Conformational Studies of Natural Products. Part 4.[1] Conformation and Absolute Configuration of Cascarosides A, B, C, D

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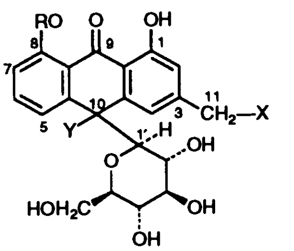
^{1}H and ^{13}C NMR spectra for the four carthartic constituents of Cascara bark, namely, the cascarosides A–D are recorded. The conformation and absolute configuration of each component of the two diastereoisomeric pairs have been determined on the basis of anisotropic effects and specific NOE (nuclear Overhauser effects) in their ^{1}H NMR spectra. CD spectra for these anthrone *C,O*-diglucosides are reported.

Cascara bark (*Rhamnus purshianus* DC bark), a well known carthartic drug,[2] has been shown to contain barbaloin **1**[3] (as a mixture of two C-10 diastereoisomers called aloin A and B),[4] 11-deoxybarbaloin **2**,[5] for which the name chrysaloin has been proposed,[6] 10-hydroxyaloins A and B **3**,[7] and two pairs of diastereoisomeric *O,C*-diglucosides, *i.e.* cascarosides A, B **4**[8,9] and C, D **5**.[10] The constitution of cascarosides has been clarified by Fairbairn and his colleagues[6,11–13] and by Wagner and Demuth[14,15] on the basis of partial hydrolyses and spectral data. In addition, these authors reached the conclusion that cascarosides A and B were 8-*O*-β-D-glucopyranosides of aloin A [formerly (+)-barbaloin] and aloin B [formerly (−)-barbaloin], respectively.



1 R = H X = OH Y = H
2 R = H X = H Y = H
3 R = H X = OH Y = OH
4 R = β-D-Glc*p* X = OH Y = H
5 R = β-D-Glc*p* X = H Y = H

Recent elucidation of the absolute configuration of the two aloins [A:**1** (10*S*) and B:**1** (10*R*); see also Fig. 2], obtained independently by Manitto *et al.*[16] and Rauwald *et al.*,[17] showed that it was possible to define the C-10 configuration of cascarosides, provided that reliable correlations were available between specific components of different diastereoisomeric pairs (**1**, **4**, **5**). Thus, the 10*S* and 10*R*-configurations were assigned to cascaroside A and cascaroside B **4**, respectively, on the basis of the following evidence: (i) separate partial hydrolyses of cascarosides A and B gave rise to a dextrorotatory aglycone[11] (assumed to be impure aloin A)[4] and to a levorotatory aglycone[11,14] (impure aloin B)[4], respectively; (ii) similarities were found in the CD spectra of aloin and cascaroside of the same A- or B-series.[15] Nevertheless, no stereochemical relationship has so far been reported for cascarosides C and D **5**.

Here we report for the first time the complete assignments of ^{1}H and ^{13}C NMR signals for all four cascarosides, isolated pure from cascara bark extracts. A spectroscopic non-correlative determination of the preferred conformation and the absolute configuration of these metabolites is also reported. In the case of cascarosides A and B, the C-10 configurations previously suggested have been confirmed.

Results and Discussion

The four cascarosides were obtained from a commercial extract of *R. purshianus* (Purselect^R, Indena) according to the procedure described in Experimental section and based on preliminary column chromatography (on resin and Si-gel) followed by preparative HPLC separation.

The chemical and physical properties listed in Table 1 (see also Fig. 1) can be used to identify each component of the diastereoisomeric pairs corresponding to the formulae **1**, **4** and **5**.

^{1}H and ^{13}C NMR data for cascarosides A–D in CD_3OD are reported in Tables 2 and 3, respectively, together with those of aloin A†, which could be used as the reference compound for chemical-shift assignments. These were further supported by homonuclear decoupling experiments, ^{1}H NOE, DEPT, and by one-bond and long-range heteronuclear 2D correlations.

In particular, the problem of how to assign the two doublets at δ 7.25–7.28 and at 7.35–7.40 to 5-H and 7-H in the four cascarosides was resolved taking into account the NOE associations. In fact, reciprocal intensity enhancements of the upfield doublet and the 10–H signals were found in NOE experiments carried out on all the compounds examined, thus proving upfield doublets to be due to the aromatic 5-H (see also Table 4). Similar NOE correlations led to the unequivocal assignments of the singlet at δ 6.64–6.85 and at 6.82–7.00 to 2-H and 4-H, respectively. (In the case of aloin A, the above alternatives were resolved by analogous reasoning.)

