Complete NMR Assignment of Digitoxigenone, a Biotransformed Derivative of Digitoxigenin by *Fusarium Ciliatum*

G. J Vieira, J. D. de Souza Filho*, A. C. Ferreira
Departamento de Química-ICEx, UFMG, Brazil
peixe@dedalus.lcc.ufmg.br

R. M. Pádua, A. B. Oliveira, F. C. Braga
Faculdade de Farmácia-UFMG, Belo Horizonte-MG, Brazil

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Abstract: *In the plant kingdom some complex chemical transformations occur due, among other factors, to enzymatic systems of high specificity. The biotransformations by plant cell cultures are dependent on the same conditions of the ones made by microorganism cells, having the disadvantage that the minor production of biomass can lead to lower yields. Some examples include biotransformations with Digitalis purpurea, Strophanthus amboensis, Strophanthus gratus, Digitalis lanata cell cultures. In a project treating biotransformation of cardenolides by fungi, digitoxigenin was allowed to be processed by Fusarium ciliatum. Apart from some hydroxylated products, digitoxigenone was obtained and high resolution NMR experiments were performed for a refined study of its structure. The HMBC experiment was performed with reduced spectral range only in dimension F1 to improve the resolution for an unambiguous assignment of the olefinic and carbonyl carbons, C-20 and C-23, respectively. The HSQC-EDIT experiment was also run with optimized sweep width in the two dimensions, F1 and F2. A forward linear prediction was applied allowing an unequivocal assignment of H-7α and H-11β; and H-6α and H-16β. Additionally, important assignments were made possible by NOE's exhibited by H-22 x [H-19, H-16β]; H-19 x [H-8, H-11β]; H-18 x [H-6β, H-8] and H-4α x [H-7α, H-9] spin systems, and also by other unambiguous heteronuclear correlations.*

In the plant kingdom some complex chemical transformations occur, among other factors, due to an enzymatic system of high specificity. Biotransformations by plant cell cultures are dependent on the same conditions of the ones produced by microorganism cells, with the disadvantage of a minor production of biomass that can lead to low yield. Some examples include biotransformations with *Digitalis purpurea*, *Strophanthus amboensis*, *Strophanthus gratus*, and *Digitalis lanata* cell cultures. In a project treating biotransformation of cardenolides by fungi, digitoxigenin (Figure 1) was allowed to be processed by *Fusarium ciliatum*. Apart from some hydroxylated products, digitoxigenone (Figure 1) was obtained, and high resolution NMR experiments[2,3] were performed for a refined study of its structure.

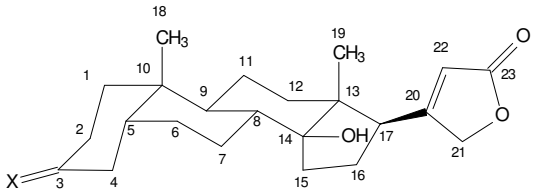

Figure 1. Structural formula of digitoxigenin (x=β-OH, H) and digitoxigenone (X=O).

Although the characterization of digitoxigenone is well known, we present here a reanalysis of ^{13}C NMR assignments[4] together with high resolution ^{1}H NMR total assignment. The experiments performed, as well as the acquisition parameters, are shown in Table 1.

Table 1. NMR experiments and acquisition parameters for digitoxigenone.

EXPERIMENT	ns(nt)	Dummy scans	Time domain in F1	Time domain in F2	Specific delays
^{1}HNMR	64	2	-	-	1s$^{\#}$
^{13}CNMR	3K	4	-	-	2s$^{\#}$
DEPT135	3K	4	-	-	2s$^{\#}$
COSY$^{\$*}$	1	8	512	2K	-
HSQC*	16	16	512	2K	862,07μs**
HSQC-EDIT$^{\$*}$	16	16	512	2K	3.4483ms**
HMBC$^{\$*}$	16	16	512	2K	70ms$^{@}$
NOESY	8	4	512	2K	400ms$^{\&}$

$^{\#}$ relaxation delay; $^{@}$evolution of the long range couplings; $^{\&}$-mixing time; **delay for multiplicity selection;
$^{\$}$ experiment also performed with optimized sweep width; *delay for gradient recovery (500μs)

The HMBC experiment was performed with reduced spectral range only in dimension F1 to improve the resolution for an unambiguous assignment of the olefinic and carbonyl carbons, C-20 and C-23, respectively (Figure 2b).

The HSQC-EDIT experiment was also run with optimized sweep width in the two dimensions, F1 and F2. A forward linear prediction was applied, and this allowed an unequivocal assignment of H-7α and H-11β and H-6α and H-16β (Figure 2a).

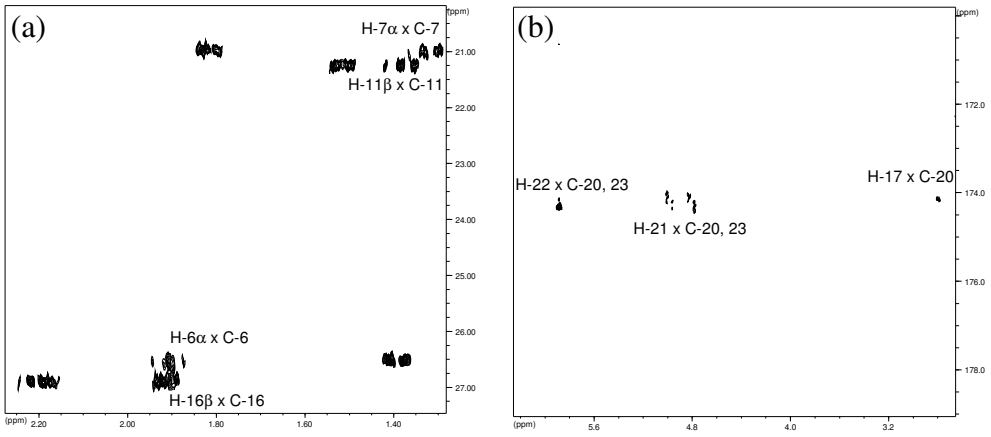

Figure 2. Section of HSQC-EDIT (a) and HMBC (b) contour plots.

Important assignments were possible also by NOE´s exhibited by H-22 x [H-19, H-16β]; H-19 x [H-8, H-11β]; H-18 x [H-6β, H-8] and H-4α x [H-7α, H-9] spin systems and by other unambiguous heteronuclear correlation.

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References

1. R. M. Pádua, *MSc. Thesis*, Universidade Federal de Minas Gerais. *Brazil,* 2003.
2. S. Braun, H. O. Kalinowsky, S. Berger, *150 and More Basic NMR Experiments of NMR,* vs. 2.0, VCH: Weinheim, 2000.
3. *Avance 1D and 2D Course*, vs. 010220, Bruker AG: Fällanden, 2001.
4. W. Robien, B. Kopp, D. Schabl, H. Schwarz, *Progress in NMR Spectroscopy* **19** (1987) 131.