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A PTEROCARPAN FROM *ULEX PARVIFLORUS*

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Abstract—A new pterocarpan has been isolated from *Ulex parviflorus*. The structure of this compound, (−)-2,3,4-trimethoxy-8,9-methylenedioxypterocarpan, was established by spectroscopic means. In addition, the known pterocarpans, (−)-maackiain, (−)-4-methoxymaackiain, (−)-2-methoxymaackiain, the isoflavone isoderrone and the triterpenoid soyasapogenol B were isolated from the same source. New ^{13}C NMR data for some of these known compounds are also included.

INTRODUCTION

Pterocarpans are constituents of plants belonging to the genus *Ulex* [1, 2] and other species of the Leguminosae. Compounds of this type are well known for their antifungal activity (e.g. as phytoalexins) [3] and their occurrence in leguminous species is of taxonomic significance. We have investigated the constituents of *Ulex parviflorus*, isolating a new pterocarpan (−)-2,3,4-trimethoxy-8,9-methylenedioxypterocarpan (**1**), together with three other compounds of this class, maackiain (**2**) [3–6], (−)-4-methoxymaackiain (**3**) [7–9] and (−)-2-methoxymaackiain (**4**) [10], the triterpenoid soyasapogenol B (**5**) [11] and the isoflavone isoderrone (**8**) [12]. We report here on the structural elucidation of the new pterocarpan (**1**), and the unequivocal assignment of the ^{13}C NMR spectra of (**2**), (**3**) and (**8**), as well as some comments on the previous ^{13}C NMR assignments for compound (**5**) [11].

RESULTS AND DISCUSSION

The ^{1}H and ^{13}C NMR spectra of (**1**) (Tables 1 and 2, respectively) showed characteristic signals for a pterocarpan structure [13]. These were similar to those of (−)-maackiain (**2**) [3–6], showing an identical pattern for the signals corresponding to rings B, C and D, thus establishing the same structural part for both compounds. In addition, compound (**1**) possessed three methoxyl groups (δ_H 3.81 *s*, 3H and 3.83 *s*, 6H; δ_C 56.3 *q*, 61.2 *q* and 61.4 *q*), which were placed on ring A (single aromatic proton at δ 6.72 *s*, and carbon signals at 107.4 *d*, 114.9 *s*, 148.1 *s*, 142.6 *s*, 143.6 *s* and 143.6 *s*), in contrast to ring A of (**2**) which contains only an OH group at C-3 (δ_{OH} 4.88 *s*, ^{1}H, H-1, H-2 and H-4 aromatic protons at δ 7.30 *d*, 6.48 *dd* and 6.35 *d*, $J_{1,2}$ = 8.4 Hz, $J_{2,4}$ = 2.0 Hz). The mass spectrum of (**1**) showed a $[M]^+$ at *m/z* 358 ($C_{19}H_{18}O_7$), which differs by 74 mu from that molecular ion of (**2**) thus confirming the above deductions based on NMR analysis.

From these data, the new pterocarpan must have three methoxyl substituents in ring A. The arrangement of these substituents was established as 2,3,4-trimethoxy, because irradiation of the signal for the H-11a proton (δ 5.39) produced NOE enhancements in the H-6a proton (δ 3.43) and in the signal corresponding to the aromatic proton at δ 6.72 *s*, thus establishing that this proton must be attached to the C-1 position and, consequently, confirming the 2,3,4-positions for the three methoxyl groups of (**1**).

The HMQC and HMBC spectra confirmed structure (**1**) for the new pterocarpan. The HMQC spectrum provided the assignment of the protonated carbon as follows: δ 40.4 *d* (C-6a), 66.8 *t* (C-6), 78.6 *d* (C-11a), 93.8 *d* (C-10), 101.3 *t* (O-CH_2-O), 104.8 *d* (C-7) and 107.4 *d* (C-1). The assignment of the quaternary carbons was achieved from the HMBC spectrum.

