Structures of Four New Oxygenated Lupanes from *Pleurostylia opposita* (Celastraceae) [1, 2]

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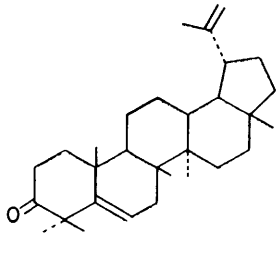
Four new oxygenated lupanes isolated from the stem bark of *Pleurostylia opposita* (Wall) Alston (Celastraceae) have been shown to be lupa-5,20(29)-dien-3-one (2), 6β,20-dihydroxylupan-3-one (5), 3β,6β-dihydroxylup-20(29)-ene (6), and 6β,28-dihydroxylup-20(29)-en-3-one (8). The structures of these compounds were established on the basis of spectroscopic (^{1}H n.m.r. and mass spectral) and chemical evidence. Deshielding of three methyl signals in the ^{1}H n.m.r. spectra of 6β-hydroxylupane derivatives has been attributed to 1,3-diaxial interaction between the relevant methyl group and the 6β-hydroxy-group.

Pleurostylia opposita (Wall) Alston (Celastraceae) is a moderately sized tree found in the dry zone of Sri Lanka. Previous work on the genus *Pleurostylia* has led to the isolation of friedelin, *epi*-friedelinol, and the spermidine celacinnine from the leaves of *P. africana*.[3] Pristimerin, α-amyrin, β-sitosterol, and eight other triterpenes were isolated from the benzene extract of the stem bark of *P. opposita*. Several lupane derivatives have been isolated from the Celastraceae.[4-7] Six of the triterpenes isolated from *P. opposita* were found to be lupane derivatives which have not been reported previously in this family. In this paper we report the isolation and structure elucidation of four new oxygenated lupane derivatives.

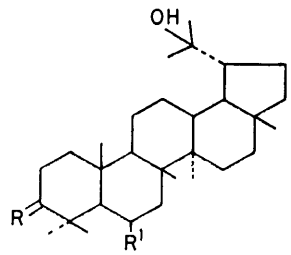
RESULTS AND DISCUSSION

Plant material used in this investigation was collected from Murugandy, in the Jaffna district of the Northern province of Sri Lanka. The benzene extract of the dried, powdered stem bark of a well matured tree of *P. opposita* yielded pristimerin, α-amyrin, β-sitosterol, and eight compounds (1)—(8), all of which gave a positive Liebermann–Burchard reaction. Compounds (2), (4), (5), (6), (7), and (8) have been identified as lupa-5,20(29)-dien-3-one (2), 20-hydroxylupan-3-one (4),[8] 6β,20-dihydroxylupan-3-one (5), 3β,6β-dihydroxylup-20(29)-ene (6), 3β,20-dihydroxylupane (7),[8] and 6β,28-dihydroxylup-20(29)-en-3-one (8), respectively. Compounds (2), (5), (6), and (8) are new lupane derivatives. Rigidenol, a lupane derivative with an α-hydroxy-substitutent at C-6 has been reported from *Maytenus rigida* (Celastraceae).[9] Our results indicate this assignment to be doubtful. Hence, (5), (6), and (8) are the first examples reported of lupanes with an oxygen substituent at C-6. Di- and tri-oxygenated friedelin derivatives with a 6β-hydroxy-substituent have been reported from *Kokoona zeylanica* belonging to the same family.[10]

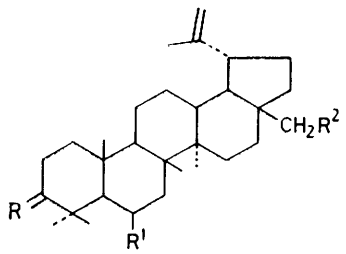
^{1}H N.m.r. and mass spectral evidence suggested the presence of a lupane skeleton in (2) and (4)—(8). Two 1-H doublets in the δ 4.6—4.5 region, and a 3-H doublet at δ 1.6—1.7 in the ^{1}H n.m.r. spectra of (2), (6), and (8) indicated the presence of a lup-20(29)-ene system in these three compounds.[11] Further, signals due to eight methyl groups, including two singlets at δ 1.1 and 1.2, in the n.m.r. spectra of (4), (5), and (7) showed the presence of a $C(Me)_2OH$ group, and indicated that these compounds were 20-hydroxylupane derivatives.[8] Fragments at *m/e* 218, 205, 203, and 189 in the mass spectra of all six compounds suggested the presence of a lupane skeleton without oxygen substitution in rings D and E.[12] Attempted acetylation of (4) gave lup-20(29)-en-3-one, and (4) was shown to be 20-hydroxylupanone by comparison with an authentic sample.[8] Reduction of (4)



(2)



