Studies on Terpenoids and Steroids. Part 3.[1] Structure and Synthesis of a New Phenolic D:A-*friedo*-24-Noroleanane Triterpenoid, Zeylasterone, from *Kokoona zeylanica*[2]

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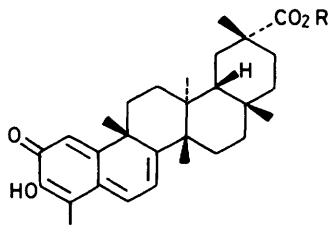
Zeylasterone, the first of a novel series of natural phenolic nortriterpenes from *Kokoona zeylanica* (Celastraceae) and 'kokum soap' has been shown to be 29-methyl hydrogen 2,3-dihydroxy-6-oxo-D:A-*friedo*-24-noroleana-1,3,5(10),7-tetraene-23,29-dioate (3) on the basis of spectroscopic and chemical evidence. Trimethylzeylasterone (8) has been synthesized from pristimerin, a quinone methide present in *K. zeylanica* and 'kokum soap'. The biosynthetic importance of some triterpenoids of *K. zeylanica* is discussed.

Kokoona zeylanica Thwaites (Celastraceae) is a tree having restricted distribution in Sri Lanka and South India. It has an outer stem bark with a brilliant yellow colour. This yellow bark is powdered and used in native medicine as a snuff to relieve headaches.[3] A paste prepared by mixing powdered yellow bark with water is dried and formed into flat pieces to produce 'kokum soap,' used by the villagers in Sri Lanka in place of toilet soap.[3,4] These applications, along with the occurrence of antibiotic [5] and antitumour [6] quinone methide triterpenes in Celastraceae plants, prompted us to investigate the yellow bark of *K. zeylanica* and 'kokum soap' for such constituents. In this paper we report the occurrence of pristimerin (1) and the isolation and structure of the first natural phenolic D:A-*friedo*-24-noroleanane triterpene which we are naming zeylasterone. Synthesis of trimethylzeylasterone (8) starting from readily available pristimerin is also reported.

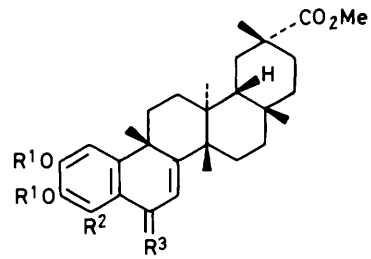
The light petroleum (b.p. 60—80 °C) extract of the outer bark of *K. zeylanica* was separated into neutral, phenolic, and acidic fractions (see Experimental section). The major compound isolated from the neutral fraction was identified as pristimerin (1). The acidic ($NaHCO_3$-soluble) fraction gave a yellow solid which, on chromatographic separation, afforded zeylasterone (3) as a colourless crystalline solid, m.p. 240—242 °C, $[\alpha]_D$ −75.4°. The light petroleum extract of 'kokum soap' when subjected to similar treatment afforded pristimerin and zeylasterone in addition to several unidentified minor constituents.

Zeylasterone analysed for $C_{30}H_{38}O_7$ and gave a positive response to both the Liebermann–Burchard test for triterpenes (orange; pristimerin gives the same colour) and the neutral iron(III) chloride test for phenols (green). I.r. bands at 3 502, 1 722, 1 707, and 1 642 cm^{-1} indicated the presence of chelated hydroxy, saturated ester carbonyl, α,β-unsaturated carboxylic acid carboxyl, and αβ-unsaturated ketone groups, respectively.

