A New Polyisoprenylated Phloroglucinol Derivative from *Hypericum perfoliatum* (Clusiaceae)

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Hyperfoliatin, a new polyisoprenylated phloroglucinol derivative was isolated from the aerial parts of *Hypericum perfoliatum* (Clusiaceae) collected in Algeria. The structure of hyperfoliatin was elucidated on the basis of its spectral data, mainly MS and multiple-pulse NMR.

Key words: Hypericum perfoliatum, Polyisoprenylated Phloroglucinol

Introduction

The genus *Hypericum* L. (Clusiaceae) comprises some 400 species of trees, shrubs, and herbs, with opposite gland-dotted leaves. It has a worldwide distribution, but most species originate from temperate regions and tropical mountains. Numerous species are used as medicinal plants. Some have been extensively studied from both chemical and pharmacological points of view, and several of their biological properties appear to be correlated with the presence of phloroglucinol-derived active constituents. Indeed, several species used in traditional medicine for the treatment of various bacterial diseases, including *Hypericum uliginosum* H. B. & K. (Parker and Johnson, 1968; Parker *et al.*, 1968; Taylor and Brooker, 1969), *Hypericum brasiliense* Choisy (Rocha *et al.*, 1995, 1996), *Hypericum japonicum* Thunb. (Ishiguro *et al.*, 1985, 1986, 1994; Hu *et al.*, 2000), and *Hypericum papuanum* Ridley (Winkelmann *et al.*, 2000) led to the isolation of antibiotic phloroglucinol derivatives. Similarly, the prenylated phloroglucinols hyperforin (Bystrov *et al.*, 1975; Brondz *et al.*, 1982) and adhyperforin (Maisenbacher and Kovar, 1992) have recently emerged as the constituents responsible for the antidepressant activity of European St. John's wort, *Hypericum perforatum* L. (Barnes *et al.*, 2001). In continuation of our studies on the chemical constituents of Algerian medicinal plants (Medjroubi *et al.*, 1998), we report here the structure determination of hyperfoliatin, a novel prenylated phloroglucinol derivative isolated from the aerial parts of *Hypericum perfoliatum* L. locally used in folk medicine for wound healing and for the treatment of various bacterial diseases.

Results and Discussion

Fractionation of the methanolic extract of *Hypericum perfoliatum* L. by solvent partition followed by repeated column chromatography resulted in the isolation of hyperfoliatin.

Hyperfoliatin (**1**) was obtained as a pale yellow viscous oil. The empirical formula was determined by accurate mass measurement as $C_{31}H_{46}O_5$. The IR spectrum showed characteristic bands at 3322, 1777, 1742, 1678, 1084, and 1058 cm $^{-1}$ associated with hydroxyl, carbonyl, alkene, and ether groups. The presence of a hemiacetal ring as a key feature

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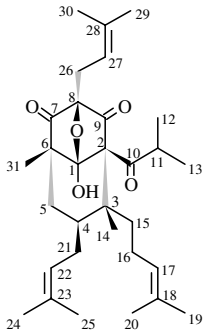

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Fig. 1.

in the structure of hyperfoliatin was deduced from both ^{1}H and ^{13}C NMR spectra, which exhibited a downfield exchangeable proton singlet at δ 7.77 ppm and a deshielded aliphatic carbon signal at δ 107.7 ppm, respectively. This observation combined with typical ^{13}C NMR resonances accounting for two carbonyls at δ 206.8 and 209.7, and three quaternary carbons at δ 51.4, 70.9, and 97.1 ppm suggested a penta-*C*-substituted phloroglucinol-derived basic core, in which the oxygen atoms are involved in two ketones and one acetal carbonyl (Verotta *et al.*, 2000). Additional ^{1}H and ^{13}C NMR signals accounted for one quaternary methyl, two prenyl, one homoprenyl, and one isopropylketone side chains. Location of these substituents on the basic skeleton was carried out using multi-impulsional COSY, HETCOR and COLOC experiments. Of particular interest were the following three bond COLOC connectivities: i) H-5ax and H-5eq at 1.34 and 1.88 ppm, and C-7 and C-1, ii) C$\underline{H}_3$-31 at 1.17 ppm and C-1, C-5, and C-7, iii) C$\underline{H}_3$-14 at 1.02 ppm and C-2, C-4, and C-15, iv) O$\underline{H}$-1 at 7.77 ppm and C-2 and C-6. Consequently, the structure of hyperfoliatin can be depicted as **1**. The oxygen bridge and the cage structure of hyperfoliatin imply the relative configurations at C-1 and C-8, and C-2 and C-6, respectively. The huge *trans*-diaxial coupling constant (J = 13 Hz) between H-5ax and H-4 gave evidence for the equatorial position of the prenyl substituent at C-4. Strong NOESY cross peaks observed between H-5ax and C$\underline{H}_3$-31 on the one hand and C$\underline{H}_3$-14 on the other hand permitted to ensure the chair conformation of the fused cyclohexane ring and to deduce the relative configurations of the remaining stereocenters at C-3 and C-4. The absolute configuration of the chiral centers could not be determined, due to the small amount of material isolated.