(4) R=O; R^1 = H
(5) R=O; R^1 = OH
(7) R=α-H, β-OH; R^1 = H

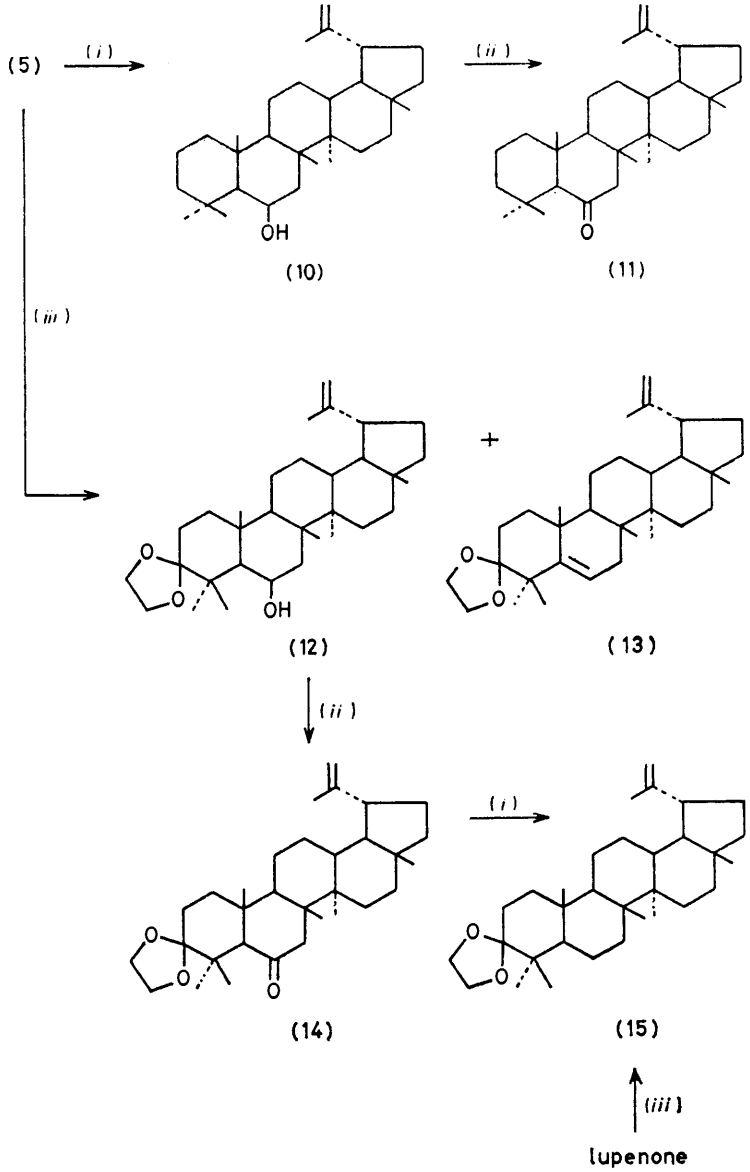


(6) R = α-H, β-OH; R^1 = OH; R^2 = H
(8) R = O; R^1 = OH; R^2 = OH
(9) R = O; R^1 = OAc; R^2 = H

gave 3β,20-dihydroxylupane,[8] which was found to be identical with compound (7).

Absorption due to a hydroxy-group ($\nu_{max.}$ 3 460 cm^{-1}) and a saturated six-membered ring carbonyl group ($\nu_{max.}$ 1 695 cm^{-1}) indicated (5) ($C_{30}H_{50}O_3$) to be a

hydroxy-ketone. The ^{1}H n.m.r. spectrum showed resonances due to eight methyl groups and a C*H*OH proton at δ 4.46 ($W_{\frac{1}{2}}$ 9.0 Hz) indicating the presence of an axial hydroxy-group. Acetylation of (5) yielded a monoacetate (9) ($C_{32}H_{50}O_3$). Doublets at δ 4.66 (1 H), 4.53 (1 H), and 1.66 in the ^{1}H n.m.r. spectrum of (9) suggested that (5) had undergone acetylation with concomitant dehydration of the t-hydroxy-group at C-20, thus giving rise to a lup-20(29)-ene system. Dehydration of (5) gave a product identical to (2). A multiplet at δ 5.45 due to a single olefinic proton and signals due to the isopropenyl system in the n.m.r. spectrum of (2) indicated it to be a lupadienone, and the hydroxy-group in (5) to be at C-6, C-11, or C-12. Huang-Minlon reduction of (5) gave (10). I.r. and n.m.r. evidence showed the absence of a carbonyl group and the presence of an isopropenyl system in (10), indicating that dehydration of the t-hydroxy-group had taken place along with reduction of the carbonyl group at C-3. Oxidation of (10) with chromium trioxide–pyridine yielded the lupenone (11) ($\nu_{max.}$ 1 710 cm^{-1}). N.m.r. evidence was used to locate the position of the carbonyl group in (11). The double doublets (*J* 12.0 Hz) centred at δ 2.36 (1 H) and 1.83 (1 H) belonging to an AB system, and a singlet at 1.98 (1 H), indicated the carbonyl group to be at C-6 and not at C-11 or C-12, where the methylene protons would have a very much more complex pattern. This assignment was supported by the Eu(fod)$_3$-induced low-field shifts of all the methyl resonances except C-28, the two double doublets, and the singlet (H-5) in the ^{1}H n.m.r. spectrum of (11). Three of the methyl resonances, including C-23 and C-24, were shifted downfield more rapidly. Hence the hydroxy-group in (5) was located at C-6, having a β-axial orientation.



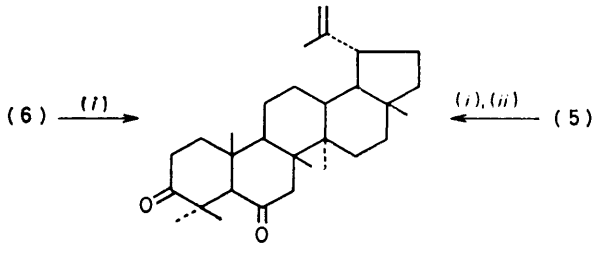
SCHEME 1 (*i*) NH_2NH_2–KOH; (*ii*) CrO_3–pyridine; (*iii*) $HOCH_2CH_2OH$, toluene-*p*-sulphonic acid

Acetalisation of (5) with ethylene glycol yielded two products. An absorption at $\nu_{max.}$ 3 450 cm^{-1}, a singlet at δ 3.93 (4 H, O[C*H*$_2$]$_2$O), and signals due to the isopropenyl system [δ 4.66 (1 H), 4.58 (1 H), 1.68 (3 H)] indicated that in the major product (12) ($C_{32}H_{52}O_3$) acetalisation had occurred along with dehydration of the t-hydroxy-group at C-20. Hence (12) was identified as 3,3-ethylenedioxy-6β-hydroxylup-20(29)-ene. A second product was also isolated. Absence of OH absorption in the i.r. spectrum of (13), and a signal due to a single olefinic proton (δ 5.40) in addition to the signals of the isopropenyl system in the n.m.r. spectrum, indicated (13) to be the dehydration product of (12), and it was assigned the structure 3,3-ethylenedioxylupa-5,20(29)-diene.

Additional support for locating the hydroxy-group in (12) and therefore in (5), at C-6, was obtained from the mass spectrum of the ethylene acetal (12). An ion at *m/e* 99, fourteen times the intensity of any other ions was observed. The intensity of this ion indicated the presence of a double bond between C-5 and C-6,[12] which could arise by dehydration of the hydroxy-group at C-6.

The ethylene acetal (12) on oxidation yielded (14) ($\nu_{max.}$ 1 700 cm^{-1}). Huang-Minlon reduction of (14) afforded (15) which was shown to be identical to the ethylene acetal of lup-20(29)-en-3-one (Scheme 1), thus locating the carbonyl group in (5) at C-3 and confirming the presence of a lupane skeleton in (5) and in (2), (4), (6), (7), and (8) which have all been related to (5) (Scheme 2). Thus (5) was assigned the structure 6β,20-dihydroxylupan-3-one.

