Oxidative Fragmentation of the Bridged β-Triketone Core of Hyperforin

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The β-triketone core of the antidepressant phloroglucinol hyperforin (**1**) undergoes a series of peroxide-induced oxidative rearrangements leading to compound **5**, which is formed by opening of ring A, and compound **6**, which is formed by removal of the C-1 carbonyl bridge. A mechanistic rationale for this process is proposed.

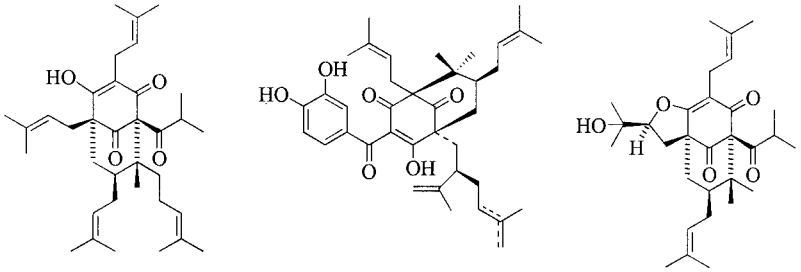
Introduction

Polyisoprenylated phloroglucinols are a class of meroterpenoids which are accumulated in Clusiaceous plants and, less systematically, in plants belonging to the Myrtaceae and Cannabaceae families.[1] Bioactivities associated to certain polyisoprenylated phloroglucinols have lead to the identification of a number of interesting targets for these compounds. Thus, hyperforin (**1**), one of the antidepressant constituents of St. John's wort (*Hypericum perforatum* L.),[2] is the most powerful ligand known for the pregnane X-receptor (PXR), a key regulator of the metabolism of endo- and xenobiotics.[3] Xanthochymol and guttiferone E (**2a**,**b**) act as Taxol® mimics on tubulin[4] while garsubellin A (**3**) induces choline acetyltransferase (ChAT) and shows powerful neurotrophic activity.[5]

All these compounds share a bridged, bicyclic β-triketone moiety (mono-enolised and etherified in garsubellin A), whose construction entails noteworthy challenge. Although no complete synthesis of **1**−**3** has yet been achieved, a number of synthetic approaches to the carbon skeleton of these compounds exemplifies the current interest in the area.[6] In the course of studies on the structure−activity relationships of hyperforin,[7] we discovered that the bridged β-triketone core of this compound is susceptible to oxidative fragmentation and we would like to report here on the structure and formation of new compounds obtained by this route.

Discussion

Reaction of hyperforin with a number of peroxidic reagents (H_2O_2, oxone, *m*CPBA, TBHP) is known to afford cyclic hemiacetal **4**. A comparative study involving various peroxidic oxidants under different reaction conditions evidenced that the course of the reaction was dramatically affected by the nature of the oxidant, the solvent, and the use of hyperforin either in a free acid form or as its ammonium salt.[7] In particular, using dioxane, the dicyclohexylammonium salt of hyperforin gave, along with **4**, two other compounds (**5**, **6**) as a result of intricate reaction pathways.



1 **2a,b** **3**

High-resolution mass spectrometry suggested the molecular formula $C_{35}H_{52}O_5$ for **5**, which corresponds to the in-

