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BIOGENETICALLY IMPORTANT QUINONEMETHIDES AND OTHER TRITERPENOID CONSTITUENTS OF *SALACIA RETICULATA**†

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Key Word Index—*Salacia reticulata* var. β-diandra; Celastraceae; celastroloids; isoiguesterinol; 30-hydroxypristimerin; salacenonal; structural elucidation; biosynthesis.

Abstract—Phytochemical investigation of the outer root bark of *Salacia reticulata* var. β-diandra (Celastraceae) has resulted in the isolation of two novel quinonemethide triterpenoids (celastroloids), isoiguesterinol and 30-hydroxypristimerin, along with salacenonal, several known celastroloids and friedo-oleanane triterpenoids. Details of the structural elucidation and ^{1}H and ^{13}C NMR spectral assignments of these compounds are presented and their biogenetic significance is discussed.

INTRODUCTION

Salacia reticulata Wight (Celastraceae) is a medicinal plant with restricted distribution in Sri Lanka and India. Two varieties of *S. reticulata* have been recognized in Sri Lanka; *S. reticulata* growing in submontane forests in the centre of the island and *S. reticulata* var. β-diandra growing in the low country rain forests of the south. In traditional medicine of Sri Lanka, an aqueous infusion of the roots of *S. reticulata* is used in the treatment of diabetes [2] and its oral hypoglycaemic activity has been demonstrated in experimental rats [3].

Salacia species are known to elaborate anthocyanidines, catechins, phenolic acids, quinones, friedo-oleananes, quinonemethide and related triterpenoids (celastroloids), mangiferin, gutta-percha and dulcitol [4–7]. Previous phytochemical studies on *S. reticulata* have resulted in the isolation of gutta-percha [8], sitosterol [8], pristimerin (**4**) [8], mangiferin [9], epi-kokoondiol [1], salacenonal (**7**) [10] and salaciquinone (**8**) [11] from the root bark, and iguesterin, pristimerin and epi-kokoondiol from the stem bark [12]. In continuing our interest in the triterpenoids of Celastraceae [1, 10, 11] and search for biosynthetic congeners of celastroloids [10, 13], we have investigated the outer root bark of *S. reticulata* var. β-diandra, which led to the isolation of two new biogenetically important celastroloids, isoiguesterinol (**1**) and 30-hydroxypristimerin (**2**) along with several known celastroloids and friedo-oleanane triterpenoids. In this paper we report the detailed structural elucidation of **1**, **2** and **7**, another biogenetically important nortriterpenoid recently reported [10]. This also constitutes the second report of the natural occurrence of netzahualcoyene (**10**) previously isolated from *Maytenus horrida* (Celastraceae) [14].

RESULTS AND DISCUSSION

The dried and powdered root outer bark of *S. reticulata* var. β-diandra was successively and exhaustively extracted with hot hexane and benzene. Although the two extracts exhibited similar TLC patterns, a close examination revealed that the hexane extract contained a higher percentage of the less polar constituents, whereas the benzene extract contained more of the polar compounds. Therefore, the hexane extract was used to isolate the less polar constituents, and the benzene extract to obtain more polar ones. Thus, the two extracts were separately fractionated by silica gel column chromatography using solvent combinations of hexane and ethyl acetate of increasing polarity. The column fractions were combined using TLC as a guide and further purified as necessary by silica gel flash chromatography and/or preparative TLC to obtain 13 compounds, of which two were new.

The hexane extract yielded (in order of increasing polarity) **7**, **8**, β-amyrin, isoiguesterin (**9**), **10**, **4**,

*Part 28 in the series 'Studies on Terpenoids and Steroids.' For part 27 see ref. [1].

†Dedicated to the memory of the late Prof. Sinnathamby Balasubramaniam, a dear friend and an eminent botanist.

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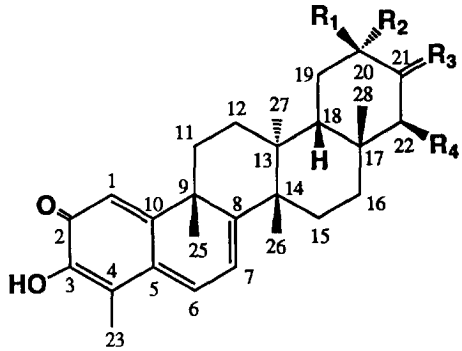


1 $R_1 = R_4 = H$; $R_2 = CH_2OH$; $R_3 = H_2$

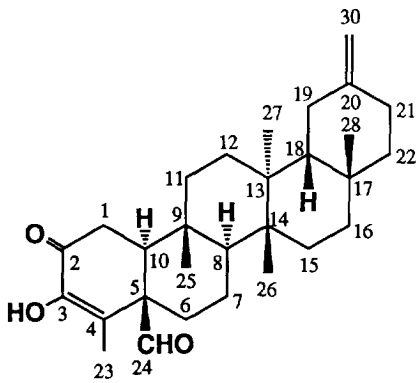
2 $R_1 = CH_2OH$; $R_2 = CO_2Me$; $R_3 = H_2$; $R_4 = H$

