 4.14 (1H, brs, 3 -H), 6.39 (1H, brs, 22-H), 7.18 (1H, brs, 21-H), 7.32 (1H, brs, 23-H). Analysis calculated for C₂₄H₃₆O₃: C, 77.38; H, 9.74. Found: C, 77.38; H, 9.76.

3β-Hydroxy-14β-methoxy-5β-card-20(22)-enolide 6b and 3β-hydroxy-14β-methoxy-5β-24-norchol-20(22)-en-21,23-lactone 7b

To a solution of 3-*tert*-butyldimethylsilyloxy-14-methoxy-17-(3-furyl)-5-androstane **3a** (6.5 g, 13.4 mmol; prepared as described above) in 320 mL of CHCl₃, AcONa (2.8 g, 33.9 mmol), AcOH (1.48 mL, 33.9 mmol) and *m*-chloroperbenzoic acid (75%, 6.85 g, 29.8 mmol) were added. The reaction mixture was stirred at room temperature for 1.5 hrs, then diluted with 500 mL of CHCl₃, washed with a 5% aq. solution of Na₂SO₃ and with a 5% aq. solution of NaHCO₃; the organic layer was dried over Na₂SO₄, evaporated to dryness under reduced pressure and the residue used for the next reduction step.

The residue obtained above was dissolved in 1.6 L of CH₂Cl₂ and 320 mL of water and to the well stirred biphasic mixture NaBH₄ (6.0 g, 158.72 mmol) was added in two portions, the second after 4 hrs. After another 18 hrs the reaction mixture was added to a 5% aq. solution of citric acid (1 L) and the organic layer extracted, separated, washed with a 5% aq. solution of NaHCO₃ and a saturated aq. solution of NaCl. The organic layer was dried over Na₂SO₄, evaporated to dryness under reduced pressure and purified by flash-chromatography (CH₂Cl₂) to give the protected derivatives **6a** (3.3 g, 49% from **2**) and **7a** (0.88 g, 13% from **2**).

The silyloxy derivative **6a** (2.4 g, 4.78 mmol) was dissolved in 100 mL of CHCl₃/MeOH 1:1, few drops of conc. HCl were added and the reaction mixture was stirred at room temperature for 24 h. The acidic solution was neutralized with solid NaHCO₃ and evaporated at reduced pressure; the residue was extracted with CH₂Cl₂ and water; the organic layer was dried over Na₂SO₄, evaporated to dryness under reduced pressure and crystallized from Et₂O to give **6b** (1.5 g, 81%) as a white solid: m.p.: 179-182 °C; ¹H-NMR (300 MHz, CDCl₃): 0.91 (3H, s, CH₃), 0.99 (3H, s, CH₃), 2.71 (1H, m, 17 -H), 3.34 (3H, s, OCH₃), 4.12 (1H, brs, 3 -H), 4.82 (2H, m, 21-H), 5.83 (1H, brs, 22-H). Analysis calculated for C₂₄H₃₆O₄: C, 74.19; H, 9.34. Found: C, 73.85; H, 9.36.

The silyloxy derivative **7a** (0.54 g, 1.07 mmol) were dissolved in 25 mL of CHCl₃/MeOH 1:1, few drops of conc. HCl were added and the reaction mixture was stirred at room temperature for 24 h. The acidic solution was neutralized with solid NaHCO₃ and evaporated at reduced pressure; the residue was extracted with CH₂Cl₂ and water; the organic layer was dried over Na₂SO₄, evaporated to dryness under reduced pressure and purified by flash-chromatography (CH₂Cl₂/AcOEt 90:10 v/v) to give **7b** (0.24 g, 58%) as a white solid: m.p.: 163-166 °C; ¹H-NMR (300 MHz, CDCl₃): 0.90 (3H, s, CH₃), 0.99 (3H, s, CH₃), 2.77 (1H, m, 17 -H), 3.32 (3H, s, OCH₃), 4.14 (1H, brs, 3 -H), 4.82 (2H, m, 23-H), 7.24 (1H, brs, 22-H). Analysis calculated for C₂₄H₃₆O₄: C, 74.19; H, 9.34. Found: C, 74.25; H, 9.33.

