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PRENYLATED AND GERANYLATED CHALCONES AND FLAVONES FROM THE AERIAL PARTS OF *DORSTENIA CILIATA*

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ABSTRACT. Investigation of the aerial parts of *Dorstenia ciliata* yielded two new prenylated flavones named ciliatins A and B and characterized as 6,7-(2"-isopropenyldihydrofurano)-5,4'-dihydroxyflavone and 6,5-(2,2-dimethyldihydropyrano)-7,4'-dihydroxy-3'-methoxyflavone, respectively. Known flavonoids: stipulin, isobavachalcone, licoflavone C, 6-prenylapigenin, dinklagin C, gancaonin P, canniflavone and poinsettifolin A were also identified. Structures of these secondary metabolites were established on the basis of spectroscopic analysis, comparison with published information and with authentic specimen for some cases and chemical evidence for the dihydropyranoflavone derivative.

KEY WORDS: *Dorstenia ciliata*, prenylated flavones, 6,7-(2"-isopropenyldihydrofurano)-5,4'-dihydroxyflavone, 6,5-(2,2-dimethyldihydropyrano)-7,4'-dihydroxy-3'-methoxyflavone, Stipulin, Isobavachalcone, Licoflavone C, 6-Prenylapigenin, Dinklagin C, Gancaonin P, Canniflavone, Poinsettifolin

INTRODUCTION

The genus *Dorstenia* (Moraceae) consists of approximately 170 mostly tropical and undergrowth species [1]. Very few of them are phytochemically or pharmacologically investigated. Several *Dorstenia* species are used in folk medicine mainly against skin diseases due to the content of furanocoumarins, which exhibit a broad range of biological activities [2]. As part of our work on isolation and identification of constituents of African marketed plants [3, 4] especially *Dorstenia* species [5], we have investigated the dichloromethane–methanol (1:1) and methanol extracts of the aerial parts of *Dorstenia ciliata* which has been neither phytochemically nor pharmacologically investigated up to now. The present paper describes the isolation and structural determination of two new prenylated flavones (**1**) and (**2**) for which the names ciliatins A and B, respectively, are proposed as well as the known stipulin (**3**) [6], isobavachalcone (**4**) [6], 8-prenylapigenin (licoflavone C) (**5**) [7], 6-prenylapigenin (**6**) [6], dinklagin C (**7**) [8], gancaonin P (**8**) [9], canniflavone (**9**) [10] and poinsettifolin A (**10**) [11].

RESULTS AND DISCUSSION

The organic extract of the aerial parts of *D. ciliata* was subjected to vacuum liquid chromatography. The non-polar portion was found to contain a mixture of hydrocarbons, which were not investigated further. The polar fractions were passed through Sephadex LH-20 column and then subjected to repeated silica gel column chromatography and preparative TLC

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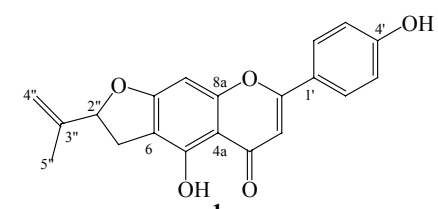
separations to give two chalcones (**3**, **4**) [7], seven flavones (**1**, **2**, **5-9**) [6-10] and one flavonol (**10**) [11].



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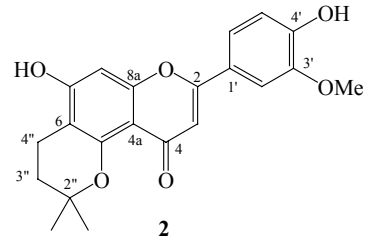

