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Triterpenoids and phenolic compounds from *Croton macrostachyus*

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1. Subject and source

The genus *Croton* belongs to the family Euphorbiaceae and consists of approximately 1300 species of trees, shrubs and herbs distributed in tropical and subtropical regions of the world (Salatino et al., 2007). *Croton macrostachyus* Hochst. ex Delile is a tall tree found in tropical regions of Africa. The plant is used in traditional medicine for the treatment of cough, tapeworm, skin rash, inflammation, epilepsy, diabete, syphilis, malaria and venereal diseases (Schmelzer and Gurib-Fakim, 2008; Kamanyi et al., 2009; Bum et al., 2012).

The twigs of *C. macrostachyus* were collected at Mount Bamboutos (West Region of Cameroon) in October 2008, and identified by Mr. Nana Victor a botanist at the Cameroon National Herbarium, Yaoundé. A voucher specimen (No 5696/SRF/CAM) is deposited.

2. Previous work

Previous phytochemical studies on the genus *Croton* resulted in the isolation of secondary metabolites such as alkaloids, terpenoids, flavonoids and other phenolic compouds. Amongst them, diterpenoids are the most widespread in this genus (Salatino et al., 2007). There is little literature concerning the phytochemical study of *C. macrostachyus*. Fatty acids, β-sitosterol, stigmasterol, lupeol, betulin and one cyclohexane diepoxide named crotepoxide were isolated from its stem barks and twigs (Addae-Mensah et al., 1992), while some clerodane diterpenes namely trachyloban-19-oic acid, trachyloban-18-oic acid, neoclerodan-5,10-en-19,6β;20,12-diolide, 3α,19-dihydroxytrachyloban and 3α,18,19-trihydroxytrachyloban and the

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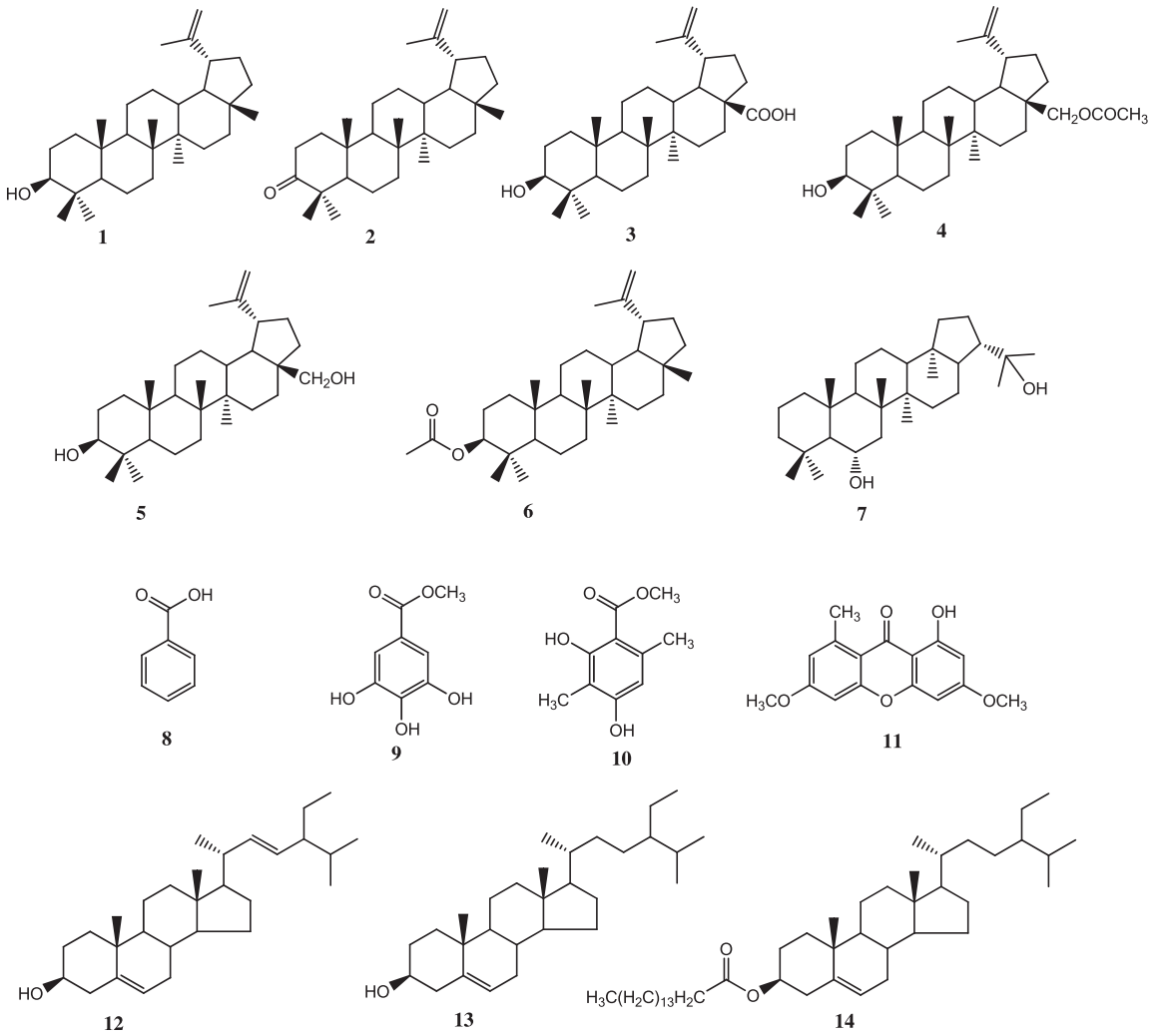