3β-Hydroxy-14β-methoxy-17β-(4-pyridazinyl)-5β-androstane 8b

To a solution of 3-*tert*-butyldimethylsilyloxy-14-methoxy-17-(3-furyl)-5-androstane **3a** (1.0 g, 2.0 mmol; prepared as described above) and AcONa (0.36 g, 2.2 mmol) in 70 mL of a dioxane/water 10:1 (v/v) mixture, NBS (0.39 g, 2.2 mmol) in 7 mL of a dioxane/water 9:1 v/v mixture were slowly added at 5 °C. After 0.5 hrs a solution of hydrazine hydrate (4 mL, 82.4 mmol) in 4 mL of water was slowly dropped while maintaining the temperature at 5 °C. The resulting mixture was kept at room temperature for 36 hrs, and then was poured into a saturated aq. solution of NaCl and extracted with Et₂O. The organic layer was dried over Na₂SO₄, evaporated to dryness under reduced pressure and purified by flash-chromatography (AcOEt/ cyclohexane 95:5 v/v, then AcOEt 100) to give **8a** (0.24 g, 24% from **2**) and **9a** (0.21 g, 20% from **2**).

9a: ¹H-NMR (300 MHz, CDCl₃): 0.04 (6H, s, OSit-BuMe₂), 0.87 (3H, s, CH₃), 0.91 (9H, s, OSit-BuMe₂), 0.96 (3H, s, CH₃), 2.81 (1H, m, 17 -H), 3.32 (3H, s, OCH₃), 3.9-4.15 (5H, m, NH₂, 3 -H and 23-H), 6.72 (1H, brs, 22-H).

A solution of **8a** (0.24 g, 0.48 mmol) in 5 mL of a 1.1 M solution of *n*-Bu₄NF (5.5 mmol) in THF was heated at 70 °C under nitrogen for 1 hr and then poured into a saturated aq. solution of NaCl and extracted with EtOAc. The organic layer was dried over Na₂SO₄, evaporated to dryness under reduced pressure and purified by flash-chromatography (AcOEt) to give **8b** (0.16 g, 81%) as a white foam. An analytical sample was obtained as the hydrogen fumarate, a white solid, m.p. 157-159 °C (dec); ¹H-NMR (300 MHz, DMSO-d₆): 0.53 (3H, s, CH₃), 0.91 (3H, s, CH₃), 2.71 (1H, m, 17 -H), 3.28 (3H, s, OCH₃), 3.89 (1H, brs, 3 -H), 6.62 (1H, s, fumarate), 7.49 (1H, m, 21-H), 9.03 (2H, m, 22-H and 23-H). Analysis calculated for C₂₄H₃₆N₂O₂ · 0.5 C₄H₄O₄: C, 70.55; H, 8.65 N 6.33. Found: C, 70.28; H, 8.60; N 6.28.

When **9a** was reacted with *n*-Bu₄NF in the same conditions described above, a complex mixture of unidentified products was obtained.

Acknowledgment: We thank Mr. G. Marazzi for spectral determinations.

References and Notes

1. Hofman, B. F.; Bigger, J. T. In *The Pharmacological Basis of Therapeutics*; Goodman Gilman, A.; Nies, A. S.; Rall, T. W.; Taylor, P., Eds.; Pergamon Press, New York, 1990, Section VII, Chapter 34.
2. Repke, K. R. H.; Schönfeld, W. *Trends Pharmacol. Sci.* **1984**, *5*, 393.
3. Schoner, W. *Trends Pharmacol. Sci.*, **1991**, *12*, 209.
4. Thomas, R.; Gray, P.; Andrews, J. *Adv. Drug Res.* **1990**, *19*, 312.
5. Yamada, A. *Chem. Pharm. Bull.* **1960**, *8*, 18.
6. Ferland, J. M.; Lefebvre, Y.; Deghenghi, R.; Wiesner, K. *Tetrahedron Lett.* **1966**, 3617.
7. Siphar S. A., DE 2,534,981; CA 85:33365f.
8. Engel, C. R.; Bach, G. *Steroids*, **1964**, *3*, 593.
9. Yoshii, E.; Koizumi, T.; Mizuno, S.; Kitatsuji, E. *Chem. Pharm. Bull.*, **1976**, *24*, 3216.
10. Pastelin, G.; Mendez, R. *Arch. Farmacol. Toxicol.* **1984**, *10*, 135.
11. Minato, H.; Nagasaki, T. *J. Chem. Soc.* **1966**, 377.
12. Use of *n*-Bu₄NF was incompatible with the presence of the , -unsaturated lactone. During the acidic hydrolysis 14-unsaturated side products were formed, as judged by TLC and ¹H-NMR of the reaction mixtures; less than 10% for **6a** and about 30% for **7a** were obtained.
13. a) Brown, L.; Erdmann, E. *Arzneim. Forsh.* **1984**, *34*, 1314. b) Jørgensen, P. L. *Biochim. Biophys. Acta* **1990**, *19*, 312.

Sample Availability: Not available.