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The Reaction of Hyperforin with Hydride Reducing Agents

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As part of a study aimed at generating analogues of hyperforin (**1**), the reaction of this prenylated phloroglucinol with various hydride reducing agents was investigated. Hyperforin contains two β-dicarbonyl systems, one of which is non-enolizable, and it was interesting to assess the relative reactivity of these structural elements in the highly compact framework of the natural product. Depending on the reducing agent employed, a surprising range of compounds could be obtained, sometimes in synthetically useful yields. The stereochemistry of the $LiAlH_4$-reduced product was secured by X-ray analysis and served as a base for elucidating the configuration of a series of reduced and deoxygenated analogues obtained with other reducing agents. The chemoselectivity observed in these reactions is apparently the result of a combination of metal-chelation and hydrogen-bonding effects.

Introduction

The biomedical relevance of hyperforin (**1**), the major lipophilic constituent of St. John's wort (*Hypericum perforatum* L.), can hardly be overestimated, since this compound shows clinical potential in several "hot" areas of pharmaceutical research, like depression, cancer, inflammation, and Alzheimer disease.[1] Though first characterized as an antibiotic, hyperforin was next identified as the major antidepressant principle of St. John's wort, while recent studies have highlighted its anticancer potential (both as an antitumor[2–4] and an antimetastatic agent[5,6]), as well as its potent antisecretase[7] and antiinflammatory[8] activity. Given the low human toxicity of hyperforin and its acceptable pharmacokinetic profile, research on this compound is burgeoning. However, only two biological targets have so far been identified for hyperforin, namely a neuronal $[Na^+]$/$[Ca^{++}]$ exchanger[9] and the pregnane X-receptors,[10] sensors for the activation of the cytochrome system. The interaction of hyperforin with these end points is seemingly involved in the antidepressant activity of St. John's wort and in its severe interactions with several drugs, while the antimetastatic activity of hyperforin is mechanistically complex and possibly related to a modulation of the expression of a series of metalloproteases-encoding genes.

Hyperforin is clearly a pleiotropic agent, capable not only of interacting with membrane receptors but also of regulating genes mediating xenobiotic metabolism, cell proliferation, and apoptosis. To dissect the various biological activities of hyperforin, we have started a systematic study of its chemical reactivity. The rationale for these investigations is that different molecular sectors of hyperforin might be involved in the interaction with its biological targets, and that the availability of analogues featuring changes in these sectors should lead to the dissection of its various pharmacophores. The unique skeletal framework of hyperforin, the presence of a host of functional groups, and the substantial lack of information on the chemical behavior of prenylated phloroglucinols combine to make it difficult to predict the reactivity of the natural product. Since hyperforin is oxygen sensitive, we first investigated its reactivity toward various oxidants,[11] building a structurally diverse library of analogues currently under biological scrutiny. To complement these investigations, we have now studied the behavior of hyperforin toward various carbonyl-reducing agents. Since functional group congestion makes the chemical behavior of hyperforin substantially unpredictable, reactivity studies on the natural product might also offer interesting clues for the planning of a total synthesis, currently pursued by many groups.[12]

Results and Discussion

Hyperforin shows four carbonyl functions with different electronic and/or steric properties, that, at least in principle, can be selectively differentiated in reduction reactions, af-