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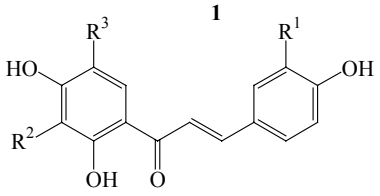
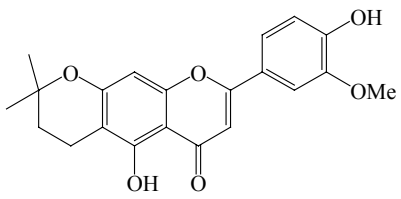
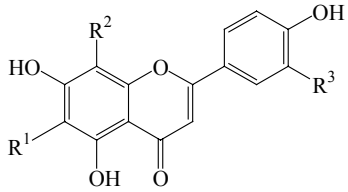
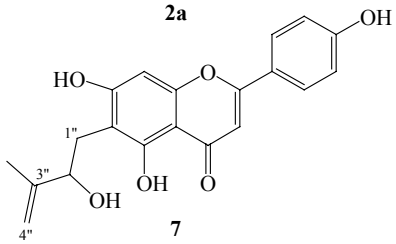


	R^1	R^2	R^3
3	Pr	H	Pr
4	H	Pr	H



2a

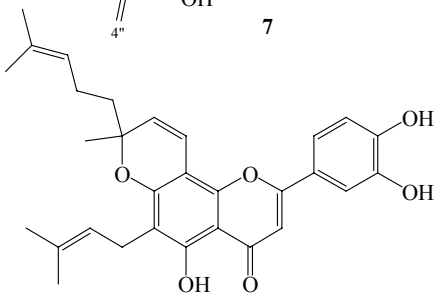




7

	R^1	R^2	R^3
5	H	Pr	H
6	Pr	H	H
8	H	H	OH
9	Pr	H	OMe

Pr = 



10

Compound **1**, obtained as yellow gum, was assigned the molecular formula $C_{20}H_{16}O_5$ from HREIMS. The brown colour produced on reaction of ciliatin A (**1**) with magnesium-hydrochloric acid together with the UV spectra data employing shift reagents (Experimental) and the NMR signals at δ_H 13.34 and δ_C 182.1 indicated that **1** was 5-hydroxyflavone [12, 13]. Its ^{1}H NMR showed two singlets of one proton each at δ 6.48 and 6.58 assignable to H-3 and H-6 or H-8. The NMR spectrum (Table 1) of **1** showed characteristic signals which revealed the presence of a *p*-disubstituted benzene ring [broad doublet of two protons each at δ_H 6.94 and 7.85 (J = 8.8 Hz), δ_C 116.0 (*d*) and 128.3 (*d*)], and one isopropenyldihydrofuran group. Thus an oxymethine, a methylene and isopropenyl signals at δ_C 87.8 (*d*), 30.2 (*t*) and 16.5 (*q*), 110.5 (*t*),

147.4 (*s*), respectively, and a broad triplet at δ_H 4.66 (J = 7.2 Hz), two broad singlets δ_H 4.82 and 4.78 and two doublets of doublet at δ_H 3.00 (J = 5.9, 13.2 Hz) and 2.90 (J = 7.3, 13.4 Hz) were also observed. Two possibilities were considered regarding the orientation of this isopropenyldihydrofuran group located in ring A: one with linear furan (**1**) or an alternative structure with an angular furan group. This question is also related to the designation of whether the singlet signal at δ_H 6.48 is appropriate for H-6 or H-8. The other singlet signal at δ_H 6.58 was undoubtedly assigned to H-3 on account of the HMBC correlations observed between this proton and the carbonyl signal at δ_C 182.1 and C-1' signal at δ_C 121.8 (*s*). The chemical shift of δ_C 93.4, which was correlated by HMQC experiments to this aromatic proton signal δ_H 6.48 is consistent with its location at C-8 and not at C-6 [14]. Thus the isopropenyldihydrofuran group is deduced to have a linear arrangement. From the foregoing data the structure of compound **1** was determined as 6,7-(2"-isopropenyldihydrofuran)-5,4'-dihydroxyflavone for which the name ciliatin A is proposed. This structure was confirmed by both ^{13}C NMR spectrum and CIMS. The ^{13}C NMR (Table 1) signals were fully assigned using DEPT spectra. The CIMS showed an important ion fragment at *m/z* 295 corresponding to the lost of isoprenyl group.

