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2″-Chartreusin-monoacetate, a New Natural Product with Unusual Anisotropy Effects from the Marine Isolate *Streptomyces* sp. B5525, and its 4″-Isomer*

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Chartreusin, Marine Streptomycetes, Anisotropy Effects

The marine Streptomycete isolates B5342 and B5525 forms, beside large amounts of the antitumor antibiotic chartreusin (**1a**), two chartreusin monoacetates as trace components. Whereas the chemical shift of the acetate methyl in **1c** is in the normal range, the methyl group of the 2″-acetate residue in **1b** and also in the tetra- and pentaacetates shows an extreme upfield shift. The structures of the monoacetates were confirmed by extensive NMR experiments, the anisotropic shift is explained by *semi*-empirical calculations.

Introduction

Chartreusin (**1a**) is a yellow well-crystallizing solid which is characterized by its poor solubility in unpolar solvents like cyclohexane, chloroform, dichloromethane, and a strong blue UV fluorescence. It was firstly isolated from *Streptomyces chartreusis* by Leach *et al.* in 1953 [1], and the structure was elucidated in the following years [2,3]. Chartreusin (**1a**) is not only antibacterially active [1], but also shows a very promising antitumor-activity against different human cell lines [4], a fact that has stimulated the search for related compounds. Due to their poor water solubility and very fast excretion through bile in case of intravenous injection, however, **1a** as well as the natural occurring derivatives D329C [5], chrymutin [5], demethyl-chartreusin [6], and the corresponding glycosides chrymutasin A–C [7] could not find a way to clinical applications [8,9].

During our screening of about 1300 strains of terrestrial and marine Streptomycetes, chartreusin (**1a**) has been found only three times. In two cultures, **1a** was accompanied by two trace components which were obviously **1a** derivatives due to their fluorescence and UV data. They were identified as isomeric chartreusin monoacetates **1b** and **1c**. The acetate group in **1b** shows an extreme upfield shift with a methyl signal at $\delta = 1.39$, whereas this of **1c** is in the normal range at $\delta = 1.97$. Even stronger upfield-shifts are observed for one of the acetate residues in the chartreusin tetra- and pentaacetates **1d** and **1e**, respectively. These properties must be due to the anisotropy effect of the aromatic ring system on one of the acetate residues. Here we report the structure of the new monoacetates and explain the acetate shift by molecular dynamics and force field calculations [10].

Results and Discussion

The marine Streptomycete isolates B5525 and B5342 produced chartreusin (**1a**) in high yields of 10–90 mg/l; the same fraction yielded the new compound 2-methyl-3-hexen-2,5-diole (**2b**), a less polar fraction afforded phenylacetic acid, streptazolin [11], and the new 5-hydroxy-5-methylhex-3-en-2-one (**2a**). After usual chromatographic work-up

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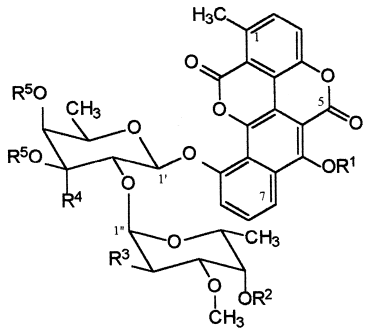
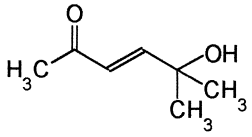


	1a	1b	1c	1d	1e	1f
R^1	H	H	H	H	Ac	H
R^2	H	H	Ac	Ac	Ac	Ac
R^3	OH	OAc	OH	OAc	OAc	NHAc
R^4	H	H	H	H	H	Me
R^5	H	H	H	Ac	Ac	Ac



2a

2b: OH instead of CO

and separating the main crop of chartreusin (**1a**) by crystallisation, the mother liquor contained traces of a less polar component with a similar colour and the same strong blue fluorescence as for **1a**.

Further purification of the mixture by chromatography on Sephadex and by HPLC afforded two components which were, according to their NMR and mass spectra, **1a** monoacetates. While MS–MS measurements of the chartreusin (**1a**) molecular ion resulted in a loss of the terminal digitalose residue (160 Dalton), both monoacetates lost a fragment of 202 Dalton, corresponding to a digitalose monoacetate unit. Obviously in both monoacetates acetylation had taken place in the terminal sugar residue (Fig. 1).