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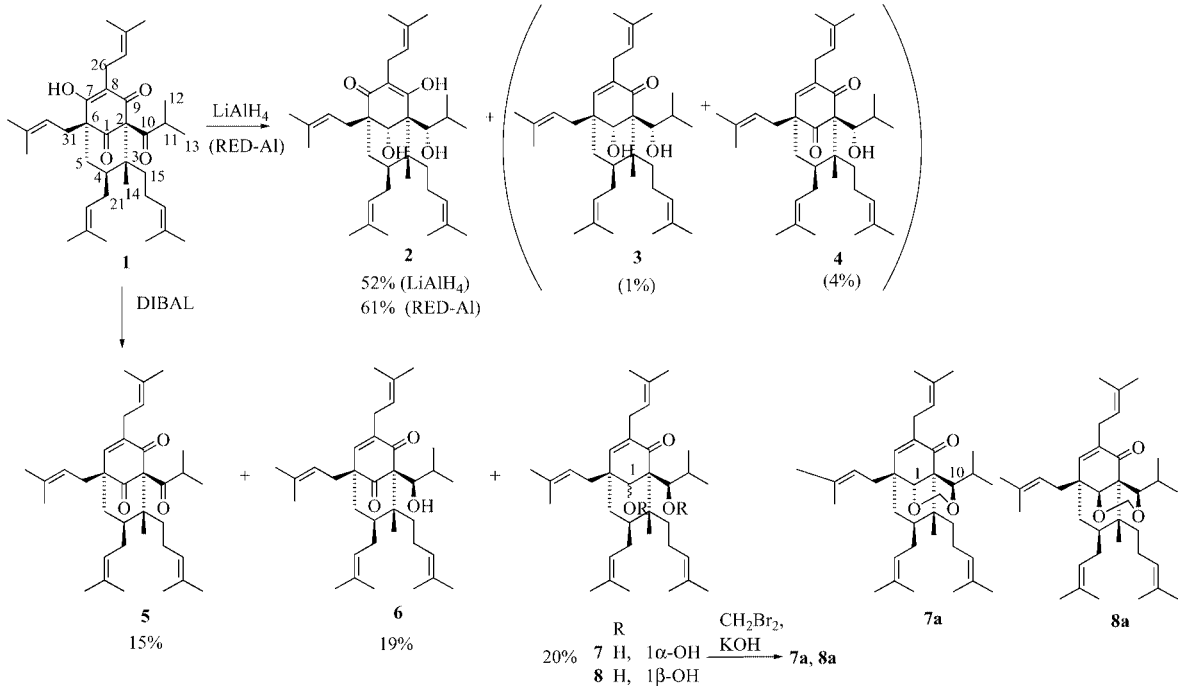
fording a series of modified templates for structure-activity studies. The enolized β-dicarbonyl system was expected to be less reactive than the two non-conjugated keto groups, with the side chain isobutyryl carbonyl being more accessible than the bridging C-1 carbonyl. Hyperforin was stable toward $NaBH_4$ under a variety of reaction conditions (methanol, ethanol, or 2-propanol and with or without additives like $CeCl_3$ or $NiCl_2$), showing that electronic (for the enolized β-dicarbonyl) and steric (for the non enolizable dicarbonyl system) factors combine to prevent hydride attack from mild reducing agents. More powerful reagents were, therefore, investigated.

$LiAlH_4$ in THF gave a clean reaction, affording, apart from unreacted starting material, the crystalline diol **2** as the only reaction product (Scheme 1). The ^{13}C NMR spectrum of **2** showed signals diagnostic for the enolized β-dicarbonyl system, while the C-1 and C-10 carbonyls were replaced by two oxymethines. These observations established that the non-enolized dicarbonyl system had been reduced, while the configuration of the newly generated stereocenters was unambiguously established by X-ray analysis. The results showed that the bridgehead ketone had been reduced with hydride attack from the side of the enolized β-dicarbonyl (*si* face), while hydride attack had taken place from the *re* face of the side chain acyl group. Given the presence of the alkyl substituents on the "southern" (*re* face) of the bridgehead keto group, the stereoselectivity observed in its reduction is predictable, while the access to only one isomer in the reduction of the side chain carbonyl was surprising. Since the enolized β-dicarbonyl is critical for the bioactivity of hyperforin, the $LiALH_4$ reaction affords a new interesting template for structure-activity relationships.

The ^1H NMR spectrum of **2** (Figure 1) showed some features worth discussion. While hyperforin shows broad NMR signals due to the slow interconversion of the two tautomeric forms of the enolized β-dicarbonyl moiety, the spectra of **2** contained sharp signals, suggesting the presence of only one tautomer. HMBC experiments verified that the tautomer present in solution is the same as that present in the crystal lattice, namely the one with the C-9 carbonyl enolized (HMBC correlations of 9-OH with C-2, C-8, and C-9). The tautomeric composition and the stereoselectivity observed in the reduction of the side chain carbonyl are presumably the result of intramolecular hydrogen bonding. Intramolecular hydrogen bonding between the side chain hydroxy and the oxygen function at C-9 is, in principle, possible with both tautomeric forms of the β-dicarbonyl system. However, intramolecular hydrogen bonding with the C-9 carbonyl tautomer involves a weak donor (the side chain alcohol hydroxy group), while in the C-7 carbonyl tautomer, the more acidic enolic hydroxy group at C-9 can act as a hydrogen bonding donor. The presence of this intramolecular hydrogen bonding acts as a conformational bias on the side chain geometry, stabilizing the 10*S* vs. the 10*R* configuration of the side chain oxymethine by minimizing steric interactions between the C-11 *gem*-dimethyl groups and the bicyclic core. Despite the poor quality of the crystals, the X-ray structure analysis gave a clear indication of intramolecular hydrogen bonding in **2**, supporting our rationalization of the stereochemical course of the reduction. Thus, the enol hydroxy group acted as a donor toward the side chain hydroxy group [O1···O4 distance: 2.526(3) Å], which in turn hydrogen bonded to the enone carbonyl of another molecule of the crystal lattice [O4···O2($1-x$, $-1/2+y$, $3/2-z$) 2.701(3) Å], while the steri-



Scheme 1. Reaction of hyperforin (**1**) with various reducing agents.

cally hindered 1-hydroxy group was engaged in weak hydrogen bonding with the crystallization solvent, *tert*-butyl methyl ether [O3···O1S distance: 2.877(6) Å].

