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Biosynthesis of Defensive Coccinellidae Alkaloids: Incorporation of Fatty Acids in Adaline, Coccinelline, and Harmonine

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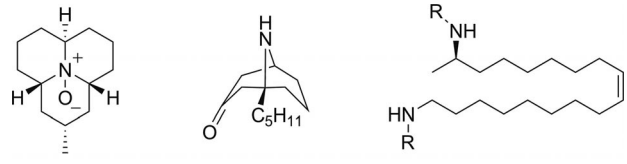
In this study, we report on in vitro incorporation experiments of several labelled fatty acids in the ladybird alkaloids coccinelline (*Coccinella 7-punctata*), adaline (*Adalia 2-punctata*), and harmonine (*Harmonia axyridis*). The obtained results clearly indicate that stearic acid is the precursor of coccinelline and harmonine, whereas myristic acid is at the origin of the carbon skeleton of adaline. Possible pathways for the biosynthesis of these alkaloids are presented.

Introduction

Much research has been carried out on the chemical defense systems of Coccinellidae, and it is now well established that many coccinellids owe their protection to the presence of alkaloids.[1–4] More than 50 ladybird alkaloids have been isolated and characterized, including perhydroazaphenalenes, homotropanes, piperidines, pyrrolidines, azamacrolides, linear amines, and so forth.[3] Despite the structural diversity of the coccinellid alkaloids, most of their skeletons are a chain of carbon atoms joined at one or more sites to a nitrogen atom, suggesting a common biogenetic origin associated with a polyacetate pathway. Until now, only a few incorporation studies supporting this hypothesis have been reported.[5] For example, incorporating labelled oleic acid and L-serine into *Epilachna varivestis* larvae and conducting subsequent GC–MS analysis of the collected pupal defensive secretion established the fatty acid origin of epilachnene.[6] Furthermore, other feeding experiments with specifically deuterated octadec-9-enoic acid indicated that only the C-15 methylene group of oleic acid was involved in the process leading to the carbon–nitrogen bond formation in epilachnene.[7]

In addition, we have shown that the adults of *Coccinella 7-punctata*[8] and *Adalia 2-punctata*[9] fed with [1-^{14}C] and [2-^{14}C]sodium acetate incorporated these precursors into coccinelline (**1**) and adaline (**2**), respectively, supporting a polyacetate origin for these alkaloids. Moreover, in vitro incubation assays using ladybird tissues demonstrated that **1** and **2** are most likely biosynthesized through a fatty acid rather than a polyketide pathway, that glutamine is the preferred source of nitrogen, and that the alkaloid biosynthesis takes place in the insect fat body.[10] In addition, the incorporation of specifically deuterated adaline into *A. 2-punctata* clearly indicates a close relationship between adaline (**2**) and adalinine,[9] the latter yielded from the former. Finally, Camarano et al.[11] reported good incorporations into the homotropane euphococcinine and two piperidine alkaloids when feeding adults of *Epilachna paenulata* on a diet enriched with [1-^{13}C] and [2-^{13}C]sodium acetate.

In our continued search for a better understanding of the biosynthetic origin of Coccinellidae alkaloids, we herein report the incorporation results into coccinelline (**1**), adaline (**2**), harmonine (**3**; see Figure 1), specifically tritiated fatty acids, namely [14,14,14-$^{3}H_3$]myristic acid (**4**), [16,16,16-$^{3}H_3$]palmitic acid (**5**), and [18,18,18-$^{3}H_3$]stearic acid (**6**).



1 **2** **3**: R = H; **10**: R = $COCH_3$

Figure 1. Structures of compounds **1**, **2**, **3**, and **10**.

Results and Discussion

The labelled fatty acids **4**–**6** were synthesized as shown in Scheme 1 by starting from diacids **7**–**9**, respectively. The starting diacids **7** and **8** are commercially available. This is not the case for heptadecanedioic acid (**9**), which had to be synthesized by a three-step sequence (Scheme 2) from tridecanedioic acid (**7**) according to the methodology described by Obaza and Smith.[12] The key step in the synthesis of the labelled fatty acids was the alkylation of mono-