While one of the monoacetates showed a normal acetate methyl signal at $\delta = 1.97$, the corresponding signal in the minor component was extremely upfield shifted to $\delta = 1.39$, an effect which must be accounted to the influence of the aromatic ring system.

Even stronger upfield shifts with signals at $\delta =$ 0.81 and 0.71, respectively, were observed for one of the acetate groups in the tetra- and pentaacetates of chartreusin, **1d** and **1e**. According to a detailed analysis of the 2D spectra, this abnormal signal was attributed to the 2″-acetate residue in all cases. In the NOESY spectrum of chartreusin pentaacetate (**1e**), indeed only the methyl signal of the 2″-acetate group showed cross signals with the aromatic protons of ring A (Fig. 2). In parallel, the structure of the high field monoacetate was assigned as **1b**, and the second must be therefore the 4″-acetate **1c**. A similar shift of one of the acetate signals has been reported for elsamicin A tetraacetate (**1f**), however, has not been commented [8,12].

Semi-empirical calculations using MACROMODEL [10] resulted in 15 minimal conformations for **1e** in a range of about 17 kJ/mol. In one of the lowest, the 2″-acetate methyl is placed indeed above the ring A of the aromatic system (Fig. 3).

At low temperature, the ^{1}H NMR spectrum of **1e** showed a splitting of all proton signals ($\Delta\delta \sim$ 0.1) with a coalition temperature at about –10 °C. Obviously at least two stable conformers with methyl/π interactions are existing.

All trials to synthesize **1b** or **1c** by partial acetylation of **1a** using various reaction conditions failed and yielded only **1d**, **1e**, and unchanged **1a**. An artificial formation of **1b/1c** during the work-up is unlikely as well, as only two of our three **1a**-producers delivered **1b** and **1c**. Both monoacetates are new natural products therefore.

