Studies on Terpenoids and Steroids. Part 19.[1] Structures of Three Novel 19(10→9)*abeo*-8α,9β,10α-Euphane Triterpenoids from *Reissantia indica* (Celastraceae)

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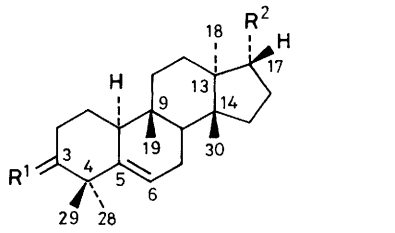
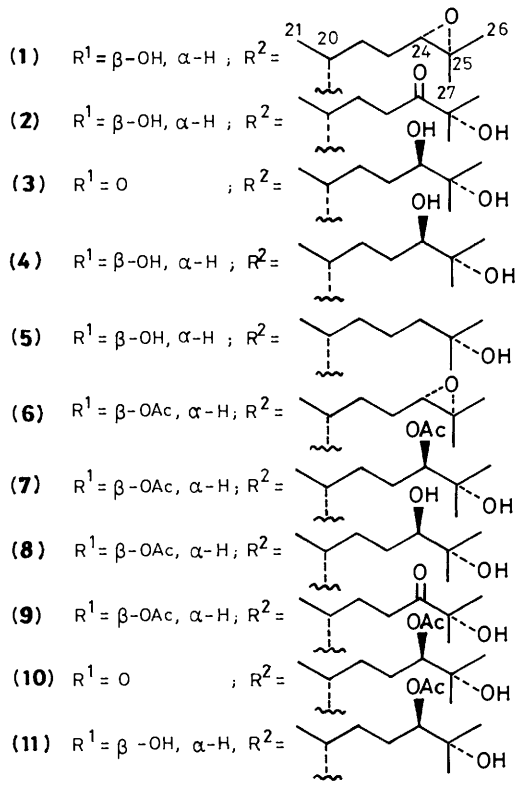
The three novel 19(10→9)*abeo*-8α,9β,10α-euphane (reissantane) triterpenoids isolated from *Reissantia indica* (Celastraceae) and related to reissantioloxide (reissantenol oxide) (**1**) have been shown to be 24-oxoreissant-5-ene-3β,25-diol (**2**), 3-oxoreissant-5-ene-24β,25-diol (**3**), and reissant-5-ene-3β,24β,25-triol (**4**). A possible biosynthetic relationship for reissantane, D:B-*friedo*-oleanane, D:A-*friedo*-oleanane, and quinone-methide triterpenoids which occur in *R. indica* is presented.

Recently we reported the *X*-ray structure of reissantiol oxide which we now rename as reissantenol oxide (**1**), the first of a series of 19(10→9)*abeo*-8α,9β,10α-euphane triterpenoids from *Reissantia indica* (Hallee) Ding Hou. [= *Hippocratea indica* Willd. = *Pristimeria indica* (Willd.) A. C. Sm.].[2] In our continuing studies on triterpenoids and steroids of Sri Lankan plants[1] we have investigated the root bark of *R. indica* and in this paper we report the details of the isolation and structure elucidation of reissantenol oxide (**1**), three further new 19(10→9)*abeo*-euphane triterpenoids along with sitosterol, 25(10→9)*abeo*-26(8→15)*abeo*-9β,14β,15α-olean-5-ene-3β,19-diol (**12**), canophyllol (**13**), pristimerin (**14**), and tingenone (**15**). Previous studies of *R.* indica had revealed the presence of only pristimerin in the root bark.[3]

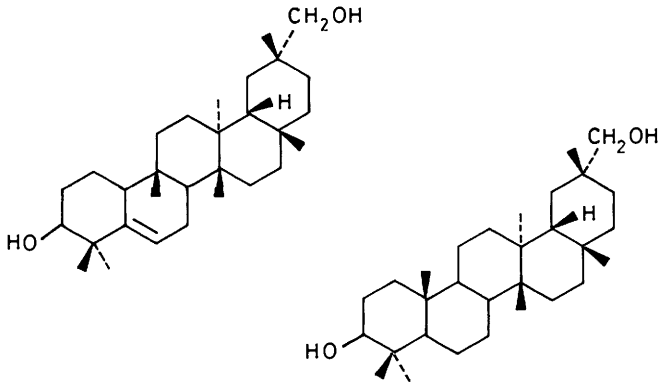
Results and Discussion

The hot hexane extract of the root bark of *R. indica* was treated with methanol to remove insoluble gutta-percha. The methanol-soluble portion was separated into acidic, phenolic, and neutral fractions by the usual procedure. The neutral fraction contained a number of triterpenoids. Four of these belonged to a new series of triterpenoids with an 19(10→9)*abeo*-euphane structure. The major triterpenoid had an epoxide system, a hydroxy group, and a double bond and was named reissantenol oxide. The names of the other three were based on reissantane [19(10→9)*abeo*-8α,9β,10α-euphane] structure. They were identified as 24-oxoreissant-5-ene-3β,25-diol (**2**), 3-oxoreissant-5-ene-24β,25-diol (**3**), and reissant-5-ene-3β,24β,25-triol (**4**) as described below. Canophyllol (**13**), pristimerin (**14**), and tingenone (**15**) were identified by direct comparison with authentic samples. Some physical and spectroscopic data of yet another triterpene were identical with those reported for 25(10→9)*abeo*-26(8→15)*abeo*-9β,14β,15α-olean-5-ene-3β,29-diol [3β,29-dihydroxy-D:B-*friedo*-olean-5-ene (**12**)] previously encounted in *Elaeoldendron balae* [=*Cassine balae*].[4] However, some ^{1}H n.m.r. chemical shifts of the methyl protons of (**12**) observed by us were found to be different from those reported by the earlier workers[4] (see Experimental section).





Reissantenol oxide (**1**), m.p. 175—177 °C, $[\alpha]_D$ +34.4°, had an analysis consistent with its formulation as $C_{30}H_{50}O_2$ and gave a positive response to the Liebermann–Burchard test for triterpenes. I.r. bands at 3 475 and 970 cm^{-1} indicated the presence of hydroxy and epoxide functions, respectively. Reissantenol oxide on acetylation afforded a crystalline monoacetyl derivative (**6**), $C_{32}H_{52}O_3$, m.p. 102—105 °C, $[\alpha]_D$ + 22.0°. With $LiAlH_4$ the epoxide ring was reduced giving the diol (**5**), $C_{30}H_{52}O_2$, as a solid which resisted crystallization. The ^{1}H n.m.r. spectra of reissantenol oxide and its acetate (**6**) (Table 1) indicated the presence of seven tertiary methyl groups and one secondary methyl group. The ^{1}H n.m.r. spectrum of the diol (**5**) was similar to that of (**1**) except that the signal at δ 2.29 due to CH of the epoxide ring was absent and that the reduction of the



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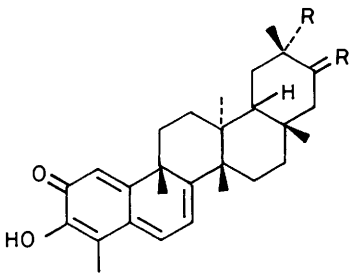


(14) R^1 = CO_2Me ; R^2 = H_2

(15) R^1 = H ; R^2 = O

epoxide ring has resulted in a tertiary alcohol. The foregoing suggested the terminal nature of the epoxide ring in a side chain pointing to a tetracyclic triterpenoid structure for reissantenol oxide. This was further confirmed by the presence of two lowfield methyl singlets (δ 1.31 and 1.27) in the ^{1}H n.m.r. spectrum comparable with C-25 gem dimethyl signals of aglaiol (δ 1.30 and 1.27), a dammarane epoxide of *Aglaia odorata* (family—Meliaceae).[5]