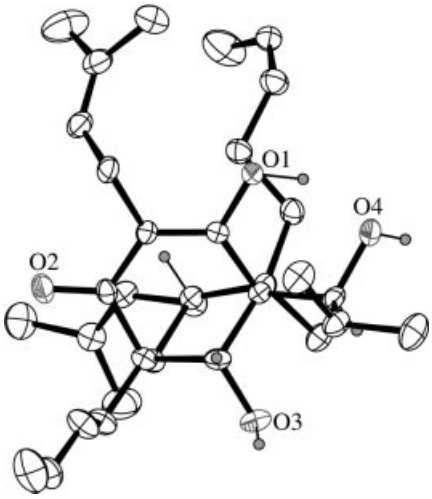
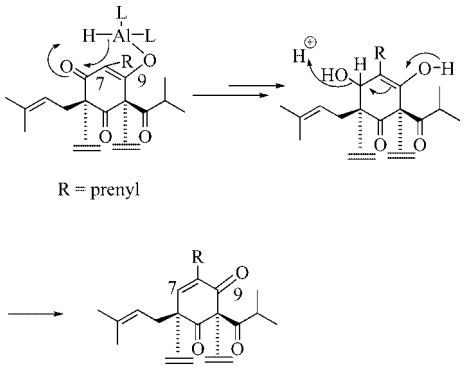


Figure 1. The conformation of **2** from the X-ray diffraction experiment; most of the H atoms are omitted for clarity; Atomic displacement parameters are at the 20% probability level and H atoms are not to scale.

The success of the reduction with $LiAlH_4$ prompted us to investigate the behavior of hyperforin with alkoxy and alkyl alanes. The reaction course with the alkoxylalane RED-Al [sodium dihydrido-bis(2-methoxyethoxy)aluminate] was somewhat similar to that with $LiAlH_4$, with diol **2** being the major reaction product, but two minor and less polar analogues were also obtained (Scheme 1). These were characterized as **3**, the 7-deoxy derivative of **2**, and **4**, the 7-deoxy derivative of the product of mono-reduction of the unconjugated β-diketone system. Deoxygenation of β-dicarbonyls with hydrides is an uncommon reaction. In hyperforin, it might occur via intramolecular hydride delivery from a C-9 aluminum chelate, as depicted in Scheme 2, a process apparently favored by the presence of alkoxy (and alkyl, vide infra) ligands on aluminum. The strong chelation of the 9-enol inhibits tautomerization (and therefore reduction) of the corresponding carbonyl tautomer during the reaction.

Deoxygenation at C-7 was also the major reaction course observed when hyperforin was treated with the alkyl alane DIBAL (diisobutylalane). A mixture of four compounds was obtained, all deoxygenated at C-7, differing in the extent and stereochemistry of reduction of the non-enolized β-dicarbonyl system (Scheme 1). Thus, in compound **5** (15% yield), the 1,10-β-dicarbonyl was still present, while in **6** (19% yield) only the side chain carbonyl was reduced. A diastereomeric mixture of bis-reduced compounds was also obtained. These compounds were separated after conversion to their methylene ethers (CH_2Br_2, KOH, DMF), whose rigid framework allowed assignment of the relative configuration by ROESY experiments. The stiffening operation locked the orientation of the oxygenated side chain, making it possible to translate into configurational terms the observed ROESY correlations. Especially diagnostic



Scheme 2. Possible mechanism for the chemoselective deoxygenation at C-7 of the 7,9-β-dicarbonyl system of hyperforin. Hydride attack is shown intramolecularly, but it could also take place intermolecularly.

were the ROESY correlations between the C-1 and C-10 oxymethines, H-1 and CH_3-14, H-10 and CH_3-14, and H-1 and H-7. These confirmed a *cis*-β relationship of the oxymethine protons of the dioxine moiety, and thus the orientation toward the homoprenyl-containing "southern" cyclohexane ring in **8a**. Conversely, in compound **7a**, NOE correlations were observed between H-1 and H-7, H-1 and H-31A, H-10 and CH_3-14, H-10 and CH_3-13, and H-1 and CH_3-12, in accordance with a *trans* relationship.

It is remarkable that in all compounds deoxygenated at C-7, the configuration of the side chain hydroxy group was opposite to that of **2**, the compound obtained from the $LiAlH_4$ reduction. This observation shows that in the reduction with the bulky reagent DIBAL, attack from the *si*-face of the side chain carbonyl was preferred, with kinetic factors apparently prevailing over thermodynamic considerations of hydrogen bonding stability. Thus, just like the deoxygenation at C-7, the reaction of the side chain carbonyl might also take place intramolecularly from a C-9 enol aluminate.

