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Isolation and Structure Elucidation of Cruentarens A and B − Novel Members of the Benzolactone Class of ATPase Inhibitors from the Myxobacterium *Byssovorax cruenta*[‡]

Larissa Jundt,[a] Heinrich Steinmetz,[a] Peter Luger,[b] Manuela Weber,[b] Brigitte Kunze,[a] Hans Reichenbach,[a] and Gerhard Höfle*[a]

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Two novel secondary metabolites, namely cruentaren A (**1**) and B (**2**), have been isolated from the myxobacterium *Byssovorax cruenta*. Their structures have been elucidated by detailed NMR spectroscopic analysis. Both compounds are isomers of a C_{22} polyketide with a 2-hydroxy-4-methoxybenzoic acid terminus, which forms a 12-membered lactone in **1** and a six-membered lactone in **2**. An amino group at C-22 is acylated by a 3-hydroxy-2-methylhexanoic acid group whose absolute configuration has been determined by GC analysis after hydrolysis and stereoselective synthesis. Degradation of **2** by olefin cross-metathesis in the presence of ethylene yields the C-12 to C-21 fragment **8**, whose relative configuration has been predicted applying Kishi's ^{13}C NMR spectroscopic data base approach, and whose absolute configuration has been proven by comparison with two synthetic stereoisomers. The configuration of C-9 and C-10 has been determined by Mosher's method and NMR spectroscopy. Degradation of cruentaren A (**1**) by olefin cross-metathesis in the presence of ethylene gives crystalline 21,22-*seco*-cruentaren (**14**). Its crystal structure analysis reveals the relative configuration and conformation of the macrocycle and enables solution conformation analysis. The high cytotoxic activity of **1** is essentially lost on derivatisation or rearrangement to **2**.

Introduction

Myxobacteria are a particularly rich source of cytotoxic secondary metabolites.[1] In addition to 12 novel inhibitors of electron transport and seven structurally diverse compounds that interact with different components of the cytoskeleton like the epothilones[2] and tubulysins,[3] we have also isolated specific inhibitors of functional proteins. Thus, ratjadon inhibits the nuclear transporter CRM1,[4] and apicularen[5] and archazolid[5] inhibit mammalian V-ATPase, while for other highly toxic compounds like the spirangiens[6] the target has not yet been identified. Here, we describe the isolation and structural elucidation of cruentaren A and B (**1** and **2**) from the myxobacterium *Byssovorax cruenta*.[7] With an IC_{50} of 1.2 ng mL^{-1} for our standard mouse fibroblast cell line L929, cruentaren A (**1**) is one of the most cytotoxic metabolites from myxobacteria.[8] From its structural resemblance to apicularen it was hypothesized that it might also be an ATPase inhibitor. Indeed, preliminary results indicate that it efficiently inhibits mitochondrial F-ATPase from baker's yeast.[8]

Results and Discussion

Byssovorax cruenta strain Ha r1 was first identified in a screening for antifungal activity, and cytotoxic activity was later also observed.[8] HPLC analysis of the culture extracts indicated the presence of several different types of metabolites, of which only one turned out to be responsible for the biological activity. A 90-L bioreactor was run in the presence of 1% of amberlite XAD adsorber resin to bind excreted secondary metabolites. Adsorber resin and cell mass were harvested by centrifugation and extracted with acetone to give 10 g of crude extract. Consecutive chromatography on silica gel and RP-18 material yielded 270 mg of cruentaren A (**1**) and 7 mg of cruentaren B (**2**) as colourless amorphous solids. HR-MS characterisation indicated that both compounds are isomers with the elemental composition $C_{33}H_{51}NO_8$. Their UV spectra, with strong bands at 216, 265, and 302 nm, are also essentially identical and indicate the presence of a substituted aromatic system. In the ^1H and ^{13}C NMR spectra of cruentaren A signals for a

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[a] Bereich Naturstoffe, Gesellschaft für Biotechnologische Forschung mbH,
Mascheroder Weg 1, 38124 Braunschweig, Germany
E-mail: gho@gbf.de
[b] Institut für Chemie und Biochemie/Kristallographie, Freie Universität Berlin,
Takustr. 6, 14195 Berlin, Germany

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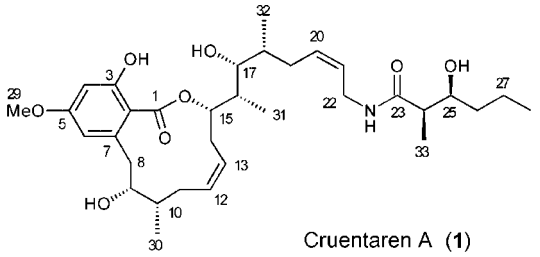
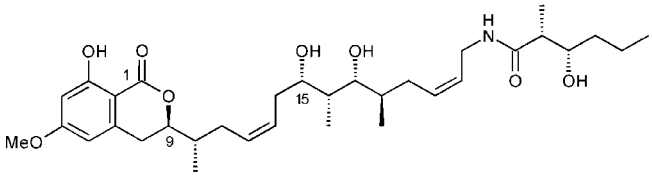
2,4,6-substituted benzoyl residue were identified. A hydroxy group, which forms a strong hydrogen bond (δ = 11.48 pm), is situated next to the carbonyl group and shifts the IR carbonyl band to 1646 cm^{-1}. A methoxy substituent at C-5 is indicated by correlation of this carbon with the methoxy protons in the HMBC spectrum. Starting from the benzylic methylene group at C-8, an extended substituted carbon chain could be derived in a straightforward manner from the combined NMR spectroscopic data in Table 1. The chain terminates with an amide group at C-22. Both the C-22 methylene and the amide protons are coupled to the carbonyl carbon of a 3-hydroxy-2-methylhexanoic acid residue. Finally, a three-bond coupling between 15-H and the benzoyl carbonyl carbon proves the presence of a 12-membered lactone in cruentaren A (**1**).



Cruentaren A (**1**)

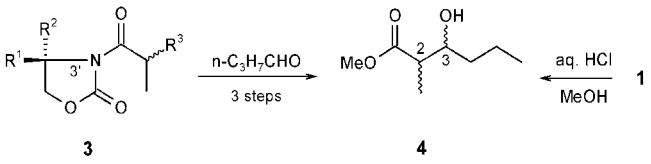


Cruentaren B (**2**)

The NMR spectroscopic data of cruentaren B (**2**) are very similar to those of **1**, with the exception that 9-H and 15-H are shifted by 0.72 ppm downfield and 1.44 ppm upfield, respectively. This indicates a six-membered lactone ring for cruentaren B (**2**), which is apparently formed by an intramolecular transesterification driven by release of ring strain. On a preparative scale, this rearrangement was achieved in quantitative yield by acid or base catalysis.

The configuration of the 12,13 double bond in **1** and **2** is *cis* according to the vicinal coupling constant of 11 Hz. Whereas the olefinic proton signals for 20-H and 21-H are not resolved, the *cis* configuration follows from the relatively high-field shifts of the allylic carbons C-19 and C-22.

After attempts to crystallise **1**, **2** and several of its derivatives failed, the relative and absolute configuration of the eight stereocentres had to be determined from NMR spectroscopic data and degradation studies. Fortunately, the stereocentres in **2** are clustered in the *N*-acyl group, the central diol part and the isochromanone system. Total acidic hydrolysis of **1** and **2** yielded 3-hydroxy-2-methylhexanoic acid (**4**) as a single isomer, as shown by chiral GC analysis on a cyclodextrin column.



All four stereoisomers of **4** were synthesised from **3** by Evans aldol (R^3 = H)[9] and chromium Reformatzky reactions (R^3 = Br).[10] The (2*R*,3*S*) configuration for the degradation product was determined by chiral GC cochromatography, and from this the (24*R*,25*S*) configuration for cruentaren A and B.