Table 1. NMR assignments of ciliatins A (**1**), B (**2**) and isociliatin B (**2a**) in CD_3COCD_3. Chemical shifts (δ) are given in ppm; multiplicities and coupling constant J (parentheses) in Hz.

C / H	**1**		**2**		**2a**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	163.4 (*s*)	----	160.3 (*s*)	----	164.4 (*s*)	
3	102.7 (*d*)	6.58 (*s*)	106.0 (*d*)	6.62 (*s*)	103.5 (*d*)	6.69 (*s*)
4	182.1 (*s*)	----	176.2 (*s*)	----	182.7 (*s*)	----
4a	102.7 (s)	----	----	----	104.2 (*s*)	----
5	159.0 (*s*)	----	158.1 (s)	----	159.5 (*s*)	----
6	104.5 (*s*)	----	107.2 (*s*)	----	105.3 (*s*)	----
7	163.2 (*s*)	----	160.2 (*s*)	----	160.6 (*s*)	----
8	93.4 (*d*)	6.48 (*s*)	94.2 (*d*)	6.44 (*s*)	95.1 (*d*)	6.47 (*s*)
8a	156.1 (*s*)	----	156.4 (*s*)	----	155.9 (*s*)	----
1'	121.8 (*s*)	----	123.7 (*s*)	----	123.2 (*s*)	----
2'	128.3 (*d*)	7.85 (*brd*, J = 8.8)	109.5 (*d*)	7.51 (*d*, J = 2.1)	109.9 (*d*)	7.60 (*d*, J = 2.1)
3'	116.0 (*d*)	6.94 (*brd*, J = 8.8)	148.2 (*s*)	---	148.3 (*s*)	----
4'	160.7 (*s*)	----	150.0 (*s*)	----	150.9 (*s*)	----
5'	116.0 (*d*)	6.94 (*brd*, J = 8.8)	115.7(*d*)	6.98 (*d*, J = 8.3)	115.9 (*d*)	7.01 (*d*, J = 8.3)
6'	128.3 (*d*)	7.85 (*brd,* J = 8.8)	120.0 (*d*)	7.48 (*dd*, J = 8.3, 2.1)	120.8 (*d*)	7.64 (*dd*, J = 8.3, 2.1)
1"	30.2 (*t*)	3.00 (*dd*, J =13.2, 5.9) 2.90 (*dd*, J =13.4, 7.3)	----	----	----	----
2"	87.8 (*d*)	4.66 (*t*, J = 7.2)	75.1 (*s*)	----	76.3 (*s*)	----
3"	147.4 (*s*)	----	17.3 (*t*)	1.83 (*t*, J = 6.8)	16.2 (t)	2.69 (*t*, J = 6.8)
4"	110.5 (*t*)	4.82; 4.78 (*brs*)	31.6 (*t*)	2.70 (*t,* J = 6.9)	31.7 (*t*)	1.88 (*t,* J = 6.8)
5"	16.5 (*q*)	1.85 (*s*)	26.4 (*q*)	1.38 (*s*)	26.4 (q)	1.38 (*s*)
6"	----	----	26.4 (*q*)	1.38 (*s*)	26.4 (q)	1.38 (*s*)
OMe	----	----	56.0 (*q*)	3.97 (*s*)	56.0 (*q*)	4.01 (*s*)
5-OH		13.34 (*s*)				13.35 (*s*)

