SHORT REPORT

A C G publications

Rec. Nat. Prod. 3:3 (2009) 165-169

records of natural products

Chemical Constituents of *Klainedoxa gabonenses and Paullinia pinnata*

Etienne Dongo[*1], Hidayat Hussain[*2], Renadin S. Miemanang [1], Dagobert Tazoo[1], Barbara Schulz[3], Karsten Krohn*[2]

[1]*Department of Organic Chemistry, Faculty of Science, Yaounde I University, P. O Box 812, Yaounde, Cameroon.*

[2]*Department of Chemistry, Universität Paderborn, Warburger Straße 100, 33098 Paderborn, Germany*

[3]*Institut für Mikrobiologie, Technische Universität Braunschweig, Spielmannstraße 7, 31806 Braunschweig, Germany*

(Received March 4, 2009; Revised April 2, 2009; Accepted April 5, 2009)

Abstract: From the whole plant of *Klainedoxa gabonenses* betulinic acid (**1**), lupeol (**2**), *β*-sitosterol (**3**), *β*-amyran-3-one (**4**) and 3,3',4'-tri-*O*-methylellagic acid (**5**) were isolated. Similarly paullinomide A (**6**), *β*-amyrin (**7**), 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-trioxaphenanthren-6-one (**8**), 5α-poriferastane-3ß,6α-diol (**9**), *β*-sitosterol (**3**), *l*-quebrachitol (**10**), and *β*-sitosterol glucopyranoside (**11**) were isolated from roots of *Paullinia pinnata.* Preliminary studies showed that 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-trioxaphenanthren-6-one (**8**) showed moderate algicidal activity against the alga *Chlorella fusca*

Keywords: *Paullinia pinnata*; *Klainedoxa gabonenses*; algicidal activity.

1. Plant Source

The African continent is endowed with one of the richest biodiversity in the world, with an avalanche of many food plants used as herbs, health foods and for therapeutic purposes. Over 5,000 different species of plant substances have been recognized to occur in these areas, and many of them have been found to be useful in traditional medicine for prophylaxis and cure of diseases [1]. As part of our systematic search for new bioactive lead structures from African medicinal plants, *Klainedoxa gabonenses* and *Paullinia pinnata* L. were selected for chemical and biological investigations.

* Corresponding authors: E- Mail:Hidayat110@gmail.com (H.Hussain), Phone +49-5251-602185 and E- Mail: k.krohn@upb.de (K. Krohn)

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 06/07/2009 EISSN: 1307-6167

The plant *K. gabonenses* (Irvingiaceae) was collected (November 2005) from Eloundem, while roots of *P. pinnata* L. (Sapindaceae) were collected (April 2004) at Obala, Central province of the Republic of Cameroon. The two African plants were identified by Dr. Louis Zapfack and voucher specimens of *P. pinnata* L. (N° 44641) and *K. gabonenses* (N° 35206/HNC) have been deposited at the National Herbarium, Yaounde, Cameroon.

2. Previous Studies

Early studies regarding the chemical constituents of *K. gabonenses* revealed the presence of one tannin, methyl gallate [2]. Later, two flavonol glycosides [3], one cerebroside and a ceramide from *P. pinnata* L. were isolated by our group [4].

