Bioorganic & Medicinal Chemistry Letters 23 (2013) 3896–3900

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Rearranged limonoids and chromones from *Harrisonia perforata* and their anti-inflammatory activity

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ARTICLE INFO

Article history:
Received 28 March 2013
Revised 21 April 2013
Accepted 23 April 2013
Available online 2 May 2013

Keywords:
Harrisonia perforata
Rearranged limonoid
Chromone
Anti-inflammatory

ABSTRACT

Two new rearranged limonoids, harperforatin (**1**) and harperfolide (**2**), and a new chromone, harperamone (**3**), were isolated from fruits and roots of *Harrisonia perforata*, together with eight known compounds. Their structures were elucidated on the basis of spectroscopic data. Harperfolide (**2**) exhibited potent anti-inflammatory activity by suppressing nitric oxide (NO) production from activated macrophages with IC_{50} value of 6.51 μM. Furthermore, its effect is mediated by reduction of iNOS protein expression, attributable to the inhibitory action of LPS-induced NO production.

Nitric oxide (NO) is one of the most important mediators in inflammatory processes. Upon inflammatory stimulation, macrophages are activated and produce NO and pro-inflammatory cytokines such as tumor necrosis factor (TNF)-alpha and interleukin (IL)-6. Overproduction of these mediators in macrophages causes many inflammatory diseases, including rheumatoid arthritis, atherosclerosis, and hepatitis.[1–3] Additionally, NO is mainly produced by inducible nitric oxide synthase (iNOS),[4] the inhibition of NO production by suppressing iNOS expression is thus an important target in the treatment of inflammatory diseases.

Harrisonia perforata (Simaroubaceae) is the only species of the genus *Harrisonia* growing in Thailand, and is applied in Thai folklore medicine. The dried root is considered to have antipyretic and anti-inflammatory effects, and it is utilized as a remedy for treatment of wound healing and diarrhea.[5] Previous investigation on chemical constituents of this plant has led to the isolation of an array of structurally diverse chromones and of highly rearranged limonoids, and most of them have been assessed for cytotoxicity against cancer cell lines.[6–10] However, investigation of their anti-inflammatory activity has not been reported yet. As part of our continuing search for biologically active compounds from Thai natural resources, we report herein the isolation and identification of one new highly rearranged limonoid (**1**) from the fruits of *H. perforata*, as well as an additional new limonoid (**2**) and a new chromone (**3**) from the roots, together with eight known compounds. Their anti-inflammatory activity was subsequently evaluated against lipopolysaccharide (LPS) induced nitric oxide production in murine macrophage cell line J774.A1. In addition, the inhibitory effect on iNOS expression was further investigated.

Repeated column chromatography on silica gel of the EtOAc extract of *H. perforata* fruits led to the isolation of a novel highly rearranged limonoid, harperforatin (**1**), while that of the root extract provided an additional new rearranged limonoid, harperfolide (**2**), and a new chromone, harperamone (**3**), along with eight known compounds classified as limonoid, chromone, coumarin, diterpene and polyketide. These includes harrisonin (**4**),[11] obacunone (**5**),[12] peucenin-7-methyl ether (**6**), perforatic acid methyl ester (**7**), *O*-methylalloptaeroxylin (**8**),[7] braylin I (**9**),[13] (+)-vouacapenic acid (**10**),[14] and harrisolanol A (**11**).[15] The structures of known compounds were determined by comparison of their NMR spectroscopic data with those in the literature. To the best of our knowledge, this is the first report of a coumarin braylin I (**9**) and a diterpene (+)-vouacapenic acid (**10**) from the genus *Harrisonia*. Structures of the isolated compounds are shown in Figure 1.