I.r. ($\nu_{max.}$ 3 390 cm^{-1}) and n.m.r. evidence indicated (6) to be a dihydroxylup-20(29)-ene. The chemical shift and half-height width (20.0 Hz) of a 1-H multiplet at δ 3.16 suggested the presence of a β-equatorial hydroxy-group at C-3. A second signal due to a C*H*OH proton was buried in the signals due to the isopropenyl system [δ 4.70 (1 H) and 4.56 (2 H)]. Acetylation of (6) gave a diacetate (16) [$\nu_{max.}$ 1 740 cm^{-1}; δ 2.05 (6 H, *s*, OCOMe)] confirming the presence of two hydroxy-groups in (6). The half-height width (8.0 Hz) of the signal at δ 5.53 due to a C*H*OAc proton indicated the acetoxy-group to have an axial orientation. Oxidation of (6) gave a lupenedione (17) ($\nu_{max.}$ 1 710—1 700 cm^{-1}) which was identical



(17)

(*i*) CrO_3-pyridine; (*ii*) $POCl_3$-pyridine

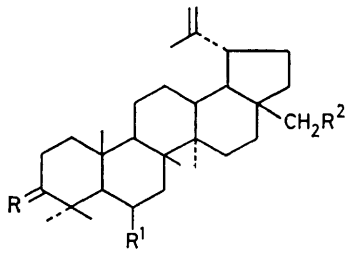
with that obtained when the oxidation product (18) of (5) was dehydrated. Thus the positions of the hydroxy-groups in (6) were established to be at C-3 and C-6, and (6) was confirmed to be 3β,6β-dihydroxylup-20(29)-ene.

Chemical and spectroscopic evidence indicated (8) to be a dihydroxylupenone. A monoacetate (19) ($C_{32}H_{50}O_4$) and a diacetate (20) ($C_{34}H_{52}O_5$) were prepared. Mass spectral evidence [*m/e* 425 (M^+ − CH_2OH, 100%)] and double doublets at δ 3.83 and 3.33 (*J* 12.0 Hz) and 4.15 and 3.73 (*J* 10.0 Hz) in the n.m.r. spectra of (8) and the diacetate (20), respectively, indicated the presence of a sterically hindered hydroxymethyl substituent in (8). The signal at δ 0.8 due to the methyl group at C-28 [13] was absent in the n.m.r. spectrum of (8), indicating the presence of a hydroxymethyl substituent at this position. The similarity in chemical shifts of the alcohol methine proton in (8) (δ 4.50) and in (5) (δ 4.46), and of the C*H*OAc proton in the diacetate (20) (δ 5.40) and the monoacetate (9) (δ 5.33) suggested that one of the hydroxy-groups in (8) most probably had the same location and orientation as the hydroxy-group in compound (5). Huang-Minlon reduction of (8) gave a lupenol (21) which was not betulin, confirming that the hydroxy-group was not at C-3. The methyl group signals in the ^1H n.m.r. spectrum of (21) were superimposable on those of (10) except for the absence of a signal at δ 0.8 due to 28-Me. Hence (8) was assigned the structure 6β,28-dihydroxylup-20(29)-en-3-one.

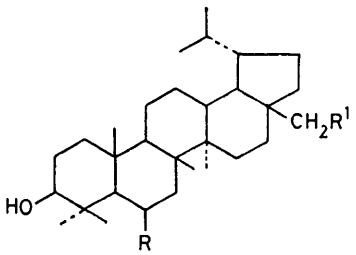
Replacement of the 6β-hydroxy-group by hydrogen in (5) and (8) was attempted by metal-amine reduction of the acetates (9) and (20), respectively, as described by Barton *et al.*[14] Reduction of (9) yielded three products, two of which were separated by preparative t.l.c. One-proton multiplets at δ 4.53 ($W_{\frac{1}{2}}$ 10.0 Hz) and 3.12 ($W_{\frac{1}{2}}$ 20.0 Hz), and eight methyl resonances indicated that the more polar product (22) ($C_{30}H_{52}O_2$) had arisen by reduction of the isopropenyl system and the carbonyl group at C-3. Hence (22) was assigned the structure 3β,6β-dihydroxylupane. Evidence for the saturation of the isopropenyl system was also seen in the mass spectrum of (22). Two frequently observed and dominant ions in the mass spectra of lup-20(29)-enes occur at *m/e* 218 and 189.[12] In the mass spectrum of (22) it was observed that ions at *m/e* 220(12%) and 191(33%) were more intense than those at *m/e* 218(8%) and 189(17%), respectively. This has been considered as evidence for the presence of a saturated isopropyl group.[12] The less polar product (23) was found to be identical to the product obtained when lupeol was reduced under similar conditions with the same reagent,[15] and was identified as 3β-hydroxylupane {m.p. 203—204 °C, $[\alpha]_D$ −20.0° (lit.,[16] 201—202 °C, $[\alpha]_D$ −17.8°)]}.

Reduction of the diacetate (20) gave a mixture of three compounds. The least polar compound [$\nu_{max.}$ 3 450 cm^{-1}, δ 3.15 (1 H, m, $W_{\frac{1}{2}}$ 20.0 Hz)] was found to be identical to (23). The next polar compound was found to be identical to (22). Hence compounds (22) and (23) have arisen by reduction of the primary acetoxy-function at C-28. This observation is in agreement with results reported previously by Gunatilaka *et al.* on the deoxygenation of sterically hindered primary alcohol groups in friedelin derivatives by metal-amine reduction of the derived acetates.[17]

The most polar component of this reduction, compound (24) ($\nu_{max.}$ 3 300 cm^{-1}), was shown to be identical (m.p., n.m.r. and i.r. comparison) to the product obtained when betulin (25) was reduced with the same reagent.[15] Double doublets at δ 3.83 and 3.30 due to a



