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STUDIES ON CASCARA, PART 2.[1] STRUCTURES OF CASCAROSIDES E AND F

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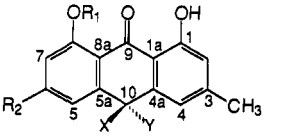
ABSTRACT.—The structures and preferred conformations of cascarosides E [**5**] and F [**6**], two new *O,C*-diglucosylanthrones from *Rhamnus purshiana* bark, have been determined by spectroscopic methods.

Continuing our chemical studies on cascara bark [*Rhamnus purshiana* DC., (Rhamnaceae)] (1), a well known cathartic drug (2), we report here the isolation and structural elucidation of two new *O,C*-diglucosylanthrones, which we have named cascarosides E [**5**] and F [**6**]. They represent the first 10-*C*-β-D-glucopyranosyl-9-anthrones of the emodin series found in Nature, with the other known examples being derivatives in the chrysophanol (1, 3–6), aloe-emodin (1, 7-13), and rhein (14) series.

The two new compounds were obtained from a commercial extract of *R. purshiana* (Purselect^{R}, Indena) according to the procedure described in the Experimental. Their uv and cd spectra revealed strong resemblances to those of cascarosides A, B, C [**1**], and D [**2**], previously isolated from the same source (1), thus suggesting a common 10-*C*-glucosyl-9-anthrone skeleton. This was further supported by inspection of the ^{1}H- and ^{13}C-nmr spectra of the compounds under investigation (Tables 1 and 2). ^{1}H- and ^{13}C-nmr data of cascarosides A–D (1) were used as references for chemical shift assignments, in addition to homonuclear decoupling experiments, ^{1}H nOe, DEPT, and one-bond and long-range heteronuclear 2D nmr correlations.



	R_1	R_2	X	Y
1	β-D-Glc*p*	H	β-D-Glc*p*	H
2	β-D-Glc*p*	H	H	β-D-Glc*p*
3	H	OH	β-D-Glc*p*, H	
4	H	OH	=O	
5	β-D-Glc*p*	OH	β-D-Glc*p*	H
6	β-D-Glc*p*	OH	H	β-D-Glc*p*

In particular, ^{1}H-nmr signals were observed, indicating the presence of an $ArCH_3$ group and of two pairs of meta-coupled aromatic protons, as well as three ^{13}C-nmr peaks due to oxygen-bearing aromatic carbons. While the protons ortho- to the methyl group (as proved by nOe and ^{1}H-^{1}H COSY nmr) showed approximately the same chemical shifts as in cascarosides C [**1**] and D [**2**] (1), the frequencies of the other pair of meta-coupled protons appeared markedly upfield shifted (ca. −0.55 ppm for H-5

[1]For Part 1, see P. Manitto *et al.* (1).

TABLE 1. ^{1}H-Nmr (300 MHz) Data (ppm) of Cascarosides E [**5**] and F [**6**] in CD_3OD at 25°.[a]

Proton[b]	Compounds	
	5	**6**
H-2	6.64 (br s)	6.61 (br s)
H-4	6.83 (br s)	6.79 (br s)
H-5	6.65 (d, 2.3)	6.70 (d, 2.1)
H-7	6.77 (d, 2.3)	6.81 (d, 2.1)
H-10	4.41 (d, 2.1)	4.44 (d, 1.5)
Me-3	2.34 (s)	2.33 (s)
H-1′	3.35 (dd, 9.5, 2.1)	[c]
H-2′	3.00 (dd, 9.5, 9.2)	3.09 (dd, 9.2, 8.7)
H-3′	3.28 (dd, 9.2, 8.4)	3.28 (dd, 9.5, 8.7)
H-4′	2.86 (dd, 9.6, 8.4)	2.95 (dd, 9.5, 8.7)
H-5′	2.89–2.96 (m)	2.83–2.88 (m)
H_a-6′	3.55 (dd, 11.7, 2.6)	3.52 (dd, 11.9, 2.5)
H_b-6′	3.36 (dd, 11.7, 5.4)	3.39 (dd, 11.9, 5.2)
H-1″	4.86 (d, 7.4)	4.87 (d, 7.4)
H-2″	3.60 (dd, 8.9, 7.4)	3.63 (dd, 8.9, 7.4)
H-3″	3.43–3.52 (m)	3.43–3.50 (m)
H-4″	3.43–3.52 (m)	3.43–3.50 (m)
H-5″	3.43–3.52 (m)	3.43–3.50 (m)
H_a-6″	3.93 (dd, 12.0, 1.8)	3.95 (br d, 11.9)
H_b-6″	3.76 (dd, 12.0, 4.7)	3.75 (dd, 11.9, 4.4)

[a]Splitting patterns and *J* values (Hz) are given in parentheses.
[b]Primed and doubly primed numbers refer to positions of the *C*- and *O*-glucosyl residues, respectively.
[c]Obscured by the solvent signal.

and −0.60 for H-7), in agreement with the presence of an additional OH group in that aromatic ring. Moreover, the negative dcims of both products were consistent with a molecular formula of $C_{27}H_{32}O_{14}$ and with the loss of an *O*-linked C_6-glycosyl residue (M−162).