The signal at δ 154.0, *s* shows a ^{3}J-coupling with the signals at δ 6.66, *s* (H-7) and δ 3.40–3.46, *m* (H-6a), so that it can only be assigned to C-10a. The signal at δ 148.1, *s* has ^{3}J-coupling with the signals at δ 6.66, *s* (H-7), δ 5.84, *d* (O-CH_2-O) and δ 3.81, *q* (OMe). Interpretation of these data is only possible if the signal at δ 148.1, *s* corresponds to two carbon

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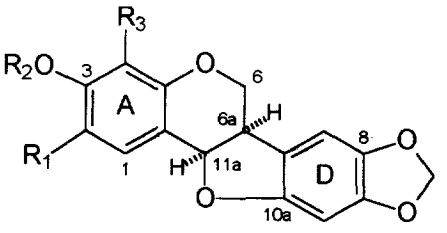

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2 R_1 = H R_2 = H R_3 = H

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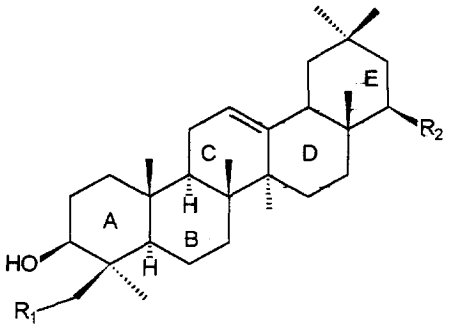
4 R_1 = OMe R_2 = H R_3 = H



5 R_1=OH R_2=OH

6 R_1=OH R_2=H

7 R_1=H R_2=OH

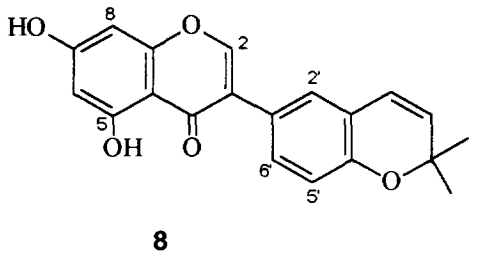


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Table 1. ^{1}H NMR spectral data of compound **1** and maackiain (**2**) (400 MHz, $CDCl_3$, TMS as int. standard)

H	1	2
1	6.72 *s*	7.30 *d*
2	—	6.48 *dd*
4	—	6.35 *d*
6βax	3.52 *t*	3.57 *t*
6βq	4.24 *dd*	4.15 *dd*
6a	3.40–3.46 *m*	3.37–3.43 *m*
7	6.66 *s*	6.65 *s*
10	6.38 *s*	6.36 *s*
11a	5.39 *d*	5.40 *d*
3-OH	—	4.88 *s*
2-OMe	3.81 *s*	—
3-OMe	3.83 *s*	—
4-OMe	3.83 *s*	—
O-CH2-O	5.84 *d*	5.84 *d*
J(Hz)		
6a/6βax	10.7	11.0
6βax/6αeq	10.7	11.0
6a/6aeq	4.4	5.0
6a/11a	6.8	6.8
1/2	—	8.4
2/4	—	2.0
O-CH_2-O	9.2	10.5

Table 2. ^{13}C NMR spectral data of compounds **1**, **3** and **4** (100 MHz, CDC_{13}, TMS as int. standard)

C	1	3	4
1	107.4 *d*	126.0 *d*	111.6 *d*
1a	114.9 *s*	113.2 *s*	110.7 *s*
2	148.1 *s*	108.9 *d*	142.1 *s*
3	142.6 *s*	149.7 *s*	147.2 *s*
4	143.6 *s*	134.9 *s*	103.6 *d*
4a	143.6 *s*	148.5 *s*	150.3 *s*
6	66.8 *t*	66.5 *t*	66.6 *t*
6a	40.4 *d*	40.0 *d*	40.4 *d*
7	104.8 *d*	104.7 *d*	104.8 *d*
7a	117.7 *s*	117.7 *s*	118.0 *s*
8	141.9 *s*	141.7 *s*	141.8 *s*
9	148.1 *s*	148.2 *s*	148.1 *s*
10	93.8 *d*	93.8 *d*	93.7 *d*
10a	154.0 *s*	154.2 *s*	154.1 *s*
11a	78.6 *d*	78.4 *d*	78.8 *d*
2-OMe	56.3 *q*	—	56.4 *q*
3-OMe	61.2 *q*	—	—
4-OMe	61.2 *q*	61.1 *q*	—
O-CH_2-O	101.3 *t*	101.3 *t*	101.3 *t*