3 $R_1 = Me$; $R_2 = CO_2H$; $R_3 = H_2$; $R_4 = H$

4 $R_1 = Me$; $R_2 = CO_2Me$; $R_3 = H_2$; $R_4 = H$

5 $R_1 = Me$; $R_2 = R_4 = H$; $R_3 = O$

6 $R_1 = Me$; $R_2 = H$; $R_3 = O$; $R_4 = OH$



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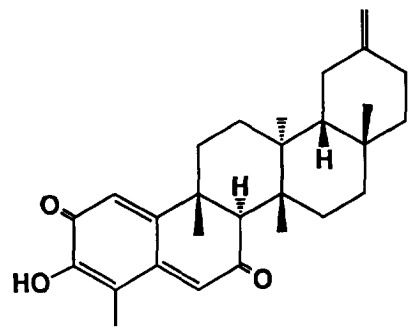

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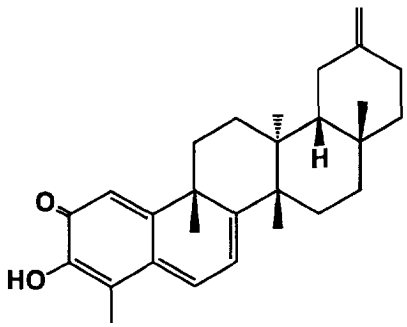
sitosterol, 29-hydroxyfriedelan-3-one, tingenone (**5**), **1** and 22β-hydroxytingenone (**6**). Column chromatography of the benzene extract afforded late fractions, which, by TLC, indicated the presence of more polar compounds not encountered in the hexane extract, and only these fractions were subjected to further purification yielding (in order of increasing polarity) epikokoondiol (21α,26-dihydroxy-D:A-friedo-olean-3-one), celastrol (**3**) and **2**. All known compounds were identified by direct comparison with authentic samples and/or by comparison of their physical data with those reported (see Experimental). Structures of the new compounds **1** and **2** were elucidated by extensive analysis of their spectral data as described below. In addition, the detailed ^1H and ^{13}C NMR spectral assignments of **7** are presented.

Compound **1**, mp 118–120°, $[\alpha]_D$ +22°, in its HR mass spectrum exhibited a molecular ion at *m/z* 422.2792 corresponding to the molecular formula $C_{28}H_{38}O_3$. The UV and IR data suggested a quinonemethide structure [15, 16], which was further supported by the ^1H NMR signals at δ 6.53 (*br s*, H-1), 7.03 (*br d*, $J = 7.0$ Hz, H-6) and 6.37 (*d*, $J = 7.0$ Hz, H-7) [15, 17]. Unlike **4**, which has six methyl signals in its ^1H NMR spectrum, **1** had only five methyl singlets; **1** also lacked the signal due to a methoxycarbonyl group (δ_H *ca* 3.55) characteristic of pristimerin, suggesting possible modification of groups attached to C-20 of the triterpenoid skeleton. Comparison of the ^1H NMR data for **1** with those reported for **4** indicated the presence of a signal due to a methine proton at δ_H 2.10 (triplet of quintet, $J = 12$ and 6 Hz), which was coupled



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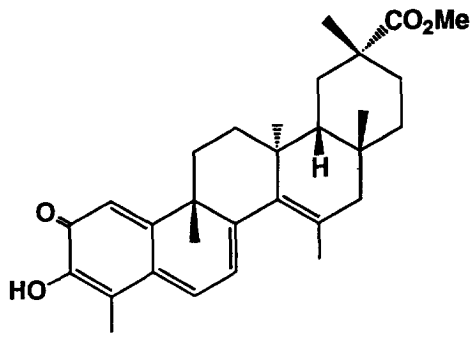

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to hydroxymethylene protons at δ_H 3.40 and 3.47 (each *dd*, $J = 10$ and 6 Hz) in **1** and this was assigned to 20-H. Careful analysis of the ^{1}H NMR spectrum of **1** with the aid of ^{1}H–^{1}H COSY and HETCOR techniques allowed assignment of all the protons in **1** (Table 1).

The ^{13}C NMR spectrum of **1**, analysed with the aid

Table 1. ^{1}H, ^{13}C and two-dimensional NMR spectral data for **1** in $CDCl_3$ (δ in ppm)