Taken together, the results of this study confirm our previous observations regarding the existence of a rich but unpredictable chemistry for hyperforin. In particular, its two dicarbonyl systems are amenable to reduction or deoxygenation upon treatment with alane reducing agents, an important finding that paves the way to new and interesting modifications of the natural product.

Experimental Section

General: Optical rotation values were recorded with a Perkin–Elmer 241 polarimeter. Column chromatography was performed with silica gel 60 (70–230 and 40–63 μm, Merck). The reactions were monitored by TLC on Merck 60 F_{254} (0.25 mm) plates, visualizing the spots with short-wave light (254 nm) and by spraying with $(NH_4)_2MoO_4$ and heating. Commercially available reagents were used without prior purification. Solvent extracts of aqueous solutions were dried with anhydrous Na_2SO_4.

Spectroscopy: ^1H NMR (500 MHz and 400 MHz) and ^{13}C NMR (125 MHz and 100 MHz) were recorded at room temperature with Bruker DRX500 and Bruker Avance 400 spectrometers with an

inverse multinuclear 5 mm probehead equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm and the coupling constants (J) in Hz. COSY, HMQC, HMBC, and NOESY experiments were recorded with gradient enhancements using sine-shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH}$ = 145 Hz and $^nJ_{CH}$ = 10 Hz. The raw data were transformed, and the spectra were evaluated with the standard Bruker XWIN-NMR software (rev. 010101). The ^1H and ^{13}C NMR resonances were assigned with the aid of 2D data based on scalar and dipolar homo- and heterocouplings. HRESI mass spectra were recorded with an FT-ICR Mass Spectrometer *APEX II* (Bruker Daltonics) equipped with a 4.7 T Magnet (Magnex).

Reaction of Dicyclohexylammonium Hyperforinate (Hyperforin DCHA) with $LiAlH_4$: Hyperforin DCHA (1.02 g, 1.4 mmol) was dissolved in dry THF (10 mL), and $LiALH_4$ (10 mol/equiv.) was added. After stirring at room temperature for 3 h, the mixture was diluted with HCl (10% solution, 10 mL) and extracted with EtOAc (3 × 10 mL). The organic phase was dried with Na_2SO_4 and evaporated to give a gum, which was purified by column chromatography over Si gel (flash, heptane/*t*BuOMe, 9:1, followed by 85:15 and 8:2). 390 mg (52% yield) of **2** were isolated from fractions 80–89.

2: Colorless crystals, m.p. 163 °C. $[\alpha]_D^{20}$ = +5.9 (c = 1.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$), δ = 10.09 (s, 1 H, 9-OH), 5.08 (t, J = 6.7 Hz, 1 H, 27-H), 4.99 (m, 1 H, 32-H), 4.97 (m, 1 H, 22-H), 4.96 (m, 1 H, 17-H), 4.61 (br. s, 1 H, 10-H), 3.99 (d, J = 3.8 Hz, 1 H, 1-H), 3.09 (dd, J = 7.3 Hz and 13.4 Hz, 1 H, 26-Ha), 2.90 (dd, J = 6.2 Hz and 13.4 Hz, 1 H, 26-Hb), 2.87 (br. s, 1 H, 10-OH), 2.63 (dd, J = 5.3 Hz and 15.2 Hz, 1 H, 21-Ha), 2.32 (m, 1 H, 15-Ha), 2.30 (m, 1 H, 11-H), 2.13 (dd, J = 8.6 Hz and 15.2 Hz, 1 H, 21-Hb), 2.00 (m, 1 H, 31-Ha), 1.92 (m, 1 H, 16-Ha), 1.74 (m, 1 H, 31-Hb), 1.73 (m, 1 H, 16-Hb), 1.68 (s, 3 H, 34-H_3), 1.68 (s, 3 H, 29-H_3), 1.67 (s, 3 H, 24-H_3), 1.66 (m, 1 H, 4-H), 1.64 (s, 3 H, 25-H_3), 1.64 (s, 3 H, 20-H_3), 1.61 (s, 3 H, 30-H_3), 1.58 (s, 3 H, 35-H_3), 1.56 (s, 3 H, 19-H_3), 1.51 (d, J = 3.8 Hz, 1 H, 1-OH), 1.40 (m, 2 H, 5-H_2) 1.38 (m, 1 H, 15-Hb), 1.26 (s, 3 H, 14-H_3), 1.09 (d, J = 6.8 Hz, 3 H, 12-H_3), 0.97 (d, J = 6.8 Hz, 3 H, 13-H_3) ppm. HRESIMS: calcd. for $C_{35}H_{56}O_4Na$ 563.40708; found 563.40682 $[M + Na]^+$, 1103.82600 $[2M + Na]^+$.