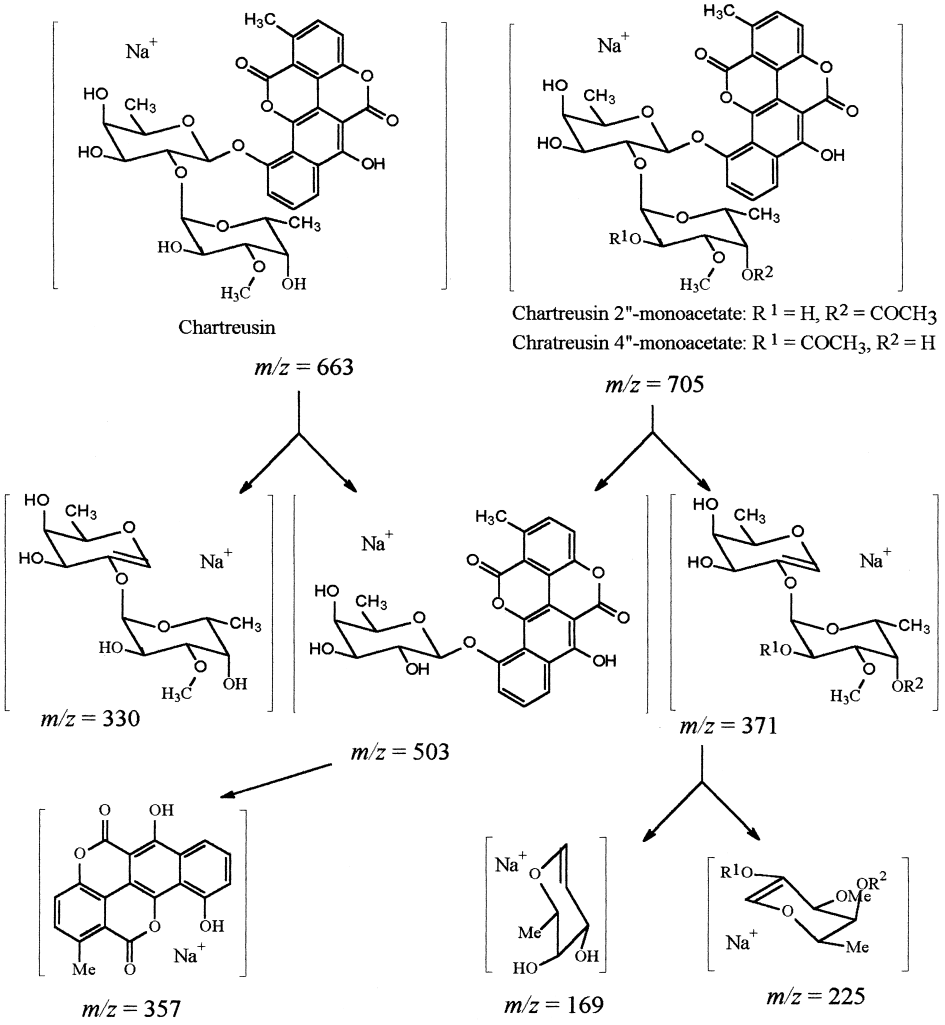


Fig. 1. Fragmentation of **1a–c** by (+)–ESI–MS–MS.

Experimental Section

^{1}H NMR, ^{13}C/APT NMR and COSY NMR spectra were recorded on Varian VXR 200 or Varian Unity 300 spectrometers. Mass spectra (EI, DCI) were measured on a Varian 311 A (70 eV) and on a Finnigan MAT 95 A mass spectrometer. HRMS data were collected on a Varian MAT 731 (peak-matching with perfluorokerosene; resolution 10,000). UV spectra were recorded on a Beckman DU-640 spectrometer. IR spectra were obtained in KBr on a Perkin Elmer 297 spectrometer. Preparative HPLC was carried out on a Eurospher C_{18} RP ($100 \times 5\ \mu$m) column.

Description of the producers

Strain B5525 has been derived from mangrove sediment of Long Island (Papua New Guinea) and was isolated on casein peptone agar [13] containing 50% natural sea water. The strain B5342 has been derived from sandy sediment of a coast site of Mauritius (Indian Ocean) and was isolated on chitin agar [13] with 50% natural seawater. The reference cultures of B5525 and B5342 are kept on yeast extract-malt extract agar [13] in the Collection of Marine Actinomycetes at the Alfred-Wegener-Institute for Polar and Marine Research in Bremerhaven.

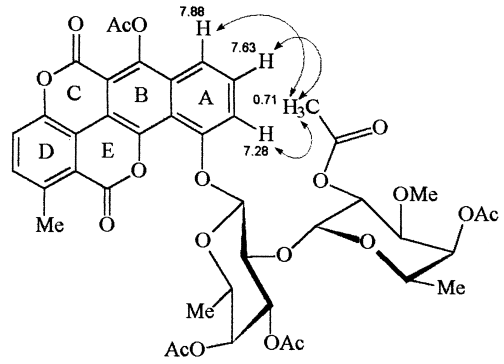


Fig. 2. NOESY couplings (↔) in chartreusin pentaacetate (**1e**).

The almost complete 16S rRNA sequence of the strain B5525 and B5342 are 100% and 99,9% respectively similar to the DNA of *Streptomyces chartreusi* (ISP 5085) belonging to the *Streptomyces cyaneus* group.

The two marine isolates show quite similar morphological and physiological features. The substrate mycelium is beige. Spores are greenish grey and are borne in mature spiral chains (*Spirales*). The surface of spores is spiny. Melanin pigment is weakly produced on peptone-yeast extract-iron agar [14] and tyrosine agar [14]. On some standard media a yellow diffusible pigment is formed. Optimum growth temperature is at about 30 °C. The strain does not reproduce at 45 °C. At 10 °C slow growth occurs. The strain does not develop in media with 7% or higher sea water salinity. Starch, casein, and esculin are degraded. Chitin and cellulose are not hydrolysed. The strain is catalase and nitrate reductase positive. H_2S is not produced. The use of carbon sources was tested with SFN2-Biolog (Hayward, CA, USA) using BMS-N without agar as basal medium [15]. Only small differences in the utilization pattern of the two strains were obtained. Both strains oxidized α-cyclodextrin, dextrin, glycogen, tween 40, tween 80, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, D-glucose, m-inositol, D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-mellobiose, β-methyl-D-glucose, D-raffinose, L-rhamnose, sucrose, D-trehalose, methyl-pyruvate, mono-methylsuccinate, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, β-hydroxybutyric acid, α-keto glutaric acid, D,L-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, succinic acid, glucuronamide, alaninamide, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, L-serine, L-threonine, γ-amino butyric acid, inosine, phenylethylamine, putrescine, 2-aminoethanol, and glycerol were oxidized by both strains. N-Acetyl-D-galactosamine, xylitol, *p*-hydroxyphenylacetic acid, urocanic acid, uridine, and D, L-α-glycerol phosphate were used only by B5525, and acetic acid only by B5342.

