Unprecedented Quassinoids with Promising Biological Activity from Harrisonia perforata**

Xin Fang, Ying Tong Di, Yu Zhang, Zhi Ping Xu, Yang Lu, Qian Qian Chen, Qi Tai Zheng, and Xiao Jiang Hao*

Abstract: Perforalactone A (1), a new 20S quassinoid with a unique cage-like 2,4-dioxaadamantane ring system and a migrated side chain, was isolated from the plant Harrisonia perforata together with two biosynthetically related new quassinoids. The structures of these natural products were elucidated by NMR spectroscopy, X-ray diffraction analysis, computational modeling, and the CD excitation chirality method. The compounds exhibited notable biological properties, including insecticidal activity against Aphis medicaginis Koch and antagonist activity at the nicotinic acetylcholine receptor of Drosophila melanogaster. The structural features of these compounds may be related to their promising biological characteristics. Their biosynthesis and an alternative origin of quassinoid-type natural products are also discussed.

Natural products are a reliable source of drug and pesticide discovery. In the course of our investigations of bioactive structurally novel natural products derived from plants, we were attracted to quassinoids, which are a class of degraded terpenoids derived from (20S)-euphol or (20S)-tirucallol from the Simaroubaceae family of plants. Quassinoids exhibit a wide range of biological activities and their total synthesis has fascinated synthetic chemists for a long time. From the twigs and stem of Harrisonia perforata (Blanco) Merr. (Simaroubaceae), we isolated two novel quassinoids: perforalactone A (1; Scheme 1), which possesses an unprecedented C25 backbone, and its postulated biosynthetic precursor perforalactone B (2). Importantly, these compounds are the first reported natural 20S quassinoids and they provide clear evidence of the previously overlooked (20R)-euphol origin of quassinoids. In the subsequent biosynthetic study, perforalactone C (3), a new C20 quassinoid with a unique C19 oxygenated functionality was discovered. This compound is presumably derived from 2. Strikingly, the compounds 1 and 2 exhibited notable biological activities related to their structural features. Herein, we describe the isolation, structural elucidation, possible biosynthesis, and biological activity of these compounds.

Perforalactone A (1) was obtained as colorless crystals. Its molecular formula, C35H32O9, was determined by HRESIMS (m/z 511.1928 [M+Na]+; calcd: 511.1944). The 1H and 13C NMR spectroscopic data of 1 indicated the presence of a methoxy group and 25 carbon atoms, including two tertiary methyl groups and one secondary methyl group (Table 1). Two carbonyl functional groups giving rise to NMR signals at δC = 168.5 and 176.7 ppm indicated the presence of δ-lactone and γ-lactone rings, which was further supported by the characteristic IR absorption bands at 1725 and 1768 cm⁻¹, respectively. Furthermore, the presence of a 2-en-1-one system bearing a methoxy group was indicated by a doublet at δH = 5.56 ppm (J = 2.5 Hz) in the 1H NMR spectrum and by the resonances at δC = 204.8, 147.5, and 121.0 ppm in the 13C NMR spectrum. This structural assignment was confirmed by HMBC correlation of the olefinic hydrogen atom with the carbonyl moiety and by the characteristic IR absorption band at 1657 cm⁻¹.

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Scheme 1. Chemical structures of (20R)-euphol, (20S)-tirucallol, and perforalactones A–C (1–3).
Table 1: $^1$H (500 MHz) and $^{13}$C NMR (100 MHz) spectroscopic data for perforalactone A (1) in CDCl$_3$.