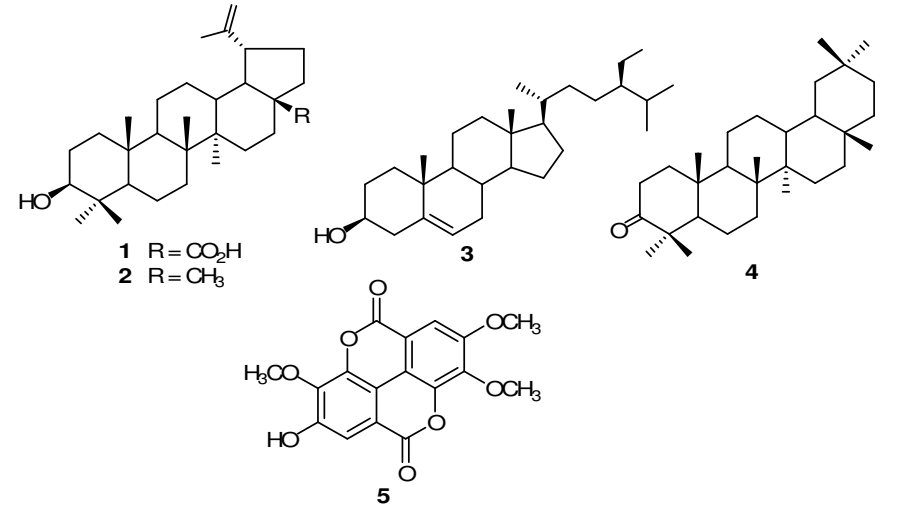
3. Present Study

The air-dried stem barks (8 kg) of *K. gabonenses* (Irvingiaceae) were exhaustively extracted with methanol at room temperature. The extract was evaporated to dryness yielding 510 g of residue. The whole extract was extracted with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. The *n*-hexane and EtOAc extract were combined (158 g) and the combined extract was subjected to column chromatography (silica gel, *n*-hexane, *n*-hexane-EtOAc and EtOAc, in order of increasing polarity) yielding 8 fractions. Fraction 1 was further separated by silica gel column chromatography and eluted with *n*-hexane-EtOAc (9:1) to give betulinic acid (**1**, 13.2 mg). Similarly, lupeol (**2**, 11.1 mg) was isolated from fraction no. 6, after elution with a mixture of *n*-hexane–EtOAc (8.5:1.5) and *β*-sitosterol (**3**, 10 mg) was isolated from fraction 7 with *n*-hexane–EtOAc (8:2). Repeated column chromatography of fraction 3 using *n*-hexane–acetone (9.5:0.5) as the eluent afforded *β*-amyran-3-one (**4**, 6.5 mg). Repeated CC of fraction 5, eluted with a mixture of petroleum ether-EtOAc (7.5:2.5), gave 3,3',4'-tri-*O*-methylellagic acid (**5**, 5.3 mg).

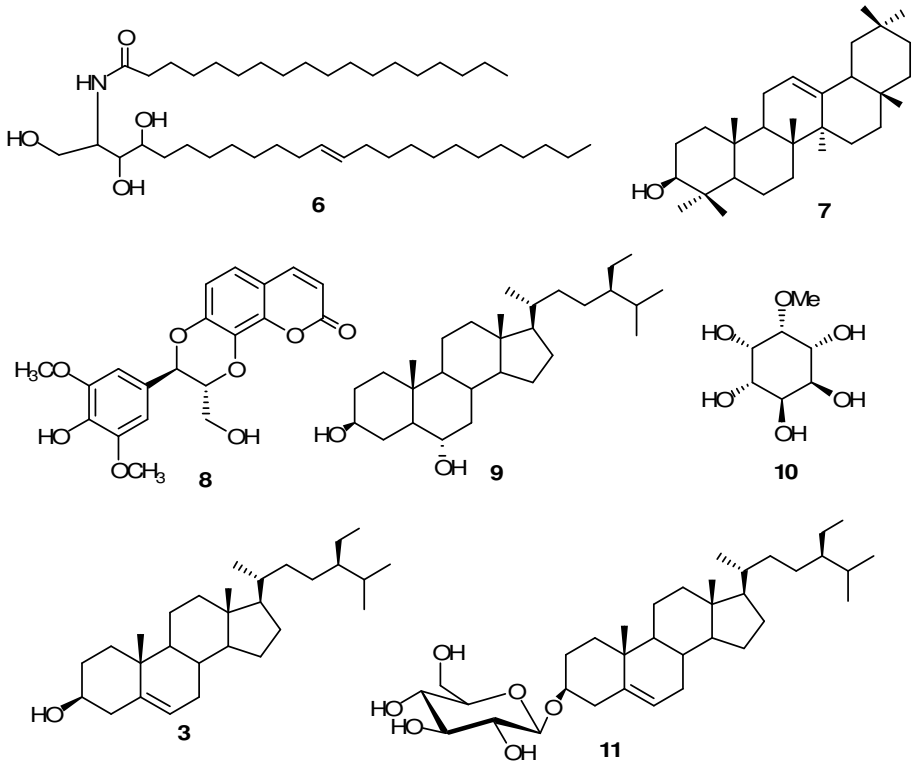
The air-dried leaves (7 kg) of *P. pinnata* L. (Sapindaceae) were exhaustively extracted with methanol at room temperature. The extract was evaporated to dryness yielding 520 g of residue. The whole extract was extracted with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. The EtOAc extract (80 g) was then subjected to column chromatography (silica gel, *n*-hexane, *n*-hexane-EtOAc and EtOAc, in order of increasing polarity) yielding 21 fractions. Fraction no. 15 was subjected to column chromatography and eluted with a mixture of *n*-hexane-EtOAc (4:6) to yield paullinomide A (**6**, 6.1 mg) (Miemanang et al., 2006), while fraction no. 3 gave *β*-amyrin (**7**, 8.0 mg) upon elution with *n*-hexane–EtOAc (9:1). Similarly, fraction 4 gave *β*-sitosterol (**3**, 11.1 mg) after elution with *n*-hexane–EtOAc (9:1). Fraction no. 13 was subjected to column chromatography and eluted with a mixture of *n*-hexane-EtOAc (6:4) to afford 5α-poriferastane-3ß,6α-diol (**9**, 6.5 mg) and impure *l*-quebrachitol (**10**). *l*-Quebrachitol (**10**, 13.5 mg) was purified by CC after elution with EtOAc-MeOH (9:1). Column fraction no. 14 was eluted with *n*-hexane–EtOAc (5:5) to afford 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-trioxaphenanthren-6-one (**8**, 6.5 mg) and fraction 17 gave *β*-sitosterol glucopyranoside (**11**, 10 mg) with *n*-hexane–EtOAc (2.5:7.5).