The relative configuration of the four stereocentres in the central part of the molecules is best analysed with cruentaren B. To predict the most probable of the eight possible diastereomers we adopted the ^{13}C NMR spectroscopic data base approach developed by Kishi et al.[11] for two contiguous propionate units in polyketide natural products. Two diastereomers with all-*syn* and *syn-syn-anti* configurations (A and B, Figure 1) were predicted with high significance.

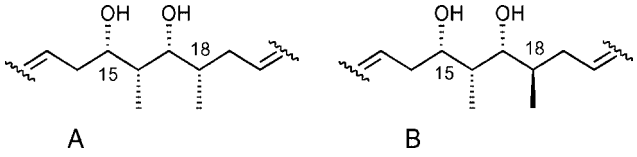
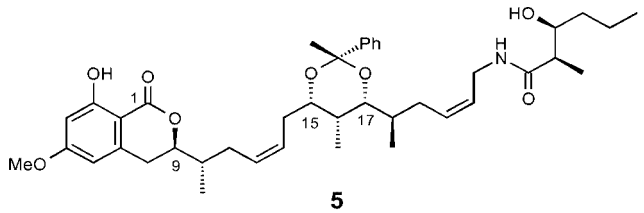


Figure 1. Relative configuration of C-15 to C-18 of cruentaren B predicted using Kishi's ^{13}C NMR chemical shift analysis.[11]

To decide which was the correct diastereomer and determine its absolute configuration, the central part of cruentaren B had to be isolated by cleavage of the adjacent double bonds and compared with reference samples from stereoselective synthesis.[7] To facilitate isolation of the degradation products, a UV-active handle was introduced by acetalisation with acetophenone to give mono- and diprotected cruentaren B **5** and **6**. Only one stereoisomer was formed at each of the newly created benzylic positions, however at this stage their configuration was not known.



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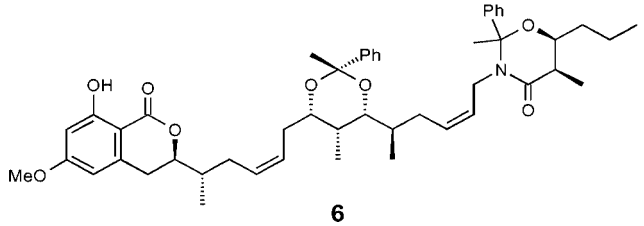

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Table 1. ^{1}H and ^{13}C NMR spectroscopic data of cruentaren A (**1**) in $CDCl_3$[a] (600 and 100 MHz).

H atom	δ [ppm] Multiplicity	J [Hz]	C atom	δ [ppm]	HMBC Correlated H atoms
3-OH	11.48 br. s		C-1	171.5	4, 15
4-H	6.35 d	2.7	C-2	105.0	4, 6, 8a,b
6-H	6.30 d	2.7	C-3	165.8	4
8-H_a	2.20–2.30 m		C-4	99.7	6
8-H_b	3.75 dd	12.8, 1.8	C-5	163.6	4, 6, 29
9-H	3.63 ddd	10.8, 3.0, 1.8	C-6	112.4	4, 8a,b
10-H	1.92–2.06 m		C-7	143.7	8a,b
11-H_a	1.92–2.06 m		C-8	36.7	6, 9, 11b
11-H_b	2.32 dt	14.3, 11.7	C-9	73.1	8a, 11b, 30
12-H	5.50 ddd	11.0, 2.9, 1.0	C-10	38.2	11b, 30
13-H	5.44 ddd	11.0, 4.5, 2.0	C-11	31.7	13, 30
14-H_a	2.20–2.30 m		C-12	132.2	11b,14b
14-H_b	2.82 dt	14.3, 11.5	C-13	125.8	11b,14b
15-H	5.29 ddd	11.7, 5.6, 2.0	C-14	29.9	12, 13, 15, 16
16-H	1.92–2.06 m		C-15	78.1	14b, 16, 17, 31
17-H	3.45 dd	9.2, 2.3	C-16	39.3	16, 31
17-OH	2.70 sbr		C-17	74.8	15, 18, 19, 31, 32
18-H	1.70 qddd	6.8, 6.8, 2.3, 2.0	C-18	36.9	17, 19, 32
19-H_2	2.20–2.30 m		C-19	30.7	18, 20, 21, 32
20-H	5.54–5.59 m		C-20	131.0	18, 19, 21, 22a,b
21-H	5.38–5.42 m		C-21	126.8	19, 20, 22a,b
22-H_a	3.80–3.84 m		C-22	36.6	20, 21
22-H_b	3.91 dddd	14.8, 7.4, 5.8, 1.5	C-23	176.5	22a,b, 24, 25, 33
22-NH	6.09 br. t	5.6	C-24	44.9	33
24-H	2.28 qd	7.2, 2.8	C-25	71.9	26a,b, 33
25-H	3.80–3.84 m		C-26	35.8	27a,b, 28
25-OH	1.58 br. s		C-27	19.3	25-OH, 26a,b, 28
26-H_a	1.28–1.32 m		C-28	14.1	26a,b, 27a,b
26-H_b	1.42–1.50 m		C-29	55.4	–
27-H_a	1.28–1.32 m		C-30	14.2	9, 11b
27-H_b	1.42–1.50 m		C-31	8.6	15, 16, 17
28-CH_3	0.92 t	7.1	C-32	16.2	18, 19
29-OCH_3	3.80 s		C-33	11.2	24
30-CH_3	1.01 d	6.8			
31-CH_3	0.89 d	7.0			
32-CH_3	0.79 d	6.8			
33-CH_3	1.14 d	7.2			

[a] Selected signals in [D_6]DMSO: δ = 10.58 (br. s, 3-OH), 4.05 (d, J = 6.0 Hz, 9-OH), 4.67 (d, J = 7.0 Hz, 17-OH), 7.84 (d, J = 5.5 Hz, 22-NH), 4.48 ppm (d, J = 5.8 Hz, 25-OH).

All attempts to oxidatively cleave the olefinic double bonds of **6**, optionally with reductive work-up, produced intractable mixtures instead of the expected dialdehyde or diol. Therefore we turned to the cleavage of double bonds by olefin metathesis in the presence of ethylene, which has worked well in a similar situation with epothilone[12] and spirangien.[7] Using the Hoveyda–Grubbs ruthenium catalyst we indeed observed cleavage of both double bonds at a similar rate. The individual fragments **7**, **8** and **9** were isolated in yields of 64, 48 and 69%.

In addition, "dimers" **10** and **11** were recovered in 25% and 24% yields. However, no non-natural dimers or oligomers were observed, which would have indicated C–C bond formation under the cleavage conditions.

Conformational analysis of **8** using proton coupling constants and selected strong NOEs proved the *syn-syn* configuration for C-15 to C-17, whereas a 10 Hz coupling between 17-H and 18-H indicated an *anti* configuration (Figure 2). At this stage the two stereoisomers of **8** in question became available as side products of an ongoing total synthesis of cruentaren B.[7] Of these, only the (15*R*,16*S*,17*S*,18*S*) isomer showed identical ^{1}H NMR chemical shifts and an optical rotation of the same magnitude but opposite sign. Thus, the central part of cruentaren B has a (15*S*,16*R*,17*R*,18*R*) configuration and that of cruentaren A has a (15*S*,16*S*,17*R*,18*R*) configuration.

