A New Prenylisoflavone from *Ulex jussiaei*

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A new naturally occurring isoflavone, derrone, was isolated from *Ulex jussiaei* (Leguminosae) together with the isoflavones ulexins A–C, lupalbigenin, isolupalbigenin, 7-*O*-methylisolupalbigenin, isoderrone, ulexone A and isochandalone, the pterocarpans (6a*R*,11a*R*)-(–)-maackiain, (6a*R*,11a*R*)-(–)-2-methoxymaackiain and (6a*R*,11a*R*)-(–)-4-methoxymaackiain, the chalcone 4-hydroxylonchocarpine and the dihydrochalcone crotaramosmine. The antifungal activity of the new compound was tested by a bioautographic method against *Cladosporium cucumerinum*, and as expected from structural features it proved to have no activity.

Introduction

Isoflavonoids have been isolated mainly from leguminous plants. The occurrence of isoflavonoid aglycones is almost restricted to the Leguminosae family, despite their large structural variation. This structural diversity arises from the different oxidation levels and also from the number and complexity of substituents. The prenylated isoflavonoids are known as complex isoflavonoids having additional carbon atoms (dimethylallyl or geranyl units) as acyclic or cyclic side chains to the basic C_{15} isoflavonoid skeleton (Tahara and Ibrahim, 1995).

Portugal is the dispersion area of the *Ulex* genus (Espírito-Santo *et al.*, 1997) (Leguminosae, subfamily Papilionoidea). These plants are the source of a great number of flavonoids, mainly isoflavonoids such as isoflavones and pterocarpans. (Harborne, 1962; Sirat and Russell, 1989; Russell *et al.,* 1990; De Rodriguez *et al.,* 1990; Máximo and Lourenço, 1998; Máximo *et al.,* 2000; Máximo *et al.,* 2002) The isoflavonoids are important as phytoalexins (Grayer, 2001) and those isolated from *Ulex* species proved to have relevant antifungal activity (Máximo *et al.,* 2000; Máximo *et al.,* 2002).

Here we report the flavonoid composition of *Ulex jussiaei*, collected at Cabo da Roca (Portugal). The new naturally occurring derrone was identified together with nine isoflanones, three pterocarpans, one chalcone and one dihydrochalcone. The structures of the metabolites were established by the analysis of their spectroscopic data, by comparison with literature data, and also with authentic samples.

Continuing our search for phytoalexins, the newly isolated derrone was tested against the fungus *Cladosporium cucumerinum* by the bioautographic TLC bioassay, as all the other compounds were, (Máximo *et al.,* 2000; Máximo *et al.,* 2002) and proved to have no activity.

Materials and Methods

Plant material

Plant material of *Ulex jussiaei* was collected at Cabo da Roca (Portugal) in April 1998. Voucher specimens are deposited in the herbarium of Museu, Laboratório, Jardim Botânico da Faculdade de Ciências da Universidade de Lisboa [LISU 171663].

Flavonoid extraction

Dried and finely powdered aerial parts of *U. jussiaei* (2.5 kg) were extracted successively with *n*-hexane (30 l) and dichloromethane (28 l) at room temperature. The dried dichloromethane extract (30.4 g) was chromatographed on a silica gel

60 column (Merck 7734) eluted with *n*-hexane–EtOAc mixtures (9:1), (8:2), (7:3) and (6:4) (v/v) to collect fractions a, b, c and d, respectively. Fractions b, c and d were successively chromatographed on silica gel columns and on silica gel 60 F_{254} TLC plates (Merck 5554) using *n*-hexane–EtOAc, *n*-hexane–Et_2O and $CHCl_3$–MeOH mixtures as eluents. After this procedure the following pure compounds were obtained in order of increasing chromatographic polarity: ulexin B (1.0 mg), 7-*O*-methylisolupalbigenin (< 0.1 mg), ulexone A (21.0 mg), isochandalone (11.1 mg), (6a*R*,11a*R*)-(–)-2-methoxymaackiain (15.4 mg), ulexin A (7.1 mg), crotaramosmine (< 0.1 mg), (6a*R*,11a*R*)-(–)-4-methoxymaackiain (36.1 mg), ulexin C (1.8 mg), 4-hydroxylonchocapin (2.4 mg), (6a*R*,11a*R*)-(–)-maackiain (130.9 mg), isoderrone (26.2 mg), lupalbigenin (11.0 mg), derrone (**1**) (2.0 mg) and isolupalbigenin (40.7 mg).