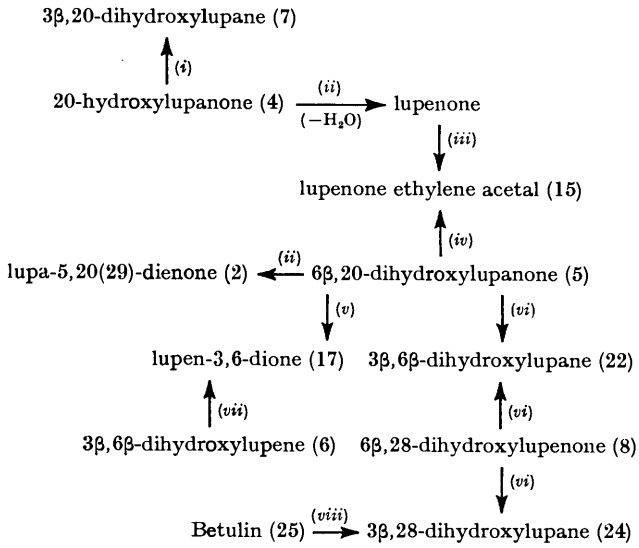
(16) R = α-H, β-OAc, R^1 = OAc, R^2 = H
(19) R = O, R^1 = OH, R^2 = OAc
(20) R = O, R^1 = R^2 = OAc
(21) R = H_2, R^1 = R^2 = OH
(25) R = α-H, β-OH, R^1 = H, R^2 = OH



(22) R = OH, R^1 = H
(23) R = R^1 = H
(24) R = H, R^1 = OH

sterically hindered hydroxymethyl group and seven methyl resonances in the n.m.r. spectrum of (24) were used to identify it as 3β,28-dihydroxylupane. These reactions related (8) to both (5) and betulin, thus confirming the assigned structure (Scheme 2).

When the methyl resonances of compounds (5), (6), and (8) were compared with the known positions of such methyl groups [13,17] in lupeol, betulin, and lupenone (Table) it was observed that three of the signals were



SCHEME 2 (*i*) $NaBH_4$; (*ii*) $POCl_3$–pyridine; (*iii*) $(CH_2OH)_2$–toluene-*p*-sulphonic acid–benzene, reflux; (*iv*) $(CH_2OH)_2$–toluene-*p*-sulphonic acid–benzene, CrO_3–pyridine, NH_2NH_2–KOH; (*v*) CrO_3–pyridine, $POCl_3$–pyridine; (*vi*) Ac_2O–pyridine, $NH_2[CH_2]_2NH_2$–Li; (*vii*) CrO_3–pyridine; (*viii*) $NH_2[CH_2]_2$-NH_2–Li

highly deshielded [*cf.* methyl signals of (5) and (8) with those of lupenone, and (6) with those of lupeol]. These three signals were assigned to the methyl groups at C-24, C-25, and C-26. Similar deshielding effects were observed in the derivatives prepared from these compounds [*e.g.* (10) and (21) in the Table]. Methyl groups which are in a 1,3-diaxial relationship to a hydroxy-group have been predicted to be deshielded strongly.[18] Deshielding of the C-24, C-25, and C-26 methyl resonances due to the presence of a 6β-hydroxy-substituent has been reported for the urs-12-ene skeleton.[19] Deshielding due to such 1,3-diaxial interaction has also been reported for a lupenediol with an 11β-hydroxy-substituent.[20] Hence the deshielding of the C-24, C-25, and C-26 methyl

TABLE

Methyl resonances (δ in $CDCl_3$) [a,c]

Compound	C-23	C-24	C-25	C-26	C-27	C-28	C-29/30
Lupeol [b]	0.97	0.73	0.84	1.05	0.97	0.80	
Betulin [b]	0.98	0.97	0.85	1.06	0.98		
Lupenone	1.03	0.98	0.95	1.06	0.98	0.80	
(5)	1.06		1.40 (3 H), 1.44 (6 H)		0.93	0.83	1.10/1.23
(6)	1.05		1.15, 1.22, 1.36		0.92	0.80	
(7)	1.00	0.76	0.86	1.06	1.00	0.83	1.10/1.23
(8)	1.13		1.43 (9 H)		0.95		
(9)	1.07		1.17, 1.27, 1.36		0.92	0.80	
(10)	0.95		1.20 (6 H), 1.36		0.95	0.80	
(12)	0.88		1.29 (6 H), 1.36		0.96	0.81	
(16)	0.90		1.03, 1.23, 1.33		0.90	0.78	
(19)	1.10		1.38, 1.43, 1.45		0.93		
(20)	1.07		1.13, 1.31, 1.38		0.95		
(21)	0.96		1.20 (6 H), 1.36		0.96		

[a] Signals assigned on the basis of known positions of methyl groups in triterpenoids. [b] Interpretation according to W.-H. Hui and M.-M. Li, *J. Chem. Soc., Perkin Trans. I*, 1976, 23. [c] Signals assigned using known effect on methyl groups of a ketone group at C-3; B. Tursch, R. Savoir, R. Ottinger, and G. Chiurdoglu, *Tetrahedron Lett.*, 1967, 539.

groups in compounds (5), (6), and (8) may be attributed to a 1,3-diaxial interaction with the β-hydroxy-group at C-6. The presence of a 6β-hydroxy-group had little effect on the chemical shift of the C-23 methyl group and on the more remote methyl groups at C-27 and C-28. The replacement of the 6β-hydroxy-group with an acetoxy-group results in an upfield shift of the C-24, C-25, and C-26 methyl resonances, in which one methyl group undergoes a more pronounced shift [*e.g.* (9), (16), and (20) in the Table].

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra were recorded for KBr discs. Identity of compounds was established by co-t.l.c., mixed m.p. and i.r. and n.m.r. comparison. T.l.c. was carried out on Merck silica gel GF_{254}. Light petroleum refers to the fraction of b.p. 40—60 °C. Optical rotations were measured at 25 °C in $CHCl_3$ with a Perkin-Elmer model 141 polarimeter. ^1H N.m.r. spectra were recorded in CCl_4 unless otherwise stated at 60 MHz with $SiMe_4$ as internal reference, on a Varian T 60 spectrometer. High-resolution mass spectra were determined at the Research School of Chemistry, The Australian National University. Microanalyses were performed by CSIRO Microanalytical Service, Melbourne.

