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Flavonoids, benzophenones and a new euphane derivative from *Clusia columnaris* Engl.

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RESUMO: "Flavonóides, benzofenonas e um novo eufano de *Clusia columnaris* Engl.". Dos frutos de *Clusia columnaris* foram isoladas as benzofenonas poliisopreniladas machuona e clusiacromeno A. Do extrato em hexano obtido de galhos e folhas novas, um novo triterpeno do tipo eufano foi isolado. Sua estrutura foi elucidada através de métodos espectroscópicos. Por outro lado, dos extratos mais polares - em acetato de etila e em butanol, foram isolados os flavonóides C-glicosilados isovitexina, vitexina e vitexina-2"-xilosídeo, além de sete bisflavonóides conhecidos como bisflavonóides do grupo da *Garcinia.*

Unitermos: *Clusia columnaris*, Guttiferae, eufanos, flavonóides C-glucosídicos, bisflavonóides.

ABSTRACT: The polyisoprenylated benzophenones machuone and clusiachromene A have been isolated from the fruits of *Clusia columnaris*. The hexane extract of the young branches with leaves afforded a new euphane derivative, whose structure was elucidated by spectroscopic methods. On the contrary, the most polar EtOAc and ButOH extracts were constituted of flavonoid C-glucosides (isovitexin, vitexin and vitexin-2"-xyloside) and seven biflavonoids of the so-called *Garcinia* group.

Keywords: *Clusia columnaris*, Guttiferae, euphane derivatives, flavonoid C-glucosides, biflavonoids.

INTRODUCTION

According to Engler, the genus *Clusia* belongs to the family Guttiferae, subfamily Clusioideae and consists of tall shrubs or trees some attaining a height of 15 meters. More than 200 species are known and their occurrence is limited to tropical and subtropical regions of Central and South America (Engler, 1925). Our chemosystematic studies (Delle Monache et al., 1988; 1991a and 1991b; Delle Monache, 1990; Martinez et al., 1994; Farfan et al., 1998; Gonzales et al., 1995) have demonstrated that the main components of the fruits are benzophenones modified by successive attach of two, three or more isoprenyl groups. Occasionally, the roots have provided dihydrophenantrenes and biphenyls (Delle Monache et al., 2002), and the leaves flavonoids C-glucosides (Delle Monache, 1991) and fukugetin (Martinez et al., 1996). Finally, triterpenes have been found both in fruits (Akihisa et al., 1999) and leaves (Hasbun et al., 1989).

This paper deals on the composition of the extracts from *C. columnaris* collected in the Venezuelan Amazonia.

MATERIAL AND METHODS

General

NMR experiments were performed on a Gemini 300 NMR spectrometer, operating at 300 MHz for ^{1}H and at 75 MHz for ^{13}C, at 25 ^{o}C. Chemical shifts are expressed in δ (parts per million) referred to the solvent peaks; coupling constants, *J*, are in Hertz.

Plant material

Young branches with leaves and fruits of *Clusia columnaris* Engl. were collected by the margins of Cataniapo River near Puerto Ayacucho (Amazonas, Venezuela) and identified by one of us (A.C.). A voucher specimen is deposited at the Herbarium of the Escuela de Biología, Universidad Central de Venezuela under the cipher AC 3351.

Fruit extract

Fruits (200 g) were extracted twice with cold

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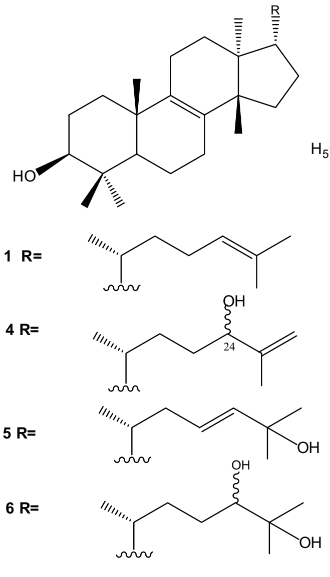
acetone and the pooled extracts evaporated yielding an oily residue (18.4 g). Part of the extract (6.8 g) was chromatographed on a SiO_2 column eluted with C_6H_6. Fraction 8-21 (250 mg) was constituted of a mixture of sesquiterpenes; fraction 22-42 (2.6 g), mainly constituted of triglycerides, was not further processed; fraction 43-80 (270 mg) was purified by CC (SiO_2, C_6H_6-hexane, 3:1) to give clusiachromene A, **3**, (62 mg); fraction 81-128 (2.1 g) was crystallized twice from hexane to afford euphol, **1**, (720 mg); finally, fraction 129-165 (130 mg), eluted with C_6H_6-EtOAc, 9:1, was again chromatographed (SiO_2, C_6H_6-EtOAc 95:5) to yield the oily machuone, **2** (65 mg).

