New Piperidine Alkaloids from Two Ladybird Beetles of the Genus *Calvia* (Coccinellidae)

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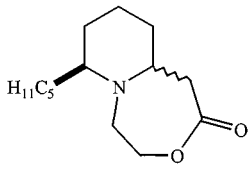
The alkaloids of two coccinellid beetles, *Calvia 14-guttata* and *C. 10-guttata* have been studied. The major alkaloid of these two species is the new piperidinic *cis*-lactone **1a**, for which the name calvine has been coined. The corresponding *trans*-lactone **1b** (2-epicalvine) is also present as a minor constituent (±10%) in both species. We report here the structure determination and the total synthesis of these compounds. Kept in methanolic solution, these lactones undergo epimerisation as well as opening of the lactone ring leading to a complex mixture of nine main components.

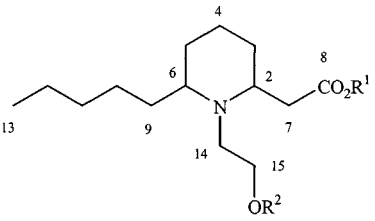
Coccinellid beetles are rarely exploited as a food source by other organisms. This has been attributed to the presence of deterrent alkaloids in their hemolymph. A survey of about 30 species of coccinellids has led to the isolation and structure determination of about 45 alkaloids belonging to many different structural families.[1][2] Little is known about the biosynthesis of these alkaloids, but several of them have in common an unbranched chain of carbon atoms joined in one or more places to one or more nitrogen atoms. This may be a consequence of closely related biosynthetic schemes. Thus, the finding of related compounds in further coccinellid species is of great interest for supporting this working hypothesis. In this context, we report the isolation and identification of the alkaloids of European specimens of two Holartic species *Calvia 14-guttata* and *Calvia 10-guttata*. These two species, although not rare, are scarce. Thus, to obtain enough material, adults of *C. 14-guttata* were collected during several successive collecting seasons and stored in methanol. Moreover, a few specimens were collected just before chemical analyses and kept in acetone.

TLC and GC analyses of the extracts indicated that only two alkaloids (**1a** and **1b**) are present in the acetonic extract. On the contrary, in the CH_3OH extract, these two compounds are accompanied by several other Dragendorff-positive compounds (**2a, 2b**, **3a**, **3b**, **4a**, **4b**, **12a**, **12b**, and **7**). Despite the complexity of the fraction and due to the amount of material available, the isolation and structure determination of these compounds were performed starting from the CH_3OH extract.

Thus, adults of *C. 14-guttata* stored in CH_3OH were exhaustively extracted with the same solvent affording a solid residue that was further fractionated by column chromatography on alumina to yield three fractions (F1−F3). Fraction F1 contained mostly lipidic material and, being devoid of alkaloids, was not further investigated. Fraction F2 yielded, after filtration through silica gel, a novel fraction (F2a) presenting several Dragendorff-positive spots in TLC and 9 main peaks in GC (peaks 1−9). For the sake of clarity, the identification of the alkaloids corresponding to

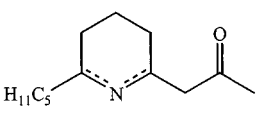


1a *cis* (calvine)
1b *trans* (2-epicalvine)



	2a, 2b	3a, 3b	4a, 4b	5a, 5b	6a, 6b
R^1	H	CH_3	CH_2CH_3	CH_3	CH_3
R^2	H	H	H	$COCH_3$	COC_6H_5

a = *cis*; b = *trans*



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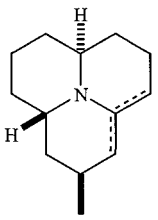

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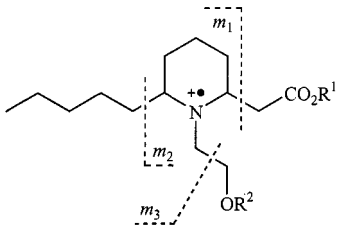
  1434−193X/99/0707−1749 $ 17.50+.50/0

these peaks will be presented together with the structure determination of the main constituents of fraction F3.

Fraction F3 was submitted to an acid/base extraction and the resulting basic portion was subjected to a reverse-phase chromatography on Sil RP-18 to yield fraction F3a, the TLC of which revealed only one Dragendorff-positive spot. In contrast, GC analysis of F3a showed that it contained two compounds (**2a** and **2b**) in approximately the same amounts. Their mass spectra, obtained by GC-MS, are superimposable suggesting that they are stereoisomers. EI-HRMS established their molecular formula to be $C_{14}H_{27}NO_3$, which requires two degrees of unsaturation. The FT-IR spectrum showed absorption bands at 1598 and 3300 cm^{-1}, attributable to carboxylate and hydroxy functions, respectively. Methylation of the mixture **2a** + **2b** with CH_2N_2 furnished, as main components, the esters **3a** and **3b** confirming the presence of a carboxylic acid. GC/EI-HRMS established the molecular formula of both compounds to be $C_{15}H_{29}NO_3$. Evidence for the presence of a hydroxy group was obtained by acetylation of a small amount of the **3a** + **3b** mixture, leading to the monoacetylated derivatives **5a** and **5b**.

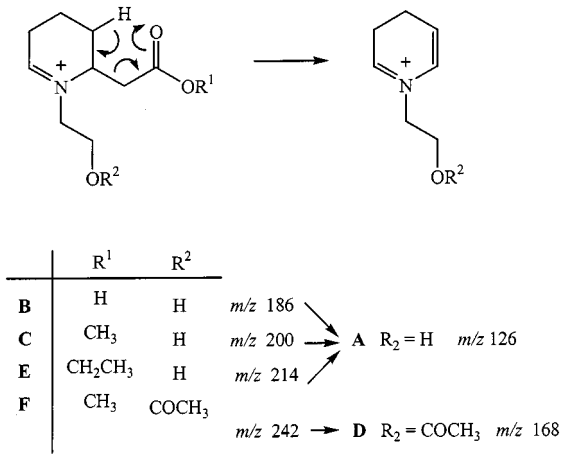
All the data presented above, together with mass-spectral data discussed below, suggested that the two main compounds (**2a** and **2b**) of F3 are the *cis* and *trans* stereoisomers of a piperidine with a 2-hydroxyethyl group attached to the ring nitrogen atom, a pentyl chain to C-6 and a carboxymethyl group to C-2. The fragment peaks observed in the mass spectra of **2a**, **2b**, **3a**, and **3b** strongly supported this structure assignment. Indeed, prominent fragments resulting from cleavages α-position to the nitrogen atom were observed (Scheme 1). Moreover, the peak at *m/z* 126 corresponding to ion **A** and appearing in the spectra of both groups of compounds can be attributed to the loss from ions **B** and **C** of CH_3COOH and CH_3COOCH_3, respectively, by a McLafferty rearrangement (Scheme 2). Similar α cleavages and McLafferty rearrangements were observed for the acetylated compounds **5a** and **5b** (Schemes 1 and 2).