All these data allowed structures of *O*-glucosides of 10-*C*-glucopyranosylemodin-9-anthrone [**3**] to be assigned to the new cascarosides (named E and F), whose epimeric relationship at C-10 resulted from their rapid base-catalyzed interconversion (7). Therefore, the position of the *O*-β-D-glucopyranosyl residue [at the C-1-OH or the C-6-OH or the C-8-OH] and the absolute configuration of C-10 in each compound remained the only structural details to be clarified.

The C-1 attachment of the *O*-glucosyl group was ruled out through consideration of the nOe association between the anomeric proton (H-1″) and H-7 (see Experimental), as well as the absence of "glycosylation shifts" for the resonance frequencies of H-2 and H-4 by comparing cascarosides C [**1**] and D [**2**] (1) and the new cascarosides. That the glucosyl group was linked to the oxygen at the C-8 position could then be inferred by inspection of the ^{1}H-nmr spectrum in the low-field region beyond δ 8. The occurrence of one sharp signal (1H) at δ 12.41 and 12.80, for cascarosides E [**5**] and F [**6**], respectively, was indicative of the presence in their molecules of only one phenolic group engaged in intramolecular hydrogen bonding with the C-9 carbonyl group. The second OH resonance appeared as a broad signal in the region δ 10.5–11.5 in both compounds. It was observed that the ^{1}H-nmr spectrum of emodin [**4**] in DMSO-d_6 shows three OH resonances at δ 12.04, 11.97 (sharp singlets), and 11.3 (br s, OH-6).

At this point, on the basis of nOe associations and the conformational preference shown in Figure 1 (1,7,8), the last ambiguity concerning the stereogenic center of C-10 was solved in favor of the

TABLE 2. ^{13}C-Nmr (75.47) Data (ppm) of Cascarosides E [**5**] and F [**6**] in CD_3OD at 25°.

Carbon[a]	Compounds	
	5	**6**
1	161.86	162.24
2	117.02	116.75
3	146.23	145.98
4	121.67	119.82
5	111.44	112.54
6	166.45	164.17
7	106.41	104.93
8	161.86	161.71
9	191.44	191.32
10	46.39	46.21
Me	21.95	21.95
1a	119.80[b]	118.60[b]
4a	140.97	145.24
5a	149.70	147.15
8a	119.34[b]	117.33[b]
1′	85.93	86.23
2′	71.80	71.83
3′	79.97	79.98
4′	72.20	71.83
5′	81.40	81.39
6′	63.58	63.11
1″	105.09	103.92
2″	74.97	74.68
3″	77.29[c]	77.66[c]
4″	71.28	71.24
5″	78.65[c]	78.56[c]
6″	62.56	62.60

[a]Primed and doubly primed numbers refer to positions of the *C*- and *O*-glucosyl residues, respectively.

[b,c]Signals with the same superscript are interchangeable.

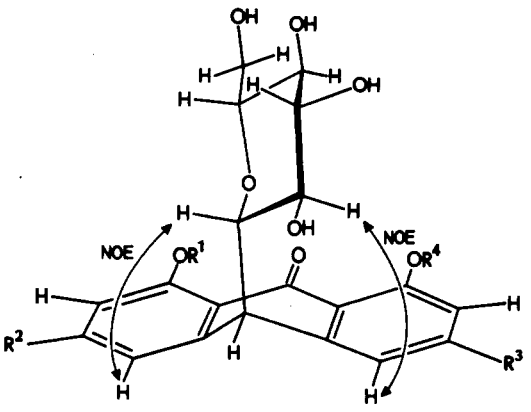
10*R*-configuration for the diastereoisomer moving more slowly by hplc. This compound was then called cascaroside E [**5**] by analogy with cascarosides A and C [**1**] having the same chirality sense (plus) of the helical array C-4a-C-10-C-1′-O in the preferred conformation shown in Figure 1 (C-4a is the angular carbon of the alkyl-substituted aromatic ring). Therefore, according to a classification suggested previously (7), and based on the relative orientation of the constitutionally non-symmetric chromophore with respect to the *C*-glucosyl moiety, cascarosides A, C [**1**] and E [**5**] belong to the *P*-helicity group.

The compound moving faster by hplc (cascaroside F [**6**]) exhibited nOe associations between protons of the two pairs H-1′,H-2′ and H-4,H-5 which did not match those of cascaroside E [**5**], as expected considering the epimeric relationship at C-10 between the two compounds. Thus, cascaroside F [**6**] is classified in the *M*-helicity group together with cascarosides B and D [**2**]. It can also be pointed out that the cd spectra of cascarosides E [**5**] and F [**6**] showed strong similarities with those of A,C and B,D, respectively (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AC 300 spectrometer using the solvent signal as internal standard: 3.30 and 49.00 ppm from TMS for ^{1}H and ^{13}C, respectively, in the case of CD_3OD, and 2.50 ppm from TMS for ^{1}H in the case of DMSO-d_6. Negative dcims were obtained on a Finnigan-MAT 4610 instrument. Cd spectra were obtained on a Jasco-500 instrument. Hptlc was carried out with Merck precoated Si gel 60 F_{254} plates (0.25 mm). Prep. hplc was carried out on a Perkin-Elmer apparatus, comprised of a Series LC 410 pump connected to a LC 95 uv detector. Analytical hplc was performed on a Hewlett-Packard 1090 L liquid chromatograph, connected to a 1040 A photodiode array detector and a HP 9000 computer station.

