Synthesis of metabolites of cis and trans apovincamine derivatives

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Dedicated to Professor Csaba Szántay on the occasion of his 80th birthday

Abstract
Synthesis of oxidative metabolites of ethyl cis- and hydroxyethyl trans-apovincamines have been described. For cis-metabolite 8 the functionalization of the 10-hydroxy group was established through key intermediate 8-methoxy-indolopyranoquinolizine 16, for trans-metabolite 10 the crucial 19-oxo-intermediate 24 was constructed from methyl 4-oxo-octahydroindoloquinolizine propionate 21a.

Keywords: Alkyl apovincaminates, oxidative metabolites, total synthesis

Introduction

Over the past decades clinical and non-clinical research on vincamine and its semi-synthetic derivatives 1−3 has confirmed their beneficial cerebrovascular effect, including a neuroprotective action (Scheme 1).1−3

Scheme 1
Previous SAR studies focused on structural modifications involving C-14 and the C/D/E ring junctions.\textsuperscript{4,5} In order to find a potent antiamnesic agent, further changes in the structure of compound 3 have been considered. To that end, a new series of substituted-alkyl esters of (3S,16R)- and (3R,16S)-\textit{trans}-apovincaminic acid was synthesized.\textsuperscript{6} From the combined results of the data obtained from in vitro and in vivo tests and metabolism studies, 2'-hydroxyethyl (3R,16S)-apovincaminate (4b, RGH-10885) was identified as the most promising compound, owing to its potent neuroprotective and antiamnesic activities.\textsuperscript{7}

Compounds 3 and 4b can be synthesized from the common intermediate 5. The direction of stereoselectivity could be modified by variation of the reaction conditions: \textit{cis}-diester 6\textsuperscript{8} or \textit{trans}-diester 7\textsuperscript{7} were isolated from the reduction, which was further transformed to the targeted endproducts (Scheme 2).

![Scheme 2](image)

**Scheme 2**

The metabolism of compounds 1-3 has been established earlier. Apovincaminic acid is the major metabolite of vinpocetine (3). Ethyl vincaminate and a compound hydroxylated on the aromatic ring were also reported as being minor metabolites of 3.\textsuperscript{9} In recent studies the latter
compound was identified as 10-hydroxyvinpocetine 8, and a new minor metabolite, vinpocetine-N-oxide 9\textsuperscript{10} was also isolated (Scheme 3).

Scheme 3. Metabolism of vinpocetine (3) and 4b.
A quite different pattern of metabolism was observed for the trans hydroxyethylester 4b. In contrast to the cis ethyl ester 3 and trans ethyl ester 4a, 4b does not appear to be affected by the esterase enzyme, but by the CYP enzymes. A series of oxidative metabolites were isolated. As a major metabolite the 19-oxo-derivative 10 was identified. Production of minor metabolites 10-hydroxy-4b (11), 6α- and 6β-hydroxy-4b (12a,b) as well as 18α- and 18β-hydroxy-4b (13a,b) were also observed (Scheme 3). 11

We synthesized cis metabolite 8, and the major metabolite of 4a trans-apovincaminate, 10 for the purpose of structural confirmation and for further studies of the biological activity.

Results and Discussion

A simple transformation of 3 into N-oxide 910 was accomplished with magnesium monoperoxy-phtalate in DMF.

The synthesis of the cis-metabolite 8 follows the route outlined in Scheme 4. Enamine 15,12 formed from 5-methoxytryptamine (14) was reacted with diethyl malonate and paraformaldehyde to obtain pyranoidoloquinolizine 16. Stereoselective reduction, followed by deformylation afforded cis-diester 17. Partial hydrolysis and reaction with sodium nitrite/acetic acid led to oximeester 18. Ring closure/deoximation yielded ethyl 10-methoxy-apovincaminate (19). Demethylation of 19 with boron tribromide afforded rac. 8(Scheme 4).

A multistep synthesis of trans-metabolite 10 was established from triptamine (20, Scheme 5).

