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Article

Isoflavonoids and Triterpenoids Isolated from *Pterodon polygalaeflorus*

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Os isoflavonóides 6,7-dimetoxi-3',4'-metilenodioxi-, 4'-hidroxi-3',6,7-trimetoxi-, 3,4,6,7-tetrametoxi-, 7-hidroxi-6-metoxi-3,4-metilenodioxi-, 2',6,7-trimetoxi-3',4'-metilenodioxi-, 2',3',4',7,7-pentametoxi- e 2',4',5,6,7-pentametoxiisoflavona, os triterpenóides lupeol e betulina e o ácido 4-metoxibenzóico foram isolados dos extratos acetônicos do alburno e do cerne de *Pterodon polygalaeflorus*. As estruturas destes produtos naturais foram caracterizadas por métodos espectrométricos, principalmente experiências de RMN 1D e 2D de hidrogênio e carbono-13, que foram também utilizados para a atribuição inequívoca dos deslocamentos químicos dos átomos de hidrogênio e carbono-13 dos isoflavonóides.

The isoflavonoids 6,7-dimethoxy-3',4'-methylenodioxy-, 4'-hydroxy-3',6,7-trimethoxy-, 3,4,6,7-tetramethoxy-, 7-hydroxy-6-methoxy-3,4-methylenodioxy-, 2',6,7-trimethoxy-3',4'-methylenedioxy-, 2',3',4',6,7-pentamethoxy- and 2',4',5,6,7-pentamethoxyisoflavone, together with the triterpenoids lupeol and betulin and 4-methoxybenzoic acid, were isolated from acetone extracts of the sapwood and heartwood of *Pterodon polygalaeflorus*. The structures of these natural products have been characterized by spectrometric methods, mainly extensive 1D and 2D NMR experiments, which were also used for complete assignments of the chemical shifts of the hydrogen and carbon-13 atoms of isoflavonoids.

Keywords: Pterodon polygalaeflorus, *Leguminosae, isoflavonoids, triterpenoids, ^{1}H- and ^{13}C-NMR data*

Introduction

In a paper published recently the isolation of diterpenes from fruits of a specimen of *Pterodon ploygalaeflorus* Benth, Leguminosae family, was reported[1]. As part of our continuing chemical investigation of this plant we have investigated the sapwood and the heartwood. The isoflavones **1-7**, *p*-methoxybenzoic acid **(8)**, lupeol **(9)**, and betulin **(10)**, have been isolated from this plant material. The structures of these natural products were established by spectral data, mainly ^{1}H- and ^{13}C-NMR including homonuclear and heteronuclear 2D experiments (**2**, **3a**, **5** and **7**) and NOE difference spectra (**1**, **2**, **3a**, **5** and **7**), which were also used for complete assignment of the hydrogen and carbon-13 atom chemical shifts of the isoflavonoid **2**, the acetyl derivatives **3a**, **5** and **7**.

Results and Discussion

The acetone extracts of heartwood and sapwood of *Pterodon ploygalaeflorus* were submitted to chromatography on silica gel column to afford the isoflavonoids **1-7**, 4-methoxybenzoic acid **(8)**, lupeol **(2)** and betulin **(10)**. The

isoflavones **1**, **2**, **5-7** were previously isolated from *Milletia dura* (**1** and **5**)[2], *Pterodon pubescens* (**2, 5** and **7**)[3,4] and *Condyla africana* (**6**)[5]. The triterpenoids **9** and **10** are frequently found in plants[6]. The structural characterization of these compounds was based on spectral data, notebly the ^{1}H- and ^{13}C-NMR spectra, including homonuclear ^{1}H- x ^{1}H-COSY and heteronuclear ^{1}H- x ^{13}C-COSY-$^{1}J_{CH}$ and ^{1}H- x ^{13}C-COSY-$^{n}J_{CH}$ (n = 2 and 3)] experiments and NOE difference spectra (^{1}H{^{1}H}-NOE) data for the isoflavonoids **1**, **2**, **3a**, **5** and **7**, in addition to corresponding ^{1}H-NMR spectral data reported in the literature [2-5] (Table 2). To the best of our knowledge, the isoflavone **3** is hitherto unreported as a natural product and ^{13}C-NMR spectral data were only found for **2** (Table 2)[7].