atoms. The former coupling can be attributed to C-9, the latter to C-2 coupling with the methoxyl protons. From these data, we can also assign the signal at δ 3.81, *q* in the ^{1}H NMR spectrum to the methoxyl group at C-2 (Table 1). The signal at δ 143.6, *s* shows ^3J-coupling with protons at δ 6.72, *s* (H-1), *d* 3.52, *t* (H-6βax), δ 4.24, *dd* (H-6αeq), δ 5.39, *d* (H-11a) and

δ 3.83, *q* (OMe). This means that the last ^3J-coupling corresponds to C-4 coupled with the protons of its attached methoxyl group (C-4-OMe). C-4a must be responsible for the other couplings, so its chemical shift is overlapped with that of C-4. The signal at *d* 141.9, *s* has ^3J-coupling with the proton signals at δ 6.38, *s* (H-10) and δ 5.84, *d* (O-CH_2-O), so it must correspond to the C-8 carbon atom. The signal at δ 142.6, *s* is assigned to C-3 because it shows ^3J-coupling with the proton signals at δ 6.72, *s* (H-1) and at δ 3.83, *q* (OMe). This confirms that the signal for the methoxyl protons on C-3 is at δ 3.83, *q* in the ^{1}H NMR spectrum overlapped with methoxyl protons on C-4.

It is known that natural pterocarpans occur in nature in two enantiomeric forms, 6a*S*,11a*S* and 6a*R*,11a*R*, which correspond to the *dextro*- and *laevo*-isomers, respectively, and that all these compounds possess the more stable *cis*-junction of rings B and C [3, 14]. Since the coupling values of the C-6 methylene and the C-6a and C-11a methine protons of (**1**) are identical (Table 1) to those found in other *cis*-pterocarpans, such as (**2**–**4**) [3–10], we conclude that (**1**) possesses the 6a*R*, 11a*R* absolute configuration, because it shows an $[\alpha]_D^{20}$ value of $-246.2°$ ($CHCl_3$; *c* 0.104). From all the above data, the new pterocarpan must possess structure (**1**) [(−)-2,3,4-trimethoxy-8,9-methylenedioxypterocarpan].

The HMQC and HMBC spectra of (**3**) and (**4**) allowed the unequivocal assignment of their ^{13}C NMR spectra (Table 2). From these data, we can also interchange the previous assignements [10] of the ^{1}H NMR for H-4 and H-10 of compound (**4**). For this compound (**4**), the absolute configuration 6a*R*,11a*R* was also confirmed from the negative sign of its optical rotation ($[\alpha]_D^{20} - 324.3°$ ($CHCl_3$; *c* 0.065)).

For the terpenoid soyasapogenol B (**5**), the reported [11] assignments for the C-3 and C-22 carbons (δ 76.6 for C-3 and δ 80.9 for C-22), respectively) must be reversed to δ 80.3 *d* (C-3) and δ 76.6 *d* (C-22). This was supported by comparing the ^{13}C NMR data of (**5**) with those of several oleonane derivatives already published [15]. In particular the chemical shifts of the A, B and C ring carbons of (**5**) (including δ 80.3 for C-3) are identical to those of oleon-12-ene-3β,24β-diol (**6**) and the carbons belonging to rings D and E (including δ 76.6 for C-22) resonated at the same field as those of oleon-12-ene-3β,22β-diol (sophoradiol, **7**) [15].

Finally, Table 3 shows the assignment of the ^{1}H NMR spectrum of isoderrone (**8**), as well as its ^{13}C NMR data, not previously reported. Unequivocal assignment of the ^{13}C NMR signals was accomplished from HMQC and HMBC spectra, which firmly supported the data in Table 3.

