

ARYLBENZOFURANS FROM *INDIGOFERA MICROCARPA*

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Key Word Index—*Indigofera microcarpa*; Leguminosae; Papilionoideae; 2-aryl-3-methylbenzofurans; structure; NMR; antimicrobial activity.

Abstract—Two new 2-aryl-3-methylbenzo [b]furans were isolated from leaves of *Indigofera microcarpa*. The structures were established by spectroscopic (particularly ^1H and ^{13}C NMR) and chemical methods.

INTRODUCTION

The Leguminosae family with 600 genera and more than 10 000 species plays an important role in the flora of all continents. Species of this family are rich in flavonoids, alkaloids, terpenes and saponins [1] which are often of pharmacological and therapeutic interest [2].

RESULTS AND DISCUSSION

In the course of our studies of biological properties of higher plants from north-eastern Brazil, we observed a pronounced antimicrobial action of the methanolic extract of leaves of *Indigofera microcarpa* Desv. [3].

The active component was isolated after solvent partitioning and column chromatography as colourless crystals which appeared homogeneous by TLC. However, ^1H NMR spectroscopy revealed the presence of two phenolic compounds in roughly equal amounts. All attempts to achieve separation by chromatography produced only partial enrichment of one of the compounds. Finally, fractional crystallization and manual selection of differently shaped crystals produced two pure products (**1** and **2**) of identical R_f .

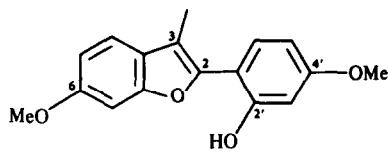
High resolution mass spectrometry indicated an elemental composition of $\text{C}_{17}\text{H}_{16}\text{O}_4$ for **1** and $\text{C}_{17}\text{H}_{14}\text{O}_5$ for **2**. Flavonoid structures were excluded because of the absence of carbonyl frequencies in the IR spectrum. The UV absorption spectra of both compounds displayed a short wave maximum at 270 nm and another strong band at longer wavelengths (313 nm for **1** and 320 nm for **2**). These bands are similar to those of some 2-phenyl-3-methylbenzofurans first isolated from *Eupomati laurina* [4, 5]. It must be pointed out, that 2-phenylbenzofurans

without substituents at C-3 show maxima between 330 and 350 nm [6, 7], probably due to a better coplanar orientation and resultant conjugation of all rings.

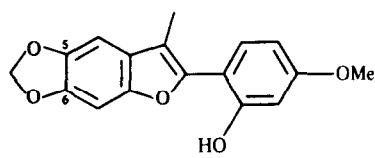
The ^1H NMR spectrum of compound **1** indicated the presence of two methoxy groups and one C-methyl group, besides the hydroxy group already evidenced by IR. These substituents had to be distributed over a skeleton containing two 1,2,4-trisubstituted aromatic rings, as shown by the presence of two different ABX systems with coupling constants of 8.5 Hz (*ortho*) and 2.5 Hz (*meta*). Comparison of the chemical shifts with data published for the 2-phenylbenzofurans **3** [8] and **4** [9] (Table 1) suggested the same oxygenation pattern in **1**. All chemical shifts were in perfect agreement except H-6', which was shifted by 0.5 ppm to higher field. This was easily explained by the better shielding due to the proximity of the methyl group at C-3.

The exact position of the hydroxy group was established from the IR spectrum; the sharp band at 3550 cm^{-1} was not influenced by dilution, indicating a strong intramolecular hydrogen bond, the formation of which was possible only if the hydroxy group was at C-2¹. A similar behaviour was described for ratanthiaphenol I(**5**) [10], which has the same substitution pattern in ring B. Further confirmation was achieved by a positive Gibbs reaction [11], showing that the *para* position to the hydroxyl group had to be unsubstituted.

