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PTEROCARPANS FROM *ONONIS VISCOSA* SUBSP. *BREVIFLORA*

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Key Word Index—*Ononis viscosa* subsp. *breviflora*; Leguminosae; alkylbenzoic acid; pterocarpans; dihidropterocarpans.

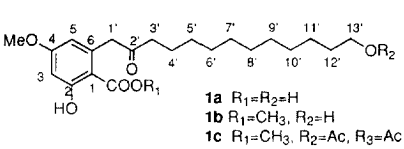
Abstract—Pterocarpin, homopterocarpin, medicarpin, maackiain, and three new compounds, 2-hydroxy-4-methoxy-6-(13-hydroxy-2-oxotridecyl)benzoic acid, 11b-hydroxy-11b,1-dihydromedicarpin and 11b-hydroxy-11b,1-dihydromaackiain were identified as minor components in a chloroform extract of *Ononis viscosa* subsp. *breviflora*.

INTRODUCTION

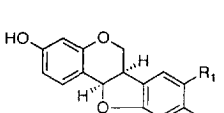
The chloroform extract of *Ononis viscosa* L. subsp. *breviflora* (Leguminosae) is mainly composed of alkylresorcinols, alkylbenzoic acids and alkylisocoumarins. Additionally, we have previously reported the presence of the pterocarpans variabilin and anhydrovariabilin [1]. Here we report the identification of 2-hydroxy-4-methoxy-6-(13-hydroxy-2-oxotridecyl)benzoic acid (**1a**) and pterocarpans **4** and **5**.

RESULTS

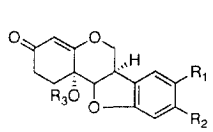
Study of the minor compounds in a chloroform extract of *O. viscosa* subsp. *breviflora* led to the identification of the known pterocarpans pterocarpin [2], homopterocarpin [2], medicarpin (**2**) [3] and maakiain (**3**) [4] the alkylbenzoic acid **1a** and two dihydro derivatives of **2** and **3**, compounds **4** and **5**, respectively.



1a R_1=R_2=H
1b R_1=CH_3, R_2=H
1c R_1=CH_3, R_2=Ac, R_3=Ac



2 R_1=H, R_2=OCH_3
3 R_1 R_2=OCH_2O



4 R_1=H, R_2=OCH_3, R_3=H
5 R_1 R_2=OCH_2O, R_3=H
5a R_1 R_2=OCH_2O, R_3=Ac

1a was isolated and characterized as its methyl ester **1b** that is an oily product for which HRMS shows a $C_{22}H_{34}O_6$ molecular formula. Its IR spectrum has absorption bands due to hydroxyl (3405 cm^{-1}) and carbonyl (1709, 1654 cm^{-1}) groups. Its ^1H NMR spectrum shows signals due to a 1,2,3,5-tetrasubstituted aromatic ring (δ 6.40 and 6.22 ppm), two methoxy groups, one on the aromatic ring (δ 3.79 ppm) and the other in the methoxycarbonyl (δ 3.81) moiety. The signal of a phenolic hydroxyl group is deshielded by intramolecular hydrogen bonding (δ 11.59). The remaining signals are assignable to an aliphatic chain with a terminal primary hydroxyl (δ 3.61, 2H, *t*, J = 7.4 Hz) and a keto function at C-2 (δ 3.88, 2H, s, H-1′ and δ 2.40, 2H, *t*, J = 7.4 Hz, H-3′). The presence of two hydroxyls was confirmed by preparation of the diacetate **1c**. Its ^1H NMR spectrum has signals of two acetate methyl groups, one aromatic acetoxyl (δ 2.27 ppm) and one aliphatic (δ 2.02), as well as the signal of the methylene which bears the last acetoxyl, which is deshielded (δ 4.05). All these data indicate **1b** the structure of methyl 2-hydroxy-4-methoxy-6-(13-hydroxy-2-oxotridecyl) benzoate, also in agreement with the signals present in its ^{13}C NMR spectrum (Table 1) and the fragmentations in the MS.

Small amounts of a mixture of the medicarpin and maackiain derivatives **4** and **5** were purified by semipreparative HPLC. A mixture of **4** and **5** in a 1:2 ratio was obtained. The HREIMS indicates that **4** has a molecular formula $C_{16}H_{16}O_5$ and **5** a $C_{16}H_{14}O_6$.