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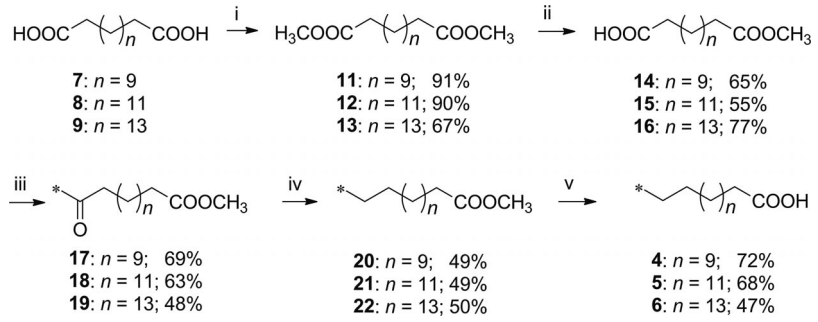
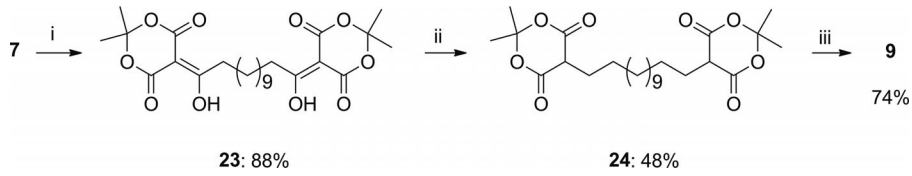


Scheme 1. Syntheses of [14,14,14-$^3H_3$]myristic acid (**4**), [16,16,16-$^3H_3$]palmitic acid (**5**), and [18,18,18-$^3H_3$]stearic acid (**6**). Reagents and conditions: (i) CH_3OH, H_2SO_4, reflux; (ii) $Ba(OH)_2$, CH_3OH for **4** and CH_3OH/THF (6:1) for **5** and **6**, then HCl; (iii) $(COCl)_2$, DMF (*N*,*N*-dimethylformamide), hexane, then [$^3H_3$]CCdCl, toluene, 0 °C; (iv) *p*-toluenesulfonylhydrazine, *p*-TsOH, DMF–sulfolane, then $NaBH_3CN$, 110 °C; (v) KOH, 18-crown-6, toluene, then HCl.



Scheme 2. Synthesis of heptadecanedioic acid (**9**). Reagents and conditions: (i) $(COCl)_2$, DMF, hexane, then Meldrum's acid, DMAP [4-(dimethylamino)pyridine], THF (tetrahydrofuran); (ii) $NaBH_3CN$, AcOH–THF, 65 °C; (iii) HCl, H_2O.

esters **14**–**16** through a coupling reaction between the corresponding acyl chlorides and [$^3H_3$]CCdCl, prepared by the action of $CdCl_2$ on [$^3H_3$]CMgI.[13–16] Ketones **17**–**19**, obtained in this way, were reduced to form methyl esters **20**–**22**, which were then hydrolyzed to yield the desired labelled fatty acids **4**–**6**.[14]

In vitro incubation experiments were carried out by using a protocol identical to the one described in our previous paper.[10] Thus, each of the three tritiated potential precursors was immersed with *C. 7-punctata, A. 2-punctata*, or *Harmonia axyridis* tissues (20 beetles each) in 1.5 mL of the saline solution for 24 h as described by Ivarsson et al.[17] This solution also contained the protease inhibitor PMSF (phenylmethylsulfonyl fluoride), NADPH (reduced nicotinamide adenine dinucleotide phosphate), and glutamine to boost the alkaloid biosynthesis. Then, the respective alkaloids, namely coccinelline (**1**), adaline (**2**), and harmonine (**3**), were isolated and repeatedly purified to a constant specific activity (SA), and the specific incorporation rates (SIR) were calculated. It should be noted that harmonine was isolated and purified as its diacetyl derivative **10**.

The results obtained are summarized in Table 1. This table shows that for *A. 2-punctata* (Entries 7–9), [14,14,14-$^3H_3$]myristic acid was about 15 times more efficiently incorporated in adaline than [16,16,16-$^3H_3$]palmitic acid and [18,18,18-$^3H_3$]stearic acid. For *C. 7-punctata*, two series of in vitro incorporation experiments were carried out during two successive springs. Whatever the incorporated precursor, the SIR measured was always higher for the first series (Entries 1, 3, and 5) than for the second one (Entries 2, 4, and 6), probably because of a better metabolic activity for the first group of beetles. In both series, [18,18,18-$^3H_3$]-stearic acid had the highest SIR in coccinelline. Finally, and not surprisingly, for *H. axyridis*, [18,18,18-$^3H_3$]stearic acid was incorporated with a much higher rate into harmonine than [14,14,14-$^3H_3$]myristic acid (Entries 10 and 11).