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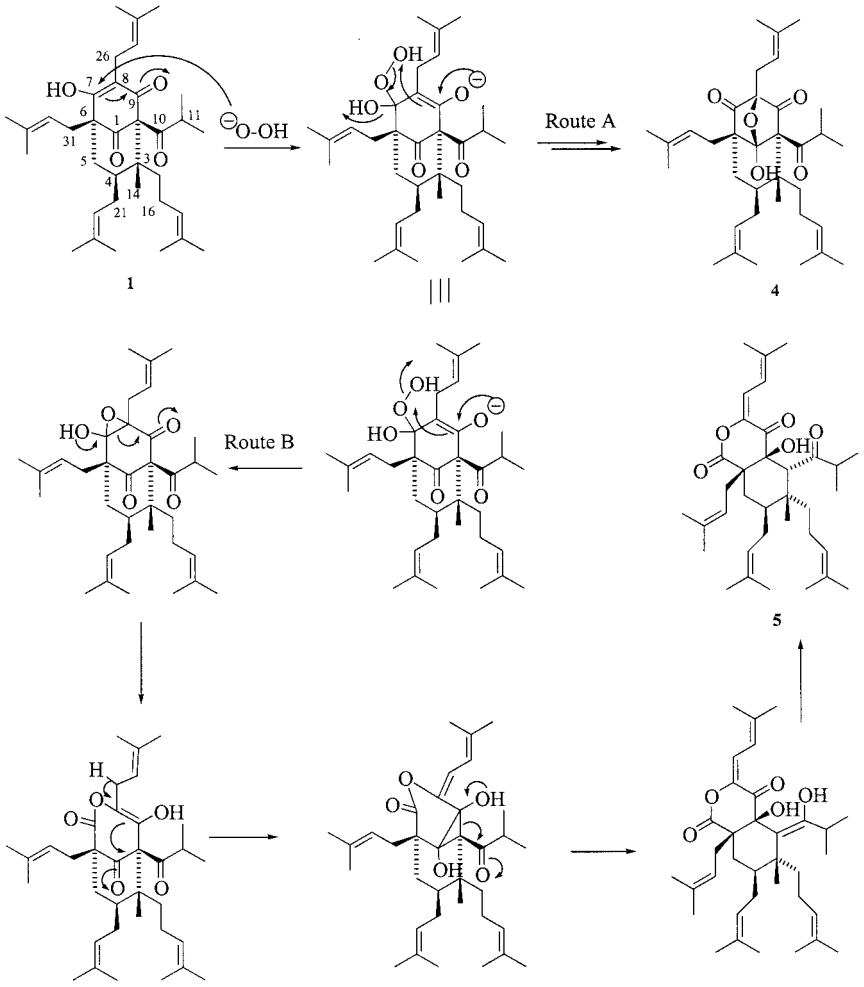
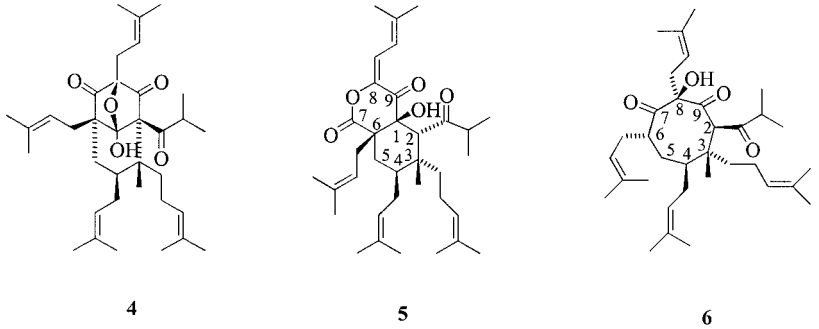


Scheme 1. Possible mechanism for the formation of compounds **4** and **5** from the H_2O_2 oxidation of hyperforin (**1**)

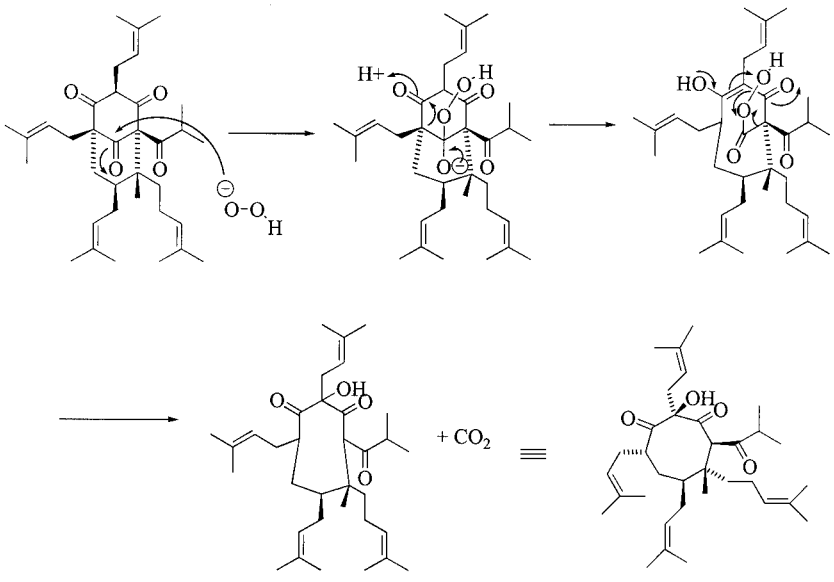


troduction of an oxygen atom in hyperforin. By comparing the ^{13}C NMR spectra of **1** and **5**, a marked (ca. 30 ppm) upfield shift of one of the ketone resonances was observed. This shift is consistent with the introduction of an extra oxygen atom adjacent to a ketone carbonyl, with overall conversion into an ester or lactone. A further striking difference is the appearance of a second aliphatic methine besides that of the isopropyl moiety. This was identified as C-2 on the basis of an HMBC correlation between this carbon and 14-H, the only aliphatic quaternary methyl, as well as between the attached proton (2-H) and the other aliphatic methine (11-H). These observations suggested a complex

rearrangement of the bicyclic core of hyperforin. Since one of the allylic methylenes in the prenyl side chains was replaced by an olefinic methine, one of the prenyl appendages must also be involved in the reaction.

