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CHEMICAL ECOLOGY OF ARTHROPODS—X[a]

THE STRUCTURE OF MYRRHINE AND THE BIOSYNTHESIS OF COCCINELLINE

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Abstract—The structure elucidation of myrrhine (**6**), a novel base isolated from *Myrrha* 18-*guttata*, is reported. The biogenesis of the Coccinellidae defensive alkaloids is discussed and coccinelline (**1**) is shown to be biosynthetized through the polyacetate pathway.

When molested, Coccinellidae emit haemolymph droplets at their joints and this well described mechanism, known as reflex bleeding[1-3] has been shown to constitute an efficient protection against predators.[4] The presence of a chemical deterrent in these droplets has long been suspected.[1,2,5] Since 1972, several papers[6-14] from this laboratory have dealt with Coccinellidae defensive substances, which have been shown to constitute a novel structural group of alkaloids.

The structure of coccinelline (**1**),[8] convergine (**4**)[14] and adaline (**7**)[11] was established by single crystal X-ray diffraction analysis on their respective hydrochloride. Precoccinelline (**2**)[7] and hippodamine (**5**)[14] have been shown by chemical correlation to be the free bases corresponding respectively to **1** and **4**. Finally, propyleine was found to be the dehydroprecoccinelline (**3**).[9]

The object of the present publication is to report the discovery of the third ring stereoisomer of the perhydro-9b-azaphenalene system, myrrhine (**6**) as well as the demonstration that coccinelline is biosynthetically derived from a polyacetate chain.

Myrrhine. In addition to precoccinelline (*cis, trans, cis* ring fusion) and hippodamine (*cis, cis, trans*) one could predict the existence of a third (*trans, trans, trans*) ring stereoisomer of the perhydro-9b-azaphenalene system common to the two hereabove ladybug alkaloids. Extensive search for this missing isomer led to its location in *Myrrha octodecimguttata* where the presence of an unknown alkaloid of molecular weight 193 had been detected earlier.[12] The name myrrhine was coined for this new alkaloid, easily distinguishable by TLC from **2** and **4**.

Myrrhine has the empirical formula $C_{13}H_{23}N$ (by MS). Its mass spectrum is virtually identical with those of **2** and **5**. This observation, together with the presence in its IR spectrum of characteristic Bohlmann bands (2·7–2·8 cm^{-1}), led to the hypothesis that myrrhine actually represents the third stereoisomer (**6**) resulting from an all-*trans* fusion of the three rings. This was fully confirmed by chemical correlation between myrrhine and coccinelline, using the Polonovski reaction.

Treatment of coccinelline in dichloromethane solution at room temperature with acetic anhydride or ethyl chloroformate led to the obtention of an unstable enamine ($\nu_{C=C}$ 1650 cm^{-1}; 1 vinylic proton multiplet at 4·4 ppm). Catalytic hydrogenation of this enamine gave a readily separable mixture of myrrhine (90%) and precoccinelline (10%), identical in all respects with authentic samples. On the basis of the known predominance of *trans* elimination in the Polonovski reaction,[16] it could be anticipated that the enamine (**8**) would preferentially be obtained from coccinelline. Had the enamine been the isomeric compound **3** or **9** resulting from cis elimination, its hydrogenation would have led to a mixture of precoccinelline and hippodamine instead of the observed mixture of myrrhine and precoccinelline.

Myrrhine N-oxide was found to be totally unreactive towards ethyl chloroformate under the conditions used for the transformation of coccinelline. This might be attributed either to an inversion of the N atom during N-oxidation (no more H atoms *trans* to the N-oxides oxygen) or, more probably, to steric hindrance (six 1,3-diaxial interactions between the oxygen and the β H atoms). This last hypothesis is preferred since myrrhine N-oxide could not be reduced catalytically under the conditions where coccinelline is quantitatively transformed into precoccinelline.

Distribution. Since their first isolation,[6,9,11,14] we have located these alkaloids in other species of Coccinellidae. Table 1 lists the species in which these compounds have been evidenced.

Biosynthesis. Coccinelline and precoccinelline have been detected in the eggs, the larvae and the adults of *Coccinella septempunctata.* Neither of these compounds

Table 1.

	1	2	3	4	5	6	7
Adalia bipunctata and var.	–	–	–	–	–	–	+
Adalia 10-*punctata*	–	–	–	–	–	–	+
Anisosticta 19-*punctata*	–	–	–	–	+	–	–
Cheilomenes propinqua (var 4-*lineata*)	+	+	–	–	–	–	–
Coccinella californica	+	–	–	–	–	–	–
Coccinella 7-*punctata*	+	+	–	–	–	–	–
Coccinella 5-*punctata*	+	+	–	–	–	–	–
Coccinella 11-*punctata*	+	–	–	–	–	–	–
Coccinula 14-*punctata*	+	+	–	–	–	–	–
Hippodamia convergens	–	–	–	+	+	–	–
Micraspis 16-*punctata*	–	+	–	–	–	–	–
Myrrha 18-*guttata*	–	–	–	–	–	+	–
Propylaea 14-*punctata*	–	–	+	–	–	–	–

[a]Part IX, see Ref. 14.

