JOC*Article*

Modulation of Chemoselectivity by Protein Additives. Remarkable Effects in the Oxidation of Hyperforin

Luisella Verotta,*,† Erminio Lovaglio,† Olov Sterner,‡ Giovanni Appendino,§ and Ezio Bombardelli||

Dipartimento di Chimica Organica e Industriale, Via Venezian 21, 20133 Milano, Italy, Department of Organic Chemistry 2, P.O.B 124, 221 00 Lund, Sweden, Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, via Bovio 6, 28100 Novara, Italy, and Indena S.p.A., V.le Ortles 12, 20139 Milano, Italy

luisella.verotta@unimi.it

Received July 6, 2004

Protein additives have a dramatic effect on the H_2O_2 oxidation of hyperforin, either protecting the enolized phloroglucinol core from oxidation (human albumin) or promoting (HRP and ovalbumin) reaction pathways derived from the intermediacy of the enollactone **4**, a minor component of the oxidation mixture in the absence of protein additives. To rationalize the exquisite specificity of several steps and their mechanistic oddity, an organocatalytic effect is postulated. The use of protein additives allows a straightforward and multigram preparation of the enollactone **6**, an interesting multifunctionalized scaffold for bioactivity induction and/or modulation.

Introduction

Hypericum perforatum L. is a traditional medicinal plant used for the topical treatment of superficial wounds, burns, and dermatitis. More recently, its efficacy in the management of mild to moderate depressive disorders has also been demonstrated,[1] with the phloroglucinol hyperforin (**1**, Figure 1)) emerging as the major psychoactive constituent of the plant.[1,2] Hyperforin shows a pleyotropic pattern of bioactivity, which includes antibacterial,[3] anticancer,[4,5] and apoptotic properties.[6] Furthermore, hyperforin can also inhibit inflammatory enzymes (cyclooxygenase-1 and 5-lipoxygenase),[7] interfere with radical chain oxidation processes,[8] and act as ultrapotent ligand for the pregnane X receptor, a nuclear factor that regulates expression of the cytochrome P450 3A4 monooxygenase.[9] PXR plays a key role in the metabolism of xenobiotics and the maintenance of chemical omeostasis, and its activation by hyperforin underlies the severe interactions with prescription drugs reported for St. John's wort extracts.[9]

No total synthesis of hyperforin and hyperforin-like compounds has yet been achieved, but this objective is currently pursued by several groups.[10] The oxidative lability of the enolized phloroglucinol core of the natural product is one of the major hurdles facing these efforts. The oxidative instability of hyperforin is especially marked toward oxygen and its activated species,[11] and the natural product is unstable under aerobic conditions in a variety of solvents, especially the apolar ones.[12]

Hyperforin could be detected in plasma after oral ingestion of St. John's wort extracts,[13] but its metabolism

* To whom correspondence should be addressed. Tel: +39 02 50314114. Fax: +39 02 503141106.
† Dipartimento di Chimica Organica e Industriale.
‡ Department of Organic Chemistry 2.
§ Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche.
|| Indena S.p.A.

(1) (a) Di Carlo, G.; Borrelli, F.; Ernst, E.; Izzo, A. A. *Trends Pharmacol. Sci.* **2001**, *22*, 292−297. (b) AA. VV. *Pharmacopsychiatry* **2001**, *34*, Suppl 1. (c) Verotta L. *Curr. Top. Med. Chem.* **2003**, *3*, 69−83.

(2) (a) Chatterjee, S. S. Bhattacharya, S. K.; Wonnemann, M.; Singer, A.; Muller, W. E. *Life Sci*. **1998**, *63*, 499−511. (b) Singer, A.; Wonnemann, M.; Muller, W. E. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 1363−1368. (c) Cervo, L.; Rozio, M.; Ekalle-Soppo, C. B.; Guiso, G.; Morazzoni, P.; Caccia, S. *Phychopharmacology* **2002**, *164*, 423−428.

(3) Schempp, C. M.; Pelz, K.; Wittmer, A.; Schopf, E.; Simon, J. C. *Lancet* **1999**, *353*, 2129.

(4) Schempp, C. M.; Winghofer, B.; Lüdtke, R.; Simon-Haarhaus, B.; Schöpff, E.; Simon, J. C. *Br. J. Dermatol.* **2000**, *142*, 979−984.

(5) Schempp, C. M.; Kirkin, V.; Simon-Haarhaus, B.; Kersten, A.; Kiss, J.; Termeer, C. C.; Gilb, B.; Kaufmann, T.; Borner, C.; Sleeman, J. P.; Simon, J. *Oncogene* **2001**, *21*, 1242−21250.

(6) Hostanska, K.; Reichling, J.; Bommer, S.; Weber, M.; Saller, R. *Eur. J. Pharm. Biopharm*. **2003**, *56*, 121−132.

(7) Albert, D.; Zündorf, I.; Dingermann, T.; Müller, W. E.; Steinhilber, D.; Werz, O. *Biochem. Pharmacol.* **2002**, *64*, 1767−1775.

(8) Heilmann, J.; Winkelmann, K.; Sticher, O. *Planta Med.* **2003**, *69*, 202−206.

(9) Watkins, R. E.; Maglich, J. M.; Moore, L. B.; Wisely, G. B.; Noble, S. M.; Davis-Searles, P. R.; Lambert, M. H.; Kliewer, S. A.; Redinbo, M. R. *Biochemistry* **2003**, *42*, 1430−1438. (b) Moore, L. B.; Goodwin, B.; Jones, S. A.; Wisely, G. B.; Serabjit-Singh, C. J.; Willson, T. M.; Collins, J. L.; Kliewer, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7500−7502.

