Studies on Terpenoids and Steroids. Part 10.[1] Structures of Four New Natural Phenolic D:A-*friedo*-24-Noroleanane Triterpenoids[2]

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The three new phenolic nortriterpenes, demethylzeylasteral, zeylasteral, and demethylzeylasterone, isolated from *Kokoona zeylanica* (Celastraceae), have been shown to be 2,3-dihydroxy-6,23-dioxo-D:A-*friedo*-24-noroleana-1,3,5(10),7-tetraen-29-oic acid (**5**), its methyl ester (**6**), and 2,3-dihydroxy-6-oxo-D:A-*friedo*-24-noroleana-1,3,5(10),7-tetraene-23,29-dioic acid (**7**), respectively. The structure of the new phenolic (9 ⟶ 8)-D:A-*friedo*-24-noroleanane triterpene obtained from 'kokum soap' has been established as 23-oxoisopristimerin III (**11**) on the basis of spectroscopic evidence. The biosynthetic significance of 23-oxoisopristimerin III is discussed.

Previous studies of *Kokoona zeylanica* Thwaites, family Celastraceae, have revealed a number of unique D:A-*friedo*-oleanane triterpenes,[3–7] *e.g.* pristimerin (**1**) and zeylasterone (**4**), the first example of a natural phenolic D:A-*friedo*-24-noroleanane triterpene.[8] The present study is concerned with the isolation and structure elucidation of demethylzeylasteral (**5**), zeylasteral (**6**), and demethylzeylasterone (**7**) related to zeylasterone (**4**), and 23-oxoisopristimerin III (**11**), a triterpene bearing a novel phenolic [9 ⟶ 8]-D:A-*friedo*-24-noroleanane skeleton. Natural occurrence of compound (**11**) is significant as its analogue, isopristimerin III (**13**), could be the possible biosynthetic precursor of two recently encountered novel natural quinone methides, pristimerinene (**16**) and hydroxypristimerinene (**17**).

Table 1. U.v. spectral data [$\lambda_{max.}$/nm (log ε)] (EtOH) of phenolic triterpenes (**4**)—(**10**)

Compd.					
(**4**)	211 (4.19)	226 (4.05)	255 (4.08)	295 (3.79)	340 (3.70)
(**5**)	207 (3.31)		247 (3.27)	304 (2.85)	388 (2.82)
(**6**)[a]	207 (3.99)		247 (3.94)	306 (3.47)	399 (3.49)
(**7**)	210 (4.15)	220 (4.00)	250 (3.99)	302 (3.67)	340 (3.64)
(**8**)	210 (4.13)	225 (4.00)	247 (4.07)	285 (3.80)	300 (3.92)
(**9**)	211 (4.32)	222 (4.29)	248 (4.20)	296 (3.91)	315 (3.97)
(**10**)	207 (4.00)	225 (3.88)	245 (3.99)	287 (3.72)	312 (3.72)

[a] Different from values reported by us earlier[8] although all other physical data (i.r., ^{1}H n.m.r., m.s., m.p., and t.l.c.) agree with those reported.

Results and Discussion

Stem Outer Bark Extractives.—The hot light petroleum and hot benzene extracts of the stem outer bark of *K. zeylanica* were separated into acidic, phenolic, and neutral fractions. The major constituents in the neutral and acidic fractions of the light petroleum extracts were pristimerin (**1**) and zeylasterone (**4**), respectively. On column and preparative layer chromatography (p.l.c.) separation, the phenolic fraction of the light petroleum extract gave (i) celastrol (**2**); (ii) a new phenolic triterpene aldehyde related to compound (**4**) and which was named zeylasteral; and (iii) another minor, new phenolic triterpenoid, 23-oxoisopristimerin III, which has also been isolated from 'kokum soap' cake. The acidic fraction of the benzene extract afforded zeylasterone (**4**) and a new triterpene acid, demethylzeylasterone.

Zeylasteral, m.p. 278—280 °C, $[\alpha]_D$ −136.0°, analysed for $C_{30}H_{38}O_6$ and gave a positive response to both the Liebermann–Burchard test for triterpenes and the neutral iron(III) chloride test for phenols. I.r. bands at 3 500—3 100br, 1 727, 1 650, and 1 642 cm^{-1} indicated the presence of chelated hydroxy, saturated ester carbonyl, aromatic aldehyde carbonyl, and α,β-unsaturated ketone carbonyl groups, repectively. Zeylasteral on methylation (Me_2SO_4–K_2CO_3) afforded a dimethyl derivative, $C_{32}H_{42}O_6$, m.p. 201—202 °C, $[\alpha]_D$ −120.3°. U.v. spectra of both zeylasteral and dimethylzeylasteral showed a close resemblance to that of dimethyl-6-oxopristimerol (**8**), suggesting the presence of a similar type of chromophore (see Table 1). H_3BO_3–NaOAc-induced shift in the u.v. spectrum of zeylasteral suggested that it contained an *ortho*-dihydroxy aromatic system.[8]