Extraction and Isolation of Compounds.—Bark material from a well matured tree was chipped, dried and powdered in a mill. Powdered bark material (4.0 kg) was extracted successively with hot light petroleum and benzene. T.l.c. examination showed both extracts to be identical. Hence the extracts were combined and evaporated to give a yellow gummy residue (248.0 g). The residue was refluxed (48 h) with methanol and filtered to remove insoluble polymeric material. The methanol extract was evaporated to dryness (28.0 g) and chromatographed in light petroleum on silica gel (25—70 mesh, 450 g). Elution of the column with light petroleum gave an unidentified triterpene (1) (0.04 g), m.p. 236—238 °C, $[\alpha]_D$ −35.0° (*c*, 1.0); light petroleum–2% ethyl acetate gave *lupa*-5,20(29)-*dien*-3-*one* (2) (0.04 g), m.p. 214—216 °C, $[\alpha]_D$ +64.4° (*c*, 1.0) (Found: C, 85.35; H, 11.0. $C_{30}H_{46}O$ requires C, 85.24; H, 10.96%); $\nu_{max.}$ 1 705, 1 450, 1 380, and 880 cm^{-1}; δ_H 5.40 (1 H, m, H-6), 4.58 and 4.71 (each 1 H, br d, $=CH_2$), 1.68 (3 H, d, *J* 1.0 Hz, vinylic Me), 1.20 (6 H, s, 2 × Me), and 1.06, 0.96, 0.83, 0.80 (s, 4 × Me); *m/e* 422 (M^+, 5%), 229(13), 218(11), 203(18), 189(19), 175(20), 161(21), 133(33), and 124(100); light petroleum–5% ethyl acetate afforded α-amyrin (0.20 g), m.p. 183—184 °C, $[\alpha]_D$ +76.4° (lit.,[16b] 186 °C, $[\alpha]_D$ +83.5°); light petroleum–10% ethyl acetate gave an unidentified triterpene (3) (0.40 g), m.p. 195—197 °C, $[\alpha]_D$ +44.8°; then β-sitosterol (0.20 g), m.p. 134—135 °C (lit.,[16c] 136—137 °C); light petroleum–15% ethyl acetate yielded 20-hydroxylupan-3-one (4) (2.80 g), m.p. 224—225 °C, $[\alpha]_D$ +30.0° (lit.,[8] 220—222 °C, $[\alpha]_D$ +27.2°); then 6β,20-*dihydroxylupan*-3-*one* (5) (0.90 g), m.p. 249—252 °C, $[\alpha]_D$ −37.5° (*c* 2.5) (Found: C, 78.4; H, 11.15. $C_{30}H_{50}O_3$ requires C, 78.55; H, 10.98%); $\nu_{max.}$ 3 450, 1 695, and 1 380 cm^{-1}; δ_H ($CDCl_3$) 4.46 (1 H, m, $W_{\frac{1}{2}}$ 9.0 Hz, 6-H), 1.43 (6 H, s, 2 × Me), and 1.40, 1.23, 1.15, 1.13, 0.93, and 0.83 (each 3 H, s, 6 × Me); *m/e* 440 ($M^+ - H_2O$, 36%), 218(36), 205(62), 203(70), 189(64), 175(46), 163(30), 149(68), 147(46), 135(61), 133(51), 123(61), and 121(90); light petroleum–20% ethyl acetate yielded 3β,6β-*dihydroxylup*-20(29)-*ene* (6) (0.09 g), m.p. 196—198 °C, $[\alpha]_D$ +4.0° (*c* 2.5) (Found: M^+, 442.58. $C_{30}H_{50}O_2$ requires *M*, 442.59); $\nu_{max.}$ 3 400, 1 360, and 880 cm^{-1}; δ_H ($CDCl_3$) 4.70 (1 H, br d, C=CH*H*), 4.56 (2 H, m, C=C*H*H and H-6), 3.16 (1 H, m, $W_{\frac{1}{2}}$ 19.0 Hz, H-3), 1.66 (3 H, d, *J* 1.0 Hz, vinylic Me), and 1.36, 1.22, 1.15, 1.05, 0.92, 0.80 (each 3 H, s, 6 × Me); *m/e* 442 (M^+, 7%), 218(16), 205(23), 203(27), 189(30), 187(40), 175(19), 161(21), 149(25), and 123(90); then 3β,20-dihydroxylupane (7) (0.15 g), m.p. 230—232 °C, $[\alpha]_D$ +26° (*c* 1.0) (lit.,[8] 237—238 °C, $[\alpha]_D$ +27.5°); light petroleum–25% ethyl acetate afforded pristimerin (4.00 g), m.p. 205 °C (lit.,[21] 197 °C), $[\alpha]_D$ −168° (*c* 1.0); light petroleum–30% ethyl acetate gave 6β,28-*dihydroxylup*-20(29)-*en*-3-*one* (8) (1.40 g), m.p. 228—229 °C, $[\alpha]_D$ −21.3° (*c* 2.0) (Found: M^+, 456.3603. $C_{30}H_{48}O_3$ requires *M*, 456.3604); $\nu_{max.}$ 3 450, 1 690, 1 450, 1 380, 1 020, and 880 cm^{-1}; δ_H ($CDCl_3$) 4.68 and 4.58 (each 1 H, br d, $=CH_2$), 4.50 (1 H, m, H-6), 3.83 and 3.33 (each 1 H, dd, J_{AB} 12.0 Hz, CH_2OH, 28-H_2), 1.70 (3 H, br s, vinylic Me), 1.43 (9 H, s, 3 × Me), and 1.13, 0.95 (each 3 H, s, 2 × Me); *m/e* 456(M^+ 40%), 425(100), 217(14), 204(27), 203(21), 189(56), 187(25), 177(32), 175(30), 161(15), 147(25), 135(33), 133(33), 121(35), and 95(40).

Characterisation and Derivatisation of 6β,20-*Dihydroxylupan*-3-*one* (5).—(a) *Acetylation of* (5). No reaction occurred on treatment of (5) with pyridine–acetic anhydride at room temperature. Treatment of (5) with these reagents at 100 °C for 24 h yielded 6β-*acetoxylup*-20(29)-*en*-3-*one* (9) (0.075 g), m.p. 183—185 °C (from chloroform–methanol), $[\alpha]_D$ −50° (*c* 0.10) (Found: C, 79.3; H, 10.4. $C_{32}H_{50}O_3$ requires C, 79.61; H, 10.43%); $\nu_{max.}$ 1 745, 1 705, 1 380, 1 235, and 880 cm^{-1}; δ_H 5.33 (1 H, m, $W_{\frac{1}{2}}$ 10.0 Hz, H-6), 4.66 and 4.53 (each 1 H, br d, $=CH_2$), 2.00 (3 H, s, OCOMe), 1.68 (3 H, br s, vinylic Me), and 1.36, 1.27, 1.17, 1.07, 0.92, 0.80 (each 3 H, s, 6 × Me); *m/e* 482 (M^+, 3%), 440(3), 218(9), 205(19), 203(14), 189(11), 175(9), 163(2), 161(8), 149(11), 137(14), 135(14), 124(8), 121(19), 95(27), and 93(97).