(10) (a) Nicolaou, K. C.; Pfefferkorn, J. A.; Kim, S.; Wei, H. X. *J. Am. Chem. Soc.* **1999**, *121*, 4724−4725. (b) Nicolaou, K. C.; Pfefferkorn, J. A.; Cao, G.-Q.; Kim, S.; Kessabi, J. *Org. Lett.* **1999**, *1*, 807−810. (c) Usuda, H.; Kanai, M.; Shibasaki, M. *Org. Lett.* **2002**, *4*, 859−862. (d) Usuda, H.; Kanai, M.; Shibasaki, M. *Tetrahedron Lett.* **2002**, *43*, 3621−3624. (e) Spessard, S. J.; Stoltz, B. M. *Org. Lett.* **2002**, *4*, 1943−1946. (f) Kraus, G. A.; Nguyen, T. H.; Jeon, I. *Tetrahedron Lett*. **2003**, *44*, 659−661.

(11) (a) Verotta, L.; Appendino, G.; Balloro, E.; Jakupovic, J.; Bombardelli, E. *J. Nat. Prod.* **1999**, *62*, 770−772. (b) Verotta, L.; Appendino, G.; Jakupovic, J.; Bombardelli, E. *J. Nat. Prod.* **2000**, *63*, 412−15. (c) Wolfender, J.-L.; Verotta, L.; Belvisi, L.; Fuzzati, N.; Hostettmann, K. *Phytochem. Anal*. **2003**, *14* (5), 290−297. (d) Verotta, L. *Phytochem. Rev*. **2002**, 389−407.

(12) Orth, H. C. J.; Rentel, C.; Schmidt, P. C. *J. Pharm. Pharmacol*. **1999**, *51*, 193−200.

10.1021/jo048857s CCC: $27.50
Published on Web 10/15/2004

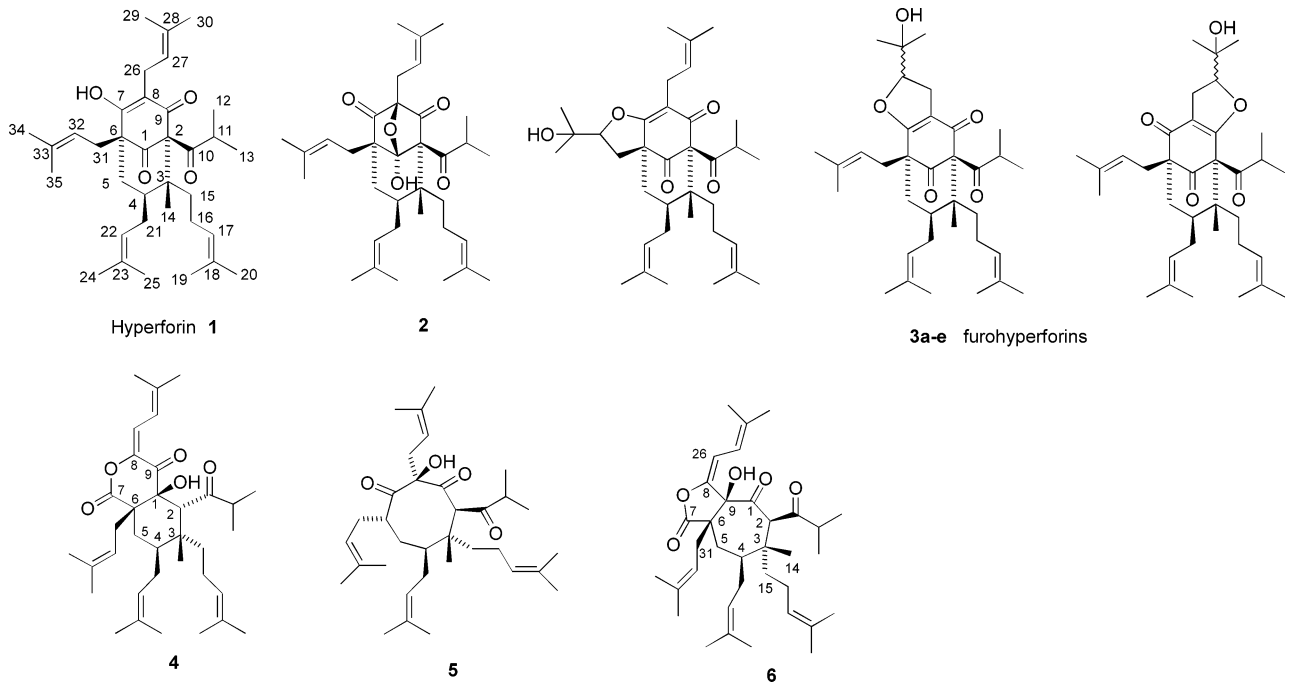


FIGURE 1.

has substantially remained a terra incognita. Recently, in vitro mimicking experiments using P450 enzymes led to the identification of a series of (pseudo)metabolites characterized by ω-oxidation of the prenyl residues and an unscathed phloroglucinol core.[14] These findings are surprising since P450 enzymes rely on activated oxygen forms, and the failure to isolate metabolites oxidized at the phloroglucinol core suggests that interaction with peptide structures (either the enzymes themselves or their cellular milieu) might have a stabilizing effect on hyperforin, echoing some observations reported in the field of ene-diine antibiotics.[15]

To test this hypothesis, we have investigated the oxidation of hyperforin by hydrogen peroxide in the presence of serum albumin, a protein known to bind lipophilic compounds, and horseradish peroxidase (HRP), a heme-containing peroxidase that catalyzes H_2O_2-mediated one- and two-electron oxidations.[16] While providing experimental support for a protein-mediated protection of the phloroglucinol core, these studies also discovered that peptide additives can have remarkable effects on the course of the oxidation reaction.

Results and Discussion

In a previous investigation, we reported that hyperforin reacts with hydrogen peroxide to afford different compounds depending on the oxidation conditions and the stoichiometry of oxidant employed.[17] The hemiacetal (**2**) was the major oxidation product, accompanied by a constellation of minor compounds that included various furohyperforins (**3a**−**e**) as well as compounds **4** and **5** (Figure 1).[17] Whereas **2**, **4**, and **5** are products of enol oxidation, furohyperforin is the result of the intramolecular nucleophilic opening of prenol epoxides by the enolic hydroxyl, a process that stabilizes the phloroglucinol core toward oxidative modification.