The homonuclear ^{1}H- x ^{1}H-COSY, heteronuclear ^{1}H- x ^{13}C-COSY-$^{n}J_{CH}$ (n = 1, spin-spin couplings between ^{13}C and ^{1}H- atoms via one bond; n = 2 and 3, COLOC = correlation via long-range couplings) 2D shift-correlated NMR spectra[8] of **2**, **3a**, **5** and **7** (Tables 1-3) and NOE difference spectra[8] of **1**, **2**, **3a**, **5** and **7** (Table 4) together with the application of the shift parameters and the observed multiplicities of the signals of the carbon atoms deduced by comparative analysis of the PND- and DEPT-^{13}C-NMR spectra[9], were also used for the complete assignment of the hydrogen and carbon-13 atom chemical shifts

Table 1. ^{13}C-NMR (50.3 MHz) data for the isoflavonoids **1**-**3**,**5** and **7** ($CDCl_3$), compared values described in the literature are in parenthesis[7] for **2**, TMS as internal standard and chemical shifts in δ (ppm).*

C	**1**	**2**[a]	**3a**[a]	**5**[a]	**7**[a]
3	124.45	123.91 (123.33)	123.86	124.27	121.54
4	175.38	175.21 (174.57)	175.14	175.76	175.24
6	147.74	147.36 (147.56)	147.68	147.29	147.30
7	154.41	154.02 (154.36)	154.35	153.33	153.98
9	152.23	151.89 (151.93)	152.12	152.27	152.05
10	117.81	117.51 (117.29)	117.72	117.08	117.55
1’	125.85	124.51 (124.91)	130.88	112.50	118.49
2’	-	-	-	152.59	151.75
3’	147.56	148.35 (148.80)	150.70	-	142.06
4’	147.74	148.65 (148.50)	139.51	148.10	153.74
5’	-	-	-	140.70	-
CH					
2	151.96	151.77 (152.84)	152.49	153.96	153.00
5	104.90	104.35 (104.42)	104.60	104.08	104.68
8	99.50	99.18 (100.05)	99.41	99.24	99.32
2’	109.72	112.13 (112.95)	113.55	-	-
3’	-	-	-	94.79	-
5’	108.29	110.75 (111.52)	122.80	-	106.96
6’	122.33	120.61 (121.21)	120.66	110.53	125.63
CH_2					
OCH_2O	101.11	-	-	100.99	-
CH_3					
MeO-6	56.38	56.50 (55.85)	56.38	55.66	56.25
MeO-7	56.31	56.16 (56.28)	56.24	55.66	56.06
MeO-2’	-	-	-	55.91	60.68
MeO-3’	-	55.62 (55.64)	55.85	-	60.53
MeO-4’	-	56.02 (55.64)	-	-	55.76

* The multiplicity of the signals was deduced by comparative analysis of the PND - and DEPT-^{13}C NMR spectra. [a] The heteronuclear 2D shift-correlated via one bond (^{1}H x ^{13}C - COSY - $^{1}J_{CH}$) and long-range couplings (^{1}Hx^{13}C-COSY-$^{n}J_{CH}$, n = 2 and 3) spectra (Table 3) were also used for these assignments.

of **2**, **3a**, **5** and **7**, and consequently of the other isoflavonoids isolated from *Pterodon polygalaeflorus* by comparison with data now unambiguously assigned (Tables 1-4).