Position	δ_H*	δ_C†‡	^{1}H–^{1}H COSY	HMBC§
1	6.53 (1H, *br s*)	119.4 (*d*)	H-6	—
2	—	178.4 (*s*)	—	—
3	—	146.0 (*s*)	—	H-1, H-23
4	—	117.2 (*s*)	—	H-23
5	—	127.4 (*s*)	—	H-1, H-23
6	7.03 (1H, *br d*, 7)	134.2 (*d*)	H-1, H-7	—
7	6.37 (1H, *d*, 7)	118.2 (*d*)	H-6	—
8	—	170.7 (*s*)		H-6, H-11β, H-25, H-26
9	–	43.3 (*s*)	—	H-11α, H-12α, H-25
10	—	164.4 (*s*)	—	H-6, H-25
11α	1.84 (1H, *td*, 13, 5)	33.1 (*t*)	H-11β, H-12α, H-12β, H-25	H-25
11β	2.12 (1H, *br d*, 12)		H-11α, H-12α, H-12β	
12α	1.66 (1H, *m*)	29.4 (*t*)	H-11α, H-11β, H-12β	H-27
12β	1.77 (1H, *m*)		H-11α, H-11β, H-12α, H-27	
13	—	40.0 (*s*)	—	H-11β, H-26, H-27
14	—	44.0 (*s*)	—	H-12β, H-18, H-26, H-27
15α	1.74 (1H, *m*)	29.7 (*t*)	H-15β, H-16α, H-16β	H-26
15β	1.64 (1H, *m*)		H-15α, H-16α, H-16β	
16α	1.54 (1H, *m*)	36.5 (*t*)	H-15α, H-15β, H-16β	H-15β, H-28
16β	1.72 (1H, *m*)		H-15α, H-15β, H-16α	
17	—	30.2 (*s*)	—	H-28
18β	1.67 (1H, *m*)	43.7 (*d*)	H-19α/β, H-27	H-19α, H-28
19α	1.08 (1H, *m*)	25.2 (*t*)	H-18, H-30	—
19β	1.80 (1H, *m*)			
20	2.10 (1H, *tq*, 12, 6)	33.0 (*d*)	H-19, H-29	H-21β
21α	1.70 (1H, *m*)	22.5 (*t*)	H-21β, H-22α, H-22β	H-19α/β, H-30
21β	1.10 (1H, *m*)		H-21α, H-22α, H-22β, H-30	—
22α	1.69 (1H, *m*)	35.2 (*t*)	H-21α, H-21β, H-22β	H-28
22β	1.20 (1H, *m*)		H-21α, H-21β, H-22α	
23	2.22 (3H, *s*)	10.3 (*q*)	—	—
25	1.47 (3H, *s*)	37.7 (*q*)	—	—
26	1.34 (3H, *s*)	23.4 (*q*)	—	H-15α
27	0.71 (3H, *s*)	17.9 (*q*)	—	H-12β
28	1.19 (3H, *s*)	36.2 (*q*)	—	H-18
29	3.40 (1H, *dd*, 10, 6)	69.3 (*t*)	—	—
	3.47 (1H, *dd*, 10, 6)			

*J in Hz.

†Multiplicity (in parentheses) deduced from a DEPT experiment.

‡Assignments based on HETCOR and HMBC.

§Long-range correlations (HMBC) between protons and indicated carbon.

of HETCOR and HMBC (heteronuclear multiple bond correlation), while showing a close resemblance to that of **4** [15], confirmed the absence of methoxycarbonyl and methyl groups at C-20 and the presence of a CH_2OH group [δ_C 69.3 (*t*)] instead. The major differences in the ^{13}C NMR spectra of **1** and **4** were recognizable in ring-E carbons (C-19, C-20 and C-21) (Table 1). The ^{13}C NMR signal due to C-20 in **1** appeared at δ_C 33.0 as a doublet and showed a long-range correlation (HMBC) to H-21β. The foregoing suggested that **1** is a quinonemethide triterpenoid in which Me-30 is lost and Me-29 is oxidized to a CH_2OH function.

The stereochemical disposition of the CH_2OH group at C-20 in **1** was examined by means of NOE difference experiments. As depicted in Fig. 1c, irradiation of the methyl singlet at δ_H 1.34 caused an enhancement of the intensity of the methyl singlet at δ_H 1.19. Since the ^1H NMR signal at δ_H 1.34 can be assigned to Me-26 on the basis of the observed long-range ^1H–^{13}C correlations (see Table 1) and the NOE observed on irradiation of Me-25, the 3H singlet at δ_H 1.19 may be assigned to Me-28. Irradiation of the signal at δ_H 1.19 caused an NOE enhancement of the C-20-H at δ_H 2.10 (Fig. 1b), suggesting a β-configuration for the latter proton. Consequently, the C-20-CH_2OH should have an α-configuration. This result and the coupling pattern of C-20-H (triplet of quintet, $J = 12$ and 6 Hz) suggested that both the rings D and E should assume a boat conformation in which C-20-H has an axial-like orientation (Fig. 2). Based on the foregoing evidence

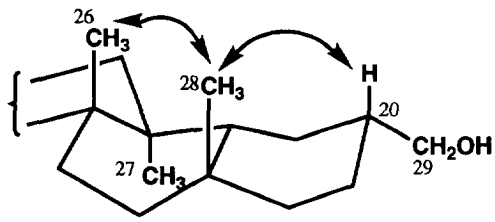


Fig. 2. D, E ring conformation showing the NOEs observed for isoiguesterinol (**1**).

the structure of the quinonemethide **1** was elucidated as the hydrated derivative of isoiguesterin (**9**) and hence named isoiguesterinol.

