The 14β-Hydroxylation in the Biosynthesis of Cardenolides in *Digitalis purpurea*. The Role of 3β-Hydroxy-5β-pregn-8(14)-en-20-one

Mónica E. Deluca, Alicia M. Seldes, and Eduardo G. Gros

Departamento de Química Orgánica y UMYMFOR, Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

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Digitalis purpurea, Cardenolide, Digitoxin, Biosynthesis, 3β-Hydroxy-5β-pregn-8(14)-en-20-one

Labelled 3β-hydroxy-5β-pregnan-20-one was incorporated by *Digitalis purpurea* plants into digitoxin while 3β-hydroxy-5β-pregn-8(14)-en-20-one was not. This result excluded the intermediary role of the latter compound as precursor of cardenolides in the mentioned plant.

It has been known for several years that the biosynthesis of cardenolides in plants of the genus *Digitalis* proceedes through the pathway cholesterol-pregnenolone-progesterone-cardenolide (*e.g.*, digitoxigenin, **1b**). An unsolved problem of cardenolide biosynthesis is the mechanism of the introduction of the 14β-hydroxy group which despite several studies [1–8] has not been clarified.

Although all the results agree with the fact that the hydroxylation at C-14 is produced before the closing of the butenolide ring, there is no coincidence about the role of an 8(14)-unsaturated steroid intermediate in this hydroxylation process. While Tschesche *et al.* [7] claimed that 5β-pregn-8(14)-ene-3,20-dione is incorporated into digitoxigenin by *Digitalis lanata* plants, in the same year Caspi *et al.* [6] demonstrated that in the same plant [8-^{3}H]cholesterol was a precursor of the same cardenolide with retention of the tritium; considering that no migration of the tritium has occurred this result discarded any intermediate with a Δ^{7}, Δ^{8} or $\Delta^{8(14)}$ double bond.

In continuing with our studies on cardenolide biosynthesis [9] and in order to test the possible role of a C_{21} steroid having a $\Delta^{8(14)}$ unsaturation, we prepared [21-^{14}C]3β-hydroxy-5β-pregn-8(14)-en-20-one (**2**) [10] and fed it to *Digitalis purpurea* plants. In a parallel experiment [21-^{14}C]3β-hydroxy-5β-pregnan-20-one (**3**) [11, 12] was also administered in similar conditions to intact specimens of the same plant.

Reprint requests to Dr. E. G. Gros.

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Results and Discussion

[21-^{14}C]3β-Hydroxy-5β-pregn-8(14)-en-20-one (**2**) and [21-^{14}C]3β-hydroxy-5β-pregnan-20-one (**3**) were administered to *D. purpurea* plants as previously described [9]. After different times digitoxin (**1a**) was isolated as already reported, hydrolyzed to digitoxigenin (**1b**) and assayed for radioactivity [9]. The results are summarized in Table I. The tabulated values clearly indicate that compound **2** was not incorporated into the cardenolide whilst compound **3** produced labelled digitoxin in an extent similar to that previously reported [5]. Hence, it may be postulated that the introduction of the 14β-hydroxy group did not involve a 20-keto-pregnane intermediate bearing a 8(14) double bond. In this respect, our results are in agreement with the finding reported by Caspi *et al.* [6] using cholesterol as a precursor.

Experimental

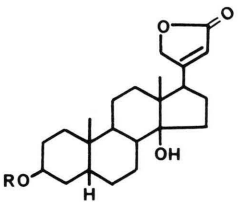
Analytical TLC was performed on silica gel G, prep. TLC on silica gel F_{254}. Labelled compounds 2 and 3 were obtained as described elsewhere [10–12]. Radioactivity was measured by liquid scintillation counting.

Feeding of tracers and isolation of digitoxin. The experiments were conducted on 3-month-old *Digitalis purpurea* plants growing in soil. The leaf wax was removed from the upper surface of the leaves by wiping with cotton wool moistened with acetone. Solns of tracers (EtOH) were applied with a glass capillary. After different times (see Table I) the plants were harvested, the leaves were washed with EtOH, and the washings were concd, analysed by TLC and measured for radioactivity. In all cases the

Table I. Data of administration of tracers to *Digitalis purpurea* plants.

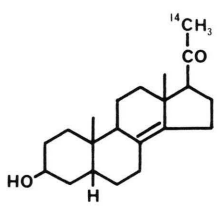
Compound administered	Amount [μg]	Total activity [dpm × 10^5]	Number of plants	Time of the experiment [days]	Activity recovered from leaves [dpm × 10^5]	Activity of diluted digitoxin [dpm × 10^3]	Activity of digitoxin after three recrystalliz. [dpm × 10^3]	Activity of digitoxigenin [dpm × 10^3]	Incorporation* [%]
[21-^{14}C]3β-Hydroxy-5β-pregnan-20-one	27	2.73	1	4	2.03	1.25	1.12	1.10	1.60
	27	2.73	1	9	1.89	0.88	0.81	0.80	0.95
	27	2.73	1	14	2.25	0.70	0.62	0.63	1.31
[21-^{14}C]3β-Hydroxy-5β-pregn-8(14)-en-20-one	34	8.66	1	4	7.76	0.68	0	–	–
	34	8.66	1	9	7.40	0.60	0	–	–
	34	8.66	1	14	6.79	0.41	0	–	–

* Incorporation is defined as the total radioactivity present in the isolated glycoside divided by the total radioactivity absorbed by the plant.

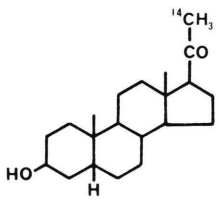


1a, R = sugar

1b, R = H



2



3

recovered radioactivity corresponded to the administered tracer.

The leaves were ground with sand and extracted with boiling EtOH (6 × 15 ml). The solvent was evaporated, the residue was redissolved in EtOH (1 ml) containing pure digitoxin (4 mg) and evaporated again. Isolation of the glycoside was performed by prep. TLC ($CHCl_3$–EtOH 93:7). The isolated digitoxin was diluted with authentic material (50 mg) and recrystallized from EtOH-water to constant specific activity.

Hydrolysis of digitoxin to digitoxigenin. In a typical experiment labelled digitoxin (25 mg) was dissolved in MeOH (10 ml), conc. H_2SO_4 (0.03 ml) was added and the soln was refluxed under N_2 for 20 min. The mixture was worked-up as described [13] and the digitoxigenin was recrystallized from EtOH-water to constant specific activity.

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