	2a, 2b	3a, 3b	4a, 4b	5a, 5b	6a, 6b
m_1	198	198	198	240	302
m_2	186	200	214	242	304
m_3	226	240	254	240	240

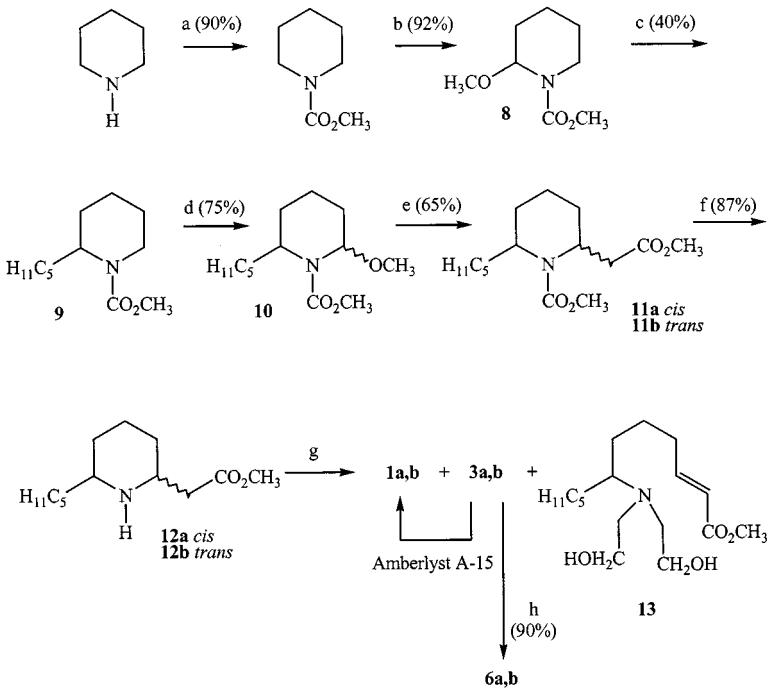
Scheme 1. Characteristic fragment ions of **2a**, **2b**, **3a**, **3b**, **4a**, **4b**, **5a**, **5b**, **6a**, and **6b** in EI-MS

To deduce the complete structure of the two stereoisomeric esters **3a** and **3b** (and consequently of the corresponding acids **2a** and **2b**) on the basis of their NMR data, we had to transform them into readily separable derivatives. This could be accomplished by formation of the benzoylated derivatives **6a** and **6b** that could cleanly be separated by column chromatography on silica gel. The EI-HRMS spectrum of the less polar benzoylated derivative **6b** was identical to that of **6a** and indicated their molecular formula to be $C_{22}H_{33}NO_4$. It also presented intense fragment ions resulting from C−C cleavages in α-position to the nitrogen atom (Scheme 1). The 1D- and 2D-NMR data of both compounds agreed with the proposed structures, and the assignments of the ^{1}H- and ^{13}C-NMR signals are reported in the Experimental Section. The assignment of the 2,6-*cis* configuration to **6a** and of the 2,6-*trans* configuration to **6b** was inferred from the comparison of the chemical shifts of C-2 and C-6, the signals of these atoms being more shielded in the *trans* (δ = 53.7 and 55.3 in **6b**) than in the *cis* (δ = 59.4 and 62.8 in **6a**) stereoisomer,[3][4] and of the chemical shifts of 2-H and 6-H which are known to be more shielded in *cis*- (δ = 3.10 and 2.55 in **6a**) than in *trans*- (δ = 3.42 and 2.75 in **6b**) 2,6-disubstituted piperidines.[5] A further argument follows from the shape of the H_2C-14 signal which reflects the magnetic nonequivalence of the methylene protons. In **6b** this signal appears as a well-defined double triplet, while in **6a** it appears as a broad, badly resolved peak.[6]



Scheme 2. Characteristic fragment ions resulting from McLafferty rearrangements in EI-MS

To prove the structure proposals for **2a** and **2b** unambiguously their synthesis was undertaken. The sequence of reactions used is outlined in Scheme 3. It is based on the regioselective introduction of substituents at the position α to the nitrogen atom of *N*-alkoxycarbonylpiperidines by anodic oxidation.[7][8] Conversion of 2-methoxy-*N*-methoxycarbonylpiperidine (**8**) into the corresponding *N*-acyliminium ion on treament with $BF_3{\cdot}OEt_2$ followed by addition of *n*-pentylmagnesium bromide yielded the 2-alkylated piperidine **9**. Compound **8** was prepared from piperidine by the anodic oxidation procedure of Shono et al.[9]. A second anodic oxidation carried out on **9** furnished regioselectively the 6-methoxylated compound **10** (*cis*/*trans* mixture, yield 75%), as expected from the steric constraints imposed by the *N*-methoxycarbamate group.[10] Nucleophilic displace-



Scheme 3. Synthetic scheme of calvine (**1a**) and 2-epicalvine (**1b**): (a) $ClCOOCH_3/K_2CO_3/H_2O$; (b) $CH_3OH/Et_4NOTs/2.63$ F/mol; (c) $CH_3[CH_2]_4MgBr/BF_3{\cdot}Et_2O/CH_2Cl_2$; (d) $CH_3OH/Et_4NOTs/3.5$ F/mol; (e) $TiCl_4$/1-methoxy-1-trimethylsilyloxyethene/CH_2Cl_2; (f) TMSI/CH_2Cl_2; (g) ethylene oxide/CH_3OH; (h) benzoic anhydride/DMAP/toluene

ment of the methoxy function of **10** by 1-methoxy-1-trimethylsilyloxyethene, prepared according to the procedure of Ainsworth and Kuo,[11] led to a 7:1 mixture of the carbamate esters **11a** and **11b** in a 65% yield. Deprotection of the secondary amine by treatment with TMSI furnished in 85% yield the stereoisomers **12a** and **12b** (ratio 86:14) that could be separated by flash column chromatography on silica gel. The *cis* configuration is attributed to the major stereoisomer on the basis of the chemical shifts of the signals of C-2 (δ = 53.5) and C-6 (δ = 57.0) that are deshielded relative to those of the minor stereoisomer (δ = 48.1 and 51.2, respectively).[3] Stereoisomers **12a** and **12b** are found to be identical (TLC, GC/EI-HRMS and GC/CI-MS) to the derivatives corresponding to peaks 1 and 2 of the GC of the fraction F2a derived from the CH_3OH extract of the beetle.