Reaction of Hyperforin DCHA with RED-Al: To a solution of hyperforin DCHA (13.3 g, 18.5 mmol) in dry toluene (100 mL), an excess (3.5 mol/equiv.) of RED-Al (3.4 M solution in toluene) was added. After stirring at room temperature for 3 h, the reaction was worked up by dilution with EtOAc and acidification with HCl (10%). The organic phase was filtered through celite, washed with water, dried with Na_2SO_4, and the solvents were evaporated. The residue was crystallized from diisopropyl ether/hexane (9:1) to obtain 4.21 g of **2**. The mother liquors were purified by column chromatography on silica gel (400 g, hexane/*tert*-butyl methyl ether, 95:5, 1.8 L, followed by 9:1, 2.4 L). Eluted material was collected in 140 × 30 mL fractions. Identical fractions were combined to give 3 main fractions: fractions 16–56 contained 386 mg of **4** (4% yield), fractions 84–96 contained 857 mg of impure **3**, and fractions 108–140 contained 1.91 g of **2** (total of 6.12 g, 61% yield). Fractions 84–96 were subjected to flash column chromatography over Si gel (65 g, Ø = 3 cm, hexane/*tert*-butyl methyl ether, 9:1, 40 × 20 mL fractions) to obtain 63 mg of **3**.

3: Amorphous solid, $[\alpha]_D^{20}$ = +3.4 (c = 0.45, $CHCl_3$); ^1H (500 MHz, $CDCl_3$): δ = 6.41 (s, 1 H, H-7), 5.14 (t, J = 7.4 Hz, 1 H, H-27), 5.09 (t, J = 7.3 Hz, 1 H, H-32), 4.99 (d, J = 10.8 Hz, 1 H, OH-10), 4.98 (m, 1 H, H-17), 4.94 (t, J = 7.1 Hz, 1 H, H-22), 4.17 (dd, J = 1.9 Hz, 10.8 Hz, 1 H, H-10), 3.83 (s, 1 H, H-1), 2.87 (m, 2 H, H-26a and H-26b), 2.35 (ddd, J = 4.0 Hz, 12.4 Hz, 15.4 Hz, 1 H, H-15a), 2.23 (dd, J = 8.2 Hz, 14.4 Hz, 1 H, H-31a), 2.10 (dd, J = 8.4 Hz, 14.4 Hz, 1 H, H-31b), 2.04 (dd, J = 4.5 Hz, 14.5 Hz, 1 H, H-21a), 1.99 (dqq, J = 1.9 Hz, 6.7 Hz, 6.7 Hz, 1 H, H-11), 1.82 (ddd, J = 9 Hz, 9 Hz, 14.5 Hz, 1 H, H-21b), 1.73 (m, 1 H, H-16a), 1.73 (s, 3 H, 34-H_3), 1.72 (s, 3 H, 29-H_3), 1.70 (s, 3 H, 24-H_3), 1.66 (s, 3 H, 35-H_3), 1.63 (s, 3 H, 19-H_3), 1.61 (m, 1 H, H-5a), 1.60 (s, 3 H, 25-H_3), 1.59 (s, 3 H, 30-H_3), 1.59 (m, 1 H, H-16b), 1.56 (s, 3 H, 20-H_3), 1.50 (m, 1 H, H-4), 1.44 (m, 1 H, H-15b), 1.30 (m, 1 H, H-5b), 1.26 (s, 3 H, 14-H_3), 1.06 (d, J = 6.7 Hz, 3 H, 12-H_3), 0.67 (d, J = 6.7 Hz, 3 H, 13-H_3) ppm. HRESIMS: calcd for $C_{35}H_{56}O_4Na$ 547.41217; found 547.41372 $[M + Na]^+$, 563.40813 $[M + K]^+$.