All of these processes were shut down by the addition of an excess of human serum albumin, even with a large excess of oxidant and with prolonged reaction times (up to 1 week!). For comparison, blank experiments conducted in otherwise similar conditions but in the absence of protein additives showed a complete oxidation in less than 5 h. These observations, while providing a possible rationalization for the oxidative stability of the phloroglucinol core of hyperforin under metabolic conditions, also suggested further experiments aimed at assessing the generality of this peptide-induced remarkable effect. The oxidative reaction was therefore carried out also with other peptide additives, namely, an oxidizing enzyme (HRP), and ovalbumine, using acetonitrile−methanol 95:5 as solvent to secure solubility of hyperforin.

HRP could not prevent the oxidation of hyperforin by H_2O_2. Nevertheless, catalytic amounts of this enzyme provided a reaction mixture containing also, apart from the hemiacetal **2**, a compound not yet identified in the previous oxidation experiments.[17] This compound, the enollactone **6** (Figure 1), could be formed also, albeit at a slower rate, in blank experiments carried out in the absence of HRP. Compared to the previous experiments,[17] the solvent system included an aqueous buffer. Compound **6** was isolated in ca. 25% yield, with the major reaction product being the hemiacetal **2**, obtained in 37% yield. Blank experiments gave comparable yields of these

(13) (a) Cui, Y., Gurley, B., Ang, C. Y. W., Leakey, J. *J. Chromatogr. B* **2002**, *780*, 129−135. (b) Cervo, L.; Rozio, M.; Ekalle-Soppo, C. B.; Guiso, G.; Morazzoni, P.; Caccia, S. *Psychopharmacology* **2002**, *164* (4), 423−8.

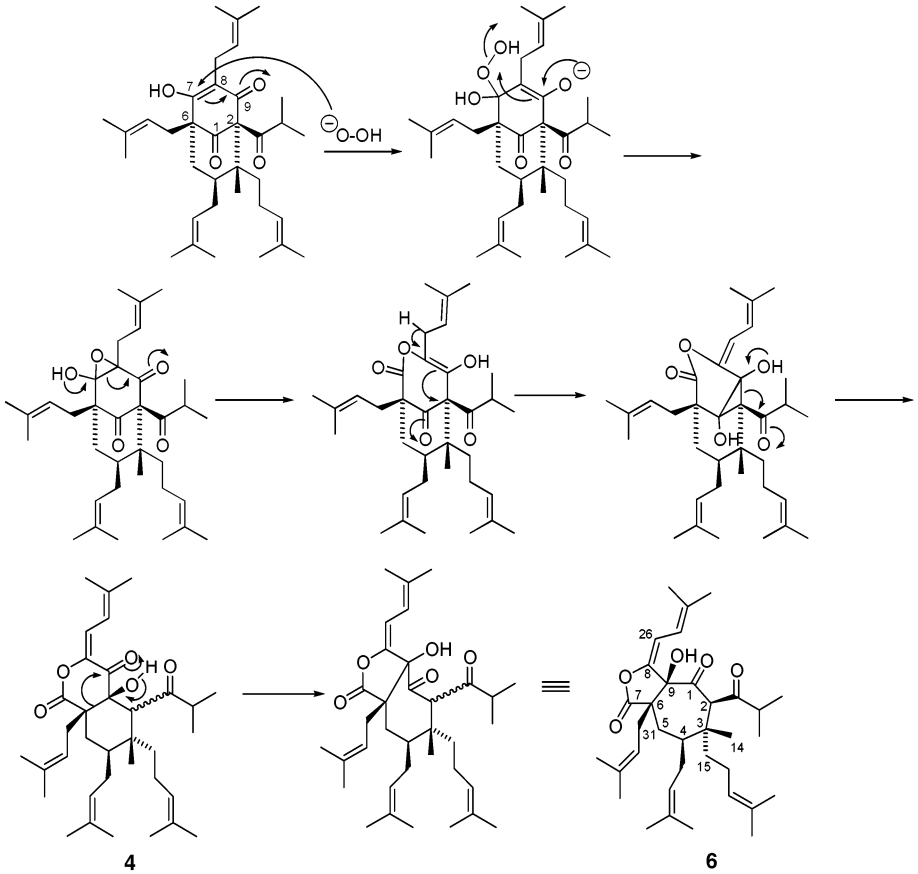
(14) Cui, Y.; Ang, C. Y. W.; Beger, R. D.; Heinze, T. M.; Hu, L.; Leakey, J. *Drug Metab. Dispos.* **2004**, *32*, 28−34.

(15) Povir, L. F.; Dattagupta, N.; Warf, B. C.; Goldberg, I. H. *Biochemistry* **1981**, *20*, 4007−4014.

(16) Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H. *Chem. Rev.* **1996**, *96*, 2841−2888.

(17) Verotta, L.; Lovaglio, E.; Sterner, O.; Appendino, G.; Bombardelli, E. *Eur. J. Org. Chem.* **2004**, 1193−1197.

SCHEME 1



4 **6**

compounds but required much longer reaction times (5 vs 1 h). High-resolution mass spectrometry suggested the elemental composition $C_{35}H_{52}O_5$ for **6**. 2D NMR experiments revealed that C-2 had become a methine group, as evident by the HMBC correlation from 14-H_3 to C-2, the HMQC correlation between 2-H and C-2, and the HMBC correlation between 2-H and C-10. Thus, the oxidation had obviously provoked a rearrangement of the carbon skeleton of hyperforin. HMBC correlations from 14-H_3 were also observed to C-3, C-4, and C-15, and COSY correlations between 4-H and 5-H as well as 21-H_2 together with HMBC correlations from 15-H_2 to C-2, C-3, C-4, and C-14, as well as from 21-H_2 to C-3, C-4, and C-5, showed that this part of the molecule was intact. The third intact 3-methyl-2-butenyl group is positioned on C-6, as shown by the HMBC correlations between 31-H_2 and C-5, C-6, and C-7. A new carbon−carbon double bond is present between C-8 and C-26. The lack of correlation to a second substituent on C-9 and the chemical shifts of the protons and carbons of the adjacent positions suggest that C-9 should be oxygenated. 26-H gave HMBC correlations to C-8 and C-9, a tertiary alcohol function, while 2-H correlated with C-1, a keto function in **6**, and the 9-OH gave HMBC correlations to C-1, C-6, C-8, and C-9. These data could be combined in the enolactone structure **6.** The stereostructure of **6** was suggested by the NOESY correlations between 9-OH and 31-Ha, 32-H, and 26-H and between 2-H and 15-H_2. A proposed mechanism for the formation of **6** is shown in Scheme 1. Thus, after formal oxygen insertion between C-7 and C-8, in a Weitz−Scheffer mode, an intramolecular Prins reaction and a retro-aldol reaction afford the δ-lactone **4**, whose carbon skeleton is remodulated by an α-ketol rearrangement.