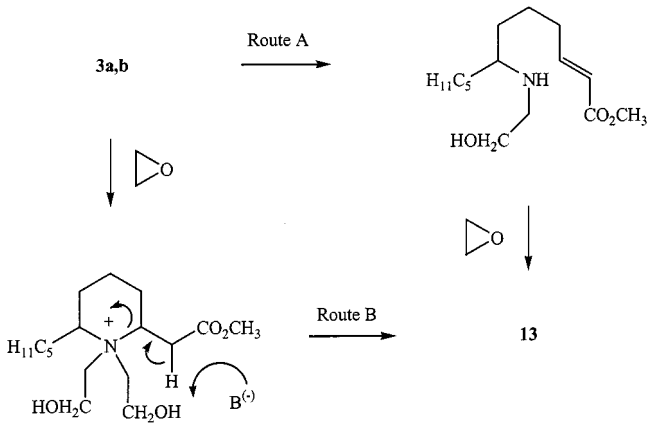
Several attempts to prepare **3a** and **3b** by direct *N*-alkylation of **12a** or **12b** with $BrCH_2CH_2OTHP$ or $BrCH_2CH_2OH$ failed, leading either to recovered starting material or to untractable mixtures. Better results were obtained by treatment of **12a** with an excess of ethylene oxide in methanol. In this case, the reaction mixture was composed of three main groups of derivatives that were separated by silica gel column chromatography. The less polar group corresponding to about 20% of the reaction mixture, is a 1:1 mixture of two compounds, the chromatographic behaviours (TLC and GC) of which are identical to those of the methyl esters **3a** and **3b**. Benzoylation of this mixture afforded, after chromatography, derivatives **6a** and **6b** identical (GC, TLC, EI-MS, and ^1H NMR) to the corresponding benzoates derived from natural **2a** and **2b**, thus confirming the proposed structures. Moreover, the chromatographic behaviour (TLC, GC/EI-HRMS and GC/CI-MS) of **3a** and **3b** is identical to that of the derivatives corresponding to peaks 4 and 5 of the GC of F2a.

The second group of compounds representing approximatively 50% of the reaction mixture, is a 2:1 mixture of the lactones **1a** and **1b** that were separated by further silica gel column chromatography. At room temperature the ^1H-NMR spectra of both lactones are badly resolved probably because of slow conformational changes in the lactone ring. Well-resolved spectra were obtained at 80°C. These spectra together with the IR and MS spectra are fully compatible with the proposed structures. Assignment of the 2,6-*cis* configuration to the lactone **1a**, which is eluted first in GC, and of the 2,6-*trans* configuration to the lactone **1b**, is inferred from the chemical shifts of the 2-H and 6-H signals, which are shielded in **1a** (δ = 2.64 and 2.35) in contrast to the corresponding protons in **1b** (δ = 3.04 and 2.88).[5] In addition, **1a** and **1b** were found to be identical (TLC, GC/EI-HRMS and GC/CI-MS) to the derivatives corresponding to peaks 8 and 9 of the GC of the fraction F2a. This confirmed also that these two lactones are the main alkaloids present in the fresh acetonic extract of the beetles.

Interestingly, when a 1:1 mixture of **3a** and **3b**, dissolved in CH_3CN, was maintained at 50°C for 2 h in the presence of Amberlyst A15, both esters were quantitatively transformed into the lactones **1a** and **1b**, respectively. In addition, a sample of **3b** submitted to the same conditions led to **1b**, thus confirming the configuration assignments based on the NMR data.

A fifth compound representing about 27% of the reaction mixture from the ethylene oxide treatment of **12a** and **12b** was systematically isolated together with the above-mentioned derivatives. Based on its spectral properties, structure

13 is attributed to this derivative. The relative amount of **13** increased with the duration of the reaction at the expense of the other derivatives. Consequently, two mechanisms, reported in Scheme 4, may be put forward to explain its formation: a retro Michael reaction on **3a** or **3b** (route A) or an elimination reaction with opening of the piperidine ring on an *N*-quaternized intermediate (route B).

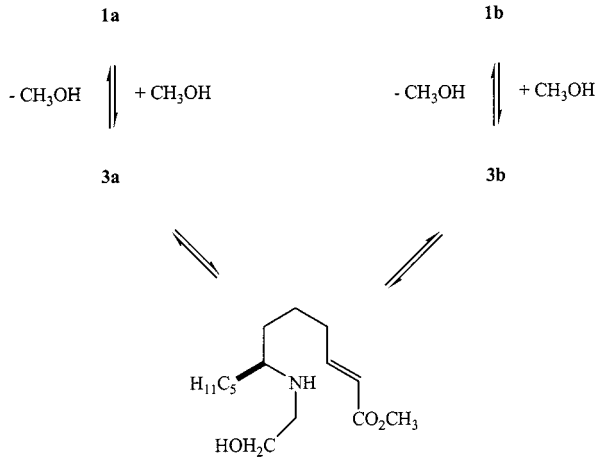


Scheme 4. Possible routes for the formation of compound **13**

Thus, besides the confirmation of the structure of the two main alkaloids present in fraction F3, this synthesis allowed us to identify in the CH_3OH extract of *C. 14-guttata* compounds **1a**, **1b**, **3a**, **3b**, **12a**, and **12b** which correspond to the GC peaks 8, 9, 5, 4, 1, and 2 of fraction F2a, respectively. The three remaining peaks were determined as follows. The mass-spectral data of the alkaloids corresponding to peaks 6 and 7 are superimposable. Their molecular formula was established as $C_{16}H_{31}NO_3$ indicating that they possessed one methylene more than **3a** and **3b**. Prominent fragments resulting from cleavages in α-position to the nitrogen atom occurred at *m/z* 198, 214, and 254 (Scheme 1). Moreover, a peak at *m/z* 126 can be attributed to the loss of $CH_3COOCH_2CH_3$ from ion **E** by a McLafferty rearrangement (Scheme 2). This information implies that they are the ethyl esters **4a** and **4b** homologous to **3a** and **3b**, respectively. It is possible that these ethyl esters arise from the storing of some beetles in ethanol rather than in methanol.

The high-resolution mass spectra of the compound associated with peak 3 established its molecular formula to be $C_{13}H_{23}NO$. Strong ion peaks were observed at *m/z* 166.1595 ($M^+ - C_2H_3O$), 153.1147 ($M^+ - C_4H_8$) and 152.1436 ($M^+ - C_3H_5O$) suggesting that compound **7** associated with peak 3 could be a Δ^1- or $\Delta^{1(6)}$-piperideine, substituted at C-2 and C-6 by an *n*-pentyl and an acetonyl group, respectively.

