A New Prenylisoflavone from *Ulex jussiaei*

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**Ulex**, Isoflavones, Derrone

A new naturally occurring isoflavone, derrone, was isolated from *Ulex jussiaei* (Leguminosae) together with the isoflavones ulexins A–C, lupalbigenin, isolupalbigenin, 7-O-methylisolupalbigenin, isolupalbigenone, ulexone A and isochandalone, the pterocarpans \((6aR,11aR)-(\_)-maackiain\), \((6aR,11aR)-(\_)-2-methoxymaackiain\) and \((6aR,11aR)-(\_)-4-methoxymaackiain\), 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 (301) and dichloromethane (281) 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 chromato-
graphed on silica gel columns and on silica gel 60
F254 TLC plates (Merck 5554) using n-hexane–
EtOAc, n-hexane–Et2O and CHCl3–MeOH mix-
tures 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 (21.0 mg), isochandalone (11.1 mg),
(6aR,11aR)-(−)-2-methoxymaackiain (15.4 mg),
ulexin A (7.1 mg), crotaramosmine (< 0.1 mg),
(6aR,11aR)-(−)-4-methoxymaackiain (36.1 mg),
ulexin C (1.8 mg), 4-hydroxylochnocaparin (2.4 mg),
(6aR,11aR)-(−)-maackiain (130.9 mg), isoderrone
(62.6 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, 1H NMR, 13C
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 physi-
cal and spectroscopic data, comparison with au-
thentic samples, and literature (ulexin B (Maxi-
mo et al., 2000, Singhal et al., 1980), 7-O-methyliso-
lupalbigenin (Maximo et al., 2002), ulexone A (Ru-
sell et al., 1990), isochandalone (Tahara et al.,
1989), (6aR,11aR)-(−)-2-methoxymaackiain (Maxi-
mo and Lourenço, 1998; Mizuno et al., 1990),
ulexin A (Maximo et al., 2000), crotaramosmine
(Rao et al., 1998), (6aR,11aR)-(−)-4-methoxyma-
ackiain (Maximo and Lourenço, 1998; Cook
et al., 1978), ulexin C (Maximo et al., 2002), 4-
hydroxylochnocaparin (Filho et al., 1975; Miyase
et al., 1980), (6aR,11aR)-(−)-maackiain (Soby
et al., 1996), isoderrone (Maximo and Lourenço,
1998); (Tahara et al., 1989), lupalbigenin and isolupalbi-
genin (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
1H NMR and 13C NMR spectra were recorded in
CDCl3 and referenced to the signal of residual
CHCl3 (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 El-
mer 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° (ace-
tone:n-hex). IR νmax cm−1 3367, 2922, 2846, 1651,
1612, 1574, 1515, 1432, 1317, 1246, 1210, 1174,
1111, 837. UV λmax NM (log ε) 266 (4.22), 299 sh,
349 sh, +NaOMe 273, 304 sh, 368, +NaOAc 267,
298, 346, +AlCl3 222, 280, 312 sh, 408. 1H NMR: Table I.

13C 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, C20H16O5
(m/z 336 [M]+ in EIMS), was obtained as yellow
crystals. Characteristic C-5—OH isoflavone signals
were present in the 1H NMR and 13C NMR
spectra (proton signals at δC-5—OH 12.90 s, δH-2
7.88 s and carbon signals at δC-2 152.6 d, δC-3 123.7
s and δC-4 181.2 s). The IR spectrum confirmed the
presence of the α,β-insaturated ketone of ring C
by the correspondent carbonyl absorption (νC=O
1651 cm−1), and establishing a hydrogen bond with
the hydroxyl group at C-7 (νC-O 1612 cm−1). The
1H NMR spectrum also showed characteristic sig-
nals of a 2,2-dimethylpyran substituent (proton
signals at δH-3,δH-4 = 10.0 Hz),
δH-4 6.83 d (JH-4,H-3 = 10.0 Hz), δC-Me/δC-Me
1.47 s and carbon signals at δC-3 127.6 d, δC-4
114.7 s and δC-5/δC-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 (λmax 266 nm →
λmax 273 nm) which is consistent with a free C-4'
hydroxyl group. Addition of NaOAc induced no
shift which proved the inexistence of a free hy-
droxyl group in C-7.