Whereas the effect of human albumin was not unexpected on account of its binding properties for lipophilic compounds, the catalysis of the α-ketol rearrangement by HRP is surprising, even though peptide additives have long been known to affect the course of oxidation reaction with H_2O_2, the most remarkable example being the Juliá−Colonna epoxidation.[18] Even more surprising was the effect of the replacement of human albumin with ovalbumine. This smaller glycopeptide analogue of HSA was not able to prevent the oxidation of hyperforin by hydrogen peroxide but had a dramatic effect on the course of the reaction, promoting the α-ketol rearrangement of **4** to the enollactone **6**, now the major reaction products (isolated yield ca. 45%), and affording a series of oxidation byproducts never observed before (compounds **7**−**10**, Figure 2) or in blank esperiments. The structural complexity and multifunctionality of **6** qualifies this compound as an interesting scaffold for the induction and/or modulation of bioactivity, and its production from the ovalbumin-mediated H_2O_2 oxidation of hyperforin allowed a multigram level scale-up.

Compound **7** ($C_{32}H_{50}O_6$, HRMS) showed proton and carbon NMR spectra similar to those of compound **4**, a byproduct of the hydrogen peroxide oxidation of hyperforin in the absence of catalysts,[17] except for the lack of the enol bond, replaced by a methoxyl (δ_H 3.44, s). The

(18) (a) Banfi, S.; Colonna, S.; Molinari, H.; Juliá S.; Guixer, J. *Tetrahedron* **1984**, *40*, 5207 and references therein. For recent reviews see: (b) Lasterra-Sánchez, M. E.; Roberts, S. M. *Curr. Org. Chem.* **1997**, 187. (c) Ebrahim, E.; Wills, M. *Tetrahedron: Asymmetry* **1997**, *8*, 3163.

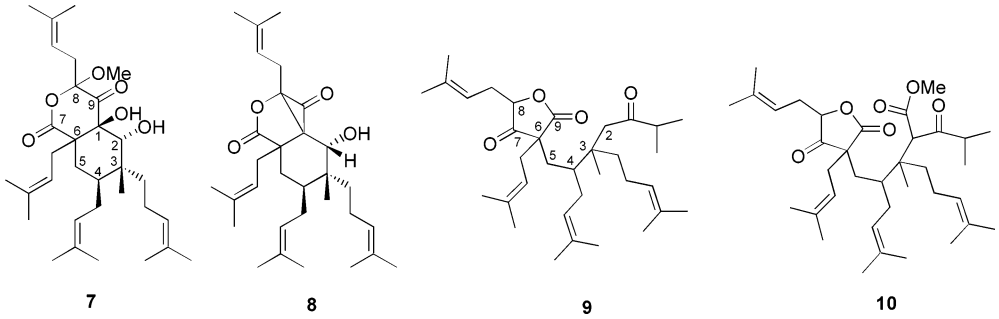
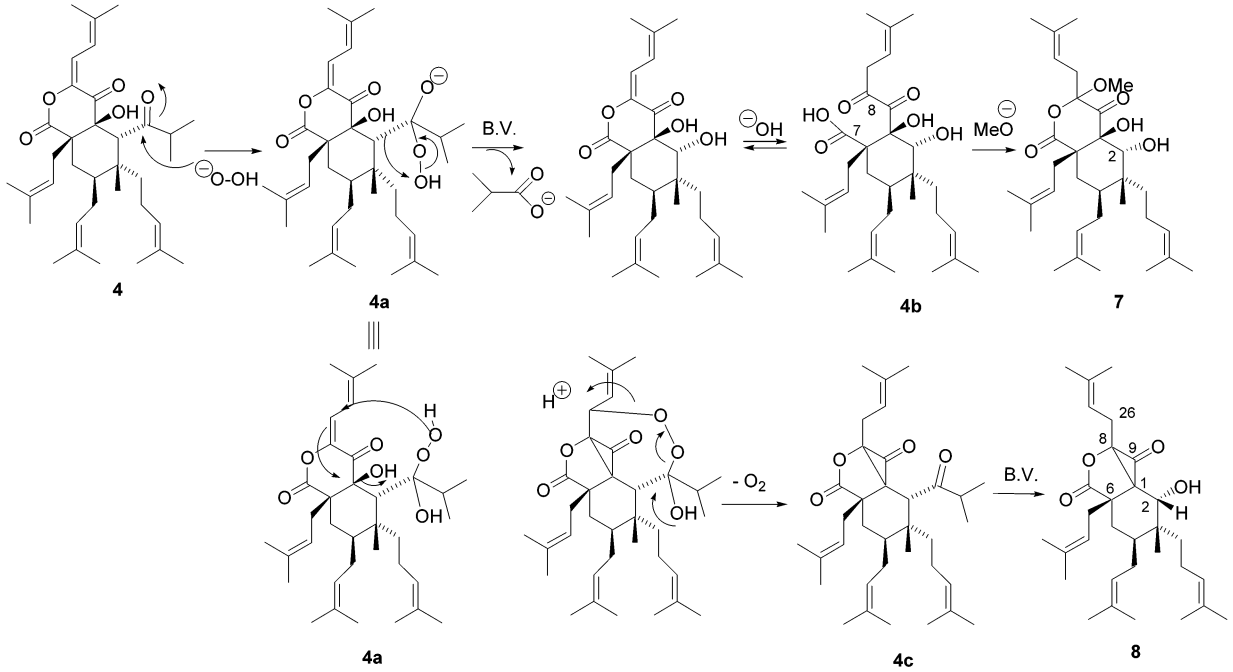


FIGURE 2.

