α -Glucosidase Inhibitors from *Millettia conraui*

Alembert TIABOU TCHINDA,^{*,a} Shamsun NAHAR KHAN,^b Victorine FUENDJIEP,^a François NGANDEU,^c Annie NGONO NGANE,^d and Muhammad Iqbal CHOUDHARY^b

^aCenter of Medicinal Plants Studies and Traditional Medicine (CRPMT), Institute of Medical Research and Medicinal Plants Studies (IMPM); P. O. Box 6163, Yaoundé, Cameroon; ^bH.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi; Karachi 75270, Pakistan; ^cDepartment of Chemistry, University of Dschang; P. O. Box 67, Dschang, Cameroon; and ^dDepartment of Biochemistry, University of Douala; P. O. Box 24157, Douala, Cameroon. Received April 25, 2007; accepted June 9, 2007

A new geranylated isoflavone, 7-*O*-geranyl-6-methoxypseudobaptigenin (1) was isolated from the stem barks of *Millettia conraui*, along with known compounds 5-methoxydurmillone (2), conrauinone A (3), β -amyrine (4), sitosterol (5), 3-*O*- β -D-glucopyranosyl sitosterol (6) and *n*-docosanol (7). Compounds 1 and 4 showed a significant α -glucosidase inhibitory activity. The structures of the compounds were determined by analysis of their spectroscopic data.

Key words *Millettia conraui*; isoflavonoid; 7-*O*-geranyl-6-methoxypseudobaptigenin; α -glucosidase inhibition

α -Glucosidase inhibitors are used as anti-obesity drugs, fungistatic compounds, inhibitors of tumour metastasis, insect antifeedants, antiviral and immune modulators¹⁾ and also for the management of the type-2 diabetes.²⁾ As part of our continuing efforts in the discovery of effective α -glucosidase inhibitors from Cameroonian plants,³⁾ we have examined the stem barks of *Millettia conraui* HARMS (Leguminosae), a tree that grows in the undergrowth of the Cameroon rainforest.⁴⁾ It is used in the treatment of intestinal parasites and colic in children.⁵⁾ This paper deals with the isolation from the methanol extract of the stem barks of a new geranylated isoflavone, 7-*O*-geranyl-6-methoxypseudobaptigenin (1) along with the known secondary metabolites 5-methoxydurmillone (2), conrauinone A (3), β -amyrine⁵⁾ (4), sitosterol (5), 3-*O*- β -D-glucopyranosyl sitosterol⁶⁾ (6) and *n*-docosa-

no⁷⁾ (7). This paper also reports the α -glucosidase inhibitory activities of compounds 1 and 4.

Isolation of Chemical Constituents Compound 1 was assigned the molecular formula C₂₇H₂₈O₇ on the basis of the EI-MS spectrum which showed an M⁺ at *m/z* 448 and further confirmed by ¹³C- and DEPT NMR spectra. Similarities were observed between its NMR data with those of 7-*O*-geranyl pseudobaptigenin⁸⁾ and conrauinone B.⁵⁾ This enabled its identification as an isoflavonoid possessing a geranyl, a methylenedioxy and methoxyl substituents. The presence of a geranyl moiety in the molecule was deduced from the presence of a mass fragment at *m/z* 312 corresponding to [M+1-geranyl]⁺. The methoxy protons signal (δ_{H} 3.95, s) showed a long-range correlation with C-6 (δ_{C} 148.0) in the HMBC spectrum (Fig. 2). The C-6 also correlated with the signal at δ 7.58 in the NOESY spectrum (Fig. 2). The OMe was thus deduced to be present at C-6, as in conrauinone B. The ¹H-NMR spectrum of 1 displayed two singlets at δ 7.58 and 6.83 assigned to H-5 and H-8, respectively. An HMBC cross-peak observed between the oxymethylenic protons at δ 4.70 (d, *J*=6.5 Hz) and C-7 showed that the geranyloxy moiety was located at C-7. A set of signals characteristic of an ABX system was observed in the ¹H-NMR spectrum with two doublets at δ 7.08 (*J*=1.6 Hz, H-2') and 6.84 (*J*=8.0 Hz, H-5') and a doublet of doublets at δ 6.95 (*J*=8.0, 1.6 Hz, H-6'). Together with the methylenedioxy group, these protons were assigned to ring B of the isoflavone. On the basis of the above spectroscopic evidences, the structure of compound 1 was deduced as 7-*O*-geranyl-6-methoxypseudobaptigenin, which is a new geranylated isoflavonoid.