Compound **2**, mp 228–229°, $[\alpha]_D$ −147°, $C_{30}H_{40}O_5$, had UV and IR data similar to those of **4** [15]. Comparison of the ^1H NMR spectral data for **2** with those reported for **4** also indicated a close resemblance; the major difference being the absence of the *tert*-Me singlet at δ_H 1.18 and the presence of two new ^1H signals at δ_H 3.39 and 3.61 (each *d*, $J = 10$ Hz) in **2** assignable to a prochiral hydroxymethylene group. The analysis of the ^1H NMR spectrum with the aid of ^1H–^1H COSY and HETCOR suggested that the CH_2OH is on C-20, leading to the proposal that **2** is presumably the 30-hydroxyl derivative of pristimerin. The ^1H NMR assignments for **2** are given in Table 2.

The ^{13}C NMR spectrum of **2**, analysed with the aid of HETCOR and HMBC, exhibited a signal at δ_C 74.1 (*t*) due to a hydroxymethylene carbon instead of the

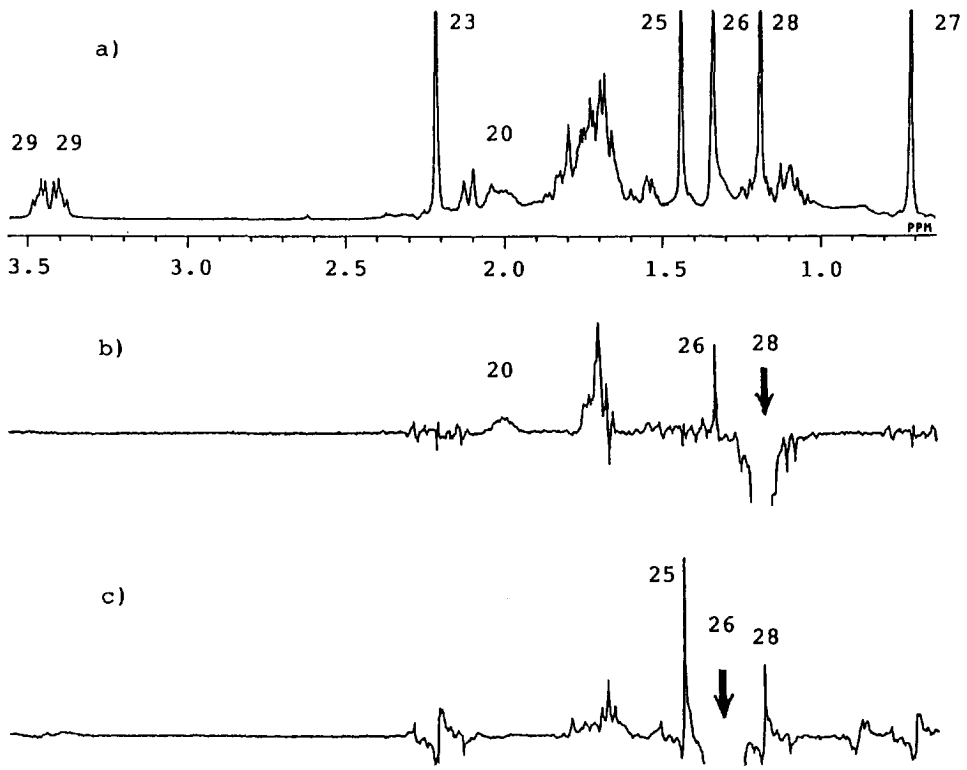


Fig. 1. NOE difference spectra of isoiguesterinol (**1**): (a) control spectrum; (b) and (c) NOE difference spectra.

Table 2. ^{1}H, ^{13}C and two-dimensional NMR spectral data for **2** in $CDCl_3$ (δ in ppm)