Finally, it should be noted that biogenetically hyperfoliatin most probably arises from a phlorisobutyrophenone polyketide, derived from a valine and three acetates, which undergo subsequent alkylations by an *S*-adenosylmethionine and four prenyl units. This biosynthesis seems closely related to those previously postulated for other *Hypericum* secondary metabolites, including the related hyperforin from *Hypericum perforatum* and the antibiotic uliginosins A and B from *Hypericum uliginosum.*

Experimental

General experimental procedures

Optical rotation was measured on a Perkin-Elmer 241 polarimeter. Mass spectra were recorded with a Nermag R-10-10C spectrometer, using electron impact (EI-MS) and desorption-chemical ionization (DCI-MS; reagent gas: NH_3) techniques. The UV spectrum (λ_{max} in nm) was recorded in spectroscopic grade EtOH on a Beckman Model 34 spectrophotometer. The IR spectrum (ν_{max} in cm^{-1}) was obtained from a sodium chloride film on a Nicolet 510 FT instrument. ^{1}H-NMR (δ[ppm], J[Hz]) and ^{13}C-NMR spectra were recorded at 300 MHz and 75 MHz, respectively, using a Bruker AC-300 spectrometer. Multiple-pulse 2D NMR experiments ($^{1}H-^{1}H$ COSY, $^{1}H-^{1}H$ NOESY, $^{13}C-^{1}H$ HETCOR, and $^{13}C-^{1}H$ COLOC) were performed using standard Bruker microprograms. Column chromatographies were carried out with silica gel 20–45 mm. Flash column chromatographies were conducted using silica gel 60 Merck (35–70 μm) with an overpressure of 300 mbar (Still *et al.*, 1978).

Plant material

Aerial parts of *Hypericum perfoliatum* L. were collected at Jijel (Algeria), in June 2001. A voucher sample (ZKNB4 06/01) is kept in the herbarium of the Laboratoire d'Obtention de Substances Thérapeutiques (LOST), Université Mentouri, Constantine (Algeria).

Extraction and isolation

Dried, pulverized aerial parts of *Hypericum perfoliatum* (1 kg) were extracted with MeOH (2 × 2 l) at room temperature and solvent was removed by evaporation under reduced pressure. The residue was taken up hot water (1 l). After filtration, the water solution was successively extracted with EtOAc (2 × 1 l), and *n*-BuOH (2 × 1 l). The solvents were removed under reduced pressure to give EtOAc and *n*-BuOH extracts (12 g and 10 g, respectively). An aliquot of the EtOAc (2 g) was subjected to flash column chromatography on silica gel, using a CH_2Cl_2-MeOH gradient of increasing polarity to yield 24 fractions. Fractions 1–5, eluted with CH_2Cl_2, were further fractionated by column chromatographies on silica gel 20–45 mm (cyclohexane-CH_2Cl_2), to give hyperfoliatin (21 mg), eluted with cyclohexane-CH_2Cl_2 6:4 v/v.

Spectroscopic data

Hyperfoliatin (**1**), $[\alpha]_D$ + 17° (1 g/100 ml, MeOH); UV (EtOH) λ_{max} (log ε) 282 (3.20), 376 (1.60) nm; IR (KBr) ν_{max} 3322, 2971, 2932, 2877, 1777, 1742, 1678, 1452, 1385, 1332, 1301, 1119, 1084, 1058 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.93 (3H, d, J = 7 Hz CH_3-13), 1.02 (3H, s, CH3-14), 1.07 (1H, m, CH-4), 1.09 (3H, d, J = 7 Hz 3 CH3–12), 1.17 (3H, s, CH_3-31), 1.34 (1H, dd, J = 14 Hz, J = 13 Hz, H-5ax), 1.54 (3H, s, CH_3-25), 1.57 (3H, s, CH_3-20), 1.60 (1H, m, CH-15a), 1.64 (6H, s, CH3-29, CH3-30), 1.67 (3H, s, CH3-19), 1.68 (1H, m, CH-21a), 1.69 (3H, s, CH3–24), 1.75 (1H, m, CH-15b), 1.82 (1H, m, CH-16a), 1.88 (1H, dd, J = 14 Hz, J = 4 Hz, H-5eq), 2.10 (1H, ddd, J = 15 Hz, J = 5 Hz, J = 2 Hz, H-21b), 2.24 (1H, m, CH-16b), 2.56 (1H, dd, J = 15 Hz, J = 7 Hz, H-26a), 2.73 (1H, dd, J = 15 Hz, J = 6 Hz, H-26b), 3.20 (1H, sept., J = 7 Hz, H-11), 4.92 (1H, m, CH-22), 4.98 (1H, m, CH-17), 5.03 (1H, m, CH-27), 7.77 (1H, s, D_2O exch., OH-1); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.3 (C-14), 16.7 (C-31), 17.5 (C-12), 17.7 (C-30), 17.9 (C-20), 18.0 (C-25), 19.1 (C-13), 23.6 (C-16), 23.8 (C-26), 25.6 (C-29), 25.7 (2C, C-19, C-24), 28.4 (C-21), 34.1 (C-5), 36.8 (C-15), 39.2 (C-11), 41.6 (C-4), 47.1 (C-3), 51.4 (C-6), 70.9 (C-2), 97.1 (C-8), 107.7 (C-1), 115.8 (C-27), 122.0 (C-22), 124.0 (C-17), 131.8 (C-18), 133.4 (C-23), 136.0 (C-28), 206.8 (C-9), 209.7 (C-7), 217.8 (C-10); DCI-MS *m/z* 516 $[M+NH_4]^+$, 499 $[MH]^+$; EIMS *m/z* (%) 498 (M+) (2), 427 (4), 415 (2), 401 (18), 383 (7), 355 (2), 345 (4), 327 (5), 315 (7), 301 (3), 277 (5), 275 (5), 247 (6), 233 (4), 221 (4), 203 (27), 161 (8), 135 (21), 123 (16), 109 (20), 95 (14), 81 (8), 69 (100); HR-EIMS found: 498.3339; calcd. for $C_{31}H_{46}O_5$, 498.3345.

