The Gonioctenina secretion completely differs from the hydrogen cyanide and benzaldehyde secretion of its sister group Paropsina[4]; these compounds are derived from mandelonitrile and the cyanogenic glucoside prunasin[5] which is probably biosynthesized by the beetle. But the *Gonioctena* larvae on the other hand produce three defensive compounds (**2**, **3**, **5**) which show biogenetic affinities for typical allomones of Chrysomelina and Phyllodectina. Phenylethanol (**5**) is a precursor for 2-phenylethyl isobutyrate and 2-phenylethyl 2-methyl butyrate in the defensive secretion of *Chrysomela interrupta*[17]. 6-Methyl-5-hepten-2-one (**2**) and the corresponding alcohol (**3**) may be biogenetically derived from citral, a major precursor of cyclopentanoid monoterpenes[11] which are present abundantly in the defensive secretions of both Chrysomelina and Phylodectina[2]. The multifarious occurrence of *Gonioctena* compounds supports the concept[8] that a larval leaf beetle gland cell may possess a fundamental capacity for several biogenetic pathways. According to this concept one or several chemical pathways may be realized by a larva, depending on food plant chemistry and the need for certain physicochemical properties of the secretions: compounds from other pathways may then be absent or present only as trace constituents. It appears that sometimes chemical defence may be of limited advantage since larval representatives of two *Gonioctena* subgenera have obviously lost their defensive glands secondarily[18].

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* Present address: Chair of Animal Ecology II, University of Bayreuth, D-8580 Bayreuth (Federal Republic of Germany)
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Cardenolide biosynthesis in chrysomelid beetles

S. Van Oycke, J.C. Braekman, D. Daloze and J.M. Pasteels

Collectif de Bio-écologie, Faculté des Sciences, Université Libre de Bruxelles, Av. F. Roosevelt 50, B-1050 Bruxelles (Belgium),
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Summary. Labeling experiments have shown that the chrysomelid beetle *Chrysolina coerulans* is able to biosynthesize its own defensive cardenolides from cholesterol, via a pathway involving a C_{21} intermediate, as in plants.
Key words. Biosynthesis; cardenolides; Coleoptera; chrysomelid beetles; [23-^{14}C]-cholesterol.

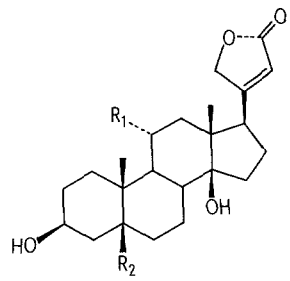
The presence of cardenolides has been reported in various insects of different orders including the aphid *Aphis nerii*[1], the bug *Oncopeltus fasciatus*[2], the grasshopper *Poekilocerus bufonius*[3] and the migratory butterfly *Danaus plexippus*[4]. All these insects feed on plants which have been shown to contain cardenolides and it is generally considered that the cardiac glycosides present in these insects are sequestered from their food plant[5].

Several insects of the family Chrysomelidae (Coleoptera) produce defensive secretions containing cardiac glycosides, thereby deriving their protection against predation[6-8]. In contrast to the aforementioned insects, all the cardenolide-containing chrysomelids studied until now feed on plants that are known to be devoid of cardiac glycosides (e.g., mint or rosemary). This suggests that these beetles are able to carry out the de novo biosynthesis of cardenolides. This hypothesis is supported by the observation[7] that adults of *Chrysolina polita* bred in the laboratory for four generations on *Mentha* × *villosa* still produce cardenolides. We wish to report here on experiments designed to supply a chemical basis for this hypothesis. Specimens of the species *Chrysolina coerulans* were fed with labeled cholesterol, a probable precursor of the cardenolides in chrysomelids. Indeed, it is well established that 1) in plants, the cardenolides arise from a C_{21} precursor resulting from the degradation of the phytosterol side chain[9,10]; and 2) phytophagous insects readily transform phytosterols into cholesterol in order to fulfill their metabolic requirements[11].

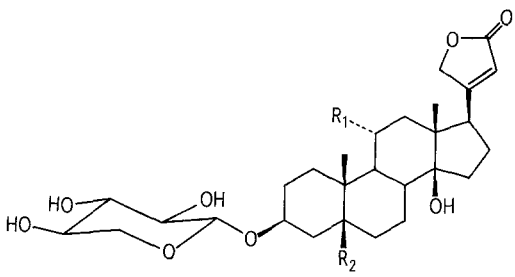
Leaves of *Mentha aquatica* were coated with 10 µl of a solution of [4-^{14}C]-cholesterol (total activity 0.1 mCi) in acetone and given as food to 45 adults of *C. coerulans*. The leaves were replaced either when decaying or when completely consumed, until administration of the precursor had been completed (approximately 2 leaves/insect/week). The secretions were collected by 'milking' on bits of filter paper. The filter papers were extracted three times with methanol and the extract evaporated in vacuo to yield 1.4 mg of dry material. It has been established in a previous work[7] that this secretion contains mainly six cardenolides, namely sarmentogenin **1**, periplogenin **2**, bipindogenin **3** and their corresponding xylosides **1a**, **2a** and **3a**.