Derrone (**1**) was identified by its physical (mp) and spectroscopic data (IV, UV, ^{1}H NMR, ^{13}C NMR, HMBC, HMQC, EIMS) and comparison with literature data for the synthetic compound (Tsukayama *et al.,* 1992). The other compounds were also identified by the analysis of their physical and spectroscopic data, comparison with authentic samples, and literature (ulexin B (Máximo *et al.,* 2000, Singhal *et al.,* 1980), 7-*O*-methylisolupalbigenin (Máximo *et al.,* 2002), ulexone A (Russell *et al.,* 1990), isochandalone (Tahara *et al.,* 1989), (6a*R*,11a*R*)-(–)-2-methoxymaackiain (Máximo and Lourenço, 1998; Mizuno *et al.,* 1990), ulexin A (Máximo *et al.,* 2000), crotaramosmine (Rao *et al.,* 1998), (6a*R*,11a*R*)-(–)-4-methoxymaackiain (Máximo and Lourenço, 1998; Cook *et al.,* 1978), ulexin C (Máximo *et al.,* 2002), 4-hydroxylonchocarpin (Filho *et al.,* 1975; Miyase *et al.,* 1980), (6a*R*,11a*R*)-(–)-maackiain (Soby *et al.,* 1996), isoderrone (Máximo and Lourenço, 1998); (Tahara *et al.,* 1989), lupalbigenin and isolupalbigenin (Tahara *et al.,* 1994).

Physical and spectroscopic measurements

Melting points were measured on a Reichert thermovar apparatus and are uncorrected. NMR spectra were recorded on a Bruker ARX 400. The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and referenced to the signal of residual $CHCl_3$ (δ 7.26 and 77.0). The EIMS were recorded on a Hewlett Packard LC/MS HP 1100 apparatus. The FTIR spectra were recorded on a Perkin Elmer Spectrum 1000 apparatus. The UV spectra were recorded on a Milton Roy Spectronic 1201. Silica gel 10% deactivated with water (Merck 7734) was used for the column chromatography separations.

Derrone (**1**)

Yellow crystals (2.0 mg), m.p. 179–181° (acetone:*n*-hex). IR υ_{max}^{NaCl} cm^{-1} 3367, 2922, 2846, 1651, 1612, 1574, 1515, 1432, 1317, 1246, 1210, 1174, 1111, 837. UV λ_{max}^{MeOH} nm (log ε) 266 (4.22), 299 sh, 349 sh, +NaOMe 273, 304 sh, 368, +NaOAc 267, 298, 346, +$AlCl_3$ 222, 280, 312 sh, 408. ^{1}H NMR: Table I. ^{13}C NMR: Table I. EIMS (70 eV) *m/z* (rel. int.): $[M]^+$ 336 (17), 321 (100), 203 (13), 160.4 (20), 152 (6), 118 (5).