4: Colorless viscous oil, $[\alpha]_D^{20}$ = +90.9 (c = 0.6, $CHCl_3$); ^1H (500 MHz, $CDCl_3$): δ = 6.47 (s, 1 H, H-7), 5.16 (t, J = 7.3 Hz, 1 H, H-27), 5.01 (m, 1 H, H-17), 4.98 (t, J = 7.0 Hz, 1 H, H-32), 4.93 (t, J = 7.1 Hz, 1 H, H-22), 4.17 (dd, J = 4.7 Hz, 12.3 Hz, 1 H, H-10), 3.05 (d, J = 12.2 Hz, 1 H, OH-10), 2.97 (d, J = 7.3 Hz, 2 H, H-26a and H-26b), 2.49 (dd, J = 6.7 Hz, 14.4 Hz, 1 H, H-31a), 2.12 (dd, J = 8.2 Hz, 14.4 Hz, 1 H, H-31b), 2.06 (dd, J = 4.7 Hz, 13.2 Hz, 1 H, H-21a), 1.93 (m, 2 H, H-16a and H-16b), 1.92 (m, 2 H, H-15a and H-15b), 1.80 (dd, J = 4.5 Hz, 13.6 Hz, 1 H, H-5aA), 1.73 (s, 3 H, 29-H_3), 1.71 (m, 1 H, H-21b), 1.70 (s, 3 H, 24-H_3), 1.67 (s, 3 H, 34-H_3), 1.66 (s, 3 H, 35-H_3), 1.65 (s, 3 H, 19-H_3), 1.62 (m, 1 H, H-4), 1.61 (m, 1 H, H-11), 1.60 (s, 3 H, 30-H_3), 1.59 (s, 3 H, 20-H_3), 1.56 (s, 3 H, 25-H_3), 1.38 (dd, J = 12.3 Hz, 13.6 Hz, 1 H, H-5b), 0.92 (s, 3 H, 14-H_3), 0.82 (d, J = 6.7 Hz, 3 H, 12-H_3) 0.75 (d, J = 6.7 Hz, 3 H, 13-H_3) ppm. HRESIMS: calcd for $C_{35}H_{54}O_3Na$ 545.39652; found 545.39693$[M + Na]^+$, 1067.80463 $[2M + Na]^+$.

Reaction of Hyperforin DCHA with DIBAL: To hyperforin DCHA (4.21 g, 5.88 mmol), magnetically stirred in dry THF (25 mL), DIBAL (1 M solution in toluene, 17.7 mL, 3 mol/equiv.) was added under nitrogen. After hyperforin disappeared (2 h), the reaction was worked up by dilution with EtOAc (10 mL), acidification with HCl (0.1 N, 10 mL), and filtration through celite. The solution was washed with H_2O (2 × 5 mL), dried with Na_2SO_4, and the solvents were evaporated. The residue was fractionated by flash column chromatography over Si gel (260 g, Ø = 6 cm, hexane/*tert*-butyl methyl ether, 20:1, 1.56 L, 13×120 mL fractions, followed by hexane/*tert*-butyl methyl ether, 9:1, 2.01 L, 67 × 30 mL fractions) to obtain three main fractions: 6–7 (483 mg, **5**, 15% yield), 8–10 (645 mg, **6**, 19% yield), and 58–80 (842 mg, **7+8**). Fractions 58–80 were purified by flash column chromatography over Si gel (270 g, Ø = 5 cm, petroleum ether/EtOAc, 18.5:1.5, 2.3 L) to obtain 302 mg of **7** and 305 mg of **8**.

A heterogeneous mixture of **7** (105 mg, calculated: 0.20 mmol), powdered KOH (10.5 mg, 10 mol/equiv.), and CH_2Br_2 (5 mol/equiv.) in dry *N*,*N*-dimethylformamide (2 mL) was stirred under nitrogen overnight. The reaction was worked up by dilution with CH_2Cl_2, filtration of the KOH, and washing with H_2O. The organic phase was dried with Na_2SO_4, and the solvents were evaporated. The residue was purified by column chromatography over Si gel (10 g, petroleum ether/EtOAc, 50:1, 48 mL, 12 × 4 mL fractions) to obtain 30 mg of **7a** (28% yield). The same reaction when applied to 115 mg (calculated: 0.22 mmol) of **8** afforded 38 mg of **8a** (33% yield).

5: Colorless viscous oil. $[\alpha]_D^{20}$ = +111.2 (c = 0.5, $CHCl_3$). ^1H (400 MHz, $CDCl_3$): δ = 6.53 (d, J = 1.0 Hz, 1 H, H-7), 5.15 (t, J = 7.1 Hz, 1 H, H-27), 5.12 (t, J = 7.1 Hz, 1 H, H-32), 5.06 (t, J =

7.1 Hz, 1 H, H-22), 4.92 (t, J = 7.0 Hz, 1 H, H-17), 3.01 (d, J = 6.2 Hz, 2 H, H-26a and H-26b), 2.47 (dd, J = 7.1 Hz, 14.6 Hz, 1 H, H-31a), 2.24 (dd, J = 7.1 Hz, 14.6 Hz, 1 H, H-31b), 2.14 (m, 2 H, H-21a and H-21b); 2.13 (m, 1 H, H-16a), 1.97 (dqq, J = 1.9 Hz, 6.7 Hz, 6.7 Hz, 1 H, H-11), 1.91 (m, 1 H, H-16b), 1.89 (m, 1 H, H-15a), 1.73 (s, 3 H, 34-H_3), 1.73 (s, 3 H, 29-H_3), 1.71 (m, 1 H, H-5a), 1.69 (s, 3 H, 24-H_3), 1.67 (s, 3 H, 35-H_3), 1.65 (s, 3 H, 19-H_3), 1.61 (s, 3 H, 25-H_3), 1.60 (s, 3 H, 30-H_3), 1.56 (s, 3 H, 20-H_3), 1.51 (m, 1 H, H-5b), 1.48 (m, 1 H, H-4), 1.36 (m, 1 H, H-15b), 1.11 (d, J = 6.8 Hz, 3 H, 12-H_3), 1.03 (s, 3 H, 14-H_3), 1.01 (d, J = 6.8 Hz, 3 H, 13-H_3) ppm. FABMS: m/z = 521 $[M + H]^+$.