Both strains were cultivated in malt extract/yeast extract/glucose medium with 50% sea water. For the upscaling, the marine strains were cultured in a 20 l jar fermentor for 72 h at 28 °C and the filtrate and the mycelium were extracted separately each three times with ethyl acetate. The combined ethyl acetate extracts were evaporated *in vacuo* at 40 °C and defatted with cyclohexane.

Preparative TLC (20×40 cm, CH_2Cl_2/5% MeOH) of the crude oily extract of strain B5342 gave 180 mg of yellow powdery **1a**. From the crude extract from the 20 l jar fermentation of strain B5525, 1.82 g **1a** were obtained as yellow precipitate.

The mother liquors obtained after filtering off the undissolved **1a** showed a light yellow spot on TLC at $R_f = 0.42$ (CH_2Cl_2/5% MeOH) with a blue fluorescence like that of chartreusin (**1a**). After purification by column chromatography on silica gel (3×30 cm, $CHCl_3$/2% MeOH), PTLC (20×20 cm, cyclohexane/46% EtOAc) Sephadex LH-20, final HPLC on RP18 (CH_3CN/ H_2O azeotrop/ 20% H_2O) resulted in 0.2 mg chartreusin-2″-acetate (**1b**, t_r = 6.1 min) and 0.3 mg chartreusin-4″-

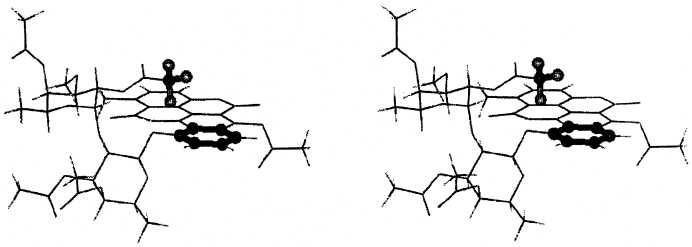


Fig. 3. Stereo-drawing of the calculated minimal conformation of chartreusin pentaacetate (**1c**) with the 2″-acetate methyl group above the aromatic ring A.

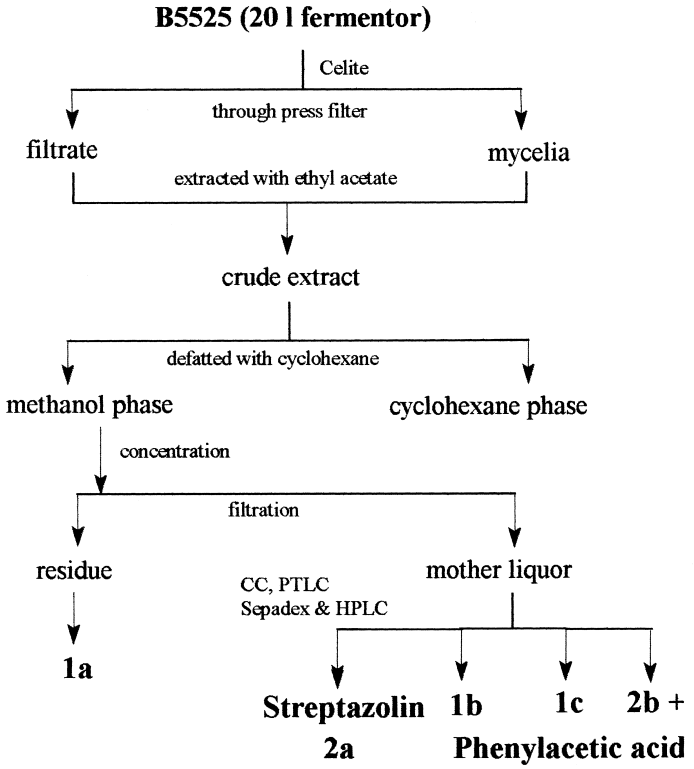


Fig. 4. Scheme of the working up of extract of isolate B5342.

acetate (**1c**, t_r = 5.3 min). On the PTLC, another UV absorbing spot with R_f = 0.55 gave a yellow colouration with anisaldehyde/sulphuric acid due to streptazolin, further spots afforded 5-hydroxy-5-methyl-hex-3-en-2-one (**2a**), 2-methylhex-3-ene-2,5-diol (**2b**), and phenylacetic acid.