Table 3. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of isoderrone **8** ($CDCl_3$, TMS as int. standard).

H	δ (ppm)	C	δ (ppm)
2	7.75 *s*	2	152.8
5-OH	12.80 *s*	3	122.8
6	6.19 *s*	4	180.9
8	6.24 *s*	5	162.7
2′	7.07 *s*	6	99.6
5′	6.74 *d*	7	162.7
6′	7.13 *d*	8	94.1
3″	5.55 *d*	9	158.0
4″	6.26 *d*	10	106.0
5″	1.36 *s*	1′	123.4
6″	1.36 *s*	2′	127.0
J (Hz)		3′	121.4
$J_{H5'-H6'}$	8.2	4′	153.3
$J_{H6'-H5'}$	8.1	5′	116.5
$J_{H3''-H4''}$	9.8	6′	129.5
$J_{H4''-H3''}$	9.6	2″	[a]
		3″	131.1
		4″	122.0
		5″	28.0
		6″	28.0

[a] Overlapped with chloroform signal.

EXPERIMENTAL

Mps: uncorr. Plant materials were collected in April 1994, at Peninha/Sintra (Portugal) and voucher specimens are deposited in the herbarium of Museu, Laboratório, Jardim Botânico da Faculdade de Ciências da Universidade de Lisboa [ASCE 2595]. The NMR spectra were recorded on a Bruker ARX 400 MHz apparatus. The MS were recorded on a Shimatzu QP1000 apparatus. The FT-IR spectra were recorded on a Perkin Elmer Spectrum 1000 apparatus. The UV spectra were recorded on a Milton Roy Spectronic 1201.

Extraction and isolation

Dried and finely powdered aerial parts of (915 g) were extracted sucessively with petrol (15 l) and CH_2Cl_2 (20 l) at room temp. The dried CH_2Cl_2 extract (6.8 g) was chromatographed on a silica gel column (Merck, No. 7734) and eluted with *n*-hexane–EtOAc mixts (9 : 1, 8 : 2, 7 : 3 and 6 : 4). The fr. collected with the most polar of these mixts was rechromatographed on an equivalent silica gel column and again eluted with *n*-hexane–EtOAc mixts (9 : 1, 8 : 2, 7 : 3 and 6 : 4). From this chromatography, frs a–c were collected. Fr. a provided five compounds after silica gel TLC eluted twice with $CHCl_3$. Compounds (**1**), (**4**), (**3**), (**2**) and isoderrone (**8**) were detected in order of increasing chromatographic polarity. Each compound was purified on silica gel columns or silica gel TLC eluted with *n*-hexane–AcO Et, $CHCl_3$–MeOH or CH_2Cl_2–MeOH mixts, before spectroscopic analysis and physical constants were obtained. From fr. c, the triterpenoid soyasapogenol (**5**) was collected and further purification was accomplished by identical means as described above for the pterocarpans. The previously known

compounds, (−)-4-methoxymaackiain (**3**) (5.1 mg) [7–9], (−)-2-methoxymaackiain (**4**) (4.1 mg) [10], maackiain (**2**) (4 mg) [3–6], soyasapogenol B (**5**) (20 mg) [11] and isoderrone (**8**) (10.2 mg) [12], were identified from their physical (mp, $[\alpha]_D$) and spectroscopic data.

(−)-2,3,4-*Trimethoxy*-8,9-*methylenedioxypterocarpan* (**1**).

White crystals (3.4 mg), mp 165–166° (MeOH). $[\alpha]_D^{20}$ −246.2° ($CHCl_3$; *c* 0.104). IR υ_{max}^{KBr} cm^{-1}: 2940, 1610, 1480, 1460, 1360, 1150, 1100, 1050, 1030, 1010, 930, 830. UV λ_{max} nm: 209, 239, 304. ^1H NMR: Table 1. ^{13}C NMR: Table 2. EIGC-MS (70 eV) *m/z* (rel. int.): [M]+ 358 (100), 357 (79), 343 (35), 311 (20), 207 (8), 191 (1), 181 (7), 179 (9), 175 (9), 162 (16), 149 (11).

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