6: Colorless viscous oil. $[\alpha]_D^{20}$ = +156.0 (c = 0.53, $CHCl_3$). ^1H (400 MHz, $CDCl_3$): δ = 6.38 (s, 1 H, H-7), 5.14 (t, J = 7.1 Hz, 1 H, H-27), 5.12 (m, 1 H, H-17), 5.10 (m, 1 H, H-32), 4.90 (t, J = 7.1 Hz, 1 H, H-22), 4.27 (dd, J = 2.8 Hz, 12.8 Hz, 1 H, H-10), 3.88 (d, J = 12.0 Hz, 1 H, 10-OH), 2.94 (d, J = 7.1 Hz, 2 H, H-26a and H-26b), 2.65 (dqq, J = 1.9 Hz, 6.7 Hz, 6.7 Hz, 1 H, H-11), 2.42 (dd, J = 6.5 Hz, 15.3 Hz, 1 H, H-31a), 2.18 (m, 1 H, H-31b), 2.13 (m, 2 H, H-21a and H-21b), 2.12 (m, 1 H, H-15a), 2.09 (m, 1 H, H-16a), 1.77 (m, 1 H, H-16b), 1.76 (m, 1 H, H-5a), 1.73 (s, 3 H, 34-H_3), 1.73 (s, 3 H, 29-H_3), 1.70 (m, 1 H, H-11), 1.69 (s, 3 H, 24-H_3), 1.69 (s, 3 H, 35-H_3), 1.69 (s, 3 H, 19-H_3), 1.61 (s, 3 H, 25-H_3), 1.58 (s, 3 H, 30-H_3), 1.56 (s, 3 H, 20-H_3), 1.55 (m, 1 H, H-5b), 1.43 (m, 1 H, H-15b), 1.41 (m, 1 H, H-4), 1.17 (s, 3 H, 14-H_3), 0.91 (d, J = 6.8 Hz, 3 H, 12-H_3), 0.67 (d, J = 6.8 Hz, 3 H, 13-H_3) ppm. FABMS: m/z = 523 $[M + H]^+$.

7a: Colorless viscous oil, $[\alpha]_D^{20}$ = +19.1 (c = 0.3, $CHCl_3$); ^1H (400 MHz, $CDCl_3$): δ = 6.37 (d, J = 2.0 Hz, 1 H, H-7), 5.18 (t, J = 7.3 Hz, 1 H, H-27), 5.06 (t, J = 7.0 Hz, 1 H, H-32), 5.06 (t, J = 7.1 Hz, 1 H, H-17), 5.01 (d, J = 5.6 Hz, 1 H, H-36a), 4.95 (t, J = 7.1 Hz, 1 H, H-22), 4.92 (d, J = 5.6 Hz, 1 H, H-36b), 4.15 (d, J = 4.0 Hz, 1 H, H-10), 4.04 (s, 1 H, H-1), 2.88 (d, J = 7.3 Hz, 2 H, H-26a and H-26b), 2.65 (dqq, J = 1.9 Hz, 6.7 Hz, 6.7 Hz, 1 H, H-11), 2.32 (dd, J = 8.9 Hz, 14.4 Hz, 1 H, H-31a), 2.04 (dd, J = 5.8 Hz, 14.4 Hz, 1 H, H-31b), 2.02 (dd, J = 4.8 Hz, 13.2 Hz, 1 H, H-21a), 1.96 (m, 1 H, H-16a), 1.83 (m, 1 H, H-21b), 1.77 (s, 3 H, 29-H_3), 1.77 (m, 1 H, H-16b), 1.74 (s, 3 H, 24-H_3), 1.73 (s, 3 H, 34-H_3), 1.68 (s, 3 H, 19-H_3), 1.64 (s, 3 H, 35-H_3), 1.62 (s, 3 H, 20-H_3), 1.60 (s, 3 H, 25-H_3), 1.60 (s, 3 H, 30-H_3), 1.57 (m, 1 H, H-15a), 1.40 (dd, J = 3.8 Hz, 12.4 Hz, 1 H, H-5a), 1.33 (m, 1 H, H-4), 1.28 (m, 1 H, H-5b), 1.27 (m, 1 H, H-15b), 1.34 (s, 3 H, 14-H_3), 1.08 (d, J = 7.0 Hz, 3 H, 12-H_3), 0.91 (d, J = 7.0 Hz, 3 H, 13-H_3) ppm. EIMS: m/z = 536 $[M^+].]^+$