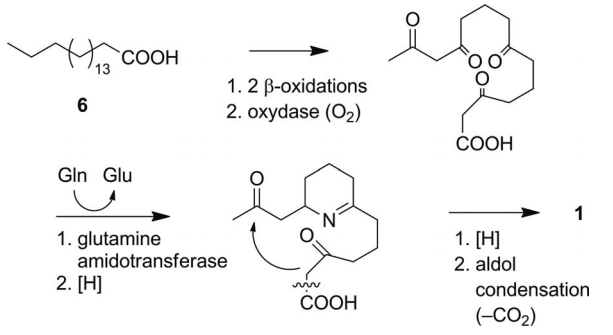
In our previous work dealing with the biosynthesis of coccinelline (**1**) and adaline (**2**), we postulated that stearic acid could be the precursor of both alkaloids.[10] However, taking into account the data presented in Table 1, we have revised our hypothesis to take into consideration the fact that although stearic acid was about 20 times more efficiently incorporated into coccinelline than myristic and palmitic acid, this was not the case for adaline. Indeed, for this alkaloid, the incorporation of myristic acid was about 15 times more efficient than the incorporation of palmitic or stearic acid. Consequently, although the pathway postulated for the biosynthesis of coccinelline remains correct (Scheme 3), the one for adaline is revised as presented in Scheme 4. The fatty acid incorporation results obtained for *A. 2-punctata* are better explained if we propose that, in this species, stearic acid does not undergo two successive β-oxidations to form myristic acid, but that instead myristic acid is detached from the fatty acid synthetase complex when the chain has grown up to 14 carbon atoms.

To refine the biosynthetic pathway of harmonine and to obtain information about the process leading to the secondary amine formed in this alkaloid, an in vitro incorporation experiment using [11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-$^2H_{17}$]stearic acid (**25**) was performed with tissues of 100 beetles and 5.1 mg of deuterated precursor. This specifically deuterated acid was synthesized by starting

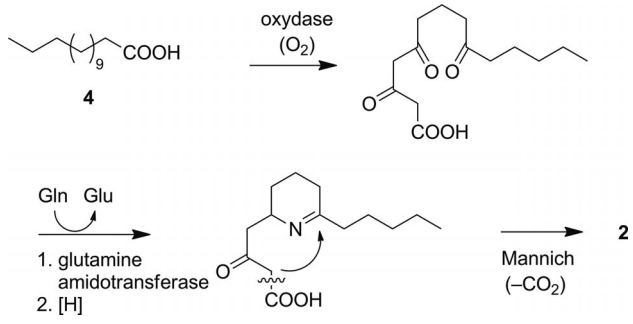
Table 1. Results of the in vitro incorporation experiments of [14,14,14-$^3H_3$]myristic acid (**4**), [16,16,16-$^3H_3$]palmitic acid (**5**), and [18,18,18-$^3H_3$]stearic acid (**6**) in coccinelline (**1**), adaline (**2**), and harmonine (**3**).[a]

Entry	Alkaloid (*Species*)	Incorporated fatty acid	RI [10^{-3} mCi]	Alkaloid amount isolated [mg]	TR [10^{-6} mCi]	SA [mCi/mmol]	SIR [%]
1	Coccinelline (**1**)	**4**	1.45	0.87	0.13	3.05 10^{-5}	0.010
2	(*C. 7-punctata*)	**4**	1.63	0.73	0.06	1.80 10^{-5}	0.005
3		**5**	1.34	1.06	0.17	3.36 10^{-5}	0.011
4		**5**	1.82	0.57	0.06	2.20 10^{-5}	0.006
5		**6**	0.81	2.07	4.63	4.68 10^{-4}	0.220
6		**6**	1.15	0.39	0.74	3.90 10^{-4}	0.136
7	Adaline (**2**)	**4**	1.55	2.96	10.74	1.59 10^{-3}	0.495
8	(*A. 2-punctata*)	**5**	1.35	2.30	0.42	1.19 10^{-4}	0.035
9		**6**	1.37	1.33	0.42	7.40 10^{-5}	0.021
10	Harmonine (**3**)[b]	**4**	1.37	2.62	1.50	2.09 10^{-4}	0.069
11	(*H. axyridis*)	**6**	0.76	2.36	28.49	4.42 10^{-3}	2.090

[a] RI = amount of radioactivity incorporated in the sample; TR = total amount of radioactivity recovered in the isolated alkaloid; SA = specific activity; SIR = specific incorporation rate. [b] Isolated as its diacetyl derivative **10**.



