Phytochemistry Letters 5 (2012) 174–176

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Retrodihydrochalcones in *Sorghum* species: Key intermediates in the biosynthesis of 3-deoxyanthocyanidins?

Ali Khalil, Raymonde Baltenweck-Guyot *, Rubén Ocampo-Torres, Pierre Albrecht

Institut de Chimie, UMR 7177 CNRS/Université de Strasbourg, 1 rue Blaise Pascal, 67000 Strasbourg, France

ARTICLE INFO

Article history:
Received 30 September 2011
Received in revised form 5 December 2011
Accepted 8 December 2011
Available online 20 December 2011

Keywords:
Sorghum bicolor (L.) Moench
Var. bicolor
Structural characterization
NMR
MS
Flavonoids
Retrodihydrochalcone

ABSTRACT

Two new open chain flavonoids were isolated from the butanediol/ethanol extract of *Sorghum bicolor* (L.) Moench leaf sheaths by fractionation and purification processes. This work led to the structural characterization of the 3-(2,4,6-trihydroxyphenyl)-1-(4-hydroxyphenyl)-propan-1-one (or 2,4,4′,6-tetrahydroxydihydrochalcone) **1**, and 3-(2,6-dihydrox-4-methoxyphenyl)-1-(4-hydroxyphenyl)-propan-1-one (or 2,4′,6-trihydroxy-4-methoxydihydro-chalcone) **2**. The structures of these flavonoids were determined by extensive spectroscopic analyses, including UV, ESIMS, HRESIMS, 1D and 2D NMR. The chemical properties of **1** were similar to those earlier described in literature for apiforol, never fully characterized. These results led us to re-question the real structure of this flavan-4-ol, which is often described to be present in *Sorghum* and has even been considered as a key intermediate in the formation of *Sorghum* 3-deoxyanthocyanidins.

1. Introduction

Sorghum bicolor (L.) Moench, the fifth-ranking cereal global production cultivated worldwide is rich in polyphenols belonging to the flavonoid and phenolic acid families (Awika and Rooney, 2004). Flavonoids are common plant secondary metabolites that may be implicated in plant growth and development processes, including protection from UV irradiation as well as in insect attraction for pollinization and seed dispersion. Some *Sorghum* flavonoids like 3-deoxyanthocyanidins (Snyder and Nicholson, 1990; Lo et al., 1999) and, more recently, flavones apigenin and luteolin (Du et al., 2010) have been identified as phytoalexins produced as response to fungal attack. In addition, numerous works on *Sorghum* plants mentioned the occurrence of red pigments called phlobaphenes; their precise structures are not defined, but they are proposed to arise as polymerization of different products such as flavan-4-ols called apiforol and luteoforol (Chopra et al., 2002; Schijlen et al., 2004). However, a reliable structure of these flavan-4-ols has never been clearly established.

The present work is based on an extract of *Sorghum* leaf sheaths with the aim to increase our knowledge of *Sorghum* 3-deoxyflavonoids. The analysis by LC-UV-ESIMS of an extract of *Sorghum bicolor* (L) Moench showed the presence of many polyphenols, some of which had a molecular mass corresponding to unknown compounds. Here we describe in particular the isolation and structural characterization of two new flavonoids, retrodihydrochalcones **1** and **2**, which may be key intermediates in the formation of *Sorghum* 3-deoxyanthocyanidins.

2. Results and discussion

The butanediol/ethanol extract of *Sorghum* leaf sheaths was initially concentrated to eliminate ethanol. It was then fractionated using a C18 endcapped cartridge into three fractions (elution by water, ethyl acetate and methanol) (Khalil et al., 2010). Subsequent chromatographic separation of ethyl acetate fraction and further purification of sub-fractions (Khalil et al., 2010) allowed the isolation of **1** and **2**. Structural assessment of these two compounds was obtained by analysis of electrospray mass spectrometry, ^{1}H and ^{13}C NMR spectroscopic data. Assignments of NMR signals were obtained especially by COSY, NOESY, HSQC and HMBC experiments.