Reaction with dimethyl formylpimelate was followed by the separation of trans isomers 21a,b.13 The desired (1S,12bR)-indoloquinolizinytl-propionate ester (21a) was isolated by means of optical resolution of acids by (−)-ephedrine. The so obtained 4-oxo-(1S,12bR)-propionic acid 22 was reesterified to 21a. The correct stereochemistry of 21a was proved by transformation to authentic 23 ester.4 Ring closure to dilactam 24 followed by oximation led to dilactam oxime 25. Hydrolysis and reaction of the so obtained trioxo compound with sodium methoxyde resulted in (3R,16S)-19-oxovincamine 26. Dehydration to 27 followed by transesterification completed the synthesis of 10 (Scheme 5).
In conclusion, different strategies were employed to synthesize oxidative metabolites of apovincaminic acid esters. The AB(C)D→ABCDE strategy (C₁₇N₂ + C₃) was applied by alkylation of the 9-methoxy-Wenkert-enamine 15 to prepare 10-hydroxy metabolite 8 of ethyl cis-apovincaminate. The skeleton of 19-oxo trans-metabolite 10 was constructed in a reaction of tryptamine and dimethyl formylpimelate (C₁₀N + C₁₀, AB→ABCDE approach). The subsequent steps to the end-products was carried out by using standard methods.¹⁵
Scheme 5. Reagents and conditions: (i) (a) toluene, 110°C, then (b) AcOH, 120°C, (c) NaOH/H₂O, CH₂Cl₂ work-up, (d) EtOH; (ii) (a) NaOH/H₂O, MeOH, (b) HCl/H₂O, (–)-ephe drine, acetone, (d) AcOH/H₂O, EtOH; (iii) (a) KOH/MeOH, (b) MeI, DMF; (iv) (a) P₂S₅, THF, (b) Raney Ni, THF; (v) tBuOK, toluene; (vi) tBuONa, tBuONO, toluene; (vii) (a) pTsOH, CH₂O, AcOH, 100°C, (b) tBuOK, MeOH; (viii) AcOCl, HCOOH, CH₂Cl₂; (ix) tBuOK, HO(CH₂)₂OH, 100°C.

Experimental Section

General Procedures. General procedures followed during the course of the work detailed herein were similar to those reported elsewhere. IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer using KBr pellets. NMR spectra were recorded on a Varian VNMRS-400 spectrometer (400 MHz for H detection). 2D NMR experiments (COSY, HSQC,
HMBC, NOESY) experiments were recorded by using the standard spectrometer software package; 0.75 s mixing time was used in the NOESY experiments. Mass spectrometric (high-resolution /HRMS/) measurements were performed on a LTQ FT Ultra (Thermo Finnigan, San Jose, CA) system. The ionization method was ESI (275 °C was the ion transfer capillary temperature and 4.2 kV was the capillary voltage). For CID experiment helium was used as the collision gas, and normalized collision energy (expressed in percentage), which is a measure of the amplitude of the resonance excitation RF voltage applied to the endcaps of the linear ion trap, was used to bring about fragmentation.

(±)-Diethyl 15a-ethyl-2,3,6,11,15,15a-hexahydro-8-methoxy-1H,5H-indolo[2,3-a]pyrano[3,2-i]quinolizine-14,14-(13H)-dicarboxylate (16). The solution of 5-methoxy-tryptamine (14) (20 g, 0.105 mol) and ethyl 3-hydroxypropylmalonic acid16 (23.2 g, 0.12 mol) in chlorobenzene (220 mL) was refluxed for 2 h, then 35 g (21 mL, 0.23 mol) phosphorus oxychloride was added at 50 °C and refluxed for 2 h. The reaction mixture was cooled to 70 °C and ethanol (40 mL) was added. The mixture was added dropwise to sodium hydroxide (31.2 g) in water (150 mL) at 70 °C. After stirring an additional 0.5 h, the separated organic phase was dried (MgSO4), filtered and concentrated under reduced pressure to give a red oil. It was solved in ethanol (50 mL), then diethyl malonate (24 g, 0.15 mol), paraformaldehyde (9 g, 0.3 mol) and triethylamine (1 mL) was charged. The reaction mixture was stirred for 2 h at 50 °C, then 2 h for 5 °C. The separated yellow crystals were filtered, washed with ethanol, to give 18.1 g (35 %) of the title 16, mp 147-148 °C. IR (cm-1): 3450, 2936, 1748, 1716, 1623, 1591, 1561, 1491, 1256, 1152, 888, 797; 1H NMR (DMSO-d6, 50 °C, δTMS=0.00 ppm): 0.77 (3H, t, CH2CH3), 1.15 (1H, dq, CHxHyCH3), 1.21 (3H, t, OCH2CH3), 1.26 (3H, t, OCH2CH3), 1.30-1.44 (2H, m, Hx-2, Hx-1), 1.54-1.68 (2H, m, Hx-2, Hx-1), 1.83 (1H, dq, CH3H2CH3), 2.49-2.80 (6H, m, H-2, H-6, Hx-3, Hx-5), 2.89 (1H, m, Hx-3), 3.31 (1H, td, Hx-5), 3.77 (s, 3H, OMe), 3.83 (1H, d, Hx-13), 4.19 (2H, q, OCH2CH3), 4.25 (2H, q, OCH2CH3), 4.43 (1H, d, H-3), 6.77 (1H, dd, H-9), 6.92 (1H, d, H-7), 7.35 (1H, d, H-10), 9.62 (1H, brs, NH); MS (ESI, CID=18 %) m/z (rel. int. %): 485(/M+H+/, 6), 455(64), 409(100), 283(14). Mass accuracy was between -0.87 and -0.21 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 485.26455 (delta: -0.13 ppm), calculated value for C27H37O6N2: 485.26461.