Starting from C-14, a sequence of HMBC correlations could be identified for **5**, showing retention of the hyperforin connectivity in the lower sesquiterpene and the C-6 prenyl moieties, while only two carbon atoms (C-3 and C-6) of the original phloroglucinol core maintain the hybridisation and atom connectivity of the starting material. The remaining resonances of the phloroglucinol moiety are a quaternary hydroxylated carbon (C-1), a lactone carbonyl (C-7), an oxygenated olefin carbon (C-8), and a methine (C-2). These data could be rationalised in an oxadecalin structure such as **5**. The presence of an NOE-correlation between 2-H and the quaternary hydroxy proton secured the relative configuration at C-1 and C-2, next related to that of C-6 by capitalising on the dipolar correlations centred around 31-$H_{a,b}$. A possible mechanism for the formation of this compound is depicted in Scheme 1. Thus, conjugated addition of H_2O_2 to the enone system affords a peroxidic semiacetal, from which both **4** and **5** could be derived. Attack on the distal oxygen leads to a C-8-hydroxylated derivative, subsequently evolving into **4** (Scheme 1, Route A). Alternatively (Scheme 1, Route B), enolate attack on the proximal oxygen atom of the peroxy group would yield a hydroxylated epoxide. Fragmentation of the epoxide carbon−carbon bond generates a seco-enolate, which evolves into a hydroxylated cyclopropane by attack on the C-1 carbonyl. Retro-aldol opening of the cyclopropane moiety would afford an enolic form of **5**, terminating a remarkable set of steps seemingly driven by the formation of a conjugated dienone in **5** and by the opening of the sterically congested bridged system of **1**. Finally, ROESY experiments also indicated epimerisation of the isobutyryl group to a β-configuration, *cis* to the angular C-3 methyl.

The HRMS spectrum of **6** showed loss of one carbon from hyperforin, suggesting introduction of two oxygen atoms and loss of the equivalent of one molecule of carbon dioxide. Detection of HMBC correlations from 14-H_3 to C-2, C-3, C-4, and C-15, as well as COSY correlations from 4-H via 5-H_2 to 6-H showed that C-2 and C-6 were both methines, identifying the extruded carbon as C-1. The pattern of HMBC correlations of the non-prenyl or homoprenyl signals (2-H to C-9, from 6-H to C-7, from 26-H_2 to C-7, C-8 and C-9, as well as from 8-OH to C-7, C-8 and C-9) could be rationalised by the presence of an eight-membered ring and C-8 as a quaternary oxygenated carbon. While the structure of **6** could be deduced in a straightforward way from 2-D experiments, translation of the observed pattern of ROESY correlations into configurational assignments was complicated by the flexibility of the medium-sized ring. Since the non-enolisable C-3 and C-4 stereocentres had apparently survived oxidation, we assumed that the hyperforin configuration was retained at these carbon atoms, which were, therefore, employed as a reference for the configurational considerations of other stereocentres. NOESY correlations between 2-H and 4-H, 16-H_2, and 26-H_2, 2H and 26H_2, 8-OH and 6-H, and the absence of NOE between 2-H and 8-OH, supported the configuration depicted in **6**. A possible mechanism for the formation of **6** from **1** is depicted in Scheme 2. Thus, hydrogen peroxide



6

Scheme 2. Possible mechanism for the formation of **6**

attack on the C-1 ketone carbonyl sets the stage for fragmentation of the adjacent C-1−C-6 bond and keto−enol tautomerization to a 7,8-enolate. Attack of the latter onto the distal peroxide oxygen might then trigger the extrusion of carbon dioxide.