Chartreusin (**1a**)

Yellow, as solid and in solution strongly blue fluorescent, R_f = 0.24 ($CHCl_3$/5% MeOH). NMR data were identical with those of an authentic sample.

Chartreusin-2″-monoacetate (**1b**)

Yellow solid, R_f = 0.42 ($CHCl_3$/5% MeOH). – ^{1}H NMR (300 MHz, $CHCl_3$; in mixture with **1c**): δ = 8.12 (d, J = 8 Hz, 1 H), 7.75 (t, J = 8 Hz, 2 H), 7.65 (d, J = 8 Hz, 1 H), 7.54 (d, J = 8 Hz, 1 H), 5.56 (br s, 1 H), 5.35 (d, J = 8 Hz, 1 H), 5.12 (br s, 1 H), 4.83 (d, J = 3 Hz, 1 H), 4.59 (d, J = 8 Hz, 1 H), 4.55–4.44 (m, 3 H), 4.02–3.87 (m, 3 H), 3.83 (m, 1 H), 3.68–3.59 (m, 3 H), 3.12 (s, 3 H), 3.84 (s, 3 H), 1.97 (s, 3 H), 1.39 (s, 3 H, 2″-OAc), 1.21 (d, J = 8 Hz, 3 H), 0.92 (d, J = 8 Hz, 3 H). – DCIMS (NH_3): *m/z* (%) = 700 (100) $[M+NH_4]^+$. – (+)–ESIMS: *m/z* (%) = 705 (100) $[M+Na]^+$. – (+)–ESIMS-MS of *m/z* = 705: *m/z* (%) = 503 (70) $[M-C_9H_{15}O_5 + H + Na]^+$, 371 (100) $[C_{15}H_{25}O_9 - H + Na]^+$.

Chartreusin-4″-monoaacetate (**1c**)

Yellow solid, R_f = 0.42 ($CHCl_3$/5% MeOH). – UV/vis (MeOH): λ_{max} (log ε) = 265 (4.42), 399(3.45), 418 nm (3.48); (MeOH + HCl): λ_{max} (log ε) = 265 (4.41), 399 (3.44), 418 nm (3.47); (MeOH + NaOH): λ_{max} (log ε) = 265 (4.49), 4.18 nm (3.48). – IR (KBr): ν = 3414 (br, OH), 2920, 2853, 1775 (CO), 1695 (CO), 1610, 1500, 1456, 1400, 1375, 1314, 1253, 1235, 1149, 1098, 963, 900, 833, 811, 777, 735, 660 cm^{-1}. – ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.12 (d, ^3J = 8 Hz, 1 H, 7-H), 7.75 (m, 2 H, 2-H, 8-H), 7.65 (d, ^3J = 8 Hz, 1 H, 3-H), 7.54 (d, ^3J = 8 Hz, 1 H, 9-H), 5.56 (s br, 1 H), 5.35 (d, ^3J = 8 Hz, 1 H), 5.12 (s br, 1 H), 4.83 (d, ^3J = 3 Hz, 1 H), 4.59 (d, ^3J = 8 Hz, 1 H), 4.55–4.44 (m, 3 H), 4.02–3.87 (m, 3 H), 3.83 (m, 1 H), 3.68–3.59 (m, 2 H), 3.12 (s, 3 H, 3″-OCH_3), 2.84 (s, 3 H,