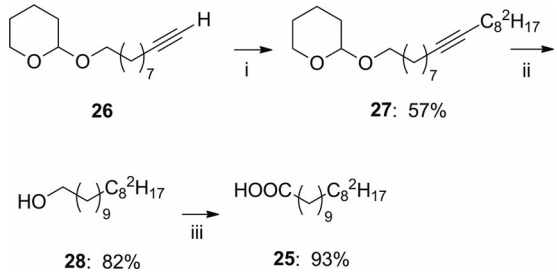
Scheme 3. Biosynthetic pathway proposed for coccinelline (**1**) in *C. 7-punctata*.



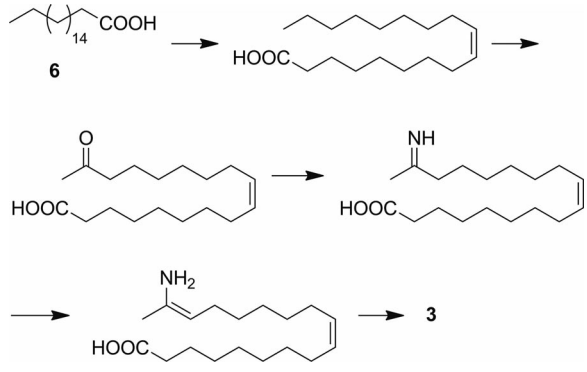
Scheme 4. Biosynthetic pathway proposed for adaline (**2**) in *A. 2-punctata*.

from protected alkynol **26**[18] and [1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-$^2H_{17}$]*n*-octyl bromide using the synthetic pathway reported by Attygalle et al.[7] and is described in Scheme 5.

The HRMS (EI) spectrum of the sample of diacetylharmonine isolated after incorporation of precursor **25** showed that besides peaks found at 367.3338 (calcd. for $C_{22}H_{43}N_2O_2$ $[M + H]^+$ 367.3325) and 323.3053 (calcd. for $C_{20}H_{39}N_2O$ $[M – CH_3CO]^{+\cdot}$ 323.3065) characteristic of unlabelled diacetylharmonine (**10**), peaks were found at 381.4182 (calcd. for $C_{22}H_{29}[^2H_{14}]N_2O_2$ 381.4203) and 337.3914 (calcd. for $C_{20}H_{25}[^2H_{14}]N_2O$ 337.3941), demonstrating the incorporation of 14 deuterium atoms. Consequently, three deuterium atoms had been lost during the incorporation experiment. A possible explanation for this observation is that two deuterium atoms were lost during the oxidation of the C-17 methylene group, and a third one was lost at the adjacent carbon atom (C-16) through an intermediary imine undergoing an imine–enamine tautomerism. Moreover, as natural harmonine occurs as a single enantiomer with the (*R*) configuration at its stereogenic center,[19] the reduction of the intermediate imine (or enamine) produced during the amination step must be stereo-



Scheme 5. Synthesis of **25**. Reagents and conditions: (i) *n*BuLi, THF, then [1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-$^2H_{17}$]*n*-octyl bromide, DMPU (*N*,*N*′-dimethyl-*N*,*N*′-propyleneurea), 0 °C; (ii) H_2, Pd/C, EtOH, then PPTS (pyridinium *p*-toluenesulfonate), EtOH, 55 °C; (iii) CrO_3, H_2SO_4, acetone.



Scheme 6. Biosynthetic pathway proposed for harmonine (**3**) in *H. axyridis*.

specific. Based on all these data, the broad outlines of the harmonine biosynthesis in *H. axyridis* can be established as depicted in Scheme 6.