Compound **2** was obtained as a yellow amorphous powder. Its NMR (Table 1) indicated the presence of 21 carbons and 20 hydrogen atoms. The molecular formula was determined as $C_{21}H_{20}O_6$ from the above observations, which was confirmed by the HREIMS. The UV spectrum of ciliatin B (**2**) exhibited maxima absorption at λ_{max} 216, 272 and 334 nm characteristic of flavone skeleton [13]. The addition of $AlCl_3$ did not show any bathochromic shift, which is indicative of the absence of a free 5-hydroxyl group, and no free *ortho*-dihydroxyl group in this flavone. The IR spectrum of **2** showed strong absorption bands for hydroxyl, carbonyl and benzene ring at ν_{max} 3350, 1635 and 1550 cm^{-1}, respectively. Its ^1H NMR spectrum was well resolved and showed signals for a 2,2-dimethyldihydropyran group (see below) and for a methoxyl group at δ 3.97. The ^1H NMR spectrum also displayed five aromatic proton signals, two of them appeared as singlet of one proton each at δ_H 6.44 and 6.62, assignable to H-3 and H-6 or H-8. The signals of the remaining three protons in ring B appeared as an ABX system [δ_H 6.98 (*d*, J = 8.3 Hz), 7.51 (*d*, J = 2.1 Hz) and 7.48 (*dd*, J = 8.3, 2.1 Hz)]. Signals that could be assigned to a 2,2-dimethyldihydropyran group were as follows: two triplets of two protons each at δ_H 2.70 and 1.83 (J = 6.8 Hz) and one singlet of six protons at δ_H 1.38 for the *gem* dimethyl groups. This dimethyldihydropyran group would have an angular arrangement (**2**) because of the upfield chemical shift of the carbonyl (δ_C 176.2) and since the chemical shift at δ_C 94.2 (*d*), which was correlated by HMQC experiments to the aromatic proton signal at δ_H 6.44, is consistent with its location at C-8. The other singlet signal of one proton at δ_H 6.62 was unambiguously assigned to H-3 from HMBC correlations observed between this proton and the carbonyl signal at δ_C 176.2 (*s*) and C-1'signal at δ_C 123.7 (*s*). The signal of the methoxyl group upon NOE irradiation caused 1.8% enhancement of the *meta* coupled doublet signal at δ_H 7.51. This finding together with the observed chemical shift of the OMe group at δ_C 56.0 suggested that the methoxyl function was located at 3'. The structure of this new derivative was determined as: 6,5-(2,2-dimethyldihydropyrano)-3'-methoxy-7,4'-dihydroxyflavone (**2**). This proposed structure was further confirmed both by CIMS spectrum and by ^{13}C NMR data (Table 1) which was fully assigned using DEPT spectra. The CIMS showed the fragment ion at *m/z* 313 $[M-55]^+$ diagnostic for the 2,2-dimethylchroman degradation [14]. The structure of ciliatin B as **2** was unequivocally established by its hemisynthesis from canniflavone (**9**) following a simple procedure reported by Hano *et al.* [15]. Ciliatin B (**2**) and its isomer isociliatin B (**2a)** were obtained from this cyclisation reaction (Experimental).

The molecular formula of compound **3**, $C_{25}H_{28}O_4$ and **4**, $C_{20}H_{20}O_4$ were deduced from the CIMS and NMR spectra measurements. They were found to be stipulin and isobavachalcone, respectively, previously isolated from *Dorstenia kameruniana* (Moraceae) [6].

The ^1H NMR of compound **5** showed a chelated hydroxyl group, a 3,3-dimethylallyl group and a *p*-disubstituted benzene ring. This compound appears to be identical with licoflavone C previously reported from *D. poinsettifolia* [11].

The NMR spectra of compound **6** displayed signals for chelated carbonyl δ_C 182.8, two protons as singlet each at δ_H 6.49 and 6.59 one prenyl group. All the spectroscopic data recorded for **6** were in agreement with those reported for 6-prenylapigenin isolated from *D. kameruniana* [6]

Compound **7** was assigned $C_{20}H_{18}O_6$ as molecular formula from HREIMS measurements. Its ^1H NMR displayed signals for two sets of *ortho* and *meta* coupled protons at δ_H 7.83 and 6.94 (2H each J = 1.2, 8.9 Hz) characteristic of a *p*-disubstituted benzene ring. The presence of 2-hydroxy-3-methyl-3-butenyl group in **7** was deduced from its NMR spectra. **7** was found to be identical with dinklagin C previously isolated from *D. dinklagei* by Ngadjui *et al.* [8].

The spectroscopic data generated for **8**, **9** and **10** (NMR, CIMS, UV, IR) were found to be similar to those reported for gancaonin P [9], canniflavone [10] and poinsettifolin A [11], respectively.