Thus, a series of 2D NMR experiments led to the assignment of all ^{1}H- and ^{13}C resonances for **2** and **3a** (*e. g.*). In the ^{1}H- x ^{13}C-COSY-$^{1}J_{CH}$ spectra of **2** and **3a** the connectivities between the protonated carbon atoms and

Table 2. ^{1}H- NMR data for the isoflavonoids **1-7** [200 MHz (**1**, **2**, **3a** , **5** and **7**), 100 MHz (**6**) and 60 MHz (**4a**)], in $CDCl_3$ and TMS as internal standard, chemical shifts in δ (ppm) and coupling constants (J, in parenthesis) in Hz.*

H	1	2	3a	4a	5	6	7
2	7.91 (*s*)	7.97 (*s*)	7.97 (*s*)	7.94(*s*)	7.88 (*s*)	8.05(*s*)	7.93(*s*)
5	7.61 (*s*)	7.63 (*s*)	7.60 (*s*)	7.74 (*s*)	7.59 (*s*)	7.67 (*s*)	7.63 (*s*)
8	6.86 (*s*)	6.88 (*s*)	6.87(*s*)	7.22 (*s*)	6.85 (*s*)	6.93 (*s*)	6.89 (*s*)
2’	7.10 (*d*, 1.7)	7.25 (*d*, 1.8)	7.34 (*d*, 1.6)	7.1-6.7 (*m*)	-	-	-
3’	-	-	-	-	6.59 (*s*)	6.68 (*s*)	-
5’	6.85 (*d*, 8.0)	6.93 (*d*, 8.3)	7.07 (*d*, 8.2)	7.1-6.7 (*m*)	-	-	6.76 (*d*, 8.5)
6’	6.98 (*dd*, 8.0, 1.7)	7.05 (*dd*, 8.3, 1.8)	7.00 (*dd*, 8.2, 1.6)	7.1-6.7 (*m*)	6.80 (*s*)	7.02 (*s*)	7.06 (*d*, 8.5)
OCH_2O	5.98 (*s*)	-	-	6.00 (*s*)	5.93 (*s*)	4.00 (*s*)	-
MeO-6	3.98 (*s*)	3.99 (*s*)	3.97 (*s*)	3.95 (*s*)	3.96 (*s*)	4.00 (*s*)	4.01 (*s*)
MeO-7	3.98(*s*)	3.99 (*s*)	3.98 (*s*)	-	3.96 (*s*)	4.00 (*s*)	4.00 (*s*)
MeO-2’	-	-	-	-	3.70 (*s*)	3.80 (*s*)	3.84 (*s*)
MeO-3’	-	3.92 (*s*)	3.86 (*s*)	-	-	-	3.95 (*s*)
MeO-4’	-	3.88 (*s*)	-	-	-	3.88 (*s*)	3.91 (*s*)
MeO-5’	-	-	-	-	-	3.97 (*s*)	-
AcO-4’	-	-	2.32 (*s*)	-	-	-	-
AcO-7’	-	-	-	2.40 (*s*)	-	-	-

*Homonuclear ^{1}Hx^{1}H-COSY and heteronuclear ^{1}Hx^{13}C-COSY-$^{1}J_{CH}$ 2D NMR spectra were also used for these assignments. Chemical shifts and coupling constants (J) were obtained from 1D ^{1}H-NMR spectra.

Table 3. Heteronuclear 2D shift-correlated via long-range couplings [^{1}H- x^{13}C - COSY- $^{n}J_{CH}$ (n = 2 and 3), COLOC] NMR spectral data for the isoflavonoids **2**, **3a**, **5** and **7**.*