In its mass spectrum (Table 3), reissantenol oxide showed significant peaks at *m/z* 152 and 290 due a retro Diels–Alder cleavage of ring B, typical of triterpenoids and steroids with 5(6)-unsaturation.[6,7] This further indicated the absence of a methyl group at C-10 suggesting a cucurbitane skeleton and ruling out the presence of a dammarane, euphane, lanostane, or protostane skeleton in reissantenol oxide. However, certain differences observed for ^{1}H n.m.r. chemical shifts of the tertiary methyl groups in (**1**) compared with 10α-cucurbita-5,24-diene-3β-diol (anhydrolitsomentol)[8] indicated that reissantenol oxide had a new skeleton and/or stereochemistry. The molecule was, therefore, examined by single-crystal *X*-ray crystallography which suggested it to have a hitherto unknown 19(10→9)*abeo*-8α,9β,10α-euphane (8,5-*friedo*-tirucallane) skeleton with the functional groups and stereochemistry as indicated in (**1**). It is interesting that the corresponding rearranged lanostanes (cucurbitanes) have been known for so long whereas the euphane and tirucallane equivalents have remained hidden, until now.

With the crystal structure in hand, we attempted to interpret the ^{1}H and ^{13}C n.m.r. spectra of reissantenol oxide (**1**). The ^{1}H n.m.r. spectrum of reissantenol oxide in $CDCl_3$ showed the presence of a 1 H broad doublet (*J* 6.1 Hz) at δ 5.64 due to 6-H, a 1 H double triplet (*J* 6.9, 3.0 Hz) at δ 3.47 due to 3-H, and a 1H triplet (*J* 6.1 Hz) at δ 2.69 due to 24-H, in addition to the signals due to seven tertiary methyl and one secondary methyl groups. These methyl groups were assigned by comparison with those of aglaiol[5] and anhydrolitsomentol[8] and were confirmed by pyridine induced shifts[9] (Table 1). The most affected are those methyl groups (4β-CH_3, 4α-CH_3, and 9β-CH_3) in the vicinity of the 3β-hydroxy group and the 5(6)-double bond.

Preliminary data for the ^{13}C n.m.r. assignment of reissantenol oxide were obtained at 50.28 MHz from a proton noise decoupled spectrum that provided the chemical shifts, and from an off-resonance decoupled spectrum that provided the multiplicity of each signal. The completely decoupled spectrum showed 30 signals between 15 and 143 p.p.m. which were found to consist of 6 singlets, 7 doublets, 9 triplets, and 8 quartets by the off-resonance decoupled spectrum. The ^{13}C n.m.r. spectrum was analysed by means of ^{1}H-^{13}C shift correlation spectroscopy (see Figure). The ^{13}C n.m.r. signals in the olefinic region were assigned to C-5 (142.2 p.p.m.) and C-6 (121.6 p.p.m.) and the three carbons bearing oxygen atoms were assigned as C-3 (76.3 p.p.m.), C-24 (64.0 p.p.m.), and C-25 (59.7 p.p.m.). The rest of the heterocosy spectrum was useful only in assigning the methyl carbons as the proton signals of these have been unambiguously assigned (see above). These assignments are given in Table 2. The remaining methine and methylene carbons were tentatively assigned by comparison with the ^{13}C chemical shift data for cholesterol[10] and (20*S*,22*R*)-20,22-epoxycholest-5-en-3β-ol.[11]

24-Oxoreissant-5-ene-3β,25-diol (**2**), obtained as a colourless crystalline solid, m.p. 129—130 °C, $[\alpha]_D$ +34.8°, had an analysis consistent with its formulation as $C_{30}H_{50}O_3$ and gave a positive response to the Liebermann-Buchard test for triterpenes. The presence of hydroxy (3 450 cm^{-1}) and keto (1 702 cm^{-1}) groups were evident from its i.r. spectrum. With Ac_2O–pyridine it gave a monoacetate (**9**), $[\alpha]_D$ +36.°, the i.r. spectrum of which indicated the presence of a free hydroxy group (3 425 cm^{-1}) suggesting its tertiary nature. The mass spectrum (m.s.) of the natural product had fragments (b), (c), (d), and (e) (Table 3) in common with reissantenol oxide (**1**); but the difference of 16 m.u. of fragment (a) and R^2 (side chain) compared with (**1**) suggested that the two oxygen atoms are present in the side chain, probably in a carbonyl and a hydroxy group. The latter group was located at C-25 due to its tertiary nature (see above). The ^{1}H n.m.r. spectra of (**2**) in [$^2H_5$]pyridine had a sharp 1 H singlet at δ 6.74 which was assigned to a chelated OH. The presence of two triple doublets (ddd) of 1 H each at δ 2.55 (*J* 16.6, 9.5, and 5.4 Hz) and 2.51 (*J* 16.6, 8.1, and 5.1 Hz) in the ^{1}H n.m.r. spectrum in $CDCl_3$ which collapsed to a multiplet at δ 2.99 in [$^2H_5$]pyridine indicated the presence of a CH_2CH_2CO moiety in (**2**). The foregoing evidence helped to locate the carbonyl group at the biogenetically favourable C-24 (see later).

The ^{1}H n.m.r. spectrum of (**2**) further indicated the presence of seven tertiary methyl groups and one secondary methyl group (Table 1). In addition, it also showed signals due to 3α-H and olefinic 6-H. The appearance of the signals due to two methyl groups at C-25 in (**2**) at a lower field (δ 1.39) compared with those of (**1**) (δ 1.27 and 1.31) may be explained as due to the presence of the carbonyl group at C-24 in (**2**). The ^{13}C n.m.r. spectrum of 24-oxoreissant-5-ene-3β,25-diol (**2**) was interpreted by comparison with that of reissantenol oxide (**1**). The significant differences observed were for C-23, C-24, C-25, and C-26 which could be explained as due to the presence of oxo and hydroxy functions at C-24 and C-25, respectively (Table 2). The structure (**2**) proposed for the natural product was further confirmed by preparation of its monoacetate (**9**) from reissantenol oxide monoacetate (**6**) by the treatment with Me_2SO–CF_3CO_2H followed by pyridine[12] (see Scheme 1 and Experimental section).

The spectral data (i.r. and ^{1}H n.m.r.) of the next polar minor

Table 1. ^{1}H N.m.r. data [δ($CDCl_3$; 400 MHz)][a] of the triterpenoids (**1**)—(**4**) and their derivatives (**5**)—(**11**)