Methylation of zeylasterone with excess of diazomethane in diethyl ether afforded the trimethyl derivative, $C_{33}H_{44}O_7$, m.p. 229—230 °C; $[\alpha]_D$ −106.9°. The i.r. spectrum of this compound indicated the absence of absorptions due to hydroxy groups and the band due to CO_2H was shifted to the αβ-unsaturated ester carbonyl region. In the highfield region of the ^1H n.m.r. spectrum of zeylasterone five 3 H singlets were present and these were assigned to methyl groups by comparison with friedelin [7] and pristimerin (see Table 1). The ^1H n.m.r. spectrum of both zeylasterone (3) and trimethylzeylasterone (8) lacked a signal around δ 2.20 due to 4-CH_3 which is present in pristimerin (1). However, the 3 H singlet at δ 3.53 was present in both (3) and (8), and this was assigned to 20α-CO_2CH_3 as in pristimerin. In the lowfield region of the ^1H n.m.r. spectrum of zeylasterone three 1 H singlets (exchangeable with D_2O) were present and these were assigned to CO_2H and two OH groups [see (9)]. The u.v. spectra of zeylasterone (3) and trimethylzeylasterone (8) (Table 2) indicated the presence of the ArC(O)C=C chromophore, and the H_3BO_3–NaOAc-induced shift in the u.v. spectrum of (3) suggested that it contained an *ortho*-dihydroxy system.[8]



(1) R = Me
(2) R = H



(3) R^1 = H, R^2 = CO_2H, R^3 = O
(4) R^1 = Me, R^2 = CO_2H, R^3 = O
(5) R^1 = R^2 = Me, R^3 = O
(6) R^1 = H, R^2 = Me, R^3 = H_2
(7) R^1 = R^2 = Me, R^3 = H_2
(8) R^1 = Me, R^2 = CO_2Me, R^3 = O

The ^{13}C n.m.r. spectrum of zeylasterone (Table 3) provided additional evidence for the proposed structure (3). Assignment of signals in the aromatic region was based on the published data for acetophenone,[9] flavones,[9,10] and xanthones.[11] Signals for the alicyclic part of the moleule were assigned by comparison with our data for D:A-*friedo*-oleananes (friedelanes).[12] The c.d. curves of pristimerin and zeylasterone are presented in the Figure.

In its mass spectrum zeylasterone (3) showed a significant peak at *m/z* 466 due to the loss of CO_2 giving the ion (10). The base peak at *m/z* 229 was probably due to ion (12) which may be produced *via* the intermediate ions (11a) and (11b) (see Scheme 1). Trimethylzeylasterone (8) showed two base peaks at *m/z* 325 and 258, the former arising from either (11a) or (11c) by the cleavage of ring D giving rise to ions (13) or (14), and the latter, due to ion (15), arising from (11c) by the cleavage of ring C.

For further proof of structure it was necessary to compare

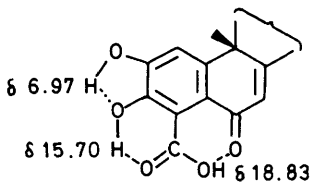
Table 1. ^{1}H N.m.r. data (δ; $CDCl_3$; 60 MHz) [a] of pristimerin (1), zeylasterone (3), dimethyl-6-oxopristimerol (5), and trimethylzeylasterone (8)

Compound	1-H	6-H	7-H	4-CH_3	9-CH_3	13-CH_3	14-CH_3	17-CH_3	20β-CH_3	4-CO_2CH_3	20α-CO_2CH_3	2,3-$(OCH_3)_2$
(1)	6.55	7.02 [c]	6.34 [c]	2.20	1.45	1.11	1.27	1.17	0.55	—	3.53	—
(3) [b]	6.50	—	7.33	—	1.60	1.11	1.32	1.17	0.55	—	3.53	—
(5)	6.17	—	6.92	2.70	1.60	1.12	1.32	1.18	0.60	—	3.55	3.78, 3.95
(8)	6.22	—	6.95	—	1.60	1.11	1.32	1.17	0.60	3.93	3.53	3.82, 3.93

[a] All singlets except for [c] where the signals were doublets (*J* 7 Hz). [b] For chemical shifts of CO_2H and OH protons, see structure (9).