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Table 1. ^{1}H NMR (500 MHz) data for **4**,* **5*** and **5a**†

Hydrogen	**4**‡	**5**‡	**5a**§
H-1α	2.20–2.28 *m*	2.20–2.28 *m*	2.40–2.95 *m*
H-1β	2.58–2.70 *m*	2.58–2.70 *m*	2.40–2.95 *m*
H-2β	2.02–2.11 *m*	2.02–2.11 *m*	2.40–2.95 *m*
H-2α	2.65–2.88 *m*	2.65–2.88 *m*	2.40–2.95 *m*
H-4	5.38 *s*	5.37 *s*	5.61 *s*
H-6α	4.79 *dd*	4.76 *dd*	4.44 *dd*
H-6β	4.22 *dd*	4.23 *dd*	4.16 *d*
H-6a	3.92 *ddd*	3.91 *ddd*	3.71 *dd*
H-7	7.21 *bd*	6.85 *bd*	6.62 *bs*
H-8	6.47 *dd*		
H-10	6.31 *d*	6.32 *s*	6.31 *s*
H-11a	4.96 *d*	4.94 *d*	5.73 *d*
OH	5.24 *s*	5.25 *s*	
OCH_3	3.73 *s*		
OCH_2O		5.92 *d*	5.92 *d*
		5.90 *d*	5.89 *d*
AcO			2.14 *s*

*Me_2CO-d_6; †$CDCl_3$; ‡$J_{6\alpha,6a}$ = 0.6 Hz, $J_{6\beta,6a}$ = 4 Hz, $J_{6\alpha,6\beta}$ = 10.9 Hz, $J_{6a,11a}$ = 10.1 Hz, $J_{7,8}$ = 8.3 Hz, $J_{8,10}$ = 2.3 Hz; §$J_{6\alpha,6a}$ = 4 Hz, $J_{6\beta,6a}$ = 0 Hz, $J_{6\alpha,6\beta}$ = 10.9 Hz, $J_{6a,11a}$ = 9.8 Hz.

Table 2. ^{13}C NMR (125 MHz) data for **4**,* **5*** and **5a**†

Carbon	**4**	**5**	**5a**
C-1	31.8‡	31.8‡	27.0
C-2	31.9‡	31.9‡	32.9
C-3	196.9	196.9	198.2
C-4	107.9	107.9	110.9
C-4a	171.4	171.4	169.5
C-6	67.5	67.2	67.7
C-6a	39.7	40.5	40.5
C-6b	120.0	119.0	117.8
C-7	107.0	104.4	109.0
C-8	124.8	141.0	142.7
C-9	161.2§	148.1	148.7
C-10	95.2	92.1	93.2
C-10a	160.5§	153.8	153.5
C-11a	83.0	83.0	79.0
C-11b	67.0	67.0	76.3
OCH_3	54.8		
OCH_2O		101.3	101.6
CH_3COO			170.1
CH_3COO			21.1

* Me_2CO-d_6; †$CDCl_3$; ‡,§may be interchanged.

Acetylation of **4**+**5** with acetic anhydride in pyridine at room temperature progressed very slowly, and refluxing conditions were necessary to complete the reaction. After work-up and chromatographic purification of the reaction residue, the monoacetate **5a** was isolated. CIMS of **5a** has an $[M+1]^+$ at *m/z* 345 in agreement with a formula of $C_{18}H_{16}O_7$. Its IR spectrum shows bands of carbonyl acetate and α,β-insaturated ketone at 1745 and 1645 cm^{-1} respectively. ^{13}C and ^{1}H NMR spectra are similar to those published for maackiain [4] but with some differences in ring A. Chemical shifts and multiplicity due to signals of the six carbons of ring D in the ^{13}C NMR (Table 2) allows a structure of 11b-acetoxy-11b,1-dihydromaackiain to be established for **5a**. Relative configuration has been established with the aid of the coupling constants of its ^{1}H NMR spectrum. So $J_{6\beta,6\alpha}$ = 0 Hz can be justified only by a *cis* union of the B and C rings and a *cis* relative disposition of H-11$_a$ and the acetoxyl group (Fig. 1).