Proton	(1)	(2)[b]	(3)	(4)	(5)[c]	(6)[c]	(7)	(8)[c,d]	(9)[c]	(10)	(11)
3α-H	3.47 dt (7.3) [3.34 dt (4, 3)]	3.47 m ($W_{\frac{1}{2}}$, 12.5) [3.73 dt (4, 3)]		3.47 m ($W_{\frac{1}{2}}$, 12)	3.50 m ($W_{\frac{1}{2}}$, 12)	4.71 m	4.77dd (10.7, 2.4)	4.67 m	4.73 m		4.77dd (10, 2)
6-H	5.64 d (6.1) [5.78 d (5.5)]	5.63 d (5.8) [5.76 d (6.0)]	5.69 d (6) [5.69 d (6)]	5.63 d (5.4)	5.66 d (6)	5.66 d (6)	5.56 d (5.4)	5.57 d (6)	5.58 d (6)	5.69 d (6)	5.65 d (6)
4α-CH_3 (28-H)	1.06 s [1.15 s]	1.06 s [1.14 s]	1.17 s [1.55 s]	1.05 s	1.06 s	1.05 s	1.05 s	1.10 s	1.08 s	1.23 s	1.15 s
4β-CH_3 (29-H)	1.15 s [1.42 s]	1.14 s [1.41 s]	1.23s [1.53 s]	1.14 s	1.16 s	1.08 s	1.08 s	1.13 s	1.25 s	1.24 s	1.26 s
9β-CH_3 (19-H)	0.88 s [0.97 s]	0.88 s [0.93 s]	0.88 s [0.82 s]	0.88 s	0.90 s	0.90 s	0.88 s	1.07 s	1.05 s	0.87 s	1.06 s
13α-CH_3 (18-H)	0.84 s [0.86 s]	0.84 s [0.87 s]	0.86 s [0.82 s]	0.84 s	0.87 s	0.83 s	0.83 s	0.90 s	0.88 s	0.83 s	0.87 s
14β-CH_3 (30-H)	0.83 s [0.84 s]	0.84 s [0.82 s]	0.81 s [0.72 s]	0.83 s	0.83 s	0.83 s	0.81 s	0.83 s	0.85 s	0.81 s	0.80 s
20-CH_3 (21-H)	0.87 d (6.6) [0.87 d (6)]	0.85 d (6.5) [0.86 d (6.5)]	0.87 d (5) [0.96 d (6)]	0.87 d (5.5)	0.87 d (6)	0.87 d (6)	0.86 d (6.4)	0.87 d (6)	0.86 d (6)	0.86 d (6.4)	0.87 d (6)
24-H	2.96 t (6) [2.77 dd (7, 5.5)]		3.29 m ($W_{\frac{1}{2}}$, 12.5) [3.72 m]	3.29 m ($W_{\frac{1}{2}}$, 16)		2.70 m	4.70 t (2.7)	3.17 m		4.75 dd (10.7, 2.4)	3.49 m
25-$(CH_3)_2$ (26-, 27-H)	1.27, 1.31 s [1.30, 1.30 s]	1.39, 1.39 s [1.56, 1.57 s]	1.23, 1.24 s [1.29, 1.31 s]	1.17, 1.22 s	1.23, 1.23 s	1.26, 1.30 s	1.21, 1.21 s	1.27, 1.27 s	1.38, 1.38 s	1.21, 1.21 s	1.21, 1.21 s
OH	1.30 d (6.9) [5.48 d (4)]	3.80 s [5.47 d (4), 6.74s]						2.60br s	3.43br s	5.30 s	
$OCOCH_3$						2.00 s	2.02, 2.11 s	1.97 s	2.00 s	2.11 s	2.12 s

[a] $J_{H,H}$/Hz in ( ); δ values in [ ] are for [$^2H_5$]pyridine solutions. [b] Other signals; 2-H, 2.55 ddd (*J* 16.6, 9.5, 5.4), 2.51 ddd (*J* 16.6, 8.1, 5.1). [c] Recorded at 60 MHz. [d] In CCl_4 solution.

Table 2. ^{13}C N.m.r. chemical shifts [δ($CDCl_3$; 50.28 MHz)] of *Reissantia* triterpenoids (**1**), (**2**), and the acetates (**7**) and (**10**)

Carbon	(1)[a]	(2)	(7)	(10)	Carbon	(1)[a]	(2)	(7)	(10)
1	34.2 t	34.1 t	34.1 t	33.9 t	16	28.0 t	28.1 t	28.0 t	28.4 t
2	30.5 t	30.2 t	30.4 t	38.0 t	17	50.2 t	49.9 d	49.6[d] d	50.1 d
3	76.3 d	76.3 d	78.5 d	215.4 s	18	16.4 q	16.4 q	16.2 q	15.9 q
4	40.9 s	40.9 s	39.2 s	50.3 s	19	24.9 q	26.6 q	26.7[c] q	26.7 q
5	142.2 s	142.2 s	142.4 s	142.9 s	20	44.7 d	44.6 d	44.5 d	44.1 d
6	121.6 d	121.7 d	119.8 d	121.1 d	21	18.9[b] q	18.9[b] q	19.1[b] q	21.0 q
7	29.0 t	28.9 t	29.0 t	30.3 t	22	32.1 t	32.2 t	31.6 t	31.6 t
8	35.2 d	34.8 d	35.0 d	35.3 d	23	25.8 t	29.1 t	29.0 t	22.0 t
9	46.2 s	46.1 s	46.0 s	46.1 s	24	64.0 d	214.9 s	80.7 d	80.6 d
10	49.6 d	49.5 d	49.7[d] d	49.6 d	25	57.9 s	76.2 s	72.4 s	72.4 s
11	25.3 t	25.2 t	25.4 t	25.0 t	26	24.9 q	26.6 q	25.0[c] q	25.0 q
12	35.7 t	35.7 t	35.6 t	35.6 t	27	18.7[b] q	18.7[b] q	18.7 q	18.5 q
13	35.2 s	35.1 s	35.0 s	35.1 s	28	25.5 q	25.5 q	26.0[c] q	24.4 q
14	47.5 s	47.4 s	47.4 s	47.4 s	29	19.0 q	18.9 q	19.4[b] q	19.1 q
15	27.9 t	27.9 t	28.0 t	28.0 t	30	15.5 q	15.3 q	15.4 q	15.4 q
					$OCOCH_3$			171.2, 170.8 s	171.2 s
					$OCOCH_3$			21.1, 20.9 q	21.0 q

[a] Assignments are based on ^1H–^{13}C shift correlated spectra (see Figure). [b–d] May be interchanged in any column.