Table 2. U.v. spectral data (EtOH) of zeylasterone (3), dimethyl-6-oxopristimerol (5), and trimethylzeylasterone (8)

Compound	λ_{max}/(log ε)				
(3)	211 (4.19)	226 (4.05)	255 (4.08)	295 (3.79)	340 (3.70)
(5)	210 (4.13)	225 (4.00)	247 (4.07)	285 (3.80)	300 (3.93)
(8)	207 (4.00)	225 (3.88)	245 (3.99)	287 (3.72)	312 (3.72)



(9)

zeylasterone (3) and/or trimethylzeylasterone (8) with a compound having similar structural features and for this purpose dimethyl-6-oxopristimerol (5) [13] was selected. The u.v., i.r., and ^{1}H n.m.r. spectra of zeylasterone (3) and trimethylzeylasterone (8) were comparable with those of synthetic dimethyl-6-oxopristimerol (5). A comparison of u.v. data is given in Table 2. The i.r. spectra of (8) and (5) were found to be superimposable except that in the former an additional band at 1 725 cm^{-1} was present and this was assigned to the aromatic ester carbonyl. Comparison of the ^{1}H n.m.r. spectra of zeylasterone (3) and trimethylzeylasterone (8) with that of dimethyl-6-oxopristimerol (5) aided the assignment of signals due to 1-H, 7-H, 20α-CO_2CH_3, and the methyl groups of the natural triterpene (see Table 1). Finally, trimethylzeylasterone (8) was synthesized from dimethyl-6-oxopristimerol (5) (see below). Trimethylzeylasterone (8), obtained by methylation of the natural sample, was shown to be identical with the above synthetic sample.

For the above comparisons, dimethyl-6-oxopristimerol (5) and trimethylzeylasterone (8) were synthesized starting from readily available pristimerin (1) by a sequence of transformations depicted in Scheme 2 (see Experimental section for details).

Biogenetic Aspects.—Two biosynthetic pathways have been proposed for the origin of natural triterpene quinone methides implicating polpunonic acid (17) as the precursor.[14,15] Conversion of friedelin into quinone methides would involve the oxidation of the A and B rings with concurrent demethylation at C-5. Co-occurrence of friedelin, 6β-hydroxy-D:A-*friedo*-oleanan-3-one [zeylanone (16)],[16] celastrol (2),[17] pristimerin (1), and zeylasterone (3) in *K. zeylanica* suggests a possible biosynthetic relationship between them as shown in Scheme 3. C-5 Demethylation *via* the intermediates proposed in our biosynthetic pathway is further supported by the recent isolation of salaspermic acid (18) from *Salacia macrosperma*, a plant belonging to the Celastraceae.[14,18]

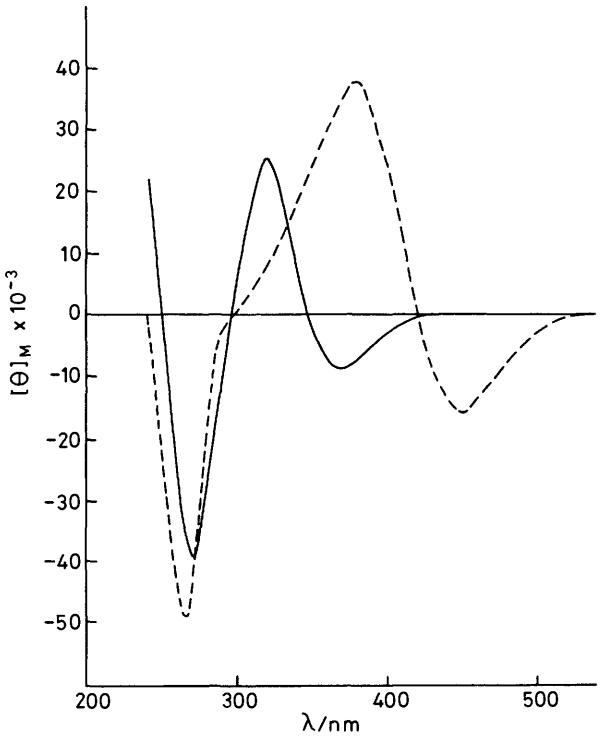
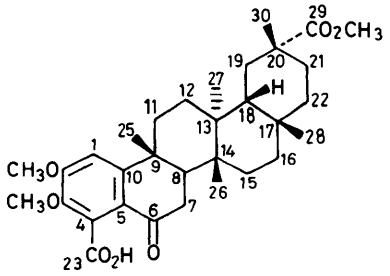


