NEW NAPHTHOPYRONE DERIVATIVES FROM CASSIA PUDIBUNDA♦,[1]

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Abstract- Further examination of the methanolic extract of the roots of Cassia pudibunda led to isolation of four new angular γ-naphthopyrones identified as 10-demethylflavasperone (1), 10-demethylflavasperone-10-sulphate (2), 10-demethylflavasperone-10-O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside (3), and cassiapyrone-10-sulphate (7-methyl-10-demethylflavasperone-10-sulphate) (4). The antimicrobial activity of the compounds is also reported.

Cassia pudibunda M. (Leguminosae, subfamily Caesalpinioideae) is a small tree growing in Brazil, in Guiana, and in Colombia. Previous examination of the methanolic extract of the roots of C. pudibunda, that showed antimicrobial activity, [2] led to the isolation of chrysophanol, physcion, cis-3,3',5,5'-tetrahydroxy-4-methoxystilbene, trans-3,3',5,5'-tetrahydroxy-4-methoxystilbene, cassiaside B (rubrofusarin-6-O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside), chrysophanol dimethyl ether, rubrofusarin-6-O-β-D-glucopyranoside, and quinquangulin-6-O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside.[3]

In this paper we report the isolation and structure determination of four new compounds, having an angular γ-naphthopyrone skeleton. Compound (1), mp 207-210 °C (from $CHCl_3$), showed a molecular ion at m/z 272 in the EI-ms spectrum. The ^1H, ^{13}C nmr and ir data of 1 were indicative of a γ-naphthopyrone skeleton. Moreover the uv spectral data, [4] and the high field position of the chelated

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♦Dedicated to Prof. G.B. Marini Bettolo on the occasion of his 75th birthday.

hydroxyl group indicated that 1 had an angular structure. As a matter of fact treatment of 1 with ethereal CH_2N_2 gave a methyl derivative that was identified with flavasperone (5), an angular γ-naphthopyrone. [5] The positive Gibbs test suggested the presence in 1 of a free OH group at C-10, locating consequently the OMe group at C-8. [6] The above assignments were confirmed by NOESY nmr techniques. In fact NOESY data showed the close proximity of both H-7 (δ 6.68) and H-9 (δ 6.42) with the methoxy group (δ 3.81). Compound (1) is thus 10-demethylflavasperone.

Compound (2) is an amorphous powder, $[M-1]^-$ at m/z 351 (FAB-ms). By acidic hydrolysis 2 gave 10-demethylflavasperone. These data and the presence in the ir spectrum of the strong bands at 1255 and 1030 cm^{-1} (S=O), and in the ^1H nmr spectrum of the resonance of a chelated hydroxy group (δ 12.84), suggested the structure of 10-demethylflavasperone-10-sulphate for 2. The structure was confirmed by direct comparison of 2 with 10-demethylflavasperone-10-sulphate obtained by treatment of 1 with sulphamic acid and pyridine. [7]

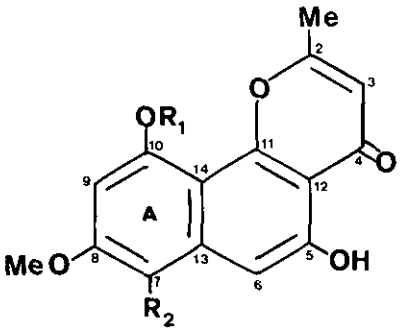
Compound (3), mp 180°C (with dec.) (from MeOH-AcOEt), $[\alpha]_D$ -53.0°, is a glycoside of a γ-naphthopyrone, as evidenced by its ^1H nmr data (see Experimental). As a matter of fact acidic hydrolysis of 3 gave two sugars and an aglycone identified as 10-demethylflavasperone. The sugar moieties were identified as glucose and apiose by co-tlc with authentic samples. ^{13}C Nmr data of 3 were in agreement with the presence of apiose and glucose in the molecule and allowed us to assign the β configuration to the glycosidic linkages on the basis of value of the anomeric carbon resonances, i.e. C-1' at 100.1 ppm., and C-1" at 109.4 ppm (Table 1). [8] The interglycosidic linkage was established to be 1→6 on the basis of the observed downfield shift of C-6 of the glucose attributed to the ether linkage with apiose, and the site of glycosylation was located at C-10 taking account of the presence of a chelated hydroxy group in the ^1H nmr spectrum of 3, that accounted for a free C-5 OH.