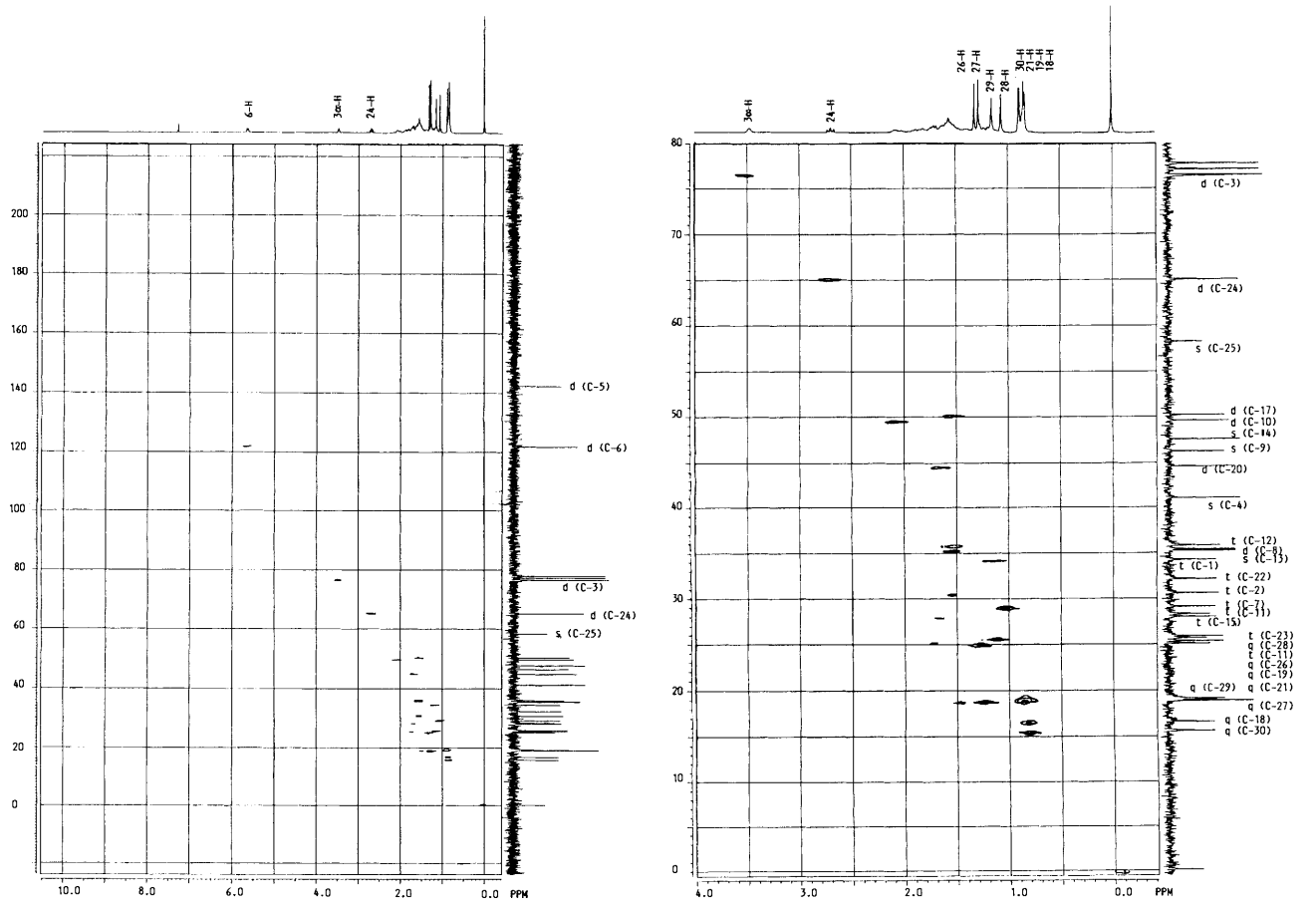
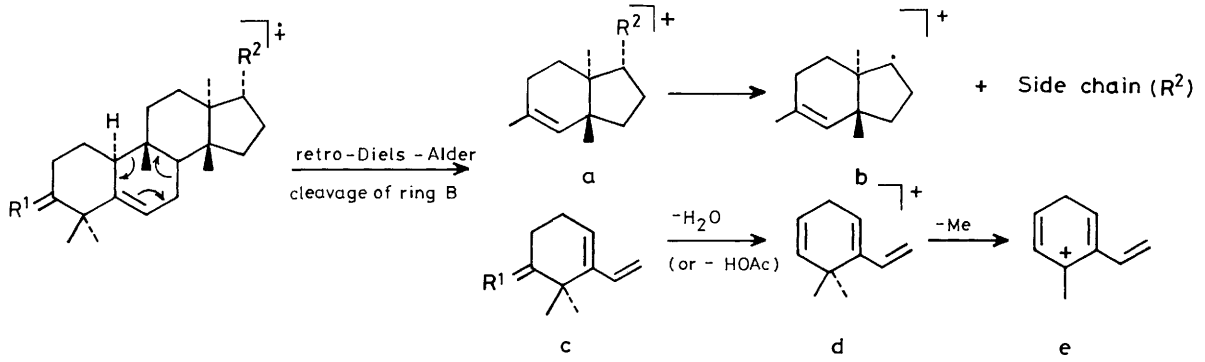


Figure 1. Contour plots of the long-range ^1H-^{13}C cosy spectra (entire and highfield region) of reissantenol oxide (**1**) [The ^1H shifts are shown on abscissa and the ^{13}C shifts on the ordinate. The multiplicities of carbon signals were determined by means of off-resonance and are indicated as s (singlet), d (doublet), t (triplet), and q (quartet)]

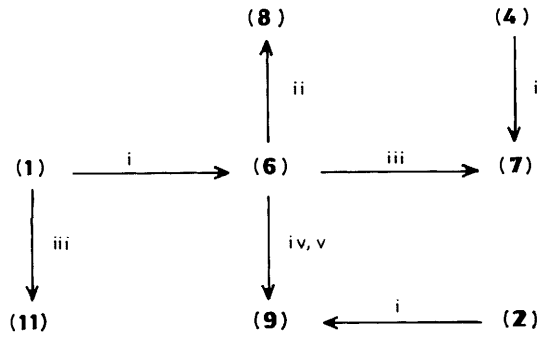
triterpene obtained as a non-crystalline solid, $C_{30}H_{50}O_3$, suggested it to be a keto-diol whose structure was elucidated as 3-oxoreissant-5-ene-24β,25-diol (**3**) from the following evidence. The presence of significant mass spectral fragments at *m/z* 308 and 150 due to retro-Diels–Alder cleavage of the molecule indicated that in contrast to (**2**) the keto group in this triterpenoid is located in ring A and the m.s. fragment at *m/z* 177 suggested the presence of both OH groups in the side chain, probably at biogenetically favourable C-24 and C-25 (Table 3). The ^1H n.m.r. spectrum of the natural triterpene in $CDCl_3$ lacked the signal at δ 3.47 (due to 3α-H) but a new 1 H multiplet was present at δ 3.29. The ^1H n.m.r. spectrum also indicated the

Table 3. Mass spectral data of triterpenoids (**1**)—(**4**) and their derivatives (**5**) and (**7**)—(**11**)



	M^+ (%)	$M^+ - H_2O$ (%)	$M^+ - Me - H_2O$ (%)	a (%)	a − Me (%)	a − H_2O (%)	a − Me − H_2O (%)	b(%)	c (%)	c − Me (%)	d (%)	e (%)	R^2 (%)
(**1**)	442 (14)	424 (9)	409 (13)	290 (71)	275 (42)	272 (19)	257 (33)	163 (95)	152 (61)	137 (30)	134 (100)	119 (62)	159 (25)
(**2**)	458 (13)	440 (3)	425 (4)	306 (100)	291 (35)	288 (6)	273 (6)	163 (90)	152 (53)	137 (28)	134 (80)	119 (51)	175 (20)
(**3**)	458 (8)	440 (6)	425 (10)	308 (38)	293 (7)	290 (23)	275 (16)	163 (74)	150 (27)	135 (27)			177 (11)
(**4**)	460 (14)	442 (4)	427 (5)	308 (71)	293 (12)	290 (39)	275 (26)	163 (100)	152 (82)	137 (30)	134 (94)	119 (51)	178 (8)
(**5**)	444 (17)	426 (15)	411 (24)	292 (84)	277 (34)	274 (93)	259 (83)	163 (96)	152 (74)	137 (84)	134 (100)	119 (86)	161 (76)
(**7**)	544 (54)	526 (12)	511 (15)	350 (85)	335 (15)	332 (33)	317 (12)	163 (100)			134 (100)		
(**8**)	502 (79)	442 (12)[a]	427 (6)	308 (69)		290 (39)[b]	275 (9)	163 (100)			134 (100)		
(**9**)	500 (69)	440 (10)[a]	425 (9)[c]	306 (100)	291 (41)		273 (7)	163 (93)			134 (89)		175 (20)
(**10**)	500 (20)	482 (11)	467 (11)	350 (70)	335 (15)	332 (31)	317 (12)	163 (100)	150 (21)	135 (52)			
(**11**)	502 (5)	484 (3)	469 (4)	350 (95)	335 (20)	332 (26)	317 (15)	163 (100)	152 (30)	137 (40)	134 (90)	119(60)	

[a] M^+ − HOAc. [b] $M^+ - H_2O$ − HOAc. [c] M^+ − Me − HOAc.