	2		3a		5		7
C	$^{2}J_{CH}$	$^{3}J_{CH}$	$^{2}J_{CH}$	$^{3}J_{CH}$	$^{2}J_{CH}$	$^{3}J_{CH}$	$^{2}J_{CH}$	$^{3}J_{CH}$
3	H-2	H-2’; H-6’	H-2’	H-2’		H-6’	H-2	H-6’
4		H-2; H-5		H-2; H-5		H-5		H-2; H-5
6	H-5	H-8; MeO-6		H-8; MeO-6		H-8; MeO-6		H-8; MeO-6
7	H-8	H-5; MeO-7	H-8	H-5; MeO-7		H-5; MeO-7		H-5; MeO-7
9	H-8	H-2; H-5	H-8	H-5		H-5	H-8	H-2; H-5
10	H-5	H-8	H-5			H-8		H-8
1’		H-2; H-5’		H-2; H-5’		H-3’		H-5’
2’				H-6’		MeO-2’		H-6’; MeO-2’
3’		H-5’; MeO-3’		H-5’; MeO-3’				H-5’; MeO-3’
4’		H-2’; H-6’; MeO-4’		H-2’; H-6’		H-6’		H-6’; MeO-4’
5’						H-3’		
6’				H-2’				

* The chemical shifts for each one of the carbon and hydrogen atoms are described in Tables 1 and 2, respectively. The corresponding 2D NMR spectra were obtained a Bruker AC-200 (^{1}H: 200 MHz; ^{13}C: 50.3 MHz).

the corresponding hydrogens that were observed are: CH-2 [**2**: δ_C 151.77(*d*) and δ_H 7.97 (*s*); **3a**: δ_C 152.49 (*d*) and δ_H 7.97 (*s*)], CH-5 [**2**: δ_C 104.35 (*d*) and δ_H 7.63 (*s*); **3a**: δ_C 104.60 (*d*) and δ_H 7.60 (*s*)], CH-8 [**2**: δ_C 99.18 (*d*) and δ_H 6.88 (*s*); **3a**: δ_C 99.41(*d*) and δ_H 6.87 (*s*)], CH-2' [**2**: δ_C 112.13 (*d*) and δ_H 7.25 (*d*, J = 1.8 Hz); **3a**: δ_C 113.55 (*d*) and δ_H 7.34 (*d*, J = 1.6 Hz)], CH-5' [**2**: δ_C 110.75 (*d*) and δ_H 6.93 (*d*, J = 8.3 Hz); **3a**: δ_C 122.80 (*d*) and δ_H 7.07 (*d*, J = 8.2 Hz); shifted downfield by only 0.14 ppm [$\Delta\delta_H$ = 7.07 (**3a**)-6.93 (**2**)] and by 12.05 ppm [$\Delta\delta_C$ = 122.80 (**3a**)-110.75 (**2**) in the acetyl derivative (**3a**), as anticipated by shielding reduction of the mesomeric_*ortho*-effect], CH-6' [**2**: δ_C 120.61 (*d*) and δ_H 7.05 (*dd*, J = 8.3 and J = 1.8 Hz); **3a**: δ_C 120.66 (*d*) and δ_H 7.00 (*dd*, J = 8.2 and J = 1.6 Hz)] and methoxy groups [**2**: δ_C 56.50 and δ_H 3.99, 56.16 and 3.99, 55.62 and 3.92, 56.02 and 3.88; **3a**: δ_C 56.38 and δ_H 3.87, 56.24 and 3.98, 55.85 and 3.86] (Tables 1 and 2).