The whole plant extract of the stem of *K. gabonenses* was fractionated by silica gel column chromatography to give several fractions, which were further chromatographed on silica gel to give three triterpenes (**1**, **2**, and **4**), one steroid (**3**), and compound **5** (Figure 1). These five compounds were identified as betulinic acid (**1**) [5], lupeol (**2**) [6], *β*-sitosterol (**3**) [7], *β*-amyran-3-one (**4**) [8], 3,3',4'-tri-*O*-methylellagic acid (**5**) [9] by comparison of 1D and 2D NMR data with reported data. Similarly, paullinomide A (**6**) [4], *β*-amyrin (**7**) [10], 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-trioxaphenanthren-6-one (**8**) [11], 5α-poriferastane-3ß,6α-diol (**9**) [12], *β*-sitosterol (**3**) [7], *l*-quebrachitol (**10**) [13], and *β*-sitosterol glucopyranoside (**11**) [14] were isolated from roots of *P. pinnata* and their structures were determined by comparison of 1D and 2D NMR data with reported values. Preliminary studies showed that 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-2,3-

dihydro-1,4,5-trioxaphenanthren-6-one (**8**) showed moderate algicidal activity against the alga *Chlorella fusca.*



Compounds (**1-5**) isolated from *Klainedoxa gabonenses*



Compounds (**3**, **6-11**) isolated from *Paullinia pinnata*

Figure 1. Compounds **1–11** isolated from *K. gabonenses* and *P. pinnata*.

*Antibacterial, Antialgal, and Antifungal activities***.** Compounds **8-10** were tested for antibacterial, antialgal and antifungal activities. Only compound **8** showed activity against the alga *Chlorella fusca,* while compounds **9** and **10** were inactive in these tests.

Chemotaxonomic significance: The small family of Irvingiaceae*,* also frequently subordinated within the Simaroubaceae*,* consists of the genera *Desbordesia, Irvingia,* and *Klainedoxa.* Whereas *Desbordesia* and *Klainedoxa* are native to tropical Africa, *Irvingia* occurs in addition in Cochinchina and Malaysia [15]. Recent treatments of the genus, variously treated as belonging to Irvingiaceae or to Simaroubaceae, have recognized two species, *K. gabonenses* Pierre ex Engl. and *K. busgenii* [16]. Early chemical studies of genus *Klainedoxa* revealed the presence of one tannin from *K. gabonenses* [2]. Continuing chemotaxonomic studies on the genus *Klainedoxa*, we report here on the results obtained for *K. gaoneneses*. Interestingly, compounds **1** and **5** were characterized for the first time from the genus *Klainedoxa* and have been isolated from genus *Irvingia* of same family [9]. This finding is evidence that the genera *Klainedoxa* and *Irvingia* are closely related taxonomically. On the other hand, compound **2**–**4** were characterized for the first time from the Irvingiaceae family, and thus isolation of compounds **2**–**4** in the present investigation is a major contribution to chemotaxonomic studies of the Irvingiaceae family.

The ceramide, paullinomide A (**6**) and *β*-amyrin (**7**), and two steroids (**3** and **11**) have been isolated previously from *P. pinnata* L. by our group [4]. The other fact which should be commented on is the isolation of a ceramide from *P. pinnata* L. This is the first time that a ceramide has been isolated from the genus *Paullinia*. On the other hand, coumarinolignoid (**8**) has been reported from genus *Daphne* of the Thymelaeaceae family [11, 17], and 5α-poriferastane-3ß,6α-diol (**9**) has been reported from the marine red alga *Garcilaria edulis* [12]. Similarly, *l*-quebrachitol (**10**) has been reported from the Asteraceae [18] and Elaeagnaceae families [19]. Thus, compounds **8**–**10** were characterized for the first time from the genus *Paullinia* as well as from the Sapindaceae family. Therefore, isolation of compounds **8**–**10** might be a useful contribution to chemotaxonomic studies of the genus *Paullinia* as well as of Sapindaceae family.