The ^{1}H n.m.r. spectrum of zeylasteral showed five 3-H singlets in the high-field region due to 5 methyl groups attached to aliphatic quaternary carbon atoms and these were assigned by comparison with the corresponding methyl signals of pristimerin (**1**), dimethyl-6-oxopristimerol (**8**), and zeylasterone (**4**) (see Table 2). The low-field signal due to 1-H at δ 11.00 was assigned to ArCHO. These ^{1}H n.m.r. comparisons further suggested the absence of the C-4 methyl group in the natural product. This along with other physical data revealed that the CHO group in zeylasteral should be at C-4. The foregoing evidence suggested the structure of zeylasteral to be methyl-2,3-dihydroxy-6,23-dioxo-D:A-*friedo*-24-noroleanan-1,3,5(10),7-tetraen-29-oate (**6**).

Further evidence for the proposed structure of zeylasteral came from its ^{13}C n.m.r. spectral data, assignment of which was made by comparison with zeylasterone (**4**) (see Table 3). As expected, changes in chemical shifts were observed for the aromatic carbons; C-2 and C-10 which are *meta* to the CHO group and are shielded by about 25 p.p.m. compared with the resonances for zeylasterone. Such large differences have not previously been observed for substituted benzenes.[9] This difference cannot be attributed to the general electron-withdrawing difference between CO_2H and CHO functions. However, owing to the close proximity of CO_2H to the carbonyl at C-6 in zeylasterone, hydrogen bonding (Figure) may decrease the electron density at C-6, which in turn may reduce the

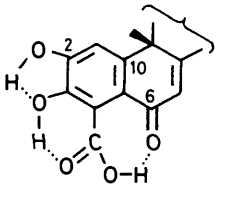
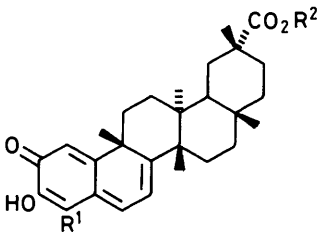


Figure.

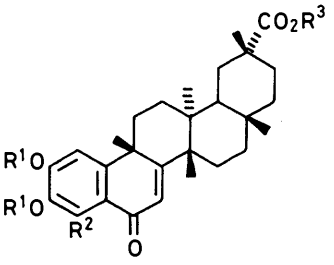
Table 2. ^{1}H N.m.r. data[a] of pristimerin (**1**) and phenolic triterpenoids (**4**)—(**10**)

Compound	1-H	6-H	7-H	4-Me	9-Me	13-Me	14-Me	17-Me	20β-Me	4-CO_2Me	4-CHO	20α-CO_2Me	2,3-$(OMe)_2$
(**1**)	6.55	7.02	6.34	2.20	1.47	1.11	1.27	1.17	0.55			3.57	
(**4**)	6.50		7.33		1.60	1.11	1.32	1.17	0.60			3.54	
(**5**)	6.33		7.23		1.56	1.11	1.31	1.20	0.73		11.00		
(**6**)	6.38		7.31		1.56	1.10	1.33	1.18	0.56		11.00	3.53	
(**7**)	6.10		7.00		1.50	1.10	1.26	1.13	0.66				
(**8**)	6.17		6.92	2.66	1.60	1.12	1.32	1.18	0.60			3.55	3.78, 3.95
(**9**)	6.36		7.03		1.61	1.13	1.33	1.18	0.63		10.31	3.58	3.86, 4.00
(**10**)	6.22		6.95		1.60	1.11	1.32	1.17	0.60	3.93		3.54	3.83, 3.93

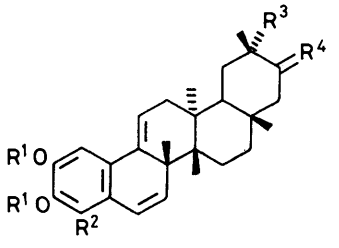
[a] δ-Values at 60 MHz in $CDCl_3$ except for demethylzeylasterone (**7**), for which data were recorded in $(CD_3)_2SO$.



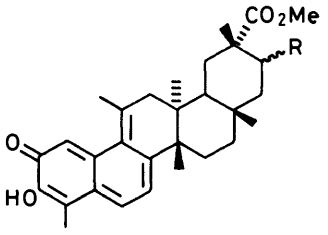
(**1**) $R^1 = R^2 = Me$
(**2**) $R^1 = Me, R^2 = H$
(**3**) $R^1 = CHO, R^2 = Me$



(**4**) $R^1 = H, R^2 = CO_2H, R^3 = Me$
(**5**) $R^1 = R^3 = H, R^2 = CHO$
(**6**) $R^1 = H, R^2 = CHO, R^3 = Me$
(**7**) $R^1 = R^3 = H, R^2 = CO_2H$
(**8**) $R^1 = R^2 = R^3 = Me$
(**9**) $R^1 = R^3 = Me, R^2 = CHO$
(**10**) $R^1 = R^3 = Me, R^2 = CO_2Me$