Scheme 1. i, Ac_2O–pyridine; ii, 1% oxalic acid–H_2O, heat; iii, glacial HOAc, heat; iv, CF_3CO_2H–DMSO; v, pyridine

presence of seven tertiary methyl groups and one secondary methyl group which were assigned by comparison with the data for (**1**). Lowfield shifts of the signals due to 4-methyl groups helped to locate the carbonyl group at the biogenetically favourable C-3 (Table 1). Acetylation (Ac_2O–pyridine) afforded the monoacetyl derivative (**10**) as a semisolid, $[\alpha]_D$ −25°, whose i.r. spectrum indicated the presence of a free OH group (3 550 cm^{-1}) suggesting its tertiary nature. In the ^1H n.m.r. spectrum of (**10**) the C*H*OAc appeared as a double doublet (*J* 10.7 and 2.4 Hz) at δ 4.75 further confirming the presence of OH group at C-24 in the natural product. Further evidence for the proposed structure of 24β-acetoxy-3-oxoreissant-5-en-25-ol (**10**) came from its ^{13}C n.m.r. spectral data, assignment of which was made by comparison with (**1**) and (**2**) (see Table 2). No attempts were made to determine the stereochemical disposition of the 24-OH group. However, by comparison with (**4**) which co-occurs with (**3**) in this plant, the stereochemistry of the 24-OH group was tentatively assigned as β.

The most polar new triterpene, $C_{30}H_{52}O_3$, $[\alpha]_D$ +35.4°, obtained as a colourless solid which resisted crystallisation, in its i.r. spectrum showed the presence of only OH group(s) (3 550—3 200 cm^{-1}). Based on the arguments presented below its structure was elucidated as reissant-5-ene-3β,24β,25-triol (**4**). On acetylation (Ac_2O–pyridine), it gave a non-crystalline diacetate (**7**), M^+, *m/z* 544 ($C_{34}H_{56}O_5$), $[\alpha]_D$ +26°, the i.r. spectrum of which indicated the presence of a free OH group (3 500 cm^{-1}) suggesting its tertiary nature. The m.s. of the parent triterpene showed the expected retro Diels–Alder cleavage leading to fragments (a), (c) and subsequent loss of the side chain from fragment (a) giving rise to fragment (b) which suggested the presence of one OH group in ring A and the remaining two OH groups in the side chain probably at the biogenetically favourable C-24 and C-25. The m.s. of the diacetate (**7**) lacked a peak due to the fragment (c); however fragment (d) (*m/z* 134) resulting from (c) by the loss of a molecule of HOAc was found to be the base peak (see Table 3). The OH in ring A was placed at C-3 based on biogenetic arguments.

In the ^1H n.m.r. spectrum of (**4**), in addition to the signals due to seven tertiary methyl groups, one secondary methyl group, and an olefinic proton, two 1 H multiplets were present at δ 3.47 and 3.29 which were assigned by comparison with (**2**) and (**3**), to C*H*OH protons at C-3 and C-24, respectively. In the diacetate (**7**) these were shifted to δ 4.75 (dd, *J* 10.7 and 2.4 Hz) and δ 4.70 (t, *J* 2.7 Hz), respectively, and the two $OCOCH_3$ singlets appeared at δ 2.11 and 2.02 (see Table 1). The ^{13}C n.m.r. spectrum of the diacetate (**7**) derived from the natural triterpene, was assigned by comparison with (**1**) and (**2**), which further supported the proposed structure. The diacetate prepared from reissantenol oxide acetate (**6**) by opening of the epoxide ring with glacial HOAc (Scheme 1) was found to be identical with the diacetate (**7**) derived from the natural triterpene. As the acid-catalysed ring opening of epoxides is known to yield products of *trans* stereochemistry,[13] the 24-OAc group should be β. Thus in the natural triterpene the 24-OH should be of β orientation.

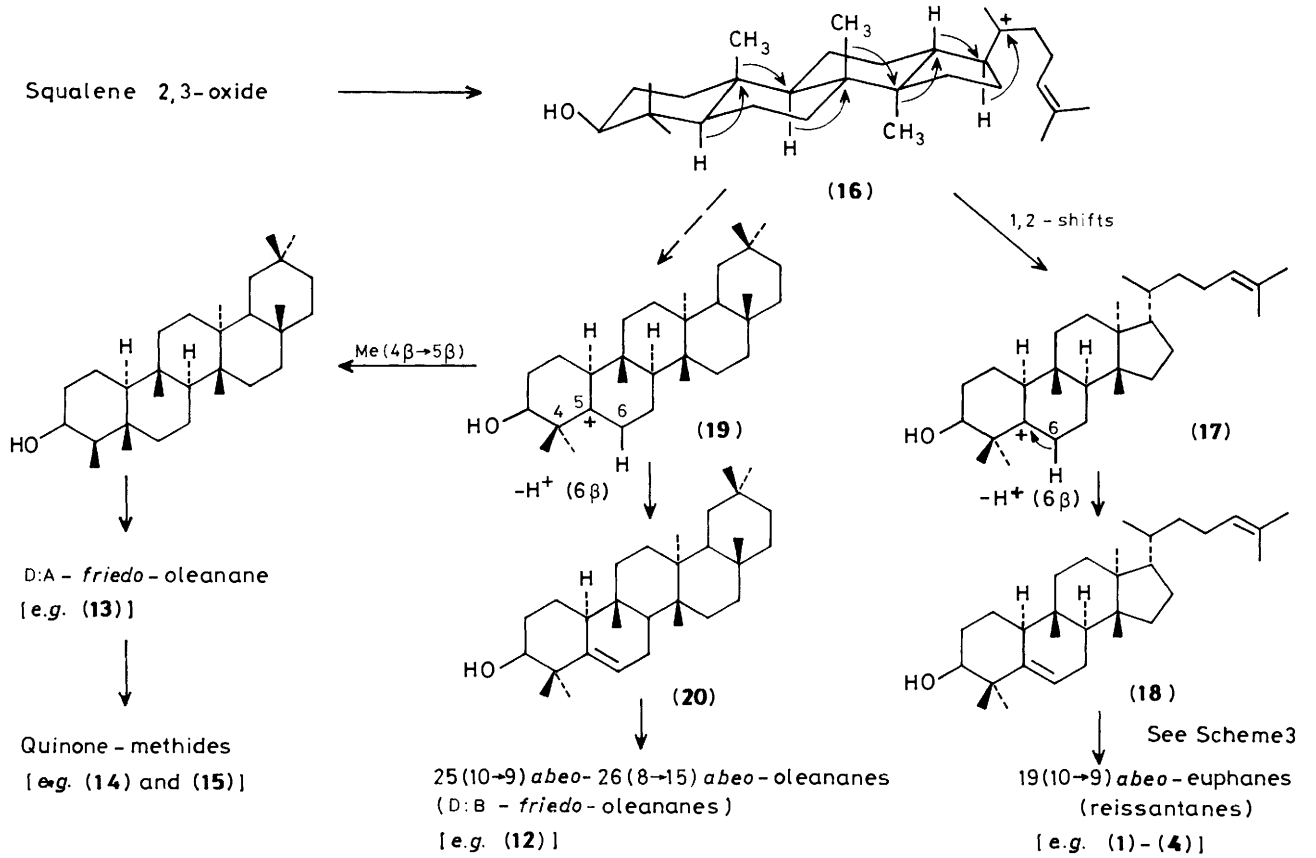
Biosynthetic Aspects.—The co-occurrence in *R. indica* of reissantane [19(10→9)*abeo*-euphane], D:B-*friedo*-oleanane-[25(10→9)*abeo*-26(8→15)oleanane], D:A-*friedo*-oleanane and quinone-methide triterpenoids may be explained by the relatively close biosynthetic relationship of the four groups as illustrated in Scheme 2. The cationic tetracyclic triterpene precursor (**16**) formed from squalene-2,3-epoxide by a *trans-anti-trans* cyclisation in a chair, chair, chair, boat conformation[14] could under a series of 1,2-shifts give the carbocation intermediate (**17**) which by the loss of 6β-H lead to 3β-hydroxyreissanta-5,24-diene (**18**), the key intermediate of the natural reissantane triterpenes (**1**)—(**4**). The loss of 6β-H from the pentacyclic intermediate (**19**) derived from (**16**) could result in 3β-hydroxy-D:B-*friedo*-oleanane (**20**), the probable precursor of (**12**) encounted in *R. indica.* Biogenetically, D:A-*friedo*-oleananes [*e.g.* canophyllol (**13**)] may arise from the same pentacyclic carbocationic intermediate (**19**) by a 1,2-shift of 4β-Me leading to 5β-Me. The possible intermediacy of D:A-*friedo*-oleananes in the biosynthesis of quinone-methides [*e.g.* pristimerin (**14**) and tingenone (**15**)] has already been predicted.[15] The biosynthetic origin of the natural triterpenes (**1**), (**2**), (**3**), and (**4**) from 3β-hydroxyreissanta-5,24-diene (**18**) (Scheme 3) parallels the biosynthesis of the cucurbitacins, bryosigenin, and bryodulcosigenin occurring in *Bryonia dioica.*[16]