We have already pointed out, that *N*-alkylation of **4a** and **4b** with an excess of ethylene oxide in MeOH, furnished the expected esters **3a** and **3b** in admixture with the lactones **1a** and **1b**. The same mixture of compounds is also obtained on methylation of **2a** and **2b** with a methanolic solution of CH_2N_2. These observations, together with the fact that the methanolic extract of the beetles is also a complex mixture of the epimeric esters **3a** and **3b**, of the corresponding lactones **1a** and **1b**, and acids **2a** and **2b**, led us to suspect that all these compounds could arise from interconversion by retro-Michael and transesterification reactions (Scheme 5). To test this hypothesis, the lactones **1a** and **1b** were dissolved in MeOH and left at room temperature for eight days. GC analysis of the resulting solutions indicated that in both cases epimerisation as well as opening of the lactone ring by transesterification had occurred. Some unidentified degradation products were also observed. Interestingly, both lactones are stable in aprotic solvents such as THF or CH_3CN. Moreover, when treated under the same conditions the secondary amines **12a** and **12b** were stable and were not subject to epimerisation. This is in agreement with the previous observations made by Galinovsky et al.[12] that 2,6-dialkylated piperidines are less readily epimerised than their *N*-alkylated analogues.



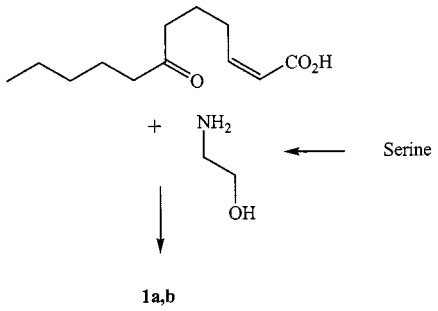
Scheme 5. Interconversion of calvine (**1a**) and 2-epicalvine (**1b**) in methanol

In view of these results, the two stereoisomeric lactones **1a** and **1b** can be considered as the true natural alkaloids produced by *C. 14-guttata*, the other alkaloids found in the methanolic extract being artifacts resulting from the extraction and storage processes. The name calvine is given to **1a** {(*R**,*R**)-3-oxo-8-*n*-pentyl-7-aza-4-oxabicyclo[5,4]-undecane} and 2-epicalvine to **1b** {(*S**,*R**)-3-oxo-8-*n*-pentyl-7-aza-4-oxabicyclo[5,4]undecane}.

Specimens of the closely related species *Calvia 10-guttata* were also collected and stored in acetone. This time, three alkaloids were recognized by GC-MS analysis: calvine (**1a**; 85%), 2-epicalvine (**1b**; 10%) and propyleine (**14**; 5%) an alkaloid we had already isolated from *Propylaea 14-punctata*.[13][14] The identification of the latter was ascertained by comparison with an authentic sample.

Biogenetically, like most other coccinellid alkaloids, the *Calvia* lactones could be derived from an unbranched chain of carbon atoms linked to one or more nitrogen atoms. In this particular case, the nitrogen atom together with carbon atoms C-14 and C-15, could arise from an ethanolamine moiety (Scheme 6) deriving from serine. Such an origin has been demonstrated by Attygalle et al.[15] for the ethanolamine moiety of epilachnene in *Epilachna varivestis*. Also interesting from a biogenetic point of view is the presence

in *Calvia 14-guttata* of trace amounts of the imino ketone **7**. Indeed, it is tempting to speculate that this alkaloid is a key intermediate in the biosynthesis of both the azaphenalene- and homotropane-type alkaloids.[2]



Scheme 6. Possible biosynthetic route to calvine (**1a**) and 2-epicalvine (**1b**)

Experimental Section

General: EI-MS, EI-HRMS, and GC/EI-HRMS were performed with a Fisons VG Autospec instrument and GC/EI-MS and GC/CI-MS analyses with a Finnigan ITD 800 apparatus coupled to a Tracor gas chromatograph equipped with a capillary column (collision gas: ammonia). − The ^{1}H- and ^{13}C-NMR spectra were recorded at 600 and 150.87 MHz, respectively, using a Varian Unity 600 instrument. Some ^{1}H-NMR spectra were recorded at 250 MHz with a Bruker WM 250 spectrometer. The chemical shifts are referred to TMS (ppm). − The IR spectra were obtained with a Bruker IFS 25 instrument as a film on an NaCl disk and the UV/Vis spectra with a Philips PU 8700 spectrophotometer. − GC analyses were performed with a Varian 3400 apparatus equipped with capillary columns (column 1: 25 m × 0.32 mm fused-silica column coated with OV1; column 2: 30 m × 0.25 mm fused-silica column coated with OV1701), carrier gas N_2, detector temperature 250 °C, injector temperature 200 °C. − Thin layer chromatography analyses (TLC) were performed with 0.25 mm Polygram silica gel SILG/UV_{254} precoated plates (Macherey−Nagel). − Column chromatography was performed on silica gel (MN Kieselgel 0.04−0.063 mm) using the flash technique.

Collection, Extraction, and Isolation: 474 adults of *Calvia 14-guttata* L. were collected in the Brussels area and stored in MeOH for several months. The sample was extracted exhaustively with an MeOH/CH_2Cl_2 mixture in a blender, filtered and the solution concentrated to dryness in vacuo to give a crude extract (1.46 g). The oily residue was separated into 3 fractions by filtration on alumina: Fraction 1 (F1; 1.2 g) eluted with CH_2Cl_2, Fraction 2 (F2; 25 mg) eluted with CH_2Cl_2/MeOH (8:2) and Fraction 3 (F3; 147 mg) eluted with MeOH/1% NH_4OH.

F3 was submitted to an acid/base extraction and the resulting basic portion (57 mg) was subjected to a reverse-phase chromatography (RP-18; gradient H_2O/MeOH) to obtain a Dragendorff-positive fraction (F3a; 23 mg) homogeneous by TLC (SiO_2; CH_2Cl_2/MeOH/NH_4OH, 8:2:0.1) but further analyses of this fraction indicated that it was a mixture of the stereoisomers **2a** and **2b**. Spectral properties of the mixture: IR (film) ν_{max} = 3300 (br.), 1598 cm^{-1}. − HR-EIMS; *m/z* (%): 257.1989 (0.6) [$C_{14}H_{27}NO_3$], 256.1911 (0.7) [$C_{14}H_{26}NO_3$], 239.1882 (1) [$C_{14}H_{25}NO_2$], 226.1810 (70) [$C_{13}H_{24}NO_2$], 198.1852 (15) [$C_{12}H_{24}NO$], 186.1129 (100) [$C_9H_{16}NO_3$], 168.1025 (66) [$C_9H_{14}NO_2$], 156.1018 (22) [$C_8H_{14}NO_2$], 126.0917 (37) [$C_7H_{12}NO$].