Results and Discussion

Derrone (**1**) isolated from *U. jussiaei*, $C_{20}H_{16}O_5$ (*m/z* 336 $[M]^+$ in EIMS), was obtained as yellow crystals. Characteristic C-5–OH isoflavone signals were present in the ^{1}H NMR and ^{13}C NMR spectra (proton signals at $\delta_{C\text{-}5-OH}$ 12.90 *s*, $\delta_{H\text{-}2}$ 7.88 *s* and carbon signals at $\delta_{C\text{-}2}$ 152.6 *d*, $\delta_{C\text{-}3}$ 123.7 *s* and $\delta_{C\text{-}4}$ 181.2 *s*). The IR spectrum confirmed the presence of the α,β-insaturated ketone of ring C by the correspondent carbonyl absorption ($\upsilon_{C=O}$ 1651 cm^{-1}), and establishing a hydrogen bond with the hydroxyl group at C-5 ($\upsilon_{C=O}$ 1612 cm^{-1}). The ^{1}H NMR spectrum also showed characteristic signals of a 2,2-dimethylpyran substituent (proton signals at $\delta_{H\text{-}3''}$ 5.90 *d* ($J_{H\text{-}3'',H\text{-}4''}$ = 10.0 Hz), $\delta_{H\text{-}4''}$ 6.83 *d* ($J_{H\text{-}4'',H\text{-}3''}$ = 10.0 Hz), $\delta_{5''\text{-}Me}/\delta_{6''\text{-}Me}$ 1.47 *s* and carbon signals at $\delta_{C\text{-}3''}$ 127.6 *d*, $\delta_{C\text{-}4''}$ 114.7 *s* and $\delta_{C\text{-}5''}/\delta_{C\text{-}6''}$ 28.1 *s*) whose location in ring A was established by UV data. Addition of NaOMe induced a bathochromic displacement of band II of the original spectrum (λ_{max} 266 nm → λ_{max} 273 nm) witch is consistent with a free C-4′ hydroxyl group. Addition of NaOAc induced no shift which proved the inexistence of a free hydroxyl group in C-7.

The ^{1}H NMR also showed two doublets of four aromatic protons with vicinal coupling constants characteristic of ring B of the isoflavone, and one proton signal at δ 6.30 *s* of aromatic proton in ring A (see Table I).

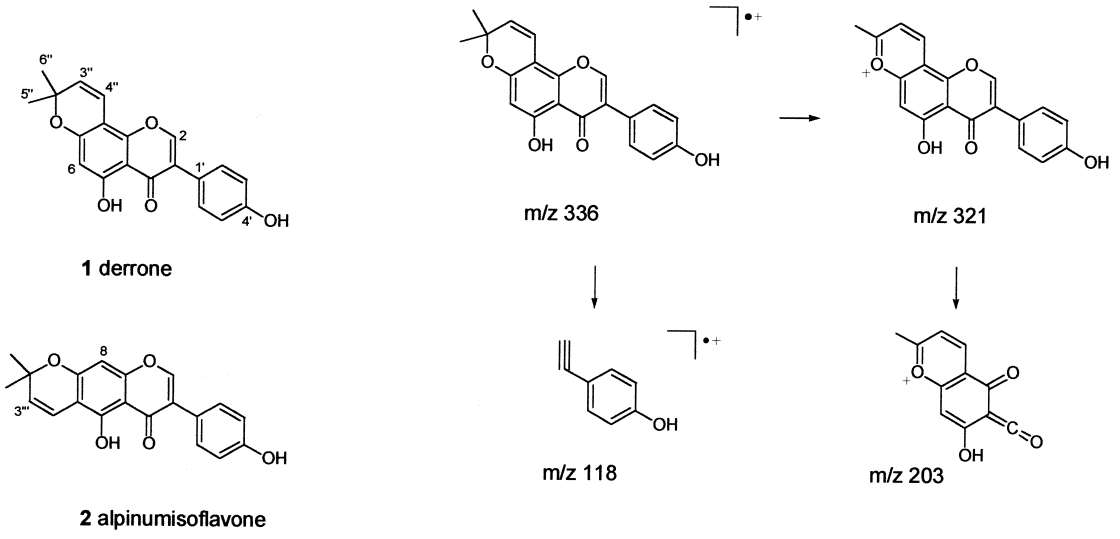

Fig. 1. Proposed EIMS fragmentation pattern of derrone (**1**).

From the above data the position of the 2,2-dimethylpyran group in ring A is not clear. To make this assignment the UV spectra are of the utmost importance since they allow angular and linear isomers to be distinguished. As publish by Tsukayama and co-workers (1992) for C-5–OH isoflavones with 2,2-dimethylpyran groups on ring A, the angular and linear structures have different behaviours by the addition of aluminum chloride. By addition of $AlCl_3$ the angular structures show a band II bathochromic shift of 8–16 nm together with a new absorption maximum at a much longer wavelength (408–415 nm). Such characteristic shift is not observed for the linear compounds. For compound **1** addition of $AlCl_3$ to the MeOH solution resulted in a band II bathochromic shift of 14 nm and the appearance of another band at 408 nm (Materials and Methods). The angular structure of compound **1** was also confirmed by the correlation observed in the HMBC spectrum between C-6 and C-5-O*H* (Table I).