It must be pointed out that the downfield glycosylation shift of the two doublets mentioned above as well as of the ^{13}C signals of the corresponding carbons (C-5, C-7)[19] with respect to aloin A gives evidence for the involvement of the 8-position in the *O*-glucoside link, as previously suggested on the basis of chemical correlations of the *O,C*-diglucosides with the corresponding 8-*O*-β-D-glucosyl anthraquinones.[15]

The two sets of protons belonging to the two carbohydrate

† No ^{1}H NMR spectral results for aloin A in methanol have previously been reported. For its spectrum in DMSO–$CDCl_3$ and DMSO, see ref. 16, 18.

Table 1 Analytical data for 10-*C*-glucosyl-9-anthrones

	1 (10S) Aloin A	**1** (10R) Aloin B	**3** (10S) Cascaroside A	**3** (10R) Cascaroside B	**4** (10S) Cascaroside C	**4** (10R) Cascaroside D
M.p. (°C)	148[a]	138–140[a]	184–187[c]	175–178[c]	183.5–185	180–182
$[\alpha]_D^{30}/10^{-1}$ deg cm^2 g^{-1}	+10.2[a]	−73.0[a]	−56.7[d]	−113.2[d]	−50.6	−94.5
(MeOH)			(*c* 0.125)	(*c* 0.093)	(*c* 0.067)	(*c* 0.092)
HPLC	4.2 min[b]	3.5 min[b]	21.6 min[e]	9.0 min[e]	22.4 min[e]	18.4 min[e]
CD	Refs. 4, 16		Fig. 1	Fig. 1	Fig. 1	Fig. 1

[a] Ref. 4. [b] LiChrosorb RP-8, MeCN-H_2O (25:75, v/v), see refs. 4, 16. [c] Ref. 15. [d] Lit.,[15] −36.8 (A); −104.4 (B). [e] See Experimental section for conditions.

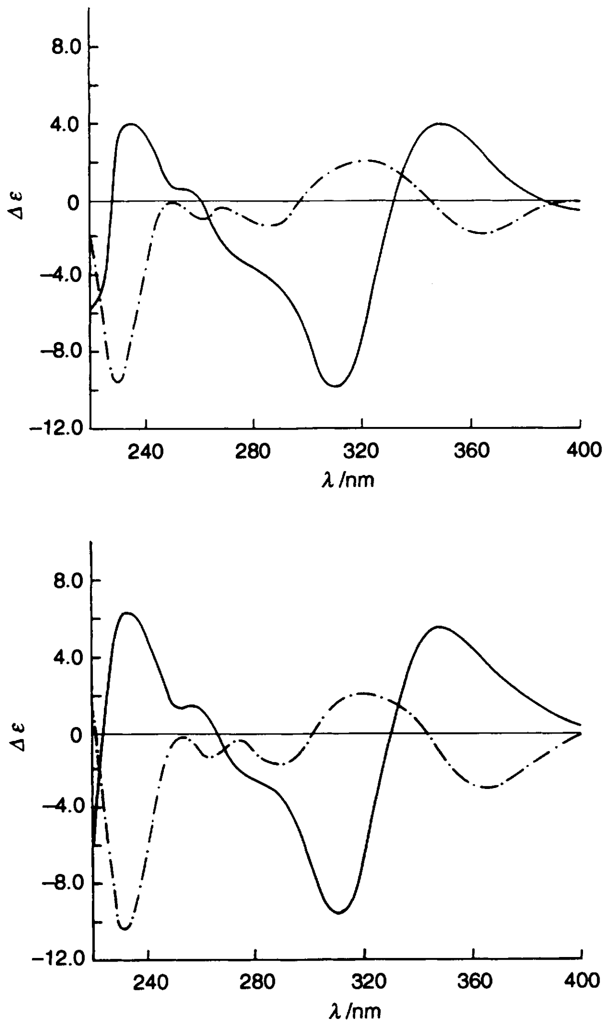


Fig. 1 CD spectra of cascarosides in MeOH at 25 °C. Top: cascarosides A (——) and B (. — . — . —). Bottom: cascarosides C (——) and D (. — . — . —). Structures are represented in Fig. 2.

units could be distinguished by internal correlations each including one anomeric proton whose chemical shift was indicative of the type of glucosidic bond.