F2 was flash-chromatographed on silica gel (CH_2Cl_2/MeOH/NH_4OH, 99:1:0.5). This led to a Dragendorff-positive fraction (F2a; 8 mg) showing 9 main peaks by GC {column 2, temperature programme from 180°C to 250°C at 5°C/min); t_R [min]; rel.%}: **12a** (5.3; tr), **12b** (5.8; tr), **7** (8.8; 5), **3b** (11.6; 45), **3a** (11.9; 25), **4b** (12.2; tr), **4a** (12.5; tr), **1a** (14.4; 10) and **1b** (14.7; 10). The mixture was analysed by GC/EI-HRMS and GC/CI-MS. − EI-HRMS of **12a**; *m/z* (%): 227.1868 (1) [$C_{13}H_{25}NO_2$], 226.1799 (2) [$C_{13}H_{24}NO_2$], 156.1029 (100) [$C_8H_{14}NO_2$], 154.1596 (20) [$C_{10}H_{20}N$], 124.0759 (17) [$C_7H_{10}NO$], 96 (7), 82 (25), 74 (19), 55 (10). − CI-MS of **12a**; *m/z* (%): 228 (100) [(M + H)$^+$]. The mass spectra of **12b** are identical to those of **12a**. − EI-HRMS of **7**; *m/z* (%): 209.1777 (10) [$C_{13}H_{23}NO$], 208.1695 (2) [$C_{13}H_{22}NO$], 180.1387 (14) [$C_{11}H_{18}NO$], 166.1595 (40) [$C_{11}H_{20}N$], 166.1231 (20) [$C_{10}H_{16}NO$], 153.1147 (67) [$C_9H_{15}NO$], 152.1436 (26) [$C_{10}H_{18}N$], 138.0918 (17) [$C_8H_{12}NO$], 124 (13), 96 (100). − CI-MS of **7**; *m/z* (%): 210 (100) [(M + H)$^+$]. − EI-HRMS of **3b**; *m/z* (%): 271.2144 (0.5) [$C_{15}H_{29}NO_3$], 270.2081 (1) [$C_{15}H_{28}NO_3$], 240.1967 (97) [$C_{14}H_{26}NO_2$], 226.1798 (4) [$C_{13}H_{24}NO_2$], 200.1289 (100) [$C_{10}H_{18}NO_3$], 198.1861 (35) [$C_{12}H_{24}NO$], 168.1028 (48) [$C_9H_{14}NO_2$], 126.0927 (30) [$C_7H_{12}NO$], 110 (7), 96 (13), 82 (11). − CI-MS of **3b**; *m/z* (%): 272 (100) [(M + H)$^+$]. The mass spectra of **3a** are identical to those of **3b**. − EI-HRMS of **4b**; *m/z* (%): 285.2275 (0.5) [$C_{16}H_{31}NO_3$], 284.2218 (1) [$C_{16}H_{30}NO_3$], 254.2108 (100) [$C_{15}H_{28}NO_2$], 214.1441 (96) [$C_{11}H_{20}NO_3$], 198.1855 (36) [$C_{12}H_{24}NO$], 184.1341 (16) [$C_{10}H_{18}NO_2$], 168.1026 (29) [$C_9H_{14}NO_2$], 126.0915 (35) [$C_7H_{12}NO$], 110 (6), 96 (9), 82 (7). − CI-MS of **4b**; *m/z* (%): 286 (100) [(M + H)$^+$]. The mass spectra of **4a** are identical to those of **4b**. − EI-HRMS of **1a**; *m/z* (%): 239.1883 (1) [$C_{14}H_{25}NO_2$], 168.1031 (100) [$C_9H_{14}NO_2$], 126.0926 (15) [$C_7H_{12}NO$], 124 (3), 110 (3), 96 (5), 82 (6). − CI-MS of **1a**; *m/z* (%): 240 (100) [(M + H)$^+$]. The mass spectra of **1b** are identical to those of **1a**.

3 adults of *C. 14-guttata* L. were collected in May 1997 in the Brussels area and stored in acetone. The acetone extract was immediately analysed by GC-MS (column 2, temperature programme: 1 min at 60°C then to 280°C at 10°C/min). Two alkaloids were identified (t_R and mass spectrum): calvine (**1a**, 95%) and 2-epicalvine (**1b**, 5%). 5 adults of *C. 10-guttata* L. were also collected in May 1997 in the Brussels area and stored in acetone. The acetone extract was immediately analysed by GC-MS (column 1, temperature programme: 2 min at 100°C then to 270°C at 5°C/min). Three alkaloids were identified (t_R and mass spectrum): calvine (**1a**, 85%), 2-epicalvine (**1b**, 10%), and propyleine (**14**, 5%).

Methylation of F3a: A freshly prepared ethereal solution of CH_2N_2 (3 mL) was added to F3a (20 mg), dissolved in MeOH (2 mL). The solution was kept at room temp for 20 min. Removal of the solvent under reduced pressure gave a solid residue which was flash-chromatographed on silica gel (CH_2Cl_2/MeOH/NH_4OH, 95:5:1) yielding a Dragendorff-positive fraction [F3m; 16 mg. − IR (film): ν_{max} = 3466 and 1742 cm^{-1}. GC-MS analysis indicated the presence of 4 compounds having retention times and mass spectra identical to those of **2b** (69%), **2a** (14%), **1a** (6%), and **1b** (6%), respectively].

Benzoylation of F2a and F3m: F2a and F3m were benzoylated separately. To 8 mg of F2a and 6 mg of F3m, dissolved in dry toluene (2 and 1 mL, respectively), were added DMAP (25 and 20 mg, respectively) and benzoic anhydride (15 and 10 mg, respectively). The solutions were vigourously stirred for 5 min and kept at room temp for 24 h. After addition of an aqueous solution of 1 M NaOH (3 mL) at 0°C, the mixtures were extracted with CH_2Cl_2 (3 × 5 mL). Removal of the solvent under reduced pressure followed by filtration on silica gel (hexane/acetone/NH_4OH, 9:1:0.1) to remove the excess of DMPA, gave two UV-positive fractions (6 and 4 mg,