SCHEME 2

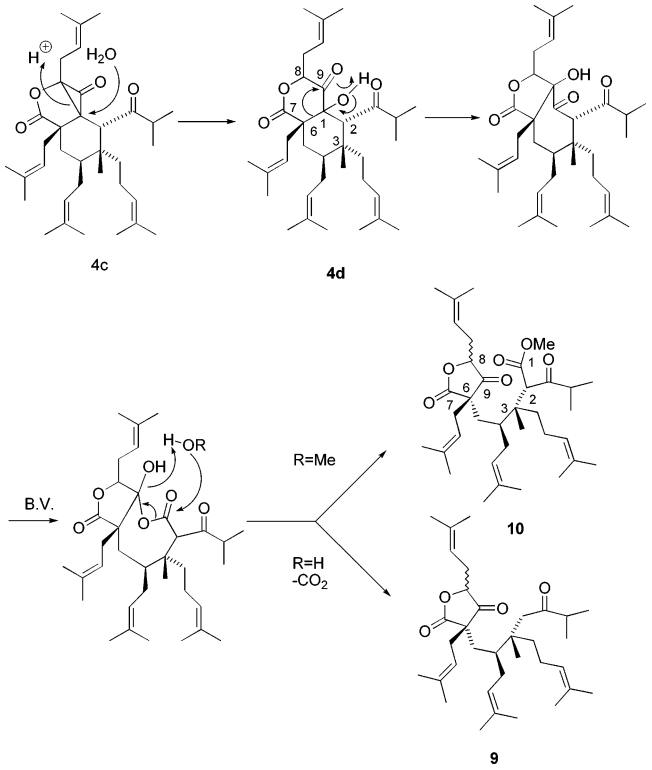


^{13}C NMR spectrum evidenced the presence of four prenyl groups and the lack of the isobutyryl chain at C-2, replaced by an OH group, as judged from the chemical shift of H-2 and C-2 (δ_H 3.40, s and δ_C 66.5, respectively). The formation of **7** from **4** involves a hydrogen peroxide mediated Bayer−Villiger rearrangement, with eventual loss of isobutyric acid and the generation of a C-2 alcohol, and the overall Markovnikov addition of methanol to the exocyclic double bond. The regiochemistry of this step suggests the intermediacy of a 8,9-diketo-7-carboxylic acid (**4b**) rather than a direct olefin addition (Scheme 2).

The high-resolution mass spectrum of compound **8** suggested a molecular formula $C_{31}H_{46}O_4$, confirmed by electrospray mass spectrometry (*m/z* 481 $[M - H]^-$). Four out of the nine unsaturation degrees required by this formula were accounted for by four double bonds (eight olefin signals in the ^{13}C NMR spectrum), and by two carbonyls (δ_C 206.4 and δ_C 180.2), showing a tricyclic structure for **8**. As with **7**, the C-2 isobutyryl chain was absent, again replaced by a hydroxyl (δ_H 3.15 for H-2, δ_C 70.4 for C-2, and δ_H 3.16 for the hydroxyl). H-2 showed a set of diagnostic long-range correlations (δ_C 76.6, C-1; δ_C 47.5, C-6; δ_C 206.4, C-9) that suggested the presence of an oxadecalin moiety, as in **4**, while the HMBC correlation between the methylene prenyl at C-8 (H-22a,b) and C-1 showed a further bond between C-1 and C-8. This cyclopropane ring accounted for the last unsaturation degree required by the molecular formula, and NOESY correlations established the orientation of its substituents. The most remarkable feature of **8** is the formal reduction of C-26 that could be explained by a process involving the Michael addition of a C-10 peroxyhemiacetal oxygen on the enone double bond of intermediate **4a**. This could trigger the formation of the bond between C-8 and C-1, while loss of oxygen accounts for the overall reduction of C-26, with Baeyer−Villiger oxidation of the isobutyryl moiety eventually affording **8** (Scheme 2).

Two further minor compounds (**9** and **10**) were isolated as a diastereomeric mixture, from which only one constituent (**9**) could be obtained as a single product by exhaustive chromatographic purification. The high-resolution mass spectrum of compound **9** suggested a molecular formula $C_{34}H_{54}O_4$, compatible with eight unsaturations and the loss of a carbon atom from hyperforin. The four prenyl chains were still present, as well as the acyl chain at C2, but the bicyclic skeleton of the natural product had extensively rearranged, since a lactone was present (ν_{max} 1800 cm-1), while two further keto groups could be detected in the ^{13}C NMR spectrum

SCHEME 3



(δ_C 214.0 and 212.8, respectively). The A-nor-A-seco structure **9** could nicely accommodate these data, and a possible derivation of **9** from **4c** is presented in Scheme 3. Thus, after addition of water to the cyclopropanone moiety to afford **4d**, α-ketol rearrangement and Baeyer−Villiger oxidation would afford a lactone intermediate, trapped by methanol to afford **10** or, alternatively, by water to give after decarboxylation the keto-lactone **9**.

In summary, our observation suggests that protein additives have a dramatic effect on the H_2O_2 oxidation of hyperforin, either protecting the enolized phloroglucinol core from oxidation (human albumin) or promoting reaction pathways derived from the intermediate enol-lactone **4**, a minor component of the oxidation mixture in the absence of additives.[17] Although it is difficult to provide a mechanistic rationale for this remarkable steering effect, it is nevertheless interesting to speculate that protein sites might provide a sort of pseudocatalysis for specific steps involved in the extensive remodulation of the carbon−carbon connectivity of the phloroglucinol core of the natural product. The organocatalytic nature of the rearrangement might well rationalize the exquisite specificity of several steps or their mechanistic oddity, such as the necessity to advocate a reductive role for H_2O_2 to account for the formation of compound **8**. Although undoubtedly specific for the substrate under investigation, these results will hopefully spur investigation on peptide-mediated organocatalytic effects, a branch of organocatalysis that, despite the relatively early discovery of the Juliá−Colonna epoxidation, has so far lagged behind amine-mediated organocatalysis.[19,20]

(19) Bentley, P. A.; Bickley, J. F.; Roberts, S. M.; Steiner, A. *Tetrahedron Lett.* **2001**, *42*, 3741−3743.