Figure. C.d. curves (in methanol) of — zeylasterone (3) and - - - - pristimerin (1)

Experimental

General Procedures.—M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra were recorded for KBr discs with a Perkin-Elmer model 257 grating i.r. spectrometer. U.v. spectra were recorded on a Unicam SP 8000 spectrophotometer. Optical rotations were measured at 27 °C in chloroform solution with a Perkin-Elmer 241 polarimeter. ^{1}H N.m.r. spectra were recorded in $CDCl_3$, unless otherwise stated, at 60 MHz with $SiMe_4$ as internal reference, on a Varian T60A spectrometer. The ^{13}C n.m.r. spectrum of zeylasterone (3) was recorded on a JEOL FX-100 spectrometer operating at 25.05 MHz. Mass spectra were obtained from the University of Aberdeen, Scotland. Microanalyses were performed by CSIRO Microanalytical Service, Melbourne. T.l.c. involved silica gel G; visualisation was by spraying with acidified anisaldehyde followed by charring in

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



Carbon	Chemical shift (multiplicity)	Carbon	Chemical shift (multiplicity)	Carbon	Chemical shift (multiplicity)
1	113.8 (d)	11	36.2 (t)	21	29.8 (t)
2	173.7 (s)	12	28.6 (t)	22	34.4 (t)
3	155.5 (s)	13	39.7 (s)	23	178.7 (s)
4	111.3 (s)	14	40.5 (s)	25	36.8 (q)
5	119.4 (s)	15	30.9 (t)	26	20.2 (q)
6	188.0 (s)	16	34.8(t)	27	32.7 (q)
7	124.4 (d)	17	43.0 (s)	28	18.3 (q)
8	153.4 (s)	18	44.2 (d)	29	179.8 (s)
9	45.6 (s)	19	29.8 (t)	30	31.6 (q)
10	152.8 (s)	20	30.5 (s)	OCH_3	51.6 (q)

heat. Preparative t.l.c. (p.l.c.) used 1.0 mm layers of silica gel G. Column chromatography involved silica gel (30—70 mesh). Light petroleum had b.p. 60—80 °C. Identity of compounds was established by co-t.l.c., mixed m.p., i.r., and ^1H n.m.r. comparisons.

Extraction of K. zeylanica *Outer Stem Bark.*—The dried and powdered outer stem bark (500 g) of *K. zeylanica* collected from Kanneliya rain forest, Sri Lanka, was exhaustively extracted with hot light petroleum. 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.

Isolation of Pristimerin (1).—The above neutral fraction (6.0 g) was chromatographed over a column of silica gel made up in light petroleum. Elution with 5% ethyl acetate in light petroleum afforded pristimerin (1) (0.903 g, 0.8%) as an orange-yellow crystalline solid, m.p. 215—217 °C (lit.,[19] 214—217 °C); $[\alpha]_D$ −168.0° (*c* 1.00); *m/z* 464 (M^+); $\nu_{max.}$ 3 370, 1 723, and 1 593 cm^{-1}; for ^1H n.m.r. data, see Table 2. This compound was shown to be identical with an authentic sample of pristimerin.