Conclusions

Based on all these results, the broad outlines of the biosyntheses of coccinelline (**1**), adaline (**2**), and harmonine (**3**) from fatty acids precursors in *C. 7-punctata*, *A. 2-punctata*, and *H. axyridis*, respectively, are now clearly established. It is highly probable that the other perhydroazaphenalenes (e.g., convergine, myrrhine, etc.), the "dimeric" alkaloids (e.g., exochomine, etc.), and the structurally related ladybird alkaloids (e.g., calvine, signatipennine, hyperaspine, etc.)[1–4] are biosynthesized along an analogous pathway that starts from a fatty acid precursor undergoing straightforward oxidation and amination reactions. Interestingly enough, the carbon skeleton of ant alkaloids such as the solenopsins[20] and the tetraponerines[21–22] have been shown to be derived from polyacetate chains, also most likely of fatty acid origin. This would indicate that, in insects, such a pathway has evolved preferentially to the amino acid pathway, which is more ubiquitous for the biosyntheses of the piperidine and pyrrolidine alkaloids of plants[23] with the noticeable exceptions of coniine in *Conium maculatum*[24] and pinidine in *Pinus spp.*[25]

Experimental Section

General Methods: Thin layer chromatography analyses were performed with 0.25 mm Polygram silica gel SILG/UV254 precoated plates (Macherey–Nagel) or with neutral alumina 60F254 precoated plates (Merck). Column chromatography was performed with silica gel columns (MN Kieselgel 60, 0.04–0.063 mm, Macherey–Nagel) by using the flash technique, and alumina filtration was realized with basic or neutral alumina (Macherey–Nagel). ^{1}H NMR spectroscopic data were recorded in $CDCl_3$ at 300 MHz with a Bruker Avance TM 300 and are reported in ppm on the δ scale by using TMS as an internal standard. ^{13}C NMR spectroscopic data were recorded in $CDCl_3$ at 75.4 MHz with a Bruker Avance TM 300 instrument. Data are reported in the format of chemical shift {multiplicity [singlet (s), broad singlet (br. s), triplet (t), multiplet (m)], coupling constants in Hz, integration}. HRMS (EI), MS (EI), and MS [CI (chemical ionization)] analyses were performed with a Fison VG Micromass 7070F Autospec instrument (70 eV). For the MS (CI) analyses, the reagent gas used was ammonia. In all cases, the peak intensities are expressed as percent relative to the base peak. The IR spectra were recorded with a Bruker IFS 25 instrument with samples as KBr pellets or as films on an NaCl disk. Radioactive compounds were assayed in a Packard Tri-Carb 1600 TR liquid scintillation analyzer. The samples were dissolved in methanol (10 mL), and 100 μL of the resulting solution was added to Packard Insta-Gel Plus liquid scintillation cocktail (10 mL). Triplicate samples of each compound were counted under comparable conditions of quenching. All chemicals were obtained from Aldrich or Acros Organics and used without further purification. [^{3}H$_3$]CI, (10 mCi, 85 mCi/mmol, 10 mCi/mL) and [1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-^{2}H$_{17}$]*n*-octanol, were purchased from Amersham (UK) and Aldrich, respectively. All synthetic steps involving tritiated compounds were developed beforehand by using the corresponding unlabelled compounds, and the spectral properties reported are those of the unlabelled derivatives.

Dimethyl Tridecanedioate (11): Concentrated sulfuric acid (0.8 mL) was added to a solution of **7** (2.002 g, 8.19 mmol) in methanol (8 mL). The mixture was heated to reflux for 2 h, cooled to room temp., and diluted with water (25 mL). The resulting mixture was exhaustively extracted with toluene. The organic layer was washed successively with water and sodium carbonate (5% aqueous solution). The toluene layer was then concentrated to dryness under reduced pressure to afford **11** (2.031 g, 7.46 mmol, 91%) as a white solid. IR (KBr): $\tilde{\nu}_{max}$ = 2930–2851, 1739, 1464, 1438, 1365, 1323, 1264, 1208, 1170, 1001, 887 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (m, 14 H), 1.61 (m, 4 H), 2.30 (t, J = 7.5 Hz, 4 H), 3.67 (s, 6 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 25.1, 29.3–29.6 (7 C), 34.2, 51.6, 174.5 ppm. MS (CI, NH_3): *m*/*z* (%) = 290 (87) [M + NH_4]$^+$, 273 (100) [M + H]$^+$, 258 (29), 243 (5), 226 (6). MS (EI): *m*/*z* (%) = 272 (<1) [M]$^{+\cdot}$, 241 (21), 208 (6), 199 (32), 167 (16), 149 (11), 126 (12), 112 (31), 98 (100), 87 (28), 84 (47), 74 (52), 55 (18).