An *anti* configuration for the isochromanone **7** degradation product followed from comparison of the vicinal 9-H, 10-H coupling of 6.6 Hz with model compounds, which show couplings of 4.2 and 5.7 Hz for the *syn* and *anti* diastereomers, respectively.[13] To apply Mosher's method for absolute configuration determination a free 9-hydroxy group is required. To this end, the phenolic hydroxy group was methylated and the lactone reduced to the diol **12a** with lithium aluminium hydride. When it turned out that a tetrahydropyran was always formed on attempted acylation, the benzylic hydroxy group was hydrogenated with palladium in acetic acid to give toluene derivative **12b**. Subsequent acylation with (*R*)- and (*S*)-Mosher's acid chloride smoothly produced the diastereomeric MTPA esters **13a** and **13b**. Although the important signals of the 10-H, 11-H_2 and 12-H_2 protons were obscured by multiplets, positive

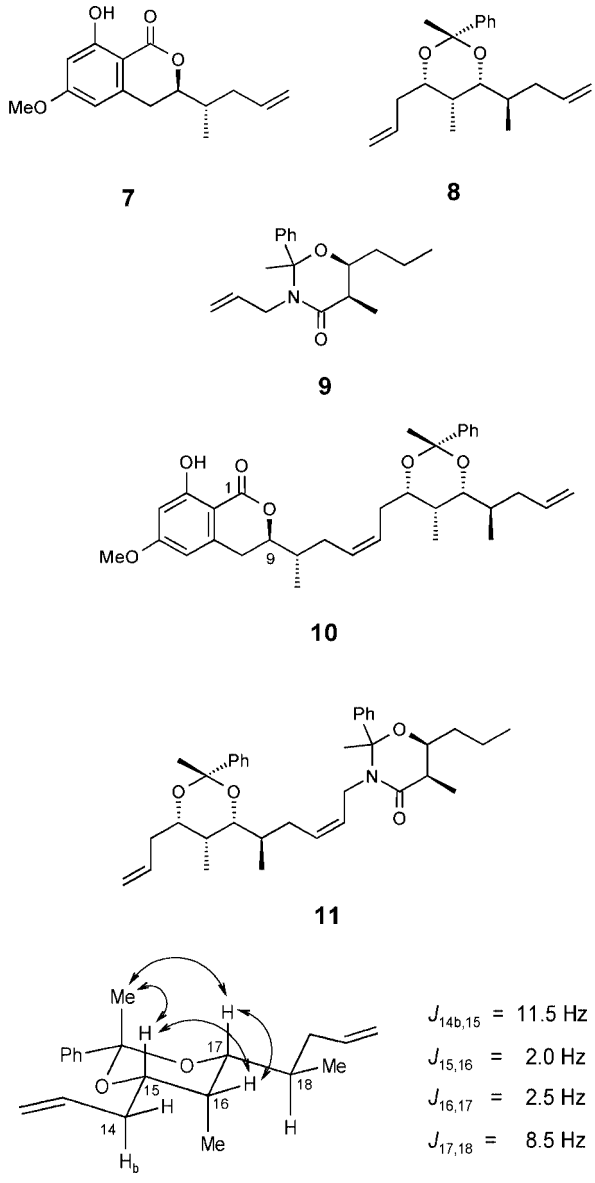
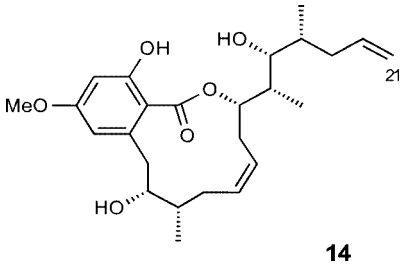


Figure 2. Relative configuration of **8** with selected NOEs and vicinal coupling constants.

$\Delta\delta$ values in the order of 0.02 to 0.08 ppm were observed for 1-H, 4-H, 6-H and 8-H_2, and a negative one of 0.12 ppm for the 30-methyl protons. This indicated a (9*R*) configuration for **13a**,**b**[14] and, according to the previously established relative configuration, a (9*R*,10*S*) configuration for cruentarens A and B.

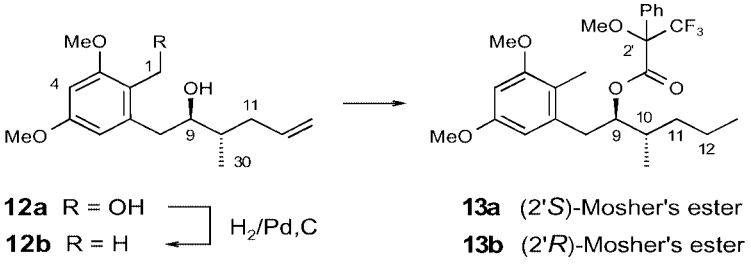
Having observed smooth cleavage of protected cruentaren B (**6**) by olefin metathesis, the behaviour of unprotected cruentaren A (**1**) in the presence of ethylene and various ruthenium catalysts was investigated. The highest conversion was observed with the Hoveyda–Grubbs catalyst, with an isolated yield of 61% of 21,22-*seco*-cruentaren A (**14**). No cleavage of the sterically hindered 12,13 double bond, and no dimer of **14** from cross metathesis was observed.



Fortunately, **14** crystallised from ethyl acetate and an X-ray crystal structure analysis could be performed. This not only proved the structure and relative configuration but also greatly facilitated solution conformation analysis. The conformation of the lactone ring in the crystal is characterised by a nearly antiperiplanar orientation of 8a-H/9-H, 11b-H/12-H, 13-H/14b-H and 14b-H/15-H. The lactone oxygen O-25 is the acceptor of an intramolecular hydrogen bond with the OH group OH-26 as donor [O-26···O-25 = 2.548(2), H-26···O-25 = 1.82(3) Å; O-26–H-26···O-25 = 147(2)°].

In chloroform solution the corresponding vicinal coupling constants are consistently in the range of 10–12 Hz. This, and the strong NOEs between 8b-H/11b-H and 14a-H/15-H, prove that the lactone ring in **14** adopts the same configuration as in the crystal (Figure 3, b). Also, the side-chain conformation seems to be retained from the 8.5 and 8.0 Hz coupling constants for 17-H/19b-H and 19b-H/20-H, whereas the C-15/C-16 bond, which connects the macrocycle with the side-chain, is rotated by about +120°, as can be seen from the 2 Hz coupling between 15-H/16-H and strong NOEs between 15-H/16-H/31-H_3 and 14a-H/31-H_3. (Figure 3, b). On the basis of this analysis it can be concluded that the lactone ring in cruentaren A (**1**) adopts the same conformation in chloroform, methanol and DMSO solution.

From a biosynthetic point of view cruentaren A is a tetradecaketide with a glycine building block inserted after the third ketide unit. From feeding of [1-^{13}C]propionate and



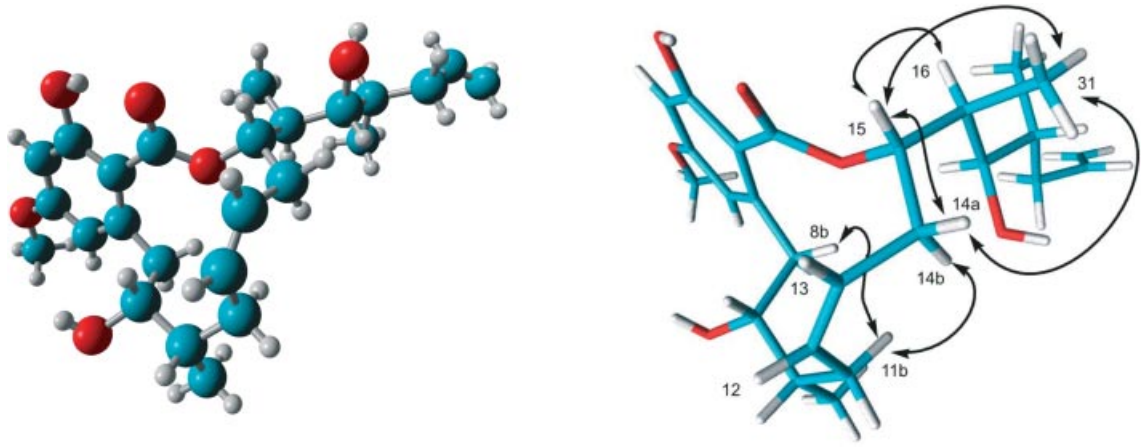
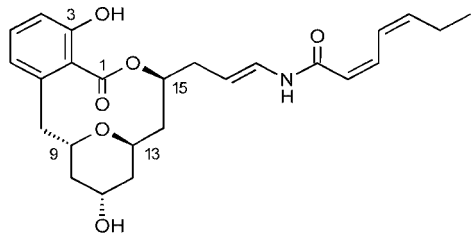
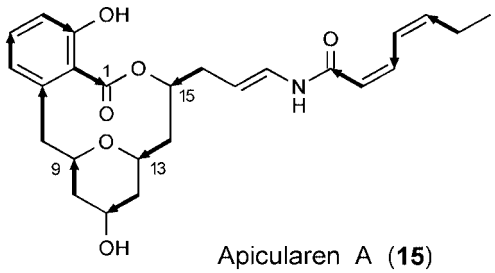