(**11**) $R^1 = H, R^2 = CHO, R^3 = CO_2Me, R^4 = H_2$
(**12**) $R^1 = Me, R^2 = CHO, R^3 = CO_2Me, R^4 = H_2$
(**13**) $R^1 = H, R^2 = Me, R^3 = CO_2Me, R^4 = H_2$
(**14**) $R^1 = R^2 = Me, R^3 = CO_2Me, R^4 = H_2$
(**15**) $R^1 = Ac, R^2 = Me, R^3 = H, R^4 = O$



(**16**) R = H
(**17**) R = OH

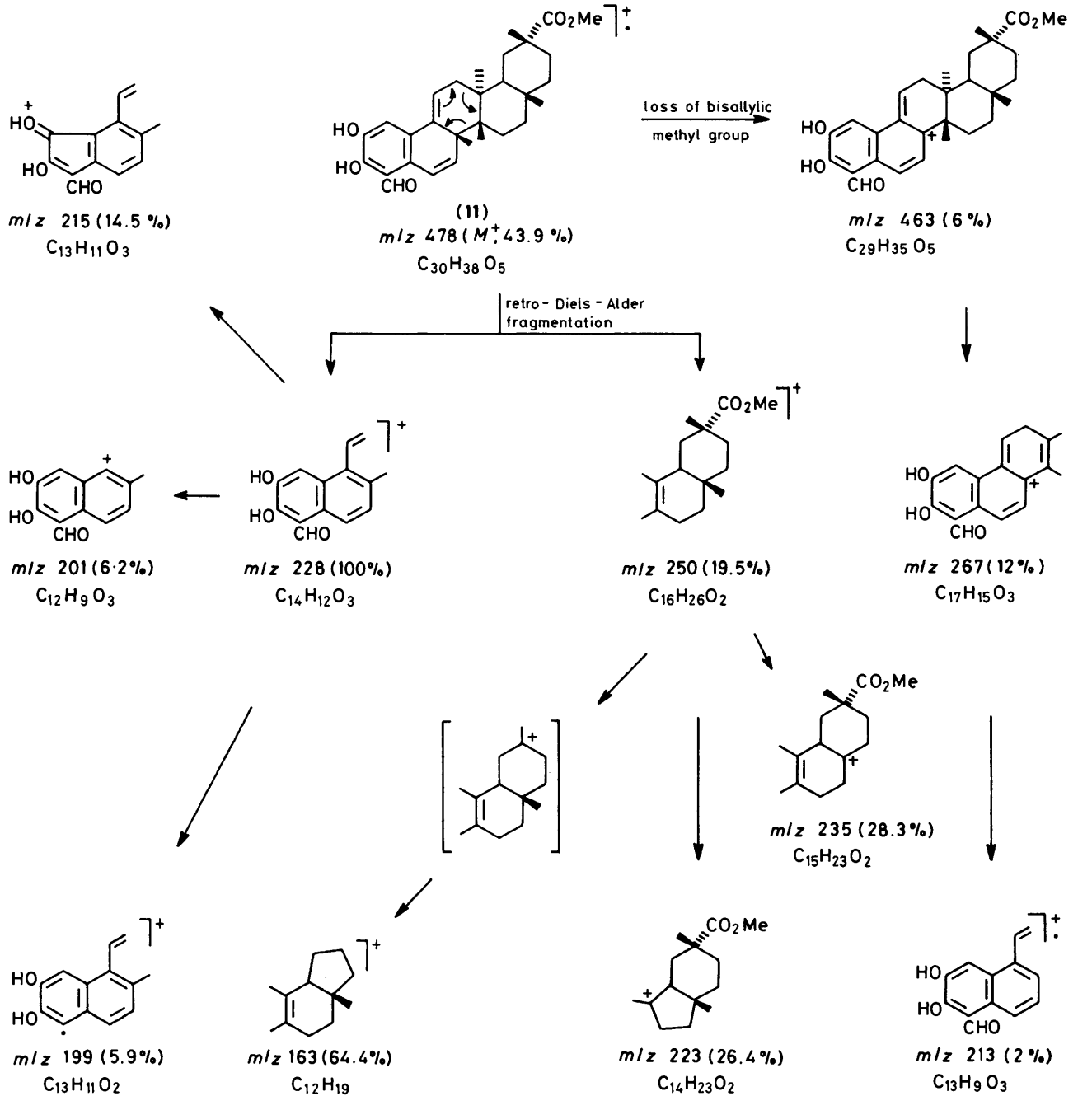
electron density at C-2 and C-10. Finally dimethylzeylasteral (**9**) was converted into trimethylzeylasterone (**10**) by oxidation with Jones' reagent followed by methylation, thus confirming the structure (**6**) proposed for zeylasteral.

