Short Communication

IDENTIFICATION OF A RARE SULFONIC ACID METABOLITE OF ANDROGRAPHOLIDE IN RATS

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ABSTRACT:

Andrographolide is widely used in clinic as an anti-inflammatory and antibiotic drug. In this paper, the metabolites of andrographolide in rats after single oral doses of 120 mg/kg were investigated. The structures of the metabolites were elucidated by high-resolution mass spectra, NMR spectroscopy including 1H NMR, 13C NMR, and two-dimensional NMR, through comparison of high-resolution mass spectra, NMR spectroscopy including 1H NMR, 13C NMR, and two-dimensional NMR, through comparison of synthetic standard. The main metabolite of andrographolide in rats was 14-deoxy-12(R)-sulfo andrographolide. In the proposed mechanism, the β-carbon of α, β-unsaturated carbonyl was attacked by sulfonic acid, to form the sulfonate compound. This was a rare metabolic reaction. It may be the main metabolic pathway of andrographolide in rats. The polarity of the sulfonate metabolite increased greatly and could be easily eliminated from body.

Andrographolide, chemically designated as 2(3H)-furanone-3, 3-[2-decahydro-6-hydroxy-5-(hydroxymethyl)-5, 8a-dimethyl-2-methylene-1-naphthalenyl]ethyldiene] dihydro-4-hydroxy-, was one of main active constituents of Andrographis paniculate (Burm) Nees, a famous traditional Chinese medicine. It was found that andrographolide had wide bioactivities, such as anti-inflammatory antibiotic (Sinha et al., 2000; Shen et al., 2002), anti-platelet aggregation (Zhang and Tan, 1997; Amroyan et al., 1999), and anti-human immunodeficiency virus activities (Calabrese et al., 2000). Andrographolide was widely used in the clinic, but it was not effective in vitro. These seemingly contradictory results have aroused the interests of many pharmacologists in this herbal product. Many experiments have been done, but much still remains to be clarified about andrographolide. Some results about the pharmacokinetics of this product have been reported after oral administration of andrographolide (Zhu and Kang, 1981; Panossian et al., 2000). However, there have been no reports on its metabolism, either in humans or animals, to our knowledge. So, isolation and identification of the metabolites of andrographolide were attempted. In this paper, results concerning the identity of andrographolide metabolites are reported, and a rare metabolic reaction was illustrated.

Experimental Procedures

Chemicals and Drugs. Andrographolide were kindly donated by Professor Fengpeng Wang, Western China Medical University. Methanol and other analytical reagents were purchased from Shenyang Chemical Company (Shenyang, China).

Animals. Wistar rats, male, weighting 300 ± 10 g were provided by The Experimental Animals Center, Shenyang Pharmaceutical University (Shenyang, China), and The Second Clinical Hospital of China Medical University (Shenyang, China).

Urinalysis. Urine within 0 to 48 h was collected at room temperature through metabolic cages (Suzhou animal experimental apparatus factory, Suzhou, China) after single oral administration of 120 mg/kg of andrographolide. The oral dose was dissolved in water, and 15 male Wistar rats were dosed for the experiments.

Isolation of Metabolites. The combined urine sample of 545 ml was filtered, and the filtrate was dried in vacuum. The residue was dissolved in water and was extracted with ethyl acetate and water-saturated N-water and was extracted with ethyl acetate and water-saturated N-water. The 5% methanol elution was subjected to Sephadex LH 20 (10 × 300 mm; Pharmacia AB, Uppsala, Sweden) and eluted with a linear gradient of water-methanol. The 5% methanol elution was subjected to Sephadex LH 20 (10 × 300 mm; Pharmacia AB) and eluted with 60% methanol. Then Rp-high performance liquid chromatography (22 × 250 mm; Alltech Associates, Deerfield, IL) was used. The mobile phase was water-methanol (95:5), and 101.1 mg of metabolite 1 was thus obtained.