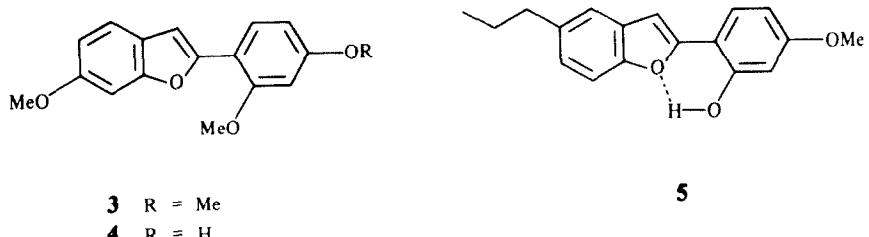
The IR spectrum of compound **2** exhibited a sharp OH band similar to that of **1**. The ^1H NMR spectrum indicated the presence of a methoxy, a methyl and a dioxymethylene group. The chemical shifts of **1** and **2** were in perfect agreement for all protons of ring B and the C-methyl group (Table 1), suggesting the same substitution pattern for this part of the molecule. The pres-



1



2

Table 1. ^1H NMR data of 2-arylbenzofurans in CDCl_3

H	1	2	3[8]	4[9]	6[12]	7[12]*
4	7.40 d(8.5)	6.90 s	7.43 d(9.0)	7.43 d(9.0)	6.81 s	7.08 s
5	6.92 dd(8.5, 2.5)	—	6.85 dd(9.0, 2.8)	6.85 dd(9.0, 2.0)	—	—
7	7.03 d(2.5)	6.98 s	7.08	7.05	6.94 s	7.08 s
3'	6.61 m	6.59 m	6.60 m	6.53 m	6.56 m	6.47 d(2.0)
5'	6.38 s	—	—	—	—	6.36 dd(8.0, 2.0)
6'	7.35 d(8.5)	7.33 d(8.2)	7.92 d(9.2)	7.85 d(9.0)	7.50 d(9.0)	7.57 d(8.0)
Me	2.34 s	2.30 s	—	—	—	—
OMe	3.85 s	3.84 s	3.55 s	3.85 s	3.83 s	—
	3.88 s		3.92 s	3.90 s		
OCH ₂ O	—	6.00 s	—	—	6.00 s	6.02 s
OH	†	6.84 s	—	†	7.15 br	†

*In $\text{DMSO}-d_6$.

†Not identified.

ence of two singlets for the protons of ring A proved their *para*-relationship and, consequently, the localization of the di-oxymethylene group at C-5 (6). Further confirmation of structure **2** was given by the ^1H NMR data [12] of the 2-phenylbenzofurans **6** and **7** (Table 1). Especially compound **6**, which differs from **2** only in the lack of the 3-methyl group and shows nearly identical chemical shifts and coupling constants.

Finally, the structure elucidation of **1** and **2** was completed by a comparison of their ^{13}C NMR data with published data for **5** [10] and **7** [12] and with those of the methyl ether of **2** (Table 2). All chemical shifts, except for C-3, showed good agreement between **7**, **2** and 2-OMe. Similarly, compounds **1** and **5** had comparable values for the carbons of ring B. In conclusion, all spectroscopic data confirm the structures of 2-phenyl-3-methylbenzo[b]furans proposed for the compounds **1** and **2**.

Some 2-arylbenzofurans such as **6** and **7**, unsubstituted at C-3, were isolated from *Sophora* species [12, 13] whilst compound **5** was found in *Krameria triandra* (Krameriaceae) [10]. Other references describe vignafuran (**4**) and similar structures, all phytoalexins produced upon fungal infection of species of *Vigna* (Leguminosae) [6, 7, 14] and *Morus alba* (Moraceae) [15]. 2-Aryl-3-methylbenzofurans like the eupomatenoids isolated from *Eupomati laurina* (Eupomatiaceae) [4, 5, 16, 17], carinatin [18] and carinatidin [19], both found in *Virola carinata* (Myristicaceae), are all of the structural type **8** and can be classified as neolignans [20] because of their composition and substitution pattern.

The 2-aryl-3-methylbenzofurans **1** and **2** described here are the first examples of a new type of natural aromatic compound which combines a neolignoid part with another phenolic part. Until now, such compounds have not been obtained, either from natural sources, or by

synthesis. They merit special attention for biogenetic reasons as well for their biological activity.

EXPERIMENTAL

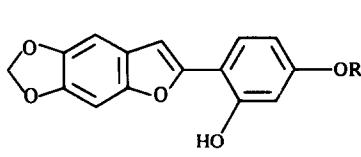
General. Mps: uncorr; IR: CCl_4 ; UV: MeOH; ^1H NMR (400 MHz) and ^{13}C NMR (100.7 MHz): CDCl_3 , TMS as int. standard; EIMS: 70 eV.