The UV spectrum (MeOH) of compound **1** showed maximum absorption at 288 nm, similarly to flavanols and flavanones. The pseudo molecular ion peak $[M+Li]^+$ (ion Li^+ from calibration of the

* Corresponding author. Present address: Métabolisme Secondaire de la Vigne, UMR 1131 INRA/Université de Strasbourg, 28 rue de Herrlisheim, 68021 Colmar, France. Tel.: +33 03 89 22 49 94; fax: +33 03 98 22 49 33.
E-mail address: baltenwe@unistra.fr (R. Baltenweck-Guyot).

1874-3900/$ – see front matter © 2011 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.phytol.2011.12.004

Table 1
^{1}H and ^{13}C NMR data for compounds **1** and **2** (500 and 125 MHz respectively, in CD_3OD), δ (ppm).

Position	Compound **1**		Compound **2**	
	δ^1H [int., mult., *J* (Hz)]	δ^{13}C	δ^1H [int., mult., *J* (Hz)]	δ^{13}C
C=O	–	203.1	–	203.3
α	3.09, 2H, t (7.5)	39.3	3.10, 2H, t (7.4)	39.3
β	2.83, 2H, t (7.5)	19.5	2.85, 2H, t (7.5)	19.8
1		107.3	–	108.6
2,6	–	157.7	–	158.1
3,5	5.84, 2H, s	95.6	5.92, 2H, s	94.2
4	–	158.3	–	160.8
1′	–	129.9	–	130.0
2′,6′	7.90, 2H, d (8.6)	132.1	7.91, 2H, d (8.8)	132.3
3′,5′	6.79, 2H, d (8.6)	116.1	6.80, 2H, d (8.7)	116.3
4′	–	163.8	–	164.0
$-OCH_3$	–	–	3.66, 3H, s	55.2

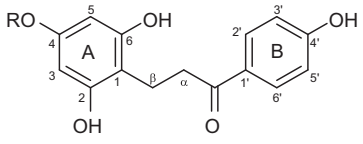
mass spectrometer) was observed for compound **1** at *m*/*z* 281.097, obtained by HRESIMS, established its formula to be $C_{15}H_{14}O_5$.

The ^{1}H NMR spectrum (500 MHz in CD_3OD, TMS) of **1** (Table 1) showed three aromatic protons signals indicative of a flavonoid skeleton. They corresponded to the H-2′/6′ equivalent protons occurring at 7.90 ppm as a doublet (*J* = 8.6 Hz) and coupled to the H-3′/5′ equivalent protons evident as a doublet (*J* = 8.6 Hz) at 6.79 ppm. An aromatic two protons singlet observed at 5.84 ppm was assigned to H-3/5 equivalent protons. Two triplets, two protons each, appearing at 3.09 (*J* = 7.5 Hz) and 2.83 ppm (*J* = 7.5 Hz) were identified as vicinal aliphatic protons H-α and H-β respectively. The ^{1}H–^{1}H COSY spectrum confirmed correlations between aromatic protons H-2′/6′ and H-3′/5′ and between aliphatic protons H-α and H-β. The major NOE effects were observed between these aliphatic hydrogens and the H-2′/6′ phenolic protons. The carbon chemical shift values corresponding to each carbon attached to respective heteronuclear hydrogens were evidenced on the basis of the HSQC spectrum.

The long-range correlation observed in the HMBC spectrum between the carbon of the carbonyl group (δ 203.1) and the aromatic protons H-2′/6′ (δ 7.90) and aliphatic protons H-α (δ 3.09) and H-β (δ 2.83), confirmed the position of the carbonyl group next to the phenyl ring B. Similarly, protons H-3/5 (δ 5.84), H-α (δ 3.09) and H-β (δ 2.83) showed correlations to carbon C-1 (δ 107.3), while protons H-3′/5′ (δ 6.79) and only H-α (δ 3.09) showed correlations to C-1′ (δ 129.9). Thus, compound **1** was characterized as a novel open chain flavonoid, the 3-(2,4,6-trihydroxyphenyl)-1-(4-hydroxyphenyl)-propan-1-one or 2,4,4′,6-tetrahydroxydihydrochalcone.