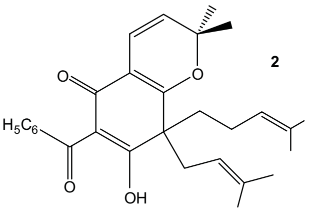
Branches with leaves extract

Young branches with leaves (500 g) were extracted twice with cold MeOH. During the concentration of the extract a solid separated, constituted mainly of waxes, which was filtered off. The soluble portion was evaporated, suspended in MeOH-H_2O, 9:1 and partitioned with hexane (hexane extract, 5.6 g). After evaporation of the MeOH, the aqueous suspension was extracted with $CHCl_3$, EtOAc and butanol saturated with water, successively, affording the $CHCl_3$ extract (6.4 g), the EtOAc extract (7.3 g) and the BuOH extract (3.8 g), respectively.

Part (5 g) of the $CHCl_3$ extract was purified by CC eluted with a gradient of acetone in $CHCl_3$. Fraction 7-11 (750 mg) was methylated with diazomethane and separated on SiO_2 column with a gradient of EtOAc in CH_2Cl_2 to give methyl-betulinate (160 mg), a mixture of methyl-oleanolate and methyl-ursolate (60 mg) (eluted with CH_2Cl_2-EtOAc, 95:5), euphane **4** (260 mg) (eluted with CH_2Cl_2-EtOAc, 9:1) and euphane **5** (205 mg), successively. Fraction 18-25 (420 mg) was again chromatographed on SiO_2 column with $CHCl_3$-MeOH, 9:1 to give euphane **6** (120 mg). Fraction 32-39 (410 mg), eluted with $CHCl_3$-acetone, 9:1, was constituted of β-sitosterol glucoside. The other fractions 1-6 (400 mg), 12-17 (680 mg), 26-31 (1.4 g) and 40-50 (770 mg), constituted of mixture of the above products and/or chlorophylls, were not furtherly processed.

The EtOAc extract was suspended in acetone, filtered and washed again with a little amount of acetone yielding a yellow solid constituted of pure vitexin (**7**) (325 mg). The pooled soluble portions were evaporated and a part (3.6 g) submitted to CC with a gradient of MeOH in $CHCl_3$. Fraction 76-90 (395 mg) by CC ($CHCl_3$-acetone, 1:1) yielded the biflavonoids GB-1a, **10** (104 mg) and volkensiflavone, **13** (25 mg). Similar purification of the





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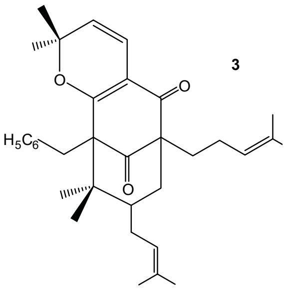

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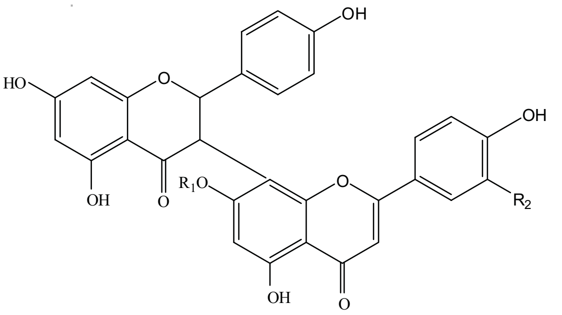
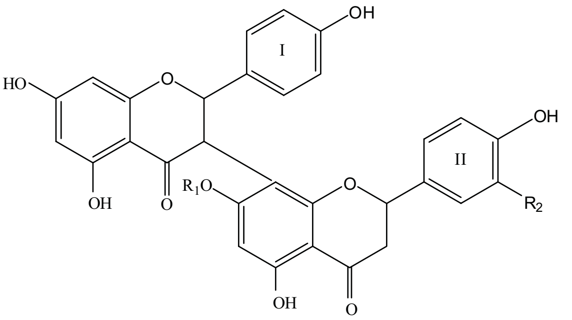
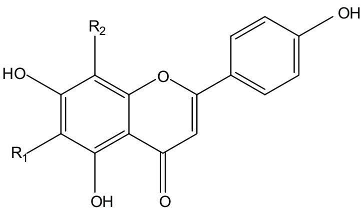