12-(Methoxycarbonyl)dodecanoic Acid (14): A solution of barium hydroxide (0.5 M in anhydrous methanol, 7 mL) was added to a solution of diester **11** (1.729 g, 6.35 mmol) in anhydrous methanol (7 mL) in a sealed tube. The medium was vigorously stirred at room temp. for 18 h. The barium salt was then removed by filtration, washed with methanol, and dissolved in HCl (4 N solution). The resulting solution was extracted with diethyl ether. The organic layers were combined, and the solvents were evaporated to dryness under reduced pressure. The solid residue was purified by flash chromatography on silica gel (hexane/AcOEt, 9:1 then 8:2) to afford **14** (1.066 g, 4.13 mmol, 65%) as a white solid. IR (NaCl disk): $\tilde{\nu}_{max}$ = 3500–2500, 2917–2849, 1733, 1709, 1177 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (m, 14 H), 1.62 (m, 4 H), 2.31 (t, J = 7.5 Hz, 2 H), 2.35 (t, J = 7.4 Hz, 2 H), 3.68 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 24.8, 25.1, 29.1–29.6 (7 C), 34.2, 34.3, 51.7, 174.9, 180.3 ppm. MS (EI): *m*/*z* (%) = 259 (<1) [M + H]$^+$, 227 (18), 208 (11), 199 (10), 167 (8), 149 (8), 126 (11), 112 (31), 98 (100), 87 (29), 84 (52), 74 (82), 69 (30), 60 (14), 55 (50).

Methyl [14,14,14-^{3}H$_3$]13-Oxotetradecanoate (17): [^{3}H$_3$]CI (112 μL, 1.8 mmol) was added to a suspension of magnesium (45.2 mg, 1.8 mmol) in anhydrous ether (1 mL) in a 5 mL sealed tube. This mixture was stirred at room temp. for 30 min and then cooled to 0 °C. $CdCl_2$ (331 mg, 1.8 mmol) was added, and the solution was stirred at room temp. for 1 h. The solvent was carefully removed under a nitrogen stream to afford the organocadmium compound [^{3}H$_3$]CCdCl. Simultaneously, ester **14** (101 mg, 0.39 mmol) was dissolved in anhydrous hexane (3 mL) in a 5 mL sealed tube. Then, DMF (30 μL, 0.39 mmol) and oxalyl chloride (200 μL, 2.32 mmol) were added. After 90 min at room temp., the hexane layer was carefully drawn off, and the solvents were evaporated to dryness. The residue was then dissolved in dry toluene, and the obtained solution was gently added at 0 °C to the organocadmium compound [^{3}H$_3$]-CCdCl. The mixture was then stirred under nitrogen at room temp. for 6 h. The reaction was quenched with sulfuric acid (2% aqueous solution), and the resulting mixture was extracted with toluene (3×). The organic layers were combined, and the solvents were evaporated to dryness under reduced pressure to give a residue, which was purified by flash chromatography on silica gel (hexane/AcOEt, 95:5) to afford **17** (69 mg, 0.27 mmol, 69%) as a white solid. IR (KBr): $\tilde{\nu}_{max}$ = 2917–2849, 1732, 1714, 1472, 1463, 1441, 1364, 1320, 1261, 1234, 1207, 1178, 888 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (m, 14 H), 1.59 (m, 4 H), 2.13 (s, 3 H), 2.30 (t, J = 7.5 Hz, 2 H), 2.41 (t, J = 7.4 Hz, 2 H), 3.66 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 23.9, 25.0, 29.2–29.6 (7 C), 29.9, 34.2, 43.9,

51.5, 174.4, 209.4 ppm. MS (EI): *m*/*z* (%) = 256 (9) $[M]^{+\cdot}$, 241 (3), 225 (35), 199 (72), 167 (62), 149 (41), 143 (8), 126 (18), 112 (22), 98 (58), 87 (49), 83 (59), 74 (47), 69 (54), 58 (100).