The acidic fraction of the benzene extract of the stem outer bark of *K. zeylanica* was separated into two major compounds. The less polar of these was zeylasterone (**4**). The more polar compound, $C_{29}H_{36}O_7$, m.p. 190—192 °C, $[\alpha]_D$ −36.5°, was a pale yellow crystalline solid. Positive Liebermann–Burchard and neutral iron(III) chloride test results indicated it to be a phenolic triterpenoid.[8] The i.r. spectrum indicated the presence of chelated hydroxyl (3 500—3 000 cm^{-1}), aliphatic and aromatic carboxylic acid (1 727 and 1 705 cm^{-1} respectively), and α,β-unsaturated carbonyl (1 633 cm^{-1}) functions. The u.v. spectrum resembled those of zeylasterone (**4**), zeylasteral (**6**), and dimethyl-6-oxopristimerol (**8**), thus suggesting the presence of the same type of chromophoric system. Further the H_3BO_3–NaOAc-induced u.v. shift indicated the presence of an *ortho*-dihydroxy aromatic system.

The ^{1}H n.m.r. spectrum $[(CD_3)_2SO]$ was almost superposable on that of zeylasterone except that the 3-H signal at δ 3.53 was absent (see Table 2). In zeylasterone the signal at δ 3.53 was assigned to 20α-CO_2Me. Therefore, it was thought that this compound may be the C-29 carboxylic acid of zeylasterone and was named demethylzeylasterone. Conversion of demethylzeylasterone into trimethylzeylasterone (**10**) with excess of diazomethane further confirmed its structure as 2,3-dihydroxy-6-oxo-D:A-*friedo*-24-noroleana-1,3,5(10),7-tetraene-23,29-dioic acid (**7**).

Root Outer Bark Extractives.—The root outer bark of *K. zeylanica* was extracted with hot light petroleum, hot benzene, and hot ethyl acetate. The hot ethyl acetate extract on column chromatographic separation afforded a new phenolic triterpene (demethylzeylasteral) and another unidentified phenolic triterpene.

Demethylzeylasteral, $C_{29}H_{36}O_6$, m.p. 158—160 °C, $[\alpha]_D$ −67.9°, gave positive Liebermann–Burchard and neutral



Scheme 1.

iron(III) chloride test results. I.r. bands at 3 400—3 100br, 1 726, 1 705, and 1 636 cm^{-1} indicated the presence of chelated hydroxy, saturated carboxylic acid carbonyl, aromatic aldehyde, and α,β-unsaturated ketone carbonyl groups, respectively. The ^1H n.m.r. spectrum was almost superposable on that of zeylasteral (**6**) except for the absence of the signal due to 20α-CO_2Me and the low-field shift of the 20β-Me signal of demethylzeylasteral (**5**) (Table 2). The foregoing evidence suggested the natural product to be 2,3-dihydroxy-6,23-dioxo-D:A-*friedo*-24-noroleana-1,3,5(10),7-tetraen-29-oic acid (**5**). This was further confirmed by methylation (Ag_2O–MeI) to give a trimethyl derivative identical with dimethylzeylasteral (**9**) obtained above.

Extractives of 'Kokum Soap' Cake.—The hot light petroleum extract of kokum soap[8] was separated into acidic, phenolic, and neutral fractions. The phenolic fraction on chromatography yielded celastrol (**2**), zeylasteral (**6**), and a new phenolic triterpenoid, $C_{30}H_{38}O_5$, m.p. 157—160 °C, identified as 23-oxoisopristimerin III (**11**) by the evidence presented below. I.r. bands at 3 500—3 200, 1 722, and 1 642 cm^{-1} indicated the presence of hydroxy, saturated ester carbonyl, and α,β-unsaturated aldehyde carbonyl, respectively. The u.v. spectrum ($\lambda_{max.}$ 263 and 277 nm) suggested the absence of both pristimerin- and zeylasterone-type chromophores,[10] but the presence of a styrene-type chromophore.[11] H_3BO_3–NaOAc-induced u.v. shifts indicated an *ortho*-dihydroxy aromatic

Table 3. ^{13}C N.m.r. chemical shifts (δ/p.p.m.; 25.05 MHz; $CDCl_3$) of zeylasterone (**4**) and zeylasteral (**6**)

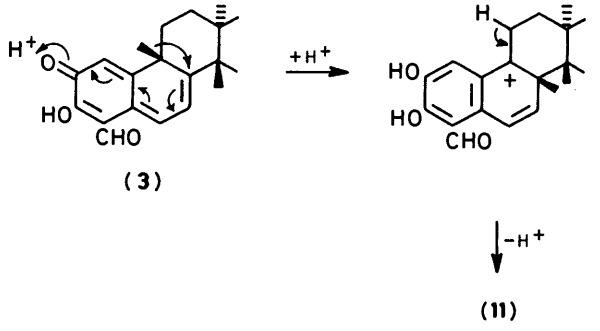
Carbon	(4)	(6)	Carbon	(4)	(6)	Carbon	(4)	(6)
1	113.8	116.5	11	36.2	36.4	21	29.8	29.8
2	173.7	150.5	12	28.6	28.6	22	34.4	33.5
3	155.5	149.7	13	39.7	39.7	23	178.7	200.3
4	111.3	116.3	14	40.5	40.5	25	36.8	36.4
5	119.4	117.1	15	30.9	31.0	26	20.2	20.6
6	188.0	186.0	16	34.8	34.8	27	32.7	32.8
7	124.4	122.8	17	43.0	44.1	28	18.3	18.3
8	153.4	149.1	18	44.2	44.1	29	179.8	178.9
9	45.6	45.3	19	29.8	29.8	30	31.6	31.8
10	152.8	125.3	20	30.5	30.7	OMe	51.6	51.6

