First International Electronic Conference on Synthetic Organic Chemistry (ECSOC-1), www.mdpi.org/ecsoc/, September 1-30, 1997

[A0011]

Synthesis and Biological Evaluation of 14 b-Methoxy Digitalis Derivatives

M. Gobbini, N. Almirante, A. Cerri, G. Padoani, and P. Melloni
Prassis Istituto di Ricerche Sigma-Tau, Via Forlanini, 3, Settimo Milanese (MI), Italy

Received: 9 July 1997 / Uploaded: 21 July 1997

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: 17b-unsaturated lactone, 3b- and 14b-hydroxy substituents and A/B and C/D *cis* ring junctions. The 14b-hydroxy group is involved in a 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 14b,15b-epoxy group (Figure 1) show high binding affinities although not as high as the 14b-hydroxy analogues (Table 1).

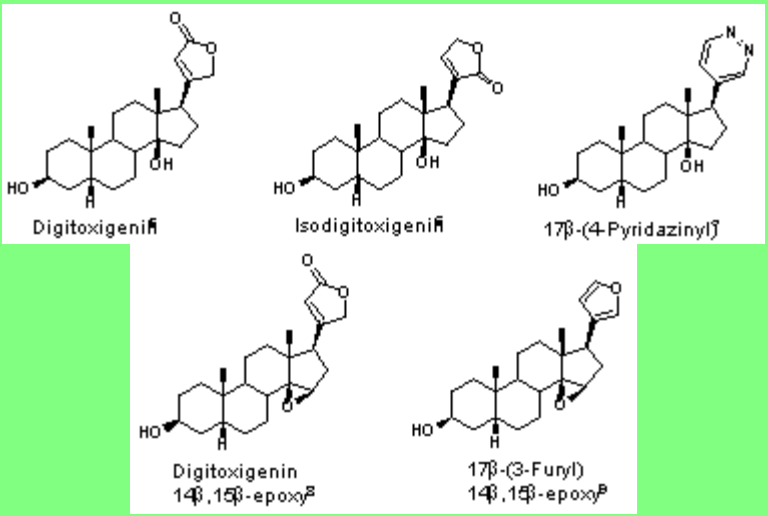
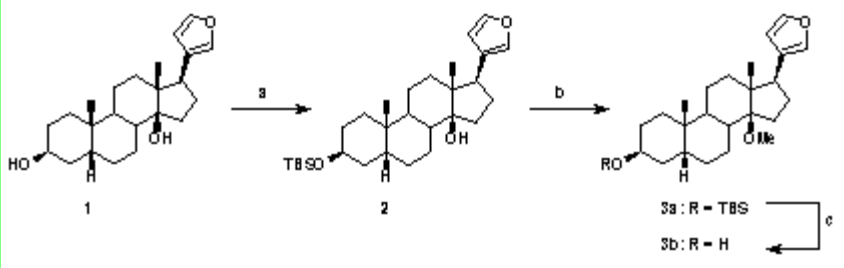


Figure 1. Known compounds synthesized using the reported procedures.

Herein, we report the synthesis and biological evaluation of unknown 14b-methoxy derivatives of digitoxygenin and of other digitalis-like compounds. These compounds have a 14b-oxygen, which can be a hydrogen bonding acceptor, as is the case of 14b,15b-epoxide derivatives, but not a hydrogen bonding donor as is the case of 14b-hydroxy derivatives. Comparison of the binding values of these three classes of compounds could allow more insight into the requirements necessary for recognition by the receptor. Only a 3b-glucoside derivative of 14b-methoxydigitoxygenin has been described;[10] for which the inotropic activity was reported to be marginal, but no synthetic route was given.

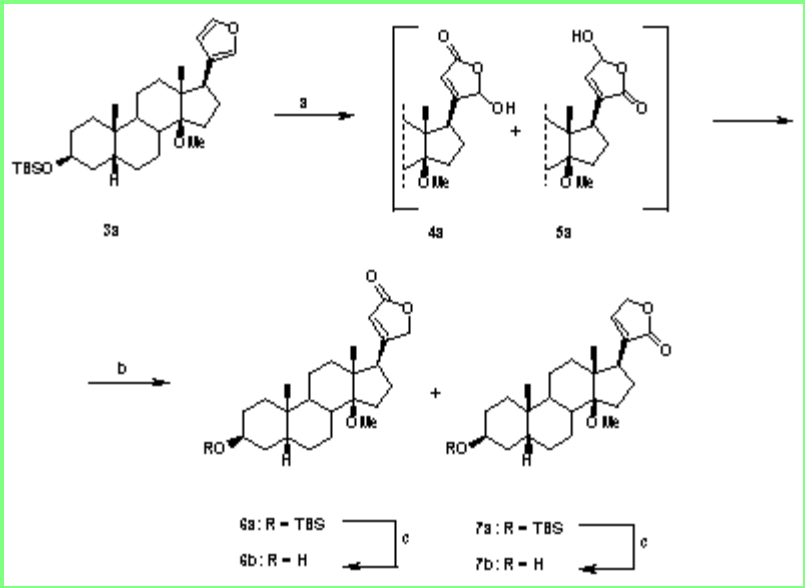
Chemistry

Attemps to introduce a methyl on the 14b-hydroxy group of digitoxigenin, with the secondary 3b-hydroxy protected, using diazomethane or dimethyl sulfate failed; diazomethane failed also when applied on the 17b-(3-furyl) analogue, while dimethyl sulfate gave low yield.
We then turned our attention to a Williamson reaction with MeI and, since the strongly basic reaction conditions proved incompatible with the presence of the a,b-unsaturated lactone of digitoxigenin, we tried the reaction on the17b-furyl derivative **2** (Scheme 1).