Compound **4** could not be isolated from its mixture with **5** and its structure has been established by comparison of the mixture spectra with those of **5a**. The EIMS shows $[M]^+$ at *m/z* 288 and 302, with base peaks at *m/z* 161 and 175 for **4** and **5** respectively. In the IR spectrum, bands due to absorption of hydroxyl groups (3352 cm^{-1}) and an α,β-unsaturated carbonyl with an alkoxy function in β-position (1620 cm^{-1}) [5] were observed. In ^{1}H and ^{13}C NMR spectra of the mixture, signals of **5** were easily assignated by comparison with those of **5a**, furthermore those corresponding to **4** are perfectly separated and also were easily assignated (Tables 1 and 2). Besides, assignations for **4** and **5** were corroborated through the correlations observed

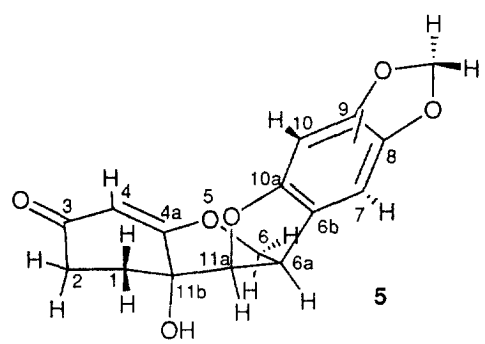

Fig. 1.

in 2D-NMR and COSY spectra (Table 3) and also in its ^{13}C-^{1}H spectrum (Table 4). So it is clear that both compounds have a pterocarpan skeleton and a similar structure for rings A, B, and C, the main differences being in the substitution pattern of the ring D. Compound **4** has a methoxyl group attached at C-9.

^{1}H-^{1}H COSY spectra for both compounds shows long range H-H correlations which are due to homoallylic couplings between H-4 and H-1α, H-11a, benzylic couplings between H-7 and H-6a, and the coupling between two aromatic protons in 1,4 position H-7 and H-10. These small couplings are in agreement with the proposed structure. Stereochemical elucidation was made in the same way as for compound **5a**, taking into account the small value of the coupling constant between H-6β and H-6a (J = 0.4 Hz).

There must be a biosynthetic connection between **4**, **5**, variabilin and anhydrovariabilin. A detoxification mechanism of the phytoalexins maackiain and medicarpin by fungal pathogens of alfalfa such as *Col-*

Table 3. Correlations observed in the COSY spectrum of **4**+**5** (500 MHz)*

4		**5**	
H-1α	H-1β, H-2α, H-2β	H-1α	H-1β, H-2α, H-2β
H-1β	H-1α, H-2α, H-2β	H-1β	H-1α, H-2α, H-2β
H-2α	H-1α, H-1β, H-2β	H-2α	H-1α, H-1β, H-2β
H-2β	H-1α, H-1β, H-2α	H-2β	H-1α, H-1β, H-2α
H-4	H-11a, H-1α	H-4	H-11a, H-1α
H-6α	H-6, H-6a	H-6α	H-6, H-6a
H-6β	H-6, H-6a	H-6β	H-6, H-6a
H-6a	H-11a	H-6a	H-11a
H-7	H-8	H-7	H-10, H-6a
H-8	H-7, H-10	H-10	H-7

* Me_2CO-d_6.

Table 4. Correlations observed in the C-H spectrum of **5** (500 MHz)*

H-1α	C-1
H-4	C-4
H-6α; H6β	C-6
H-6a	C-6a
H-7	C-7
H-10	C-10
H-11	C-11
O—CH_2—O	101.3

* Me_2CO-d_6.

letotrichum trifolii, *C. dematium* f. *truncatum*, *C. destructivum*, *Stemphylium alfalfae* and others [6] has been recently reported. These fungi are able to metabolize those phytoalexins to less toxic compounds, as the 1a epimer of **5**. Additionally, several fungi such as *Botrytis cinerea* and *Colletotrichum* species are able to transform medicarpin to several hydroxylated compounds, including 6a-hydroxymedicarin, also as a detoxification mechanism [7]. The presence of several pterocarpans as homopterocarpin, medicarpin and maackiain together with products of hydroxylation-reduction, variabilin, anhydrovariabilin, **4** and **5** in *Ononis viscosa* subsp. *breviflora*, free from fungal attack, shows that some legumes have an enzymatic system similar to that of some phytopathogenic fungi which are able to lower the concentration of pterocarpan phytoanticipins.