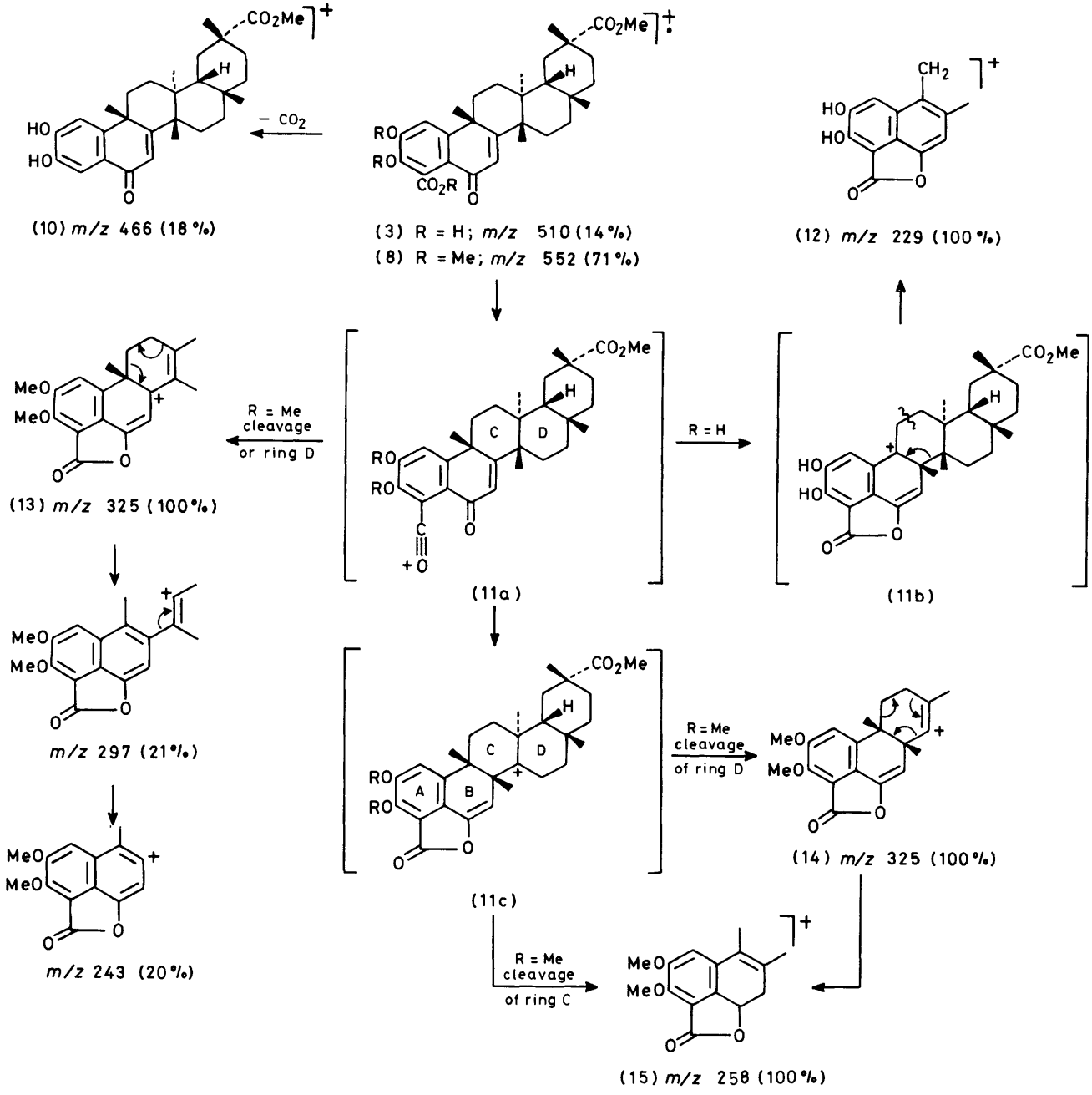
Isolation of Zeylasterone (3).—The acidic fraction (0.5 g) obtained above was chromatographed over a column of silica gel made up in chloroform. Elution with 0.5% acetic acid in chloroform under pressure gave *zeylasterone* (3) (0.22 g, 0.41%) as crystals, m.p. 240—242 °C (from aqueous methanol); $[\alpha]_D$ −75.4 ° (*c*, 2.03) (Found: C, 70.4; H, 7.1. $C_{30}H_{38}O_7$ requires C, 70.56; H, 7.50%); $\nu_{max.}$ 3 502, 3 300—3 100, 2 442, 1 722, 1 707, 1 642, 1 592, 1 463, 1 383, 1 313, 1 300, 1 272, 1 230, 1 205, 1 180, 1 162, 1 100, 1 082, 1 060, 1 045, 1 035, 1 018, 995, 945, 918, 883, 870, 810, 773, 753, 723, 703, 685 and 643 cm^{-1}; for ^1H n.m.r., u.v., and ^{13}C n.m.r. data, see Tables 1, 2, and 3, respectively; *m/z* 510 (M^+, 14%), 446 (18), 281 (13), 267 (16), 255 (23), 243 (40), 230 (52), 229 (100), 228 (11), 217 (16), 215 (16), 189 (32), 187 (10), 147 (32), 135 (17), 121 (38), 119 (15), 110 (34), 108 (34), 95(51), 83 (31), 81 (27), 79 (13), and 55 (37).