	R_1	R_2	
10	H	H	GB1a
11	H	OH	GB2a
12	Glu	OH	GB2a glucoside



	R_1	R_2	
13	H	H	Volkensiflavone
14	Glu	H	Volkensiflavone glucoside
15	H	OH	Fukugetin
16	Glu	OH	Fukugiside



	R_1	R_2
7	H	Glu
8	Glu	H
9	H	Glu-2"-Xyl

Table 1. NMR data ($CDCl_3$) of euphanes **1, 4, 5** and **6**.

	1	**4**	**5**	**6**
1	35.2	35.1	35.1	35.2
2	27.9	27.7	27.7	27.7
3	78.9	78.8	78.7	78.6
4	38.8	38.8	38.7	38.8
5	50.9	50.8	50.8	50.8
6	18.9	18.8	18.8	18.8
7	27.6	27.5	27.6	27.6
8	134.0	133.9	133.7	133.9
9	33.5	133.3	133.3	133.3
10	37.2	37.1	37.1	37.1
11	21.5	21.4	21.3	21.4
12	30.8	30.7	30.8	30.8
13	44.0	44.0	44.0	44.0
14	49.9	49.8	49.8	49.9
15	29.7	29.6	29.6	29.7
16	28.1	28.0	27.8	28.1
17	49.6	49.5	49.4	49.8
18	15.6	15.5	15.5	15.6
19	20.1	20.0	20.0	20.0
20	35.8	35.8	36.1	35.7
21	18.8	18.9	18.9	18.7
22	35.3	31.3	38.0	33.4
23	24.7	30.9, 30.8	125.4	29.7,28.6
24	125.1	76.4, 76.3	139.1	78.9,78,7
25	130.7	147.6, 147.5	70.5	73.3,73.2
26	17.6	111.0, 110.9	29.7	23.1
27	25.9	17.3	29.6	26.4
28	28.0	27.9	27.9	28.0
29	15.5	15.4	15.5	15.5
30	24.4	24.3	24.3	24.4

fraction 91-102 (595 mg) gave further GB-1a (245 mg) and GB-2a, **11** (180 mg). Fraction 103-126 (600 mg) was not furtherly processed and fraction 127-139 (230 mg) by CC with acetone gave fukugetin, **15** (83 mg). Extended chromatography (SiO_2; EtOAc-Acetone-H_2O, 25;8;2 upper phase) of the fraction 140-158 (210 mg) afforded volkensiflavone glucoside, **14** (22 mg), GB-2a glucoside, **12** (23 mg) and isovitexin, **8,** (12 mg).

Analogous separation proceedings were used to purify the BuOH extract (3.8 g). Washing with acetone gave a yellow solid (650 mg) constituted of a 1:2 mixture of vitexin and vitexin-2”- xyloside, **9** (650 mg). Repeated column chromatography of the filtrate gave further vitexin (600 mg), volkensiflavone glucoside, **14** (105 mg) and vitexin-2”-xyloside (540 mg).

Euphane-8, 25-diene-3β, 24-diol **(4):** ^{1}H NMR spectrum ($CDCl_3$), *δ*; 4.92 and 4.83 (br s; H-26), 4.00 (t, *J* 6.1 Hz; H-24), 3.23 (dd, *J* 11.2 and 4.7 Hz; H-3), 1.72 (s, Me-27), 0.95 (s, Me-19), 0.86 (s, Me-30), 0.79 (s, Me-18), 0.78 (d, *J* 6.1 Hz; Me-21). Long-range connectivities (L.R. spectrum); C-24 with *δ* 4.92/4.83; C-25 with *δ* 1.72 (Me-27). Long-range connectivities (INEPT); H-26 with *δ*c 17.3 (Me-27); Me-27 with *δ*c 147.6/147.5 (C-25) and *δ*c 111.0/110.9 (C-26).

