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MECHANISM OF 14β HYDROXYLATION IN THE BIOSYNTHESIS OF CARDENOLIDES: THE ROLE OF 14β-CHOLEST-5-EN-3β-OL

MARIO ANASTASIA* and FIAMMA RONCHETTI†

*Istituto di Chimica della Facoltà di Medicina e Chirurgia, Via Saldini 50, 20133 Milan, Italy; † Istituto di Chimica Organica della Facoltà di Scienze, Centro Nazionale per lo Studio della Chimica, delle Sostanze Organiche Naturali del C.N.R., Via Saldini 50, 20133 Milan, Italy

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Key Word Index—*Digitalis lanata*; biosynthesis; cardenolides; digitoxigenin; 14β-cholest-5-en-3β-ol.

Abstract—The intermediary role of 14β-cholest-5-en-3β-ol in the biosynthesis of cardenolides in *Digitalis lanata* was excluded. This rules out one plausible biogenetic mechanism for the 14β-hydroxylation, according to which the epimerization at C-14 of cholesterol preceeds the introduction of the OH-group at the same centre.

In the biosynthesis of cardenolides, e.g. digitoxigenin (1), which is known [1] to proceed through the pathway: cholesterol → pregnenolone → progesterone → digitoxigenin, a 14β–OH compound originates from a 14α–H precursor.

Despite several studies [2–6] the mechanism of this unusual hydroxylation still waits clarification.

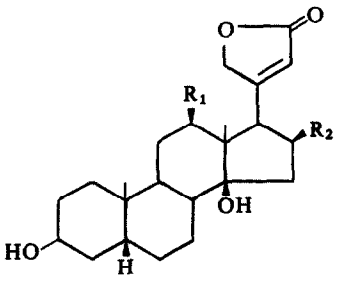
The involvement of the adjacent positions has been excluded [2, 3], but the possibility remained to be tested that this hydroxylation with inversion of configuration derives from an inversion at C–14 followed by 'normal' hydroxylation with retention of configuration*.

In fact an enzymic or photochemical epimerization at C–14 during the biosynthesis of cardenolides is not an unreasonable process, as it has been observed to occur with steroids irradiated in the presence of bromine [7]. Moreover, 5β,14β–pregnane–3β–ol–20–one (4) is incorporated into cardenolides by *Digitalis lanata* [6], although only to a very small extent.

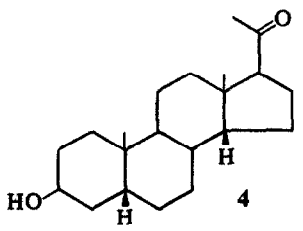
We have checked the possibility that the inversion at C–14 can occur at an early stage of cardenolides biosynthesis by administration of 14β-cholest-5-en-3β-ol – [15β-^{3}H-] (5) [8].

A mixture of 14β-cholest-5-en-3β-ol-[15β-^{3}H] (5) and cholesterol-[4-^{14}C] (1.16 × 10^8 dpm of ^{14}C, ^{3}H:^{14}C ratio = 1.36) was administered to three *Digitalis lanata* plants, six months old, dissolved in acetone, once a week for four weeks. After five weeks the plants were harvested, dried, pulverized and processed as previously described [3]. The labelled cardenolides were isolated,

* Owing to the change in the priority order the designation of configuration at C-14 remains *S* when the 14α-H of a 14*S*-pregnane is replaced with a 14β-OH, whereas in the substitution of the 14β-H of a 14*R*-pregnane with a 14β-OH it changes from *R* to *S*.



1 R_1=R_2=H
2 R_1=H; R_2=OH
3 R_1=OH; R_2=H



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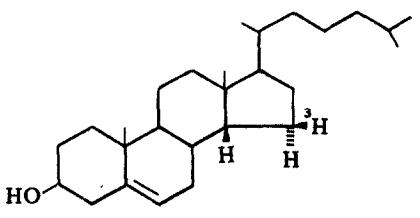

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Table 1. Incorporation of 14β-cholest-5-en-3β-ol-[15β-^{3}H] and cholesterol-[4-^{14}C] (1.16×10^8 dpm of ^{14}C, ^{3}H:^{14}C ratio = 1.36) into cardenolides in *Digitalis lanata*

Products	Specific activity (dpm of ^{14}C/mM)	^{3}H:^{14}C ratio
Digitoxigenin (1)	1.086×10^6	0.055
Gitoxigenin (2)	6.404×10^5	0.099
Digoxigenin (3)	1.026×10^5	0.050

diluted with cold material, purified and crystallized to constant specific activity. The results are summarized in Table 1.

The ^{3}H:^{14}C ratio of the labelled cardenolides undoubtedly shows that 14β-cholest-5-en-3β-ol-[15β-^{3}H] is not incorporated by *Digitalis lanata*, whereas in the same experiment cholesterol-[4-^{14}C] is transformed in the usual yields.

This strongly suggests that the epimerization at C-14, provided that this process is on the main biosynthetic pathway, does not occur with cholesterol itself, but with a more advanced precursor such as pregnenolone or progesterone.

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INHIBITION OF LIMONOID BIOSYNTHESIS IN LEAVES OF *CITRUS LIMON* BY TRIETHYLAMINE DERIVATIVES

SHIN HASEGAWA, HENRY YOKOYAMA and JOHN E. HOAGLAND
Fruit and Vegetable Chemistry Laboratory, 263 South Chester Avenue, Pasadena, California 91106, U.S.A.

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Key Word Index—*Citrus limon*; limonoids; triethylamine derivatives; inhibition; biosynthesis; metabolism.

Abstract—Both 2-(4-ethylphenoxy)triethylamine and 2-(3,4-dimethylphenoxy)triethylamine markedly inhibited the biosynthesis of limonoids in lemon leaves. However, neither significantly affected the biodegradation of limonoids.

INTRODUCTION

Limonin (1) is an intensely bitter, tetracyclic, triterpenoid dilactone present in citrus seeds [1, 2]. Fruit and leaf tissues do not contain 1 but contain a precursor, limonoate A-ring lactone (2), which is gradually converted to 1 after juice extraction [3, 4]. The limonin bitterness in certain citrus juices and other processed products continues to be an important economic problem in citrus industry.

Limonoids have been shown to be synthesized in citrus leaves and translocated to the fruit [5]. Therefore, citrus leaves should be especially suitable for the study of limonoid biochemistry.

Derivatives of triethylamine, such as 2-(4-chlorophenylthio)triethylamine chloride and many others, have been shown to inhibit the cyclase(s) in carotenogenesis in citrus [6, 7] and microorganisms [8, 9]. Since limonoids are cyclic terpenoids, we believe that cyclase(s) must be involved in the biogenesis of limonoids. If so, triethylamine derivatives should inhibit the formation of limonoids in citrus.

We report the effects of two such derivatives,