Position	δ_H*	δ_C†‡	^{1}H–^{1}H COSY	HMBC§
1	6.52 (1H, *d*, 1.4)	119.4 (*d*)	H-6	—
2	—	178.4 (*s*)	—	—
3	—	146.0 (*s*)	—	H-1, H-23
4	—	117.1 (*s*)	—	H-23
5	—	127.5 (*s*)	—	H-1, H-7, H-23
6	7.01 (1H, *dd*, 7, 1.4)	133.9 (*d*)	H-1, H-7	—
7	6.35 (1H, *d*, 7)	118.2 (*d*)	H-6	—
8	—	169.8 (*s*)	H-25	H-25, H-26
9	–	42.9 (*s*)	—	H-1, H-7, H-11β, H-12α, H-25
10	—	164.7 (*s*)	—	H-6, H-25
11α	1.86 (1H, *td*, 14, 5)	33.6 (*t*)	H-11β, H-12α, H-12β, H-25	H-25
11β	2.16 (1H, *ddd*, 14, 4.5, 2)		H-11α, H-12α, H-12β	
12α	1.76 (1H, *ddd*, 14, 5, 2)	29.8 (*t*)	H-11α, H-11β, H-12β	H-27
12β	1.68 (1H, *td*, 14, 4.5)		H-11α, H-11β, H-12α, H-27	
13	—	39.4 (*s*)	—	H-11α, H-26, H-27
14	—	45.0 (*s*)	—	H-16α, H-26, H-27
15α	1.68 (1H, *td*, 13.5, 5)	28.6 (*t*)	H-15β, H-16α, H-16β	H-26
15β	1.58 (1H, *ddd*, 13.5, 6.2)		H-15α, H-16α, H-16β	
16α	1.52 (1H, *ddd*, 13.5, 5, 2)	36.4 (*t*)	H-15α, H-15β, H-16β	H-28
16β	1.90 (1H, *td*, 13.5, 6)		H-15α, H-15β, H-16α	
17	—	30.8 (*s*)	—	H-28
18β	1.62 (1H, *br d*, 8)	43.6 (*d*)	H-19α/β, H-27	H-27, H-28
19α	2.25 (1H, *br d*, 15)	25.5 (*t*)	H-18, H-30	—
19β	1.69 (1H, *dd*, 15, 8)			
20	—	46.2 (*s*)	—	H-19α, H-22
21α	2.26 (1H, *ddd*, 14, 4, 2)	25.1 (*t*)	H-21β, H-22α, H-22β	H-19α/β, H-30
21β	1.41 (1H, *td*, 14, 5)		H-21α, H-22α, H-22β, H-30	—
22α	2.08 (1H, *td*, 14, 4)	34.0 (*t*)	H-21α, H-21β, H-22β	H-28
22β	1.05 (1H, *ddd*, 14, 5, 2)		H-21α, H-21β, H-22α	
23	2.21 (3H, *s*)	10.3 (*q*)	—	—
25	1.46 (3H, *s*)	38.4 (*q*)	—	—
26	1.28 (3H, *s*)	21.7 (*q*)	—	—
27	0.56 (3H, *s*)	18.4 (*q*)	—	H-12β
28	1.09 (3H, *s*)	31.5 (*q*)	—	H-22α
29	—	177.4 (*s*)		19β, 21β
30	3.39 (1H, *d*, 10)	74.1 (*t*)	H-19α/*b*, H-21β	—
	3.61 (1H, *d*, 10)			
OCH_3	3.61 *s*	51.9 (*q*)		—
3-OH	6.98 (1H, *br s*)	—		—

*J in Hz.

†Multiplicity (in parentheses) deduced from a DEPT experiment.

‡Assignments based on HETCOR and HMBC.

§Long-range correlations (HMBC) between protons and indicated carbon.

Me-30 carbon [δ_H 32.7 (*q*)] in **4** [15]. Apart from this difference and significant shifts in some ring-E carbons (C-19, C-20 and C-21), the rest of the ^{13}C NMR spectrum of **2** (Table 2) resembled that of **4** [15]. It remained to determine the stereochemical disposition at C-20 and this was done by difference NOE spectroscopy. Irradiation of the methyl protons at δ_H 0.56 (s, Me-27) enhanced the CO_2Me singlet at δ_H 3.61 and vice versa. Therefore, the CO_2Me group should have the 20α-configuration and the CH_2OH group 20β-configuration. Irradiation of the methyl singlets at δ_H 1.09 (Me-28) and 1.28 (Me-26) caused NOE enhancements of the signals at δ_H 1.62 (18-H), 1.69 (19-Hβ) and 1.90 (16-Hβ) and at δ_H 1.46 (Me-25), 1.62 (18-H) and 1.90 (16-Hβ), respectively. Based on the evidence presented above the structure of **2** was determined as 30-hydroxypristimerin, in which the rings C, D and E are slightly flattened and this agrees well with the previously reported X-ray structure of pristimerol-bis-*p*-bromobenzoate [18].

In a recent communication we provided evidence for the structure of salacenonal (**7**), a suspected biogenetic precursor of celastroloids occurring in *S. reticulata* var. β-diandra [10]. Herein we wish to report the complete ^{1}H and ^{13}C NMR assignments of **7** by the application of 2D NMR techniques. By postulating a friedelane skeleton it was possible to assign some of the carbons and protons of rings D and E by comparison with the NMR spectra of **9** [11]. The ^{1}H NMR spectrum of **7** had signals due to a CHO group (δ_H 9.62, *d*, J = 1.8 Hz, H-24), two olefinic protons of an exomethylene group (δ_H 4.59 and 4.58, each *br s*, H_a-30 and H_b-30), a

methyl group on an unsaturated carbon (δ_H 1.65, *s*, Me-23) and four methyl groups on quaternary carbons [δ_H 1.11 (*s*, Me-28), 1.01 (*s*, Me-27), 0.89 (*s*, Me-25) and 0.84 (*s*, Me-26)], all of which were readily assigned based on their chemical shift values and/or coupling constants. However, the remaining resonances required more rigorous analysis and this was done by the use of ^{1}H–^{1}H COSY, HETCOR and HMBC experiments as described below.