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_{H}$ [ppm] ($J$ [Hz])</th>
<th>$\delta_{C}$</th>
<th>Position</th>
<th>$\delta_{H}$ [ppm] ($J$ [Hz])</th>
<th>$\delta_{C}$ [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>204.8</td>
<td>15$\alpha$</td>
<td>2.31 (dd, 19.0, 13.0)</td>
<td>28.4</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>147.5</td>
<td>15$\beta$</td>
<td>2.64 (dd, 19.0, 7.0)</td>
<td>168.5</td>
</tr>
<tr>
<td>3</td>
<td>5.56 (d, 2.5)</td>
<td>121.0</td>
<td>16</td>
<td>–</td>
<td>97.7</td>
</tr>
<tr>
<td>4</td>
<td>2.51$^\text{[H]}$</td>
<td>31.1</td>
<td>17</td>
<td>–</td>
<td>68.5</td>
</tr>
<tr>
<td>5</td>
<td>1.96$^\text{[H]}$</td>
<td>42.8</td>
<td>18</td>
<td>1.10 (s)</td>
<td>24.2</td>
</tr>
<tr>
<td>6$\alpha$</td>
<td>2.04 (td, 14.5, 3.5)</td>
<td>24.1</td>
<td>19</td>
<td>1.56 (s)</td>
<td>13.8</td>
</tr>
<tr>
<td>6$\beta$</td>
<td>1.77 (m)</td>
<td>20</td>
<td>2.74 (m)</td>
<td>43.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4.17 (s)</td>
<td>81.2</td>
<td>21$\alpha$</td>
<td>4.45 (dd 9.0, 6.0)</td>
<td>68.1</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>35.5</td>
<td>21$\beta$</td>
<td>4.33 (t, 8.0)</td>
<td>28.8</td>
</tr>
<tr>
<td>9</td>
<td>2.15 (s)</td>
<td>39.0</td>
<td>22$\alpha$</td>
<td>2.55 (dd, 17.0, 6.0)</td>
<td>11</td>
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<tr>
<td>10</td>
<td>–</td>
<td>48.4</td>
<td>22$\beta$</td>
<td>2.50 (dd, 17.0, 9.5)</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>–</td>
<td>96.2</td>
<td>23</td>
<td>–</td>
<td>176.7</td>
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<tr>
<td>12$\alpha$</td>
<td>1.90 (d, 13.5)</td>
<td>44.6</td>
<td>28</td>
<td>1.13 (d, 6.5)</td>
<td>19.3</td>
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<tr>
<td>12$\beta$</td>
<td>1.73$^\text{[H]}$</td>
<td>30a</td>
<td>1.95$^\text{[H]}$</td>
<td>36.4</td>
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<td>13</td>
<td>–</td>
<td>75.2</td>
<td>30b</td>
<td>–</td>
<td>14</td>
</tr>
<tr>
<td>14</td>
<td>1.93$^\text{[H]}$</td>
<td>46.2</td>
<td>OMe</td>
<td>1.20 (d, 12.0)</td>
<td>55.4</td>
</tr>
</tbody>
</table>

(a) Overlapped signals; the multiplicity could not be observed.

Figure 1. a) Key $^1$H–$^1$H COSY and HMBC correlations, b) key ROESY correlations, and c) X-ray single-crystal structure of 1.

and absolute configurations of 1 were also determined by ROESY and X-ray crystallographic experiments (Figure 1b). Thus, the full structure of this novel quassinoid named perforalactone A (1) with an unprecedented skeleton was elucidated. Notably, 1 contains a unique cagelike 2,4-dioxoadamantane ring system not usually encountered in the natural products of the plant kingdom. Previously, only two polyketides and two marine natural products, one of which is tetrodotoxin, were known to feature this structural moiety. Furthermore, compound 1 is the first naturally occurring 20S quassinoid, thus suggesting an alternative origin of quassinoids.