respectively) whose TLC indicated that both fractions contained the same two major compounds. They were combined and chromatographed on a silica gel Lobar column (Lichroprep Si 60, 24 × 1 cm, 40−63 μm; hexane/THF/NH_4OH, 95:5:1). This yielded the benzoylated derivatives **6a** (5 mg) and **6b** (6 mg) as colourless gums. **6a**: GC (column 2, temperature programme: 10 min at 200°C then to 260°C at 10°C/min): t_R = 26.7 min; R_f (SiO_2, hexane/THF, 7:3, 0.1% NH_4OH) = 0.69. − IR (film): ν_{max} = 1733, 1717, 1266, 739 cm^{-1}. − UV (CH_3OH): λ_{max} (ε) = 228 (9541). − EI-HRMS: molecular ion at *m/z* (%) 375.2394 (0.2) [$C_{22}H_{33}NO_4$], fragment ions at *m/z* (%) 374.2310 (0.4) [$C_{22}H_{32}NO_4$], 304.1549 (100) [$C_{17}H_{22}NO_4$], 302.2122 (24) [$C_{19}H_{28}NO_2$], 240.1965 (37) [$C_{14}H_{26}NO_2$]. − NMR (600 MHz; $CDCl_3$; COSY; HMQC): (^1H *J*; ^{13}C) HC-2 (3.10 m; 59.4), H_2C-3 (1.50 m, 1.30 m; 27.7), HC-6 (2.55 m; 62.8), H_2C-7 (2.74 dd 9/15, 2.33 dd 4/15; 39.8), C-8 (172.5), H_2C-12 (1.25 m; 22.7), H_3C-13 (0.86 t 6.5; 14.1), H_2C-14 (2.84 m, 2.78 m; 44.6), H_2C-15 (4.23 m), 64.4), H_3C-16 (3.65 s; 51.6), C-17 (166.5), C-18 (130.2), 2× HC-19 (8.02 d 8; 129.6), 2× HC-20 (7.41 dd 7/8; 128.4), HC-21 (7.53 dd 7/7; 133.0), C-4, -5, -9 to -11 (34.2, 32.1, 29.4, 26.3, and 23.4). **6b**: GC (column 2, temperature programme: 10 min at 200°C then to 260°C at 10°C/min): t_R = 25.9 min; R_f (SiO_2, hexane/THF, 7:3, 0.1% NH_4OH) = 0.72. − IR (film): ν_{max} = 1733, 1717, 1266, 740 cm^{-1}. − UV (CH_3OH): λ_{max} (ε) = 228 nm (11560). − EI-HRMS: molecular ion at *m/z* (%) 375.2366 (0.5) [$C_{22}H_{33}NO_4$], fragment ions at *m/z* (%) 374.2305 (0.4) [$C_{22}H_{32}NO_4$], 304.1541 (100) [$C_{17}H_{22}NO_4$], 302.2119 (25) [$C_{19}H_{28}NO_2$], 240.1969 (71) [$C_{14}H_{26}NO_2$]. − NMR (600 MHz; $CDCl_3$; COSY; HMQC): (^1H *J*, ^{13}C) HC-2 (3.42 m; 53.7), H_2C-3 (1.70 m, 1.28 m; 24.6), HC-6 (2.75 m; 55.3), H_2C-7 (2.69 dd 8/14, 2.40 dd 7/14; 37.2), C-8 (172.8), H_2C-12 (1.25 m; 22.7), H_3C-13 (0.86 t 6.5; 14.1), H_2C-14 (2.92 ddd 7/7/14, 2.78 ddd 7/7/14; 45.5), H_2C-15 (4.26 dd 7/7; 64.8), 64.8), H_3C-16 (3.63 s; 51.4), C-17 (166.6), C-18 (130.5), 2× HC-19 (8.04 d 7; 129.6), 2× HC-20 (7.43 dd 7/7; 128.3), HC-21 (7.55 dd 7/7; 132.8), C-4, -5, -9 to -11 (32.6, 32.0, 26.1, 25.9, and 20.3).

Acetylation of F3m: 10 mg of F3m was treated with the mixture pyridine/acetic anhydride (1:1) (1 mL). The solution was kept at room temp. for 14 h. Water (3 mL) was added and the mixture was stirred for 15 min. Removal of the solvent under reduced pressure followed by flash chromatography of the residue on silica gel (gradient CH_2Cl_2/MeOH) gave 8 mg of a 85:15 mixture of **5a** and **5b** [GC (column 1, temperature programme: 2 min at 100°C then to 270°C at 5°C/min): t_R = 30.8 and 30.5 min, respectively]. After a second flash chromatography, 4 mg of almost pure **5b** were isolated as a colourless gum. − IR (film): $\tilde{\nu}_{max}$ = 1748, 1254 cm^{-1}. − EI-MS; *m/z* (%): 313 (3), 256 (5), 254 (5), 242 (100), 240 (82), 170 (12), 168 (11), 87 (63). − CI-MS; *m/z* (%): 314 (100) [$(M + H)^+$].

2-Pentyl-*N*-methoxycarbonylpiperidine (9): To a stirred suspension of Mg (1.1 g; 46 mmol) in dry ether (16 mL) was added dropwise a solution of 1-bromopentane (7.8 g; 52 mmol) in ether (10 mL). The mixture was stirred at room temp under nitrogen for 3 h. After a quick filtration through cotton, the volume of the solution was reduced to about 10 mL. CH_2Cl_2 (20 mL) was added and the temperature kept at −78°C (dry ice in acetone). Then, a solution of *N*-methoxycarbonylpiperidine (0.8 g; 4.6 mmol) in CH_2Cl_2 was added, followed by dropwise addition of $BF_3 \cdot Et_2O$ (1.1 mL). The resulting mixture was stirred at −78°C for 20 min and kept at room temp. overnight. The reaction was quenched by adding cold water (14 mL) and the aqueous layer extracted twice with CH_2Cl_2. The organic layers were combined, washed with brine, dried with $MgSO_4$, and concentrated to leave a colourless oil. Purification of the oil by flash chromatography on silica gel (hexane/EtOAc, 9:1) gave **9** (350 mg, yield 40%): GC (column 1, temperature programme from 100°C to 270°C at 10°C/min): t_R = 11.1 min. − IR (film): $\tilde{\nu}$ = 1698 cm^{-1}. − EI-MS: molecular ion at *m/z* (%) 213 (43), fragment ions at *m/z* (%) 198 (2), 182 (4), 170 (1), 156 (21), 142 (100). − ^{13}C NMR (62.9 MHz; $CDCl_3$; DEPT): δ = 156.7 (C), 52.7 (CH), 51.2 (CH), 39.5 (CH_2), 32.2 (CH_2), 30.0 (CH_2), 29.7 (CH_2), 26.3 (CH_2), 26.0 (CH_2), 23.0 (CH_2), 19.4 (CH_2), 14.4 (CH_3). − ^1H NMR (250 MHz; $CDCl_3$): δ = 4.15 (m, 1 H), 3.90 (m, 1 H), 3.61 (s, 3 H), 2.73 (dt, 1 H, *J* = 3, 13), 1.6−1.05 (m, 14 H), 0.79 (t, 3 H, *J* = 6.5).