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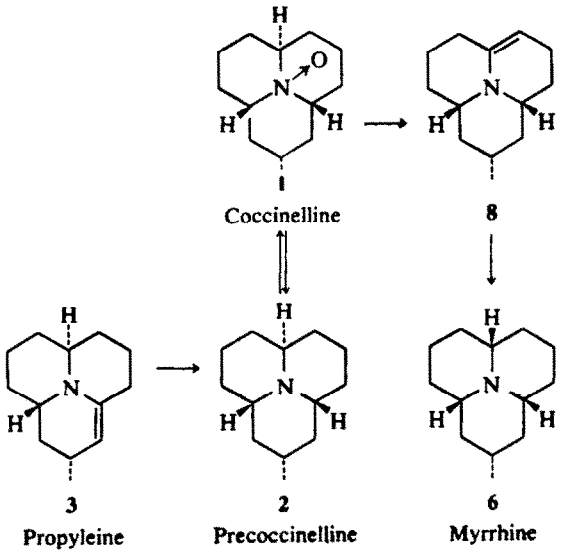

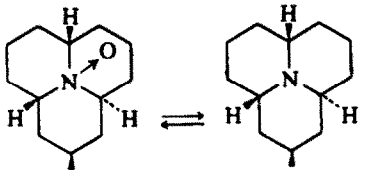

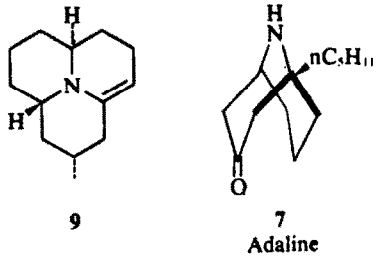

could be detected in the aphids that constitute the prey of the beetle, thus indicating that these alkaloids are most probably synthetized by the insect itself.

A plausible hypothesis to explain the origin of Coccinellidae alkaloids implies their formation from the β-polyketoacid (13) produced by linear combination of seven acetate units as illustrated in Chart 1. An intermediate such as (14) would rationalize the existence of the different structures known so far.

To test the validity of this hypothesis, specimens of *Coccinella septempunctata* were fed with $CH_3{}^{14}COONa$ and $^{14}CH_3COONa$ (Experimental). These experiments are summarized in Table 2.

The labelled samples of coccinelline obtained from each feeding experiment were transformed into their hydrochlorides for purification and then submitted to a Kuhn-Roth oxidation (Chart 2).

This degradation yielded acetic acid (corresponding to carbon atoms C-10 and C-2), which was isolated and purified as its 2-aminoaphthalene derivative **16**.[17] In the two experiments **16** was found to contain about 16% of the activity of the original intact coccinelline (Table 3).

Table 3.

Experiment No.	Specific activity of 15	Specific activity of 16	Observed relative specific activity	Expected relative specific activity
1	$53{\cdot}3\ 10^3$	$9{\cdot}0\ 10^3$	16%	16·7%
2	$104{\cdot}2\ 10^3$	$17{\cdot}6\ 10^3$	16·8%	14·3%

It follows that the observed mode of incorporation of acetate in coccinelline is fully consistent, within experimental errors, with the pathway proposed in Chart 1. Consequently one can exclude, amongst others the biogenetic scheme involving the condensation of six acetate units and subsequent Me addition, since the expected relative specific activities would then be 16·7% and 0% in experiments 1 and 2 respectively. A similar polyketide origin is well established for coniine and the related *Conium* alkaloids.[18]

Notes. Small amounts of alkaloids of much higher molecular weight have been detected in several other species of Coccinellidae. Isolation and chemical study of these compounds in view of their biological evaluation are under way in our laboratory.

dl-hippodamine (and hence *dl*-convergine) and myrrhine, identical with our natural compounds, have recently been obtained by total synthesis by Ayer and Dawe.[19]

EXPERIMENTAL

The following instruments were used for measuring the physical data: IR: Pye Unicam SP 1000; NMR: Varian T 60; MS: Hitachi

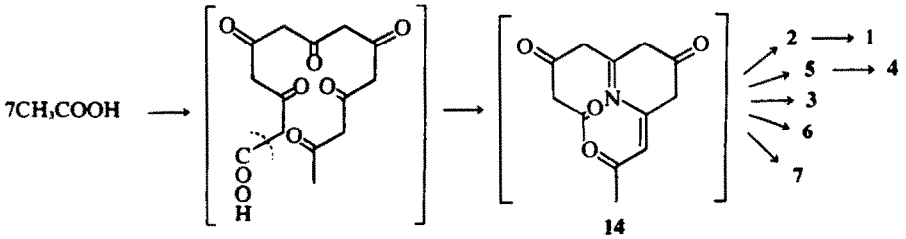

Chart 1

Table 2.