(±)-cis-1-(2',2'-Diethoxycarbonylthethyl)-1-ethyl-9-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizine(17). A solution of 16 (30 g, 62 mmol) in DMF (60 mL) was hydrogenated over 10 % palladium on activated carbon (0.6 g) for 2 h. After filtration, 25% NH4OH solution (12 mL) was added and the mixture was stirred for 1 h. EtOH (20 mL) then water (180 mL) was added. The precipitated crystals were separated, washed with water, then suspended in a mixture of EtOH/water 2:1 (110 mL) to yield 19.4 g (68 %) of 17, mp 120-121 °C (recrystallization from EtOH/water). IR (cm-1): 3435, 2940, 1756, 1728, 1627, 1588, 1461, 1271, 1224, 1025, 807; 1H NMR (DMSO-d6, 25 °C, δTMS=0.00 ppm): 1.01 (3H, t, CH2CH3), 1.03 (3H, t, OCH2CH3), 1.11 (3H, t, OCH2CH3); 1.40-1.57 (4H, m, H-2, Hx-3, CH3H2CH3); 1.70 (1H, m, Hx-3); 1.76 (1H, dd, CH3H2CH(COOEt)2), 1.86 (1H, dq, CH3H2CH3), 2.28 (1H, m, Hx-4), 2.34
(1H, dd, CH$_2$H$_5$CH(COOEt)$_2$), 2.42 (1H, td, H$_x$-6), 7.50 (1H, m, H$_y$-7), 2.72 (1H, m, H$_z$-7), 2.91-2.98 (2H, m, H$_y$-6, H$_y$-4), 3.24 (1H, t, CH$_2$H$_5$CH(COOEt)$_2$), 3.27 (1H, s, H-12b), 3.73 (3H, s, OMe), 3.91-4.06 (4H, m, 2x(OCH$_2$CH$_3$)), 6.66 (1H, dd, H-10), 6.84 (1H, d, H-8), 7.33 (1H, d, H-11), 9.62 (1H, s, NH); MS (ESI, CID=19 %) m/z (rel. int. %): 457/(M+H$^+$, 15), 440(1), 411(100), 365(5), 284(88), 238(6), 192(1). Mass accuracy was between -0.91 and -0.25 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 457.26962 (delta: -0.17 ppm), calculated value for C$_{26}$H$_{37}$O$_5$N$_2$: 457.26970.

(±)-cis-Ethyl-1-ethyl-9-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizine-1-(2’-hydroxyimino)-propionate (18). To 17 (6 g, 13 mmol) in EtOH (100 mL) a solution of potassium hydroxide (0.9 g, 16 mmol) in water (9 mL) was added and stirred for 3 h at 25-30 °C. The pH was adjusted to 7 with AcOH and the solution was evaporated in vacuo. The residue was dissolved in AcOH (18 ml). Nitrogen oxide was evolved from sodium nitrite (2.6 g) in water (7 mL) and concd. HCl (6 mL) and bubbled to the solution for 0.5 h at 10-15 °C, then a solution of sodium nitrite (1.1g) in water (5 mL) was added. The pH was adjusted with 1:2 mixture of concd HCl and water to 3. The precipitated 18 HCl crystals were filtered, stirred in a mixture of ethanol (20 mL), dichloromethane (1 mL) and 25% NH$_4$OH (5 mL) to give 18 (2.6 g, 48% yield), mp 178-180 °C (recrystallization from EtOH). IR (cm$^{-1}$): 3457, 2951, 1702, 1625, 1592, 1491, 1297, 1157, 1024, 800; $^1$H NMR (DMSO-d$_6$, 25 °C, $\delta$$_{TMS}$=0.00 ppm): 0.98 (3H, t, CH$_2$CH$_3$), 1.05 (3H, t, OCH$_2$CH$_3$), 1.30 (1H, m, H$_z$-2); 1.41 (2H, m, H$_x$-3, CH$_3$H$_2$CH$_3$), 1.49 (1H, m, H$_y$-2), 2.07 (1H, dq, CH$_3$H$_2$CH$_3$), 2.16 (1H, m, H$_x$-3), 2.22 (1H, d, CH$_3$H$_2$CH(COOEt)(NOH)), 4.01-4.16 (2H, m, OCH$_2$CH$_3$), 6.66 (1H, dd, H-10), 6.85 (1H, d, H-8), 7.35 (1H, d, H-11), 9.68 (1H, s, NH), 11.97 (1H, s, OH); MS (ESI, CID=22 %) m/z (rel. int. %): 414/(M+H$^+$, 12), 396(74), 381(9), 340(3), 324(39), 284(97), 253(14), 241(100). Mass accuracy was between -0.68 and -0.31 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 414.23881 (delta: 0.18 ppm), calculated value for C$_{23}$H$_{32}$O$_4$N$_3$: 414.23873.