The four major compounds present in the secretion, **1**, **1a**, **2a** and **3a** were purified by HPLC (C-18 reverse phase column, acetonitrile/water 1/3). Their specific activity was measured using a liquid scintillation counter (table 1).

The values reported in table 1 clearly show that [4-^{14}C]-cholesterol is incorporated into cardenolides, thus confirming our previous hypothesis of a de novo biosynthesis starting from cholesterol. These results prompted us to devise further incorporation



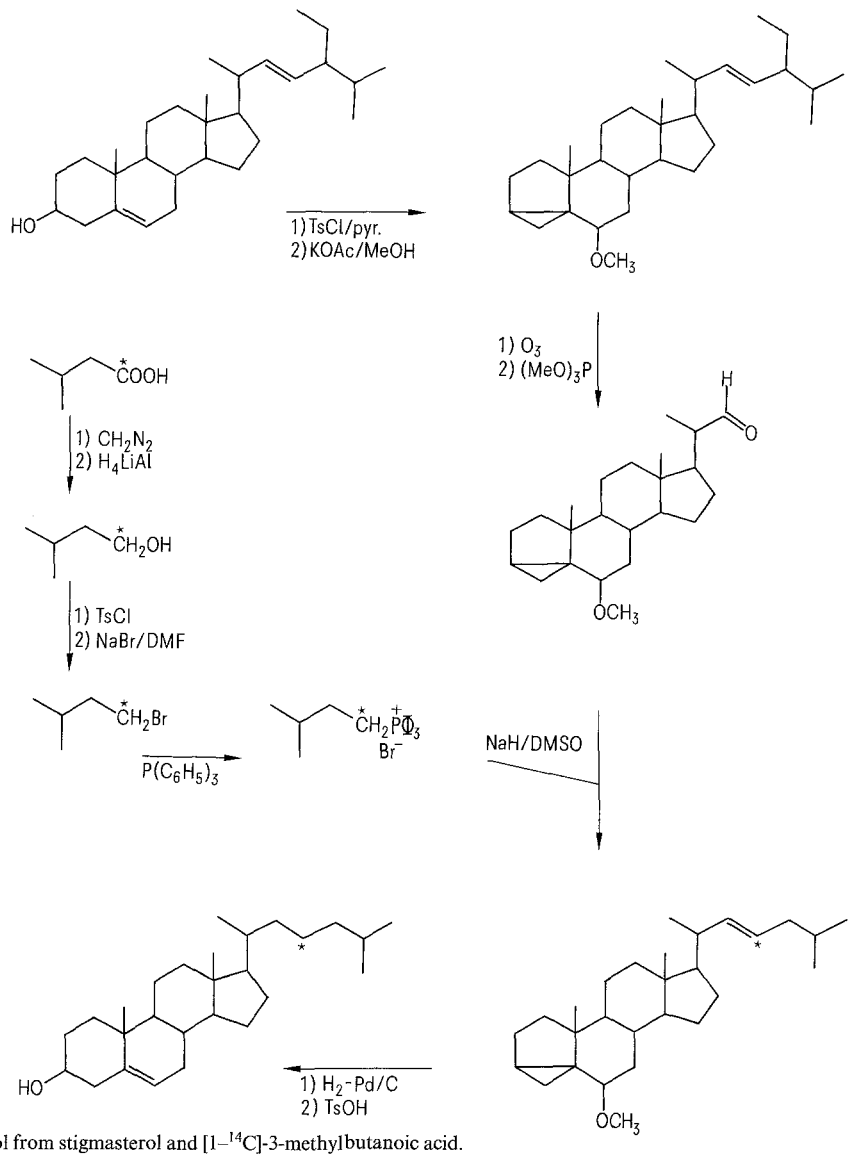
1: R1 = OH R2 = H
2: R1 = H R2 = OH
3: R1 = OH R2 = OH



1a: R1 = OH R2 = H
2a: R1 = H R2 = OH
3a: R1 = OH R2 = OH

experiments in order to define more accurately the biotransformation of cholesterol into cardiac glycosides by the chrysomelids. The biosynthesis of cardenolides in plants has been widely investigated[12]. It has been shown that the side chain of the phytosterols is cleaved between C-20 and C-22, leading to an intermediate whose precise nature still remains unclear[8]. It appears that several pathways leading to cardenolides coexist and that different C_{21} intermediates could be involved[8]. The two missing carbon atoms which are required for building the characteristic lactone ring of the cardenolides originate from acetate[14].