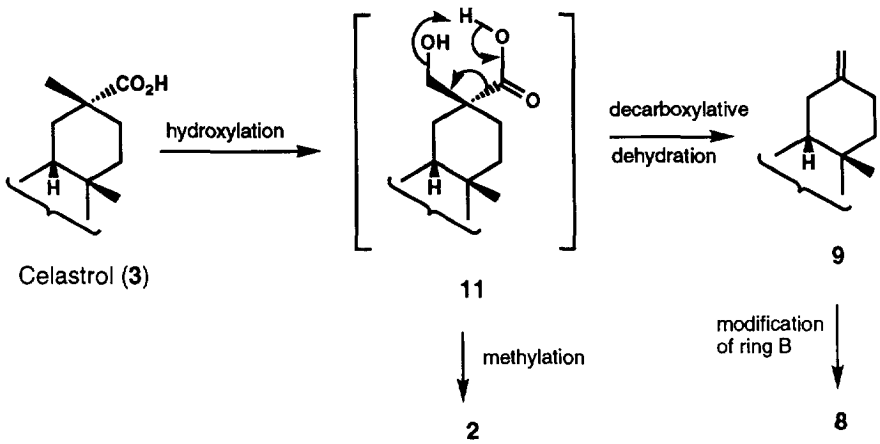
In the ^{1}H-^{1}H COSY spectrum of **7**, the two sets of *dd* at δ_H 2.68 (J = 18 and 5 Hz, and 2.87 (J = 18 and 15 Hz) (assigned to H-1α and H-1β based on their chemical shifts and coupling constants) showed cross peaks with the methine proton at δ_H 2.19 (*dd*, J = 15 and 5 Hz), indicating this proton to be H-10. The methylene protons at C-6 appeared as distinctly separated groups of signals at δ_H 1.20 (*ddd*, J = 13, 4.5 and 1.8 Hz, H-6α) and 2.66 (*dt*, J = 13 and 2.5 Hz, H-6β) as a result of the anisotropic effect due to the CHO group on C-5. The signal due to this CHO group appeared at δ_H 9.62 as a doublet (J = 1.8 Hz) due to the long-range coupling with H-6α at δ_H 1.20. Both protons of the CH_2-6 group coupled vicinally with the protons at δ_H 1.61 (*m*, 7-Hα) and 1.42 (*m*, 7-Hβ), which also revealed a correlation with the proton at δ_H 1.40 (*m*, 8-H). On the other hand, the methyl protons Me-25, Me-26, Me-27 and Me-28 showed long-range correlations with CH_2-11 (δ_H 1.35, *m*), CH_2-15 (δ_H 1.30, *m*), H-12β (δ_H 1.33, m) and H-18 (δ_H 1.51, *br d*, J = 6 Hz) and H-22α (δ_H 2.01, *td*, J = 13.5 and 5 Hz), respectively. By following correlations of these protons, all the other protons (CH_2-16, H-12α, CH_2-19, H-22β and CH_2-21) were unambiguously assigned (Table 3).

The ^{13}C NMR spectrum of **7**, analysed with the aid of the DEPT spectrum, showed a total of 29 carbons which consisted of five methyl, 11 methylene, four methine and nine quaternary carbons. All protonated carbons were assigned based on the correlations observed in the HETCOR spectrum. Assignment of the quaternary carbons, however, required careful analysis of the HMBC spectrum. The correlations observed in the HMBC spectrum are given in Table 3. The sp^3 quaternary carbons, C-5, C-9 and C-17, were assigned based on the correlations with H-24, Me-25 and Me-28, respectively. Two other sp^3 quaternary carbons at δ_C 40.7 and 39.5 both showed correlations with Me-26 and Me-27. The former also correlated with H-12, H-18 and H-19, and the latter with H-7, H-8 and H-16. Thus, the carbon at δ_C 40.7 was assigned to C-13 and the one at δ_C 39.5 to C-14. The sp^2 quaternary carbon at δ_C 149.1 was readily assigned to C-20 based on the HMBC correlations with H-18, H-19 and H-21. The remaining two sp^2 quaternary carbons at δ_C 146.5 and 127.0 both revealed correlations with 3-OH and Me-23. They were assigned to C-3 (δ_C 146.5) and C-4 (δ_C 127.0) based on their chemical shifts and the HMBC correlations with H-1 and H-10, respectively. The complete ^{13}C NMR assignments for **7** are given in Table 3.

The co-occurrence of **2** together with **9** and **8**, in *S. reticulata* var. β-diandra suggests the possible intermediacy of 30-hydroxycelastrol (**11**) in the biosynthetic conversion of **3** into **2** and **9** as depicted in Scheme 1.

EXPERIMENTAL

General experimental methods. Mps (uncorr.) were determined on a Kofler hot stage apparatus. Optical rotations were measured in $CHCl_3$ solns at 25° with a Perkin Elmer 241 polarimeter. UV spectra were recorded for EtOH with a Shimadzu UV 160 spectrometer, and IR spectra for KBr discs with a Shimadzu IR 408 spectrometer. The MS were recorded on a JEOL JMS-D 300 mass spectrometer with a direct inlet system. Unless otherwise stated, instrumentation and conditions used for NMR measurements and processing were the same as those described previously [17]. The NMR spectra were recorded as *ca* 10% solns in $CDCl_3$



Scheme 1. Proposed biogenetic relationship between celastrol (**3**), 30-hydroxypristimerin (**2**), isoiguesterin (**9**) and salaciquinone (**8**).

