Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 68 (2007) 646–651
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

C-methylated and C-prenylated isoflavonoids from root extract of *Desmodium uncinatum*

Salome M. Guchu [a,b], Abiy Yenesew [b,*], Muniru K. Tsanuo [c], Nicholas K. Gikonyo [d], John A. Pickett [e], Antony M. Hooper [e], Ahmed Hassanali [a]

[a] *International Centre of Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya*
[b] *Department of Chemistry, University of Nairobi, P.O. Box 30197, Nairobi, Kenya*
[c] *Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, Nairobi, Kenya*
[d] *Department of Pharmacy and complementary/Alternative medicine, Kenyatta University, P.O. Box 43844, Nairobi, Kenya*
[e] *Biological Chemistry Division, Rothamsted Research, Harpenden,Herts A15 2JQ, UK*

Received 12 October 2006; received in revised form 18 November 2006
Available online 17 January 2007

Abstract

A pterocarpan, 1,9-dihydroxy-3-methoxy-2-methylpterocarpan (named uncinacarpan) and two isoflavanones, 5,7-dihydroxy-2′,3′,4′-trimethoxy-6-(3-methylbut-2-enyl)isoflavanone (named uncinanone D) and 5,4′-dihydroxy-7,2′-dimethoxy-6-methylisoflavanone (named uncinanone E), were isolated from the CH_2Cl_2 root extract of *Desmodium uncinatum* (Jacq.) DC and characterised by spectroscopic methods. In addition, a rare pterocarpan edudiol and two known abietane diterpenes, 7-oxo-15-hydroxydehydroabietic acid and 7-hydroxycallitrisic acid were identified. The fraction of the root extract that was analysed induced germination of *Striga hermonthica* seeds, but none of the isolated compounds showed this activity.

Keywords: *Desmodium uncinatum*; Fabaceae; Isoflavonoids; Pterocarpans; Uncinacarpan; Edudiol; Isoflavanones; Uncinanone D; Uncinanone E; *Striga hermonthica*; Germination stimulation

1. Introduction

The genus *Desmodium* (Fabaceae) is used in erosion control, ground cover and wildlife protection in lands cleared of vegetation (Trout, 2004). Many of the species are also highly valued as fodder and in folk medicine. The fodder legumes *Desmodium uncinatum* (silver leaf) and *Desmodium intortum* (green leaf), when deployed as intercrops, have been found to reduce damage to maize by stem borers such as *Busseola fusca* (Noctuidae) and *Chilo partellus* (Pyralidae) (Khan et al., 2000). Volatile repellent emissions from these legumes have been shown to be responsible. When these intercrops are located in areas infested with parasitic witch-weeds such as *Striga hermonthica* (Scrophulariaceae), dramatic reduction in the infestation of maize is observed (Khan et al., 2002, 2006). In addition to effects of increased nitrogen and shading associated with maize-desmodium intercrops, an allelopathic effect is largely responsible for the control of *S. hermonthica*. The root exudate of *D. uncinatum* has been found to stimulate germination of striga seeds and to inhibit radical growth of the resulting seedlings, and this combination represent the allelopathic mechanism associated with striga control (Khan et al., 2002; Tsanuo et al., 2003). It also accounts for the fact that intercropping maize with desmodium over successive years results in a continual and rapid depletion of striga seed bank in the soil (Khan et al., 2002).

In a previous investigation of the aqueous root exudates of *D. uncinatum* (Tsanuo et al., 2003), three isoflavanones

* Corresponding author. Tel.: +254 20 4440044x2170; fax: +254 20 4446138.
E-mail address: ayenesew@uonbi.ac.ke (A. Yenesew).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.11.035

and an isoflavone were isolated and characterized. Of these, 4″,5″-dihydro-5,2′,4′-trihydroxy-5″-isopropenylfurano-(2″,3″;7,6)-isoflavanone (uncinanone B) and 4″,5″-dihydro-2′-methoxy-5,4′-dihydroxy-5″-isopropenylfurano-(2″,3″;7,6)-isoflavanone (uncinanone C) were active, as a moderate striga germination stimulant and as a moderate post-germination radical inhibitor, respectively. The present paper, which reports on the isolation and structure elucidation of three new isoflavonoids from a fraction of CH_2Cl_2 extract of *D. uncinatum* roots, represents part of our current studies to comprehensively examine root chemistry of this plant and to bioassay constituents of different polarity range.

2. Results and discussion

Unlike the aqueous root exudates of *D. uncinatum*, which exhibited both striga germination stimulation and post-germination radicle growth inhibition activities (Tsanuo et al., 2003), the CH_2Cl_2 extract of *D. uncinatum* (fresh roots) only caused germination stimulation of *S. hermonthica* seeds. At 10 μg/ml and 100 μg/ml, the CH_2Cl_2 extract of *D. uncinatum* (roots) stimulated the germination of *S. hermonthica* seeds by $51.5 \pm 2.2\%$ and $61.5 \pm 0.9\%$ respectively. Chromatographic fractionation of the extract on silica gel yielded four fractions with fraction D (eluted with 50% acetone in hexane) giving a potent germination stimulation activity (Table 1). In the same bioassays, GR-24, a synthetic sesquiterpene known to stimulate striga germination (Johnson et al., 1981), caused germination stimulation of $50.2 \pm 4.7\%$ at 5 μg/ml. Isolation and purification of some of the compounds from the fraction of the CH_2Cl_2 extract of *D. uncinatum* (roots) yielded four isoflavonoids (compounds **1**–**4**, Fig. 1), together with two abietane diterpenes. However, none of these compounds exhibited significant striga germination stimulation activities.