Perforalactone B (2) possesses the molecular formula C$_{20}$H$_{24}$O$_6$, as determined by HRESIMS (m/z 495.1999 [M+Na]$^+$; calcd: 495.1994). Its IR, $^1$H NMR, and $^{13}$C NMR spectroscopic data (see Table S2) were analogous to those of 1, thus suggesting that 1 and 2 are structurally similar. The primary difference between 1 and 2 is that 2 has four methyl groups, and its γ-lactone ring is connected to C13 through a ketone group at C17, as suggested by the HMBC correlations of H$_2$-18/C17, H-12/C17, H-20/C17, and H-21/C17. Detailed 2D NMR spectroscopic analysis (see Figure S7a and Table S1) provided the structure of 2. However, the ROESY correlations of H$_2$-21/H$_2$-18, and H$_2$-22/H$_2$-30 only provided a clue to assign the key relative configuration at C20 because of rotation about the C13–C17 and C17–C20 bonds. Thus, computational modeling was used to help determine the orientation of H-20. The potential-energy surface of 2 with the 20-αH configuration was scanned at the AM1 level of theory by rotation about the C13/C17 and C17/C20 bonds, which resulted in 11 minimum-energy points (see Figure S1). All stable conformations were then optimized by DFT at the B3LYP/6-31G* level, and the harmonic vibrational frequencies of each structure were calculated by the same method in GAUSSIAN03. Seven stable conformations were identified, of which conformers 2a (accounting for 60% of the conformations in the gas phase) and 2b (accounting for 38%) are predominantly populated. By the same manner, the two predominant conformers 20-epi-2a (accounting for 51%) and 20-epi-2b (accounting for 47%) of the C20 epimer of 2 were found (see Table S3). The distances between the H$_2$-21 and H$_2$-18 and the H$_2$-22 and H$_2$-30 pairs in conformers 2a and 2b are shorter than those in 20-epi-2a and 20-epi-2b (Figure 2). Notably, 2 yielded the aforementioned ROEY correlations, whereas 20-epi-2 did not yield analogous ROSEY correlations. Thus, the α orientation of H-20 was unambiguously established. Furthermore, the absolute configuration of 2 was determined to be identical to that of 1, as evidenced by their nearly congruent circular dichroism curves (see Figures S6b and S11b).

Both perforalactones A (1) and B (2) have the S configuration at C20, which gives us an opportunity to analyze the origin of quassinoids. Previously, quassinoids were suggested to be biogenetically derived from (20R)-euphol or (20S)-tirucallol. However, only C$_{25}$ quassinoids contain a γ-lactone-ring side chain that includes C20. This side chain is absent in the C$_{20}$, C$_{19}$, and C$_{18}$ types. Thus, the true origin of quassinoids must be deduced from the configuration at C20 of C$_{25}$ quassinoids. Because all reported C$_{25}$ quassinoids have the
in C synthase abstracts a hydrogen atom from a methyl group in its substrate penicillin N to produce an ethylene sulfide.[16] Subsequently, the produced oxygen radical attacks the C11 carbonyl moiety to generate the C17/C11 ether bridge. Finally, the newly generated oxygen radical reabstracts the hydrogen atom from the active-site residue of the enzyme to produce 1 and thereby return the enzyme to its activated state for another reaction cycle.

To further investigate this possible biogenetic pathway, we used LC–MS to identify the proposed intermediates in fresh plant extracts. However, after several attempts, no ion peaks of the possible intermediates were detected. Instead, a compound with an ion peak at m/z 417.2 was observed to co-occur with compound 1 as a minor constituent; its UV spectrum was similar to that of 1. This compound was isolated, and its 1H and 13C NMR spectroscopic data were observed to be very similar to those of compound 2 (see Table S2), except for the absence of the side chain and the presence of an oxygenated methylene and an acetyl group, thus suggesting that it is a C20 quassinoid related to 2 that bears a acetyl group. Its HRESIMS spectrum (m/z 439.1737 [M+Na]+; calcd: 439.1732) gave the molecular formula C20H29O6, according to its 1D NMR spectroscopic data. Detailed 2D NMR spectroscopic analysis (see Figure S12a and Table S1) gave structure 3 in Scheme 1. The absolute configuration of the compound was determined by applying the CD excitation chirality method.[17,24] The CD spectrum exhibited a positive split (231 nm, Δε = -0.48; 250 nm, Δε = 6.56) due to the excitation coupling between the two chromophores of the 2-en-1-one and 12-en-11-one systems;[17] this result indicated a clockwise orientation of the transition dipole moments of the two chromophores (see Figure S16b). Thus, the absolute configuration of this compound, perforalactone C (3), a C20 quassinoid with a 19-OAc group, was determined and is identical to that of 1 and 2.