(20) Bentley, P. A. In *Biotransformations*; Kelly, D. R., Ed.; Wiley-VCH: Weinheim, 2000; Vol. 8b, Chapter 12, pp 491−517.

Finally, it is not unconceivable to assume that the protective effect of HSA underlies the formation of ω-hydroxylated pseudometabolites of hyperforin with an intact phloroglucinol core under biological conditions that mimic cytochrome metabolism.

Experimental Section

Oxidations of Hyperforin DCHA Salt with H_2O_2. General Procedure. Under nitrogen atmosphere, to a magnetically stirred solution of hyperforin DCHA salt in CH_3CN−MeOH (95:5, 30 mL/100 mg salt) was added an aqueous phosphate buffer solution (pH 8.2) (20 mL/100 mg salt) containing the catalytic reagent, followed by H_2O_2 (1 mol/equiv). In blank experiments, the buffer solution was added as such.

Blank Experiments. After 5 h the solution (100 mg of starting hyperforin salt) was partially evaporated, diluted with 1 N HCl, and extracted with CH_2Cl_2 (3 × 15 mL). After drying with Na_2SO_4 and solvent evaporation, 84 mg of a crude residue was obtained. This was purified by flash column chromatography over silica gel (ϕ 1.5 cm, h 17 cm) using petrol/*tert*-butyl-methyl ether 20/0.5 (300 mL, 30 × 10 mL fractions), petrol/*tert*-butyl-methyl ether (260 mL, 26 × 10 mL fractions), and AcOEt (30 mL, 1 × 30 mL fraction). Fractions 3−5 contained 27 mg of hemiacetal (**2**) (35.7%), fractions 46−55 contained 19 mg of (**6**) (20%), and fraction 57 contained 29 mg of furohyperforins (**3a**−**e**) (39%).

Reaction Promoted by HRP (2×10^{-3} mol/equiv). After 1 h the reaction mixture (100 mg of starting hyperforin salt) was worked up and purificated as described for the blank experiments; 28 mg of hemiacetal (**2**) (37%), 15 mg of (**6**) (25%), and 32 mg of furohyperforins (**3a**−**e**) (42%) were obtained.

Reaction Promoted by Ovalbumine (10^{-1} mol/equiv). After stirring for 4 days at room temperature and under nitrogen, the reaction mixture (7.9 g of hyperforin starting salt) was worked up by filtration of the albumin, dilution with 1 N HCl, and extraction with CH_2Cl_2 (3 × 1 L). The organic phase was dried over Na_2SO_4 and evaporated. The residue was fractionated by flash column chromatography over Si gel (300 g, ϕ = 6 cm) using petrol/*tert*-butyl-methyl ether 20:1 (6.75 L, 45 × 150 mL fractions) as an eluent, to obtain four main fractions: 2−4 (390 mg, hemiacetal (**2**)), 5−7 (330 mg), 17−24 (840 mg), 25−44 (g **6**, 2.72 g, 45%). Fractions 5−7 were purified by flash column chromatography over Si gel (80 g, ϕ = 3 cm,) using petrol/ *tert*-butyl-methyl ether 30:1 (1.4 L, 70 × 20 mL fractions) to afford an additional 31 mg of hemiacetal (**2**) (overall yield 421 mg, 6.9%), 91 mg of (**7**) (1.5%), and a mixture of the two isomers (**9**) (fr 41−70, 74 mg, 1.3%). The two isomers were purified over Si gel (flash, 10 g, ϕ = 1 cm) with petrol/*tert*-butyl-methyl ether 40:1 (200 mL, 50 × 4 mL fractions) to obtain 34 mg of **9a** (one isomer) (fr 35−43) and 30 mg of **9b** (one isomer) (fr 45−50). Fractions 17−24 were purified by flash column chromatography over Si gel (200 g, ϕ = 5 cm) using petrol/*tert*-butyl-methyl ether 20:1 (3 L, 150 × 20 mL fractions) to obtain 316 mg of (**8**) (5.9%) in fractions 60−84 and 522 mg of impure (**10**) in fractions 85−150, which were further purified over Si gel (flash 80 g, ϕ = 3 cm) using petrol/*tert*-butyl-methyl ether 20:1 (1.85 L, 185 × 10 mL fractions), giving, in fractions 118−142, 120 mg of (**10**) (two isomers) (overall yield 260 mg, 4.0%).

Enollactone 6: colorless viscous oil; $[\alpha]^{25}_D = -86.3$ (*c* 0.30, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ (mult, *J* (Hz)) 6.13, 1H, dq, *J* = 11.3 and 1.3, 27-H; 5.57, 1H, d, *J* = 11.3, 26-H; 5.11, 1H, s, 2-H; 5.10, 1H, m, 32-H; 5.04, 1H, m, 22-H; 5.01, 1H, m, 17-H; 2.98, 1H, s, 9-OH; 2.77, 1H, dd, *J* = 10.0 and 15.1, 31-Ha; 2.49, 1H, sept., *J* = 6.8, 11-H; 2.16, 1H, d, *J* = 15.1, 31-Hb; 2.04, 1H, m, 21-Ha; 1.98, 2H, m, 16-H_2; 1.82, 3H, s, 30-H_3; 1.76, 1H, m, 4-H; 1.74, 3H, s, 34-H_3; 1.74, 3H, s, 25-H_3; 1.72, 1H, m, 21-Hb; 1.71, 3H, s, 29-H_3; 1.70, 3H, s, 24-H_3; 1.68, 3H, s, 20-H_3; 1.63, 3H, s, 35-H_3; 1.62, 3H, s, 19-H_3; 1.61, 1H, m, 15-Ha; 1.60, 2H, m, 5-H_2; 1.51, 1H, m, 15-Hb; 1.08, 3H, d,