Plant material. Leaves of *Indigofera microcarpa* Desv. were collected in Paulista/Pernambuco and identified by A. A. Chiappetta. Voucher specimens were deposited at the Herbarium of the Departamento de Antibioticos under no. 5221.

Isolation of active fraction. Air-dried leaves (1 kg) were extracted at room temp. with MeOH (x 4). The solvent-free extract (109 g) was partitioned between MeOH-H₂O (9:1) and hexane. Evaporation of the hexane gave a lipophilic, inactive fraction (37.7 g). The MeOH phase was concd, suspended in H₂O and extracted with EtOAc. The aq. phase yielded a hydrophilic, inactive fraction (28 g) and the organic phase after evapn gave a brown solid (28 g), active against several gram-positive microorganisms. The latter was purified by CC silica gel, toluene), yielding colourless crystals (14 g, mp 96°) which were separated into two pure compounds (**1** and **2**) by careful recrystallization from MeOH and manual selection of differently formed crystals.

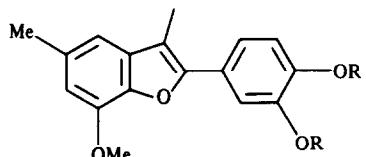
2-(2'-hydroxy-4'-methoxyphenyl)-3-methyl-6-methoxybenzo[b]furan (1). Colourless prisms. mp 109°; $\text{C}_{17}\text{H}_{16}\text{O}_4$, M^+ found 284.1047, calc. 284.1048; IR ν_{max} cm⁻¹: 3550, 1640, 1620, 1275, 1200, 1150, 1120, 800; UV λ_{max} nm (ϵ): 313 (17045), 270 (15909); EIMS (probe) 70 eV, m/z (rel. int.): 285 (8), 284 (100), 269 (95), 225 (4), 198 (7), 170 (5), 152 (8), 142 (19).

2-(2'-hydroxy-4'-methoxyphenyl)-3-methyl-5,6-dioxymethyl-ene-benzo[b]furan (2). Colourless needles, mp 123°; $\text{C}_{17}\text{H}_{14}\text{O}_5$, M^+ found 298.0844, calculated 298.0841; IR ν_{max} cm⁻¹: 3525, 1640, 1515, 1260, 1250, 1180, 1115, 1050, 970, 955, 840, 800; UV λ_{max} nm (ϵ): 320 (29850), 270 (19104); EIMS (probe) 70 eV, m/z



6 R = Me

7 R = H



8

Table 2. ^{13}C NMR data of 2-arylbenzofurans in CDCl_3

C	1	2	2-OMe	5[11]	7*
2	148.02	148.29 ^a	148.83 ^a	†	147
3	111.74	112.13	113.26	†	107
3a	123.78	123.70	124.12	†	122
4	119.38	106.94	104.80	†	102 ^a
5	111.66	144.49 ^b	143.99 ^b	†	143 ^b
6	158.14	145.97 ^b	145.58 ^b	†	144 ^b
7	107.08	101.80	99.02	†	103 ^a
7a	154.26	148.47 ^a	149.35 ^a	†	152
1'	109.65	109.60	112.72	109.32	108
2'	155.34	155.14	158.35	155.08	155 ^c
3'	95.81	93.18	93.28	101.46	92
4'	161.29	161.14	161.44	161.84	157 ^c
5'	101.68	97.78	97.77	108.02	98
6'	129.77	129.61	131.76	128.30	126
Me	9.17	9.11	9.05	—	—
OMe	55.38	55.25	55.37	55.51	—
			55.80	55.54	
OCH ₂ O	—	101.28	100.98	—	100

*In $\text{DMSO}-d_6$; approximate values taken from the spectrum shown in [12].

†Not comparable.

^{a-c}Values in the same column may be interchanged.

(rel. int.): 299 (33), 298 (100), 297 (14), 284 (19), 283 (53), 269 (14), 255 (10), 225 (17), 197 (15), 169 (16), 152 (12), 139 (18), 115 (30). Methylation (CH_2N_2) of **2** followed by CC (silica gel hexane-EtOAc, 9:1) yielded 2-(2',4'-dimethoxyphenyl)-3-methyl-5,6-dioxymethylenebenzo[b]furan.

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