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Prenylated flavanone derivatives isolated from *Erythrina addisoniae* are potent inducers of apoptotic cell death

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ABSTRACT

Extracts of *Erythrina addisoniae* are frequently used in the traditional medicine of Western Africa, but insufficient information about active compounds is available. From the stem bark of *E. addisoniae*, three (**1**, **2**, **4**) and three known (**3**, **5**, **6**) flavanones were isolated: addisoniaflavanones I and II, containing either a 2″,3″-epoxyprenyl moiety (**1**) or a 2″,3″-dihydroxyprenyl moiety (**2**) were shown to be highly toxic (MTT assay: EC_{50} values of 5.25 ± 0.7 and 8.5 ± 1.3 μM, respectively) to H4IIE hepatoma cells. The cytotoxic potential of the other isolated flavanones was weaker (range of EC_{50} values between 15 and >100 μM). Toxic effects of addisoniaflavanone I and II were detectable after 3 h (MTT assay). Both compounds induced an apoptotic cell death (caspase-3/7 activation, nuclear fragmentation) in the hepatoma cells and, at high concentrations, also necrosis (membrane disruption: ethidium bromide staining). Formation of DNA strand breaks was not detectable after incubation with these compounds (comet assay). In conclusion, the prenylated flavanones addisoniaflavanones I and II may be of interest for pharmacological purposes due to their high cytotoxic and pro-apoptotic potential against hepatoma cells.

1. Introduction

Prenylated flavonoids were reported to possess interesting biological and pharmacological properties: For example, Ren et al. (2010) demonstrated that prenylated flavonoids (e.g. artorigidin A) isolated from *Artocarpus rigida* twigs were potent inhibitors of NF-κB activity in low micromolar range. Prenylated flavonoids from *Artocarpus altilis* are capable to inhibit the production of melanin (Lan et al., 2013). Tabopda et al. (2008) demonstrated that dorsilurin F, a triprenylated flavonoid from *Dorstenia psilurus* possess a potent alpha-glucosidase inhibitory properties. Systematic reviews on biological activities of prenylated flavonoids are given by Chen et al. (2014) and Šmejkal (2014).

The genus *Erythrina* is known to contain prenylated flavonoid derivatives (Krukoff and Barneby, 1974). In traditional medicine, extracts prepared from *Erythrina* spp. are used for several important diseases in their respective area of distribution (Cox, 1993; Ghosal et al., 1972; Saiduh et al., 2000). *Erythrina addisoniae* Hutch. & Dalziel, which occurs in Ghana and other West-African states, is used to treat dysentery, hepatitis, rheumatic disorders and pain, as well as swellings and cancer (Burkill, 1995; Hartwell, 1970). In two previous papers we have reported the occurrence of bioactive pterocarpans and flavanones from the stem bark of *E. addisoniae* showing cytotoxic activities in micromolar ranges (Wätjen et al., 2007, 2008). Prenylated stilbenoids and isoflavanoids from the root bark of *Erythrina* species have also been reported to possess inhibitory activities against neuraminidases from influenza viruses and have shown activities against breast cancer lines and protein tyrosine phosphatase 1B (Bae et al., 2006; Cui et al., 2010; Nguyen et al., 2010, 2012). We now describe the isolation and structure elucidation of three new and three known prenylated flavanones and their cytotoxic and pro-apoptotic activities on H4IIE hepatoma cells.

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2. Results and discussion

2.1. Isolation and identification of the compounds

Purification of four major fractions of the dichloromethanic extract of the stem bark of *E. addisoniae* Hutch. & Dalz. rendered six compounds (**1**–**6**). The ^{1}H and ^{13}C NMR spectra of compound **1** showed resonances for 25 carbons and 28 protons. Nine carbon and seven proton signals were found at shift values characteristic for a 5,7-dihydroxy-2,3-dihydro-benzopyran-4-one ring system, representing the moiety of flavanones in ring A and C (Wätjen et al., 2007; Hauschild et al., 2010). Interpretation of the 2D NMR data led to the assignment of six further carbon resonances of a 3,4,5-trisubstituted benzoyl moiety attached to C-2 of the pyranone ring, which represents the ring B of a flavanoid. The downfield shift of the resonance of C-4′ (δ 157.0 ppm) indicated a 4′-OH group, accompanied by two aliphatic substituents at C-3′ and C-5′. Accordingly, five of the remaining 10 carbon resonances could be identified as signals of a 3,3-dimethylallyl substituent (prenyl side chain) by means of the HMBC-, HMQC- and COSY-spectra. The five remaining ^{13}C resonances indicated the presence of a second C-5 unit, whose methylene and methine resonances significantly shifted upfield to δ 53.9 and 54.6, respectively. Moreover the corresponding proton resonances of the terminal methyl groups were shifted to a higher field. In comparison to the regular prenyl side chain the methylene protons were no longer represented by one signal (δ 3.04 and 2.75) indicating a stereochemical effect, which has been reported to be characteristic for a 3,3-dimethyloxyranylmethyl side chain (Harborne and Mabry, 1982; Paulson et al., 1975; Tahara and Ibrahim, 1995; Wandji et al., 1994). Therefore, compound **1** was identified as 3′-(2″,3″-epoxy-3″-methylbut-3″-enyl)licoflavanone. The structure was additionally confirmed by its LCMS spectrum, displaying the quasimolecular ion at 425 $[M+H]^{+}$. Due to the small amount of the compound all trials to determine the absolute configuration at C-2″ failed. However, this compound was found for the first time in nature to the best of our knowledge. According to the formerly used trivial names it may be named addisoniaflavanone I.