(b) *Dehydration of* (5). A solution of (5) (0.05 g) in pyridine (5 ml) was refluxed with phosphoryl chloride (0.05 ml) for 30 min. *Lupa*-5,20(29)-*dien*-3-*one* (2) was obtained as white crystals (0.04 g) (from chloroform–methanol), m.p. 212—213 °C, $[\alpha]_D$ +63° (*c* 0.60).

(c) *Huang-Minlon reduction of* (5). A mixture of ethylene glycol (10 ml), hydrazine hydrate (95%, 1 ml), sodium hydroxide (0.20 g), and (5) were refluxed at 140 °C for 2 h and at 205 °C for 4 h. 6β-*Hydroxylup*-20(29)-*ene* (10) was obtained as white needles (0.06 g) (from chloroform–methanol), m.p. 174 °C, $[\alpha]_D$ +15.3° (*c* 0.22) (Found: M^+, 426.3861. $C_{30}H_{50}O$ requires *M*, 426.3860); $\nu_{max.}$ 3 400, 1 380, and 880 cm^{-1}; δ_H 4.66 (1 H, br d), 4.56 (2 H, m, $=CH_2$ and H-6), 1.68 (3 H, br s, vinylic Me) 1.36 (3 H, s, Me), 1.20 (6 H, s, 2 × Me), 0.95 (6 H, s, 2 × Me), and 0.80 (3 H, s, Me); *m/e* 426 (M^+, 100%), 233(10), 229(8), 218(18), 205(18), 203(31), 189(79), 174(17), 163(10), 161(10), 149(23), 137(14), 135(24), 124(8), 123(29), 121(28), 95(34), and 93(22).

(d) *Oxidation of* (10). Compound (10) (0.05 g) was treated with chromium trioxide–pyridine at room temperature for 12 h, to yield *lup*-20(29)-*en*-6-*one* (11) as white needles (from chloroform–light petroleum) (0.035 g), m.p. 197—198 °C, $[\alpha]_D$ 0.0° (*c* 2.15) (Found: C, 84.8; H, 11.7. $C_{30}H_{48}O$ requires C, 84.84; H, 11.39%) (Found: M^+, 424.3704. $C_{30}H_{48}O$ requires *M*, 424.3705); $\nu_{max.}$ 1 710, 1 380, and 880 cm^{-1}; δ_H 4.66 and 4.58 (each 1 H, br d, $=CH_2$), 1.68 (3 H, br s, vinylic Me), 2.36 and 1.83 (each 1 H, dd, *J* 11.0 Hz, C-7-H_2), 1.98 (1 H, s, H-5), and 1.20, 1.06,

1.03, 0.86, 0.83, and 0.78 (each 3 H, s, 6 × Me); *m/e* 424 (M^+, 14%), 218(17), 205(24), 203(19), 189(31), 175(16), 151(28), 147(26), 135(28), 123(58), and 121(40).

(e) *Oxidation of* (5). Chromium trioxide (0.06 g), pyridine (5 ml) and (5) (0.10 g) were stirred at room temperature for 12 h. White needles (0.08) of 20-*hydroxylupane*-3,6-*dione* (18) were obtained from chloroform–methanol, m.p. 193—195 °C, $[\alpha]_D$ −9.3° (*c* 0.30) (Found: C, 78.8; H, 10.15. $C_{30}H_{48}O_3$ requires C, 78.89; H, 10.59%); $\nu_{max.}$ 3 450, 1 740, 1 700, and 1 160 cm^{-1}; δ_H 2.36 (2 H, m, C-7-H_2), 2.0 (1 H, m, H-5), 1.20 (6 H, s, 2 × Me), 1.13 (6 H, s, 2 × Me), and 1.08, 1.05, 1.03, 0.80 (each 3 H, s, 4 × Me).

(f) *Dehydration of* (18). The dione (18) (0.06 g) in pyridine (5 ml) was refluxed with phosphoryl chloride (0.5 ml) for 1.5 h. White needles (0.05 g) of *lup*-20(29)-*ene*-3,6-*dione* (17) were obtained from chloroform–methanol, m.p. 174—175 °C, $[\alpha]_D$ −29° (*c* 1.3) (Found: C, 82.05; H, 10.5. $C_{30}H_{46}O_2$ requires C, 82.13; H, 10.56%); $\nu_{max.}$ 1 710, 1 700, and 880 cm^{-1}; δ_H 4.66 and 4.58 (2 H, br d, =CH_2), 2.33 (2 H, m, C-7-H_2), 2.0 (1 H, m, H-5), 1.68 (3 H, br s, vinylic Me), 1.10 (9 H, s, 3 × Me), and 1.03, 10.2, 0.80 (each 3 H, s, 4 × Me); *m/e* 438 (M^+, 15%), 370(9), 258(8), 218(8), 205(26), 203(12), 189(19), 175(13), 161(21), 149(38), 137(37), 123(52), 121(67), 109(88), and 95(100).