Euphane-8, 23-diene-3β , 25-diol **(5):** ^{1}H NMR spectrum ($CDCl_3$), *δ*; 5.57 (br s; H-23 and H-24), 3.23 (dd, *J* 11.2 and 4.7 Hz), 1.31 (s; Me-26 and Me-27), 1.00 (s, Me-28), 0.95 (s, Me-19), 0.80 (s, Me-18), 0.78 (d, *J* 6.0 Hz; Me-21), Long-range connectivities (L.R. spectrum); C-24 with *δ* 1.31 (Me-26/Me-27); C-25 with *δ* 1.31 (Me-26/Me-27).Long-range connectivities (INEPT); H-23/H-24 with *δ*c 70.5 (C-25); Me-26/Me-27 with *δ* 139.1 (C-24) and *δ*c 70.5 (C-25).

Euphane-8-ene-3β, 24, 25-triol **(6):** ^{1}H NMR spectrum ($CDCl_3$), *δ*; 3.30 (m, H-24), 3.23 (dd, *J* 11.2 and 4.7; H-3), 1.21 (s, Me-27), 1.16 (s, Me-26), 0.94 (s, Me-19), 0.86 (s, Me-30), 0.79 (s, Me-18), 0.71 (d, *J* 6.0 Hz; Me-21). Long-range connectivities (L.R. spectrum); C-24 with *δ* 1.21 (Me-27); C-25 with *δ* 1.21 (Me-27) and *δ* 1.16 (Me-26); Me-27 and *δ* 1.16 (Me-26). Long-range connectivity (INEPT); Me-27 with *δ*c 73.3/73.2 (C-25).

Vitexin-2”-xyloside **(9):** NMR data ($DMSO-d_6$) in Table 2. ^{1}H NMR spectrum (C_5D_5N), *δ* 14.08 (s, OH-5), 8.35 (d, *J* 8.8 Hz; H-2’,6’), 7.28 (d, *J* 8.8 Hz; H-3’,5’), 6.94 (s, H-3), 6.79 (s, H-6), 5.97 (d, *J* 10 Hz; G-1), 4.55 (d, *J* 7.72 Hz; X-1). ^{13}C NMR spectrum (C_5D_5N), *δ* 183.1 (C-4), 164.9, 164.6 (C-3, C-7), 162.6, 162.3 (C-4’, C-6), 157.9 (C-9), 129.4 (C-2’,6’), 122.4 (C-1’), 116.8 (C-3’,5’), 106.9 X-1), 105.22, 105.17 (C-10, C-8), 103.3 (C-3), 99.2 (C-6), 83.3 (G-2), 82.1 (G-5), 80.5 (G-3), 77.5, 75.0 (X-3, X-2), 73.2 (G-1), 71.7 (G-4), 70.6 (X-4), 66.7 (X-5), 62.5 (G-6).

GB-1a **(10)**, *GB-2a* **(11)**, *Volkensiflavone* **(13)**, *Fukugetin* **(15):** ^{1}H NMR spectra (C_5D_5N) in Table 3. ^{13}C NMR spectra (C_5D_5N) in Table 4.

GB-2a glucoside **(12),** *Volkensiflavone glucoside*, **(12)**, *Fukugiside* **(16):** The identification

Table 2. NMR data (C_5D_5N, *J* in Hertz in parenthesis) for vitexin-2”-xyloside, **9**.