system. Methylation yielded a dimethyl derivative, $C_{32}H_{42}O_5$, m.p. 178—181 °C.

The ^1H n.m.r. spectrum of the natural product showed six 3-H singlets, 5 in the high-field region for Me groups attached to aliphatic quarternary centres and one in the OMe region. In the low-field region, singlets at δ 11.93 and 10.33 were assigned to OH (exchangeable with D_2O) and CHO, respectively. Information on the aromatic and vinylic protons were obtained by comparison of the ^1H n.m.r. spectra (Table 4) of this compound and its dimethyl derivative with those of dimethylisopristimerin III (**14**)[12] and di-*O*-acetylisotingenone III (**15**),[13] both of which have been obtained by the acid-catalysed rearrangement of the corresponding natural products (pristimerin and tingenone) followed by derivatization. Assignment of some significant ^1H n.m.r. signals for 23-oxoisopristimerin III and its dimethyl derivative (**12**) are given in Table 4.

The mass spectrum of 23-oxoisopristimerin III further supported the proposed structure (**11**). The m.s. fragmentation pattern of this follows essentially two pathways[13] similar to di-*O*-acetylisotingenone III: (a) formal retro-Diels–Alder fragmentation of ring C to give the cation at *m/z* 228 (100%)[13,14] and (b) loss of a bisallylic methyl group to give an ion at *m/z* 463 followed by cleavage of ring D to form an ion at *m/z* 267. Formation of these ions, along with the origin of other possible fragments *via* the above two pathways, are depicted in Scheme 1.

It is possible at 23-oxoisopristimerin III (**11**) may be an artefact arising from a pristimerin analogue (**3**) by an acid-catalysed rearrangement (Scheme 2) during the isolation



Scheme 2.

process. However, the absence of isopristimerin III in the same extract containing pristimerin as the major constituent and the failure of pristimerin to undergo rearrangement under the conditions employed in the isolation process rules out the artefactual origin of 23-oxoisopristimerin III (**11**).

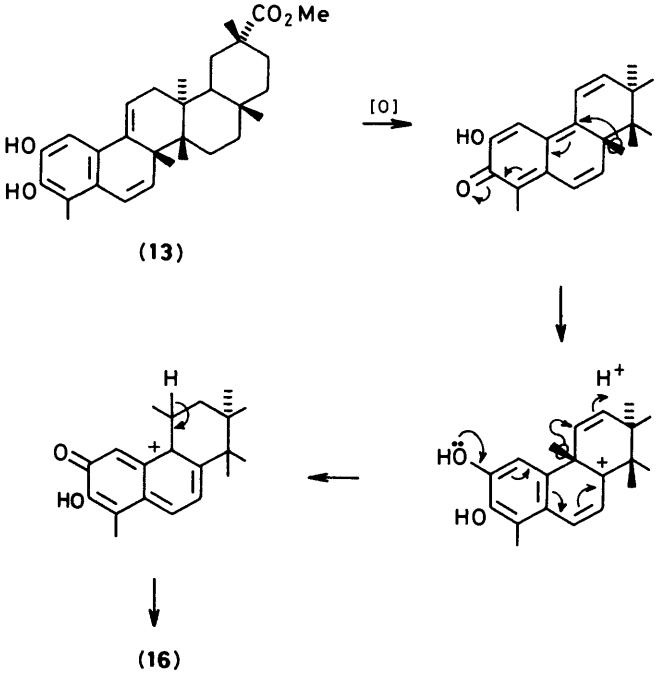
Table 4. ^1H N.m.r. data of 23-oxoisopristimerin III (**11**), its dimethyl derivative (**12**) and some related triterpenes

Compound	1-H	6-H,7-H	11-H
(**11**)[a]	7.11 s	7.00 m	5.56 m
(**12**)[a]	7.00 s	7.15 d, 6.48 d (*J* 10 Hz)	5.57 dd(*J* 6 and 2 Hz)
(**14**)[b]	6.76 s	6.49 d, 6.30 d (*J* 8.6 Hz)	
(**15**)[c]	6.97 s	6.43 q(*J* 10 Hz)	5.63 dd(*J* 6 and 2.2 Hz]

[a] δ-Values at 60 MHz in CCl_4. [b] Data from ref. 12. [c] Data from ref. 13.