Conclusions

Hyperforin undergoes a series of peroxide-induced oxidative rearrangements of its tricarbonyl moiety, resulting in the transformation of the bridged, bicyclic core to a monocyclic or linearly fused bicyclic system. These reactions are reminiscent of acid-catalysed cascade-rearrangements of polyprenoids, with enol/carbonyl rather than cation/olefin interactions as chain carriers. In view of the key role of hydrogen peroxide in cellular metabolism, one might speculate on the possible formation of compounds **5** and **6** in vivo, as a result of hyperforin metabolism. Finally, it seems reasonable to assume that the oxidative lability of the tricarbonyl core is a common hallmark of polyprenylated phloroglucinols. This feature should be taken into consideration in the synthetic plans aimed at their total synthesis.

Experimental Section

General: Optical rotation values were recorded with a Perkin−Elmer 241 polarimeter. The IR spectra were recorded with an FTIR Perkin−Elmer 1725 X spectrometer. Column chromatography was performed using silica gel 60 (70−230 and 40−63 mesh, Merck). The reactions were monitored by TLC on Merck 60 F_{254} (0.25 mm) plates, visualising the spots with fluorescent 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: ^{1}H 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 probe head equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$ and the solvent signals (δ = 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, DQFCOSY, HMQC, HMBC and ROESY experiments were recorded with gradient enhancements using sine-shaped gradient pulses. For the 2-D heteronuclear correlation-spectroscopy, the refocusing delays were optimised for $^1J_{C,H}$ = 145 Hz and $^nJ_{C,H}$ = 10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker XWIN NMR software (rev. 010101). The ^{1}H and ^{13}C NMR resonances were assigned with the aid of 2-D data based on scalar and dipolar homo- and heterocouplings. FAB mass spectra were recorded with a Jeol SX102 spectrometer.

Oxidation of Hyperforin: Hydrogen peroxide 30% (2 mL) was added to hyperforin dicyclohexylammonium salt (500 mg, 0.70 mmol), suspended in dioxane (5 mL), whilst stirring at room temperature. After 4 h, the mixture was diluted with CH_2Cl_2 and extracted. After drying and solvent evaporation, a crude residue was obtained (498 mg), which was purified by column chromatography on silica gel (flash, petroleum ether/*t*BuOMe, 24:1, followed by 13:1). In order of increasing polarity, 135 mg of **4** (0.244 mmol, 35 %), 69.5 mg of **5** (0.126 mmol, 21 %), and 37 mg of **6** (0.07 mmol, 10 %) were isolated. The column was then washed with EtOAc giving 82 mg as a mixture of furohyperforins (14.8 %). The same reaction, stopped after 30 min, worked-up and immediately purified gave **4** (95 %), which was stable for months in CH_2Cl_2. Part of the crude material gave furohyperforins after one week at room temperature and after exposure to light.

Hemiacetal **4** (90 mg) was dissolved in dioxane and H_2O_2 30% (0.8 mL) was added. Trifluoroacetic acid and diethylamine were added at room temperature to the hemiacetal **4** dissolved in CH_2Cl_2. No changes were evident after a few weeks.

Data for **4** are reported in ref.[8]

Compound **5** was obtained as a colourless oil. $[\alpha]_D^{25}$ = +49.4 (*c* = 0.42, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 0.97 (s, 3 H, 14-H_3), 1.14 (d, *J* = 6.8 Hz, 3 H, 13-H_3), 1.29 (m, 1 H, 15-H_b), 1.48 (d, *J* = 6.8 Hz, 3 H, 12-H_3), 1.49 (m, 1 H, 4-H), 1.56 (s, 3 H, 35-H_3), 1.63 (s, 3 H, 25-H_3), 1.64 (s, 3 H, 19-H_3), 1.64 (m, 1 H, 15-H_a), 1.70 (s, 3 H, 34-H_3), 1.71 (s, 3 H, 24-H_3), 1.72 (m, 1 H, 21-H_b), 1.72 (s, 3 H, 20-H_3), 1.90 (s, 3 H, 29-H_3), 1.92 (m, 2 H, 5-H_2), 1.93 (s, 3 H, 30-H_3), 2.04 (dd, *J* = 5.9, 14.5 Hz, 1 H, 31-H_b), 2.13 (m, 3 H, 16-H_2 and 21-H_a), 2.27 (dd, *J* = 8.9, 14.5 Hz, 1 H, 31-H_a), 2.75 (sept., *J* = 6.8 Hz, 1 H, 11-H), 3.23 (s, 1 H, 2-H), 4.95 (t, *J* = 6.9 Hz, 1 H, 32-H), 5.00 (t, *J* = 6.6 Hz, 1 H, 22-H), 5.05 (t, *J* = 6.8 Hz, 1 H, 17-H), 6.26 (s, 1 H, OH), 6.33 (d, *J* = 12.0 Hz, 1 H, 27-H), 6.79 (d, *J* = 12.0 Hz, 1 H, 26-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 17.8 (q, C-19), 18.0 (q, C-25), 18.0 (q, C-35), 18.2 (q, C-13) 19.2 (q, C-29), 19.7 (q, C-14), 21.9 (q, C-12), 22.1 (t, C-16), 24.9 (t, C-5), 25.7 (q, C-20), 25.9 (q, C-34), 25.9 (q, C-24), 26.8 (t, C-31), 26.9 (q, C-30), 27.1 (t, C-21), 36.0 (d, C-4), 38.5 (t, C-15), 40.5 (s, C-3), 43.8 (d, C-11), 46.6 (d, C-2), 51.2 (s, C-6), 79.8 (s, C-1), 116.2 (d, C-32), 116.2 (d, C-26), 117.8 (d, C-27), 122.8 (d, C-22), 122.8 (d, C-17), 132.5 (s, C-23), 133.1 (s, C-18), 136.1 (s, C-33), 141.2 (s, C-8), 148.3 (s, C-28), 168.6 (s, C-7), 188.8 (s, C-9), 223.0 (s, C-10) ppm. FAB HRMS: *m*/*z* = 552.3811 (calcd. for $C_{35}H_{52}O_5$, 552.3814).