Biological Activity Compounds 1 and 4 showed potent *in vitro* α -glucosidase inhibitory activities with IC₅₀=35.5 $\pm$ 0.5 and 10.3 $\pm$ 0.5 μ M, respectively, compared to the standards

Fig. 1. Structures of Compounds 1–7

Fig. 2. Key HMBC and NOESY Correlations of Compound 1

* To whom correspondence should be addressed. e-mail: alembertt2002@yahoo.fr

deoxynojirimycin ($IC_{50}=425\pm 8.14\ \mu M$), a known potent α -glucosidase inhibitor and acarbose ($IC_{50}=780\pm 28\ \mu M$), a clinically used drug for type-2 diabetes. Compounds **2** and **3** precipitated and its exact IC_{50} values could not be measured. The significant potency of compound **1** is probably due to the presence of a geranyl moiety and it may interact with the enzyme binding sites through hydrophobic interactions.

Experimental

General Melting points were determined on a Büchi 535 melting point apparatus. IR spectra were recorded on a Jasco 302-A spectrophotometer. UV spectra were obtained on a Hitachi UV 3200 spectrophotometer. EI-MS (70 eV) was measured on a Varian MAT 312 A spectrometer. 1D and 2D spectra were recorded on Bruker AMX 300 and 400 MHz NMR spectrometers. The chemical shifts are given in ppm (δ), relative to TMS as internal standard, and coupling constants are in Hz. Column chromatography (CC) was carried out on silica gel (70–230 mesh, Merck). TLC and preparative TLC were performed on Merck precoated silica gel 60 F254 aluminium foil and Merck silica (Si) gel 60 plates (e 0.5 mm) respectively and spots were detected using ceric sulphate as spray reagent. A Molecular Device spectrophotometer was used for measurement of enzyme inhibition.

Plant Material The stem barks of *M. conraui* HARMS were collected at Kumbo (North-west province Cameroon) in September 2004. A voucher specimen was deposited at the National Herbarium of Yaounde, Cameroon.

Extraction and Isolation The air-dried and powdered stem barks of *M. conraui* (2 kg) were extracted with MeOH (8 l $\times$ 2) at room temperature for 48 h and 120 g of a viscous brown extract was obtained after filtration and evaporation of solvent under reduced pressure. The extract was further treated with a mixture of $CHCl_3$ –MeOH– H_2O (2:2:1) (31). The concen-

trated organic extract (70 g) was subjected to Si gel CC eluted with gradients of petrol–EtOAc, EtOAc and EtOAc–MeOH. Fractions of 250 ml were collected and regrouped on the basis of their TLC profile. The 10% fraction was further purified by repeated CC using increasing amounts of EtOAc in petrol to obtain *n*-docosanol (12 mg), sitosterol (48 mg) and β -amyrin (68 mg). From the 20% EtOAc fraction, a crystalline mixture containing two compounds was obtained and separated over a Si gel CC using petrol–EtOAc (10%) as eluent to give 5-methoxydurmillone (5 g). The mother liquid was further purified by prep. TLC [eluent: petrol–EtOAc (9:1)] and conrauinone A (8 mg) was obtained. The 30% EtOAc fraction was purified through a Si gel CC to afford 7-*O*-geranyl-6-methoxybaptigenin (50 mg). 3-*O*- β -D-glucopyranosyl sitosterol (78 mg) was obtained from the 5% EtOAc–MeOH fraction.

7-*O*-Geranyl-6-methoxypseudobaptigenin (**1**): White crystals. R_f 0.2 (petrol–EtOAc 7:3). mp 150 °C. $[\alpha]_D^{26} -41.8^\circ$ ($c=0.1$, $CHCl_3$). IR (KBr) γ_{max} cm^{-1} : 3433, 2916, 1631, 1496, 1422, 1384, 1330, 1257, 1176, 1107, 1026, 987, 926, 825. UV λ_{max} (MeOH) nm (log ϵ): 318 (2.2), 293 (2.6), 263 (2.7). EI-MS m/z (rel. int.): 448 (2) (M)⁺, 394 (2), 312 (57), 298 (6), 282 (13), 252 (2), 166 (3), 146 (8), 95 (6). ¹H- and ¹³C-NMR ($CDCl_3$, 300 and 75 MHz): see Table 1.