Cassiapyrone-10-sulphate (4) was obtained as an amorphous powder. The FAB-ms spectrum of 4 showed the molecular ion peak at m/z 365 $[M-1]^-$. The formation of a white precipitate on addition of $BaCl_2$ to a solution of 4 refluxed with HCl 0.1N suggested for the compound the structure of a sulphate derivative. In the ^1H nmr spectrum of 4 the signals of three aromatic protons (singlets), a chelated hydroxyl group, a methoxy group and two methyls were present. The above data indicated for 4 the structure of a γ-naphthopyrone sulphate with a pattern

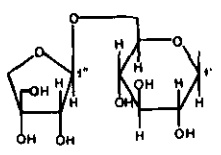
Table 1. ^{13}C Nmr spectral data of compounds (1), (2), (3), and (6).

	1*	2*	6#	3*		3*
C-2	167.6	168.4	166.5	168.3	C-1'	100.1
C-3	109.8	109.5	110.1	109.6	C-2'	73.4
C-4	182.1	182.3	182.1	182.3	C-3'	75.6[c]
C-5	157.0	157.0	155.3[a]	156.2	C-4'	69.9
C-6	104.9	104.8	101.7	104.9	C-5'	76.0[c]
C-7	97.6[a]	101.4	107.5	99.7[a]	C-6'	67.7
C-8	161.3	160.5	158.4	161.1	C-1"	109.4
C-9	99.9[a]	108.5	96.4	100.7[a]	C-2"	76.9
C-10	155.6[b]	151.4	155.0[a]	155.6[b]	C-3"	78.7
C-11	155.3[b]	155.3	156.5[a]	155.1[b]	C-4"	73.6
C-12	103.8	104.5	103.4	103.2	C-5"	63.5
C-13	140.7	139.9	138.5	140.3		
C-14	107.6	109.7	107.8	108.1		
Me-2	20.1	19.8	20.4	19.8		
Me-7	-	-	10.3	-		
OMe	55.2	55.5	55.6	55.5		

Solvents: * = DMSO-d_6; # = $CDCl_3$-DMSO-d_6, 1:1
a,b,c, These signals may be interchanged in the same column.



	R_1	R_2
1	H	H
2	SO_3^-	H
3	A	H
4	SO_3^-	Me
5	Me	H
6	H	Me
7	Me	Me



A

of substitution similar to that of 2 except for the presence of an additional aromatic methyl group on the A-ring. By treatment with ethereal CH_2N_2 the hydrolysed derivative of 4, 6, was transformed into the monomethyl derivative (7). The absence in the ^{13}C nmr spectrum of 7 of signals at ca. 60 ppm attributable to a ortho disubstituted methoxy group suggested the location of the additional methyl group of cassiapyrone-10-sulphate at C-7.

2D INEPT long-range experiments [9] carried out on compound 6 allowed us to assign the signal at 158.4 ppm to the carbon bearing the OMe group. Moreover, selective irradiation of the methyl protons at C-7 (δ 2.28 in $CDCl_3$-DMSO-d_6) gave a 6 Hz coupling with the carbon at 107.5 ppm (C-7), and 4 Hz couplings with the carbons at 138.5 ppm and 158.4 ppm , respectively. These long-range coupling values, in agreement with those found for toluene, [10] confirmed the location of the additional methyl group at C-7 and allowed us to assign the OMe group to C-8.

To our knowledge γ-naphthopyrone sulphates were so far isolated only from crinoids, where they are probably involved in chemical defence processes against fish. [11]

BIOLOGICAL ACTIVITY.

Compounds (1), (2), (3), (4), and (6) were tested against Candida albicans, C. tropicalis, C. parapsilosis, C. krusei, Enterococcus faecalis, Serratia marcescens, Escherichia coli, and Acinetobacter sp. They showed the following activities against Acinetobacter sp. (MIC: 10, 50, 100, 100, 10 µg/ml, respectively). 10-Demethylflavasperone was tested for cytotoxicity in vitro against KB cells (ED_{50}: 1.5 µg/ml) as previously described. [12]

EXPERIMENTAL

^1H and ^{13}C nmr spectra were registered at 400 and 100 MHz, respectively, on a Bruker AM 400 (TMS as internal standard). The NOESY experiment was performed using a Bruker AC 200.