Compound **6** was obtained as a colourless oil. $[\alpha]_D^{25}$ = −85.8 (*c* = 0.72, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.00 (d, *J* = 6.8 Hz, 3 H, 13-H_3), 1.04 (d, *J* = 6.8 Hz, 3 H, 12-H_3), 1.13 (m, 1 H, 15-H_b), 1.14 (s, 3 H, 14-H_3), 1.34 (m, 1 H, 4-H), 1.34 (m, 1 H, 5-H_b), 1.45 (m, 1 H, 15-H_a), 1.56 (m, 1 H, 5-H_a), 1.59 (s, 6 H, 35-H_3 and 29-H_3), 1.62 (s, 3 H, 25-H_3), 1.62 (s, 3 H, 19-H_3), 1.64 (s, 3 H, 30-H_3), 1.66 (s, 6 H, 20-H_3 and 34-H_3), 1.70 (s, 3 H, 24-H_3), 1.78 (m, 1 H, 21-H_b), 1.79 (m, 1 H, 31-H_b), 1.93 (m, 1 H, 16-H_2), 1.97 (m, 1 H, 21-H_a), 2.39 (m, 1 H, 31-H_a), 2.48 (dd, *J* = 6.8, 15.6 Hz, 1 H, 26-H_b), 2.75 (dd, *J* = 7.1, 15.6 Hz, 1 H, 26-H_a), 2.88 (sept., *J* = 6.8 Hz, 1 H, 11-H), 3.39 (m, 1 H, 6-H), 4.19 (s, 1 H, 2-H), 4.34 (s, 1 H, OH), 4.76 (t, *J* = 6.6 Hz, 1 H, 27-H), 4.89 (t, *J* = 6.7 Hz, 1 H, 17-H), 4.93 (t, *J* = 6.8 Hz, 1 H, 32-H), 5.02 (t, *J* = 6.8 Hz, 1 H, 22-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 16.7 (q, C-14), 17.8 (q, C-35), 17.9 (q, C-25), 17.9 (q, C-19), 18.2 (q, C-29), 19.3 (q, C-13), 19.4 (q, C-12), 21.6 (t, C-16), 25.7 (q, C-34), 25.7 (q, C-20), 25.9 (q, C-30), 26.0 (t, C-24), 26.8 (t, C-31), 29.9 (t, C-5), 30.1 (t, C-21), 33.2 (t, C-26), 39.3 (d, C-4), 39.5 (d, C-15), 39.5 (d, C-15), 41.2 (d, C-11), 47.0 (d, C-6), 48.9 (s, C-3), 63.8 (d, C-2), 88.3 (s, C-8), 115.8 (d, C-27), 122.3 (d, C-32), 123.1 (d, C-17), 123.3 (d, C-22), 132.2 (s, C-18), 132.5 (s, C-33), 133.0 (s, C-23), 136.8 (s, C-28), 202.8 (s, C-9), 207.8 (s, C-10), 210.1 (s, C-7). FAB HRMS: *m*/*z* = 527.4089 (calcd. for $C_{34}H_{55}O_4$, 527.4100).

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