Figure 3. a) Crystal structure and b) proposed solution conformation with selected NOEs of 21,22-*seco*-cruentaren A (**14**).



Apicularen A (**15**)

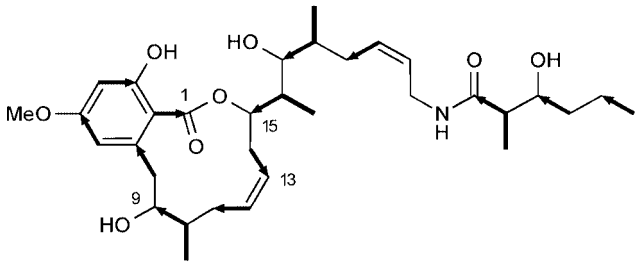


Apicularen A (**15**)

[$^{13}CH_3$]methionine it follows that all four *C*-methyl groups originate from incorporation of propionate. Contrary to that, the structurally related apicularen A (**15**) is an undecaketide assembled solely from acetate.[15] The glycine building block in apicularen is transformed into an *N*-acylvinylamine, whereas in cruentaren it is transformed into an *N*-acylallylamine. It can be speculated whether the PKS/NRPS synthases have evolved from a common ancestor or were derived from each other.

Both apicularen A and cruentaren A (Figure 4) are 12-membered lactones with the difference that 9-OH in apicularen is blocked in an ether ring which prevents isomerisation to a six-membered lactone like cruentaren A. The bicyclic lactone structure forces apicularen into a fundamentally different conformation, which also prevents hydrogen-bond formation between the lactone carbonyl and the 3-OH.[16] As a consequence, it is very unlikely that cruentaren A and apicularen A, in spite of their planar structural similarity, occupy the same protein binding site.

Preliminary data on the structure–activity relationship indicate that an intact macrolactone, both C=C double bonds and the acylamino part of the side-chain are essential for cytotoxic activity. However, it is not known what the exact structural requirements for the side-chain are. Preliminary experiments to obtain side-chain-modified cruentarens by olefin cross-metathesis of **14** with synthetic building blocks have failed so far.



Cruentaren A (**1**)

Figure 4. Acetate and propionate labelling patterns of apicularen A (**15**) and cruentaren A (**1**).

Experimental Section

General Procedures: Optical rotations were determined with a Perkin–Elmer 241 instrument. UV spectra were recorded with a Shimadzu UV-2102 PC scanning spectrometer. IR spectra were measured with a Nicolet 20DXB FT-IR spectrometer. NMR spectra were recorded in $CDCl_3$ with a Bruker DMX-600 spectrometer. For structural analogues only a selection of significant NMR spectroscopic data are given. EI and DCI mass spectra (reactant gas ammonia) were obtained with a Finnigan MAT 95 spectrometer; high resolution data were acquired using peak matching (M/DM = 10000). Pure compounds were characterised by analytical HPLC on Nucleosil C 18 (column 125 × 2 mm, 5 μm, flow 0.3 mL min^{-1}),

acetonitrile/water, isocratic 55:45, diode array detection. Preparative HPLC was performed on Nucleosil (column 250 × 21 mm, 7 μm, flow 18 mL min^{-1}), acetonitrile/water, 55:45, detection by UV absorption at 254 nm. Analytical TLC [TLC aluminium sheets silica gel Si 60 F_{254} (Merck), solvent: ethyl acetate], detection by UV absorption at 254 nm, dark blue spots on staining with cerium(IV) sulfate/phosphomolybdic acid in sulfuric acid followed by charring. Precoated silica gel Si 60 F_{254} plates of 0.25-mm layer thickness were used for preparative TLC. GC analysis: Shimadzu GC-17 A gas chromatograph with FID detection; capillary column HYDRODEX® β-PM [heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin], 25 m × 0.25 mm ID, temperature gradient: 5 min 80 °C, 6 °C min^{-1} to 180 °C. The Hoveyda–Grubbs catalyst [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(*o*-isopropoxyphenylmethylene)ruthenium was purchased from Aldrich.

Isolation of Cruentarens A (1) and B (2) from *Byssovorax cruenta*: A 90-L fermentation batch of strain Ha r1 grown in the presence of 0.9 L of Amberlite XAD 16 adsorber resin was harvested by centrifugation. The wet cell mass and adsorber resin mixture was extracted with four 3-L batches of acetone by stirring, sedimentation and decanting. The extract was analysed by HPLC to contain 290 mg of cruentaren A and about 10 mg of cruentaren B. It was then concentrated on a rotary evaporator at 30 °C/200 mbar to the water phase, which was extracted with three 0.5-L portions of ethyl acetate. The organic phase was dried with magnesium sulfate and evaporated at 30 °C/150 mbar. The resulting crude extract (10.0 g) was separated by chromatography on 180 g of silica gel Si 60 (15–40 μm, Merck) in an open glass column of 6 cm diameter. After elution of lipophilic components with 0.8 L of ethyl acetate/*n*-heptane (2:1) and 0.3 L of ethyl acetate the cruentarens were eluted with 0.7 L of ethyl acetate/methanol (8:2) at a flow of 10 mL min^{-1}. Cruentaren-containing fractions were identified by TLC, combined and the solvents evaporated in vacuo (320 mg). This material was further purified and separated by HPLC (RP-18, Kronlab ODS AQ 120 A, 16 μm, 3×48 cm, detection at 254 nm) with acetonitrile/water (55:45) at 18 mL min^{-1}. Cruentaren A and B eluted at 68–77 min and 100–110 min, respectively. The fractions were concentrated in vacuo to the water phase, extracted with two portions each of 100 mL of ethyl acetate, evaporated and dried to give 270 mg of cruentaren A and 7 mg of cruentaren B.

Preparation of Cruentaren B (2) from Cruentaren A (1): Compound **1** (100 mg) was dissolved in 1 mL of methanol, 0.8 mL of 1 N HCl was added and the mixture kept overnight at 45 °C. After dilution with water the mixture was adjusted to pH 7 with 1 N NaOH, the methanol evaporated in vacuo, the water phase extracted with ethyl acetate, dried with magnesium sulfate, and the solvents evaporated to dryness. Yield: 99 mg (99%) of a slightly yellow oil.