Biogenetic Aspects.—Although no experimental proof has thus far been provided for the biogenetic origin of quinone methides, their co-occurrence with D:A-*friedo*-oleananes in several plants of the Celastraceae has led to postulation of four biosynthetic pathways implicating zeylanol and polpunonic acid as precursors.[9,15–17] Recent isolation of salaspermic acid[18] and orthosphenic acid[17] from plants of the Celastraceae further supports our proposed pathway.[8]

The natural occurrence of 23-oxoisopristimerin III (**11**) is significant as its analogue, isopristimerin III (**13**), could be the possible biosynthetic precursor of recently encountered novel quinone methides in nature, *viz.* pristimerinene (**16**) and hydroxypristimerinene (**17**)[19] (see Scheme 3).



Scheme 3.

Experimental

General Procedures.—The general experimental details were the same as those described previously.[8]

Extraction of K. zeylanica *Outer Stem Bark.*—The dried and powdered outer stem bark (500 g) of *K. zeylanica* was exhaustively extracted with hot light petroleum, benzene, and ethyl acetate. The total light petroleum extract (50 g) was separated into acidic (4.7 g), phenolic (13.4 g), and neutral (24.0

g) fractions in the usual manner.[8] Similar treatment of the benzene extract (35 g) gave 2.7 g, 7.5 g, and 23.1 g of the corresponding fractions. The hot ethyl acetate extract weighed 4.7 g.

Isolation of Zeylasteral (**6**).—The phenolic fraction of the light petroleum extract (0.7 g) was chromatographed over a silica gel (G type) column (20 g) made up in 2% methanol in dichloromethane under medium pressure. Elution with the same solvent under pressure yielded zeylasteral (**6**) as a pale yellow solid, which was recrystallized from methanol to yield *pale yellow crystals* (0.05 g, 0.19%); m.p. 278—280 °C; $[\alpha]_D^{27}$ − 136.04° (*c* 0.86 in $CHCl_3$); for u.v. data see Table 1; $\nu_{max.}$(KBr) 3 500—3 100br, 2 940, 1 727, 1 650sh, 1 642, 1 597, 1 467, 1 456, 1 447, 1 383, 1 301, 1 218, 1 204, 1 161, 1 138, 1 101, 1 086, 1 058, 1 006, 996, 929, 886, 801, 776, 757, 702, and 696 cm^{-1}; δ($CDCl_3$) 12.85 (1 H, br s, OH, exchangeable with D_2O), 11.00 (1 H, s, CHO), 7.31 (1 H, s, 7-H), 6.38 (1 H, s, 1-H), 3.53 (3 H, s, CO_2Me), and 1.56, 1.33, 1.18, 1.10, and 0.56 (3 H, s, 5 × Me); for ^{13}C n.m.r. data, see Table 3; *m/z* 494 (M^+, 16.6%), 479 (100), 466 (3.3), 451 (3.6), 435 (2.2), 410 (2), 351 (1.3), 323 (1.3), 297 (3.6), 283 (3.5), 281 (2.2), 271 (9.5), 255 (8), 243 (8), 231 (14), 217 (11.1), 204 (52.7), 189 (14), 146 (5.5), 121 (11.1), 109 (13.8), 107 (11), 105 (5.5), 95 (16.6), 93 (8.3), 91 (5.5), 81 (11.1), 79 (8.3), 77 (2.7), 69 (22.2), 67 (19.5), and 55 (39) (Found: C, 73.1; H, 7.8%; M^+, 494. $C_{30}H_{38}O_6$ requires C, 72.84; H, 7.74%; *M*, 494).

Methylation of Zeylasteral.—Zeylasteral (0.05 g) was dissolved in anhydrous acetone (5 ml) and refluxed with dimethyl sulphate (0.1 ml) in the presence of anhydrous potassium carbonate (0.05 g) for 3 h (t.l.c. control). The reaction mixture was filtered and acetone was removed under reduced pressure. Usual work-up and subsequent purification on p.l.c. followed by recrystallization from light petroleum and chloroform afforded *dimethylzeylasteral* (**9**) (0.045 g, 85%) as white crystals, m.p. 201—202 °C; $[\alpha]_D^{27}$ −120.27° (*c* 0.7 in $CHCl_3$); for u.v. data see Table 1; $\nu_{max.}$(KBr) 2 930, 1 727, 1 707, 1 642, 1 580, 1 485, 1 463, 1 453, 1 417, 1 380, 1 345, 1 307, 1 280, 1 265, 1 217, 1 207, 1 175, 1 147, 1 102, 1 090, 1 080, 1 060, 1 035, 995, 965, 955, 895, 880, 855, 775, 757, and 695 cm^{-1}; δ($CDCl_3$) 10.3 (1 H, s, CHO), 7.03 (1 H, s, 7-H), 6.36 (1 H, s, 1-H), 4.00 and 3.86 (3 H, each s, 2 × OMe), 3.58 (3 H, s, CO_2Me), and 1.61, 1.33, 1.18, 1.13, and 0.63 (3 H, each s, 5 × Me); *m/z* 522 (M^+, 32.1%), 507 (100), 493 (21.4), 479 (19.9), 325 (5.7), 309 (7.8), 295 (20.7), 283 (31.4), 269 (13.5), 257 (22.4), 255 (6.4), 243 (13.5), 232 (26.7), 217 (14.9), 203 (12.8), 189 (5.7), 163 (4.9), 147 (8.2), 135 (8.2), 133 (4.9), 121 (18.2), 109 (14.9), 107 (13.2), 95 (20.3), 93 (7.4), 81 (9.6), 69 (6.4), 55 (7.1), and 44 (13.5) (Found: M^+, 522.2979. $C_{32}H_{42}O_6$ requires *M*, 522.2980).