The chemical shift assignments of the quaternary carbon atoms were established by ^{1}H- x ^{13}C-COSY-^{n}J$_{CH}$ (n = 2 and 3, COLOC) spectra . Thus, the spectrum of **2** showed long-range correlations (Table 3): C-3 (δ_C 123.91) with H-2 (δ_H 7.97, ^{2}J$_{CH}$), H-2' (δ_H 7.25, ^{3}J$_{CH}$) and H-6' (δ_H 7.05, ^{3}J$_{CH}$); C-4 with H-2 (δ_H 7.97, ^{3}J$_{CH}$) and H-5 (δ_H 7.63, ^{3}J$_{CH}$); C-6 (δ_C 147.36) with H-5 (δ_H 7.63, ^{2}J$_{CH}$), H-8 (δ_H 6.88, ^{3}J$_{CH}$) and MeO-6 (δ_H 3.99, ^{3}J$_{CH}$); C-7 (δ_C 154.02) with H-8 (δ_H 6.88, ^{2}J$_{CH}$), H-5 (δ_H 7.63, ^{3}J$_{CH}$) and MeO-7 (δ_H 3.99, ^{3}J$_{CH}$); C-9 (δ_C 151.89) with H-8 (δ_H 6.88, ^{2}J$_{CH}$), H-2 (δ_H 7.97, ^{3}J$_{CH}$) and H-5 (δ_H 7.63, ^{3}J$_{CH}$); C-10 (δ_C 177.51) with H-5 (δ_H 7.63, ^{2}J$_{CH}$) and H-8 (δ_H 6.88, ^{3}J$_{CH}$); C-1' (δ_c 124.51) with H-2 (δ_H 7.97, ^{3}J$_{CH}$) and H-5' (δ_H 6.93, ^{3}J$_{CH}$); C-3' (δ_C 148.33) with H-5' (δ_H 6.93, ^{3}J$_{CH}$) and MeO-3' (δ_H 3.92, ^{3}J$_{CH}$); C-4' (δ_C 148.65) with H-2' (δ_H 7.25, ^{3}J$_{CH}$), H-6 (δ_H 7.05, ^{3}J$_{CH}$) and MeO-4' (δ_H 3.88, ^{3}J$_{CH}$). The spectra of **3a, 5** and **7** (δ_H 7.97, ^{3}J$_{CH}$) revealed analogous results as shown in Table 3.

Table 4. NOE difference spectra (^{1}H-{^{1}H}-NOE) data for the isoflavonoids **1, 2, 3a, 5** and **7**.

	{H}		NOE enhancements		
Compound	**H**	δ_H	**H**	δ_H	**%**
1	(MeO)$_2$-6,7	3.98	5	7.61	5
			8	6.86	10
	5	7.61	MeO-6	3.98	15
	2	7.91	2'	7.10	7
			6'	6.98	10
2	MeO-4'	3.88	5'	6.93	6
	MeO-3'	3.92	2'	7.25	8
	(MeO)$_2$-6,7	3.99	5	7.63	6
			8	6.88	5
3a	MeO-3'	3.86	2'	7.34	9
	(MeO)$_2$-6,7	3.97, 3.98	5	7.60	6
			8	6.87	6
5	MeO-2'	3.70	3'	6.59	11
			2	7.88	> 1
	2MeO-6,7	3.96	5	7.59	4
			8	6.85	12
7	MeO-2'	3.84	2	7.93	5
	MeO-4'	3.91	5'	6.76	7
	2 MeO-6,7	4.00, 4.01	5	7.63	7
			8	6.89	9

Homonuclear NOE difference (^{1}Hx{^{1}H}-NOE) spectra (Table 4) of compounds **1, 2, 3a, 5** and **7** contributed to the assignments (Tables 1 and 2). The EIMS and IR spectra were also used (*vide* experimental).

Experimental

General experimental procedures

Mps were determined on a Mettler PF-5 melting point analyser. IR spectra were recorded in KBr using a Perkin-Elmer 720 infrared spectrometer. UV spectra were recorded in MeOH on a Varian-UV/ VIS 634-5 spectrometer. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker AC-200 spectrometer with standard pulse sequences operating at 200 MHz and 50.3 MHz, respectively, except the ^{1}H- NMR of **4a** and **6** which were recorded on a Varian EM-360 (60 MHz) and Varian XL-100 spectrometers, respectively. The chemical shift values are reported in δ (ppm) and the coupling constants (J) are in Hz; carbon multiplicities were determined by DEPT experiments; ^{1}H-x ^{1}H- - COSY, ^{1}H- x ^{13}C - COSY - $^1J_{CH}$, ^{1}H- x ^{13}C - COSY - $^nJ_{CH}$ (n = 2 and 3, COLOC), NOE difference spectra NMR experiments were carried out using Bruker commercial microprograms. Low-resolution EIMS (70 eV) data were obtained on a GC/MS Finningan 3300F/ 9500 apparatus. Chromatography was performed using Merck Kieselgel 0.05-0.20 mesh and TLC with Merck Kieselgel 60 F_{254}. TLC plates were examined under UV illumination and after exposure to iodine vapour.