Plant material. Roots of C. pudibunda were collected in Pernambuco (Brazil). A voucher sample is kept at the Departamento de Antibioticos, Recife, Brazil (n. 5461).

Extraction and purification. The roots of C. pudibunda (1.5 Kg) were extracted with MeOH (3x1.5l) at room temperature. The residue after evaporation (40 g) was chromatographed on SiO_2 using a gradient $CHCl_3$-AcOEt as eluent to give the following substances: chrysophanol (160 mg), physcion (24 mg), 10-demethylflava-

sperone (160 mg), chrysophanol dimethyl ether (36 mg), cis-3,3',5,5'-tetrahydroxy-4-methoxystilbene (160 mg), and trans-3,3',5,5'-tetrahydroxy-4-methoxystilbene (480 mg). The fraction eluted with MeOH (28 g) was rechromatographed on Sephadex LH-20 (MeOH) to afford a mixture of 1 and 4 (0.8 g), and a mixture of glycosides (0.7 g). The latter was resolved using LiChroprep RP-8 (MeOH/H_2O, 7:3) into 10-demethylflavasperone-10-O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside (120 mg), rubrofusarin-6-O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside (240 mg), rubrofusarin-6-O-β-D-glucopyranoside (40 mg), quinquangulin-6-O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside (120 mg), and quinquangulin-6-O-β-D-glucopyranoside (20 mg). Preparative paper chromathography of crude 10-demethylflavasperone-10-sulphate using n-BuOH/EtOH/H_2O (4:1:2.2) gave pure 10-demethylflavasperone-10-sulphate (0.6 g), and cassiapyrone-10-sulphate (56 mg).

10-Demethylflavasperone, 1. mp 207-210°C (from $CHCl_3$). Uv (MeOH), λ_{max} nm (log ε): 373 (3.52), 283 (4.27), 241 (4.53); ir (KBr), ν_{max} cm^{-1}: 1660, 1615, 1550, 1470, 1400, 1360, 1310, 1160, 960, 850. Positive to the Gibbs test. ^1H Nmr (DMSO-d_6), δ: 2.45 (3H, s, Me), 3.81 (3H, s, OMe), 6.42 (1H, d, J=2.1 Hz, H-9), 6.43 (1H, s, H-3), 6.68 (1H, d, J=2.1 Hz, H-7), 6.85 (1H, s, H-6), 12.80 (1H, s, exchang. with D_2O, OH). ^{13}C Nmr: see Table 1. EI-ms, m/z (%): 272 (M^+, $C_{15}H_{12}O_5$, 100), 243 (20), 232 (15), 229 (12), 201 (10).

Methylation of 1 (10 mg) with ethereal CH_2N_2 gave flavasperone identified by comparison of its uv and ^1H nmr data with those reported in the literature. [4]

10-Demethylflavasperone-10-sulphate, 2. Amorphous powder. Uv (MeOH), λ_{max} nm: 385, 315 sh, 278, 250 sh, 234 . Ir (KBr), ν_{max} cm^{-1}: 3600-3450, 1660, 1615, 1560, 1425, 1320, 1255, 1230, 1030. ^1H Nmr (DMSO-d_6), δ: 2.52 (3H, s, Me), 3.86 (3H, s, OMe), 6.46 (1H, s, H-3), 6.94 (1H, s, H-6), 7.01 and 7.02 (2H, 2d, J=2.0 Hz, H-7 and H-9), 12.84 (1H, s, exchang. with D_2O, OH). ^{13}C Nmr: see Table 1. FAB-ms, m/z: 351 $(M-1)^-$.

Hydrolysis of 2 with 1N HCl gave 1.

Product of sulphation. 10-Demethylflavasperone (10 mg), sulphamic acid (20 mg), and pyridine (2 ml) were refluxed for 1 h. The reaction mixture was evaporated, and the residue after evaporation partitioned between water saturated with sodium bicarbonate and n-BuOH. The purification of the organic phase with preparative paper chromatography using n-BuOH/EtOH/H_2O (4:1:2.2) gave pure 2 (5 mg) identified by direct comparison.