Isolation of Pristimerin (1) *and Zeylasterone* (3) *from* ' *Kokum Soap* '.—Coarsely ground ' kokum soap ' cake (65 g) purchased from Gilimale, Sri Lanka, was extracted with hot light petroleum, and the total light petroleum extract (28.5 g) was separated into neutral (15 g), acidic (1.05 g), and phenolic (10.8 g) fractions. Separation of the neutral fraction as above afforded pristimerin (0.9 g, 1.38%), identical with an authentic sample. The acidic fraction on combined column chromatography and p.l.c. gave zeylasterone (0.665 g, 1.02%), identical with the sample obtained from *K. zeylanica* outer stem bark.

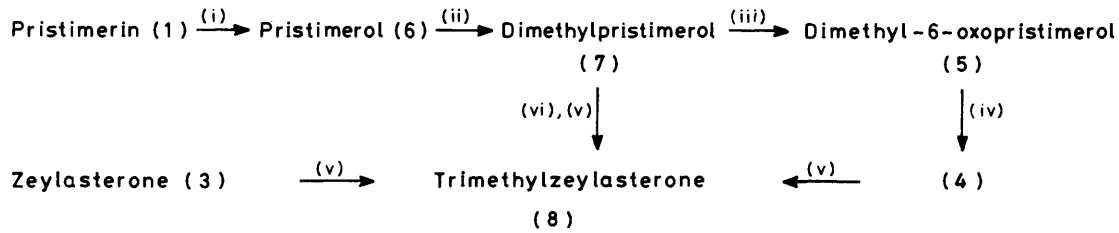
Trimethylzeylasterone (8).—Methylation of zeylasterone with excess of ethereal diazomethane afforded a quantitative yield of *trimethylzeylasterone* (8) as a crystalline solid, m.p. 229—230 °C (from chloroform–light petroleum); $[\alpha]_D$ −106.9° (*c*, 1.02); $\nu_{max.}$ 2 930, 1 735, 1 725, 1 655, 1 650, 1 585, 1 480, 1 460, 1 450, 1 420, 1 345, 1 310, 1 285, 1 260, 1 232, 1 203, 1 155, 1 145, 1 095, 1 065, 1 025, 990, 960, 885, 870, 860, 795, 767, and 710 cm^{-1}; for ^1H n.m.r. and u.v. data, see Tables 1 and 2, respectively; *m/z* 552 (M^+, 71%), 537 (32), 520 (14), 505 (10), 493 (9), 477 (5), 465 (5), 461 (4), 337 (5), 325 (100), 309 (10), 297 (21), 295 (11), 283 (14), 271 (29), 258 (100), 243 (20), 203 (21), 184 (8), 163 (10), 161 (7), 147 (8), 133 (5), 121 (18), 108 (16), 106 (15), 95 (21), 93 (11), 91 (8), 81 (14), 78 (7), 68 (9), 66 (9), 53 (17), and 41 (15).

Synthesis of Dimethyl-6-oxopristimerol (5) *and Trimethylzeylasterone* (8).—(a) *Reduction of pristimerin* (1) *to pristimerol* (6).[20] Pristimerin (1) (500 mg) was dissolved in methanol (75 ml) and sodium borohydride was added portionwise until the yellow colour disappeared. The solvent was evaporated under reduced pressure and the residue partitioned between diethyl ether and water. The ether phase was washed with water, dried, and evaporated to yield crude pristimerol (6).