(±)-Ethyl 10-methoxy-apovincaminate (19). A mixture of p-toluenesulfonic acid hydrate (5.3 g, 27 mmol) 18 (4.6 g, 11 mmol), toluene (55 mL) and EtOH (5 ml) was stirred and distilled until the temperature of the overhead rose to 108 °C. The reaction mixture was refluxed for 2 h. The reaction mixture was cooled to 20 °C and washed with Na$_2$CO$_3$ 7% solution (110 mL). The organic layer was dried over MgSO$_4$, clarified with Al$_2$O$_3$ (2 g), and evaporated. Crystallization of the residue from ethanol (15 mL) afforded ester 19 (3.2 g, 74%), mp 148-150 °C. IR (cm$^{-1}$): 2945, 1719, 1629, 1607, 1474, 1254, 1225, 1077, 830, 797; $^1$H NMR (DMSO-d$_6$, 25 °C, $\delta$$_{TMS}$=0.00 ppm): 0.80 (1H, td, H$_x$-17), 0.93 (3H, t, H$_z$-21), 1.30 (3H, t, OCH$_2$CH$_3$), 1.33 (1H, m, H$_{eq}$-18), 1.48 (1H, m, H$_{eq}$-17), 1.57 (1H, m, H$_{ax}$-18), 1.83 (2H, q, H$_2$-20), 2.39 (1H, m, H$_{eq}$-6), 2.41 (1H, m, H$_{ax}$-19), 2.52 (1H, m, H$_{eq}$-19), 3.00 (1H, m, H$_{ax}$-6), 3.08-3.24 (2H, m, H$_2$-5), 3.77 (3H, s, OMe), 4.05 (1H, s, H-3), 4.29-4.41 (2H, m, OCH$_2$CH$_3$), 6.06 (1H, s, H-15), 6.72 (1H, d, H-11), 6.94 (1H, d, H-9), 7.07 (1H, d, H-12); MS (ESI, CID=22 %) m/z (rel. int. %): 381/(M+H$^+$, 62), 364(6), 353(87), 338(100), 324(44), 309(31), 296(6), 266(8). Mass accuracy
was between -0.45 and -0.23 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 381.21736 (delta: 0.24 ppm), calculated value for C_{23}H_{29}O_{3}N_{2}: 381.21727.

**(±)-Ethyl 10-hydroxy-apovincaminate (8).** A solution of 19 (1 g, 2.6 mmol) in chloroform (50 mL) was treated with BBr$_3$ (2.4 mL) at 0-5 °C, under nitrogen. The mixture was warmed to 20 °C, then water (50 mL) was added and the pH was adjusted to 9 with NH$_4$OH 10% solution. The organic layer was separated, the aqueous was extracted with chloroform (20 ml). The combined organic solutions were evaporated, the residue was crystallized from methanol, to give 8 (0.6 g, 63%), mp 209-210 °C. IR (cm$^{-1}$): 3042, 2931, 2860, 1731, 1636, 1614, 1570, 1298, 1266, 1203, 1078, 1023, 782; $^1$H NMR (DMSO-d$_6$, 25 °C, $\delta$$_{TMS}$=0.00 ppm): 0.80 (1H, td, H$_{ax}$-17), 0.93 (3H, t, H$_3$-21), 1.30 (3H, t, OCH$_2$CH$_3$), 1.33 (1H, m, H$_{eq}$-18), 1.46 (1H, m, H$_{eq}$-17), 1.56 (1H, m, H$_{ax}$-18), 1.81 (2H, q, H$_2$-20), 2.32 (1H, m, H$_{eq}$-6), 2.43 (1H, m, H$_{ax}$-19), 2.51 (1H, m, H$_{eq}$-19), 2.94 (1H, m, H$_{ax}$-6), 3.05-3.21 (2H, m, H$_2$-5), 4.02 (1H, s, H-3), 4.28-4.40 (2H, m, OCH$_2$CH$_3$), 6.00 (1H, s, H-15), 6.58 (1H, dd, H-11), 6.74 (1H, d, H-9), 6.97 (1H, d, H-12), 8.89 (1H, brs, OH); MS (ESI, CID=20 %) m/z (rel. int. %): 367(/M+H$/^+$, 33), 350(8), 339(81), 324(100), 310(40), 295(31), 293(10), 282(5), 252(8). Mass accuracy was between -0.46 and -0.24 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 367.20164 (delta: 0.06 ppm), calculated value for C$_{22}$H$_{27}$O$_3$N$_2$: 367.20162.