Plant material

A specimen of *Pterodon polygalaeflorus* was collected in Monte Alegre - Bom Jesus, Piauí State, Brazil and identified by Professor Afrânio Gomes Fernandes (Universidade Federal do Ceará, Fortaleza, Ceará, Brazil). A voucher specimen is deposited at the Herbarium Prisco Bezerra of the Departamento de Biologia - Universidade Federal do Ceará.

Isolation of pterodon polygalaeflorus constituents

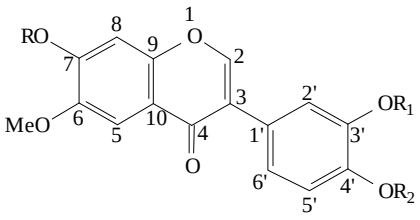
Acetone extraction of the heartwood

Dried and powdered heartwood (4.4 Kg) was continuously extracted with hot acetone. Upon removal of the solvent a residue (189 g) remained. This residue was chromatographed on a silica gel (756 g) column giving fractions H-1 to H-5, in this order, by elution with *n*-hexane-$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$ - acetone (1:1), acetone and MeOH. Fraction H-2 (43 g, eluted with $CHCl_3$) was rechromatographed on a silica gel column using cyclohexane- $CHCl_3$ (1:1) and $CHCl_3$ -acetone (8:2, 7:3 and 1:1) as eluents to obtain fraction H-2a to H-2d, respectively. Chromatography of fraction H-2a furnished **1** (56 mg), H-2b afforded **1** (75 mg) and **7** (284 mg), H-2c yielded **1** (74 mg) and **2** (605 mg) and H-2d funished **2** (310 mg) and **5** (560 mg).

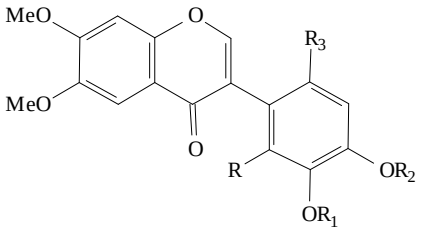
Fraction H-3 (20 g), was eluted with $CHCl_3$-acetone (1:1) and rechromatographed on a sílica gel column furnishing fractions H-3a to 3c, in this order, by elution with $CHCl_3$-EtOAc (9.5:0.05, 9:1 and 7.5:2.5). These fractions afforded **1** (61 mg), **2** (398 mg) and **5** (544 mg), **3** (200 mg), **6** (283 mg) and **4** as acetyl derivative [**4a** (100 mg), obtained by treatment with Ac_2O/Py], respectively, after rechromatographed on silica gel columns.

Acetone extraction of the sapwood

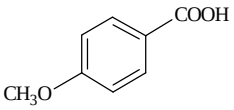
Dried and powdered sapwood (4.4 Kg) was continuously extracted with hot acetone. The residue (200 g) obtained was chromatographed on a silica gel column using $CHCl_3$, $CHCl_3$-acetone (1:1), acetone and MeOH as eluents to furnish fractions 5-1 to 5-4. Fraction 5-1 (10 g) was rechromatographed on a sílica gel column to give **1** (50 mg), **8** (20 mg), **9** (539 mg) and **10** (130 mg).