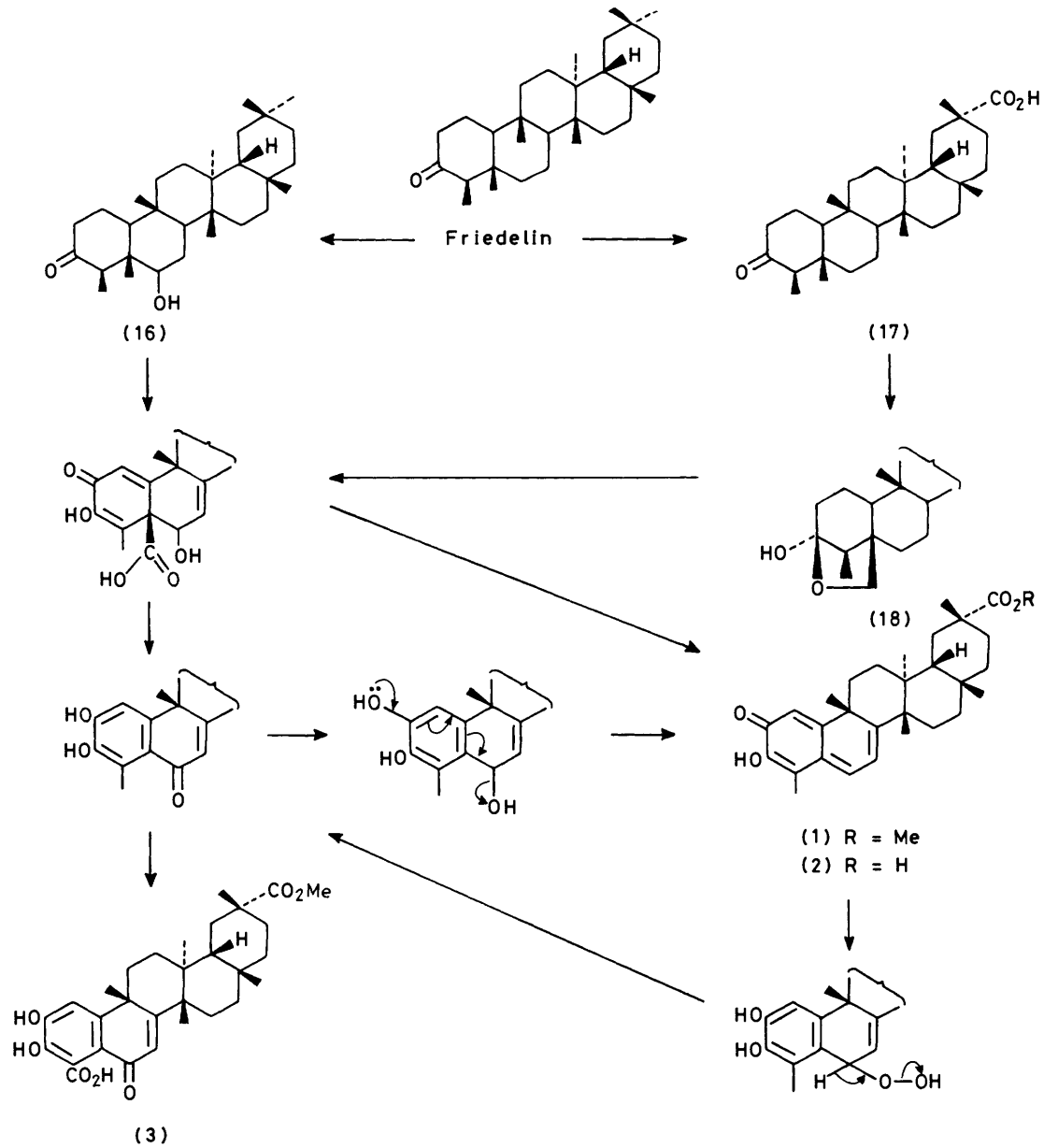
(b) *Methylation of* (6). A solution of the crude pristimerol in anhydrous acetone (15 ml) was refluxed overnight with dimethyl sulphate (2 ml) and anhydrous potassium carbonate (500 mg). The usual work-up, purification by p.l.c., and recrystallisation from acetone afforded dimethylpristimerol (7) (330 mg, 66%), m.p. 210—212 °C (lit.,[13] 214—215 °C); $[\alpha]_D$ −15.4° (*c*, 0.9).