The UV spectrum (MeOH) of compound **2** was similar to that of compound **1**. The pseudo molecular ion peak $[M-H]^-$ observed for compound **2** at *m*/*z* 287.091, obtained by HRESIMS, established the molecular formula of **2** to be $C_{16}H_{16}O_5$. Consequently, compound **2** corresponded to the same molecular formula as **1** with one additional CH_2 methylene group. Fig. 1

NMR experiments showed that compound **2** had structural similarity with **1**: the chemical shifts of all aromatic and aliphatic protons of **2** were analogous to those of compound **1**. A notable difference was the presence of a three proton singlet at 3.66 ppm,



1, R = H
2, R = CH_3

Fig. 1. Structure of compounds **1** and **2**.

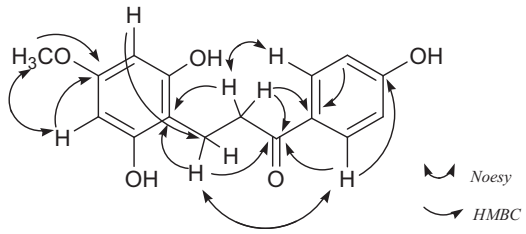


Fig. 2. Major HMBC and NOE correlations observed on compound **2**.

corresponding to protons of a methoxyl group. Moreover, these hydrogens were spatially near to H-3/5 (NOE effect, see Fig. 2). The long-range correlation observed in the HMBC spectrum between the C-4 (δ 160.8) and these protons (δ 3.66) confirmed the presence of a methoxyl group linked at C-4. Thus, compound **2** was identified as the dihydrochalcone 3-(2,6-dihydrox-4-methoxy-phenyl)-1-(4-hydroxyphenyl)-propan-1-one (or 2,4′,6-trihydroxy-4-methoxydihydrochalcone). It corresponds to an analogue of the 7-*O*-methyl-deoxyflavonoids (7-*O*-methylapigenin, 7-*O*-methyl-luteolin and 7-*O*-methylapigeninidin) previously identified in the *Sorghum* leaves (Muller-Harvey and Reed, 1992; Misra and Seshadri, 1967; Pale et al., 1997).

These two dihydrochalcones **1** and **2** are named retrodihydrochalcones because the typical O-substitution patterns of the A- and B-rings of flavonoid structures are apparently reversed and the carbonyl group shifted from C-1 to C-3.

Few plants containing retrodihydrochalcones are known: other retrodihydrochalcones were earlier identified from *Dracaena loureiri* (Meksuriyen and Cordell, 1988) and from the stem bark of *Uvaria mocoli* (Fleicher et al., 1998).

It is notable that new elucidated retrodihydrochalcone **1** has the same molecular weight (MW 274), the same molecular formula ($C_{15}H_{14}O_5$) and a similar UV spectrum as those attributed in the literature to apiforol (see structure in Fig. 3) (Watterson and Buttler, 1988). However, even if the latter compound has never been fully characterized in plants, the hypothetical structure of apiforol has been proposed as a potential precursor of *Sorghum* 3-deoxyanthocyanidins (Kambal and Bate-Smith, 1976; Styles and Ceska, 1989). Therefore, the reliability of the apiforol structure should be questioned. To compare the structure of apiforol with that of retrodihydrochalcone **1**, the latter was treated in the same reaction conditions as used by Watterson and Buttler (1988) on the proposal apiforol: a hot aqueous concentrated solution of HCl (Bate-Smith reaction, Bate-Smith, 1969). As for apiforol detected by Watterson and Buttler (1988), we observed the solution change from colorless to yellow. Analysis of the yellow solution by electrospray ionization mass spectrometry in positive mode and by ^{1}H NMR spectroscopy indeed demonstrated the presence of apigeninidin **3** by comparison with a reference sample (Fig. 3), already reported in *Sorghum*, a result identical with that obtained by Watterson and Buttler (1988) for apiforol.

Identical experiments were carried out on compound **2**, and similar results were obtained, i.e. the formation of 7-*O*-methyla-pigeninidin **4** by the Bate-Smith reaction. The similarity between our results and those of Watterson and Buttler (1988) on proposed apiforol led us to question the real structure of the latter and its

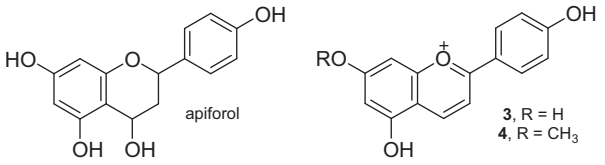


Fig. 3. Structure of apiforol, apigeninidin **3** and 7-*O*-methyl-apigeninidin **4**.

real existence, as well as that of other flavan-4-ols in *Sorghum* plant. The two novel retrodihydrochalcones could indeed be key intermediates in the biosynthesis pathway of 3-deoxyanthocyanidins in *Sorghum* plants. This hypothesis is now subjected to further investigations.