Cruentaren A (1): Colourless amorphous solid; t_R = 3.9 min (Nucleosil C18, 125 × 2 mm, 5 μm, acetonitrile/water 0.3 mL min^{-1}, diode array detection); R_f = 0.53 (silica gel Si 60 aluminium sheets, ethyl acetate). $[\alpha]_D^{22}$ = –3.4 (c = 13.5 mg mL^{-1}, MeOH). UV (MeOH): λ_{max} (lg ε) = 216 (4.26), 265 (3.98), 302 (3.66), 346 nm (1.94). IR (KBr): $\tilde{\nu}_{max}$ = 3396, 2959, 2932, 2863, 1646, 1614 cm^{-1}. NMR see Table 1. HRMS (DCI, NH_3) for $C_{33}H_{51}NO_8$: calcd. 589.3615; found 589.3645.

Cruentaren B (2): Colourless amorphous solid; t_R = 5.1 min (Nucleosil C18, 125 × 2 mm, 5 μm, acetonitrile/water 0.3 mL min^{-1}, diode array detection); R_f = 0.40 (silica gel Si 60 aluminium sheets, ethyl acetate). $[\alpha]_D^{22}$ = –9.1 (c = 6.6 mg mL^{-1}, MeOH). UV (MeOH) λ_{max} (lg ε) = 210 (4.36), 215 (4.38), 267 (4.14), 301 nm (3.77). IR (KBr): $\tilde{\nu}_{max}$ = 3416, 2963, 2930, 2869, 1661 cm^{-1}. NMR see Table S1 in the Supporting Information. HRDMS (DCI, NH_3) for $C_{33}H_{52}NO_8$: calcd. 590.3714; found 590.3693.

^{13}C-Labeled Cruentaren A: Two days after inoculation each of two 2-L batches of strain Ha r1 in the presence of Amberlite XAD16 (10 mL) was fed with [^{13}C-CH_3]methionine or sodium [1-^{13}C]propionate (1 g each, added in 5 portions, 95% enrichment) and cultivated for 7 and 8 d, respectively. Cell mass and adsorber resin were collected by centrifugation and extracted with acetone. From the crude extracts of 0.24 g and 0.12 g pure cruentaren A samples (7.7 and 2.1 mg) were isolated by RP18 HPLC as described above.

Methionine-Labelled Cruentaren A: ^{13}C NMR ($CDCl_3$): δ = 55.4 ppm (C-29) 2.8% ^{13}C.

Propionate-Labelled Cruentaren A: ^{13}C NMR ($CDCl_3$): δ = 73.1 (C-9, 3.1%), 78.1 (C-15, 3.0%), 74.8 (C-17, 2.4%), 176.4 (C-23, 3.5% ^{13}C).

Acetalisation of Cruentaren B (2): a) Cruentaren B (30 mg, 51 μmol), 1,1-dimethoxyethylbenzene (83 mg, 500 μmol) and pyridinium *p*-toluenesulfonate (17 mg, 71 μmol) were dissolved in 1 mL of dried dichloromethane under nitrogen. After stirring overnight saturated sodium hydrogen carbonate solution was added and the mixture extracted three times with diethyl ether. The ether solution was then washed with saturated sodium chloride solution, dried with magnesium sulfate and the solvents evaporated in vacuo. The residue was separated by chromatography on silica gel with diethyl ether as eluent to yield monoacetal **5** (5.6 mg, 16%) and diacetal **6** (8.7 mg, 21%).

b) Cruentaren B (**2**) (87 mg, 147 μmol), 1,1-(dimethoxyethyl)benzene (485 mg, 2.95 mmol) and pyridinium *p*-toluenesulfonate (51 mg, 206 μmol) were dissolved in 1 mL of dichloromethane. The rest of the procedure was performed as above to give diacetal **6** as a colourless solid (86.3 mg, 74%).

Cruentaren B Monoacetal (5): R_f = 0.58 (Et_2O). UV (MeOH): λ_{max} (lg ε) = 204 (4.5), 266 (4.2), 301 nm (3.8). IR (KBr): $\tilde{\nu}$ = 2961, 2874, 1666 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.83 (d, J = 6.61 Hz, 31-H_3, 32-H_3), 0.92 (t, J = 7.12 Hz, 28-H_3), 1.04 (d, J = 7.12 Hz, -H_3), 1.11 (d, J = 7.12 Hz, 33-H_3), 1.23–1.52 (m, 26-H_2, 27-H_2), 1.55 (s, 35-H_3), 1.60 (m, 1 H, 16-H), 1.68–1.83 (m, 18-H), 2.03 (ddd, J = 13.86, 8.39, 8.27 Hz, 10-H), 2.10–2.25 (m, 11b-H, 4b-H, 9b-H), 2.28–2.45 (m, 11a-H, 14a-H, 19a-H), 2.47–2.59 (m, 24-H), 2.71–2.84 (m, 8b-H), 2.83–2.98 (m, 8a-H), 3.67 (dd, J = 9.92, 1.78 Hz, 17-H), 3.81 (s, 29-H_3), 3.84–4.00 (m, 15-H, 22-H_2), 4.01–4.09 (m, 25-H), 4.36 (ddd, J = 12.21, 6.10, 3.05 Hz, 9-H), 5.40–5.70 (m, 12-H, 13-H, 21-H), 5.74 (br. t, 22-NH), 6.24 (d, J = 2.54 Hz, 6-H), 6.36 (d, J = 2.03 Hz, 4-H), 7.26–7.37 (m, 3 H, phenyl), 7.56 (d, J = 8.65 Hz, 2 H, phenyl), 11.21 (s, 3-OH) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 4.8 (C-31), 11.0 (C-33), 14.0 (C-28), 14.2 (C-32), 15.0 (C-30), 19.2 (C-27), 21.2 (C-35), 29.9 (C-8), 30.1 (C-11), 30.9 (C-19), 31.1 (C-14), 32.7 (C-16), 34.8 (C-18), 35.8 (C-26), 36.5 (C-22), 37.3 (C-10), 44.6 (C-24), 55.6 (C-29), 71.7 (C-25), 73.8 (C-15), 77.2 (C-17), 82.2 (C-9), 99.0 (C-34), 99.5 (C-4), 101.9 (C-2), 106.4 (C-6), 124.6 (C-37,C-38), 125.5, 127.60, 127.62, 128.0 (2 C), 128.6, 132.4 (C-12, C-13, C-20, C-21, C-39, C-40, C-41), 141.2 (C-7), 145.9 (C-36), 164.6 (C-3), 165.8 (C-5), 170.0 (C-1), 176.4 (C-23) ppm. EI-MS: *m*/*z* (%) 691 (16) [M$^+$], 676 (70), 554 (55), 409 (50), 256 (83). HREI-MS for $C_{41}H_{57}N_1O_8$: calcd. 691.4084; found 691.4079.

Cruentaren B Bisacetal (6): R_f = 0.75 (Et_2O). UV (MeOH): λ_{max} (lg ε) = 204 (4.5), 266 (4.2), 301 nm (3.8). $[\alpha]_D^{22}$ = –0.2 (c = 0.5, MeOH). IR (KBr): $\tilde{\nu}_{max}$ = 2962, 2874, 1665 cm^{-1}. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 0.73 (d, J = 6.76 Hz, 32-H_3), 0.78 (d, J = 7.03 Hz, 3-H_3), 0.91 (t, J = 7.17 Hz, 28-H_3), 1.02 (d, J = 6.76 Hz,