Scheme 1. *Reagents and conditions*: **a**: *tert*-butyldimethylsilyl chloride, TEA, DMF, rt (90%); **b**: KH, dry THF, reflux; then MeI; **c**: *n*-Bu_4NF, THF, reflux (quantitative from **2**).

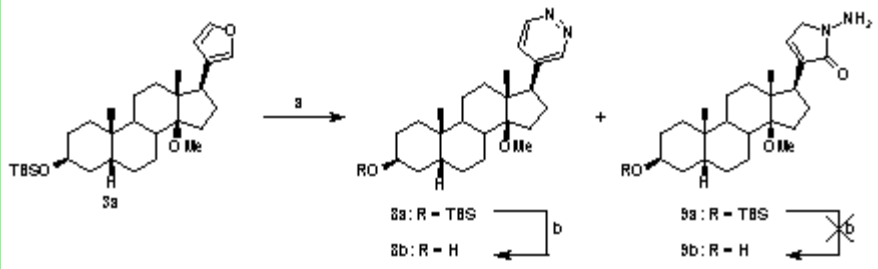
The known 17b-(3-furyl)-5b-androstane-3b,14b-diol **1**[11] was reacted with *tert*-butyldimethylsilyl chloride in DMF in the presence of triethylamine to give the protected derivative **2** (90%); this TBS derivative and KH were kept at reflux temperature for one hour in dry THF; the addition of MeI instantaneously gave the desired 14b-methoxy derivative **3a**. The crude **3a** was deprotected with *n*-Bu4 NF in THF at reflux temperature to give **3b** in quantitative yield from **2**. From the 14b-methoxy derivative **3a** the 14b-methoxy digetoxigenin **6b** could be obtained by the oxidative/reductive procedure[6] shown in Scheme 2. The crude **3a** was reacted with *m*-chloroperbenzoic acid in $CHCl_3$ in the presence of AcOH and AcONa; the crude hydroxy lactone intermediates **4a** and **5a** were reduced with $NaBH_4$ in CH_2Cl_2 to give a mixture of the desired digitoxigenin derivative **6a** and of the isomeric isodigitoxigenin derivative **7a** in a 8:2 ratio. The two compounds were separated by flash chromatography to give **6a** (49% from **2**) and **7a** (13% from **2**) and then deprotected by acidic hydrolysis with dil. HCl in a $CHCl_3$/MeOH mixture; **6b** (81%) and **7b** (58%).[12]



Scheme 2. *Reagents and conditions*: **a**: *m*-chloroperbenzoic acid, AcOH, AcONa, $CHCl_3$, rt; **b**: $NaBH_4$, CH_2Cl_2, rt, (**6a** 49%; **7a** 13%); **c**: 5% aq. HCl, $CHCl_3$/MeOH, rt (**6b** 81%; **7b** 58%).

The 17b-(4-pyridazinyl) derivative **8a** was prepared by reacting the 17b-(3-furyl) derivative **3a** with NBS in THF 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*-Bu4 NF in THF at reflux temperature (81% yield), while **9a** degraded to a complex mixture under the same conditions.



Scheme 3. *Reagents and conditions*: **a**: NBS, AcONa, THF, 5 deg.C; then hydrazine, water, rt, (**8a** 24%; **9a** 20%); **b**: *n*-Bu_4NF, THF, reflux (**8b** 81%; **9b** degradation).

Biological Data

All the synthesized compounds were evaluated, in comparison with 14b,15b-epoxy and/or 14b-hydroxy analogues, for displacement of the specific [^{3}H]-ouabain binding[13] on Na^+,K^+-ATPase (Table 1).

Table 1

Compound	Binding[a]	Compound	Binding[a]
Digitoxigenin	7.2	17b-(3-furyl) derivative **1**	6.6
Digitoxigenin 14b,15b-epoxy	6.6	17b-(3-furyl)-14b,15b-epoxy	5.2
Digitoxigenin 14b-methoxy **6b**	5.4	17b-(3-furyl)-14b-methoxy **3b**	4.3
Isodigitoxigenin	5.4	17b-(4-pyridazinyl) derivative	7.0
Isodigitoxigenin 14b-methoxy **7b**	17% at 10^{-4}M	17b-(4-pyridazinyl)-14b-methoxy **8b**	4.9

[a]Average of three values (-log IC_{50}). The affinity for the receptor site of Na^+,K^+-ATPase was evaluated by the displacement of the specific [^{3}H]-ouabain binding from Na^+,K^+-ATPase receptor[13a] isolated from dog kidney and purified according to J&#65533;rghensen.[13b]

All the new 14b-methoxy derivatives show a considerable reduced binding affinity when compared with the 14b-hydroxy analogues and also with the 14b,15b-epoxy derivatives; the reduction in the affinity varies from 65 times for **6b**, the most potent 14b-methoxy derivative, to 200 times for **3b**; the 14b-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 14b region, even of relatively small volume like the methyl group. In fact the reduced binding affinities of the 14b-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 14b-hydroxy analogues.

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.; Sch&#65533;nfeld, 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 Letters*, **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*-Bu_4NF was incompatible with the presence of the a,b-unsaturated lactones; during the acidic hydrolysis the 14-unsaturated side products were formed: less than 10% for **4a** and 30% for **5a**.
13. a) Brown, L.; Erdmann, E. *Arzneim. Forsh.*, **1984**, *34*, 1314. b) Jrghensen, P. L. *Biochim. Biophys. Acta*, **1990**, *19*, 312.