EXTRACTION AND ISOLATION.—Preliminary fractionation of cascarosides A/B and C/D was performed as previously reported (1) using 5 g of commercial *R. purshiana* extract (Purselect^R). Fractions containing mixtures of cascarosides C [**1**], D [**2**], E [**5**], and F [**6**] were evaporated to dryness, and the residue dissolved in 0.03% aqueous HOAc-MeCN (92:8), and subjected to prep. hplc [column, Merck Lichrosorb RP-18 (7 μm, 10 mm i.d.×25 cm); mobile phase, solvent A: 0.03% aqueous HOAc-MeCN (92:8), solvent B: MeOH, linear gradient of 10–100% B for 35 min; flow rate, 6 ml/min; detector, uv (254 nm)]. Four fractions corresponding to single peaks having *R*,s of 20.9 min (cacaroside D) [**2**], 23.1 min, 27.8 min (cascaroside C) [**1**], and 32.7 min were collected. The eluate containing the compound with *R*, 23.1 min was concentrated under reduced pressure and lyophilized to give cascaroside F [**6**] (32 mg), which appeared pure by analytical hplc [column, LiChrosorb RP-18 (5 μm, 4.6 mm i.d.×12.5 cm);



	R^1	R^2	R^3	R^4
5	β-D-Glc*p*	OH	CH_3	H
6	H	CH_3	OH	β-D-Glc*p*

FIGURE 1. Preferred conformation of cascarosides E [**5**] and F [**6**]. Double arrows indicate relevant nOe correlations.

mobile phase, solvent A: 0.03% aqueous HOAc, solvent B: MeCN, solvent C: MeOH, linear gradients from 92% (A) and 8% (B) to 78% (A), 8% (B), and 14% (C) in 15 min, then to 70% (A), 15% (B), and 15% (C) in 10 min; flow rate, 1.5 ml/min; detector, uv (254 nm)].

The eluate containing the compound with R_f 32.7 min was further purified by hptlc [$CHCl_3$-MeOH-H_2O (7:3:0.5)]. The bands with R_f 0.2 were collected and eluted with MeOH-$CHCl_3$ (2:3). After removal of the solvent under reduced pressure, addition of H_2O and lyophilization, cascaroside E [**5**] (20 mg) was obtained and shown to be pure by analytical hplc (chromatographic conditions as above).

Cascaroside E [**5**].—Mp 197–199°; $[\alpha]^{25}D$ −95.4° (*c*=0.1, MeOH); R_t 23.7 min (analytical hplc); uv λ max (MeOH) (log ε) 210 (4.32), 246 (3.94), 272 (3.83), 335 nm (4.00); cd Δε nm (*c*=0.091 mM, MeOH) 235 (−1.99), 265 (+4.84), 310 (−11.11), 355 (+4.56), 400 (+0.14); ^{1}H-nmr data (300 MHz, CD_3OD), see Table 1; ^{13}C-nmr data (75.47 MHz, CD_3OD), see Table 2; nOe associations (% intensity enhancement) from H-1′ to H-5 (3.7), from H-2′ to H-4 (6.4), from H-1″ to H-7 (9.7), from H-4 to H-2′ (7.3), from H-5 to H-1′ (2.3), from H-7 to H-1″ (8.5); dcims *m/z* 580 $[M]^-$, 442, 418, 280, 256.

Cascaroside F [**6**].—Mp 176–178°; $[\alpha]^{25}D$ −69.5° (*c*=0.1, MeOH); R_t 15.9 min (analytical hplc); uv λ max (MeOH) (log ε) 210 (4.33), 246 (3.96), 270 (3.82), 336 nm (4.00); cd Δε nm (*c*=0.089 mM, MeOH) 230 (−3.42), 245 (−5.13), 255 (−4.27), 265 (−5.13), 315 (+9.69), 355 (−5.13), 400 (−0.28); ^{1}H-nmr data (300 MHz, CD_3OD), see Table 1; ^{13}C-nmr data (75.47 MHz, CD_3OD), see Table 2; nOe associations (% intensity enhancement) from H-1′ to H-4 (2.0), from H-2′ to H-5 (3.1), from H-1″ to H-7 (7.1), from H-4 to H-1′ (not calculated due to overlap of the solvent signals in the reference spectrum), from H-5 to H-2′ (5.3), from H-7 to H-1″ (6.5); dcims *m/z* 580 $[M]^-$, 442, 418, 280, 256.

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