EXPERIMENTAL

General. Melting points are uncorrected and were obtained on a micro melting point apparatus. UV spectra were taken in methanol solution on Shimadzu UV-210 PC UV-vis spectrophotometer. IR spectra were measured as KBr disk. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD or CD_3COCD_3 on a Bruker spectrometer operating at 300 and 75 MHz, respectively, with the residual solvent peaks as internal references. HMBC, HMQC and selective NOESY experiments were carried out with gradient enhancements. CIMS were recorded by direct inlet (70 eV) on a Finnigan SSQ-7000 Single Quadrupole mass spectrometer.

Plant material. The aerial parts of *Dorstenia ciliata* were collected at Ngombuku in South West Province of Cameroon. Dr Achoundong, director of the National Herbarium in Yaounde identified the plant. Voucher specimen (No. 43992) is deposited at the National Herbarium, Yaounde, Cameroon.

Extraction, isolation and characterization. The air dried and powdered aerial parts of *D. ciliata* (1.5 kg) were soaked in the mixture of CH_2Cl_2/MeOH (1:1) followed by pure MeOH for 24 h and 2 h, respectively, at room temp. Concentration of the combined organic extract under reduced pressure yielded a greenish dark residue (125 g). Part (80 g) of this extract was subjected to column chromatography (silica gel 60, 270 g) and eluted with *n*-hexane followed by *n*-hexane-EtOAc gradient. The first fractions eluted with 10% EtOAc contained mainly hydrocarbons and were not investigated further. Elution with 25% EtOAc afforded five fractions, which were separated by repeated CC on Sephadex LH-20, and silica gel, preparative TLC to give **1** (8 mg), **2** (10 mg) and **7** (9 mg). The following fractions eluted with 40% EtOAc gave, after repeated CC on Sephadex and silica gel: **3** (25 mg), **4** (75 mg), **5** (10 mg), **6** (45 mg), **8** (10 mg), **9** (120 mg) and **10** (65 mg).

*6,7-(2''-Isoprepenyldihydrofurano)-5,4'-dihydroxyflavone, ciliatin (**1**).* Yellow gum; $[\alpha]_D$ +16.5 (MeOH, c 0.012). UV λ_{max}^{MeOH} nm (log ε): 215 (4.00), 274 (3.83), 335 (3.82); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 217 (4.02), 283 (3.79), 302 (3.78), 355 (3.89); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 215 (4.01), 285 (3.78), 304 (3.77), 355 (3.88); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 216 (4.71), 277 (3.81), 331 sh (3.71), 390 (3.95); $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 212 (4.70), 278 (3.82), 335 sh (3.73), 389 (3.93); IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1640 (C=O), 1610, 1550, 1500, 1440, 1285, 1110; ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 1; CIMS (isobutane, probe), 200 eV, *m/z* (rel. int): 337 $[M+H]^+$ (100), 311 (15), 295 $[M\text{-isopropenyl}]^+$ (10), 285 (20), 271 (14). HREIMS: found 336.0990; calc. for $C_{20}H_{16}O_5$: 336.0998.

*6,5-(2,2-Dimethyldihydropyrano-7,4'-dihydroxy-3'-methoxyflavone, ciliatin B (**2**).* Yellow amorphous powder. UV λ_{max}^{MeOH} nm (log ε): 216 (4.39), 272 (4.10), 334 (4.17); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): no change; $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 217 (4.96), 274 (4.21), 322 (3.98), 388 (4.23); $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 213 (4.88), 263 (4.16), 274 (4.16), 330 (3.93), 392 (4.32); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1640 (C=O), 1610, 1580, 1550, 1450, 1320, 1210, 1190; ^{1}H NMR (300 MHz, CD_3COCD_3) and ^{13}C NMR (75 MHz, CD_3COCD_3): Table 1; CIMS (isobutane, probe), 200 eV, *m/z* (rel. int.): 369 $[M+H]^+$ (100), 313 $[M\text{-}55]^+$ (98), 298 $[M\text{-}55\text{-}CH_3]^+$ (40), 270 $[M\text{-}55\text{-}CH_3\text{-}CO]^+$ (10); HREIMS: found 368.1264 calc. for $C_{21}H_{20}O_6$: 338.1260.