Barnes J., Anderson L. A., and Phillipson J. D. (2001), St. John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. J. Pharm. Pharmacol. **53**, 583–600.

Bystrov N. S., Chernov B. K., Dobrynin V. N., and Kolosov M. N. (1975), The structure of hyperforin. Tetrahedron Lett. 2791–2794.

Brondz I., Greibrokk T., Groth P. A., and Aasen A. J. (1982), The relative stereochemistry of hyperforin – An antibiotic from *Hypericum perforatum* L. Tetrahedron Lett. **23**, 1299–1300.

Ishiguro K., Yamaki M., Takagi S., Yamagata Y., and Totima K. (1985), *X*-Ray crystal structure of sarothralin, a novel antibiotic compound from *Hypericum japonicum*. J. Chem. Soc., Chem. Commun. 26–27.

Ishiguro K., Yamaki M., Kashihara M., and Takagi S. (1986), Sarothralen A and B, new antibiotic compounds from *Hypericum japonicum.* Planta Med. ⏩, 288–290.

Ishiguro K., Nagata S., Fukumoto H., Yamaki M., and Isoi K. (1994), Phloroglucinol derivatives from *Hypericum japonicum.* Phytochemistry **35**, 469–471.

Hu L.-H., Khoo C.-W., Vittal J. J., and Sim K.-Y. (2000), Phloroglucinol derivatives from *Hypericum japonicum.* Phytochemistry **53**, 705–709.

Maisenbacher P. and Kovar K.-A. (1992), Adhyperforine: a homologue of hyperforin from *Hypericum perforatum*. Planta Med. **58**, 291–293.

Medjroubi K., Benayache F., Benayache S., Akkal, S., Kaabeche M. , Tillequin F., and Seguin E. (1998), Eudesmanolide from *Centaurea granata*. Phytochemistry **49**, 2425–2427.

Parker W. L. and Johnson F. (1968), The structure determination of antibiotic compounds from *Hypericum uliginosum*. I. J. Am. Chem. Soc. **90**, 4716–4723.

Parker W. L., Flynn J. J., and Boer F. P. (1968), The structure determination of antibiotic compounds from *Hypericum uliginosum*. II. The molecular and crystal structure of bromouliginosin B. J. Am. Chem. Soc. **90**, 4723–4726.

Rocha L., Marston A., Potterat O., Kaplan M. A. C., Stoeckli-Evans H., and Hostettmann K. (1995), Antibacterial phloroglucinols and flavonoids from *Hypericum brasiliense.* Phytochemistry **40**, 1447–1452.

Rocha L., Marston A., Potterat O., Kaplan M. A. C., and Hostettmann K. (1996), More phloroglucinols from *Hypericum brasiliense. Phytochemistry* **42**, 185–188.

Still W. C., Kahn M., and Mitra A. (1978), Rapid chromatographic technique for preparative separations with moderate resolution. J. Org. Chem. **43**, 2923–2925.

Taylor H. L. and Brooker R. M. (1969), The isolation of uliginosin A and B from *Hypericum uliginosum*. Lloydia **32**, 217–219.

Verotta L., Appendino G., Jacupovic J., and Bombardelli E. (2000), Hyperforin analogues from St. John's Wort (*Hypericum perforatum*). J. Nat. Prod. **63**, 412–415.

Winkelmann K., Heilmann J., Zerbe O., Rali T., and Sticher O. (2000), New phloroglucinol derivatives from *Hypericum papuanum.* J. Nat. Prod. **63**, 104–108.