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A New Glucuronidated Metabolite of Andrographolide in Human

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Abstract: A new andrographolide metabolite **1** was isolated from human urine samples after oral administration. The structure was determined to be 3-carbonylandrographolide-19-*O*-β-D-glucuronide on the basis of chemical evidences and spectral analysis, especially by 2D-NMR techniques.

Keywords: Andrographolide, metabolite in human urine, 3-carbonyl-andrographolide-19-*O*-β-D-glucuronide, oxygenated metabolite, glucuronide conjugate.

Andrographolide, one of the main bioactive constituents of *Andrographis paniculata* (Burm) Nees, a famous traditional Chinese medicine, was chemically designated as 3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylene-1-napthalenyl] ethylidene] dihydro-4-hydroxy-2(3H)-furanone. Andrographolide showed a wide biological activities, including antiinflammatory[1,2,3], anti-allergic[4,5], anti-platelet aggregation[6], hepatoprotective[7,8], anti-human immunodeficiency virus(HIV) activities[9,10] and was widely used in clinic for the treatment of fever, cold, inflammation, diarrhea and other infectious diseases. The pharmacokinetic studies showed that it absorbed and intensely metabolized in rats and human quickly[11]. Recently, our reasearch group found that one of the metabolites of andrographolide in rats after oral administration, 14-deoxy-12(R)-sulfoandrographolide, was a antiinflammatory drug (Lianbizhi) used in clinic as an injection[12,13]. So the metabolites of andrographolide in human urine were further investigated. The present paper describes the structural elucidation of a new andrographolide metabolite (see **Figure 1**).

Metabolite **1**[14], white amorphous power, was positive for the Legal and Kedde reactions, suggesting the presence of an α,β-unsaturated lactone. The negative ESI-MS showed the quasi-molecular ion peak $[M-H]^-$ at *m/z* 523. Combined with the ^{1}H-NMR and ^{13}C-NMR spectral data, the molecular formula of **1** was determined to be $C_{26}H_{36}O_{11}$. The MS^2 spectrum of the $[M-H]^-$ ion *m/z* 523 provided a fragment ion at *m/z* 505 $[M-H-H_2O]^-$. In the MS^3 spectrum, the $[M-H-H_2O]^-$ ion *m/z* 505 eliminated 176 amu (glucuronic acid $-H_2O$) to give *m/z* 329, suggesting that **1** was a glucuronide conjugate

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Figure 1 Structures of andrographolide and metabolite **1**

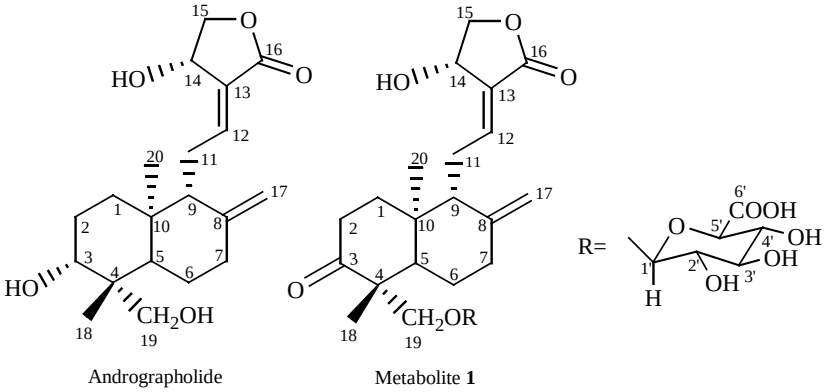
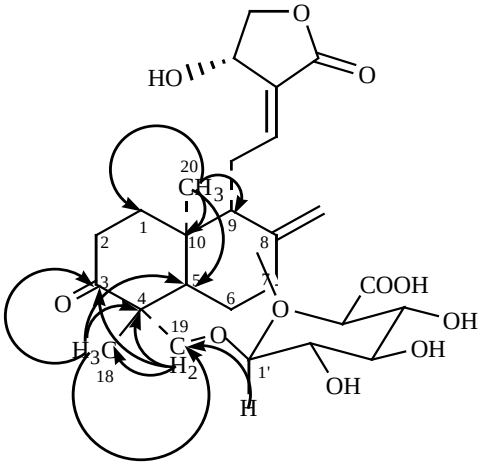


Figure 2 Key HMBC correlations for **1**



of andrographolide. The ^{13}C-NMR data also showed the existence of a glucuronic acid [δ 73.6 (C-4′), 74.8 (C-2′), 76.1 (C-5′), 77.7 (C-3′), 104.8 (C-1′), 176.8 (C-6′)]. The linkage site of the glucuronic acid moiety was determined to be at C-19 by analysis of the HMBC spectrum (see **Figure 2**), in which the signals of H-19 (δ 4.56, d, 1H, *J*=9.7 Hz; δ 3.30, m, 1H) correlated with C-1′ (δ 104.8) and the anomeric proton of glucuronide (δ 4.13, d, 1H, *J*=7.8 Hz) correlated with C-19 (δ 73.5). The β-form anomeric configuration of the glucuronic acid was judged from its coupling constant of the anomeric proton (*J*=7.8 Hz).