EXPERIMENTAL

LREIMS and LRCIMS (CH_4) were determined on a Hewlett-Packard 5988A mass spectrometer. The NMR spectra were recorded on a Bruker ARX 500 and a Bruker AMX 300 spectrometer (δ values given in ppm relative to internal $Me_4Si(=0)$ and J values in Hz). 2D NMR were made on a Bruker ARX 500 spectrometer using the sequences COSYDFST (^{1}H-^{1}H) and INVBSTF3 (^{1}H-^{13}C) of the Pulse Program Bruker Library. Assignments of ^{13}C-NMR signals were made with the aid of additivity rules, DEPT and 2D experiments. Column chromatography was carried out using Si gel Merck 60 (70–230 mesh), eluting with mixtures of hexane/$CHCl_3$ or $CHCl_3$/Me_2CO of increasing polarity. HPLC was performed with a KONIC Model 500B apparatus equipped with a diode array UV detector. Analytical TLC was performed on silica gel Merck 60 G layers of 0.25 mm thickness, using a 7% phosphomolybdic acid solution (EtOH) for compound visualization.

Extraction and isolation

Ononis viscosa subsp. *breviflora* (D.C.) Nyman was collected at Castell de Ferro (Granada, Spain) in April of 1990. It was identified by Prof. F. Valle of the Department of Botany, University of Granada. A voucher specimen is available for inspection at the Herbarium of the Faculty of Sciences of the University of Granada.

The air-dried aerial parts (2 kg), were extracted in a Soxhlet with $CHCl_3$ (24 h). From the chloroform extract (66 g, 3.3% of dried plant) fatty acids (18 g, 27% of extract) were removed by precipitation in MeOH at low temperature. The defatted extract (48 g, 73%) was then column chromatographed, eluting using a hexane/$CHCl_3$ gradient up to 100% $CHCl_3$ and $CHCl_3$/Me_2CO gradient, up to 100% acetone. Medicarpin (25 mg, 0.05%), maackiain (37 mg, 0.08%) and 2-hydroxy-4-methoxy-6-(13-hydroxy-2-oxotridecyl)benzoic acid (**1a**) (120 mg, 0.25%) were eluted with $CHCl_3$. CH_2N_2 methylation of **1a** and further column chromatography allowed to isolate methyl 2-hydroxy-4-methoxy-6-(13-hydroxy-2-oxotridecyl)benzoate (**1b**). From fractions eluting with $CHCl_3$/Me_2CO 96:4, homopterocarpin (69 mg, 0.14%) and pterocarpin (43 mg, 0.08%) were isolated. The fraction eluting with $CHCl_3$/Me_2CO 9:1 was submitted to HPLC purification on a 250 × 10 mm Nucleosil ODS column, eluting with CH_3CN:H_2O 3:8 to afford a clean mixture of 11b-hydroxy-11b,1-dihydromedicarpin (**4**) and 11b-hydroxy-11b,1-dihydromaackiain (**5**) (10 mg, 0.03%). The mixture was then refluxed with Ac_2O in pyridine. Usual work-up and further column chromatography using gradient of hexane:Et_2O (up to 100% Et_2O) allowed to isolate 11b-acetoxy-11b,1-dihydromaackiain (**5a**).

Methyl 2-hydroxy-4-methoxy-6-(13-hydroxy-2-oxotridecyl) benzoate (**1b**). Powder: mp 96–98° ($CHCl_3$–hexane); IR ν_{max}^{KBr} cm^{-1} 3405 (OH), 2921, 2850, 1709 (C=O), 1654, 1622 (Ar), 1439, 1373, 1331, 1260, 1196, 1061, 950, 842, 776, 688; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.59 (1H, *s*, OH), 6.40 (1H, *d*, J = 2.6 Hz, H-3), 6.22 (1H, *d*, J = 2.6 Hz, H-5), 3.88 (2H, *s*, H-1′), 3.81 (3H, *s*, COOMe), 3.79 (3H, *s*, C—4—OMe), 3.61 (2H, *t*, J = 7.4 Hz, H-13′), 2.40 (2H, *t*, J = 7.4 Hz, H-3′), 1.50 (4H, *m*, $W_{1/2}$ = 15 Hz, H-4′, H-12′), 1.25 (12H, *bs*, H-4′-H-12′); ^{13}C NMR

($CDCl_3$, 75 MHz) δ 207.3 (C, C-2′), 170.8 (C, COOMe), 165.6 (C, C-4), 164.0 (C, C-2), 138.8 (CH, C-6), 112.8 (CH, C-5), 105.0 (C, C-1), 99.9 (CH, C-3), 62.9 (CH_2, C-13′), 55.3 (MeO-C-4), 51.7 (COOMe), 51.2 (CH_2, C-1′), 41.8 (CH_2, C-3′), 32.7 (CH_2, C-12′), 29.4, 4*29.3, 29.1 (6*CH_2, C-5′-C-10′), 25.6 (CH_2, C-11′), 23.5 (CH_2, C-4′); LREIMS (70 eV) *m/z* $[M]^+$ 394 (2), 362 (2) $[M\text{-MeOH}]^+$, 219 (5), 206 (12), 196 (50), 165 (15), 164 (100), 135 (15), 97 (20), 83 (25), 81 (13), 69 (33), 57 (13), 55 (57), 43 (32); HRFABMS (positive ion mode) *m/z* $[MH]^+$ 395.2428 (calcd for $C_{22}H_{35}O_6$ 395.2435).