Methyl [14,14,14-$^3H_3$]Tetradecanoate (20): *p*-Toluenesulfonic acid (1 mg) and *p*-toluenesulfonylhydrazine (56 mg, 0.58 mmol) were added to a solution of **17** (66 mg, 0.26 mmol) dissolved in a 1:1 mixture of DMF and sulfolane (1 mL). The mixture was stirred at room temp. for 24 h before the addition of $NaBH_3CN$ (25 mg, 0.37 mmol). After stirring at 110 °C for 5 h, the crude mixture was diluted with water, and the resulting mixture was extracted with hexane (3 ×). The organic layers were combined and concentrated under reduced pressure to give a residue, which was purified by flash chromatography on silica gel (hexane/AcOEt, 99:1) to afford **20** (31 mg, 0.13 mmol, 49%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.7 Hz, 3 H), 1.25 (m, 20 H), 1.61 (m, 2 H), 2.30 (t, *J* = 7.5 Hz, 2 H), 3.67 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.3, 22.8, 25.1, 29.3–29.8 (8 C), 32.1, 34.3, 51.6, 174.5 ppm. MS (EI): *m*/*z* (%) = 242 (17) $[M]^{+\cdot}$, 211 (5), 143 (6), 87 (47), 74 (100), 69 (18), 59 (22), 55 (39).

[14,14,14-$^3H_3$]Myristic Acid (4): Ester **20** (29 mg, 0.12 mmol), potassium hydroxide (25 mg, 0.44 mmol), and 18-crown-6 (9 mg, 0.03 mmol) were placed in a 1 mL sealed flask containing anhydrous toluene (0.5 mL). The mixture was stirred at room temp. overnight and then acidified with HCl (4 N solution), and the resulting mixture was extracted with toluene (3 ×). The combined organic layers were concentrated under reduced pressure, and the obtained residue was purified by flash chromatography on silica gel (hexane/AcOEt, 9:1) to afford **4** (20 mg, 0.09 mmol, 72%) as a white solid. IR (KBr): $\tilde{\nu}_{max}$ = 3500–2500, 2918–2849, 1699, 1471, 1411, 1290, 927, 720 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.7 Hz, 3 H), 1.27 (m, 20 H), 1.61 (m, 2 H), 2.35 (t, *J* = 7.5 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.3, 22.8, 24.8, 29.2–29.8 (8 C), 32.1, 34.2, 180.0 ppm. MS (EI): *m*/*z* (%) = 228 (58) $[M]^{+\cdot}$, 211 (11), 199 (5), 185 (32), 171 (10), 143 (11), 129 (49), 115 (15), 101 (9), 87 (19), 85 (30), 83 (18), 73 (100), 71 (40), 69 (31), 60 (78), 57 (55), 43 (63). SA = 0.3034 mCi/mmol.

[16,16,16-$^3H_3$]Palmitic Acid (5): The same synthetic scheme was applied to prepare acid **5**, but in this case, pentadecanedioic acid (**8**) was used as the starting material, and the solvent for the selective hydrolysis was CH_3OH/THF (6:1) instead of CH_3OH. SA of **5** = 0.3193 mCi/mmol. The yields for each step in the synthesis are reported in Scheme 1. The spectral properties of all the intermediates are compatible with those reported in the literature.

1,13-Bis(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)tridecane-1,13-dione (23): Diacid **7** (100 mg, 0.41 mmol) was dissolved in anhydrous hexane (3 mL) in a 5 mL sealed tube, and DMF (64 μL, 0.82 mmol) and oxalyl chloride (422 μL, 4.91 mmol) were added. The mixture was kept at room temp. under nitrogen for 90 min. Then, the hexane layer was carefully drawn off, and the solvents were evaporated to dryness. The diacyl dichloride thus obtained was dissolved in dry THF, and the obtained solution was added dropwise at room temp. to a solution containing Meldrum's acid (261 mg, 1.81 mmol) and DMAP (150 mg, 1.22 mmol) in anhydrous THF (0.5 mL). The mixture was stirred at room temp. under nitrogen overnight and quenched with water (6 mL). The pH was adjusted to 1 with HCl, and the organic solvent was removed under reduced pressure. Through filtration of the resulting aqueous layer, the desired product **23** (178 mg, 0.36 mmol, 88%) was obtained as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28 (m, 14 H), 1.69 (m, 4 H), 1.73 (s, 12 H), 3.06 (t, *J* = 7.6 Hz, 4 H), 15.29 (br. s, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 26.3, 26.9, 29.3–29.4 (7 C), 35.9, 91.4, 104.9, 160.3, 170.7, 198.5 ppm.