Table 3. ^1H, ^{13}C NMR and HMBC data for **7** in $CDCl_3$ (δ in ppm)

Position	δ_H*	δ_C†‡	HMBC [^1H (l.r. coupl.)]§	
			$^3J_{(CH)}$	$^2J_{(CH)}$
1α	2.68 (1H, *dd*, 18, 5)	31.4 (*t*)	—	H-10
1β	2.87 (1H, *dd*, 18, 15)		—	—
2	—	193.2 (*s*)	H-10, 3-OH	H-1
3	—	146.5 (*s*)	H-1, H-23	3-OH
4	—	127.0 (*s*)	H-10, 3-OH	H-23
5	—	54.7 (*s*)	H-1, H-23	H-6, H-10, H-24
6α	1.20 (1H, *ddd*, 13, 4.5, 1.8)	30.6 (*t*)		—
6β	2.66 (1H, *dt*, 13, 2.5)		—	—
7α	1.61 (1H, *m*)	18.3 (*t*)	—	—
7β	1.42 (1H, *m*)		—	—
8	1.40 (1H, *m*)	48.7 (*d*)	H-6, H-10, H-25, H-26	—
9	—	37.0 (*s*)	H-1, H-7	H-8, H-10, H-25
10	2.19 (1H, *dd*, 15, 5)	56.0 (*d*)	H-6, H-8, H-25	H-1
11α,β	1.35 (2H, *m*)	33.3 (*t*)	H-25	—
12α	1.56 (1H, *m*)	28.8 (*t*)	H-27	—
12β	1.33 (1H, *m*)		—	—
13	—	40.7 (*s*)	H-19, H-26	H-12, H-18, H-27
14	—	39.5 (*s*)	H-7, H-16, H-27	H-8, H-26
15α,β	1.30 (2H, *m*)	28.2 (*t*)	H-26	H-16
16α	1.27 (1H, *m*)	35.9 (*t*)	H-22, H-28	H-15
16β	1.74 (1H, *m*)		—	—
17	—	31.4 (*s*)	H-19	H-16, H-18, H-22, H-28
18	1.51 (1H, *br d*, 6)	45.3 (*d*)	H-12, H-27, H-28	H-19
19α	2.31 (1H, *m*)	29.8 (*t*)	H-21, H-30	H-18
19β	2.34 (1H, *br d*, 15, 5)		—	—
20	—	149.1 (*s*)	H-18	H-19, H-21
21α	2.12 (1H, *m*)	30.7 (*t*)	H-19, H-30	—
21β	2.31 (1H, *m*)		—	—
22α	2.01 (1H, *td*, 13.5, 5)	38.0 (*t*)	H-16, H-28	—
22β	1.14 (1H, *m*)		—	—
23	1.65 (3H, *s*)	10.7 (*q*)	—	—
24	9.62 (1H, *d*, 1.8)	194.9 (*d*)	—	—
25	0.89 (3H, *s*)	18.1 (*q*)	H-10	—
26	0.84 (3H, *s*)	15.1 (*q*)	H-10	—
27	1.01 (3H, *s*)	17.9 (*q*)	—	—
28	1.11 (3H, *s*)	31.4 (*q*)	H-16, H-18, H-22	—
30	4.58 (1H, *br s*)	107.6 (*t*)	H-19, H-21	—
	4.59 (1H, *br s*)			
3-OH	6.43 (1H, *s*)	—	—	—

*J in Hz.

†Multiplicity (in parentheses) deduced from a DEPT experiment.

‡Assignments based on HETCOR and HMBC.

§Long-range correlations (HMBC) between protons and indicated carbon.

at ambient temp. NOE difference spectra were determined with a JEOL standard pulse sequence with 5 sec irradiation. TLC involved silica gel GF; visualization was by UV (254 nm) and by spraying with acidified anisaldehyde followed by charring with heat. Flash CC involved silica gel of mesh 230–400 ASTM. Prep. TLC used 0.25 mm layers of silica gel GF_{254}.

Plant material. Root outer bark of *S. reticulata* var β-diandra was collected at the Sinharaja Forest in Sri Lanka by the late Prof S. Balasubramaniam of the Department of Botany, University of Peradeniya, Sri Lanka, where a voucher specimen (SB-SRBD-1) is deposited.

Extraction and fractionation of the hexane extract. The dried and pulverized root outer bark (375 g) of *S. reticulata* var. β-diandra was sequentially and exhaustively extracted with hot hexane and C_6H_6. Evapn afforded hexane (35 g) and C_6H_6 (65 g) extracts. The hexane extract (25 g) was subjected to flash CC over silica gel with solvent gradients ranging from hexane to hexane containing increasing amounts of EtOAc. A total of 75 frs were collected and combined based on their TLC patterns. Column frs 4–5 on further purification by prep. TLC (hexane–EtOAc, 10:1) afforded **7** [10] (0.012 g, 0.0032%). Combined column frs 6–7 were further sepd by flash CC over silica gel followed by prep. TLC (C_6H_6–EtOAc, 20:1) giving **8** [11] (0.01 g, 0.002%) and β-amyrin. Combined column frs 8–9 on standing pptd an orange crystalline solid, the major constituent of which was isolated by prep. TLC

(hexane–EtOAc, 5:1) giving **9** [11] (0.05 g, 0.013%). Combined column frs 12–14 on flash CC followed by prep. TLC afforded **10** (0.023 g, 0.006%), **4** [19] (0.05 g, 0.013%) and sitosterol (0.04 g, 0.01%). Combined column frs 19–21 were further sepd by prep. TLC (hexane–EtOAc, 4:1) affording 29-hydroxyfriedelan-3-one [20] (0.021 g, 0.006%) and **5** [19] (0.035 g, 0.009%). Combined column fr. 22–25 on flash CC followed by prep. TLC (hexane–EtOAc, 5:1) yielded **6** [19] (0.021 g, 0.006%). Combined column frs 34–40 were further purified by prep. TLC (CH_2Cl_2–MeOH, 50:1) affording **1** (0.05 g, 0.013%).