J = 6.8, 12-H_3; 1.07, 3H, s, 14-H_3; 1.02, 3H, d, J = 6.8, 13-H_3; ^{13}C NMR (125 MHz, $CDCl_3$) δ 207.3 C-10, 201.2 C-1, 175.2 C-7, 147.8 C-8, 137.5 C-33, 137.0 C-28, 134.3 C-23, 131.8 C-18, 123.6 C-17, 122.8 C-22, 118.3 C-32, 117.0 C-27, 102.4 C-26, 87.0 C-9, 61.0 C-2, 49.8 C-6, 43.4 C-3, 42.9 C-11, 39.2 C-4, 37.7 C-15, 33.4 C-5, 32.7 C-31, 28.2 C-21, 26.1 C-30, 26.0 C-34, 25.9 C-24, 25.7 C-20, 21.8 C-16, 18.5 C-12, 18.3 C-29, 18.1 C-25, 18.1 C-13, 18.1 C-35, 17.7 C-19, 16.4 C-14; FABHRMS *m/z* 552.3835 $[M]^+$ (calcd for $C_{35}H_{52}O_5$ 552.3814).

Compound 7: colorless viscous oil; $[\alpha]_D^{20}$ +9.4 (*c* 1.1 $CHCl_3$); IR (KBr) ν_{max} 3466, 1781, 1742, 1448, 1374, 1240, 1099, 1048, 938, 847 cm^{-1}; UV (EtOH) λ_{max} 205 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ (mult, J (Hz)) 5.25, 2H, m, 27-H and 32-H; 5.06, 2H, m, 17-H and 22-H; 3.44, 3H, s, 8-OMe; 3.40, 1H, s, 2-H; 2.59, 1H, dd, J = 6.5 and 15.0, 26-Ha; 2.51, 1H, dd, J = 7.7 and 15.0, 26-Hb; 2.42, 1H, m, 31-Ha; 2.37, 1H, m, 31-Hb; 2.14, 1H, m, 21-Ha; 2.01, 2H, m, 16-H_2; 1.84, 2H, d, J = 7.3, 5-H_2; 1.73, 3H, s, 35-H_3; 1.72, 3H, s, 30-H_3; 1.70, 3H, s, 25-H_3; 1.69, 3H, s, 20-H_3; 1.63, 1H, m, 21-Hb; 1.57, 1H, m, 15-Ha; 1.63, 3H, s, 34-H_3; 1.62, 3H, s, 19-H_3; 1.62, 3H, s, 29-H_3; 1.58, 3H, s, 24-H_3; 1.44, 1H, m, 4-H; 1.24, 1H, m, 15-Hb; 0.93, 3H, s, 14-H_3; ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.9 C-9, 180.0 C-7, 136.1 C-28, 135.6 C-33, 133.0 C-23, 132.1 C-18, 123.8 C-17, 122.7 C-22, 119.0 C-32, 116.0 C-27, 97.6 C-8, 80.8 C-1, 66.5 C-2, 54.3 8-OMe, 47.6 C-6, 42.0 C-15, 41.6 C-4, 37.2 C-3, 35.3 C-31, 33.8 C-5, 27.9 C-21, 25.9 C-26, 26.1 C-35, 25.9 C-30, 25.8 C-25, 25.7 C-20, 22.4 C-16, 17.9 C-14, 17.9 C-19, 17.9 C-24, 17.9 C-29, 17.7 C-34; EIMS *m/z* (%) 512 (15), 495 (11), 472 (14), 459 (20), 443 (42), 397 (38), 371 (40), 316 (55), 151 (100).

Compound 8: colorless viscous oil; $[\alpha]_D^{20}$ +15.7 (*c* 0.1, $CHCl_3$); IR (liquid film) ν_{max} 3467, 1786, 1751, 1707, 1673, 1450, 1378, 1310, 1262, 1232, 1164, 1106, 1065, 1038, 851 cm^{-1}; UV (EtOH) λ_{max} 206 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ (mult, J (Hz)) 5.25, 1H, t, J = 6.8, 27-H; 5.20, 1H, t, J = 6.6, 32-H; 5.10, 1H, t, J = 6.8, 17-H; 5.05, 1H, t, J = 6.9, 22-H; 3.15, 1H, s, 2-H, 3.06, 1H, s, OH; 2.65, 1H, dd, J = 16 and 9.4, 31-Ha; 2.55, 2H, bd, J = 7.1, 26-H_2; 2.39, 1H, m, 31-Hb; 2.10, 1H, m, 21-Ha; 1.95, 2H, m, 16-H_2; 1.80, 2H, m, 5-H_2; 1.77, 3H, s, 34-H_3; 1.76, 6H, s, 30- and 35-H_3; 1.71, 6H, s, 20- and 24-H_3; 1.66, 3H, s, 29-H_3; 1.63, 3H, s, 19-H_3; 1.53, 1H, m, 4-H; 1.56, 3H, s, 25-H_3; 1.52, 1H, m, 21-Hb; 1.49, 1H, m, 15-Ha; 1.30, 1H, m, 15-Hb; 1.15, 3H, s, 14-H_3; ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4 C-9, 180.2 C-7, 138.1 C-33, 137.4 C-28, 133.4 C-23, 132.1 C-18, 124.2 C-17, 123.0 C-22, 120.1 C-32, 115.4 C-27, 96.9 C-8, 76.6 C-1, 70.4 C-2, 47.5 C-6, 41.7 C-15, 40.8 C-4, 38.0 C-3, 35.3 C-31, 31.8 C-21, 27.4 C-5, 26.4 C-26, 26.3 C-30 or 34, 26.2 C-34 or 30, 26.0 C-24 or 20, 25.9 C-20 or 24, 22.3 C-16, 19.9 C-14, 18.3 C-35, 18.2 C-29, 18.1 C-25, 17.8 C-19; ESIMS (negative mode) *m/z* 481 $[M - H]^-$, MS^2 412, MS^3 329, MS^4 260; EIHRMS *m/z* 482.3333 $[M]^+$ (calcd for $C_{31}H_{46}O_4$ 482.3396).

