J Nat Med (2009) 63:58–60
DOI 10.1007/s11418-008-0275-7

NOTE

A guanidine derivative from seeds of *Plantago asiatica*

Yukihiro Goda · Nobuo Kawahara · Fumiyuki Kiuchi · Kazuhiro Hirakura · Yuichi Kikuchi · Hiroaki Nishimura · Masaki Takao · Masahiko Marumoto · Hironori Kitazaki

Received: 8 April 2008 / Accepted: 5 June 2008 / Published online: 31 July 2008

Abstracts A new guanidine derivative named plantagoguanidinic acid was isolated from the seeds of *Plantago asiatica*. The structure was elucidated by two-dimensional (2D) nuclear magnetic resonance (NMR) spectral and other spectral methods.

Keywords *Plantago asiatica* · Plantaginaceae · Guanidine derivative · NMR · Plantagoguanidinic acid

Introduction

The seeds of *Plantago asiatica* are used as a crude drug for diuretic, antitussive, expectorant, and antiphlogistic purposes. Fatty acids [1, 2], polysaccharides [3, 4], aucubin [5], geniposidic acid [6], and acteoside [6] were reported as the components of the seeds. In our study of the seeds, we detected a specific spot that was not identified as the above mentioned compounds by thin-layer chromatography (TLC) experiments. Therefore, we isolated the component corresponding to the spot from the seeds. This paper deals with the structural elucidation of a new guanidine derivative named plantagoguanidinic acid (**1**).

Y. Goda · N. Kawahara
National Institute of Health Sciences (NIHS),
1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

F. Kiuchi
Research Center for Medicinal Plant Resources National Institute of Biomedical Innovation, 1-2 Hachimandai, Tsukuba, Ibaraki 305-0843, Japan

K. Hirakura (✉) · Y. Kikuchi · H. Nishimura · M. Takao · M. Marumoto · H. Kitazaki
Tsumura & Co, 3586 Yoshiwara, Ami-Machi, Inashiki-gun, Ibaraki 300-1192, Japan
e-mail: hirakura_kazuhiro@mail.tsumura.co.jp

Results and discussion

An aqueous extract of the seeds of *Plantago asiatica* was extracted with *n*-butanol (*n*-BuOH). The *n*-BuOH extract was washed with hexane to remove fat, then chromatgraphed on silica gel, Sephadex LH-20, and NH-silica gel, successively to give **1** (Fig. 1).

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{11}H_{19}N_3O_2$ by high-resolution electrospray-ionization mass spectrometry (HRESI-MS). The infrared (IR) spectrum of **1** indicated the presence of a hydroxyl group (3,192 cm^{-1}) and a carboxylic group (1,691 cm^{-1}).

Compound **1** was positive to Dragendorff's reagent [7], sodium nitroprusside reagent [8], and pentacyanoaquoferriate reagent [9], but negative to Ninhydrin and Sakaguchi reagent [9]. The ^{1}H NMR and ^{13}C NMR spectra of **1** showed the presence of a prenyl group (C-4 to C-8), a guanidine carbon (C-2′) [10], a carboxylic carbon (C-1), two methine carbons (C-2 and C-4′), and two methylene carbons (C-3 and C-5′). The connections of these carbons were clarified by double quantum filter (DQF), ^{13}C-^{1}H, and heteronuclear multiple-bond connectivity (HMBC) spectra (Fig. 2). The DQF correlation spectroscopy (COSY) spectrum of **1** indicated ^{1}H-^{1}H connections from H-5 to H-5′ to establish the carbon skeleton. The HMBC correlations of H-2, H-3, and H-4′ to C-1 showed that a carboxylic group was attached to C-2. H-4′ and H-5′ showed HMBC correlations to C-2′ guanidine carbon, indicating that the guanidine group together with the C-4′ and C-5′ form an imidazoline ring. In the nuclear Overhauser effect