On inspection of Table 2 it clearly appears that chemical shifts and coupling constants of the C-bonding β-D-glucopyranosyl group in all cascarosides are very close to those exhibited by aloin A. In addition, a comparison of the corresponding ^{1}H chemical shifts of the two carbohydrate residues in the single cascaroside reveals that all the resonances, except that of the anomeric proton, are upfield shifted on going from the *O*- to the *C*-linked glucose moiety. Similar shielding effects due to the anthrone nucleus were previously observed in aloins (when compared with glucose in DMSO) and explained in terms of conformational preference.[16] The preferred conformation represented in Fig. 2 was first suggested for both aloins A and B in solution[15] on the basis of (i) NMR data (magnetic anisotropy effects and the value of *ca.* 2.0 Hz for the coupling constant between 1′-H and 10-H); (ii) steric interactions typical of 10-substituted 9-anthrones; and (iii) force-field calculations. It was further supported by X-ray analysis of aloin B.[17]

The close similarity of the NMR spectra of the aloins and cascarosides, in particular the chemical shifts of the H-C(10)-glucose moiety, is a clear indication that the same conformation as that of Fig. 2 is preferred by all these *C*-glucosyl anthrones in solution (as well as by other aloin derivatives).[7,20,21]

Taking account of this conformational predominance, the absolute configuration of C-10 in the cascarosides A, B, C, D can be inferred from the NOE associations listed in Table 4. In fact, the NOE correlations of 1′-H with 5-H (and of 2′-H with 4-H) in anthrones A and C together with those of 1′-H with 4-H (and of 2′-H with 5-H) in anthrones B and D, are indicative not only of the prevalent conformation shown in Fig. 2, but also of a 10*S*-configuration for cascarosides A(III) and C(V), and a 10*R*-configuration for cascarosides B(IV) and D(VI). In addition, the significant intensity enhancements of the 1″-H signal observed by irradiation of the 7-H in all four cascarosides (and those of the 7-H signal by irradiation of the 1″-H is consistent with the glycosylation of the 8-OH and could be interpreted in terms of rotameric preferences of the C(8)–O and O–C(1″) bonds causing 7-H and 1″-H to be in close proximity.[22]

Experimental

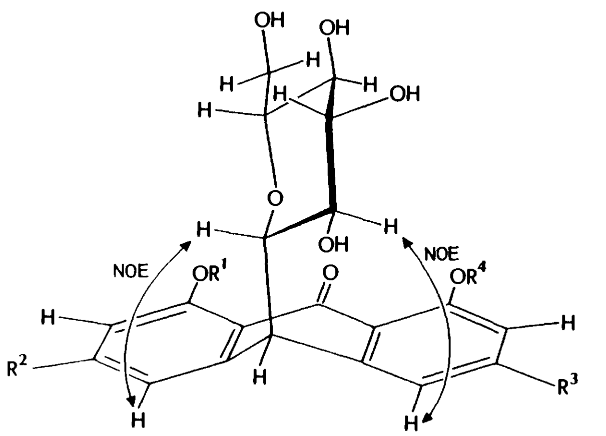
General Experimental Details.—M.p.s are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and UV spectra on a Perkin-Elmer 554 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer, equipped with an ASPECT 3000 computer, in CD_3OD, using the solvent signal as internal standard (3.30 and 49.00 ppm from $SiMe_4$ for ^{1}H and ^{13}C, respectively). NOE difference experiments were performed by using standard Bruker software (DISNMR version 91). Negative DCI mass spectra were obtained on a Finnegan-MAT 4610 instrument with a SuperINCOS data system using ammonia at 0.35 mbar. CD spectra were obtained on a Jasco 500 instrument. Column chromatography was performed using Merck silica gel 60 (0.040–0.063 mm). HPTLC was carried out with Merck precoated silica gel 60 F_{254} plates (0.25 mm). Plates were visualized with UV light. Analytical HPLC was performed on: (a) a Waters 600 E liquid chromatograph, connected to a Waters WISP 712 autosampler, a Waters 484 UV detector and a HP 3396 integrator; (b) a Hewlett Packard 1090 L, connected to a 1040 A photodiode array detector and a HP 9000 computer station. Preparative HPLC was carried out on (a) a Waters DELTA PREP 4000 connected to a Waters 490 E preparative UV detector; (b) a Perkin-Elmer apparatus, composed of a Series LC 410 pump connected to a UV detector LC 95.