2-Methoxy-*N*-methoxycarbonyl-6-pentylpiperidine (10): A solution of **9** (700 mg; 3.28 mmol) and Et_4NOTs (94 mg; 0.31 mmol) as electrolyte in methanol (35 mL) was placed into an electrolysis cell equipped with 4 carbon electrodes. A constant current (30−32 mA) was passed through the solution. After 3.5 F/mol was consumed, the solvent was evaporated under reduced pressure, then diluted ammonia was added to the residue and the aqueous phase extracted with CH_2Cl_2 (3× 50 mL). The organic layer was washed with water, dried with $MgSO_4$, and concentrated to dryness under reduced pressure. Purification of the oil by flash chromatography on alumina (hexane/EtOAc, 95:5) afforded compound **10** (*cis*/*trans* mixture; 600 mg; yield 75%): GC (column 1, temperature programme from 100°C to 270°C at 10°C/min): t_R = 11.9 and 12.7 min. − IR (film): $\tilde{\nu}$ = 1709 cm^{-1}. − EI-MS: molecular ion at *m/z* (%) 243 (0.3), fragment ions at *m/z* (%) 228 (3), 212 (35) 172 (85), 140 (20), 71 (100).

***cis*- and *trans*-*N*-Methoxycarbonyl-2-methoxycarbonylmethyl-6-pentylpiperidine (11a and 11b):** To a solution of **10** (80 mg; 0.33 mmol) in CH_2Cl_2 (1.5 mL) was added slowly a solution of $TiCl_4$ (0.1 mL; 0.9 mmol) in CH_2Cl_2 (1.5 mL) at −78°C under nitrogen. After stirring (15 min), a solution of 1-methoxy-1-trimethylsilyloxyethene (330 mg; 2.4 mmol) in CH_2Cl_2 (1.5 mL) was added and the stirring maintained for 3 h at −78°C. (1-Methoxy-1-trimethylsilyloxyethene was prepared according to the procedure of Ainsworth and Kuo.[9] Under these conditions a 1:1 mixture of *O*- and *C*-silylated derivatives was obtained. The solution was allowed to warm to room temp and stirred overnight. After addition of 25 mL of cold water, the solution was basified to pH = 9 with ammonia and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, filtered on a filter paper and concentrated under reduced pressure. Purification of the resulting oil by flash chromatography on silica gel (hexane/AcOEt, 95:5) afforded a 7:1 mixture of **11a** and **11b** (60 mg; yield 65%). GC (column 1, temperature programme from 100°C to 270°C at 10°C/min): t_R = 14.8 min (**11a**, 87%) and 14.4 min (**11b**, 13%). **11a** and **11b**: IR (film): ν_{max} = 1737, 1698 cm^{-1}. − EI-HRMS; *m/z* (%): 285.1937 (1) [$C_{15}H_{27}NO_4$], 254.1766 (2) [$C_{14}H_{24}NO_3$], 214.1081 (80) [$C_{10}H_{16}NO_4$], 212.1651 (20) [$C_{12}H_{22}NO_2$], 182.0816 (24) [$C_9H_{12}NO_3$], 140.0717 (100) [$C_7H_{10}NO_2$]. − ^1H NMR of **11a** (250 MHz; $CDCl_3$): δ = 4.65 (m, 1 H), 4.13 (m, 1 H), 3.69 (s, 3 H), 3.67 (s, 3 H), 2.62 (dd 10/14, 1 H), 2.52 (dd 5/14, 1 H), 0.89 (t 6, 3 H). − ^{13}C NMR of **11a** (62.8 MHz; $CDCl_3$): δ = 172.3, 156.9, 52.9, 52.0, 51.1, 47.8, 39.4, 35.0, 32.2, 28.6, 27.7, 27.4, 23.0, 14.5. − ^1H NMR of **11b** (250 MHz; $CDCl_3$): δ = 3.93 (m, 1 H), 3.80 (m, 1 H), 3.67 (s, 3 H), 3.66 (s, 3 H), 2.88 (dd 5.5/15.5, 1 H), 2.57 (dd ?/15.5, 1 H), 0.89 (t 6, 3 H).

***cis*- and *trans*-2-Methoxycarbonylmethyl-6-pentylpiperidine (12a and 12b):** To a solution of **11a** + **11b** (180 mg; 0.63 mmol) in CH_2Cl_2 (8 mL) was added TMSI (0.35 mL; 2.5 mmol) under nitrogen. The mixture was refluxed for 2 h. After addition of MeOH (2 mL) and stirring for 10 min, the solution was concentrated to dryness under reduced pressure. The solid residue was distributed between water/1% NH_4OH and CH_2Cl_2. The water layer was extracted twice with

CH_2Cl_2. The combined organic layers were filtered on a paper filter and concentrated to dryness. GC and TLC analyses of the solid residue (125 mg; yield 87%) indicated the presence of 2 compounds (6:1) which were separated by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/MeOH/NH_4OH, 99:1:0.1 to 97:3:0.4). **12a**: Colourless gum (99 mg). − GC (column 2, temperature programme: 10 min at 180°C then to 250°C at 10°C/min): t_R = 6.5 min. − IR (film): ν_{max} = 3328, 1728 cm^{-1}. − EI-HRMS; *m/z* (%): 227 (1) [M^+], 226 (2), 168 (2), 156 (100), 142 (15), 124 (15), 96 (10), 82 (31). − CI-MS (NH_3); *m/z*: 228 [M + H]$^+$. − NMR (600 MHz, $CDCl_3$, COSY, HMBC, HMQC): ($^{1\,H,\,J;\,13}C$) NH (2.80 bs), OCH_3 (3.63 s), HC-2 (2.93 m; 53.5), H_2C-3 (ax 1.12 dq 13/4.3, eq 1.55 bdd 13/2.5; 32.1), H_2C-4 (ax 1.32 m, eq 1.75 dq 13.6/3.1; 24.4), H_2C-5 (ax 1.01 dq 13/4.3, eq 1.63 bdd 13/2.5; 31.8), HC-6 (2.50 m; 57.0), H_2C-7 (2.39 d 6.2; 41.0), C-8 (172.8), H_2C-9 (1.32 m; 37.0), H_2C-10 (1.25 m; 25.4), H_2C-11 (1.25 m; 31.9), H_2C-12 (1.25 m; 22.5), H_3C-13 (0.83 t 7; 14.0). **12b**: colourless gum (16 mg); GC (column 2, temperature programme: 10 min at 180°C then to 250°C at 10°C/min): t_R = 7.6 min. − IR (film): ν_{max} = 1740 cm^{-1}. − EI-HRMS; *m/z* (%): 227.1854 (0.5) [$C_{13}H_{25}NO_2$; M^+], 226.1807 (2) [$C_{13}H_{24}NO_2$], 156.1020 (100) [$C_8H_{14}NO_2$], 154.1591 (25) [$C_{10}H_{20}N$], 124.0761 (19) [$C_7H_{10}NO$], 82.0656 (31) [C_5H_8N]. − NMR (600 MHz, $CDCl_3$, COSY, HMBC, HMQC): ($^{1\,H,\,J;\,13}C$) NH (4.70 bs), OCH_3 (3.65 s), HC-2 (3.45 m; 48.1), H_2C-3 (ax 1.44 m, eq 1.73 m; 29.8), H_2C-4 (ax 1.54 m, eq 1.58 m; 18.9), H_2C-5 (ax 1.36 m, eq 1.73 m; 29.4), HC-6 (2.95 m; 51.2), H_2C-7 (2.45 dd 16/8.6, 2.73 dd 16/5.5; 37.5), C-8 (172.4), H_2C-9 (1.44 m, 1.54 m; 33.1), H_2C-10 (1.25 m; 25.6), H_2C-11 (1.25 m; 31.7), H_2C-12 (1.25 m; 22.5), H_3C-13 (0.85 t 7; 13.9).