Methyl 2-acetoxy-4-methoxy-6-(13-acetoxy-2-oxotridecyl) benzoate (**1c**). Colourless oil; IR ν_{max}^{film} cm^{-1} 2926, 2852, 1770, 1725, 1611, 1572, 1459, 1433, 1366, 1320, 1274, 1239, 1199, 1152, 1098, 1041, 958, 889, 799; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.62 (1H, *d*, $J = 2.6$ Hz, H-3), 6.58 (1H, *d*, $J = 2.6$ Hz, H-5), 4.05 (2H, *t*, $J = 7.4$ Hz, H-13′), 3.82 (2H, *s*, H-1′), 3.80 (3H, *s*, COOMe), 3.78 (3H, *s*, C—4—OMe), 2.42 (2H, *t*, $J = 7.4$ Hz, H-3′); 2.27 (3H, *s*, AcO—C-2), 2.02 (3H, *s*, AcO—C—13′), 1.58 (4H, *m*, $W_{1/2} = 15$ Hz, H-4′, H-12′), 1.22 (12H, *bs*, H-5′—H-11′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.2 (C, C-2′), 171.4 (CH_3COO—C-13′), 169.3 (CH_3COO—C-2), 166.1 (C, COOMe), 160.8 (C, C-4), 155.5 (C, C-2), 138.2 (CH, C-6), 117.6 (CH, C-1), 115.8 (C, C-5), 107.8 (CH, C-3), 64.7 (CH_2, C-13′), 55.7 (MeO—C-4), 52.1 (COOMe), 48.9 (CH_2, C-1′), 42.4 (CH_2, C-3′), 29.6, 29.5, 29.3, 29.2 (CH_2, C-5′—C-10′), 28.7 (CH_2, C-12′), 26.0 (CH_2, C-11′), 23.7 (CH_2, C-4′), 21.1, 21.0 (CH_3COO—C-12′, CH_3COO—C-2); LREIMS (70 eV) *m/z* $[M]^+$ 446 (1), 404 (10), 388 (4), 280 (7), 241 (18), 206 (34), 199 (60), 196 (28), 181 (26), 164 (69), 135 (16), 97 (20), 83 (27), 69 (34), 55 (60), 43 (100); HRFABMS (positive ion mode) *m/z* $[MH]^+$ 479.2634 (calcd for $C_{26}H_{39}O_8$ 479.2645).

Mixture of 11b-hydroxy-11b,1-dihydromedicarpin (**4**) *and 11b-hydroxy-11b-1-dihydromaackiain* (**5**). Colorless oil; IR ν_{max}^{film} cm^{-1} 3325, 2929, 1620, 1498, 1472, 1340, 1174, 1150, 1069, 1035, 1006, 938, 857, 748. EIMS (70 eV) *m/z* $[M]^+$; 302 (62), 288 (25), 176 (50), 175 (100), 164 (49), 163 (27), 162 (43), 161 (85), 151 (12), 147 (16), 138 (18), 133 (26), 89 (16), 69 (51); HREIMS *m/z* $[M]^+$ 298.0999 (calcd for $C_{16}H_{16}O_5$ 298.0997) and *m/z* $[M]^+$ 302.0783 (calcd for $C_{16}H_{14}O_6$ 302.0790).

11b-Acetoxy-11b-1-dihydromaackiain (**5a**). Colorless oil; UV λ_{max}^{MeOH}, nm (log ε) 256 (3.75), 305 (3.30); IR ν_{max}^{film} cm^{-1} 2927, 2855, 1745, 1654, 1626, 1498, 1473, 1394, 1368, 1336, 1220, 1180, 1150, 1069, 1016, 942, 896, 850, 803, 756. LRCIMS (CH_4) *m/z* $[M+1]^+$ 345 (100), 285 (52), 175 (11), 85 (16).

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