Fig. 1. Compounds (**1–14**) isolated from twigs of *Croton macrostachyus*.

triterpene 3β-acetoxytaraxer-14-en-28-oic acid were identified in its roots (Kapingu et al., 2000). In addition to lupeol, betulin and crotepoxide, further diterpenes namely crotomacrine, floridolide A, hardwickic acid and 12-oxo-hardwickic acid were obtained from the fruits and stem barks of *C. macrostachyus* (Tane et al., 2004; Tene et al., 2009).

3. Present study

To complete our phytochemical study on *C. macrostachyus* (Tane et al., 2004; Tene et al., 2009), we describe herein the isolation of seven triterpenes (**1–7**), four phenolic compounds (**8–11**) and three sterols (**12–14**) from its twigs (Fig. 1).

The fine powder (2.0 Kg) of the twigs of *C. macrostachyus* was extracted with MeOH (3 × 4 L, 72 h) at room temperature. Evaporation under reduced pressure afforded a crude extract (70.4 g). A portion (68 g) of the crude extract was subjected to a column chromatography over silica gel ($CHCl_3$–MeOH 10:0 → 10:5) to give 35 fractions. The collected fractions were combined on the basis of their TLC profiles into 4 major fractions (FrA–FrD). FrB was rechromatographed on a silica gel column eluted with a gradient solvent system of Et_2O–Me_2CO (100:0 → 100:4) to yield five subfractions (FrB-1 to FrB-5). FrB-2, FrB-5 and FrB-4 crystallized from MeOH to give compounds **1** (200 mg), **12** (3 mg) and **13** (15 mg) respectively and three residues. The residue obtained from FrB-2 was applied to a silica gel column using an isocratic system of Et_2O–Me_2CO (100:0.5) to afford compound **2** (40 mg). Similarly, the residue obtained from FrB-5 was purified on a silica gel column (Et_2O–Me_2CO 100:0.5) to give compound **11** (13 mg). The residue from FrB-4 was separated by a Sephadex LH-20 column eluted with $CHCl_3$–MeOH (3:7) to yield compound **6** (10 mg). FrC was subjected to a silica gel column (Et_2O–Me_2CO 100:0 → 100:10) to produce four subfractions (FrC-1 to FrC-4). FrC-2 was purified on a silica gel column using an isocratic system of Et_2O–Me_2CO

(100:1) to afford compounds **3** (5 mg) and **4** (13 mg). FrC-3 and FrC-4 were also purified over silica gel column (Et_2O–Me_2CO 100:2) to give compounds **6** (8 mg) and **8** (4 mg), respectively. Fraction D was fractionated by a silica gel column ($CHCl_3$–MeOH 100:0 → 100:2) to afford five subfractions (FrD-1 to FrD-5). FrD-1 was applied to a silica gel column (Et_2O–Me_2CO 100:1) to give compound **14** (15 mg). FD-4 was further separated on a silica gel column using an isocratic system of Et_2O–Me_2CO–$CHCl_3$ (100:4:1) to yield compounds **5** (8 mg) and **7** (20 mg). Compounds **9** (7 mg) and **10** (11 mg) were obtained from the purification of FrD-5 on a Sephadex LH-20 column ($CHCl_3$–MeOH 1:9).