The 1H 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).
From the above data the position of the 2,2-di- 
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

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).

<table>
<thead>
<tr>
<th>H</th>
<th>$\delta_H$</th>
<th>C</th>
<th>$\delta_C$</th>
<th>HMBC</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>7.88 s</td>
<td>2</td>
<td>152.6 d</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>123.7 s</td>
<td>3</td>
<td>159.8 s</td>
<td>H-2',H-6'</td>
</tr>
<tr>
<td>4</td>
<td>181.2 s</td>
<td>4</td>
<td>–</td>
<td>H-2</td>
</tr>
<tr>
<td>5-OH</td>
<td>12.90 s</td>
<td>5</td>
<td>162.5 s</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>6.30 s</td>
<td>6</td>
<td>100.4 d</td>
<td>C$_5$-OH</td>
</tr>
<tr>
<td>7</td>
<td>159.8 s</td>
<td>7</td>
<td>104.1 d</td>
<td>H-4'</td>
</tr>
<tr>
<td>8</td>
<td>101.2 s</td>
<td>8</td>
<td>101.2 s</td>
<td>H-6,H-3'</td>
</tr>
<tr>
<td>9</td>
<td>152.6 s</td>
<td>9</td>
<td>115.7 d</td>
<td>H-2</td>
</tr>
<tr>
<td>10</td>
<td>106.1 s</td>
<td>10</td>
<td>123.1 s</td>
<td>H-2,H-4'</td>
</tr>
<tr>
<td></td>
<td>1'</td>
<td></td>
<td>123.1 s</td>
<td>H-2,H-4',3',H-5'</td>
</tr>
<tr>
<td>2'</td>
<td>7.40 d</td>
<td>2'</td>
<td>130.5 d</td>
<td>H-6'</td>
</tr>
<tr>
<td>3'</td>
<td>6.87 d</td>
<td>3'</td>
<td>115.7 d</td>
<td>H-5'</td>
</tr>
<tr>
<td>4'</td>
<td>156.1 s</td>
<td>4'</td>
<td>115.7 d</td>
<td>H-2',H-6'</td>
</tr>
<tr>
<td>5'</td>
<td>6.87 d</td>
<td>5'</td>
<td>115.7 d</td>
<td>H-3'</td>
</tr>
<tr>
<td>6'</td>
<td>7.40 d</td>
<td>6'</td>
<td>130.5 d</td>
<td>H-2'</td>
</tr>
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<td></td>
<td>2'</td>
<td></td>
<td>78.1 s</td>
<td>H-4'</td>
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<tr>
<td>3''</td>
<td>5.90 d</td>
<td>3''</td>
<td>127.6 d</td>
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</tr>
<tr>
<td>4''</td>
<td>6.83 d</td>
<td>4''</td>
<td>114.7 d</td>
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<tr>
<td>5''-Me</td>
<td>1.47 s</td>
<td>5''</td>
<td>28.1 q</td>
<td>H-6''</td>
</tr>
<tr>
<td>6''-Me</td>
<td>1.47 s</td>
<td>6''</td>
<td>28.1 q</td>
<td>H-5''</td>
</tr>
</tbody>
</table>

$\delta$ values for compound 1 are referenced to the signal of residual CHCl$_3$ ($\delta$ 7.26 ppm and $\delta$ 77.0 ppm).

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-OH (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.
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: $\delta$ 152.6 $d$ (C-2), $\delta$ 130.5 $d$ (C-2', C-6'), $\delta$ 127.6 $d$ (C-3'), $\delta$ 115.7 $d$ (C-3', C-5'), $\delta$ 114.7 $d$ (C-4'), $\delta$ 100.4 $d$ (C-6), $\delta$ 28.1 $d$ (C-5', C-6'). From the HMBC spectrum it was possible to determine the chemical shifts of C-8 ($\delta$ 101.2) and C-10 ($\delta$ 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-dimethylpyran)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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