Identification of the Metabolites. The metabolites were dissolved in CD3OD (99.9% D; Cambridge Isotope Laboratories, Andover, MA). All NMR spectra were recorded on APEX II (Bruker Daltonics Inc., Billerica, MA). ESI-MS spectra were recorded on a Finnigan LCQ system (Thermo Finnigan Mat, San Jose, CA), Melting points were determined on a Yanaco MP-3 micro-melting point apparatus (Yanaco Corporation, Kyoto, Japan). IR spectra were determined on a BRUKER IFS 55 spectrometer (Bruker Daltonics Inc.) in KBr pellets. UV spectra were measured on a SHIMADZU UV-2201 spectrometer (Shimadzu, Kyoto, Japan).

Chemical Syntheses. Compound A (synthetic metabolite 1) was synthesized using the published procedures (Meng, 1981) as described below. Identity and purity of compound A were confirmed by examining the mass spectra and NMR spectra.

Synthesis of Compound A (Metabolite 1 Reference). Andrographolide (1.0 g) was dissolved in 15 ml of 95% ethanol on heating at 50°C (solution 1). To 4 ml, 1 M Na2SO3, 4.8 ml of 2% H2SO4 (M/M) and 8 ml of water was
added (solution 2). Solution 1 was poured into solution 2 and refluxed for 30 min. The pH value of the reaction solution was adjusted to 6 to ~7 by adding 2% H2SO4 solution and evaporated to dryness. Addition of 20 ml of water to dissolve the residue was followed by extraction with the same volume of chloroform for three times. The water layer was evaporated, dissolved in 10 ml of methanol, and filtered. The filtrate was then evaporated to dryness and gave 0.6 g product with a yield of 50.7%.

Results

Identification of Metabolite 1. The structure of metabolite 1 was elucidated by chemical and spectroscopic methods.

Metabolite 1, colorless needle crystal, mp > 300°C (decomposed), was positive to Legal and Kedde reactions, suggesting it was an α, β-unsaturated lactone. In the 13C NMR, there were 20 carbon signals. In the IR spectrum, 1200.1 cm⁻¹ was the characteristic absorption of the sulfonate group. The molecular weight was 414 on fast atom bombardment-mass spectrum. The molecular formula of C20H30O7S was thus derived based on the 1H NMR, 13C NMR and IR data. The high resolution second ionization-mass spectrum showed the quasi-molecular ion [M+H]⁺ at m/z 413.1634 (calculated 413.1639), which confirmed the molecular formula.

The obvious changes in the chemical shifts at C9 and C11 to ~C16 were observed when metabolite 1 was compared with the parent drug, andrographolide, suggesting that the structural changes of metabolite 1 occurred only in the side chain of the lactone, without any changes in rings A and B (Fig. 1). The carbon signal of carbonyl (C-14) shifted from 172.6 to 177.2 ppm, whereas the oxygen-linked carbon C-15 changed from 76.1 to 73.3 ppm. These changes in chemical shifts showed that the carbon-carbon double bond at 12(13) of andrographolide changed into 13(14), and the double bond transferred from outside to inside the ring of the lactone. In HMBC spectra (Fig. 2), the signal of H-15 (4.95, 2H) correlated with the resonances at 132.5 (C-13) and 152.1 (C-14). That meant that the carbon-carbon double bond was located at 13(14). In the UV spectrum, the maximal absorption of metabolite 1 was at 204 nm, which was different from that of andrographolide at 225 nm. The hypsochromic shift in the UV spectrum suggested the change of conjugated system. On the other hand, the resonance of H-14 (7.65, H, t, J = 1.8 Hz) correlated with 56.8 (C-12), and H-12 (3.92, H, dd, J = 12.2, 1.8 Hz) correlated with 132.5 (C-13), 152.1 (C-14), and 177.2 (C-16). These correlated peaks showed that the sulfonate group was linked at C-12.

To confirm the metabolite 1 structure further, we synthesized compound A with a known method (Meng, 1981). The 1H and 13C NMR of compound A and metabolite 1 were essentially identical and thus suggested us that metabolite 1 and compound A have the same structures.