(g) *Acetalisation of* (5). Ethylene glycol (0.5 ml) and compound (5) (0.15 g) were refluxed in benzene (20 ml) containing toluene-*p*-sulphonic acid (0.05 g) using a Dean–Stark trap for 4 h, to afford two products. The major product was identified as 3,3-*ethylenedioxy*-6β-*hydroxylup*-20(29)-*ene* (12), white crystals (0.075 g) from methanol, m.p. 210—211 °C, $[\alpha]_D$ +11.7° (*c* 0.20) (Found: M^+, 484.3910. $C_{32}H_{52}O_3$ requires *M*, 484.3916); $\nu_{max.}$ 3 450, 1 060, and 880 cm^{-1}; δ_H 4.66 and 4.58 (each 1 H, br d, =CH_2), 3.93 (4 H, s, O[CH_2]$_2$O), 1.68 (3 H, br s, vinylic Me), 1.29 (6 H, s, 2 × Me), and 1.36, 0.96, 0.88, and 0.81 (each (3 H, s, 4 × Me); *m/e* 484 (M^+, 7%), 385(6), 383(8), 231(2), 229(2), 218(3), 205(2), 203(5), 189(3), 175(2), 163(3), 161(4), 144(4), 137(4), 135(5), 124(1), 123(5), 121(10), 99(100), 95(9), and 93(7). The second product was identified as 3,3-*ethylenedioxylupa*-5,20(29)-*diene* (13) which was crystallised from methanol (0.03 g), m.p. 225—227 °C, $[\alpha]_D$ +5.5 (*c* 0.19); $\nu_{max.}$ 1 060 and 880 cm^{-1}; δ_H 5.40 (1 H, t, *J* 3.0 Hz, H-6), 4.66 and 4.58 (each 1 H, br d, =CH_2), 3.88 (4 H, s, O[CH_2]$_2$O), 1.68 (3 H, s, vinylic Me), 1.12, 1.10, 0.98 (each 3 H, s, 3 × Me), 0.96 (6 H, s, 2 × Me), and 0.80 (3 H, s, Me).

(h) *Oxidation of the ethylene acetal* (12). A mixture of (12) (0.07 g), pyridine (5 ml), and chromium trioxide (0.10 g) was kept at room temperature for 12 h; this gave crystals (0.05 g) of 3,3-*ethylenedioxylup*-20(29)-*en*-6-*one* (14) from chloroform–methanol, m.p. 214—217 °C, $[\alpha]_D$ −12.2° (*c* 0.23) (Found: M^+, 482.3774. $C_{32}H_{50}O_3$ requires *M* 482.3756); $\nu_{max.}$ 1 700, 1 060, and 880 cm^{-1}; δ_H 4.66 and 4.58 (each 1 H, br d, =CH_2), 3.90 (4 H, s, O[CH_2]$_2$O), 1.68 (3 H, br s, vinylic Me), and 1.15, 1.13, 1.08, 0.93, 0.83, 0.80 (each 3 H, s, 6 × Me); *m/e* 482 (M^+, 6%) 438(25), 218(5), 205(19), 203(13), 189(12), 175(6), 163(7), 161(10), 149(6), 137(13), 135(23), 123(20), 121(27), 99(100), 95(33), and 93(23).

(i) *Huang-Minlon reduction of* (14). Ethylene glycol (10 ml), hydrazine hydrate (4 ml), potassium hydroxide (0.20 g), and (14) (0.77 g) were refluxed at 140 °C for 2 h, then at 205 °C for 4 h. Lupenone ethylene acetal (15) was obtained as white needles (0.04 g) from chloroform–light petroleum, m.p. 202—203 °C, $[\alpha]_D$ +3.1 (*c* 0.81); δ_H 4.68 and 4.55 (each 1 H, br d, =CH_2), 3.94 (4 H, s, O[CH_2]$_2$O), and 1.07, 1.03, 0.91, 0.87, 0.83, 0.78 (each 3 H, s, 6 × Me), shown to be identical with an authentic sample of the ethylene acetal of lupenone.

Characterisation and Derivatisation of 3β,6β-*Dihydroxylup*-20(29)-*ene* (6).—(a) *Acetylation of* (6). Treatment of (6) (0.03 g) with pyridine–acetic anhydride at 100 °C for 24 h afforded white crystals (0.03 g) of 3β,6β-*diacetoxylup*-20(29)-*ene* (16) from chloroform–methanol, m.p. 191—192 °C, $[\alpha]_D$ +3.7° (*c* 0.47) (Found: M^+, 526.4827. $C_{34}H_{54}O_4$ requires *M*, 526.4868; $\nu_{max.}$ 1 740, 1 360, 1 250, and 880 cm^{-1}; δ_H ($CDCl_3$) 5.53 (1 H, m, $W_{\frac{1}{2}}$ 8.0 Hz, H-6), 4.66 and 4.58 (each 1 H, br d, =CH_2), 4.40 (1 H, m, overlapped by signal at 4.58), 2.16 (6 H, s, OCOMe), 1.68 (3 H, br s, vinylic Me), and 1.33 (3 H), 1.23 (3 H), 1.03 (3 H), 0.90 (6 H), 0.78 (3 H) (6 × Me); *m/e* 526 (M^+, 7%), 466(8), 406(6), 258(15), 218(100), 205(14), 203(20), 189(15), 187(35), 135(11), 133(11), and 121(14).

(b) *Oxidation of* (6). Chromium trioxide (0.05 g), pyridine (5 ml), and (6) (0.04 g) were stirred at room temperature for 12 h. Dilution of the reaction mixture with cold water, extraction with diethyl ether, and successively washing with dilute hydrochloric acid (2M) and water afforded white needles (0.03 g) of *lup*-20(29)-*en*-3,6-*dione* (17) from chloroform–light petroleum, m.p. 171—172 °C, $[\alpha]_D$ −32.0° (*c* 1.5).

Characterisation and Derivatisation of 6β,28-*Dihydroxylup*-20(29)-*en*-3-*one* (8).—(a) *Acetylation of* (8). Acetic anhydride (5.0 ml), sodium acetate (0.20 g), and (8) (0.10 g) were refluxed for 24 h. White needles (0.07 g) of 6β,28-*diacetoxylup*-20(29)-*en*-3-*one* (20) were obtained from chloroform–methanol, m.p. 240—241 °C, $[\alpha]_D$ −19.0° (*c* 0.40) (Found: M^+, 540.3817. $C_{34}H_{52}O_5$ requires M^+, 540.3815); $\nu_{max.}$ 1 735, 1 725, 1 705, 1 380, 1 220, and 880 cm^{-1}; δ_H 5.40 (1 H, m, $W_{\frac{1}{2}}$ 9.0 Hz, 6-H), 4.68 and 4.58 (each 1 H, br d, =CH_2), 4.15 and 3.93 (each 1 H, dd, *J* 1.10 Hz, CH_2OH), 2.0 (6 H, s, 2 × OCOMe), 1.68 (3 H, br s, vinylic Me), and 1.38, 1.31, 1.13, 1.07, and 0.95 (each 3 H, s, 5 × Me); *m/e* 540 (M^+, 22%), 480(60), 467(36), 405(22), 218(10), 217(27), 205(25), 203(100), 189(50), 175(25), 161(19), 149(13), 135(23), 121(35), 95(33), and 93(27).