30-H_3), 1.25–1.33 (m, 27-H_2), 1.36 (d, J = 7.30 Hz, 33-H_3), 1.43–1.48 (m, 26b-H), 1.48–1.55 (m, 16-H, 26a-H), 1.56 (s, 43-H_3), 1.62–1.72 (m, 18-H), 1.80 (ddd, J = 14.00, 8.25, 8.05 Hz, 19b-H), 1.92 (s, 35-H_3), 1.99–2.05 (m, 10-H), 2.12–2.23 (m, 11b-H, 14bH), 2.28–2.39 (m, 11a-H, 14a-H, 19a-H), 2.50 (dq, J = 7.30, 2.71 Hz, 1 H, H24), 2.74 (dd, J = 16.23, 2.98 Hz, 1 H, H8b), 2.91 (dd, J = 16.10, 12.31 Hz, 1 H, H8a), 3.17 (dd, J = 15.15, 5.68 Hz, 1 H, H22b), 3.56 (dd, J = 9.87, 2.03 Hz, 1 H, H17), 3.81 (s, 3 H, H29), 3.96–4.04 (m, 2 H, H15, H22a), 4.19 (ddd, J = 8.25, 4.87, 2.84 Hz, 1 H, H25), 4.35 (ddd, J = 12.38, 6.29, 2.98 Hz, 1 H, H9), 5.32–5.58 (m, 12-H, 13-H, 20-H, 21-H), 6.23 (m, 6-H), 6.35 (d, J = 2.16 Hz, 4-H), 7.22–7.37 (m, 6 H, phenyl) 7.41–7.52 (m, 4 H, phenyl), 11.21 (s, 3-O*H*) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 4.8 (C-31), 12.0 (C-33), 13.9 (C-28), 14.0 (C-32), 14.9 (C-30), 19.0 (C-27), 21.1; 21.7 (C-43, C-35), 30.0 (C-8), 30.4 (C-11), 30.5 (C-19), 31.1 (C-14), 32.6 (C-16), 33.4 (C-26), 34.5 (C-18), 37.3 (C-10), 41.2 (C-24), 41.7 (C-22), 55.6 (C-29), 71.1 (C-25), 73.8 (C-15), 77.2 (C-17), 82.3 (C-9), 91.4; 98.9 (C-34, C-42), 99.5 (C-4), 101.9 (C-2), 106.3 (C-6), 124.6, 126.5 (4 C, phenyl), 127.5, 127.6, 127.7, 128.6, 128.9 (6 C, phenyl), 127.9 (C-13, C-20, C-21), 129.5 (C-12), 141.2 (C-7), 142.3, 145.9 (C-36, C-44), 164.6 (C-3), 165.8 (C-5), 170.0 (C-1), 172.2 (C-23) ppm. DCI-MS: m/z (%) = 811 (100) [M + NH_4^+]. HRDCI-MS for $C_{49}H_{64}NO_8$: calcd. 794.4614; found 794.4631.

Degradation of Cruentaren B Bisacetal (6) by Cross Metathesis with Ethylene: Cruentaren B Bisacetal (**6**; 66 mg, 83 μmol) and Hoveyda–Grubbbs catalyst (11 mg, 17 μmol) were dissolved in dried dichloromethane (10 mL). The atmosphere above the light-green solution was then replaced with ethylene. After stirring for four hours the solvent was evaporated in vacuo and the residue separated by preparative RP-18 HPLC (MeCN/H_2O, 57:43). The following compounds were obtained as black oils: **7** (13.8 mg, 63%), **8** (11.9 mg, 48%), **9** (16.4 mg, 69%), **10** (11.0 mg, 25%) and **11** (11.1 mg, 24%). Filtration through a bed of silica gel (PE/Et_2O, 1:1) gave light-yellow oils without loss of material.

Isochromanone 7: R_f = 0.69 (PE/Et_2O, 1:1). UV (MeOH): λ_{max} (lgε) = 215 (4.3), 267 (4.1), 301 nm (3.7). $[\alpha]_D^{22}$ = +32.6 (c = 0.7, MeOH). IR (KBr): $\tilde{\nu}$ = 3430 (w), 2922 (m), 2851 (w), 1667 (s), 1627 cm^{-1} (s). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.02 (d, J = 6.42 Hz, 14-H_3), 2.02 (td, J = 13.12, 6.61 Hz, 10-H), 2.05–2.12 (m, 11b-H), 2.33–2.46 (m, 11a-H), 2.77 (dd, J = 16.24, 3.02 Hz, 8b-H), 2.92 (dd, J = 16.05, 12.28 Hz, 8a-H), 3.81 (s, 15-H_3), 4.35 (ddd, J = 12.18, 6.33, 3.02 Hz, 9-H), 4.97–5.18 (m, 13-H_2), 5.64–5.92 (m, J = 17.14, 10.06, 6.99 Hz, 12-H), 6.25 (s, 6-H), 6.35 (d, J = 2.27 Hz, 4-H), 11.20 (d, 3-O*H*) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 14.7 (C-14), 29.9 (C-8), 36.5; 36.7 (C-11, C-10), 55.6 (C-15), 82.1 (C-9), 99.5 (C-4), 101.9 (C-2), 106.3 (C-6), 117.1 (C-13), 135.8 (C-12), 141.2 (C-7), 165.8 (C-5), 169.9 (C-1) ppm. MS (DCI): m/z (%) 280.9 (100) [M + NH_4^+], 102 (65). HRMS (DCI) for $C_{15}H_{18}O_4$: calcd. 262.1205; found 262.1199.

Diol Fragment 8: R_f = 0.79 (PE/Et_2O, 10:1). UV (MeOH): λ_{max} (lgε) = 209 (4.1), 249 (2.9), 255 (2.9), 262 nm (2.8). $[\alpha]_D^{22}$ = –4.0 (c = 0.2, MeOH). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 0.82 (d, J = 6.80 Hz, 1-H_3, 5-H_3), 1.57 (qt, J = 6.85, 2.28 Hz, 2-H), 1.63 (s, 14-H_3), 1.75–1.84 (m, J = 10.09, 7.94, 6.79, 3.38 Hz, 4-H), 2.03 (dt, J = 13.70, 8.26 Hz, 6b-H), 2.16–2.22 (m, 10b-H), 2.38–2.45 (m, 10a-H), 2.47–2.54 (m, J = 13.69, 6.14, 3.32, 1.60, 6a-H), 3.67 (dd, J = 9.96, 2.22 Hz, 3-H), 4.10 (ddd, J = 7.91, 6.02, 2.31 Hz, 9-H), 5.04 (ddd, J = 9.89, 2.22, 1.25 Hz, 8b-H), 5.06–5.10 (m, J = 9.02, 2.28, 1.17, 1.17 Hz, 8a-H), 5.15 (ddd, J = 17.15, 3.43, 1.65 Hz, 12-H), 5.70–6.02 (m, 7-H, 11-H), 7.27 (ddd, J = 7.25, 1.79, 1.68 Hz, 20-H), 7.33 (td, J = 7.29, 1.09 Hz, 16-H, 17-H), 7.58 (d, J = 7.03 Hz, 18-H, 19-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 4.8 (C-1), 13.9 (C-5), 21.3 (C-14), 32.6 (C-2), 33.9 (C-4), 37.4 (C-6), 37.5 (C-10), 73.7 (C-9), 77.3 (C-3), 98.9 (C-13), 116. (C-8), 116.8 (C-12), 124.7 (C-16, C-17), 127.4 (C-20), 127.9 (C-18,C-19), 134.9 (C-11), 136.9 (C-7), 146.2 (C-15) ppm. MS (DCI): m/z (%) 301 (100), [M + H^+] 198 (20). HRMS (DCI) for $C_{20}H_{28}O_2$: calcd. 300.2089; found 300.1949.