Experimental

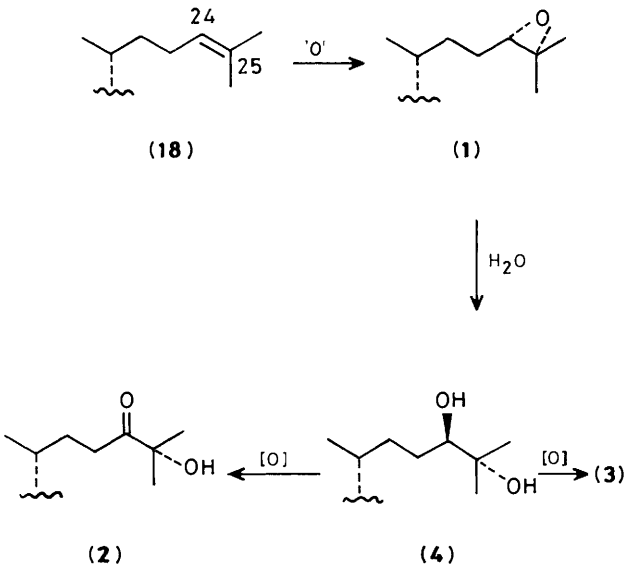
General Procedures.—The general experimental details were the same as those described previously.[15a] Medium pressure liquid chromatography (m.p.l.c.) was performed over silica gel (mesh 70—230). Unless otherwise stated i.r. spectra were recorded in $CHCl_3$ with a Shimadzu IR-408 spectrometer. Optical rotations were measured at 23 °C in $CHCl_3$ solutions with a Perkin-Elmer 241 polarimeter. Mass spectra were obtained using JEOL D-300 mass spectrometer (ionization voltage, 70 eV; accelerating voltage, 3 kV) using a direct inlet system. ^1H N.m.r. spectra were recorded in $CDCl_3$ and [$^2H_5$]pyridine at 400 MHz with a Bruker WH 400 spectrometer unless otherwise stated, with $SiMe_4$ as internal reference. ^{13}C N.m.r. spectra were obtained using a Bruker WH 400 spectrometer operating at 50.28 MHz and the multiplicity of carbon signals established using DEPT experiments. ^{13}C N.m.r., 2-D n.m.r. spectra and microanalysis were obtained from Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan, and ^1H n.m.r. and mass spectra were obtained from Otsuka Pharmaceutical Co. Ltd., and University of Sydney, Australia.

Extraction of R. indica *Root Bark.*—The dried and powdered root bark (4.0 kg) of *R. indica* collected at Wilpattu, Sri Lanka, was exhaustively extracted with hot hexane. Evaporation afforded a hexane extract (46 g) which was re-extracted with hot methanol to remove gutta-percha. Evaporation of methanol yielded a reddish brown semisolid (32 g). This (10 g) was separated into acidic ($NaHCO_3$ soluble, 0.11 g), phenolic (NaOH soluble, 1.61 g), and neutral (8.14 g) fractions. The neutral fraction (8.0 g) was subjected to m.p.l.c. with solvent gradients ranging from light petroleum to dichloromethane to methanol. Similar (by t.l.c.) eluates were combined to obtain a total of six fractions labelled A—F.

Isolation of Reissantenol Oxide (**1**) *and Sitosterol.*—The column fraction A contained two compounds and these were separated by m.p.l.c. (eluant: 5% ethyl acetate in light



Scheme 2. Possible biosynthetic relationships of reissantane, D:B-*friedo*-oleanane, D:A-*friedo*-oleanane and quinone-methide teriterpenoids occurring in *Reissantia indica*



Scheme 3.

petroleum) to yield *reissantenol oxide* (250 mg, 0.02%) and sitosterol (35 mg, 0.003%). Reissantenol oxide was obtained as colourless prisms, m.p. 175 °C (from light petroleum–ethyl acetate), $[\alpha]_D$ +34.4° (*c* 1.2); $\nu_{max.}$($CHCl_3$) 3 475 (OH), 2 900, 1 455, 1 380, 1 110, 97 (epoxide), and 890 cm^{-1}; for ^1H n.m.r., ^{13}C n.m.r. and m.s. data, see Tables 1 2, and 3, respectively (Found: C, 81.4; H, 11.1%; M^+, 442.3807. $C_{30}H_{50}O_2$ requires C, 81.39; H, 11.38%; *M*, 442.3848).

Sitosterol was obtained as white needles, m.p. 135—137 °C (from light petroleum–chloroform), $[\alpha]_D$ −35° (*c* 1.0); identity was confirmed by comparison (mixed m.p., co-t.l.c.) with an authentic sample.

Isolation of 25(10→9)abeo-26(8→15)abeo-9β,14β,15α-*Olean*-5-*ene*-3β,29-*diol* (**12**).—The m.p.l.c. column fraction B was found to be a mixture of two compounds. This was subjected to flash chromatography (eluant: 10% ethyl acetate in light petroleum) followed by p.l.c. (eluant: 40% ether in light petroleum) to yield two triterpenoids. The less polar compound was obtained as a colourless crystalline solid (15 mg, 0.0012%) and was found to be identical with authentic material,[4] m.p. 279—280 °C, $[\alpha]_D$ +54° (*c* 1.2) (lit.,[4] m.p. 276—279 °C; $[\alpha]_D$ + 55.7°); δ(400 MHz; $CDCl_3$) 5.63 (1 H, d, *J* 6 Hz, 6-H), 3.47 (1 H, m, 3-H), 3.30 and 3.24 (1 H, each d, *J* 10.4 Hz, CH_2OH), 1.41, 1.21, 1.13, 1.04, 1.02, 1.00, and 0.85 (3 H each, s, Me groups on quaternary carbons) [lit.,[4] δ(60 MHz; $CDCl_3$) 5.56 (m, $W_{\frac{1}{2}}$ 8 Hz, 6-H), 3.46 (m, $W_{\frac{1}{2}}$ 5.6 Hz, 3-H), 3.28 (s, CH_2OH), 0.91, 0.86, 0.83, 0.83, 0.73, 0.73, 0.70 (3 H each, s, Me groups on quaternary carbons)]; *m/z* 442 (M^+, 7%), 290 (65), 275 (43), 259 (100), 221 (18), 152 (58), and 134 (60) (Found: M^+, 442.3820. $C_{30}H_{50}O_2$ requires *M*, 442.3848).

Isolation of Canophyllol (**13**).—The more polar compound (20 mg, 0.0016%) obtained from the above p.l.c. separation was identified as canophyllol, m.p. 277—278 °C, $[\alpha]_D$ −21° (*c* 1.02) (lit.,[17] m.p. 278—280 °C, $[\alpha]_D$ −22.3°); δ(400 MHz) 3.29 and 3.24 (1 H each, dd, *J* 10 and 4.8 Hz, CH_2OH), 2.24 (1 H, q, *J* 6.4 Hz, 4-H), 0.88 (3 H, d, *J* 6.4 Hz, C4-Me), 1.22, 1.05, 1.033, 1.028, 0.87, and 0.73 (3 H each, s, Me groups on quaternary carbons) (Found: M^+, 442.3824. $C_{30}H_{50}O_2$ requires *M*, 442.3848).