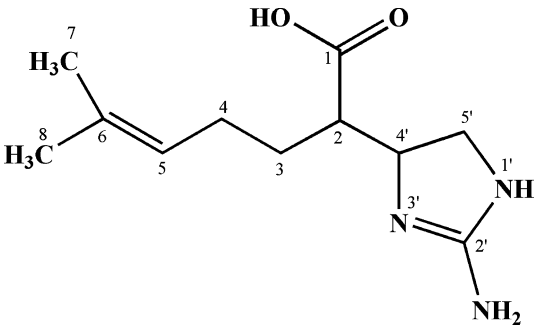

Fig. 1 Structure of compound **1**

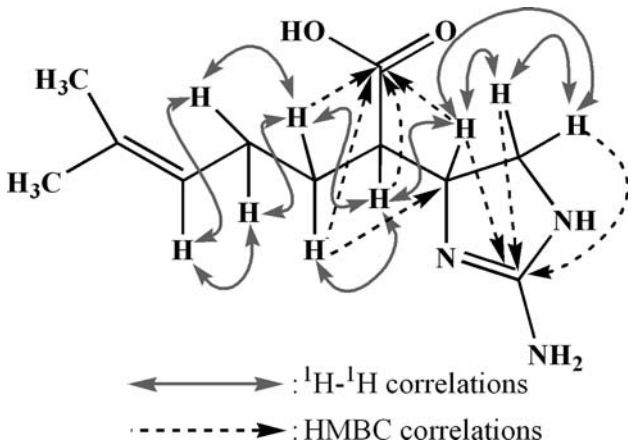

Fig. 2 Significant 2D NMR correlations of **1**

spectroscopy (NOESY) spectrum of **1**, H-4′ showed correlations to both of H-5′ protons, so the relative configuration of H-4′ and H-5′ could not be determined, and X-ray crystallographic analysis could not performed because compound **1** gave an amorphous powder. Based on these results, the planar structure of compound **1** was identified as 2-(2-amino-4,5-dihydro-1*H*-imidazol-4-yl)-6-methylhept-5-enoic acid (Fig. 1). Although similar compounds that have an imidazoline skeleton in the molecule have been isolated from some plants [11, 12], compound **1** has not been reported. Considering the plant source and the guanidinic group, we proposed the name of compound **1** as plantagoguanidinic acid (**1**).

Experiment

General

IR spectrum was measured using a JASCO FT/IR-4200 Fourier-transform infrared spectrometer. Specific rotation was recorded on a JASCO DIP-1000 digital polarimeter. NMR spectra were recorded on a JEOL JNM AL-400 FT NMR spectrometer. ESI mass spectrum was obtained using on a MICROMASS Q-Tof micromass spectrometer. For the NMR data, chemical shifts are expressed in δ ppm from tetramethylsilane (TMS) as an internal standard and coupling constants (J) are given in Hz. Silica gel 60 (70–230 mesh, Merck), Chromatorex NH DM1020 (NH-silica gel, 100–200 mesh, FUJI SILYSIA CHEMICAL Ltd.) and Sephadex LH-20 (Pharmacia) were used for column chromatography. Silica gel 60 F_{254} (0.25 mm, Merck) was used for TLC.

Extraction and isolation

Crushed seeds (1.7 kg) of *Plantago asiatica* (Jiangsu province, China) were extracted twice by stirring with water (5 L) at room temperature for 2 days. The aqueous solution was extracted three times with *n*-BuOH (3 L), and then concentrated under reduced pressure to give the *n*-BuOH extract (121.7 g). The *n*-BuOH extract was dissolved in 90% MeOH, and then partitioned with hexane (1 L) three times to give 90% MeOH extract (58.7 g). The 90% MeOH extract was chromatographed on silica gel (8.5 cm i.d. × 46 cm) eluted with $CHCl_3$–MeOH–H_2O (7:4:0.5) to give fraction A (2.6 g), which contained compound **1**. Fraction A was rechromatographed on Sephadex LH-20 (7.0 cm i.d. × 70 cm) eluted with MeOH, and Chromatorex NH 20 (7.5 cm i.d. ×62 cm) eluted with EtOAc–MeOH–H_2O (14:5:2), successively to give compound **1** (1.2 g).