α -Glucosidase Inhibition Assay The assay was performed according to the slightly modified method of Matsu *et al.*⁹⁾ α -Glucosidase (E.C. 3.2.1.20) from *Saccharomyces* sp. was purchased from Wako Pure Chemical Industries Ltd. (Wako 076-0284). The enzyme inhibition studies were carried out spectrophotometrically in 96 well plates. The optimum pH 6.9 was maintained and the assay was performed at 37 °C. 0.7 mM *p*-nitrophenyl α -D-glucopyranoside (PNP-G) (Sigma) as a synthetic substrate and 250 units/ml of enzyme, in 50 mM sodium phosphate buffer containing 100 mM NaCl were used. 1-Deoxynojirimycin (0.425 mM) and acarbose (0.78 mM) (Sigma) were used as positive controls. The increment in absorption at 400 nm due to the hydrolysis of PNP-G by α -glucosidase was monitored continuously with a spectrophotometer (SpectroMax Molecular Devices, U.S.A.). The results are expressed with the Standard Error of the Mean (S.E.M.) equals to standard deviation/ $\sqrt{n}$, where n represents the number of replicates for IC_{50} value. Three replicates were used for all compounds.

Acknowledgments We are grateful to TWAS (Academy of Science for Developing World) Trieste, Italy and UNESCO, for a travel grant to T.T.A. through the 'TWAS-UNESCO Associateship Scheme at the Centres of Excellence in the South' fellowship program to H.E.J. Research Institute of Chemistry, University of Karachi, Pakistan, and to TWOWS (Third World Organisation for Women in Science) for the Ph.D. fellowship to S.N.K.

References

- 1) El Ashry E. S. H., Rashed N., Shobier A. H. S., *Pharmazie*, **55**, 251–262 (2000).
- 2) Sou S., Mayumi S., Takahashi H., Yamasaki R., Kadoya S., Sodeoka M., Hashimoto Y., *Bioorg. Med. Chem. Lett.*, **10**, 1081–1084 (2000).
- 3) Kouam S. F., Khan S. N., Krohn K., Ngadjui B. T., Kapche D. G. W. F., Yapna D. B., Zareem S., Choudhary M. I., *J. Nat. Prod.*, **69**, 229–233 (2006).
- 4) Mbenkum T. F., Systematic studies in genus *Milletia*. Ph. D. Thesis, University of Reading, U.K., 1986, pp. 45–50.
- 5) Fuendjiepe V., Nkenfack E., Fomum Z. T., Sondengam B. L., *J. Nat. Prod.*, **61**, 380–383 (1998).
- 6) Sadikun A., Aminah I., Ismail N., Ibrahim P., *Nat. Prod. Sci.*, **21**, 19–23 (1996).
- 7) Fuendjiepe V., "Contribution à l'Etude Chimique d'une Plante Médicinale Camerounaise: *Milletia conraui*, Légumineuse," 'Doctorat d'Etat' Thesis, University of Yaounde I, Cameroon, 1994, pp. 133–134.
- 8) Yankep E., Fomum Z. T., Dagne E., *Phytochemistry*, **46**, 591–593 (1997).
- 9) Matsu T., Yoshimoto C., Osajima K., Oki T., Osajima Y., *Biostat. Biotech. Biochem.*, **60**, 2019–2022 (1996).

Table 1. NMR Data of Compound **1**

Carbon No.	δ_C	δ_H (mult.), J (Hz)	HMBC correlations
2	151.9	7.88 s	C-3, C-4, C-8a
3	124.4		
4	175.3		
4a	117.7		
5	104.9	7.58 s	C-8a
6	148.0		
7	153.6		
8	100.6	6.83 s	C-4, C-4a, C-7
8a	152.1		
1'	125.9		
2'	109.8	7.08 d (1.6)	C-4', C-5'
3'	147.6		
4'	147.5		
5'	108.3	6.84 d (8.0)	C-1', C-3'
6'	122.3	6.95 dd (8.0, 1.6)	C-2', C-4', C-5'
1''	66.4	4.70 d (6.5)	C-7, C-2'', C-3''
2''	118.4	5.50 t (6.5)	C-1'', C-3'', C-4''
3''	142.1		
4''	39.5	2.08 ov	C-2'', C-3'', C-5'', C-6'', C-10''
5''	26.2	2.09 ov	C-3'', C-4'', C-6'', C-7''
6''	123.6	5.05 ov	C-4'', C-5'', C-8''
7''	131.9		
8''	16.9	1.63 s	C-6'', C-7'', C-9''
9''	17.7	1.57 s	
10''	25.6	1.63 s	C-2'', C-3'', C-4''
6-OMe	56.3	3.95 s	C-6
OCH ₂ O	101.1	5.96 s	C-3'