***N*-Alkylation of 12a and 12b:** Typically, a solution of **12a** (50 mg) in anhydrous MeOH (2 mL) was added to ethylene oxide (1 mL) at −78°C in a sealed tube under nitrogen. The solution was heated at 50°C for 22 h and then cooled to room temp. Removal of the excess of ethylene oxide at atmospheric pressure and of the solvent at reduced pressure furnished a solid residue which was flash-chromatographed on silica gel (CH_2Cl_2 then CH_2Cl_2/MeOH/NH_4OH, 95:5:0.3). The separations were monitored by GC and TLC. This yielded 3 main fractions containing a 1:1 mixture of **3a** and **3b** (10 mg), a 2:1 mixture of **1a** and **1b** (25 mg), and pure **13** (15 mg), respectively. A similar reaction mixture was obtained when this alkylation reaction was performed with **12b**. Benzoylation of the mixture of **3a** and **3b** (10 mg), using the same procedure as mentioned above, yielded **6a** (5 mg) and **6b** (6 mg) whose chromatographic behaviours (GC, TLC) and spectral properties (IR, MS, ^1H and ^{13}C NMR) were identical to those of the corresponding benzoates derived from natural **2a** and **2b**. Silica gel column chromatography of the 2:1 lactone mixture led to pure **1a** and **1b**. Moreover, successive flash chromatographies on silica gel of a fraction resulting from the gathering of several chromatographic fractions containing **3a** and **3b** led to the isolation of a pure sample of **3b**.

3b: Same EI-HRMS as the natural compound. − NMR (600 MHz, $CDCl_3$, COSY, HMBC, HMQC) ($^{1\,H,\,J;\,13}C$): OCH_3 (3.68 s; 51.6), HC-2 (3.41 m; 52.6), H_2C-3 (ax 1.25 m, eq 1.70 m; 24.1), H_2C-4 (ax 1.57 m, eq 1.70 m; 20.4), H_2C-5 (ax 1.20 m, eq 1.50 m; 25.6), HC-6 (2.79 m; 54.5), H_2C-7 (2.35 dd 15/6, 2.79 m; 37.0), C-8 (173.0), H_2C-9 (1.28 m, 1.48 m; 32.9), H_2C-10 (1.25 m, 1.35 m; 26.2), H_2C-11 (1.25 m; 31.9), H_2C-12 (1.25 m, 1.35 m; 22.6), H_3C-13 (0.87 t 7; 14.0), H_2C-14 (2.60 dt 13.8/4.2, 2.77 m; 47.1), H_2C-15 (3.43 m, 3.59 ddd 11.4/9/4.2; 59.5).

1a: GC (column 2, temperature programme: 10 min at 180°C then to 250°C at 10°C/min): t_R = 20.4 min. − EI-HRMS; *m/z* (%): 239.1883 (1) [M^+, calcd. for $C_{14}H_{25}NO_2$ 239.1885], 168.1031 (100) [calcd for $C_9H_{14}NO_2$ 168.1025], 126.0926 (15) [calcd. for $C_7H_{12}NO$ 126.0919]; CI-MS; *m/z* (%): 240 (100) [(M + H)$^+$]. − IR (film): ν_{max} = 1738 cm^{-1}. − ^1H NMR (250 MHz, COSY, $CDCl_2CDCl_2$, 80°C): δ = 4.34 (t, *J* = 4, H_2C-15), 3.35 (dt, *J* = 4/15, HC-14), 2.82 (m, H_2C-7), 2.64 (m, H_2C-2), 2.50 (dt, *J* = 4/15, HC-14), 2.35 (m, HC-6), 0.95 (t, *J* = 6, H_3C-13).

1b: GC (column 2, temperature programme: 10 min at 180°C then to 250°C at 10°C/min): t_R = 21.0 min. − EI-HRMS and CI-MS identical to those of **1a**. − IR (film): ν_{max} = 1739 cm^{-1}. − ^1H NMR (250 MHz, COSY, $CDCl_2CDCl_2$, 80°C): δ = 4.46 (dd, *J* = 5/14, HC-15), 4.34 (dd, *J* = 7/15, HC-15), 3.15 (m, HC-14), 3.04 (m, HC-2 + HC-14), 2.88 (m, HC-6 + HC-7), 2.70 (m, HC-7), 0.95 (t, *J* = 6, H_3C-13).

13: Colourless gum. − IR (film): ν_{max} = 3408, 1732, 1658 cm^{-1}. − UV (CH_3OH): λ_{max} (ε) = 212 nm (13500). − EI-HRMS; *m/z* (%): 316 (8) [M + H]$^+$, 315 (3) [M^+], 284 (100), 244 (77), 188 (83), 144 (11). − ^1H NMR (250 MHz, $CDCl_3$): δ = 6.95 (ddd, *J* = 15.5/7, HC-6), 5.83 (bd, *J* = 15.5, HC-7), 3.73 (s, OCH_3), 3.58 (t, 5.4, H_2C-15 + H_2C-17), 2.66 (t, 5.4, H_2C-14 + H_2C-16), 2.45 (m, HC-2), 2.21 (m, H_2C-5), 0.89 (t, *J* = 7, H_3C-13).

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