1-CH_3), 1.97 (s, 3 H, 4″-$OCOCH_3$), 1.21 (d, ^3J = 6.2 Hz, 3 H, 6′-CH_3), 0.92 (d, ^3J = 6.2 Hz, 3 H, 6″-H_3). – EIMS (70 eV): *m/z* (%) = 334 (6), 317 (29), 229 (19), 214 (18), 195 (32), 163 (42), 140 (50), 135 (100), 122 (21), 107 (19), 95 (24), 93 (29), 79 (34), 68 (20), 55 (19), 41 (20). – DCIMS: *m/z* (%) = 700 (89), 366 (95), 242 (100), 236 (93). – DCIMS (NH_3): *m/z* (%) = 700 (100) $[M + NH_4]^+$. – (+)–ESIMS: *m/z* (%) = 705 (100) $[M + Na]^+$. – (+)–ESIMS-MS of *m/z* = 705: *m/z* (%) = 503 (70) $[M-C_9H_{15}O_5 + H + Na]^+$, 371 (100) $[C_{15}H_{25}O_9-H + Na]^+$. – (+)–ESIMS-MS of *m/z* = 503: *m/z* (%) = 485 (100), 429 (12), 357 (26) $[M-C_{15}H_{25}O_9 + H + Na]^+$. – (+)–ESIMS-MS of *m/z* = 371: *m/z* (%) = 225 (18) $[C_9H_{15}O_5-H + Na]^+$, 169 (100) $[C_6H_{10}O_4 + Na]^+$.

Chartreusin-3′,4′,2″,4″-tetraacetate (**1d**)

Chartreusin (**1a**, 180 mg) was treated with acetic acid anhydride (0.8 ml) in pyridine (1 ml) with a catalytic amount of 4-dimethylaminopyridine for 4 h at room temp. After hydrolysis with MeOH and water, **1d** was extracted with ether and the solution dried over magnesium sulphate. Purification of the reaction product by PTLC (CH_2Cl_2/2.5% MeOH) yielded 7 mg **1d** as a yellow powder. – UV (MeOH): λ_{max} = 421, 400, 267.5, 237.5 nm. – IR (KBr): ν = 2970, 1740, 1695, 1500, 1375, 1232, 1070, 780 cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 11.74 (s, 1 H, 6-OH), 8.31 (dd, ^3J = 8 Hz, ^4J = 1 Hz, 1 H, 7-H), 7.65 (t, ^3J = 8 Hz, 1 H, 8-H), 7.57 (d, ^3J = 8 Hz, 1 H, 3-H), 7.52 (dt, ^3J = 8 Hz, ^4J = 0.5 Hz, 1 H, 2-H), 7.35 (d, br, ^3J = 8 Hz, ^4J = 1 Hz, ^5J = 0.2 Hz, 1 H, 9-H), 6.18 (d, ^3J = 4 Hz, 1 H, 1″-H), 5.49 (d, br, 3J = 7.5 Hz, ^5J = 0.2 Hz, 1 H, 1′-H), 5.40 (dd, ^3J = 3.5 Hz, ^4J = 0.5 Hz, 1 H, 4′-H), 5.32 (dd, ^3J = 3 Hz, ^3J = 1 Hz, 1 H, 4″-H), 5.20 (dd, ^3J = 10 Hz, ^3J = 3.5 Hz, 1 H, 3′- H), 4.95 (dd, ^3J = 10.5 Hz, ^3J = 4 Hz, 1 H, 2″-H), 4.57 (dd, ^3J = 10 Hz, ^3J = 7.5 Hz, 1 H, 5 Hz, 2′-H), 4.13 (dq, ^3J = 7 Hz, ^3J = 1 Hz, 1 H, 5″-H), 4.10 (dq, ^3J = 7 Hz, ^3J = 0.5 Hz, 1 H, 5′-H), 3.56 (dd, ^3J = 10.5 Hz, ^3J = 3.5 Hz, 1 H, 3″-H), 3.24 (s, 3 H, 3″-OCH_3), 2.96 (s, br, 3 H, 1-CH_3), 2.24, 2.11, 2.08 (3 s, each 3 H, COOCH*3*), 1.31 (d, ^3J = 7 Hz, 3 H, 5″-CH_3), 1.24 (d, ^3J = 7 Hz, 3 H, 5′-CH_3), 0.81 (s, 3 H, 2″-$COOCH_3$). – ^{13}C/APT NMR (125.7 MHz, $CDCl_3$): δ = 171.1, 171.08, 170.6, 169.6, 165.1, 159.0 (6 CO), 157.1, 153.1, 146.8, 140.4, 139.0, (5 C_q), 133.2 (C-2), 127.8 (C-8), 127.0 (C^q), 121.0 (C-3), 120.0, 118.7 (2 C^q), 118.5 (C-7), 118.2 (C^q), 113.5 (C-9), 109.2 (C^q), 99.5 (C-1′), 98.1 (C-1″), 97.3 (C^q), 75.4 (C-3″), 74.2 (C-2″), 72.6 (C-3′), 70.0 (C-4′), 69.7 (C-4″), 69.5 (C-5′), 69.0 (C-2″), 65.0 (C-5″), 57.4 (3-OCH_3), 22.4 (1-CH_3), 20.9 (3 × $COOCH_3$), 19.5 (2″-$OOCCH_3$), 16.6 (CH_3-6′), 16.2 (CH_3-6″). – FDMS: *m/z* = 808.