Notably, although nearly all other positions of a quassinoid could be oxygenated, a C19 methyl moiety has never been previously observed to be oxygenated. This functionality, together with the co-occurrence of 3 with 1 in this experiment as a minor isolate and the sole C20 quassinoid, strongly suggests that 3 shares a common intermediate I and a similar radical mechanism with 1 in its biosynthesis, instead of the involvement of a P450 enzyme. Clearly, the shift of a C30 methyl radical generated by the enzymatic radical to the C19 methyl group and the coupling of a free hydroxyl radical could lead to this unique oxygenation at C19 (see Scheme S1 in the Supporting Information).

The scaffold and structural features of these compounds are unique and differ significantly from those previously reported; thus, these compounds might display unprecedented biological profiles. The two major isolates 1 and 2 were subjected to insecticidal assays because other quassinoids have exhibited insecticidal activity.[23] Compounds 1 and 2 demonstrated significant activity against the agriculturally relevant sucking pest insect Aphis medicaginis Koch, with LC50 values of 86.4 and 7.23 μM, respectively; however, they demonstrated no activity against the chewing insects Spodoptera exigua (Hübner) and Mythimna separate Walker. Moreover, 1 and 2 showed no evident cytotoxicity against...
a series of cell lines (see the Supporting Information), thus suggesting the mode of action (MoA) of their insecticidal effect is not attributable to their cytotoxicity, unlike the MoAs of other reported quassinoids.[18]

In an attempt to preliminarily investigate the MoA of these quassinoids, we focused on the insect nicotinic acetylcholine receptor (nAChR) because it is a major and ubiquitous target for insecticide action.[19] Thus, compounds 1 and 2 were tested for their activity at the nAChR on isolated neurons from Drosophila melanogaster, which is a model insect commonly used in this assay.[19,20] Both compounds exhibited significant nAChR activity (IC50 = 15.8 nm for 1 and IC50 = 1.26 nm for 2). In particular, 2 showed an IC50 value approximating that of the positive reference imidacloprid (IC50 = 0.79 nm), which is the most commercially important insecticide targeting an insect nAChR. Further biochemical electrophysiological tests suggested that they are all antagonists at the nAChR of the insect.[21] Owing to the paucity of the sample, compound 3 was only evaluated for its cytotoxicity against the HL-60 cell line, and showed moderate activity with an IC50 value of 8.7 μm.

Notably, 2 showed higher activities by the same order of magnitude against the D. melanogaster nAChR (12.5-fold) and A. medicaginis (11.9-fold) as compared with 1. These data indicate that the insecticidal activity of both compounds might arise from their activity at the nAChR of the insect.[22] However, the binding site and affinity of these compounds may differ from those of neonicotinoid pesticides. Indeed, their structures are quite different; compounds 1 and 2 lack the negatively charged nitro groups that contribute to the activity of neonicotinoid pesticides.[19] These results imply that the isolates could be novel lead compounds as pesticides, especially because neonicotinoid pesticides have been suggested to cause unwanted environmental impact.[23] Although their structures are too complex to be synthesized easily, they could provide valuable inspiration for the development of new pesticides. Furthermore, the remarkable insecticidal and insect-nAChR activity of these quassinoids may be related to the defense system of plants.

**Keywords**: biosynthetic pathways · insecticidal activity · natural products · nicotinic acetylcholine receptors · structure elucidation

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[6] CCDC 768066 (1) 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.


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