**(±)-Methyl trans-1-ethyl-4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizine-1-propionate ((±)-21).** Tryptamine (20) (57 g, 0.35 mol) and dimethyl 3-ethyl-3-formyl-pimelate$^{14}$ (100 g, 0.41 mol) was refluxed in toluene (450 mL) for 2 h. After evaporation in vacuo, the residue was dissolved in acetic acid (200 mL) and refluxed for 2 h. Water (500 mL) and dichloromethane was added to the cooled reaction mixture and the pH was adjusted at 25 °C to 9 with 20% sodium hydroxide solution. The organic layer was washed with water (100 mL) and evaporated. The residue was refluxed in ethyl acetate (530 mL), then stirred for 2 h at r.t. The separated crystals of cis and trans (40:60 ratio, according to HPLC) methyl indoloquinolizine propionates (60 g) was refluxed in ethanol (600 mL) for 1 h, then stirred for 40 °C for 1 h to obtain 24 g (20%) of (±)-21, mp 199-200 °C. IR (cm$^{-1}$): 3300, 2920, 1443, 1628, 1467, 1304, 1168, 736; $^1$H NMR (CDCl$_3$, 25 °C, $\delta$$_{TMS}$=0.00 ppm): 0.72 (3H, t, CH$_2$CH$_3$), 0.99 (1H, dq, CH$_3$H$_2$CH$_3$), 1.48 (1H, dq, CH$_3$H$_2$CH$_3$), 1.64 (1H, m, H$_{ax}$-2), 1.78 (1H, m, H$_{ax}$-2), 2.07 (1H, m, CH$_3$H$_2$CH$_2$COOMe), 2.28 (1H, m, CH$_3$H$_2$CH$_2$COOMe), 2.40-2.58 (2H, m, H$_2$-3), 2.54-2.66 (2H, m, CH$_2$CH$_2$COOMe), 2.70 (1H, m, H$_{ax}$-6), 2.72-2.80 (2H, m, H$_2$-7), 3.80 (1H, s, OMe), 4.77 (1H, s, H-12b), 5.14 (1H, m, H$_{eq}$-6), 7.19 (1H, td, H-9), 7.19 (1H, td, H-10), 7.43 (1H, d, H-11), 7.51 (1H, d, H-8), 9.28 (1H, brs, NH); MS (ESI, CID=19 %) m/z (rel. int. %): 355(/M+H$/^+$, 30); 337(23), 323(100), 305(5), 295(1), 244(3), 212(8), 144(2). Mass accuracy was between -0.96 and -0.32 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 355.20165 (delta: 0.09 ppm), calculated value for C$_{21}$H$_{27}$O$_3$N$_2$: 355.20162.

**(1S,12bR)-1-Ethyl-4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizine-1-propionic acid (22).** A solution of (±)-21 (24 g, 67 mmol) in methanol (108 mL) and sodium hydroxide 20% solution (18.5 mL) was stirred for 2 h. The pH was adjusted to 6 with concd HCl solution, the solvent was evaporated in vacuo, then the solution was further acidified to pH 3. the
precipitated crystals were filtered to obtain 22.5 g of racem o xoacid, which was suspended in acetone (375 ml). (–)- Ephedrine (11 g, 67 mmol) in acetone (110 mL) was added and the mixture was stirred for 2.5 h, the separated (–)-oxoacid ephedrine salt was filtered and the filtrate was evaporated in vacuo, the residue was treated with water (190 mL) and acetic acid (7.5 mL). the precipitated crystals were refluxed in ethanol (110 mL), filtered, and evaporated to 15 mL, and stirred for 2 h at 0 °C to yield (+)-22 (6 g, 26%), mp 191-192°C, \([\alpha]_D^+145\) (c 1, AcOH). IR (cm–1): 3401, 2943, 1708, 1596, 1468, 1414, 1309, 1227, 741; \(^1\)H NMR (DMSO-\(d_6\), 25 °C, \(\delta\)TMS=0.00 ppm): 0.64 (3H, t, CH\(_2\)CH\(_3\)), 0.89 (1H, dq, CH\(_x\)HyCH\(_3\)), 1.25 (1H, dq, CH\(_x\)H\(_y\)CH\(_3\)), 1.49 (1H, m, H\(_x\)-2), 1.82 (1H, m, H\(_y\)-2), 1.93-2.10 (2H, m, CH\(_2\)CH\(_2\)COOMe), 2.23 (1H, m, H\(_x\)-3), 2.32-2.44 (2H, m, H\(_y\)-3, CH\(_x\)H\(_y\)COOMe), 2.48-2.74 (4H, m, H\(_ax\)-6, H\(_2\)-7, CH\(_2\)CH\(_x\)H\(_y\)COOMe), 4.85 (1H, s, H-12b); 5.86 (1H, m, H\(_{eq}\)-6), 6.97 (1H, td, H-9), 7.06 (1H, td, H-10), 7.42 (2H, d, H-8, H-11), 7.51 (1H, d, H-8); 10.45 (1H, brs, NH); MS (ESI, CID=17 %) m/z (rel. int. %): 341(+/M+H/+ 15), 323(100), 305(1.1), 198(2.2), 144(1.4). Mass accuracy was between -0.82 and -0.25 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 341.18597 (delta: 0 ppm), calculated value for C\(_{20}\)H\(_{25}\)O\(_3\)N\(_2\): 341.18597.