In order to determine whether a C_{21} pathway takes place in chrysomelid beetles, [1,2-^{3}H, 23-^{14}C]-cholesterol was incorporated. The [23-^{14}C]-cholesterol was synthesized through a Wittig reaction between the aldehyde obtained by ozonolysis of stigmasterol-i-methylether with the phosphonium salt of [1-^{14}C]-3-methyl-1-bromobutane obtained in five steps from [1-^{14}C]-3-methylbutanoic acid (see scheme). After catalytic reduction of the double bond and deprotection, the [23-^{14}C]-cholesterol obtained was mixed with a commercial sample of [1,2-^{3}H]-cholesterol to obtain a doubly labeled precursor having a ^{3}H/^{14}C isotopic ratio of 7.94 and a ^{14}C specific activity of 24 nCi/mmol. This precursor was presented to a group of beetles and the secretion collected and worked up as described above.



Synthesis of [23-^{14}C]-cholesterol from stigmasterol and [1-^{14}C]-3-methylbutanoic acid.

The specific activities of the purified cardenolides are reported in table 2. No significant variation in the isotope ratio was observed after a second HPLC purification of the compounds.
These data clearly show that the radioactivity associated with the ^{14}C located at C-23 of the starting cholesterol has been lost, thus suggesting the passage through an intermediate containing 21 carbon atoms, as in plants.
A sample of ^{3}H labeled sarmentogenin xyloside **1a** coming from the incorporation experiment was subjected to a mild acid hydrolysis (H_2SO_4 0.5N). No significant radioactivity could be detected in the carbohydrate fraction. This confirmed our results and showed that no radioactivity was associated with the glycosidic part of the compounds.
We may conclude from our results that *Chrysolina coerulans* is able to synthesize cardenolides from cholesterol most probably via a C_{21} precursor. Such a pathway is similar to that occurring in plants. It is reasonable to suppose that this scheme may be extended to all the other cardenolide-producing chrysomelids. Chemical similarity between insect and plant defensive compounds has often been stressed[16]. The production of cardenolides by leaf beetles offers another striking example of such convergence, not only in the class of compounds synthesized, but also in the biosynthetic pathway followed.

Table 1. Specific activities of the major cardiac glycosides of *C. coerulans* after incorporation of [4-^{14}C]-cholesterol

Compound	Amount collected (mg)	Specific activity (dpm/mmol) × 10^{-6}	Incorporation (%) (%) × 10^2
1	0.15	0.7	1
1a	0.21	7.4	19
2a	0.36	3.2	20
3a	0.34	7.3	9

Table 2. Specific activities and $^{3}H/^{14}C$ isotopic ratio of *C. coerulans* major cardiac glycosides after incorporation of [1,2–^{3}H, 23–^{14}C]-cholesterol

Compound	Amount collected (mg)	Isotope	Specific activity (dpm/mmol) × 10^{-6}	Isotope ratio $^{3}H/^{14}C$	Inc. % ×10^3
1	0.1	^{14}C	0.1	189	1
		^{3}H	26.5		30
1a	0.8	^{14}C	0.1	159	8
		^{3}H	17.5		160
2a	0.3	^{14}C	0.4	323	10
		^{3}H	135.8		410
3a	0.5	^{14}C	0.04	182	2
		^{3}H	7.03		40
Precursor		^{14}C	53	7.94	
		^{3}H	420		

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Total synthesis of orellinine, a minor toxic component of the fungus *Cortinarius orellanus* Fries[1]

M. Tiecco, M. Tingoli, L. Testaferri, D. Chianelli and E. Wenkert*

*Istituto di Chimica Organica, Facoltà di Farmacia, Università di Perugia, I-06100 Perugia (Italy), and *Department of Chemistry, University of California – San Diego, La Jolla (California 92093, USA), 18 December 1985*

Summary. The 3,3′,4,4′-tetrahydroxy-2,2′-bipyridyl-N-oxide has been synthesized by dealkylation of the corresponding tetramethyl derivative. The chemical properties of this compound are identical to those reported for the minor fungal toxin of *Cortinarius orellanus*, orellinine.
Key words. Orellinine; orelline; orellanine; *Cortinarius orellanus; Cortinarius speciossimus.*

The toxic properties of *Cortinarius orellanus* Fries were attributed by Grzymala[2] to a crystalline, colorless substance which could be isolated from the fungus and which was called orellanine. This compound, when heated above 270 °C, undergoes a vigorous decomposition to afford a yellow, non toxic, sublimable compound. More recently, Antkowiak and Gessner[3] isolated the toxic substance in pure form and showed that it exhibited physicochemical properties and biological activity identical to those of the orellanine isolated by Grzymala. On the basis of chemical and spectral data these authors proposed the structure of 3,3′,4,4′-tetrahydroxy-2,2′-bipyridyl-N,N′-dioxide (**6**) for orellanine and that of 3,3′,4,4′-tetrahydroxy-2,2′-bipyridyl (**4**)