Isolation of Pristimerin (**14**).—The column fraction C on

further purification by flash chromatography (eluant: 10% ethyl acetate in light petroleum) afforded pristimerin as orange needles (1.2 g, 0.096%), m.p. 214—215 °C (from acetone) (lit.,[18] m.p. 214—217 °C), identical with an authentic sample.[15a]

Isolation of 24-*Oxoreissant*-5-*ene*-3β,25-*diol* (**2**).—The column fraction D on purification by p.l.c. (eluant: 50% ether in light petroleum, double development) gave 24-*oxoreissant*-5-*ene*-3β,25-*diol* as a colourless crystalline solid (72 mg, 0.0058%), m.p. 129—130 °C (from light petroleum–dichloromethane), $[\alpha]_D$ + 34.8° (*c* 0.89) (Found: C, 78.45; H, 10.85%; M^+, 458.3770. $C_{30}H_{50}O_3$ requires C, 78.55; H, 10.99%; *M*, 458.3760); for ^{1}H n.m.r., ^{13}C n.m.r., and m.s. data, see Tables 1, 2, and 3, respectively.

Isolation of Tingenone (**15**).—The column fraction E contained an orange red pigment which on further purification by p.l.c. (eluant: 40% ethyl acetate in light petroleum) afforded tingenone (79 mg, 0.0063%) as reddish orange plates, m.p. 135—137 °C (from ethyl acetate) identical (mixed m.p., co-t.l.c., ^{1}H n.m.r. and co-i.r.) with an authentic sample.[19]

Isolation of 3-*Oxoreissant*-5-*ene*-24α,25-*diol* (**3**).—The column fraction E contained two new triterpenoids and these were separated by flash chromatography (eluant: 0.5% methanol in chloroform) to afford 3-oxoreissant-5-ene-24α,25-diol as a colourless amorphous solid which resisted crystallisation (86 mg, 0.007%); $\nu_{max.}$ 3 500—3 100, 2 975, 1 705, 1 460, 1 375, 1 110, 1 065, and 750 cm^{-1}; for ^{1}H n.m.r., ^{13}C n.m.r. and m.s. data, see Tables 1, 2, and 3, respectively.

Isolation of Reissant-5-*ene*-3β,24α,25-*triol* (**4**).—The more polar triterpenoid separated during the purification of (**3**) above, was found to be the *triol* (**4**) which was obtained as a colourless non-crystallizable amorphous solid (94 mg, 0.0075%), $[\alpha]_D$ +35.4° (*c* 1.0); $\nu_{max.}$ 3 550, 3 200 (OH), 2 905, 1 455, 1 380, 968, 950, and 752 cm^{-1}; for ^{1}H n.m.r., ^{13}C n.m.r. and some m.s. data, see Tables 1, 2, and 3, respectively; *m/z* 460 (M^+, $C_{30}H_{52}O_3$), 445 ($C_{29}H_{49}O_3$), 442 ($C_{30}H_{50}O_2$), 427 ($C_{29}H_{47}O_2$), 424 ($C_{30}H_{48}O$), 409 ($C_{29}H_{45}O$), 402 ($C_{27}H_{46}O_2$), 384 ($C_{27}H_{44}O$), 308 ($C_{20}H_{36}O_2$), 293 ($C_{19}H_{33}O_2$), 290 ($C_{20}H_{34}O$), 275 ($C_{19}H_{31}O$), 257 ($C_{19}H_{29}$), 250 ($C_{17}H_{30}O$), 191 ($C_{14}H_{23}$), 189 ($C_{14}H_{21}$), 175 ($C_{13}H_{19}$), 173 ($C_{13}H_{17}$), 163 ($C_{12}H_{19}$), 152 ($C_{10}H_{16}O$), 134 ($C_{10}H_{14}$), 127 ($C_8H_{15}O$), 123 (C_9H_{15}), and 109 (C_8H_{13}) (Found: M^+, 460.3917. $C_{30}H_{52}O_3$ requires *M*, 460.3916).

$LiAlH_4$ *Reduction of Reissantenol Oxide* (**1**).—To a well stirred solution of reissantenol oxide (28 mg) in anhydrous THF (2 ml) under an atmosphere of dry N_2 was added $LiAlH_4$ (50 mg) portionwise over a period of 15 min. The resulting mixture was stirred for a further 3 h. Customary work-up followed by p.l.c. purification afforded *reissant*-5-*ene*-3β,25-*diol* (**5**) as an amorphous solid (21 mg, 75%), $[\alpha]_D$ +37.5° (*c* 1.1); for ^{1}H n.m.r. and some m.s. data see Tables 1 and 3, respectively; *m/z* 444 (M^+, $C_{30}H_{52}O_2$), 429 ($C_{29}H_{49}O_2$), 426 ($C_{30}H_{50}O$), 411 ($C_{29}H_{47}O$), 393 ($C_{29}H_{45}$), 297 ($C_{22}H_{33}$), 292 ($C_{20}H_{36}O$), 277 ($C_{19}H_{33}O$), 274 ($C_{20}H_{34}$), 259 ($C_{19}H_{31}$), 219 ($C_{16}H_{27}$), 192 ($C_{13}H_{20}O$), 191 ($C_{13}H_{19}O$), 175 ($C_{13}H_{19}$), 173 ($C_{13}H_{17}$), 165 ($C_{11}H_{17}O$), 163 ($C_{12}H_{19}$), 152 ($C_{10}H_{16}O$), 149 ($C_{11}H_{17}$), 137 ($C_{10}H_{17}$), 136 ($C_{10}H_{16}$), 134 ($C_{10}H_{14}$), and 123 (C_9H_{15}) (Found: M^+, 444.3964. $C_{30}H_{52}O_2$ requires *M*, 444.3967).

Acetylation of Reissantenol Oxide (**1**).—Reissantenol oxide (44 mg) was acetylated with Ac_2O–pyridine (1:1) (6 ml) overnight at room temperature. Customary work-up followed by p.l.c. purification afforded reissantenol oxide acetate (**6**) (36 mg, 75%), m.p. 102—105 °C, $[\alpha]_D$ +22.0° (*c* 0.8); $\nu_{max.}$ 2 865, 1 735 (acetate), 1 455, 1 375, 1 245, and 1 115 cm^{-1}; for ^{1}H n.m.r. data, see Table 1.

Conversion of Reissantenol Oxide Acetate (**6**) *into* 3β-*Acetoxy*-24-*oxoreissant*-5-*en*-25-*ol* (**9**).[12]—To a stirred solution of the acetate (**6**) (48 mg) in dry Me_2SO (2 ml) under N_2 was added CF_3CO_2H (0.5 ml) and the mixture stirred for 30 min; dry pyridine (1 ml) was then added. After the mixture had been stirred for a further 1 h, it was worked-up by addition of an excess of ether and brine. The aqueous layer was extracted several times with ether and the combined ether extracts were washed with brine, dried ($MgSO_4$), and evaporated to yield the crude product which was purified by p.l.c. to give 3β-acetoxy-24-oxoreissant-5-en-25-ol (**9**) as a colourless semisolid (15 mg, 31%) which was identical (i.r., ^{1}H n.m.r., m.s. and $[\alpha]_D$) with the above acylated product of 24-oxoreissant-5-ene-3β,25-diol (**2**).