Chartreusin-pentaacetate (**1e**)

1a (250 mg) was triturated with a mixture of acetic acid anhydride (2 ml), pyridine (2 ml), and a catalytic amount of 4-dimethylaminopyridine and left for 24 h at room temperature. The reaction mixture was hydrolysed with methanol. After hydrolysis, the yellow solid was filtered off, washed with water and dried to yield 267 mg (80%) of chromatographically pure **1e**. – R_f = 0.64 ($CHCl_3$/5% MeOH). – ^1H NMR (500 MHz, DMSO-d_6): δ = 8.03 (d, ^3J = 8.6 Hz, 1 H, 7-H), 7.84 (t, ^3J = 8.3 Hz, 1 H, 8-H), 7.78 (d, ^3J = 8.2 Hz, 1 H, 3-H), 7.72 (d, ^3J = 8.3 Hz, 1 H, 2-H), 7.61 (d, ^3J = 8.3 Hz, 1 H, 9-H), 5.83 (d, ^3J = 7.5 Hz, 1 H, 1′-H), 5.79 (d, ^3J = 3.7 Hz, 1 H, 1″-H), 5.32 (d, ^3J = 3.1 Hz, 1 H), 5.30 (d, ^3J = 3.3 Hz, 1 H), 5.18 (dd, ^3J = 9.8, 3.4 Hz, 1 H), 4.63 (dd, ^3J = 10.9, 3.8 Hz, 1 H), 4.44 (d, ^3J = 6.1 Hz, 1 H), 4.22 (m, 2 H), 3.57 (dd, ^3J = 11.0, 3.4 Hz, 1 H), 3.11 (s, 3 H, 3″-OCH_3), 2.86 (s, 3 H, 1-CH_3), 2.56 (s, 3 H), 2.20 (s, 3 H), 2.05 (s, 3 H), 2.01 (s, 3 H), 1.13 (d, ^3J = 6.4 Hz, 1 H), 1.09 (d, ^3J = 6.4 Hz, 1 H), 0.55 (s, 3 H). – ^1H NMR (500 MHz, $CDCl_3$): δ = 7.90 (d, ^3J = 7.9 Hz, 1 H, 7-H), 7.66 (d, ^3J = 8.3 Hz, 1 H, 8-H), 7.53 (d, ^3J = 8.3 Hz, 1 H, 2-H), 7.52 (d, ^3J = 8.3 Hz, 1 H, 3-H), 7.29 (d, ^3J = 7.9 Hz, 1 H, 9-H), 6.12 (d, ^3J = 3.8 Hz, 1 H, 1″-H), 5.46 (d, ^3J = 7.9 Hz, 1 H, 1′-H), 5,37 (d, ^3J = 3.0 Hz, 1 H, 4′-H), 5.28 (d, ^3J = 2.3 Hz, 1 H, 4″-H), 5.17 (d, ^3J = 9.8, 3.4 Hz, 1 H, 3′-H), 4.88 (d, ^3J = 10.6, 3.8 Hz, 1 H, 2″-H), 4.05 (d, ^3J = 6.8 Hz, 1 H, 5″-H), 4.02 (d, ^3J = 6.8 Hz, 1 H, 5′-H), 3.53 (d, ^3J = 8.3 Hz, 1 H, 1-H), 3.20 (s, 3 H, 3″-OCH_3), 2.94 (s, 3 H, 1-CH_3), 2.60 (s, 3 H, 6-O-$COCH_3$), 2.21 (s, 3 H, 3′-$OCOCH_3$), 2.10 (s, 3 H, 4′-$OCOCH_3$), 2.08 (s, 3 H, 4″-$OCOCH_3$), 1.48 (s br, D_2O-exchangeable, 1 H), 1.27 (s, 3 H, 6′-CH_3), 1.20 (s, 3 H, 6″-CH_3), 0.71 (s, 3 H, 2″-$OCOCH_3$). – ^{13}C/APT NMR (500 MHz, $CDCl_3$): δ 170.9 (CO-4′), 170.8 (CO-4″), 170.4 (CO-3′), 169.4 (CO-2″), 169.0 (CO-6), 158.4 (C_q-12/5), 156.7 (C_q-5/12), 153.2 (C_q-10), 146.8 (C_q-3a/10b), 145.6 (C_q-6), 144.3 (C_q-10b/3a), 139.7 (C_q-1), 133.4 (CH-2), 130.5 (C_q-6a), 128.8 (CH-8), 120.9 (CH-3), 118.8 (C_q-3b), 117.6 (C_q-10a), 117.3 (C_q-12a), 117.2 (CH-7), 112.4 (CH-9), 111.2 (C_q-10a), 108.7 (C_q-5a), 99.5 (CH-1′), 98.0 (CH-1″), 75.1 (CH-3″), 74.0 (CH-2′), 72.4 (CH-3′), 69.8 (CH-4′), 69.5 (CH-4″), 69.4 (CH-5′), 68.9 (CH-2″), 64.9 (CH-5″), 57.3 (3″-OCH_3), 22.4 (1-CH_3), 21.0 (4′-$OCOCH_3$), 20.8 (3′-, 4″-, 6-$OCOCH_3$), 19.2 (11″-$OCOCH_3$), 16.6 (6″-CH_3), 16.1 (6′-CH_3).