In the nuclear overhauser enhancement spectroscopy spectra, H-12 correlated with H-14, H-9, and H-11α, but it had no NOE effect with H-1a and H-1e. Therefore, the C-12 configuration of metabolite 1 could be drawn in R-form, which is in accordance with the literature assignment (Matsuda et al., 1994; Zhang and Masayoshi, 1997). Combined with the finding at 20-CH3 had NOE effects with 19-CH3OH, H5, H6a, H7a, H11, and H11β, and 19-CH3OH correlated with H2a and H6a, it is suggested that 20-CH3 and 19-CH3OH were located at axial orientations. Similarly, the signal of H-3 had NOE effects with 18-CH3, H1α, and H5α, which indicated that H-3 was located at an axial orientation. Based on the above data, the stereochemical structure of metabolite 1 was assigned as 14-deoxy-12(R)-sulfo andrographolide. The full assignments of the signals were summarized in the Table 1.

Discussion

Metabolite 1 was a newly identified metabolite of andrographolide. It could be presumed that the β-carbon (C-12 of andrographolide) of the α, β-unsaturated carbonyl was attacked by the sulfur atom (HSO3⁻) with lone-paired electrons and formed this sulfonate compound. In addition, the hydroxyl group (14-hydroxyl of andrographolide) at position 14 was dehydrated, and a new carbon-carbon double bond was formed at 14(13). This appears to be a rare metabolic reaction, but it has been cited in the literature (Wikberg and Taskinen, 1993; Yoshino et al., 1993). It appears that this may be the main metabolic pathway of andrographolide in rats and somehow could explain why the drug could be easily eliminated from the body.

To confirm where the sulfonate reaction occurred, another experiment was done. We collected and then analyzed the bile and the contents of the small intestines immediately after oral administration of andrographolide to rats. Metabolite 1 was detected in the contents of the small intestine but not in bile. So we excluded the possibility that the metabolite in the small intestines immediately after oral administration of andrographolide to rats. Metabolite 1 was detected in the contents of the small intestine but not in bile. So we excluded the possibility that the metabolite in the small intestines immediately after oral administration of andrographolide to rats.

![Fig. 1. Proposed metabolic scheme of andrographolide in rats.](image)

![Fig. 2. Primary HMBC correlations of metabolite 1.](image)
intestine was produced from bile and, therefore, that the sulfonate metabolite was produced in the small intestine in rats.

In conclusion, these results demonstrate that andrographolide undergoes a sulfonate reaction at C-12 and then appears to dehydrate to form metabolite 1. Understanding of this somewhat unique metabolic pathway may shed light on its clinical efficacy (Meng, 1981) and also may assist in molecular structure-activity studies for new antibiotic and anti-inflammatory drug development efforts in the future.

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TABLE 1

All spectra were recorded on Inova 600, in CD3OD, 600 MHz for 1H and 150 MHz for 13C. The carbon and proton signals were assigned with additional 1H NMR, 13C NMR, correlation spectroscopy, total correlation spectroscopy, nuclear overhauser enhancement spectroscopy, heteronuclear multiple quantum coherence, and HMBC experiments.

<table>
<thead>
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<th>No.</th>
<th>δC</th>
<th>δH (mult, J in Hz)</th>
<th>δH</th>
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<tr>
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<td>38.9</td>
<td>38.1</td>
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<tr>
<td>2</td>
<td>29.8</td>
<td>29.0</td>
<td>1.02(H, m) 1.28(H, m)</td>
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<td>3</td>
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<td>80.9</td>
<td>1.71(2H, m) 1.78(2H, m)</td>
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<td>57.2</td>
<td>56.3</td>
<td>1.10(H, dd, 12.6, 2.4) 1.32(H, m)</td>
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<tr>
<td>6</td>
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<tr>
<td>11</td>
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<td>27.0</td>
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<tr>
<td>12</td>
<td>56.8</td>
<td>149.3</td>
<td>3.92(H, dd, 12.2, 1.8) 6.83(H, dd, 6.7, 5.4)</td>
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<tr>
<td>13</td>
<td>132.5</td>
<td>129.8</td>
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<tr>
<td>14</td>
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<td>4.95(2H, o) 4.46(H, dd, 10.2, 6.1)</td>
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<td>15.5</td>
<td>0.68(3H, s) 0.74(3H, s)</td>
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m, multiple split; o, overlapped peaks.

References