Preliminary Fractionation of Cascarosides A/B and C/D.—A solution of commercial *R. purshianus* extract (Purselect[R],

Table 2 ^1H NMR (300 MHz) peaks (ppm) of aloin A and cascarosides A–D in CD_3OD at 25 °C. Splitting patterns and *J* values (Hz) are given in parentheses.

Proton	Aloin A	Cascaroside A	Cascaroside B	Cascaroside C	Cascaroside D
2-H	6.87 (br s)	6.85 (d, 1.4)	6.83 (br s)	6.69 (br s)	6.64 (br s)
4-H	7.05 (br s)	7.00 (d, 1.4)	6.98 (br s)	6.89 (d, 1.6)	6.82 (br s)
5-H	7.03 (d, 7.9)	7.26 (dd, 7.7, 1.0)	7.27 (d, 7.4)	7.28 (dd, 7.7, 1.1)	7.25 (d, 7.6)
6-H	7.48 (dd, 8.3, 7.9)	7.54 (dd, 8.3, 7.7)	7.53 (dd, 8.3, 7.4)	7.57 (dd, 8.3, 7.7)	7.51 (dd, 8.2, 7.6)
7-H	6.84 (d, 8.3)	7.37 (dd, 8.3, 1.0)	7.36 (d, 8.3)	7.40 (dd, 8.3, 1.1)	7.35 (d, 8.2)
10-H	4.58 (d, 2.0)	4.58 (d, 2.2)	4.59 (d, 1.9)	4.56 (d, 2.2)	4.55 (d, 1.9)
11-H_2	4.65 (AB syst., 15.5)	4.60 (AB syst., 15.2)	4.62 (br s)		
11-H_3				2.36 (s)	2.34 (s)
1′-H	3.40 (dd, 9.7, 2.0)	3.34 (dd, 9.6, 2.2)	3.31 (dd, 9.6, 1.9)	3.34 (dd, 9.6, 2.2)	3.30[a]
2′-H	3.00 (dd, 9.7, 8.7)	2.94 (dd, 9.6, 8.8)	2.99 (dd, 9.6, 8.7)	2.98 (dd, 9.6, 8.9)	2.98 (dd, 9.6, 8.7)
3′-H	3.25 (dd, 8.7)	3.24 (dd, 8.8)	3.26 (dd, 8.7)	3.26 (dd, 8.9)	3.26 (dd, 8.7)
4′-H	2.85–2.96 (m)	2.79–2.90 (m)	2.84–2.93 (m)	2.82–2.93 (m)	2.83–2.92 (m)
5′-H	2.85–2.96 (m)	2.79–2.90 (m)	2.85–2.93 (m)	2.82–2.93 (m)	2.83–2.92 (m)
6′-H_a	3.56 (dd, 11.7, 2.1)	3.50 (dd, 11.7, 2.4)	3.51 (dd, 11.9, 2.1)	3.52 (dd, 11.8, 2.3)	3.51 (dd, 11.9, 2.0)
6′-H_b	3.37 (dd, 11.7, 5.2)	3.32 (dd, 11.7, 5.2)	3.36 (dd, 11.9, 4.9)	3.35 (dd, 11.8, 5.1)	3.36 (dd, 11.9, 4.9)
1″-H		4.91 (d, 7.5)	4.95 (d, 7.7)	4.93 (d, 7.5)	4.92 (d, 7.7)
2″-H		3.60 (dd, 7.5, 9.1)	3.65 (dd, 7.7, 8.7)	3.61 (dd, 7.5, 9.2)	3.64 (dd, 7.7, 8.9)
3″-H		3.51 (dd, 9.1)	3.41–3.49 (m)	3.53 (dd, 9.2)	3.41–3.48 (m)
4″-H		3.37–3.47 (m)	3.41–3.49 (m)	3.40–3.48 (m)	3.41–3.48 (m)
5″-H		3.37–3.47 (m)	3.41–3.49 (m)	3.40–3.48 (m)	3.41–3.48 (m)
6″-H_a		3.89 (dd, 12.0, 2.2)	3.93 (dd, 12.1, 1.8)	3.92 (dd, 12.0, 2.1)	3.92 (dd, 12.1, 1.7)
6″-H_b		3.70 (dd, 12.0, 5.2)	3.72 (dd, 12.1, 5.4)	3.72 (dd, 12.0, 5.3)	3.72 (dd, 12.1, 5.1)

[a] Obscured by the solvent signals.



