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]

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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**)

for its yellow decomposition product, orelline[4]. We have confirmed the correctness of these structural attributions by effecting two unambiguous syntheses of these two compounds[5] (scheme). Similar conclusions were also reached by Dehmlow and Schulz[6] who have independently carried out the synthesis of orellanine and orelline using a procedure substantially identical to one of the two methods employed by us.

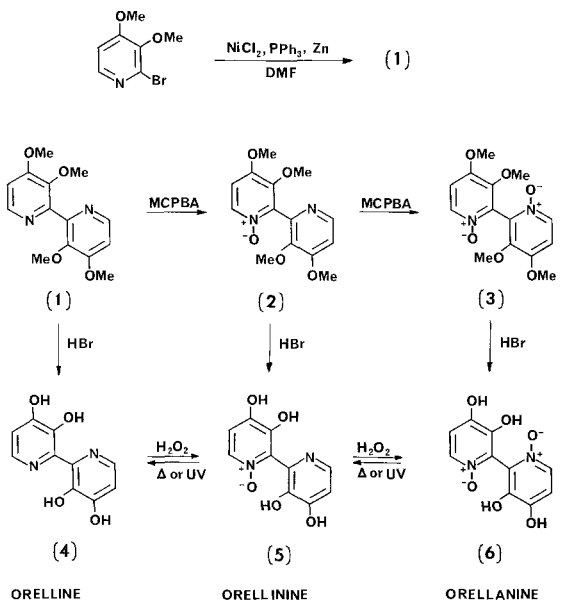
Chromatographic analysis of cold methanolic extracts of *Cortinarius orellanus* showed that, together with orellanine, small amounts of orelline and of a third component were also present[3]. These three compounds were also found in the methanolic extracts of *Cortinarius speciossimus*[7]. This third compound was found to have a toxicity similar to that of orellanine. By thin layer chromatography it was also observed that the same compound is formed, together with orelline, from the thermal or the UV-induced decomposition of orellanine and that it also decomposes to orelline. On the basis of these observations Antkowiak and Gessner proposed the structure of 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl-N-oxide (**5**) for this compound, and called it orellinine. These authors suggest that, on heating or by UV irradiation, orellanine (**6**) suffers a chemical transformation consisting in a two-stage successive loss of the two oxygen atoms in the N-oxide functions to give orellinine (**5**) and orelline (**4**).

We now report the synthesis of orellinine (**5**) and the study of its chemical properties.

The key step of the synthesis of orellinine is represented by the preparation of 3,3',4,4'-tetramethoxy-2,2'-bipyridyl (**1**) which we have obtained in 87% yield from the nickel-promoted homo coupling of 2-bromo-3,4-dimethoxy pyridine[8]. Oxidation of **1** with one equivalent of *m*-chloroperbenzoic acid in chloroform at room temperature yielded, after column chromatography on deactivated alumina (eluant: chloroform and methanol, 97:3), pure 3,3',4,4'-tetramethoxy-2,2'-bipyridyl-N-oxide (**2**) (85% yield), m.p. 171–173 °C (found: C, 57.95; H, 5.45; N, 10.03. $C_{14}H_{16}N_2O_5$ requires C, 57.52; H, 5.53; N, 9.58%); ^{1}H-NMR δ ($CDCl_3$) 8.3 (d, $H_{6'}$, J = 5.5 Hz), 8.05 (d, H_6, J = 7.0 Hz), 6.9 (d, $H_{5'}$, J = 5.5 Hz) 6.85 (d, H_5, J = 7.0 Hz), 3.95 (s, 3H), 3.94 (s, 3H), 3.90 (s, 3H), 3.70 (s, 3H); ^{13}C-NMR δ ($CDCl_3$) 158.8, 151.8, 146.2, 145.7, 144.8, 143.7, 135,4, 108.5, 61.2, 60.9, 56.4, 55.4. Mass m/e 294 (32), 278 (45), 264 (50), 263 (100), 262 (44) 261 (45), 247 (52), 218 (47), 202 (43), 190 (35), 177 (38), 166 (33), 151 (38), 150 (45), 145 (72), 135 (21), 114 (45).