The HREIMS of compound **1** gave a molecular ion peak at *m/z* 300.0993 corresponding to the molecular formula of $C_{17}H_{16}O_5$. The ^1H NMR and ^1H,^1H-COSY spectra showed four aliphatic protons in a single spin system at δ 4.22 (*dd*, $J = 4.4$, -10.4 Hz), 3.50 (*dd*, $J = -10.4$, 11.0 Hz), 3.43 (*ddd*, $J = 4.4$, 6.6, 11.0 Hz) and 5.65 (*d*, $J = 6.6$ Hz), attributed to CH_2-6, H-6a and H-11a of a pterocarpan skeleton, respectively (Table 2). The presence of a pterocarpan skeleton was supported by ^{13}C NMR spectrum, which showed the corresponding carbons at δ 66.5 (C-6), 39.5 (C-6a) and δ 76.3 (C-11a). In the ^1H NMR spectrum further signals were observed which showed the presence of a methyl group (δ 2.07, s), a methoxyl group (δ 3.80, s), an aromatic singlet (δ 6.13) and aromatic protons with an ABX spin system (δ 6.33, *d*, $J = 2.2$ Hz; δ 6.36, *dd*, $J = 2.2$, 8.2 Hz and δ 7.12, *d*, $J = 8.2$ Hz). The molecular formula $C_{17}H_{16}O_5$ and the presence of five oxygenated aromatic carbon atoms (δ 155.2, 155.8, 158.8 and 159.8, 160.8) in the ^{13}C NMR spectrum are consistent with two hydroxyl substituents, in addition to the methyl and methoxy groups in the pterocarpan skeleton. Formation of a di-acetate, whose EIMS gave a molecular ion peak at *m/z* 384 confirmed the presence of the two hydroxyl groups in this compound. In the HMBC spectrum (Table 2), correlation of methyl protons (δ 2.07) with the signals at δ 155.8, 105.6 and 159.8 are consistent with a tri-substituted A-ring with the methyl at C-2 and oxygenations at C-1 and C-3. HMBC correlation of the methoxyl protons (δ 3.80) with the signal at δ 159.8 and that of H-11a (δ 5.65) with the signal at δ 155.8 allowed placement of the methoxyl group at C-3 and hydroxyl at C-1. The substitution pattern in A-ring was confirmed by 1D-GOESY experiments, which showed interaction between the methyl protons (δ 2.07) and the methoxyl group (δ 3.80); and between the methoxyl group (δ 3.80) and an aromatic singlet at δ 6.13 (H-4). The ABX spin system corresponds to D-ring protons with the biogenetically expected oxygenation at C-9, and this was confirmed by the HMBC experiments (Table 2). Natural pterocarpans are known to occur in *cis* configuration (Dewick, 1988, 1994). In agreement with this, the coupling constant between H-6a and H-11a ($J = 6.6$ Hz) and the strong NOE between H-11a (δ 5.65) and H-6a (δ 3.43) are consistent with *cis*-geometry at the ring junction (Van Aardt et al., 1999, 2001). This compound showed high negative optical rotation ($[\alpha]_D = -250°$) consistent with 6a*R*:11a*R* absolute configuration (Yenesew et al., 1998). Thus compound **1** was characterized as (6a*R*:11a*R*)-1,9-dihydroxy-3-methoxy-2-methylpterocarpan (**1**) for which the trivial name uncinacarpan is suggested. All the ^1H and ^{13}C NMR spectroscopic signals of **1** (Table 2) were assigned on the basis of ^1H–^1H COSY, GOESY, HMQC and HMBC spectra.

Compound **2** was assigned a molecular formula of $C_{21}H_{22}O_5$ (*m/z* 354.1467) from HREIMS. The ^1H and ^{13}C NMR spectra (Table 2) showed this compound to be a pterocarpan derivative with a 3-methylbut-2-enyl, a methoxyl and two hydroxyl substituents. Comparison of the NMR data of compound **2** with those of compound **1** (Table 2) showed that **2** differs from **1** by the presence of a 3-methylbut-2-enyl group in place of a methyl group

Table 1
Germination response of *Striga hermonthica* seeds to fractions of CH_2Cl_2 extract of *Desmodium uncinatum* roots

Test solution	Percentage mean germination (±SE), $n = 10$			
	100 ppm	10 ppm	5 ppm	1 ppm
CH_2Cl_2 extract	61.5 (1.0)^{a,b}	51.5 (2.2)^{b,c,d}		36.8 (2.1)^{e,f}
Fraction A	0.6 (0.6)^{g}	0.4 (0.4)^{g}		0.0 (0.0)^{g}
Fraction B	46.2 (1.9)^{c,d,e}	42.6 (1.2)^{d,e}		29.4 (2.4)^{f}
Fraction C	57.6 (3.9)a,b,c	50.2 (2.8)^{b,c,d}		35.8 (0.7)^{e,f}
Fraction D	67.8 (3.6)^{a}	53.8 (4.3)^{b,c,d}		41.4 (3.1)^{d,e,f}
GR-24 (Johnson et al., 1981)			50.2 (4.7)^{b,c,d}	

Means with the same letter are not significantly different ($P < 0.05$) by Tukey's studentized range test.