References

[1] M. M. Iwu (1993). Hand book of African medicinal plants. Pp 1 CRC press, Inc Florida.
[2] A. Ekouya, G. B. Itoua, A. Ouabonzi and J. M. Ouamba (2005). Tannin isolated from an antibacterial extract of *Klainedoxa gabonensis*. *J. Soc. Ouest-Afr. Chim*. **10**, 1–9.
[3] E. A. Abourashed, N. J. Toyang, J. J. Choinski and I. A. Khan. (1999). Two New Flavone Glycosides from *Paullinia pinnata. J. Nat. Prod.* **62**, 1179–1181.
[4] T. M. Tsakala, O. Pen R. S. Miemanang, K. Krohn, H. Hussain and E. Dongo (2006). Paullinoside A and Paullinomide A: A New Cerebroside and a New Ceramide from leaves of *Paullinia pinnata. Z. Naturforsch.* **61b**, 1123–1127.
[5] A. Ikuta, K. Kamiya, T. Satakek and Y. Saiki (1995). Triterpenoids from callus tissue cultures of *Paeonia* species. *Phytochemistry* **38**, 1203–1207.
[6] M.G. Carvalho, C. R. X. Velloso, R. Braz-Filho and W. F. Costa (2001). Acyl-lupeol Esters from *Parahancornia amapa* (Apocynaceae). *J. Barz. Chem. Soc*. **12**, 556–559.
[7] I. Rubinstein, L. J. Goad, A. D. H. Clague (1976). The 200 MHz spectra of phytosterols. *Phytochemistry* **15**, 195–200.
[8] K. S. Krishnaveni and J. V. S. Rao (2000). A new triterpene from callus of *Pterocarpus santalinus. Fitoterapia* **71**, 10–13.
[9] V. Kuete, G. F. Wabo, B. Ngameni, A. T. Mbaveng, R. Metuno, F.-X. Etoa, B. T. Ngadjui, V. P. J. J. Benga, M. Meyer and N. Lall. (2007). Antimicrobial activity of the methanolic extract, fractions and compounds from the stem bark of *Irvingia gabonensis* (Ixonanthaceae). *J. Ethnopharmacol*. **114**, 54–60
[10] R. C. Heupel (1985). Varietal similarities and differences in the polycyclic isopentenoid composition of sorghum. *Phytochemistry* **24**, 2929–2937.

[11] F. Cottiglia, L. Bonsignore, G. Loy, D. Garau, C. Floris and M. Casu (2002). Structure elucidation and antibacterial activity of a new coumarinolignoid from *Daphne gnidium* L. *Magn. Reson. Chem.* **40**, 551–553.
[12] B. Das and K. V. N. S. Srinivas (1992). Dihydroxysterols from the marine red alga, *Gracilaria edulis*. *Phytochemistry* **31**, 4371–4373.
[13] I. K. Dowd and E. D. Stevens (2002). The crystal structure of D-pinitol and l-querachitol by low temperature X-ray diffraction. *J. Carbohydrate Chem.* **21**, 373–383.
[14] S. Seo, Y. Tomita, K. Tori and Y. Yoshimura (1978). Determination of the absolute configuration of a secondary hydroxy group in a chiral secondary alcohol using glycosidation shifts in carbon-13 nuclear magnetic resonance spectroscopy. *J. Amer. Chem. Soc*. **100**, 3331–3339.
[15] D. A. Link (1992). The floral nectaries in the*Irvingiaceae*. *Pl. Syst. Evol*. **180**, 235–242.
[16] A. H. Gentry (1984). *Klainedoxa* (Irvingiaceae) at Makokou, Gabon: Three Sympatric Species in a Putatively Monotypic Genus. *Ann. Missouri Bot. Gard.* **71**, 166–168.
[17] S-G. Liao, B-L. Zhang, Y. Wu, J-M. Yue (2005). New Phenolic Components from *Daphne giraldii*. *Helv. Chim. Acta* **88**, 2873–2878.
[18] M. Y. Bouberte, K. Krohn, H. Hussain, E. Dongo, B. Schulz and Hu, Q (2006). Tithoniaquinone A and Tithoniamide B: A New Anthraquinone and a New Ceramide from the leaves of *Tithnonia diversifolia*. *Z. Naturforch.* **61b**, 78–82.
[19] K-F. Huang and S-J. Chen (1995). Constitution of the roots of *Elaeagnus obovata*. *Chin. Pharm. J.* **47**, 235–242.