The carbon signals of the aglycone moiety of **1** and its parent drug andrographolide were very similar except for the signals at C_1~C_5, C_{18} and C_{19}, indicating that the varieties of **1** occurred at ring A only. It was obvious that the hydroxyl-linked carbon signal at δ 80.9 (C-3) of andrographolide had disappeared and a new carbon signal of carbonyl at δ 217.4 could be observed in **1**, suggesting that the hydroxyl at C-3 of andrographolide might be oxygenated to carbonyl. Due to the occurrence of the

oxygenation at C-3 and the glucuronidation at C-19, the signals of C-2, C-4, C-5 and C-19 of **1** shifted downfield from δ 29.0 to δ 39.8, δ 43.6 to δ 54.5, δ 56.3 to δ 58.4 and δ 64.9 to δ 73.5, while the signals of C-1 and C-18 shifted upfield from δ 38.1 to δ 36.9 and from δ 23.3 to δ 20.8, respectively. In the HMBC spectrum (also see **Figure 2**), the signal of H-18 (δ 1.19, 3H, s) had peaks correlated with δ 217.4 (C-3), δ 73.5 (C-19), δ 58.4 (C-5) and δ 54.5 (C-4), the signal of H-2β (δ 2.14, m, 1H) correlated with δ 217.4 (C-3) and the signals of H-19 (δ 4.56, d, 1H, *J*=9.7 Hz; δ 3.30, m, 1H) had correlations with δ 217.4 (C-3), δ 54.5 (C-4) and δ 20.8 (C-18). These correlated peaks further confirmed that the carbonyl was located at C-3. Thus, the structure of the ring A could be established. Based on the above chemical and spectroscopic evidences, the structure of **1** was elucidated to be 3-carbonyl-andrographolide-19-*O*-β-D-glucuronide.

References and Notes

1. Y. C. Shen, C. F. Chen, W. F. Chiou, *Planta Med.*, **2000**, *66*, 314.
2. Y. C. Shen, C. F. Chen, W. F. Chiou, *Br. J. Pharmacol.*, **2002**, *135*(2), 399.
3. S. Madav, S. K. Tandan, J. Lal, *Fitoterapia*, **1996**, *67*, 452.
4. S. Madav, C. F. Tripathi, S. K. Tandan, *et al*., *Indian J. Pharm. Sci.*, **1998**, *60*, 176.
5. P. P. Gupta, J. S. Yandon, G. K. Patnaik, *Pharm.Biol.*, **1998**, *36*, 72.
6. E. Amroyan, E. Gabrielian, A. Panossian, *et al*., *Phytomedicine*, **1999**, *6*, 27.
7. B. Shukla, P. K. S. Visen, G. K. Patnaik, B. N. Dhawan, *Planta Med.*, **1992**, *58*, 146.
8. P. Pramyothin, W. Udomuksorn, S. Poungshompoo, C. Chaichantipyuth, *Asia Pac. J. Pharmacol.*, **1994**, *9*, 73.
9. S. S. Handa, A. Sharma, *Indian J. Med. Res.*, **1990**, *92*, 284.
10. A. Basak, S. Cooper, A G. Roberge, *et al*., *Biochem. J.*, **1999**, *338*, 107.
11. A. Panossian, A. Hovhannisyan, G. Mamikonyan, *et al*., *Phytomedicine*, **2000**, *7*, 351
12. X. J. He, J. K. Li, H. Gao, *et al*., *Tetrahedron*, **2003**, *59*, 6603.
13. X. J. He, J. K. Li, H. Gao, *et al*., *Chem. Pharm. Bull.*, **2003**, *51*, 586.
14. selected data of **1**: white amorphous powder, $C_{26}H_{36}O_{11.}$ Legal and Kedde reactions: red. UV λ_{max}(MeOH) nm: 202, 225. ^{1}H-NMR (400 MHz, CD_3OD, δ ppm, J Hz) δ6.84 (dt, 1H, *J*=6.7, 1.7, H-12), 5.02 (br.d, 1H, *J*=6.1, H-14), 4.94 (s, 1H, H-17), 4.73 (s, 1H, H-17), 4.56 (d, 1H, *J*=9.7, H-19), 4.45 (dd, 1H, *J*=10.2, 6.1, H-15), 4.15 (dd, 1H, *J*=10.2, 2.1, H-15), 4.13 (d, 1H, *J*=7.8, H-1′), 3.50 (d, 1H, *J*=9.4, H-5′), 3.37 (overlapped, 1H, H-4′), 3.33 (overlapped, 1H, H-3′), 3.30 (overlapped, 1H, H-19), 3.12 (m, 1H, H-2′), 3.04 (m, 1H, H-1β), 2.65 (m, 1H, H-11β), 2.46 (m, 1H, H-7β), 2.26 (m, 1H, H-1α), 2.14 (m, 1H, H-2α), 2.06 (m, 1H, H-7α), 2.00 (m, 1H, H-9), 1.83 (m, 1H, H-6β), 1.66 (m, 1H, H-5), 1.64 (m, 1H, H-11), 1.60 (m, 1H, H-2β), 1.57 (m, 1H, H-6α), 1.19 (s, 3H, H-18), 1.08 (s, 3H, H-20); ^{13}C-NMR (100 MHz, CD_3OD) δ 217.4 (s, C-3), δ 176.8 (s, C-6′), 172.6 (s, C-16), 148.9 (d, C-12), 148.2 (s, C-8), 129.9 (s, C-13), 110.0 (t, C-17), 104.8 (d, C-1′), 77.7 (d, C-3′), 76.1 (d, C-5′), 76.0 (t, C-15), 74.8 (d, C-2′), 73.6 (d, C-4′), 73.5 (t, C-19), 66.6 (d, C-14), 58.4 (d, C-5), 56.8 (d, C-9), 54.5 (s, C-4), 40.1 (s, C-10), 39.8 (t, C-2), 38.6 (t, C-7), 36.9 (t, C-1), 25.9 (t, C-11), 25.9 (t, C-6), 20.8 (q, C-18), 15.2 (C-20).

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