C	^{13}C	^{1}H	Long-range connectivities	
			^{2}J	**^{3}J**
2	164.2		H-3	H-2’,6’
3	102.8	6.80s		
4	182.4		H-3	
5	160.9		H-6	OH-5
6	98.5	6.27s		
7	163.1		H-6	G-1
8	104.0		G-1	H-6
9	156.9			G-1
10	104.1			H-3, OH-5
1’	121.9			H-3’,5’
2’,6’	129.2	8.04 d (8.6)		
3’,5’	116.3	6.94 d (8.6)		
4’	161.5			H-2’,6’
glc-1	71.9	4.83 d (9.8)		
glc-2	82.1	3.32 m		
glc-3	78.6	3.58*		
glc-4	70.5	3.54*		
glc-5	81.6	4.09 br t (9.0)		
glc-6	61.3	3.79 br d; 3.60		
xyl-1	106.1	3.91 d (7.0)		
xyl-2	74.0	2.80 dd (7.0; 8.0)		
xyl-3	76.2	2.90 br t (8.0)		
xyl-4	69.7	3.02+		
xyl-5	65.8	3.02+; 2.39 t (12.6)		

*, + Overlapped signals

Table 3. ^{1}H NMR data (C_5D_5N) of the biflavonoids **10**, **11**, **13** and **14**.

	GB1a (**10**)	GB2a (**11**)	Volkensiflavone (**13**)	Fukugetin (**14**)
I-2	6.22	6.26	6.54	6.48
3	5.20	5.26	5.56	5.34
5-OH	13.18	13.18	13.20	13.20
6	6.53	6.53	6.55	6.58
8	6.49	6.47	6.50	6.47
2',6'	7.69	7.74	7.72	7.67
3',5'	7.31	7.33	6.99	7.00
6'	7.69	7.74	7.72	7.67
II-2	5.76	5.55	-	-
3	2.83	2.93	6.70	6.80
5-OH	12.74	12.75	12.87	12.87
6	6.42	6.53	6.82	6.73
2'	7.56	6.43	8.12	7.99
3'	7.24	-	7.34	-
5'	7.24	7.33	7.34	7.38
6'	7.56	7.12	8.12	7.96

Table 4. ^{13}C NMR data (C_5D_5N) for the biflavonoids **10**, **11**, **13** and **14**.

	GB1a (**10**)	GB2a (**11**)	Volkensiflavone (**13**)	Fukugetin (**14**)
I-2	82.6	82.7	82.3	82.5
3	48.9	48.8	50.2	50.5
4	197.8	197.9	197.9	197,5
5	165.3	165.4	165.5	166.0
6	97.2	97.3	97.5	98.1
7	168.0	168.1	168.3	168.5
8	96.0	96.0	96.4	96.9
9	164.2	164.1	164.4	164.6
10	102.7	102.8	102.9	103.5
1'	129.8	129.6	129.7	129,9
2'	130.0	130.1	130.0	129.6
3'	116.3	116.0	116.9	116.2
4'	159.5	159.4	159.3	159.6
5'	116.3	116.0	116.9	116.2
6'	130.0	130.1	130.0	129.6
II-2	79.5	79.9	164.2	165.0
3	43.6	43.8	103.6	104.3
4	196.3	196.4	182.8	183.2
5	166.3	166.4	162.7	162.7
6	96.6	96.8	98.9	100.2
7	168.0	168.1	168.3	168.5
8	102.7	102.7	102.1	102.4
9	161.8	161.9	162.4	157.2
10	102.3	102.4	104.7	105.4
1'	132.3	131.0	122.3	123.1
2'	128.6	118.5	129.2	120.0
3'	115.7	147.6	115.8	148.0
4'	159.3	147.9	156.8	151.9
5'	115.7	115.8	115.8	115.1
6'	128.6	116.8	129.2	117.2

was performed by acid hydrolysis which gave the corresponding aglicone with NMR data agreeing with those of Table 3 and 4. For fukugiside the identification was confirmed by comparison with an authentic specimen (Martinez et al., 1996). The presence of glucose suggested by the appropriate ^{13}C NMR signals in the regions 103.0-102.0 ppm, 80.0-79.0 ppm, 76.0-75.0 ppm and 62.0-61.8 ppm, was confirmed by co-TLC of the hydrolyzed sugar with glucose.