The dealkylation of **2** was effected with 48% hydrobromic acid at 120 °C for 5 h. The excess acid was removed by distillation in vacuum, the solid residue was dissolved in diluted ammonia and the pH was adjusted to 4 by addition of acetic acid. The almost colorless solid so obtained was filtered off and dried under vacuum. This product was identified as 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl-N-oxide (orellinine) (**5**) (70% yield) (found: C, 50.15; H, 3.44; N, 11.55. $C_{10}H_8N_2O_5$ requires C, 50.85; H, 3.42; N, 11.86%); ^{1}H-NMR δ (DMSO-d_6) 9.2–8.2 broad (s, 2H), 8.05 (d, 1H, J = 7.0 Hz), 8.0 (d, 1H, J = 5.5 Hz), 7.0 (d, 1H, J = 5.5 Hz), 6.95 (d, 1H, J = 7.0 Hz); ^{13}C-NMR δ (DMSO-d_6) 159.2, 151.8, 147.3, 147.1, 136.0, 133.4, 131.1, 130.5, 110.8, 110.4. Mass m/e 237 (50), 221 (27), 192 (46), 164 (51), 138 (27), 137 (19), 111 (13), 95 (13), 70 (11). UV (MeOH), 213, 267, 342; (0.1 N NaOH), 223, 305; (0.1 N HCl) 209, 267, 281 (sh). This product is soluble in alkaline and acid solutions and in DMSO; it is slightly soluble in methanol and insoluble in water and in all the most common organic solvents. The product is stable until 160–170 °C; at higher temperature it slowly decomposes to give the subliming yellow orelline (**4**). Sublimation of **5** under reduced pressure (0.01 mm/Hg, 220 °C) produced orelline (**4**) (60% yield), whereas oxidation with 35% hydrogen peroxide at room temperature gave orellanine (**6**) (scheme). In the previous work[5] we have shown that **6** was also obtained by oxidation of **4** with excess H_2O_2 and that **4** was produced when **6** was decomposed by sublimation; as indicated in the scheme, both these transformations very probably proceed through the intermediate formation of orellinine (**5**).

The behavior of the synthetic compounds **4**, **5**, and **6** towards UV irradiation was investigated by TLC according to the procedure reported by Antkowiak and Gessner for the three natural products **4**, **5**, and **6**[7]. The TLC experiments were carried out on cellulose plates using a mixture of isopropanol, conc. HCl and water (85:22:18) as eluant. It was observed that the TLC spot of the synthetic orelline (**4**) presented a light blue fluorescence under UV irradiation, whereas those of the synthetic orellinine (**5**) and orellanine (**6**) showed no fluorescence. Both these spots, however, showed the characteristic fluorescence of orelline after exposure to UV light for a few min. Thus, if an unexposed plate was eluted and then observed under UV light, each compound gave rise to a single spot (having similar fluorescence) with R_f 0.78 (**6**), 0.66 (**5**), and 0.48 (**4**). On the contrary, if the TLC plate was first exposed to UV light for about 10 min and then eluted, compound **4** gave rise to a single spot (R_f 0.48), compound **5** gave two spots (R_f 0.66 and 0.48) and compound **6** gave three spots (R_f 0.78, 0.66 and 0.48). These results indicate that the mono-N-oxide **5** and the di-N-oxide **6** are very sensitive to UV light, which causes their decomposition to the deoxygenated product **4** (scheme). The chromatographic properties of the synthetic compounds **4**, **5**, and **6**, as well as their behavior towards UV irradiation are identical to those reported for the naturally occurring orelline, orellinine and orellanine, respectively[9]. Thus the present results, together with those already described in our previous work[5], fully confirm the correctness of structural attributions made for the natural products[4,7].



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