Conversion of Dimethylzeylasteral (**9**) *into Trimethylzeylasterone* (**10**).—Dimethylzeylasteral (10 mg) was treated with Jones' reagent (0.2 ml) in acetone at 0 °C for 15 min. The reaction mixture was filtered, the acetone was evaporated off under reduced pressure, and the residue was extracted with ether. The crude reaction mixture was then treated with excess of ethereal diazomethane. Purification of the product by p.l.c. and subsequent crystallization with light petroleum and chloroform yielded trimethylzeylasterone (0.007 g), m.p. 228—229 °C, whose identity was confirmed by comparison (m.p., mixed m.p., i.r., t.l.c.) with a synthetic sample.[8]

Isolation of Celastrol (**2**).—Elution of the above column (from which zeylasteral was isolated) with 3% methanol in dichloromethane yielded impure compound (**2**), which on purification by p.l.c. yielded celastrol (0.05 g, 0.019%), m.p. 200—202 °C (lit.,[20] 205 °C), whose identity was confirmed by comparison (m.p., mixed m.p., co-t.l.c.) with an authentic sample.

Isolation of Demethylzeylasterone (**7**).—The acidic fraction (0.3 g) of the above benzene extract was chromatographed on a column of silica gel G (10 g) made up in 1% acetic acid in chloroform. The first few fractions contained zeylasterone (**4**) (0.102 g, 0.18%). Elution of the column with chloroform–methanol–acetic acid (97:2:1) afforded demethylzeylasterone (**7**), which on crystallization from methanol and water yielded *pale yellow crystals* (0.070 g, 0.13%), m.p. 190—192 °C; $[\alpha]_D^{27}$ −36.48° (*c* 2.46 in $CHCl_3$); for u.v. data see Table 1; $\nu_{max.}$(KBr) 3 500, 3 200—3 000br, 2 920, 1 727, 1 705sh, 1 633, 1 585, 1 465, 1 444, 1 375, 1 360, 1 315, 1 205, 1 158, 1 095, 1 050, 990, 885, 875, 802, 773, 745, 705, and 640 cm^{-1}; δ[$(CD_3)_2SO$] 7.00 (1 H, s, 7-H), 6.10 (1 H, s, 1-H), and 1.50, 1.26, 1.13, 1.10, and 0.66 (3 H, each s, 5 × Me); *m/z* 496 (M^+, 2.8%), 478 (23.2), 452 (30.9), 437 (10.5), 391 (12.6), 277 (9.8), 269 (10.5), 229 (30.3), 204 (87.3), 202 (12.5), 189 (10.5), 121 (12.6), 109 (17), 95 (21.1), 85 (100), 81 (12.3), and 55 (20) (Found: M^+, 496.2459. $C_{29}H_{36}O_7$ requires *M*, 496.2460).

Conversion of Demethylzeylasterone (**7**) *into Trimethylzeylasterone* (**10**).—Demethylzeylasterone (0.015 g) was treated with an excess of diazomethane in ether. Evaporation of the ether, subsequent purification of the residue by p.l.c., and recrystallization from light petroleum and chloroform yielded trimethylzeylasterone (0.013 g, 79%), m.p. 229—230 °C, whose identity was confirmed by comparison (m.p., mixed m.p., i.r., co-t.l.c.) with a synthetic sample.[8]