From this experimental evidence we disagree with other authors (Chibber and Sharma, 1980; Tanaka *et al.,* 1998) that published the natural occurrence of derrone. In fact they are describing the isomeric linear structure, alpinumisoflavone (**2**), as have already been discussed by Tsukayama *et al.* (1992). The EIMS spectrum is in agreement with derrone (**1**) structure (Fig. 1). It presents a fragment at *m/z* 321 amu due to the loss of a methyl radical of the 2,2-dimethylpyran substituent, and the fragments at *m/z* 203 amu and *m/z* 118 amu that result from the *retro* Diels-Alder rupture of ring C.

Table I. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data for compound **1** ($CDCl_3$, coupling constants (*J*) in Hz).

H	δ_H	C	δ_C	HMBC
2	7.88 *s*	2	152.6 *d*	–
		3	123.7 *s*	H-2′,H-6′
		4	181.2 *s*	H-2
5-OH	12.90 *s*	5	162.5 *s*	–
6	6.30 *s*	6	100.4 *d*	C_5-O*H*
		7	159.8 *s*	H-4″
		8	101.2 *s*	H-6,H-3″
		9	152.6 *s*	H-2
		10	106.1 *s*	H-6, C_5-O*H*
		1′	123.1 *s*	H-2,H-3′,H-5′
2′	7.40 *d* (8.0)	2′	130.5 *d*	H-6′
3′	6.87 *d* (8.4)	3′	115.7 *d*	H-5′
		4′	156.1 *s*	H-2′,H-6′
5′	6.87 *d* (8.4)	5′	115.7 *d*	H-3′
6′	7.40 *d* (8.0)	6′	130.5 *d*	H-2′
		2″	78.1 *s*	H-4″
3″	5.90 *d* (10.0)	3″	127.6 *d*	H-5″,H-6″
4″	6.83 *d* (10.0)	4″	114.7 *d*	
5″-Me	1.47 *s*	5″	28.1 *q*	H-6″
6″-Me	1.47 *s*	6″	28.1 *q*	H-5″

δ values for compound **1** are referenced to the signal of residual $CHCl_3$ (δ 7.26 ppm and δ 77.0 ppm).

All the ^{13}C NMR signals of derrone (**1**) were assigned from HMQB and HMBC spectra (Table I). From the HMQC spectrum the chemical shifts of the protonated carbon atoms were assigned, as follows: δ 152.6 *d* (C-2), δ 130.5 *d* (C-2′, C-6′), δ 127.6 *d* (C-3″), δ 115.7 *d* (C-3′, C-5′), δ 114.7 *d* (C-4″), δ 100.4 *d* (C-6), δ 28.1 *d* (C-5″, C-6″). From the HMBC spectrum it was possible to determinate the chemical shifts of C-8 (δ 101.2) and C-10 (δ 106.1) and assign all the remaining quaternary carbon atoms.

From the above reasons we can establish that compound **1** is derrone (5,4′-dihydroxy-7,8-(2,2-dimethylpyrano)isoflavone), now isolated for the first time from a natural source.

The antifungal activity of derrone (**1**) was tested against *Cladosporium cucumerinum* by the bioautographic TLC bioassay. (Máximo *et al.,* 2000) Considering the structure/activity relations proposed from the tests performed, with the same fungus, over fourteen isoflavones isolated from *Ulex* species (Máximo *et al.,* 2000, Máximo *et al.,* 2002), it was predictable that derrone (**1**) was inactive. From the previous studies it was clear that the prenyl substitution of isoflavonoids is important for activity, and that open chains substituted structures are more active then the cyclic ones. It was also observed that for isoflavones just with a 2,2-dimethylpyran substitution, the absence of this group in ring B implies no activity. The same result as for derrone (**1**) was observed for alpinumisoflavone (**2**). (Máximo *et al.,* 2002)

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