Amide Fragment 9: UV (MeOH): λ_{max} (lgε) = 209 (3.9), 250 nm (2.5). $[\alpha]_D^{22}$ = –40.1 (c = 4.2, MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.92 (t, J = 7.06 Hz, 6-H_3), 1.26–1.48 (m, 5-H_2), 1.36 (d, J = 7.35 Hz, 7-H_3), 1.48–1.64 (m, 4-H_2), 1.92 (s, 12-H_3), 2.51 (ddd, J = 14.55, 7.30, 2.83 Hz, 2-H), 2.98–3.20 (m, J = 15.59, 6.55, 1.27 Hz, 8b-H), 3.90–4.05 (m, J = 15.61, 5.02, 1.55 Hz, 8a-H), 4.16–4.26 (m, 3-H), 4.85–5.03 (m, 10-H_2), 5.60–5.80 (m, J = 17.07, 10.38, 6.45, 5.09 Hz, 9-H), 7.29–7.51 (m, 5 H, phenyl) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 12.0 (C-7), 14.0 (C-6), 19.0 (C-5), 21.9 (C-12), 33.3 (C-4), 41.2 (C-2), 46.8 (C-8), 71.1 (C-3), 91.5 (C-11), 116.3 (C-10), 126.5 (2-C)/128.6 (2-C)/128.9 (1-C) (C14–C-18), 134.0 (C-9), 142.2 (C-13), 172.2 (C-1) ppm. MS (EI): m/z (%) 287.0 (100) [M$^+$]. HRMS for $C_{18}H_{25}NO_2$: calcd. 287.1885; found 287.1905.

***O*-Methylation and Reduction of 7:** Isochromanone **7** (6 mg, 23 μmol), silver(I) oxide (5.6 mg, 240 mmol) and 1.5 mL of methyl iodide were stirred under an atmosphere of nitrogen at room temperature overnight. The reaction mixture was filtered through a bed of Celite, the Celite washed with dichloromethane and the combined solutions evaporated. The residue (6.1 mg) was dissolved in dried THF (200 μL) and a 1 M solution of lithium aluminium hydride in THF (43 μL, 43 μmol) was added with stirring at 0 °C under an atmosphere of nitrogen. After two hours the reaction was quenched with water. The mixture was then filtered through a bed of Celite, the Celite washed with THF and the combined solutions evaporated. The residue was purified by preparative TLC (silica gel, diethyl ether) to yield **12a** (5.2 mg, 85%) as a light-yellow oil. R_f = 0.62 (PE/Et_2O, 1:1). UV (MeOH): λ_{max} (lgε) = 210 (4.2), 229 (3.6), 283 nm (3.0). $[\alpha]_D^{22}$ = +78.1 (c = 0.8, MeOH). IR (KBr): $\tilde{\nu}$ = 3417 (w), 2959 (w), 2934 (w), 1749 (w), 1606 cm^{-1} (m). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.02 (d, J = 6.78 Hz, 14-H_3), 1.64–1.89 (m, 10-H), 1.95–2.18 (m, 11b-H), 2.23–2.51 (m, 11a-H), 2.67–3.03 (m, 8-H_2), 3.62 (ddd, J = 9.04, 5.37, 4.05 Hz, 9-H), 3.79, 3.82 (s, 15-H_3, 16-H_3), 4.54 (d, J = 11.49 Hz, 1b-H), 4.82 (d, J = 11.87 Hz, 1a-H), 4.97–5.15 (m, 13-H_2), 5.84 (td, J = 17.14, 7.16 Hz, 12-H), 6.32 (d, J = 2.26 Hz, 6-H), 6.36 (d, J = 2.45 Hz, 4-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 14.7 (C-14), 31.3 (C-8), 37.3 (C-11), 37.7 (C-10), 55.2, 55.4 (C-15, C-16), 77.7 (C-9), 96.1 (C-4), 104.4 (C-6), 116.1 (C-13), 116.4 (C-2), 135.7 (C-7), 137.1 (C-12), 156.5, 159.1 (C-3, C-5) ppm. MS (EI): m/z (%) 281 (70) [M + H^+], 280.1 (100) [M$^+$]. HRMS for $C_{16}H_{24}O_4$: calcd. 280.1675; found 280.1666.

2,4-Dimethoxytoluene Derivative 12b: A mixture of diol **12a** (15 mg), Pd/C (15 mg) and acetic acid (25 μL) in methanol (1 mL) was stirred under an atmosphere of hydrogen overnight. The mixture was then filtered through a bed of Celite, evaporated and separated by TLC on silica gel plates (PE/Et_2O, 1:1) to yield **12b** as a colourless oil (9.5 mg, 67%). R_f = 0.63 (PE/Et_2O, 1:1). UV (MeOH): λ_{max} (lgε) = 209 (4.3), 220 (sh, 3.9), 280 nm (3.5). IR (KBr): $\tilde{\nu}$ = 3464 (w), 2956 (s), 2933 (m), 2872 (m), 1607 cm^{-1} (s). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.93 (t, J = 7.12 Hz, 13-H_3), 0.99 (d, J = 6.61 Hz, 14-H), 1.16–1.35 (m, 11b-H, 12b-H12b), 1.43–1.60 (m, 11a-H, 12a-H), 1.66–1.74 (m, 10-H) 2.10 (s, 1-H_3), 2.54 (dd, J = 13.73, 10.17 Hz, 8b-H), 2.88 (dd, J = 13.73, 2.54 Hz, 8a-H), 3.62 (ddd, J = 9.92, 5.09, 2.80 Hz, 9-H), 3.78, 2.79 (s, 15-H_3, 16-H_3), 6.35 (s, 6-H), 6.36 (s, 4-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 11.1 (C-1), 14.4 (C-13), 15.1 (C-14), 20.5 (C-11), 34.6 (C-12), 37.9 (C-8), 38.5 (C-10), 55.4, 55.6 (C-15, C-16), 75.6 (C-9), 96.9 (C-4),

106.6 (C-6), 117.6 (C-2), 139.1 (C-7), 158.4, 159.0 (C-3, C-5) ppm. MS (DCI): *m/z* (%) 284 (100) [M + NH_4^+], 267 (82) [M + H^+]. HRMS for $C_{16}H_{26}O_3$: calcd. 267.196; found 267.192.

Mosher Esters 13a and 13b: Alcohol **12b** (3.5 mg, 13 μmol), (*S*)-MTPA-chloride [or (*R*)-MTPA-chloride] (6.2 μL, 33 μmol), DMAP (3 mg,26 μmol) and pyridine (15 μL) were mixed in dichloromethane and stirred at room temperature for 2 h. The solvents were evaporated in vacuo and the residue separated by preparative TLC on silica gel plates (PE/Et_2O, 1:1). (*R*)-MTPA-ester **13a** and (*S*)-MTPA-ester **13b** were obtained as a colourless oils (5.4 mg, 87% and 5.0 mg, 80%, respectively).

13a: R_f = 0.79 (PE/Et_2O, 1:1) UV (MeOH): λ_{max} (lg ε) = 209 (4.5), 225 (4.1), 282 nm (3.6). $[a]_D^{22}$ = +13.2 (*c* = 0.56, MeOH). IR (KBr): $\tilde{\nu}$ = 3439 (m), 2959 (m), 2874 (w), 1741 cm^{-1}(s). ^1H NMR ($CDCl_3$, 300 MHz): δ = 0.93 (t, *J* = 7.12 Hz, 14-H_3), 1.00 (d, *J* = 6.78 Hz, 13-H_3), 1.15–1.37 (m, 11b-H, 12b-H), 1.41–1.64 (m, 11a-H, 12a-H), 1.79–1.95 (m, 10-H), 2.06 (s, 1-H_3), 2.86 (d, *J* = 6.97 Hz, 8-H_2), 3.34 (s, 17-H_3), 3.65 (s, 15-H_3), 3.76 (s, 16-H_3), 5.34 (td, *J* = 7.02, 3.67 Hz, 9-H), 6.22 (d, *J* = 2.45 Hz, 6-H), 6.29 (d, *J* = 2.45 Hz, 4-H), 7.25–7.42 (m, 5 H, phenyl) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 11.0 (C-1), 14.3 (C-13), 15.2 (C-14), 20.5 (C-12), 33.9, 34.2 (C-8, C-11), 36.2 (C-10), 55.1 (C-15, C-16), 55.5 (C-17), 80.7 (C-9), 85.0 (C-19), 97.1 (C-4), 106.4 (C-6), 117.7 (C-2), 121.5, 125.3 (C-26), 127.5/128.2/129.3 (5 C, C-21 to C-25), 132.1 (C-20), 137.1 (C-7), 158.0 (C-3), 166.2 (C-18) ppm. MS (EI): *m/z* (%) 483.1 (30) [M + H^+], 482.0 (100) [M^+]. HRMS for $C_{26}H_{33}F_3O_5$: calcd. 482.2280; found 482.2274.