	R^1	R^2	R^3	R^4
I	H	H	CH_2OH	H
II	H	CH_2OH	H	H
III	β-D-Glc*p*	H	CH_2OH	H
IV	H	CH_2OH	H	β-D-Glc*p*
V	β-D-Glc*p*	H	CH_3	H
VI	H	CH_3	H	β-D-Glc*p*

Fig. 2 Preferred conformation of aloins A (I) and B (II),[16,17] and of cascarosides A (III), B (IV), C (V) and D (VI). Arrows indicate relevant NOE correlations.

Indena) (5 g) in water (50 cm^3) was acidified to pH 4 with concentrated H_2SO_4 and extracted with BuOH–toluene (7:3). The aqueous layer was chromatographed on XAD-4 Amberlite^R resin (300 cm^3), eluting with water and then with methanol. The eluate was evaporated to dryness and the residue (1.8 g) column chromatographed [30 g of silica gel, ethyl acetate–methanol–water (100:17:13)] to give a mixture of cascarosides A/B (350 mg), followed by a mixture of cascarosides C/D (170 mg).

Isolation of Cascarosides A and B.—Four portions (2.5 cm^3 each) of a solution of cascarosides A/B mixture (320 mg) in water (10 cm^3) were subjected to preparative HPLC [system (a) column, Waters Preppak Cartridge Bondapak C-18 (15-20 μm, 47 mm i.d. × 30 cm); mobile phase, MeOH–water (26:74); flow rate, 80 cm^3 min^{-1}; detector, UV (310 nm); t_R (cascaroside A) 23.2 min, t_R (cascaroside B) 11.9 min]. Eluates were collected, concentrated under reduced pressure and lyophilized to give cascarosides A and B (100 mg each), which were shown to be pure by analytical HPLC [system (a) column, LiChrosorb RP-18 (7 μm, 4.6 mm i.d. × 25 cm); mobile phase, MeOH–water (26:74); flow rate, 1 cm^3 min^{-1}; detector, UV (254 nm); t_R (cascaroside A) 21.6 min, t_R (cascaroside B) 9.0 min].

(i) Cascaroside A: λ_{max}(MeOH)/nm 213 (log ε 4.37), 266 (3.86), 294 (4.03) and 320 (3.97); δ_H(300 MHz, CD_3OD) see Table 2; δ_C(75.47 MHz, CD_3OD) see Table 3; *m/z* 580 ($M^{-\bullet}$), 442, 418 and 255.

(ii) Cascaroside B: λ_{max}(MeOH)/nm 212 (log ε 4.35), 263 (3.87), 293 (4.02) and 323 (3.96); δ_H(300 MHz, CD_3OD) see Table 2; δ_C(75.47 MHz, CD_3OD) see Table 3; *m/z* 580 ($M^{-\bullet}$), 442, 418 and 255.

Isolation of Cascarosides C and D.—Repeated injections (150 mm^3 each) of a saturated solution of cascarosides C/D mixture in 0.03% aqueous acetic acid–acetonitrile (92:8) were performed on the preparative HPLC system (b) [column, Merck Lichrosorb RP-18 (7 μm, 10 mm i.d. × 25 cm); mobile phase, solvent A: 0.03% aqueous acetic acid–acetonitrile (92:8), solvent B: MeOH, linear gradient of 10–100% B during 35 min; flow rate, 6 cm^3 min^{-1}; detector, UV (254 nm); t_R (cascaroside C) 27.8 min, t_R (cascaroside D) 20.9 min]. Eluates were collected, concentrated under reduced pressure and lyophilized to give pure cascaroside C (80 mg) and slightly impure cascaroside D (34 mg). The latter was further purified by HPTLC [dichloromethane–MeOH (80:25)] for three repeated elutions (3.5, 7.9 and 9.5 cm, respectively). The bands corresponding to cascaroside D (R_f 0.55) were collected and eluted with MeOH–chloroform (1:1). After removal of the solvent under reduced pressure, addition of water and lyophilization, cascaroside D (23 mg) was obtained and shown to be pure by analytical HPLC [system (b) column, LiChrosorb RP-18 (5 μm, 4.6 mm i.d. × 12.5 cm); mobile phase, solvent A: 0.03% aqueous acetic acid, solvent B: acetonitrile, solvent C: MeOH, linear gradients from 92% (A) and 8% (B) to 78% (A), 8% (B), and 14% (C) in 15