Compound 9a: colorless viscous oil; $[\alpha]_D^{20}$ −11.1 (*c* 0.5, $CHCl_3$); IR (liquid film) ν_{max} 2854, 1800, 1754, 1714, 1461, 1377, 1213, 1047, 854; ^{1}H NMR (500 MHz, $CDCl_3$) δ (mult, J (Hz)) 5.21, 1H, m, 27-H; 5.08, 1H, m, 22-H; 5.02, 1H, m, 17-H; 4.98, 1H, m, 32-H; 4.32, 1H, dd, J = 4.3 and 8.7, 8-H; 2.56, 1H, m, 26-Ha; 2.54, 1H, m, 11-H; 2.48, 2H, m, 31-H_2; 2.45, 1H, m, 26-Hb; 2.44, 2H, m, 2-H_2; 2.04, 1H, m, 21-Ha; 1.88, 1H, m, 4-H; 1.87, 1H, m, 21-Hb; 1.87, 1H, m, 16-Ha; 1.85, 1H, m, 5-Ha; 1.77, 1H, m, 16-Hb; 1.74, 3H, s, 30-H_3; 1.71, 1H, m, 5-Hb; 1.67, 3H, s, 35-H_3; 1.66, 3H, s, 20-H_3; 1.65, 3H, s, 25-H_3; 1.65, 3H, s, 29-H_3; 1.59, 3H, s, 19-H_3; 1.58, 3H, s, 24-H_3; 1.58, 3H, s, 34-H_3; 1.47, 1H, m, 15-Ha; 1.34, 1H, m, 15-Hb; 1.07, 3H, d, J = 6.8, 12-H_3; 1.06, 3H, d, J = 6.8, 12-H_3; 0.94, 3H, s, 14-H_3; ^{13}C NMR (125 MHz, $CDCl_3$) δ 214.0 C-10, 212.8 C-9, 176.1 C-7, 138.4 C-33, 136.4 C-28, 131.1 C-18, 130.7 C-23, 124.7 C-17, 124.3 C-22, 117.3 C-27, 115.8 C-32, 84.7 C-8, 54.3 C-6, 46.2 C-2, 42.0 C-11, 40.5 C-4, 39.4 C-3, 37.1 C-31, 36.3 C-15, 35.3 C-5, 29.6 C-21, 29.6 C-26, 25.9 C-35, 25.8 C-25, 25.8 C-30, 25.7 C-20, 22.6 C-16, 22.0 C-14, 18.4 C-12, 18.3 C-13, 18.0 C-29, 17.9 C-24, 17.8 C-34, 17.6 C-19; EIMS *m/z* (%) 526 (M^+, 28), 508 (66), 483 (57), 457 (36), 429 (42), 415 (36), 371 (87), 330 (51), 315 (54), 303 (65), 263 (100); EIHRMS *m/z* 526.4092 $[M]^+$, (calcd for $C_{34}H_{54}O_4$ 526.4022).

Compound 10. Major isomer: colorless viscous oil; IR (liquid film) ν_{max} 2855, 1799, 1752, 1717, 1455, 1377, 1239, 1214, 1048, 845; ^{1}H NMR (500 MHz, $CDCl_3$) δ (mult, J (Hz)) 5.19, 1H, m, 27-H; 5.17, 1H, m, 22-H; 5.03, 1H, m, 17-H; 4.99, 1H, m, 32-H; 4.36, 1H, dd; J = 4.4 and 8.5, 8-H; 4.03, 1H, s, 2-H; 2.67, 1H, m, 11-H; 2.58, 1H, m, 26-Ha; 2.48, 1H, m, 21-Ha; 2.48, 1H, m, 31-Ha; 2.42, 1H, m, 26-Hb; 2.39, 1H, m, 31-Hb; 2.35, 1H, m, 21-Hb; 2.00, 1H, m, 5-Ha; 1.96, 1H, m, 16-Ha; 1.85, 1H, m, 4-H; 1.84, 1H, m, 16-Hb; 1.73, 3H, s, 25-H_3; 1.73, 3H, s, 30-H_3; 1.69, 1H, m, 5-Hb; 1.68, 3H, s, 35-H_3; 1.66, 3H, s, 20-H_3; 1.64, 3H, s, 29-H_3; 1.62, 3H, s, 19-H_3; 1.62, 3H, s, 24-H_3; 1.60, 3H, s, 34-H_3; 1.55, 2H, m, 15-H_2; 1.08, 3H, m, 34-H_3; 1.04, 3H, m, 34-H_3; ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.4 C-9, 208.4 C-10, 42.6 C-11, 175.7 C-7, 169.6 C-1, 137.4 C-33, 136.4 C-28, 136.2 C-23, 131.2 C-18, 124.7 C-17, 117.4 C-22, 117.3 C-27, 116.8 C-32, 84.6 C-8, 61.4 C-2, 53.7 C-6, 52.0 1-OMe, 44.0 C-3, 40.5 C-4, 37.1 C-5, 35.8 C-21, 35.5 C-15, 35.5 C-31, 29.6 C-26, 25.8 C-35, 25.7 C-25, 25.7 C-30, 25.6 C-20, 22.8 C-16, 21.1 C-14, 18.2 C-12, 18.5 C-13, 17.9 C-24, 17.9 C-29, 17.8 C-34, 17.7 C-19; EIMS *m/z* (%) 584 $[M]^+$, (5), 566 (9), 553 (5), 541 (10), 440 (19), 371 (22), 237 (35), 109 (100).

Acknowledgment. Dr. N. Fuzzati (Indena SpA, Milano) is gratefully acknowledged for the ESIMS spectrum. Work supported in part by MIUR, Italy.

Supporting Information Available: General experimental procedures and ^{1}H and ^{13}C NMR spectra for compounds **6**−**10** in $CDCl_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

JO048857S