5-Hydroxy-5-methyl-hex-3-en-2-one

Oily, 2 mg, R_f = 0.33 ($CHCl_3/CH_3OH$: 93/7), brown with anisaldehyde/sulphuric acid. – IR (KBr): ν = 3427 (br, OH), 2926, 1632, 1384, 1122 cm^{-1}. – ^1H NMR ($CDCl_3$, 200 MHz): δ = 6.84, 6.27 (AB, ^3J = 16 Hz, each 1H, 4-H, 3-H), 2.29 (s, 3 H, 1-H_3), 1.40 (s, 6 H, 5-CH_3, 6-H_3). – ^{13}C/APT NMR ($CDCl_3$, 125.7 MHz): δ = 198.9 (C_q-2), 153.2 (CH-4), 126.7 (CH-3), 70.8 (C_q-5), 29.4 (CH_3-6), 29.4 (5-CH_3), 27.6 (CH_3-1). – HMQC ($CDCl_3$, inverse CH-COSY, INVBTP, F1 75.5 MHz, F2 300.1 MHz): all expected couplings were found. – EIMS (70 eV): *m/z* (%) = 113 (19), 85 (100), 67 (31), 43 (84). – DCIMS (NH_3): *m/z* (%) = 163 ($[M + NH_4 + NH_3]^+$, 23), 146 ($[M + NH_4]^+$, 100), 129 ($[M + H]^+$, 1).

2-Methylhex-3-ene-2,5-diol (**2b**)

Oil (7 mg), R_f = 0.63 ($CHCl_3/CH_3OH$: 90/10) with anisaldehyde/sulphuric acid green, then yellow and finally pink. – ^1H NMR ($CDCl_3$, 200 MHz): δ = 5.83 (d, ^3J = 15.6 Hz, 1 H, 3-H), 5.71 (dd, ^3J = 15.6 Hz, ^3J = 6.4 Hz, 1 H, 4-H), 4.33 (dq, ^3J = 6.4 Hz, ^3J = 6.4 Hz, 1 H, 5-H), 1.33 (s, 6 H, 1-H_3, 2-CH_3), 1.27 (d, ^3J = 6.4 Hz, 3 H, 6-H_3). – ^{13}C/APT NMR ($CDCl_3$, 125.7 MHz): δ = 137.8 (CH), 130.8 (CH), 70.5 (C_q-2), 68.4 (CH-5), 29.8 (CH_3-1), 29.7 (2-CH_3), 23.5 (CH_3-6). – EIMS (70 eV): *m/z* (%) = 115 (22), 97 (59), 85 (17), 69 (43), 59 (23), 43 (100). – DCIMS (NH_3): *m/z* (%) = 148 ($[M + NH_4]^+$, 45), 147 ($[M + NH_3]^+$, 25), 130 (M^+, 100), 113 (87).

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