The NMR spectra of compound **2** resembled those of **1**. Differences were found for the signals of one of the prenyl side chains. The proton signals of the two methyl groups appeared in a higher field and the methylene and methine proton resonances were found to be shifted downfield. Thus, the signals were found at shift values characteristic for a 2″,3″-dihydroxy prenyl moiety, often found in similar compounds. This was confirmed by the mass spectrometric data, indicating a molecular weight of 426 u instead of 424 u for **1**. Thus, **2** was identified as the equally new natural product 3′-(2″,3″-dihydroxy-3″-methylbutyl)licoflavanone, for which the name addisoniaflavanone II may be suggested.

The NMR spectra of compound **3** in analogy to the spectra of 1 and 2 showed the carbon and proton resonances of a 5,7-dihydroxyflavanone with 3′, 5′ substituted ring B. One of the substituents could be identified as a 3,3-dimethylallyl side chain. In comparison with **1** and **2** the ^{13}C NMR signals of C-3′ and C-4′ appeared at smaller shift values indicating a different prenyl substituent, a methylation of the C-4′-OH or a ring closure with a second prenyl substituent, forming a 2,2-dimethylpyran system, often found in prenylated flavanones. According to the remaining proton resonances and the 2D NMR (COSY; HMQC and HMBC) spectra it was found that the second prenyl moiety at C-3′ formed a 2,2-dimethyl-3-hydroxy-tetrahydropyran moiety. Compound **3** was therefore identified as 5,7-dihydroxy-5′-prenyl-[2″,2″-(3″-hydroxy)-dimethylpyrano]-(5″,6″:3′,4′)flavanone. This compound was already reported by Cui et al. in the stem bark of *Erythrina abyssinica* (Cui et al., 2008), all analytical data found by us are identical to those reported there.

Compound **4** was found to be different from the other flavonoids, although its NMR data clearly indicated the presence of a 5,7-dihydroxy-flavanone. However, it obviously only contains a 3,3-dimethylallyl side chain, which could be deduced from the five carbon and the corresponding proton signals. In contrast to **1** and **2** the proton and carbon spectra of **4** showed two additional resonances typical for a methylated hydroxyl group (3.96 s and 65.0 ppm, respectively) and an aldehyde substituent (10.36 and 190.4 ppm, respectively). From the cross peaks found in the 2D NMR HMBC spectrum of **4** (see Fig. 1B) the aldehyde group was found to be attached to C-3′ of the ring B of the flavanone. The spectrum also showed that the C-4′ hydroxyl was methylated, while the hydroxyl groups at C-5 and C-7 remained unsubstituted. The positions of the substituents at C-3′, C-4′ and C-5′ were unambiguously determined by the cross peaks found in the long range HMQC and HMBC spectra. All connections (1J, 2J and 3J) contacts found in the HMBC are shown in Fig. 1B. The compound was thus identified as 3′-formyllicoflavone-4′-methylether, which was also found for the first time in nature to the best of our knowledge. It could therefore be named addisoniaflavanone III.

Compounds **5** and **6** were found to be abyssinin II and abyssinoflavanone V, previously isolated from *Erythrina milbrandii* (Jang et al., 2008) and *E. abyssinica* (Cui et al., 2007).

Each of the isolated substances shows substituents at C-3′ and C-5′. In the case of **1**, **2**, **3** and **6**, the molecules possess one 3,3-dimethylallyl side chain and a further substituent derived from a second 3,3-dimethylallyl moiety both *ortho* to the 4′-OH group. Biogenetic investigations have already been made for the biotransformation of 3,3-dimethylallyl side chains in prenylated phenols (Paulson et al., 1975). The prenyl substituents seem to be part of a series of enzymatic reactions which start with a 3,3-dimethylallyl side chain and may lead to the hydroxylated side chain in compound **3** via the 2,3-epoxy-3-methylbut-3-enyl derivative (**1**) (see Fig. 1). This possible biogenetic pathway also includes 3′-(2-hydroxy-3-methyl-but-3-enyl)-4′-O-methyllicoflavanone, 3′-(2-hydroxy-3-methyl-but-3-enyl)abyssinone II and abyssinoflavanone VII, previously reported as constituents of *E. addisoniae* (Wätjen et al., 2007). Side chains can also form 2,2-dimethylchromene derivatives by cyclisation with phenolic hydroxyl groups as found in **3** and **6**. So far, eight of the various partial structures formed by such prenyl side chains in natural products have been found in *E. addisoniae* (Hartwell, 1970; Wätjen et al., 2007). Their structures and substitution patterns were unequivocally resolved through 2D-HMBC spectra.

Although we carefully tried to e.g. keep the temperature of solvents as low as possible by reducing the pressure during evaporation and by a frequent change of the solvent during Soxhlet extraction, it is generally possible, that the compounds found here were built as artefacts during the extraction and isolation process. However, to rule this out, we extracted a small portion of the bark material with acetone at room temperature and checked for the presence of 1–5 by LC–MS. Since, compounds showing the correct molecular weight at the particular retention time were fund (see M&M), we strongly believe that all reported compounds are genuine natural product in *E. addisoniae* not being artefacts built by extraction.

2.2. Bioactivity of the compounds

Since *Erythrina* species are used to treat cancer in traditional medicine of Western Africa, we analysed the cytotoxic potential of distinct compounds on H4IIE hepatoma cells (Fig. 2): incubation