(1S,12bR)-Methyl 1-ethyl-4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizine-1-propionate (21a). A mixture of 22 (6 g, 17.6 mmol), methanol (50 mL), and KOH (1 g, 18 mmol) was stirred for 0.5 h, then evaporated in vacuo to dryness. The residue was dissolved in DMF (24 mL) and stirred with MeI (5 g, 35 mmol) for 3 h. The reaction mixture was diluted with water (120 mL), stirred for 1 h at 10 °C, the separated crystals were filtered to give 21a (6.1 g, 97%), mp 190-191 °C (MeOH), \([\alpha]_D^+131\) (c 1, MeOH). IR (cm–1): 3362, 2934, 1736, 1648, 1620, 1463, 1415, 1306, 1195, 739; \(^1\)H NMR (CDCl\(_3\), 25 °C, \(\delta\)TMS=0.00 ppm): 0.72 (3H, t, CH\(_2\)CH\(_3\)), 0.99 (1H, dq, CH\(_x\)HyCH\(_3\)), 1.48 (1H, dq, CH\(_x\)HyCH\(_3\)), 1.64 (1H, m, H\(_x\)-2), 1.78 (1H, m, H\(_x\)-2), 2.07 (1H, m, CH\(_x\)H\(_y\)CH2COOMe), 2.28 (1H, m, CH\(_x\)H\(_y\)CH2COOMe), 2.40-2.58 (2H, m, H\(_2\)-3), 2.54-2.66 (2H, m, CH\(_2\)CH\(_2\)COOMe), 2.70 (1H, m, H\(_{eq}\)-6), 2.72-2.80 (2H, m, H-2), 3.80 (1H, s, OMe), 4.77 (1H, s, H-12b), 5.14 (1H, m, H\(_{eq}\)-6), 7.19 (1H, td, H-9), 7.19 (1H, td, H-10), 7.43 (1H, d, H-11), 7.51 (1H, d, H-8); 9.28 (1H, brs, NH); MS (ESI, CID=19 %) m/z (rel. int. %): 355(+/M+H/+ 20), 337(22), 323(100), 305(3), 244(2), 212(43), 144(2). Mass accuracy was between -0.93 and -0.39 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 355.20162 (delta: 0 ppm), calculated value for C\(_{21}\)H\(_{27}\)O\(_3\)N\(_2\): 355.20162.

(1S,12bR)-Methyl 1-ethyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizine-1-propionate (23) from (21a). A solution of 21a (1 g, 2.8 mmol) in THF (10 mL) was stirred with P\(_2\)S\(_5\) (0.7 g, 1.6 mmol) for 1 h. The reaction mixture was filtered, the filtrate was added to Raney-Nickel (4 g) in THF (10 mL), the mixture was stirred for 1 h, filtered, evaporated in vacuo, the residue was crystallized from methanol, to obtain 23 (0.4 g, 42%), mp 106-107°C, \([\alpha]_D^+65\) (c 1, CHCl\(_3\)) \{lit\(^4\) mp 108-109°C, \([\alpha]_D^+69\)\}.