Fourteen compounds were isolated and identified as lupeol (**1**), lupenone (**2**), betulinic acid (**3**), 28-*O*-acetylbetulin (**4**), betulin (**5**), lupeol acetate (**6**), zeorin (**7**) (Mahato and Kundu, 1994; Prachayasittikul et al., 2010), benzoic acid (**8**) (Mizyuk et al., 2008), methyl gallate (**9**) (Ekaprasada et al., 2009), methyl 2,4-dihydroxy-3,6-dimethylbenzoate (**10**) (Chen et al., 2010), lichexanthone (**11**) (Carvalho et al., 2009), stigmasterol (**12**), β-sitosterol (**13**), and β-sitosterol palmitate (**14**) (Dupon et al., 1997). This is the first report of the presence of compounds **2**–**4**, **6**–**11**, and **14** in *C. macrostachyus*.

The structures of compounds **1**–**11** and **14** were determined on the basis of their spectral data (MS, ^{1}H NMR, ^{13}C NMR and 2D NMR including HSQC, HMBC and H–HCOSY) and by comparison of the data with those reported in the literature. Compounds **12** and **13** were identified by Co-TLC with authentic samples.

4. Chemotaxonomic significance

The role of triterpenoids in the chemotaxonomy of species of *Croton* has been neglected. Knowledge about their occurrence in the genus is increasing, especially lupane and hopane triterpenoids. In the present study, the identification of compounds **1**, **5**, **12**, and **13** was in agreement with the previous report from the twigs of *C. macrostachyus* (Addae-Mensah et al., 1992), while **2**–**4**, **6**,**7**, and **14** were isolated from this species for the first time. It should be noted that, lupeol (**1**), lupenone (**2**), betulinic acid (**3**), and betulin (**5**) were previously found in other species of *Croton*, such as *Croton megalocarpus* Huch. (Addae-Mensah et al., 1989), *Croton betulaster* Müll. Arg. (Barbosa et al., 2003), *Croton hieronymi* Griseb. (Catalán et al., 2003), and *Croton gratissimus* Burch.var. *gratissimus* (syn *Croton zambesicus* Muell. Arg.) (Mohamed et al., 2009). To the best of our knowledge, 28-*O*-acetylbetulin (**4**) and lupeol acetate (**6**) have never been reported from the genus *Croton*. Zeorin (**7**) is a hopane-type triterpene also found in this genus for the first time. However, compounds belonging to this same class of metabolite have been reported from *C. betulaster* (Barbosa et al., 2003) and *C. hieronymi* (Catalán et al., 2003). Amongst the isolated sterols, β-sitosterol palmitate (**14**) is reported from this genus for the first time, while stigmasterol (**12**) and β-sitosterol (**13**) are common and occur in high amount in species of *Croton* (Salatino et al., 2007).

Some hydroxybenzoic acids and methyl benzoate derivatives have been reported from species of the genus *Croton* such as *Croton panamensis* (Klotzsch) Müll.Arg. (Kostova et al., 1999), *Croton draco* Cham. & Scldh. (Murillo et al., 2001), *C. hieronymi* (Catalán et al., 2003), and *Croton xalapensis* Kunth. (Arevalo et al., 2009). However, compounds **8**–**10** were identified in *C. macrostachyus* for the first time. Furthermore, this is the first report of benzoic acid (**8**) from this genus. Methyl gallate (**9**) and methyl 2,4-dihydroxy-3,6-dimethylbenzoate (**10**) were previously obtained from *C. xalapensis* (Arevalo et al., 2009) and *Croton hutchinsonianus* Hos. (Li et al., 1990), respectively. The occurrence of xanthones in Euphorbiaceae family is very rare, although lichexanthone (**11**) has been previously isolated from *Croton cuneatus* Klotzsch (Suárez et al., 2004).

Diterpenes are often reported to occur in Euphorbiaceae plants and have previously been reported from the roots, stem barks and fruits of *C. macrostachyus* (Kapingu et al., 2000; Tane et al., 2004; Tene et al., 2009). Associating our findings with those reported by Addae-Mensah et al. (1992), the apparent absence of diterpenoids in its twigs could be due to the lack of enzymes responsible for their biosynthesis in these plants' organs. Thus the ability to produce or accumulate such compounds in this part of the plant might be lost in the evolutionary process and substituted by other classes of metabolites (Salatino et al., 2007). Since the knowledge about the co-occurrence of lupane- and hopane-type triterpenes as well as methyl benzoate derivatives in different species of *Croton* is increasing and species vary in the trirpenes, the isolation of compounds **2**–**4** and **6**–**10** in the present investigation might be a useful contribution to the chemotaxonomic of the genus *Croton*.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2013.08.001.

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