8a: Colorless viscous oil, $[\alpha]_D^{20}$ = +8.8 (c = 0.8, $CHCl_3$); ^1H (400 MHz, $CDCl_3$): δ = 5.94 (d, J = 2.0 Hz, 1 H, H-7), 5.21 (t, J = 7.1 Hz, 1 H, H-27), 5.22 (t, J = 7.3 Hz, 1 H, H-32), 5.00 (t, J = 7.1 Hz, 1 H, H-17), 5.05 (d, J = 5.6 Hz, 1 H, H-36a), 4.95 (t, J = 7.1 Hz, 1 H, H-22), 4.66 (d, J = 5.6 Hz, 1 H, H-36b), 3.32 (s, 1 H, H-10), 3.75 (d, J = 1 Hz, 1 H, H-1), 3.00 (d, J = 14.5 Hz, 1 H, H-26a), 2.94, (dd, J = 7.2 Hz, 14.5 Hz, 1 H, H-26b), 2.38 (dqq, J = 1.9 Hz, 6.7 Hz, 6.7 Hz, 1 H, H-11), 2.28 (dd, J = 8.2 Hz, 14.4 Hz, 1 H, H-31a), 2.10 (dd, J = 5.8 Hz, 14.4 Hz, 1 H, H-31b), 2.04 (dd, J = 4.5 Hz, 14.5 Hz, 1 H, H-21a), 1.82 (m, 2 H, H-16a and H-16b), 1.75 (m, 1 H, H-21b), 1.72 (s, 3 H, 29-H_3), 1.76 (s, 3 H, 24-H_3), 1.80 (s, 3 H, 34-H_3), 1.70 (s, 3 H, 19-H_3), 1.64 (s, 3 H, 35-H_3), 1.58 (s, 3 H, 20-H_3), 1.58 (s, 3 H, 25-H_3), 1.59 (s, 3 H, 30-H_3), 1.75 (m, 1 H, H-15a), 1.38 (m, 1 H, H-5a), 1.33 (m, 1 H, H-4), 1.30 (m, 1 H, H-5b), 1.33 (m, 1 H, H-15b), 0.94 (s, 3 H, 14-H_3), 1.31 (d, J = 6.7 Hz, 3 H, 12-H_3), 1.09 (d, J = 6.7 Hz, 3 H, 13-H_3) ppm. EIMS: m/z = 536 $[M^+].]^+$

Crystal Structure of 2: $C_{35}H_{56}O_4 \cdot C_5H_{12}O$, Mr = 628.94. The material was crystallized from different solvents, but always with poor results; the best crystals were obtained from *tert*-butyl methyl ether, yielding opaque prismatic crystals, probably due to the partial loss of solvated ether. In spite of the apparently good quality of the crystals, they diffracted quite poorly and at relatively low angle. A first attempt to collect data was affected by decay. Attempts to collect at lower temperature failed probably due to a phase transition. In the end, data were collected at room temperature with a crystal completely covered by epoxy glue to prevent decay. Orthorhombic, space group $P2_12_12_1$, a = 12.161(2), b = 17.046(2), c = 19.465(2) Å, V = 4035.0(9) Å^3, Z = 4, D_c = 1.034 g cm^{-3}, Mo-K_α radiation (λ = 0.71073 Å), μ(Mo-K_α) = 0.066 mm^{-1}, and dimensions 0.32 × 0.25 × 0.24 mm. Data were recorded with a Bruker APEX CCD area-detector diffractometer with 34689 reflections (θ < 25°), 3974 reflections after averaging (R_{av} = 0.0411), and 2981 reflections with $I > 2\sigma(I)$. The structure was solved by SIR2002[13] and refined by full-matrix least-squares based on F^2 by SHELXL.[14] Heavy atoms were refined anisotropically; only hydrogen atoms on stereocenters were refined isotropically, all the others being fixed in a calculated position. Final results: wR = 0.1570, R = 0.0740 (0.1487 and 0.583, respectively on observed reflections); $\Delta\rho_{max}$ = 0.40 e·Å^{-3}. Due to the poor quality of the crystals and the large ADPs, especially for the solvate ether molecule, the structural data are of low quality; in spite of that, the relative configuration of the molecule is surely correct; the absolute configuration was assigned on the biogenetic configuration of the starting molecule.

CCDC-609005 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting Information (see footnote on the first page of this article): ^1H and ^{13}C NMR spectra of reported compounds.

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