(3R,16S)-14,16-Dioxo-E-homoeburnane (24). To a solution of 21a (6 g, 17 mmol) in toluene potassium tert-butoxide (3 g, 27 mmol) was charged at 30 °C, under nitrogen, and stirred for 1 h. The reaction mixture was treated with a solution of water (60 mL) and acetic acid (2 mL), filtered, the organic layer was evaporated and the residue was crystallized from methanol to give...
24 (4.2 g, 77%) mp 146-148 °C, [α]D +121 (c 1, DMF). IR (cm⁻¹): 3319, 2929, 1707, 1650, 1622, 1458, 1414, 1317, 759; ¹H NMR (CDCl₃, 25 °C, δTMS=0.00 ppm): 0.77 (3H, t, H₃-21), 0.91 (1H, dq, Hₓ-20), 1.38 (1H, dq, Hₜ-20), 1.70 (1H, dt, Hₓ⁻15), 1.78 (1H, td, Hₜ⁻17), 1.89 (1H, ddd, Hₑq⁻17), 2.12 (1H, td, Hₓ⁻15), 2.39 (1H, ddd, Hₜ⁻18), 2.53 (1H, ddd, Hₑq⁻18), 2.71-2.86 (4H, m, Hₑq⁻14a, Hₜ⁻5, H₂⁻6), 3.00 (1H, td, Hₜ⁻14a), 4.95 (1H, s, H⁻3), 5.17 (1H, m, H⁻9), 7.28-7.40 (2H, m, H⁻10, H⁻11), 7.47 (1H, d, H⁻9); MS (ESI, CID=21 %) m/z (rel. int. %): 323/(M+H⁺, 14), 305(23), 295(100), 280(22), 266(53), 252(29), 238(11), 212(11), 184(4). Mass accuracy was between -0.72 and -0.47 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 323.17545 (delta: 0.14 ppm), calculated value for C₂₀H₂₃O₂N₂: 323.17540.

(3R,16S)-14,16-Dioxo-15-hydroxyimino-E-homoeburnane (25). To a solution of 24 (4 g, 12.4 mmol) in toluene (60 mL) tert-butyl nitrite (2.1 g, 20 mmol) in toluene (5 mL), then sodium tert-butoxide (1.9 g, 20 mmol) was added under nitrogen, at 28 °C, and stirred for 3 h. The reaction mixture was treated with a solution of water (30 mL) and acetic acid (3 mL), then diluted with water (150 mL). The separated crystals were filtered to yield 25 (2 g, 46%), mp 233-234 °C, [α]D +119 (c 1, DMF). IR (cm⁻¹): 3159, 2928, 1698, 1614, 1457, 1324, 1259, 1008, 855, 759; ¹H NMR (DMSO-d₆, 25 °C, δTMS=0.00 ppm); the spectrum shows two signal sets owing to the slowly exchanging oxime isomers in a ca. 1.2 : 0.8 Z/E ratio; characteristic signals: 2.19 (d, Hₓ⁻15'), 2.39 (d, Hₓ⁻15), 2.82 (d, Hₓ⁻15'), 3.25 (d, Hₓ⁻15), 4.78-4.96 (m, H⁻3, H⁻3', Hᵧ⁻5, Hᵧ⁻5'), 7.58 (d, H⁻9, H⁻9'), 8.28 (d, H⁻12'), 8.40 (d, H⁻12), 11.79 (brs, NOH), 12.64 (brs, NOH'); MS (ESI, CID=20 %) m/z (rel. int. %): 352/(M+H⁺, 17), 335(40), 323(11), 307(100), 295(62), 266(13), 251(28), 239(47), 224(25), 211(5). Mass accuracy was between -0.91 and -0.76 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 352.16558 (delta: 0.03 ppm), calculated value for C₂₀H₂₂O₃N₃: 352.16557.

(3R,16S,14R)-19-Oxovincamine (26). A solution of 25 (2 g, 5.7 mmol), p-toluenesulfonyl acid hydrate (1.3 g, 6.7 mmol), and paraformaldehyde (1.24 g, 40 mmol) in acetic acid (38 mL) was stirred at 100 °C. After 4 h the reaction mixture was cooled, treated with water (120 mL) and extracted with dichloromethane (2×30 mL). The organic layer was washed with water (2×20 mL) and NaHCO₃ 1% solution (2×20 mL), dried (MgSO₄) and evaporated. The residue was dissolved in tertBuOK/methanol (0.2 g in 6 mL) and stirred under nitrogen for 10 h. The reaction mixture was treated with acetic acid (0.1 mL), then filtered, the filtrate was evaporated to 5 mL and poured to water (20 mL) to obtain 26 (1.35 g, 64%), mp 133-134 °C (MeOH), [α]D +99 (c 1, CHCl₃). IR (cm⁻¹): 3342, 2952, 1746, 1626, 1440, 1305, 1137, 1034, 744; ¹H NMR (CDCl₃, 25 °C, δTMS=0.00 ppm): 0.86 (3H, t, H₃-21), 1.20 (1H, dq, Hₓ-20), 1.37 (1H, dq, Hₓ⁻20), 1.56 (1H, td, Hₓ⁻17), 2.00 (1H, ddd, Hₑq⁻17), 2.18 (1H, d, Hₓ⁻15), 2.41 (1H, d, Hₓ⁻15), 2.46-2.60 (2H, m, H₂⁻18), 2.76-2.84 (2H, m, H₂⁻6), 3.06 (1H, m, Hₓ⁻5), 3.88 (3H, s, OMe), 4.44 (1H, s, H⁻3), 4.93 (1H, m, Hₑq⁻5), 7.10-7.20 (3H, m, H⁻10, H⁻11, H⁻12), 7.50 (1H, m, H⁻9); MS (ESI, CID=19 %) m/z (rel. int. %): 369/(M+H⁺, 6), 351(3), 337(34), 322(11), 309(100), 292(11), 250(8). Mass accuracy was between -0.53 and -0.24 ppm for the fragment ions. The protonated molecular ion
peak can be detected at m/z 369.18085 (delta: -0.09 ppm), calculated value for C_{21}H_{25}O_{4}N_{2}: 369.18088.