The C_6H_6 extract (35 g) of *S. reticulata* var. *β*-diandra was subjected to flash CC over silica gel with solvent gradients ranging from hexane to hexane containing increasing amounts of EtOAc. A total of 100 frs were collected and combined according to their TLC patterns. TLC examination of column frs 1–30 indicated the presence of minor amounts of compounds encountered in the hexane extract and, therefore, were not further investigated. Combined column frs 32–37 on further CC followed by prep. TLC (CH_2Cl_2–MeOH, 50:1) gave epi-kokoondiol [1] (0.023 g, 0.006%) and **3** [21] (0.02 g, 0.0053%). Combined column frs 51–54 on flash CC followed by two successive purifications by prep. TLC (hexane–Me_2CO, 4:1) afforded **2** (0.025 g, 0.007%).

Netzahualcoyene (**10**). Red crystalline solid, mp 211–212°, lit. [14] 150–152° and 176–178°, $[\alpha]_D$ +210° ($CHCl_3$, *c* 0.1); IR ν_{max} cm^{-1} 3400–3200, 1715, 1642, 1545, 1500; UV λ_{max} EtOH (log ε) 259 (4.00), 444 (4.05); HREIMS *m/z* (rel. int.): $[M]^+$ 462.2786 (100) [$C_{30}H_{38}O_4$ requires 462.2770; ^1H NMR δ_H ($CDCl_3$): 0.82 (3H, *s*, Me), 1.20 (3H, *s*, Me), 1.21 (3H, *s*, Me), 1.28 (3H, *s*, Me), 1.72 (3H, *s*, Me), 2.26 (3H, *s*, Me), 3.68 (3H, *s*, Me), 6.15 (1H, *d*, $J = 7.0$ Hz), 6.60 (1H, *s*), 7.20 (1H, *d*, $J = 7.0$ Hz); ^{13}C NMR δ_C ($CDCl_3$): 179.4 (*s*, C-29), 178.1 (*s*, C-2), 160.1 (*s*, C-10), 159.7 (*s*, C-8), 146.3 (*s*, C-5), 135.4 (*s*, C-14), 134.9 (*d*, C-6), 128.4 (*s*, C-4), 127.6 (*s*, C-15), 121.6 (*d*, C-1), 119, 9 (*d*, C-7), 116.8 (*s*, C-3), 51.8 (*q*, OMe), 44.5 (*s*, C-13), 44.0 (*d*, C-18), 43.2 (*s*, C-17), 42.7 (*s*, C-9), 37.8 (*t*, C-22), 37.6 (*t*, C-16), 36.1 (*t*, C-21), 35.7 (*t*, C-12), 34.0 (*t*, C-11), 33.8 (*s*, C-20), 31.5 (*q*, C-30), 29.7 (*t*, C-19), 29.5 (*q*, C-25), 24.0 (*q*, C-26), 22.0 (*q*, C-27), 19.8 (*q*, C-28), 10.4 (*q*, C-23).

Isoiguesterinol (**1**). Orange crystalline solid, mp 118–120°; $(\alpha)_D$ +22° ($CHCl_3$, *c* 1.8), IR ν_{max} cm^{-1} 3325, 1602; UV λ_{kax} EtOH (log ε) 244 (4.01), 424 (4.14); HREIMS *m/z* (rel. int.): 422 $[M]^+$ (60) ($C_{28}H_{38}O_3$), 253 (28) ($C_{17}H_{17}O_2$), 241 (33) ($C_{16}H_{17}O_2$), 201 (100) ($C_{13}H_{13}O_2$); $[M]^+$ 422.2792; $C_{28}H_{38}O_3$ requires 422.2821; ^1H and ^{13}C NMR: Table 1.

30-*Hydroxypritimerin* (**2**). Orange crystalline solid, mp 228–229° (CH_2Cl_2); $[\alpha]_D$ −147° ($CHCl_3$, *c* 1.1); IR ν_{max} cm^{-1} 3375, 1720, 1603; UV λ_{max} EtOH (log ε) 249 (3.68), 417 (3.90); HREIMS *m/z* (rel. int.): 480 $[M]^+$ (100) ($C_{30}H_{40}O_5$), 253 (33) ($C_{17}H_{17}O_3$), 241 (62) ($C_{16}H_{17}O_2$), 227 (16) ($C_{15}H_{15}O_2$), 215 (20) ($C_{14}H_{15}O_2$), 201 (91) ($C_{13}H_{13}O_2$); $[M^+]$ 480.2880; $C_{30}H_{40}O_5$ requires 480.2875; ^1H and ^{13}C NMR: Table 2.

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