*6-(2-Hydroxy-3-methyl-3-butenyl)-5,7,4'-trihydroxyflavone,dinklagin C(**7**).* Yellow crystals from hexane-EtOAc; m.p. 236 °C $[\alpha]_D^{25}$ +13.5° (MeOH, c 0.020); UV λ_{max}^{MeOH} nm (log ε): 217 (4.66), 274 (4.40), 335 (4.50); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 220 (4.60), 287 (4.42), 304 (4.40), 358 (4.50); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): no change; $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 206 (4.80), 276

(4.55), 300 (4.35), 380 (4.43); IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1650 (C=O), 1620, 1440, 1350, 1250, 1180, 1101; ^{1}H NMR (300 MHz, CD_3OD): δ 7.85 (2 H, *br, d*, J = 8.8 Hz, H-2', H-6'), 6.93 (2 H, *br, d*, J = 8.8 Hz, H-3', H-5'), 6.56 (1H, *s*, H-3), 6.44 (1 H, *s,* H-8), 4.81(1H, *br, s*, H-4''a), 4.73 (1H, *br, s*, H-4''b), 4.38 (1H, *br, t*, J = 6.6 Hz, H-2''), 3.04 (1H, *dd*, J = 5.3, 13.6 Hz, H-1''a), 2.91 (1H, *dd*, J = 7.5, 13.6 Hz, H-1''b), 1.85 (3H, *s*, 3H-5''); ^{13}C NMR (75 MHz , CD_3OD): δ 182.8 (*s*, C-4), 164.9 (*s*, C-2), 164.4 (*s*, C-7), 161.7 (*s*, C-4'), 159.6 (*s*, C-5), 156.7 (*s*, C-8a), 147.7 (*s*, C-3''), 128.3 (*d*, C-2', C-6'), 122.3 (*s*, C-1'), 116.1 (*d*, C-3',C-5'), 110.0 (*t*, C-4''), 109.7 (*s*, C-6), 103.8 (*s*, C-4a), 102.7 (*d*, C-3), 94.0 (*d*, C-8), 75.6 (*d*, C-2''), 28.5 (*t*, C-1'') and 16.7 (*q*, C-5''); CIMS (isobutane, probe), 200 eV, *m/z* (rel. int.): 355 $[M+H]^+$ (100), 337 $[(M+H)-H_2O]^+$ (80); HREIMS: found 354.1106; calc. for $C_{20}H_{18}O_6$: 354.1103.

*Cyclisation of canniflavone (**9**)*. Canniflavone (50 mg) dissolved in methanol (15 mL) was heated under reflux with HCl (10%, 5 mL) at 50 °C for 3 h. The reaction mixture was diluted with distilled water and extracted with $CHCl_3$. The residue was submitted to CC and preparative TLC to give two compounds (10 mg and 25 mg). The smallest isolated compound was identical (NMR, TLC) to ciliatin B (**2**). The second compound named isociliatin (**2a**) was characterized as follows: yellow gum. UV λ_{max}^{MeOH} nm (log ε): 215 (4.05), 273 (3.72), 344 (3.84); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 215 (4.08), 261 sh (3.67), 2.82 (3.74), 368 (3.82); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): no change; $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 213 (4.79), 262 (3.86), 269 sh (3.80), 299(3.53), 408 (4.01); $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 210 (4.66), 262 (3.86), 301 sh (3.53), 408 (4.01). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1645 (C=O), 1600, 1580,1550, 1490, 1260, 1210, 1160, 1100; ^{1}H NMR (300 MHz, CD_3COCD_3) and ^{13}C NMR (75 MHz, CD_3COCD_3): Table 1; CIMS (isobutane, probe), 200 eV, *m/z* (rel. int.): 369 $[M+H]^+$ (100), 313 $[M-55]^+$ (95); HREIMS: found 368.12596 calc. for $C_{21}H_{20}O_6$: 368.12599.

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