(3R,16S)-19-Oxoapovincamine (27). To vincamine 26 (1.3 g, 3.5 mmol) in a mixture of dichloromethane (25 mL) and formic acid (2 mL) acetyl chloride (1.2 mL, 16 mmol) was added and stirred for 2 h. The reaction mixture was treated with water (15 mL), the organic layer was washed with 10% NaHCO₃ solution (5 mL) and evaporated in vacuo. The residue was crystallized from methanol, to yield 27 (0.95 g, 77.5%), mp 119-120 °C (MeOH), [α]D +36 (c 1, CHCl₃). IR (cm⁻¹): 3418, 2933, 1730, 1650, 1439, 1264, 1194, 1085, 745; ¹H NMR (CDCl₃, 25 °C, δ_TMS=0.00 ppm): 0.51 (1H, dq, Hₓ-20), 0.72 (3H, t, H₃-21), 1.43 (1H, dq, Hᵧ-20), 1.90 (1H, m, Hₓ-17), 2.16 (1H, m, Hₑq-17), 2.52 (1H, ddd, Hₓ-18), 2.63 (1H, dd, Hₑq-18), 2.78-2.86 (2H, m, H₂-6), 3.10 (1H, m, Hₓ-5), 3.98 (3H, s, OMe), 4.52 (1H, s, H-3); 4.98 (1H, m, Hₑq-5), 6.39 (1H, s, H-15), 7.16-7.24 (2H, m, H-10, H-11), 7.34 (1H, m, H-12); 7.49 (1H, m, H-9); MS (ESI, CID=19 %) m/z (rel. int. %): 351/(M+H⁺, 52), 319(100), 291(20), 240(61), 208(26). Mass accuracy was between -0.67 and -0.48 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 351.17035 (delta: 0.09 ppm), calculated value for C_{21}H_{23}O_{3}N_{2}: 351.17032.

(3R,16S)-2’Hydroxyethyl 19-oxo-apovincaminate (10). To the solution of 27 (0.9 g, 2.5 mmol) in ethylene glycol (9 mL) potassium tert-butoxide (0.05 g, 0.4 mmol) was added. The reaction mixture was stirred at 100 °C for 2 h. After cooling to r.t. the solution was treated with acetic acid/water (0.05ml/45 ml) to obtain 0.77 g (80% yield) of 10, mp 115-117 °C (MeOH), [α]D +25 (c 1, MeOH). IR (cm⁻¹): 3401, 2965, 1725, 1631, 1438, 1265, 1194, 1079, 746; ¹H NMR (CDCl₃, 25 °C, δ_TMS=0.00 ppm): 0.51 (1H, dq, Hₓ-20), 0.67 (3H, t, H₃-21), 1.37 (1H, dq, Hᵧ-20), 1.98 (1H, m, Hₓ-17), 2.08 (1H, m, Hₑq-17), 2.32-2.52 (2H, m, Hₑq-6), 2.68 (1H, m, Hₓ-6), 2.80 (1H, m, Hₑq-6), 3.06 (1H, m, Hₓ-15), 3.71 (2H, m, CO₂CH₂CH₂OH), 4.37 (2H, m, CO₂CH₃CH₂OH), 4.68 (1H, s, H-3), 4.76 (1H, dd, Hₑq-5), 4.50 (1H, brs, OH), 6.55 (1H, s, H-15), 7.10-7.18 (2H, m, H-10, H-11), 7.35 (1H, m, H-12), 7.50 (1H, m, H-9); MS (ESI, CID=20 %) m/z (rel. int. %): 381/(M+H⁺, 100), 363(5), 352(8), 337(53), 319(96), 291(28), 270(33), 208(91). Mass accuracy was between -0.55 and -0.34 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 381.18080 (delta: -0.22 ppm), calculated value for C_{22}H_{25}O_{4}N_{2}: 381.18088.

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