1,13-Bis(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)tridecane (24): $NaBH_3CN$ (1.9 g, 28.42 mmol) was added to a solution of **23** (3.528 g, 7.10 mmol) in acetic acid (28 mL) and THF (42 mL). The mixture was stirred at room temp. for 5 min and then warmed at 60 °C for 2 h. The crude mixture was then diluted with water (160 mL), and the resulting mixture was acidified with HCl. The obtained precipitate was removed by filtration and purified by flash chromatography on silica gel (CH_2Cl_2) to afford **24** (1.602 g, 3.42 mmol, 48%). IR (NaCl disk): $\tilde{\nu}_{max}$ = 2918–2849, 1743, 1339, 1208, 1061, 984 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.25 (m, 18 H), 1.44 (m, 4 H), 1.75 (s, 6 H), 1.79 (s, 6 H), 2.08 (m, 4 H), 3.52 (t, *J* = 5.0 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 26.6, 26.8, 27.0, 28.5, 29.3–29.6, 46.2, 104.9, 165.8 ppm. MS (CI, NH_3): *m*/*z* (%) = 486 (1) $[M + NH_4]^+$, 326 (12), 309 (15), 299 (7), 282 (100), 265 (22), 112 (13). MS (EI): *m*/*z* (%) = 468 (<1) $[M]^{+\cdot}$, 142 (8), 128 (6), 112 (18), 98 (100), 84 (23), 67 (23), 58 (64), 55 (72).

Heptadecanedioic Acid (9): Compound **24** (101 mg, 0.22 mmol) was dispersed in HCl (6 N aqueous solution, 2 mL), and the reaction mixture was heated to reflux overnight. After cooling, the solid obtained was removed by filtration, washed with water, and dried to afford diacid **9** (48 mg, 0.16 mmol, 74%) as a white solid. IR (NaCl disk): $\tilde{\nu}_{max}$ = 2919–2849, 1699, 1471, 1411, 1290, 927, 720 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.24 (m, 22 H), 1.48 (m, 4 H), 2.18 (t, *J* = 7.3 Hz, 4 H), 11.92 (br. s, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 24.5, 28.5–29.0 (11 C), 33.7, 174.5 ppm. MS (CI, NH_3): *m*/*z* (%) = 318 (64) $[M + NH_4]^+$, 300 (92), 282 (100), 264 (10), 223 (5), 126 (7), 112 (22). MS (EI): *m*/*z* (%) = 300 (<1) $[M]^{+\cdot}$, 256 (9), 126 (9), 112 (34), 98 (100), 84 (66), 73 (26), 69 (37), 60 (31), 55 (60).

[18,18,18-$^3H_3$]Stearic Acid (6): The labelled acid **6** was prepared from diacid **9** according to the same procedure as described for acids **4** and **5**. The solvent for the selective hydrolysis was CH_3OH/THF (6:1). SA of **9** = 0.2107 mCi/mmol. The yields for each step in the synthesis are reported in Scheme 1. The spectral properties of all the intermediates are compatible with those reported in the literature.

[11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-$^2H_{17}$]Stearic Acid (25): The labelled acid **25** was prepared according to the procedure described by Attygalle et al.[7] The yields for each step in the synthesis are reported in Scheme 5.

In Vitro Incubation Assays: Adults of *A. 2-punctata* were purchased from Horpi Systems (Verlaine, Belgium). Adults of *C. 7-punctata* and of *H. axyridis* were field-collected in Belgium. The experimental setup for the in vitro incubation assays has already been described.[10] The purifications of each alkaloid were carried out until a constant radioactivity was measured. For adaline (**2**), the crude extract was purified by successive flash chromatography procedures on silica gel by using AcOEt then AcOEt/MeOH/NH_4OH (95:5:1) as eluents, and on neutral alumina by using hexane/CH_2Cl_2 (5:5) then pure CH_2Cl_2 as eluents. For coccinelline (**1**), the crude extract was purified by successive flash chromatography procedures on silica gel by using AcOEt then AcOEt/MeOH/NH_4OH (5:5:0.1), and CH_2Cl_2 then CH_2Cl_2/MeOH/NH_4OH (9:1:0.1) as eluents. For harmonine (**3**), the crude extract was filtered through neutral alumina by using CH_2Cl_2 then CH_2Cl_2/MeOH/NH_4OH (8:2:0.2) as eluents. The sample of harmonine thus obtained was then treated with a 1:1 mixture of pyridine/acetic anhydride (1 mL) at room temp. for 2 h. After the addition of MeOH, the mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography on silica gel (AcOEt then AcOEt/MeOH, 95:5) to afford pure *N*,*N*-diacetylharmonine (**10**).

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