Table 3 ^{13}C NMR (75.47 MHz) peaks (ppm) of aloin A and cascarosides A–D in CD_3OD at 25 °C.

Carbon	Aloin A	Cascaroside A	Cascaroside B	Cascaroside C	Cascaroside D
1	163.37	161.94	162.03	161.81	161.93
2	114.39	114.41	114.06	117.15	116.77
3	151.49	150.33	151.01	147.12	147.75
4	119.11	118.68	117.24	121.89	119.92
5	119.94	123.67	124.64	123.69	124.73
6	137.00	135.79	134.65	135.68	134.59
7	116.79	118.56	116.75	118.61	117.40
8	162.90	159.16	159.06	159.14	159.02
9	195.48	192.40	192.16	192.36	192.13
10	45.86	46.14	46.19	45.99	45.96
CH_2OH	64.51	64.53	64.56		
CH_3				22.04	22.03
1a	117.70	120.55	120.22	119.48	119.11
4a	143.22	141.29	143.48	141.03	143.47
5a	146.53	147.25	145.49	147.25	145.37
8a	118.61	125.06	125.00	125.12	125.02
1′	86.60	85.07	85.60	85.14	85.69
2′	71.83	71.78	71.79	71.75	72.02
3′	79.95	79.85	79.89	79.85	79.85
4′	71.96	72.02	72.03	72.08	71.76
5′	81.66	81.44	81.38	81.40	81.33
6′	63.20	63.55	63.09	63.39	63.06
1″		104.90	103.23	104.98	103.30
2″		75.00	74.76	75.01	74.73
3″		77.24	77.87	77.24	77.79
4″		71.26	71.27	71.30	71.21
5″		78.63	78.51	78.63	78.46
6″		62.55	62.63	62.57	62.51

Table 4 Selected proton associations based on NOE difference spectroscopy for cascarosides A–D[a]

From	to			
	Cascaroside A	Cascaroside B	Cascaroside C	Cascaroside D
4-H	2′-H (6.2)	1′-H[b]	2′-H (6.6)	1′-H[b]
5-H	1′-H (2.1)	2′-H (5.6)	1′-H (1.8)	2′-H (7.5)
7-H	1″-H (8.1)	1″-H (7.1)	1″-H (6.8)	1″-H (8.9)
1′-H	5-H (2.8)	4-H (4.1)	5-H (3.3)	4-H (4.0)
2′-H	4-H (5.6)	5-H (6.2)	4-H (6.0)	5-H (5.9)
1″-H	7-H (10.8)	7-H (9.1)	7-H (9.2)	7-H (12.8)

[a] In CD_3OD at 25 °C. Intensity enhancements (%) are given in parentheses. [b] Not calculated due to overlap of the solvent signals in the reference spectrum.

min, then to 70% (A), 15% (B) and 15% (C) in 10 min; flow rate, 1.5 cm^3 min^{-1}; detector, UV (254 nm); t_R (cascaroside C) 22.4 min, t_R (cascaroside D) 18.4 min].

(i) Cascaroside C: λ_{max}(MeOH)/nm 209 (log ε 4.45), 266 (3.88), 296 (4.05) and 318 (4.00); δ_H(300 MHz, CD_3OD) see Table 2; δ_C(75.47 MHz, CD_3OD) see Table 3; m/z 564 ($M^{-\bullet}$), 426, 402 and 239.

(ii) Cascaroside D: λ_{max}(MeOH)/nm 210 (log ε 4.45), 262 (3.87), 294 (4.02) and 320 (3.96); δ_H(300 MHz, CD_3OD) see Table 2; δ_C(75.47 MHz, CD_3OD) see Table 3; m/z 564 ($M^{-\bullet}$), 426, 402 and 239.

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