3. Experimental

3.1. General experimental procedures

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured in CD_3OD on a Bruker AMX500 spectrometer at 298 K and with TMS as internal standard. ^{1}H chemical shifts were assigned using 1D and 2D ^{1}H NMR (COSY and NOESY), whereas ^{13}C resonances were assigned using 2D NMR techniques (HMBC and HSQC). An Esquire 3000+ and a MicroTOF (Bruker Daltonics, Bremen, Germany) mass spectrometers equipped with an electrospray source for ionization was used for the ESIMS and HRESIMS. Samples were introduced into the spectrometers with a syringe pump (200 μl/min). Spectra were recorded in positive and negative mode between *m*/*z* 80 and 1000. The capillary voltage was ±4 kV, the capillary temperature 180 °C and the capillary exit 100 V.

3.2. Plant material

The *Sorghum* extract was obtained by extraction of red *Sorghum* leaf sheath [*Sorghum bicolor* var. *bicolor* (Moench)], cultivar Gervex 1296 (CIRAD) from Burkina Faso.

3.3. Extraction and isolation

The *Sorghum* extract was obtained using a mixture of 1,3-butanediol and ethanol (1:2) as solvent. Ethanol was then removed by evaporation; the Sorghum extract contained 5% of dry total extract by weight. 5 ml of the butanediol solution was diluted with water (1:3) and separated in several fractions according to their polarity, using a Macherey-Nagel Chromabond® C18 endcapped cartridge (C18 ec, 6 ml, 500 mg). Eluting with 10 ml of water permits to eliminate salts and acids; the flavonoids were then eluted with 15 ml of ethyl acetate which gave a pink fraction. The latter was concentrated and then separated by HPLC (Waters, Milford, MA, USA) used together with an autosampler (Waters 717 plus) on a reverse phase C18 Zorbax-SB semi preparative column (250 × 9.6 mm, 5 μm, Agilent, Santa Clara, CA, USA). The detection was carried out using a diode array detector (Waters 996) with a flow of 4 ml/min. The eluents **A** (H_2O) and **B** (CH_3CN) were used with a 45 min gradient to separate compounds **1** and **2**: 0–15 min, 25–35% B; 15–33 min, 35–42% B, 33–40 min, 42–100% B, 40–45 min, 100% B. Compounds **1** (retention time 14.1 min) and **2** (retention time 17.5 min) were collected separately and then concentrated under vacuum to give ca. 0.5 mg of compound **1** and ca. 0.4 mg of compound **2**.

3.4. Compounds characterization

3.4.1. 3-(2,4,6-Trihydroxyphenyl)-1-(4-hydroxyphenyl)-propan-1-one (1)

Pale yellow solid; UV (MeOH) λ_{max}: 280 nm; ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD): see Table 1; ESIMS (+) *m*/*z* 275.1 $[M+H]^+$, 297.1 $[M+Na]^+$, ESIMS (−) *m*/*z* 273.1 $[M-H]^-$; HRESIMS (+) *m*/*z* 281.097 $[M+Li]^+$ (calcd. for $C_{15}H_{14}O_5Li^+$ 281.099).

3.4.2. 3-(2,6-Dihydrox-4-methoxyphenyl)-1-(4-hydroxyphenyl)-propan-1-one (2)

Pale yellow solid; UV (MeOH) λ_{max}: 280 nm; ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD): see Table 1; ESIMS (+) *m*/*z* 289.1 $[M+H]^+$, 311.1 $[M+Na]^+$, ESIMS (−) *m*/*z* 287.1 $[M-H]^-$; HRESIMS (−) *m*/*z* 287.091 $[M-H]^-$ (calcd. for $C_{16}H_{15}O_5^-$ 287.091).