Conversion of Reissantenol Oxide Acetate (**6**) *into* 3β,24α-*Diacetoxyreissant*-5-*en*-25-*ol* (**7**).—The acetate (**6**) (50 mg) was treated with glacial HOAc (3 ml) under reflux in an atmosphere of N_2 for 5 h (t.l.c. control). Excess of ether was added followed by brine. The aqueous layer was extracted several times with ether and the combined ether extracts were washed with brine, dried ($MgSO_4$), and evaporated to yield the crude product which was purified by p.l.c. to give 3β,24α-diacetoxyreissant-5-en-25-ol (**7**) as a colourless semisolid (28 mg, 55%) which resisted crystallization, $[\alpha]_D$ +26° (*c* 0.8); $\nu_{max.}$($CHCl_3$) 3 400 (OH) and 1 723 cm^{-1} (acetate); for ^{1}H n.m.r., ^{13}C n.m.r., and m.s. data, see Tables 1, 2, and 3, respectively (Found: M^+, 544.4135. $C_{34}H_{56}O_5$ requires *M*, 544.4128).

Conversion of Reissantenol Oxide Acetate (**6**) *into* 3β-*Acetoxyreissant*-5-*ene*-24α,25-*diol* (**8**).—The acetate (**6**) (24 mg) was treated with an aqueous solution of 1% oxalic acid (2 ml) under reflux in an atmosphere of N_2 for 3 h (t.l.c. control). Work-up as for (**7**) above, followed by p.l.c. purification afforded 3β-acetoxyreissant-5-ene-24α,25-diol (**8**) as a colourless semisolid (10 mg, 42%), $[\alpha]_D$ +43.2° (*c* 0.9); $\nu_{max.}$($CHCl_3$), 3 475 (OH) and 1 750 cm^{-1} (acetate); for ^{1}H n.m.r. and m.s. data, see Tables 1 and 3, respectively (Found: M^+, 502.4012. $C_{32}H_{54}O_4$ requires *M*, 502.4023).

Conversion of Reissantenol Oxide (**1**) *into* 24α-*Acetoxyreissant*-5-*ene*-3β,25-*diol* (**11**).—Treatment of compound (**1**) (22 mg) with glacial HOAc as for the conversion of (**6**) into (**7**), above followed by work-up and purification by p.l.c. afforded 24α-*acetoxyreissant*-5-*ene*-3β,25-*diol* (**11**) as a colourless amorphous solid (10 mg, 45%) which resisted crystallization, $[\alpha]_D$ +20 °C (*c* 0.6); $\nu_{max.}$($CHCl_3$) 3 100 (OH) and 1 733 cm^{-1} (acetate); for ^{1}H n.m.r. and m.s. data, see Tables 1 and 3, respectively (Found: M^+, 502.4007. $C_{32}H_{54}O_4$ requires *M*, 502.4023).

Acetylation of 24-*Oxoreissant*-5-*ene*-3β,25-*diol* (**2**).—The diol (**2**) (46 mg) was treated with Ac_2O–pyridine (1:1) (4 ml) at room temperature for overnight. Work-up gave the crude product which was purified by p.l.c. to yield 3β-*acetoxy*-24-*oxoreissant*-5-*en*-25-*ol* (**9**) as a colourless semisolid (40 mg, 80%), $[\alpha]_D$ +36.7° (*c* 0.8); $\nu_{max.}$($CHCl_3$) 3 450 (OH), 1 735 (acetate), and 1 702 cm^{-1} (ketone carbonyl); for ^{1}H n.m.r. and m.s. data, see Tables 1 and 3, respectively (Found: M^+, 500.3850. $C_{32}H_{52}O_4$ requires *M*, 500.3866).

Acetylation of Reissant-5-*ene*-3β,24α,25-*triol* (**4**).—The triol (**4**) (27 mg) was treated with Ac_2O–pyridine (1:1) (2 ml) at room temperature for 24 h. Work-up followed by purification by p.l.c. afforded 3β,24α-diacetoxyreissant-5-en-25-ol (**7**) (19 mg, 79%),

identical (i.r., ^{1}H n.m.r., m.s., and $[\alpha]_D$) with the product obtained above by the treatment of reissantenol oxide acetate (**6**) with glacial HOAc.

Acetylation of 3-*Oxoreissant*-5-*ene*-24α,25-*diol* (**3**).—The diol (**3**) (11 mg) on acetylation with Ac_2O–pyridine (1:1) (1 ml), subsequent work-up and purification as above yielded 24α-acetoxy-3-oxoreissant-5-en-25-ol (**10**) as a colourless semisolid (8 mg, 62%), $[\alpha]_D$ −25° (*c* 0.5); $\nu_{max.}$($CHCl_3$) 3 350 (OH), 1 735 (acetate), and 1 702 cm^{-1} (ketone carbonyl); for ^{1}H n.m.r., ^{13}C n.m.r., and m.s. data, see Tables 1, 2, and 3, respectively.

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References

1 For part 18 see H. C. Fernando, A. A. L. Gunatilaka, Y. Tezuka, and T. Kikuchi, *Tetrahedron*, in the press.
2 C. B. Camlath, A. A. L. Gunatilaka, and E. O. Schlemper, *J. Chem. Soc. Chem. Commun.*, 1988, 249.
3 R. Bruning and H. Wagner, *Phytochemistry*, 1978, **17**, 1821.
4 G. Weeratunga and V. Kumar, *Phytochemistry*, 1985, **24**, 2369.
5 D. Schiengthong, A. Verasarn, P. NaNonggai-Suwanrath, and E. W. Warnhoff, *Tetrahedron*, 1963, **21**, 917.
6 H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, 1963, **85**, 3688.
7 H. E. Audier and B. C. Das, *Tetrahedron Lett.*, 1966, 2205.
8 T. Itoh, T. Tamura, T. M. Jeong, T. Tamura, and T. Matsumoto, *Lipids*, 1980, **15**, 122.
9 T. Iida, T. Tamura, N. Miura, and T. Matsumoto, *Steroids*, 1977, **29**, 453.
10 J. N. Shoolery, *J. Nat. Products*, 1984, **47**, 226.
11 W. G. Anderson, C. Y. Byon, M. Gut, and F. H. Bissett, *Tetrahedron Lett.*, 1976, 2193.
12 T. M. Santosusso and D. Swern, *Tetrahedron Lett.*, 1968, 4261.
13 R. E. Parker and N. E. Isaacs, *Chem. Rev.*, 1959, **59**, 737.
14 A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, 1955, **38**, 1890.
15 (*a*) G. M. K. B. Gunaherath and A. A. L. Gunatilaka, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2845; (*b*) J. P. Kutney, W. H. Beale, P. J. Salisbury, K. L. Stuart, B. R. Worth, P. M. Townsely, W. T. Chalmers, K. Nilsson, and G. G. Jacoli, *Phyochemistry*, 1981, **20**, 653; (*c*) G. B. Marini-Bettolo, *Rev. Latinoam. Quim.*, 1979, **10**, 97.
16 G. P. Moss, *Planta Medica*, 1966, **14** (suppl), 86; L. Cattel, G. Ballino, O. Caputo, and F. Viola, *ibid.*, 1981, **41**, 328.
17 T. R. Govindachari, N. Viswanathan, B. R. Pai, U. Rama Rao, and M. Sirinivasan, *Tetrahedron*, 1967, **23**, 1901.
18 A. G. Gonzalez, C. G. Francisco, R. Friere, R. Hernandez, J. A. Salazer, and E. Suarez, *Phytochemistry*, 1975, **14**, 1067.
19 F. D. Monache, G. B. Marini-Bettolo, M. Pompani, J. F. de Mello, O. G. de Lima, and R. H. Thomson, *J. Chem. Soc., Perkin Trans. 1*, 1979, 3127.

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