Scheme 1.



Scheme 2. *Reagents and conditions*: (i) $NaBH_4$, CH_3OH, 25 °C; (ii) Me_2SO_4, K_2CO_3, acetone, reflux, overnight; (iii) NBS, $CaCO_3$, dioxane–H_2O, *hν* (W filament), 3 h; (iv) NBS, $(PhCO_2)O_2$, $CaCO_3$, dioxane–H_2O, *hν* (i.r.), 6 h; (v) CH_2N_2, diethyl ether; (vi) NBS, $(PhCO)_2O_2$, $CaCO_3$, dioxane–H_2O, *hν* (i.r.), 7 h



Scheme 3. Possible biosynthetic routes to pristimerin (1) and zeylasterone (3)

(c) *Oxidation of* (7) *to dimethyl-6-oxopristimerol* (5).[21] A stirred suspension of dimethylpristimerol (150 mg) and calcium carbonate (150 mg) in dioxane (18 ml) containing water (4.5 ml) was irradiated at room temperature with a 60 W tungsten filament lamp. *N*-Bromosuccinimide (NBS) (150 g) was added all at once and the mixture was irradiated and stirred for a further 3 h (t.l.c. control), after which the reaction mixture was filtered into water (60 ml) and extracted with diethyl ether, and the extract was dried and evaporated. Purification by p.l.c. gave pure dimethyl-6-oxopristimerol (5) (60 mg, 40%), m.p. 211—213 °C (from chloroform–light petroleum) (lit.,[13] 217—218 °C); $[\alpha]_D$ −79.4° (*c*, 1.07).

(d) *Conversion of* (5) *into trimethylzeylasterone* (8). To a stirred suspension of dimethyl-6-oxopristimerol (20 mg) and calcium carbonate (20 mg) in dioxane (5 ml) and water (0.8 ml) were added a crystal of dibenzoyl peroxide and NBS (20 mg). The mixture was irradiated with an i.r. lamp for 6 h (t.l.c. control). The reaction mixture was filtered into water (10 ml) and extracted with diethyl ether. The extract was washed twice with 10% aqueous sodium hydrogencarbonate. The combined sodium hydrogencarbonate washings were acidified and extracted with diethyl ether, and the extract was dried and evaporated to obtain the crude acidic fraction (12 mg) containing (4). Methylation of this with diazomethane in diethyl ether and subsequent purification by p.l.c. afforded trimethylzeylasterone (8) (7 mg, 35%), indistinguishable (m.p., mixed m.p., $[\alpha]_D$, i.r., and co-t.l.c.) from the sample obtained by methylation of zeylasterone (see above).

(e) *Direct oxidation of dimethylpristimerol* (7) *to trimethylzeylasterone* (8). To a stirred suspension of dimethylpristimerol (20 mg) and calcium carbonate (20 mg) in dioxane (5 ml) and water (0.8 ml) were added a crystal of dibenzoyl peroxide and NBS (30 mg). This mixture was irradiated with an i.r. lamp for 7 h (t.l.c. control). The reaction mixture was filtered into water (15 ml) and extracted with diethyl ether. The extract was washed twice with 10% aqueous sodium hydrogencarbonate,

and the combined washings were acidified with 2M HCl and extracted with diethyl ether. The extract was dried and evaporated to obtain the crude acid fraction (10 mg). This was methylated with excess of ethereal diazomethane and subsequent purification by p.l.c. yielded trimethylzeylasterone (8) (6 mg, 30%), identical with the sample obtained above.

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