Harperforatin (**1**)[16] was obtained as colorless crystals and assigned the molecular formula $C_{27}H_{32}O_{10}$ from its HRESIMS (*m/z* 517.1947 $[M+H]^+$, Calcd 517.2068). The ^1H NMR spectrum of **1** (Table 1) displayed signals attributable to four tertiary methyls (δ_H 1.35, 1.51, 1.57, 1.59), one secondary methyl (δ_H 1.40, d, J = 8.0 Hz), one methoxy (δ_H 3.74), two olefinic protons (δ_H 6.30, 6.63, each d, J = 10.0 Hz), and a β-furanyl ring (δ_H 6.77, 7.40, 8.04, each br s). The ^{13}C NMR (Table 1) and HSQC data revealed the presence of five methyls (four tertiary, one secondary), three methylenes, eight methines (six olefinic), five quaternary carbons (three

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http://dx.doi.org/10.1016/j.bmcl.2013.04.064

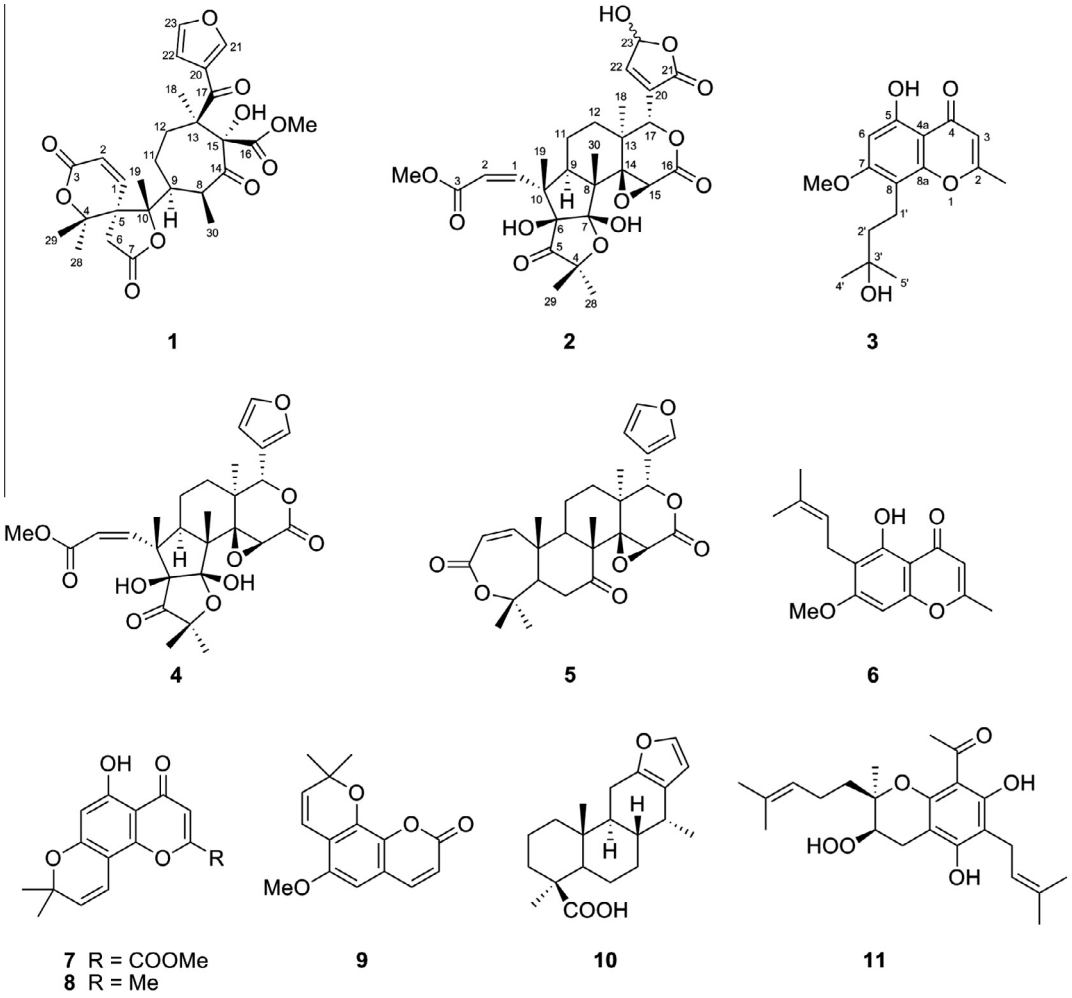

Figure 1. Structures of isolated compounds from *Harrisonia perforata* fruits and roots.