	R	R1	R2
1	Me	—CH2—	
2	Me	Me	Me
3	Me	Me	H
3a	Me	Me	Ac
4	H	—CH2—	
4a	Ac	—CH2—	



	R	R1	R2	R3
5	H	—CH2—		OMe
6	H	Me	Me	OMe
7	OMe	Me	Me	H



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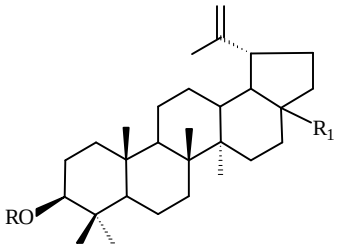


	R	R_1
9	H	Me
10	H	CH_2OH
10a	Ac	CH_2OAc

6,7-Dimethoxy-3',4'-methylenedioxyisoflavone **(1)**

Colorless crystals from MeOH, m.p. 240-241 °C. Spectral data are in accordance with values described in the literature[2]. ^{13}C-NMR: Table 1. ^{1}H-NMR: Table 3. NOE difference spectra (^{1}H-{^{1}H}-NOE) data: Table 4.

3',4',6,7-Tetramethoxyisoflavone **(2)**

Colorless crystals from MeOH, m.p. 188-189 °C. Spectral data are in accordance with literature values[3,4]. ^{13}C-NMR: Table 1. ^{1}H- NMR: Table 2. Heteronuclear 2D ^{1}H- x ^{13}C shift-correlated via long-range coupling (^{1}Hx^{13}C-COSY-$^{n}J_{CH}$, n = 2 and 3): Table 3. NOE difference spectra data: Table 4.

4-Hydroxy-3',6,7-trimethoxyisoflavone **(3)**

Colorless crystals, m.p. 279-281 °C. IR ν_{max} cm $^{-1}$: 3240 (OH), 1620 (C=O), 1590, 1510 (aromatic). ^{1}H-NMR (60 MHz, CF_3COOH) δ_H: 8.80 (*s*, H-2), 7.84 (*s*, H-5), 7.50 (*s*, H-8), 7.10 (*m*, H-2', H-5' and H-6'), 4.27(*s*, MeO), 4.27 (*s*, MeO) and 4.04 (*s*, MeO). EIMS *m/z* (rel. int.): 328 (100, $[M]^{\cdot+}$), 313 (6, $[M\text{-}Me^{\cdot}]^{+}$), 285 (5, $[M\text{-}Me^{\cdot}\text{-}CO]^{+}$), 181 (8, **3b**), 180 (7, **3c**), 148 (19, **3d**).

4'-O-Acetyl-3',6,7-trimethoxyisoflavone **(3a)**

Treatment of the isoflavone **3** (100 mg) with (Ac_2O) (2 mL) and pyridine (2 mL) at room temperature for 24 h, and usual work-up, produced **3a** (98 mg), colorless crystals, m.p. 116-118 °C. IR ν_{max} cm $^{-1}$: 1760 (ester), 1625 (C=O), 1600, 1510 (aromatic). EIMS *m/z* (rel. int.): 370 (3, $[M]^{\cdot+}$), 328 (100, $[M\text{-}CH_2C{=}O]^{\cdot+}$), 327 (20, $[M\text{-}Ac^{\cdot}]^{+}$), 181 (**3**, **3b**), 180 (**4**, **3c**). Heteronuclear 2D ^{1}H- x ^{12}C shift-correlated via long-range compling (^{1}Hx^{13}C-COSY-$^{n}J_{CH}$, n = 2 and 3): Table 3. ^{13}C-NMR: Table 1. ^{1}H-NMR: Table 2. NOE dfference spectra (^{1}H-{^{1}H}-NOE) data: Table 4.

Methylation of **3**

A solution of **3** (100 mg) in anhydrous acetone (40 mL) was treated with Me_2SO_4 (0.5 mL) in the presence of calcinated K_2CO_3, under reflux during 24 h. After filtration, the acetone was evaporated and the residue washed with 50% NH_4OH. The remaining residue was crystallized from MeOH to give **2**.