13b: R_f = 0.79 (PE/Et_2O, 1:1). UV (MeOH): λ_{max} (lg ε) = 209 (4.5), 224 (4.0), 282 nm (3.5). $[a]_D^{22}$ = –19.0 (*c* = 0.56, MeOH). IR (KBr): $\tilde{\nu}$ = 3444 (m), 2959 (m), 2874 (w), 1743 cm^{-1} (s). ^1H NMR ($CDCl_3$, 300 MHz): δ = 0.88 (d, *J* = 6.78 Hz, 14-H_3), 0.93 (t, *J* = 6.97 Hz, 13-H_3), 1.14–1.36 (m, 11b-H, 12b-H), 1.40–1.57 (m, 11a-H, 12a-H), 1.83–2.01 (m, 10-H), 2.09 (s, 1-H_3), 2.80–2.93 (m, 8-H_2), 3.35 (d, *J* = 0.75 Hz, 17-H), 3.66 (s, 15-H), 3.79 (s, 16-H_3), 5.31 (ddd, *J* = 8.43, 5.04, 3.11 Hz, 9-H), 6.30 (d, *J* = 2.45 Hz, 6-H), 6.36 (d, *J* = 2.45 Hz, 4-H), 7.08–7.38 (m, 5 H, phenyl) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 11.0 (C-1), 14.3, 14.4 (C-13, C-14), 20.6 (C-12), 33.0 (C-8), 34.5 (C-11), 35.9 (C-10), 55.1 (C-15, C-16), 55.6 (C-17), 81.1 (C-9), 84.8 (C-19), 97.1 (C-4), 106.4 (C-6), 121.5, 125.4 (C-26), 127.5/128.2/129.3 (5C, C-21 to C-25), 132.3 (C-20), 137.3 (C-7), 158.2 (C-5), 158.8 (C-3), 166.4 (C-18) ppm. MS (EI): *m/z* (%): 483.01 (47) [M + H^+], 482.0 (100) [M^+]. HRMS for $C_{26}H_{33}F_3O_5$: calcd. 482.2280; found 482.2296.

21,22-*seco*-Cruentaren A (14): Cruentaren A (**1**; 100 mg, 170 μmol) and the Hoveyda–Grubbs catalyst (21 mg, 34 μmol) were dissolved in dried dichloromethane (50 mL). This solution was stirred under an ethylene atmosphere for 24 h then evaporated in vacuo and the residue separated by preparative HPLC (MeOH/H_2O, 70:30). 21,22-*seco*-Cruentaren A was obtained as a light-yellow viscous oil (42 mg, 61%). Thin needles formed on slow evaporation of an NMR sample in $CDCl_3$, and these were recrystallised from ethyl acetate by slow evaporation at 4 °C. M.p. 170 °C (from ethyl acetate). t_R = 6.5 min (MeOH/H_2O, 75:25). UV (MeOH): λ_{max} (lg ε) = 202 (3.6), 302 (3.9), 353 nm (1.48). $[a]_D^{22}$ = +7.3 (*c* = 0.9, MeOH). IR (KBr): $\tilde{\nu}_{max}$ = = 3438, 2960, 2929, 1646, 1614 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz): δ = 0.77 (d, *J* = 6.8 Hz, 32-H_3), 0.91 (d, *J* = 7.1 Hz, 31-H_3), (d, *J* = 6.9 Hz, 30-H_3), 1.62–1.69 (m, 18-H), 1.90 (dt, *J* = 14.0, 8.0 Hz, 19a-H), 1.95 (d, *J* = 14.8 Hz, 11a-H), 1.99–2.05 (m, 16-H, 10-H), 2.17–2.23 (m, 14a-H, 8a-H), 2.29–2.34 (m, 19b-H, 11b-H), 2.83 (dt, *J* = 14.3, 11.5 Hz, 14b-H), 3.45 (dd, *J* = 8.5, 2.5 Hz, 17-H), 3.63 (ddd, *J* = 10.8, 3.1, 1.7 Hz, 9-H), 3.74 (dd, *J* = 12.8, 1.7 Hz, 8b-H), 3.79 (s, 29-H_3), 5.02–5.04 (m, 21-H_2), 5.26 (ddd, *J* = 11.7, 5.2, 2.0 Hz, 15-H), 5.42 (ddd, *J* = 11.1, 4.7, 2.1 Hz, 13-H), 5.49 (ddd, *J* = 11.2, 2.9, 1.3 Hz, 12-H), 5.72–5.80 (m, 20-H_2), 6.30 (d, *J* = 2.6 Hz, 6-H), 6.36 (d, *J* = 2.6 Hz, 4-H), 11.46 (s, 3-OH) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 8.7 (C-31), 14.1 (C-30), 16.0 (C-32), 29.8 (C-14), 31.7 (C-11), 36.7 (C-18), 36.7 (C-8), 37.5 (C-19), 38.3 (C-10), 39.2 (C-16), 55.4 (C-29), 73.0 (C-9), 75.8 (C-17), 77.8 (C-15), 99.7 (C-4), 104.8 (C-2), 112.4 (C-6), 116.4 (C-21), 125.6 (C-13), 132.3 (C-12), 137.1 (C-20), 143.7 (C-7), 163.6 (C-5), 165.9 (C-3), 171.5 (C-1) ppm. EI-MS: *m/z* (%) 433.1 (32) [M + $H]^+$, 432.1 (100) [M^+]. HRMS (EI) for $C_{25}H_{35}O_6$: calcd. 432.2505; found 432.2511.

X-ray Crystal Structure Determination of 14:[16] Diffraction data were collected at 293 K on a Bruker SMART 1000 diffractometer with Mo-K_α radiation (graphite monochromator, λ = 0.7107 Å) and CCD area detector.[17] The crystal lattice is orthorhombic, space group $P2_12_12_1$, with unit cell parameters *a* = 9.216(1), *b* = 13.524(1), *c* = 19.278(1) Å, *V* = 2402.8(2) $Å^3$, *Z* = 4, D_{calc} = 1.196 $g\,cm^{-3}$, μ = 0.084 mm^{-1}. 11599 Reflections were collected up to $2\theta_{max}$ = 56.5° and reduced to 5527 independent reflections (R_{int} = 0.028). The phase problem was solved by direct methods (SHELXS-97)[18] and the structure was refined on F^2 with SHELXL-97 with anisotropic displacement parameters for the non-H atoms.[19] The hydrogen atoms located from difference syntheses were given isotropic displacement parameters. After convergence of the refinements discrepancy factors were R_1 = 0.0406, wR_2 = 0.0852 for 4268 reflections with $F_o > 4\sigma(F_o)$ and R_1 = 0.0553, wR_2 = 0.0900 for all data; Gof = 0.930.

Supporting Information (see also the footnote on the first page of this article): The synthesis and GC analysis of 3-hydroxy-2-methylhexanoic acids **4**, Kishis's ^{13}C-NMR chemical shift analysis of cruentaren B (**2**), and spectroscopic data of 12,13,20,21-tetrahydrocruentaren A, 20,21-dihydrocruentaren A and cruentaren B (**2**).

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