Experiment No.	Precursor	Nominal total activity (mCi)	No. of specimens fed	Feeding period (h)	mg of (1)	% of incorporation
1	$CH_3{}^{14}COONa$	0·3	46	48	15	$2\ 10^{-4}$
2	$^{14}CH_3COONa$	0·1	50	48	14	$3\ 10^{-4}$

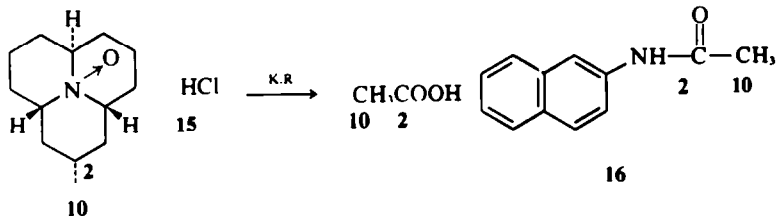

Chart 2

Perkin Elmer RMU 60 or Finnigan 3000 D; m.p.: Arthur Thomas heating stage.

The ^{1}H NMR spectra were recorded in $CDCl_3$ soln. Chemical shifts are quoted in δ-values (ppm) downfield from TMS as internal standard.

Radioactivity was measured by liquid scintillation counting (Packard tri Carb instrument). Samples were dissolved in methanol (1 ml) and the solution was dispersed in 15 ml of insta-gel emulsifier (Packard).

The TLC were performed on 0·2 mm aluminium oxid N plates (Alox N Macherey-Nagel).

Isolation of myrrhine. Myrrhine (2 mg) was obtained from *Myrrha octodecimguttata* (50 specimens) using the standard procedure.[12] Myrrhine: IR 2800–2700 cm^{-1}; MS: 193 (46, M^+), 192 (base peak), 178 (27), 164 (33), 151 (54), 150 (52), 137 (41), 136 (22), 123 (33).

Myrrhine from coccinelline

(a) *Polonovski reaction.* A soln of 40 mg of coccinelline in 2 ml dry methylene chloride was cooled to 0° and treated with 100 mg ethyl chloroformate in 2 ml methylene chloride. The mixture was left at room temp. for 2 hr and then evaporated to dryness.

In one experiment, the residue was submitted to catalytic hydrogenation (*vide infra*). In an otherwise similar experiment, the residue was dissolved in chloroform and the soln filtered through alumina. Evaporation of the solvent led to the unstable enamine (**8**): IR ($CHCl_3$): 1650 cm^{-1} (ν C=C); ^{1}H NMR: 0·9 ppm (3H, d, J = 5 Hz, secondary Me), 4·4 ppm (1H, m, vinylic proton).

(b) *Catalytic hydrogenation.* The residue from the Polonovski reaction was dissolved in MeOH (5 ml) and immediately submitted to hydrogenation (3 atm) during 2 hr in the presence of Pt. After filtration and evaporation of the solvent, the residue was fractionated by preparative TLC on alumina (Aluminium oxid Merck F 254; eluent: chloroform/ethanol 98/2). 24 mg of myrrhine and 2 mg of precoccinelline were obtained and identified by TLC, IR and MS.

Oxidation of myrrhine

Myrrhine (20 mg) in 2 ml dichloromethane and 0·5 ml *m*-chloroperbenzoic acid in ether were stirred at 0° for 30 min. Diluted NH_4OH (10 ml) and chloroform (10 ml) were added. After the separation of the organic layer, the aqueous phase was extracted with two 10 ml portions chloroform. The organic phases were combined, dried and evaporated. Myrrhine N-oxide was obtained as white needles charring above 210° without melting. MS: M^+ 209.

Administration of labelled sodium acetate to Coccinella septempunctata

The insects, collected around Brussels, were kept at 20° for 3 days and then fed (twice a day during 2 days) with small pieces of bananas impregnated with the labelled precursors dissolved in 0·5 ml of water ($c = 1 \cdot 10^{-5}$ m/ml). The third day, the ladybugs were thrown in MeOH. A summary of the feeding experiments which were carried out is presented in Table 2. The tracers were commercial products (Institut National des Radioéléments).

Isolation and purification of labelled coccinelline

Labelled coccinelline from each experiment was isolated using the standard procedure, dissolved in acetone and transformed into its hydrochloride by bubbling gaseous HCl through the soln. After addition of inactive coccinelline hydrochloride (30 mg), each sample was purified to constant activity by repeated crystallization from dichloromethane/hexane, m.p.: 230–240° (dec).

Kuhn-Roth oxidation of coccinelline hydrochloride

To 20 mg of labelled coccinelline hydrochloride were added 5 ml of a stock soln of chromic acid [10 g of CrO_3 in a soln of sulfuric acid (5 ml) and water (20 ml)]. The mixture was refluxed during 2 hr. After cooling and addition of 10 ml water, the aqueous soln was distilled until 50 ml distillate had been collected. During distillation, repeated additions of water (5 ml) were effected. The distillate was then neutralized with 0·01 N NaOH and evaporated to dryness under vacuum. To the solid residue dissolved in 3 ml water was added 15 mg β-naphtylamine (in 1 ml water) and 100 mg 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl (also in 1 ml water). After standing at room temp for 12 hr, the crystals of N-acetyl-β-naphtylamine were filtered off, dried under vacuum, sublimed and recrystallized from benzene/hexane m.p. 131°.

In Table 3 are listed the obtained specific activities.

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