Isolation and Structure of 23-Oxoisopristimerin III (**11**).—Coarsely powdered kokum soap cake (65 g) was successively and exhaustively extracted with hot light petroleum and hot benzene. The light petroleum extract (28.5 g) was separated into acidic (1.05 g), phenolic (10.74 g), and neutral (15 g) fractions by the usual procedure.[8] The acidic fraction on combined column and p.l.c. yielded zeylasterone (**4**) (0.665 g, 1.02%), identical with the sample obtained above. The neutral fraction on column chromatography afforded pristimerin (**1**) (0.9 g, 1.38%), identical with an authentic sample. The phenolic fraction (10.74 g) was separated as for *K. zeylanica* stem outer bark to yield zeylasteral (**6**) (0.107 g, 0.16%), celastrol (**2**) (0.015 g, 0.02%), and *23-oxoisopristimerin III* (**11**) (0.071 g, 0.11%), m.p. 157—160 °C; $\lambda_{max.}$ 277sh (log ε 3.17) and 263 nm (3.15); $\nu_{max.}$(KBr) 3 500—3 200br, 1 722, 1 642, 1 462, 1 448, 1 380, 1 342, 1 307, 1 275, 1 250, 1 232, 1 212, 1 187, 1 105, 1 087, 1 050, 1 025, 1 002, 887, 857, 803, 772, 730, and 710 cm^{-1}; δ(CCl_4) 11.93 (1 H, s, OH, exchangeable with D_2O), 10.33 (1 H, s, CHO), 7.11 (1 H, s, 1-H), 7.00 (2 H, m, 6- and 7-H), 5.56 (1 H, m, 11-H), 3.53 (3 H, s, CO_2Me), 1.17 (3 H, s, Me), 1.07 (6 H, s, 2 × Me), 0.97 (3 H, s, Me), and 0.80 (3 H, s, Me); *m/z* 478 (M^+, $C_{30}H_{38}O_5$, 43.9%), 463 (6.1, $C_{29}H_{35}O_5$), 419 (3.9, $C_{28}H_{35}O_3$), 281 (5.7, $C_{18}H_{17}O_3$), 267 (12, $C_{17}H_{15}O_3$), 250 (19.5, $C_{16}H_{26}O_2$), 241 (13, $C_{15}H_{13}O_3$), 235 (28.3, $C_{15}H_{23}O_2$), 228 (100, $C_{14}H_{12}O_3$), 223 (26.4, $C_{14}H_{23}O_2$), 215 (14.5, $C_{13}H_{11}O_3$), 210 (23.7, $C_{14}H_{10}O_2$), 201 (6.2, $C_{12}H_9O_3$), 199 (5.9, $C_{13}H_{11}O_2$), 181 (2, $C_{13}H_9O$), 175 (12, $C_{13}H_{19}$), 163 (64.4, $C_{12}H_{19}$), 149 (10, $C_{11}H_{17}$), 121 (14, C_9H_{13}), 107 (13.4, C_8H_{11}), and 95 (16.4, C_7H_{11}) (Found: M^+, 478.242 27. $C_{30}H_{38}O_5$ requires *M*, 478.271 93).

Methylation of Compound (**11**).—23-Oxoisopristimerin III (0.020 g) was refluxed with dimethyl sulphate (0.02 ml) in the presence of anhydrous potassium carbonate (0.02 g) in anhydrous acetone (3 ml). The usual work-up after 6 h of reflux, followed by purification by p.l.c., afforded the dimethyl derivative (**12**) (0.018 g, 85%), m.p. 178—181 °C; $\nu_{max.}$(KBr) 1 722, 1 660, 1 582, 1 450, 1 380, 1 312, 1 245, 1 202, 1 152, 1 102, 1 087, 1 045, 960, 870, 820, and 762 cm^{-1}; δ(CCl_4) 10.43 (1 H, s, CHO), 7.15 (1 H, d, *J* 10 Hz, 6-H), 7.00 (1 H, s, 1-H), 6.48 (1 H, d, *J* 10 Hz, 7-H), 5.57 (1 H, dd, *J* 2 and 6 Hz, 11-H), 3.90 and 3.86 (3

H, each s, 2 × OMe), 3.50 (3 H, s, CO_2Me), and 1.15, 1.05, 1.01, 0.95, and 0.81 (each 3 H, each s, 5 × Me).

Isolation of Demethylzeylasteral (**5**).—The ethyl acetate extract (4 g) of outer stem bark was chromatographed over a column of silica gel made up in chloroform and was eluted with chloroform containing increasing amounts of methanol. The fraction eluted with 3% methanol in chloroform was further purified by p.l.c. [eluant methanol–chloroform–benzene (5:94:1)] to yield *compound* (**5**) as a yellow solid (63 mg, 0.012%), m.p. 158—160 °C; $[\alpha]_D^{27}$ −67.9° (*c* 1.06 in $CHCl_3$); $\nu_{max.}$(KBr) 3 400—3 100, 2 925, 1 726, 1 705, 1 636, 1 575, 1 455, 1 285, 1 140, 870, and 740 cm^{-1}; for ^1H n.m.r. data see Table 2; *m/z* 480 (M^+, 8%), 465 (22), 437 (1), 280 (6), 265 (6), 204 (21), 162 (22), 149 (100), 115 (11), and 69 (32) (Found: M^+ 480.2475. $C_{29}H_{36}O_6$ requires *M*, 480.2414).

Methylation of Demethylzeylasteral.—A solution of demethylzeylasteral (5 mg) in chloroform was stirred with Ag_2O (30 mg) and iodomethane (0.5 ml) for 2 h. After filtration, the reaction mixture was evaporated and the product, on crystallisation from acetone, afforded dimethylzeylasteral (**9**), m.p. 200—202 °C, identical with the sample obtained above.

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