10-Demethylflavasperone-10-O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside, 3.

Mp 180°C (with dec.) (MeOH-AcOEt), $[\alpha]_D$ -53.0° (c 0.3, MeOH). Uv (MeOH), λ_{max} nm (log ε): 370 (3.35), 310 sh (3.77), 279 (4.16), 244 (4.26), 239 (4.36). ^{1}H Nmr (DMSO-d_6), δ: 2.53 (3H, s, Me), 3.87 (3H, s, OMe), 4.81 (1H, d, J=3.0 Hz, H-1"), 5.08 (1H, d, J=7.8 Hz, H-1'), 6.48 (1H, s, H-3), 6.72 and 6.91 (2H, 2d, J=2.1 Hz, H-7 and H-9), 6.94 (1H, s, H-6), 12.93 (1H, s, exchang. with D_2O, OH). ^{13}C Nmr: see Table. FAB-ms, m/z: 589 $(M+Na)^+$, 567 $(M+1)^+$.

Hydrolysis of 3: 1. Compound 3 (20 mg) was refluxed with 0.1N H_2SO_4 (3 ml) for 20 min. After neutralization with $Ba(OH)_2$ and filtration, the aqueous phase was extracted with AcOEt. The residue of the organic phase (10 mg) was chromathographed on SiO_2 (n-hexane-AcOEt, 1:1) to give pure 1, that was identified by direct comparison. In the aqueous phase the sugars were identified as apiose and glucose by co-tlc with authentic samples (SiO_2, n-BuOH/AcOH/H_2O, 6:3:1, revealed with thymol).

Cassiapyrone-10-sulphate, 4. Amorphous powder. Uv (MeOH), λ_{max} nm (log ε): 370 (3.48), 320 sh (3.83), 284 (4.34), 240 sh (4.42), 236 (4.43), 220 sh (4.39). ^{1}H Nmr (DMSO-d_6), δ:2.39 (3H, s, Me-7), 2.55 (3H, s, Me-2), 3.88 (3H, s, OMe), 6.48 (1H, s, H-3), 6.95 (1H, s, H-6), 7.27 (1H, s, H-9), 12.80 (1H, s, exchang. with D_2O). FAB-ms, m/z: 365 $(M-1)^-$, 285 $(M-SO_3-1)^-$.

Hydrolysis of 4:6. Compound 4 (40 mg) was refluxed in 0.1 N HCl (4 ml) for 20 min. After neutralization with $Ba(OH)_2$ and filtration, the aqueous phase was extracted with AcOEt. The residue of the organic phase (22 mg) was chromatographed on SiO_2 (n-hexane-AcOEt, 1:1) to give pure 6.

Cassiapyrone, 6. Amorphous powder. Uv (MeOH), λ_{max} nm (log ε): 387 (3.54), 292 (4.16), 246 (4.50), 218 (4.16). ^{1}H Nmr (DMSO-d_6), δ: 2.20 (3H, s, Me-7), 2.49 (3H, s, Me-2), 3.84 (3H, s, OMe), 6.43 (1H, s, H-3), 6.64 (1H, s, H-9), 6.79 (1H, s, H-6), 10.15 (1H, bs, OH, exchang. with D_2O), 12.76 (1H, s, OH, exchang. with D_2O). ^{13}C Nmr: see Table 1. EI-ms, m/z (%): 286 (70), 256 (10), 246 (30), 231 (30), 203 (20), 149 (44), 135 (50), 119 (30), 105 (30), 91 (50), 83 (100).

Methylation of 6: 7. Compound 6 was treated with ethereal CH_2N_2 overnight to give pure 7. mp 209-213°C (MeOH). ^{1}H Nmr ($CDCl_3$), δ: 2.34 (3H, s, Me-7), 2.49 (3H, s, Me-2), 3.96 and 4.00 (6H, 2s, 2 OMe), 6.27 (1H, s, H-3), 6.53 (1H, s, H-9), 7.04 (1H, s, H-6), 12.73 (1H, s, OH, exchang. with D_2O). ^{13}C Nmr ($CDCl_3$): 55.9 and 56.1 ppm (OMe).

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