3.5. Bate-Smith reaction

30 μg of each retrodihydrochalcone **1** and **2** were reacted individually with 2–3 ml of conc. HCl (37%, 12 M). The mixture was heated for 15 min in a water bath at 100 °C. The resulting 3-deoxyanthocyanidins (apigeninidin **3** and 7-*O*-methylapigeninidin **4**) were then analyzed by UV-visible spectroscopy, MS and NMR.

Acknowledgments

We wish to thank L'OREAL Research and Development, Aulnay-sous-Bois, France for providing us with a sample of the *Sorghum* extract; the Centre National de la Recherche Scientifique (CNRS) and the Université de Strasbourg (UdS) for financial support; Dr G. Hussler, L'OREAL, for helpful discussion; Dr R. Graff for NMR measurements. One of us (A.K.) thanks the Ministère de l'Enseignement Supérieur et de la Recherche (France) for a doctoral fellowship.

References

Awika, J.M., Rooney, L.W., 2004. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 65, 1199–1221.
Bate-Smith, E.C., 1969. Luteoforol (3′,4,4′,5,7-pentahydroxyflavan) in *Sorghum vulgare* L. Phytochemistry 8, 1803–1810.
Chopra, S., Gevens, A., Svabek, C., Wood, K., Peterson, T., Nicholson, R., 2002. Excision of the transposon from a hyper-mutable allele shows that the sorghum gene controls the biosynthesis of both 3-deoxyanthocyanidin phytoalexins and phlobaphene pigments. Physiol. Mol. Plant Pathol. 60, 321–330.
Du, Y., Chu, M., Wang, M., Chu, I., Lo, C., 2010. Identification of flavone phytoalexins and a pathogen-inducible flavone synthase II gene (*SbFNSII*) in Sorghum. J. Exp. Bot. 61, 983–994.
Fleicher, T., Waigh, R., Waterman, P., 1998. A novel retrodihydrochalcone from the stem bark of *Uvaria mocoli*. Phytochemistry 47, 1387–1391.
Kambal, A.E., Bate-Smith, E.C., 1976. A genetic and biochemical study on pericarp pigments in a cross between two cultivars of grain sorghum, *Sorghum bicolor*. Heredity 37, 413–416.
Khalil, A., Baltenweck-Guyot, R., Ocampo, R., Albrecht, P., 2010. A novel symmetrical pyrano-3-deoxyanthocyanidin from a *Sorghum* species. Phytochem. Lett. 3, 93–95.
Lo, S.C., De Verdier, K., Nicholson, R.L., 1999. Accumulation of 3-deoxyanthocyanidin phytoalexins and resistance to *Colletotrichum sublineolum* in *Sorghum*. Physiol. Mol. Plant Pathol. 55, 263–273.
Meksuriyen, D., Cordell, G.A., 1988. Retrodihydrochalcones from *Dracaena loureiri*. J. Nat. Prod. 51, 1129–1135.
Misra, K., Seshadri, T., 1967. Chemical components of *Sorghum durra* glumes. Indian J. Chem. 5, 409–412.
Muller-Harvey, I., Reed, J., 1992. Identification of phenolic compounds and their relationships to in vitro digestibility of Sorghum leaves from bird-resistant varieties. J. Sci. Food Agric. 60, 179–196.
Pale, E., Koudas-Bonafos, M., Nacro, M., Vanhaelen, M., Vanhaelen-Fastré, R., Ottinger, R., 1997. 7-*O*-Methylapigeninidin, an anthocyanidin from *Sorghum caudatum*. Phytochemistry 45, 1091–1092.
Schijlen, E., de Vos, R., van Tunen, A., Bovy, A., 2004. Modification of flavonoid biosynthesis in crop plants. Phytochemistry 65, 2631–2648.
Snyder, B.A., Nicholson, R.L., 1990. Synthesis of phytoalexins in sorghum as a site-specific response to fungal ingress. Science 248, 1637–1639.
Styles, E.D., Ceska, O., 1989. The genetic control of flavonoid synthesis in maize. Can. J. Genet. Cytol. 19, 289–302.
Watterson, J., Buttler, L., 1988. Occurrence of an unusual leucoanthocyanidin and absence of proanthocyanidins in sorghum leaves. J. Agric. Food Chem. 31, 41–45.