6-Methoxy-7-0-acetyl-3',4'-methylenedioxyisoflavone (**4a**)

Colorless crystals from MeOH, m.p. 204-205 °C. IR ν_{max} cm^{-1}: 1740 (ester), 1650 (C=O), 1610, 1480 (aromatic). EIMS ***m/z*** (rel. int.) 354 (42, $[M]^{\cdot+}$), 312 (100, $[M\text{-}CH_2C{=}O]^{\cdot+}$), 311 (20, $[M\text{-}Ac]^{+}$), 166 (10, **4b**), 146 (18, **4c**). ^{1}H- NMR: Table 2.

2',6,7-Trimethoxy-4',5'-methylenedioxyisoflavone (**5**)

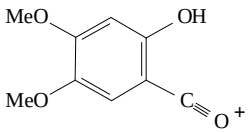
Colorless crystals from $CHCl_3$ + MeOH, m.p. 237-239 °C. Spectral data are in accordance with literature values[2-4] ^{13}C-NMR: Table 1. ^{1}H-NMR: Table 2. Heteronuclear 2D ^{1}H- x ^{13}C shift-correlated via long-range coupling (^{1}H x ^{13}C-COSY-$^{n}J_{CH}$, n = 2 and 3): Table 3. NOE difference spectra (^{1}H-{^{1}H}-NOE): Table 4.

2',4',5',6,7-Pentamethoxyisoflavone (**6**)

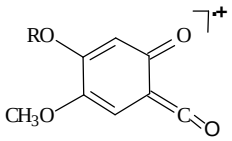
Colorless crystals from MeOH, m.p. 169-172 °C. Spectral data are in accordance with literature values[5]. ^{1}H-NMR: Table 2.

2',3',4',6,7- Pentamethoxyisoflavone (**7**)

Colorless crystals, m.p. 170-172°C. Spectral data are in accordance with literature values[3.4]. ^{13}C-NMR: Table 1. ^{1}H-NMR: Table 1. Heteronuclear ^{1}H- x ^{13}C 2D shift-correlated via long-range coupling (^{1}Hx^{13}C-COSY-$^{n}J_{CH}$, n = 2 and 3): Table 3. NOE difference spectra (^{1}H-{^{1}H}-NOE) data: Table 4.

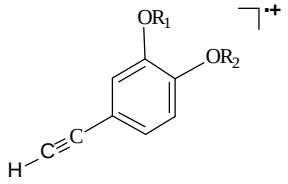


3b *m/z* 181



3c R=Me, *m/z* 180

4b R= H, *m/z* 166



3d R_1=Me R_2= H, *m/z* 148

4c R_1,R_2= CH_2, *m/z* 146

4-Methoxybenzoic acid (**8**, p-anisic acid)

Colorless crystals, m.p. 182-184 °C. (Lit.[10] m.p. 184 °C). ^{1}H-NMR (60 MHz, CF_3COOH) δ: 8.15 (*d*, J = 9.0 Hz, 2H-2,6), 7.08 (*d*, J = 9.0 Hz, 2H-3,5), 4.02 (*s*, MeO-4). EIMS *m/z* (rel. int.): 152 (98, $[M]^{+\cdot}$), 135 (100, $[M\text{-}OH]^+$), 107 (13, [M-OH-CO and/or M-COOH]$^+$).

Lupeol (**9**)

Colorless crystals from MeOH, m.p. 211-214 °C. [Lit.[11] m.p. 215-216 °C (Me_2CO). Spectral data, mainly the chemical shifts and multiplicities of the signals of the carbon-13 deduced by comparative analysis of the PND- and DEPT-^{13}C-NMR, and comparison with literature values[12] were used in the identification of this natural product.

Betulin (**10**)

Colorless crystals from MeOH, m.p. 249-251 °C. [Lit.[13] m.p. 251-252 °C (EtOH)]. Spectral data, mainly the chemical shifts and multiplicities of the signals of the carbon-13 deduced by comparative analysis of the PND- and DEPT-^{13}C-NMR, including the diacetyl derivative (**10a**) and comparison with literature values[12] were used in the identification of this compound.

Acknowledgments

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