Plantagoguanidinic acid (**1**)

White amorphous powder. $[\alpha]_D^{20}$+53.0° (c 1.1, MeOH), IR (KBr) cm^{-1}: 3,192(br), 2,968, 1,691, 1,585, 1,397. HR ESI-MS *m/z*: 226.1553 (Calcd. for $C_{11}H_{20}N_3O_2$:226.1556). ^{1}H NMR (CD_3OD) δ: 1.51 (1H, m, H-3), 1.60 (1H, m, H-3), 1.61(3H, brs, H-7), 1.67(3H, brs, H-8), 2.01 (1H, m, H-4), 2.11 (1H, m, H-4), 2.33 (1H, ddd, J = 4, 8, 10, H-2), 3.53 (1H, dd, J = 6, 9, H-5′), 3.74 (1H, t, J = 9, H-5′), 4.09 (1H, ddd, J = 6, 8, 9, H-4′), 5.11 (1H, brt, J = 7, H-5). ^{13}C NMR (CD_3OD) δ: 17.9 (C-7), 25.9 (C-8), 27.1 (C-4), 30.6 (C-3), 48.4 (C-5′), 59.1 (C-4′), 59.4 (C-2), 125.3 (C-5), 132.8 (C-6), 161.2 (C-2′), 180.4 (C-1).

Acknowledgements We are grateful to Mr. K. Kano for mass spectrum measurement. This work was supported in part by a Health and Labour Sciences Research Grant.

References

1. Kashimoto T (1955) Studies of Vegetable Oils and Fats. III. Nippon Kagaku Zasshi 76:664–667
2. Ogata A, Nishioji R (1924) Uber die Samen von Japanischen Wegerich. Yakugaku Zasshi 44:1040–1049

3. Tomoda M, Uno M (1971) Plant mucilages. I. Isolation and property of a mucous polysaccharide, "plantasan", from *Plantago major* L. var. *asiatica* seeds. Chem Pharm Bull 19:1214–1217
4. Tomoda M, Yokoi M, Ishikawa K (1981) Plant mucilages. XXIX. Isolation and characterization of a mucous polysaccharide, "Plantago-mucilage A", from the seeds of *Plantago major* var. *asiatica*. Chem Pharm Bull 21:2877–2884
5. Syukuya S (1922) Yakugaku Zasshi 42:1163
6. Taguchi H, Aburada M (1983) Chemistry and pharmacological actions on Chuling, Plantago seed and Gentiana. Gendai Toyo Igaku 4:47–55
7. Wagner H, Bladt S, Zgainski EM (1984) Plant drug analysis. Springer-Verlag, Berlin
8. Hofmann ED, Wünsch A (1958) Verwendung von Nitro-prussidreagenz in der Papierchromatographie. Naturwiss 45:338
9. Smith I (1960) Chromatographic and electrophoretic techniques. Heinemann-Interscience, London
10. Charles JP, Jacqlynn B (1993) The Aldrich library of ^{13}C and ^{1}H FT NMR spectra, vol. 1, 1st edn. Aldrich Chemical Company, Milwaukee, USA, pp 1280–1281, 1329
11. Fellows LE, Hider RC, Bell EA (1977) 3-[2-Amino-2-imidazolin-4(5)-yl]alanine (Enduracididine) and 2-[2-amino-2-imidazolin-4(5)-yl]acetic acid in seeds of *Lonchocarpus sericeus*. Phytochemistry 16:1957–1959
12. Evans SV, Fellows LE, Bell EA (1985) Distribution and systematic significance of basic non-protein amino acids and amines in the Tephrosieae. Biochem Syst Ecol 13:271–302