oxygenated), one methoxy, three ester and two ketone carbonyls. On the basis of the above NMR data, compound **1** had a tetracyclic skeleton due to eight units of the 12 unsaturations coming from five carbonyl groups and three carbon–carbon double bonds. One ketone (δ_C 196.2) was connected to the C-20 of a β-furanyl ring because of the downfield shift of H-22 (δ_H 8.04), and its HMBC correlation with Me-18 (Fig. 2). The other ketone moiety (δ_C 208.4) was assigned to C-14 by HMBC correlation between Me-30/C-14 and H-8/C-14. Observed HMBC correlations of Me-18/C-12, Me-18/C-13, Me-18/C-15, H-12/C-15 and Me-30/C-14, coupled with the connectivity of the partial structure $-CH_2(12)-CH_2(11)-CH(9)-CH(8)Me-$ by $^1H-^1H$ COSY correlations, suggested the existence of seven-membered ring in **1** (Fig. 2). In addition, the presence of two lactone rings connecting together through the C-5 spiro carbon were corroborated by HMBC cross peaks between H-1/C-5, Me-28/C-5, Me-29/C-9, H_2-6/C-7, H_2-6/C-10 and Me-19/C-10. This unit was further connected to a seven-membered ring at C-9 by a strong HMBC correlation from Me-19 to C-9. The complete structure and relative configuration of **1** was finally established by single-crystal X-ray diffraction analysis using Mo K_α radiation as shown in Figure 3.[17] To the best of our knowledge, the structure of **1** possesses a very unique two lactones connecting together through a spiro carbon.

Harperfolide (**2**)[18] was obtained as colorless crystals and had the molecular formula as $C_{27}H_{32}O_{12}$ by HRESIMS (*m/z* 547.1886 $[M-H]^-$, Calcd 547.1810), implying 12° of unsaturation. The NMR data of **2** (Table 1) showed that 6 U of unsaturation came from two carbon–carbon double bonds, three ester and one ketone carbonyls. Therefore, the remaining degrees of unsaturation required **2** to be pentacyclic. A combined anaylsis of 1D and 2D ($^1H-^1H$ COSY, HSQC and HMBC) NMR spectra (Fig. 2) indicated the presence of an α,β-unsaturated methyl ester [δ_H 5.77 (d, *J* = 12.4 Hz), 6.00/6.01 (d, *J* = 12.4 Hz), 3.78 s; δ_C 52.1 CH_3, 123.2/123.3 CH, 153.6/153.7 CH, 166.8 qC], a γ-hydroxybutenolide ring [δ_H 6.18 (d, *J* = 4.0 Hz)/6.22 (d, *J* = 12.0 Hz), 7.32/7.34 (br s); δ_C 96.6/97.4, 134.3/134.6, 149.5/150.3, 169.3], and five tertiary methyls (δ_H 1.24/1.25, 1.15, 1.19, 1.24/1.25, 1.37 s; δ_C 14.6, 17.3, 17.9, 24.1, 27.4). The existence of an α,β-epoxy-δ-lactone ring was confirmed by HMBC cross peaks from H-17 to both bridgehead carbons (C-13 and C-14) and the C-16 ester carbonyl, from Me-18 to C-13, C-14 and C-17, and from H-15 to C-14 and C-15. Actually, the NMR data of **2** were similar to those of harrisonin (**4**), a known rearranged limonoid isolated from this plant. This indicated they must share the same basic skeleton, except for the presence of a γ-hydroxybutenolide moiety instead of a furanyl ring in **4**. Moreover, observed HMBC correlations from H-17 to C-20, C-21 and C-22 of a butenolide group clarified the location of a γ-hydroxybutenolide at C-17. In addition, the appearance of pairs of most proton and carbon resonances in the NMR spectra of **2** (Table 1) suggested the presence of C-23 epimers, the same as those in moluccensin N.[19] Thus the structure of **2** was established as shown.

Harperamone (**3**)[20] was obtained as a light yellow solid. Its molecular formula was determined as $C_{16}H_{20}O_5$ by HRESIMS (*m/z* 293.1383 $[M+H]^+$, Calcd 293.1316). The UV absorption maxima at