RESULTS AND DISCUSSION

The fruit extract was subjected to extensive separation to give a great amount (more than 0.3% from the fresh fruits) of euphol, **1**, and two polyisoprenylated benzophenones, machuone (Martinez et al., 1994), **2** and clusiachromene A, **3**. The structure **3** was firstly proposed for a compound isolated from *Clusia grandiflora* (Delle Monache, 1992 and 1993), than assigned to a compound named chamone II obtained from the same species (Lokvam et al., 2000).

The $CHCl_3$ soluble portion of the MeOH extract of branches with leaves yielded β-sitosterol glucoside, betulinic acid, an unseparable mixture of oleanolic and ursolic acids, and three euphanes (**4-6**), whose ^{13}C NMR spectra are reported in Table 1. ^{13}C NMR shifts relative to ring carbons and methyls of the compounds **4-6** are virtually identical to those of euphol (**1**), suggesting a same skeleton. In addition, the ^{1}H NMR spectrum of euphane **4** (see Experimental) displayed, *inter alia*, signals for a secondary methyl (δ 0.78), an unsaturated methyl (δ 1.72), an oxigen-bearing methine (δ 4.0) and a terminal methylene (δ 4.92 and 4.83), therefore it was assigned the structure of eupha-8, 25-diene-3β, 24-diol, previously attributed to a product obtained by photo-oxidation of euphol (Leong and Harrison, 1999), but never reported as a natural product. Doubling of carbon resonances associated to C-24 and adjacent carbons is consistent with the presence of a C-24 epimer. Analogously, the ^{1}H NMR spectrum of the euphane **5** (see Experimental) showed for the chain signals attributable to a secondary methyl (δ 0.79), a disubstituted double bond (δ 5.57, broad s, 2H) and two deshielded methyls (δ 1.31; 6H), consequently it was attributed the structure of eupha-8, 23 diene-3β, 25 diol (**5**), previously assigned to a compound isolated from *Tripetalum cymosum* (Guttiferae) (Leong and Harrison, 1999). Finally, the most polar euphane, **6,** isolated from the leaves, exhibited in the ^{1}H NMR spectrum (see Experimental) signals for a methyl doublet (δ 0.79) and two methyl singlets (δ 1.16 and 1.21). In addition to the three above methyls (δc 18.7, 23.1 and 26.9) in the ^{13}C NMR spectrum (Table 1) the euphane **6** displayed for the chain two methylenes (δc 33.4 and 29.7), a methyne (δ 35.7) and two signals at δc 78.9 and 73.3 for a secondary and a tertiary alcohol, respectively.

Therefore, it was assigned the structure of eupha-8-ene-3β, 24, 25 triol, **6**. Doubling of signals of carbons near to C-24 was again attributed to the presence of an epimer in that position. The chain depicted in **6** is fairly common in fungal lanostanes (De Bernardi et al., 1981) and in cycloartanes (Anjaneyulo et al., 1985), but not yet in euphanes.

Repeated chromatography of the EtOAc and

ButOH soluble portions of the MeOH extract afforded compressively three flavonoid C-glucosides and seven biflavonoids. Vitexin, **7**, and isovitexin, **8,** were obtained from the EtOAc extract and identified by NMR data and co-TLC with authentic specimens (Leitão et al., 1994), while the ButOH soluble portion gave vitexin-2”-xyloside (**9**) whose NMR data are reported in Table 2, as a result of Hetcor spectrum enriched by long-range connectivities. Our results agreed with the data reported by Gluchoff-Fiasson et al. (1989) except for those of C-5 and C-4’, which should be reversed.

Finally, six biflavonoids of the *Garcinia* group (**10-16**), namely GB 1a (**10**), GB 2a (**11**) and its glucoside (**12**),volkensiflavone (**13**) and its glucoside (**14**), fukugetin (morelloflavone) (**15)** and its glucoside (**16**) were isolated. The biflavonoids were identified by co-TLC with authentic samples (Botta et al., 1984) and by NMR data. As known, (Chari etr al., 1977) NMR spectra of biflavonoids present, at room temperature, two set of signals for the presence of conformational equilibrium due to inhibition of free rotation about the C-C bond in the interflavonoid linkage. The data of Tables 3 and 4 refer to the most abundant conformer present in the estimated following ratio; GB-1a, 2:1; GB-2a, 5:1; volkensiflavone, 3:2; fukugetin, 3.5:2.

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