When compound (8) (0.05 g) was refluxed with acetic anhydride (5 ml) and sodium acetate (0.1 g) for 2 h, 28-*acetoxy*-6β-*hydroxylup*-20(29)-*en*-3-*one* (19) was obtained as colourless plates (0.04 g) from methanol, m.p. 111—112 °C, $[\alpha]_D$ −50° (*c* 0.10) (Found: M^+, 498.3729. $C_{32}H_{50}O_4$ requires *M*, 498.3756); $\nu_{max.}$ 3 450, 1 735, and 880 cm^{-1}; δ_H 4.66 and 4.58 (each 1 H, br d, =CH_2, C-29-H_2), 4.43 (1 H, m, $W_{\frac{1}{2}}$ 7.0 Hz, H-6), 4.32 and 3.73 (each 1 H, dd, *J* 10.0 Hz, CH_2OAc), 2.02 (3 H, *s*, OCOMe), 1.68 (3 H, br s, vinylic Me), and 1.45, 1.43, 1.38, 1.10, and 0.93 (each 3 H, *s*, 5 × Me); *m/e* 498 (M^+, 32%), 480(37), 425(50), 229(13), 218(20), 205(23), 203(100), 189(84), 175(31), 163(13), 161(31), 149(20), 137(12), 135(43), 124(11), 123(17), 121(56), 95(49), and 93(39).

(b) *Huang-Minlon reduction of* (8). A mixture of (8) (0.10 g), ethylene glycol (5 ml), hydrazine hydrate (100%, 1 ml), and sodium hydroxide (0.2 g) was refluxed at 140 °C for 5 h, and then at 205 °C for 4 h. Crystallisation from methanol gave 6β,28-*dihydroxylup*-20(29)-*ene* (21) as white needles (0.06 g), m.p. 212—214 °C, $[\alpha]_D$ +110° (*c* 0.10); $\nu_{max.}$ 3 450 and 880 cm^{-1}; δ_H 4.66 and 4.58 (each 1 H, br d, =CH_2), 3.70 and 3.28 (each 1 H, dd, *J* 10.0 Hz, CH_2OH), 1.68 (3 H, br s, vinylic Me), 1.36 (6 H, s, 2 × Me), 1.20 (3 H, s, Me), and 0.96 (6 H, s, 2 × Me) (Found: M^+, 442.3823. $C_{30}H_{50}O_2$ requires *M*, 442.3811); *m/e* 442 (M^+, 40%), 424(31), 411(32), 233(20), 229(7), 218(4), 216(84), 205(25), 203(37),

201(40), 191(57), 189(100), 185(80), 177(39), 175(31), 161(19), 147(28), 149(15), 137(20), 135(31), 133(37), 123(50), and 121(27).

Reduction with Lithium–Ethylenediamine.—(a) *Reduction of* 6β-*acetoxylup*-20(29)-*en*-3-*one* (9). Compound (9) (0.88 g) was refluxed with fresh lithium metal (0.20 g) in ethylenediamine (10 ml) until a blue colour appeared. The mixture was kept at this temperature for 20 min. Excess of lithium was destroyed by the addition of t-butyl alcohol. Dilution with cold water, extraction with diethyl ether, and successively washing with dilute hydrochloric acid (2N) and water yielded a yellowish solid which consisted of three components. Two components were isolated by preparative t.l.c. The more polar compound, 3β,6β-*dihydroxylupane* (22) was obtained as white needles (0.04 g) from chloroform–methanol, m.p. 220—221 °C, $[\alpha]_D$ −28.5° (*c* 0.36) (Found: M^+, 444.3934. $C_{30}H_{52}O_2$ requires M, 444.3967); $\nu_{max.}$ 3 400 and 750 cm^{-1}; δ_H 4.53 (1 H, m, $W_{\frac{1}{2}}$ 10.0 Hz, 6-H), 3.12 (1 H, m, $W_{\frac{1}{2}}$ 20.0 Hz, 3-H), 1.40, 1.23, 1.16, 1.13, 1.06, 0.93, 0.80, and 0.76 (each 3 H, s, 8 × Me); *m/e* 444 (M^+, 24%), 231(14), 220(12), 218(8), 205(50), 203(8), 191(33), 189(17), 187(66), 175(10), 163(23), 161(8), 149(28), 137(17), 135(19), 124(12), 123(100), 121(22), 95(33), and 93(14).

The less polar compound was crystallised (0.015 g) from light petroleum and identified as 3β-hydroxylupane (23), m.p. 203—204 °C, $[\alpha]_D$ −20.0° (*c* 0.50) (lit.,[22] 201—202 °C, $[\alpha]_D$ −17.8°); $\nu_{max.}$ 3 450 cm^{-1}; δ_H 3.15 (1 H, m, $W_{\frac{1}{2}}$ 20.0 Hz, H-3), and 1.03 (3 H), 0.95 (6 H), 0.92 (3 H), 0.83 (3 H), 0.78 (9 H) (8 × Me).

(b) *Reduction of* 6β-28-*diacetoxylup*-20(29)-*en*-3-*one* (20). The diacetate (20) (0.08 g) was treated with ethylenediamine (10 ml) and fresh lithium metal as described in (a) above. The usual work-up afforded a brownish residue, consisting of three components. These were separated by preparative t.l.c. The least polar compound was crystallised (0.015 g) from light petroleum and identified as 3β-hydroxylupane (23) m.p. 203—204 °C, $[\alpha]_D$ −18.4° (*c* 0.40). The next compound was crystallised from chloroform–methanol (0.015 g) and found to be identical with (22), m.p. 224—225 °C, $[\alpha]_D$ −33.3°.

The most polar compound was crystallised from methanol (0.02 g) and identified as 3β,28-dihydroxylupane (24), m.p. 275—276 °C, $[\alpha]_D$ +32.0° (*c* 0.22) (Found: M^+, 444.3971. $C_{30}H_{52}O_2$ requires M, 444.3967); $\nu_{max.}$ 3 275—3 375 cm^{-1}; δ_H 3.83 and 3.30 (each 1 H, *J* 11.0 Hz, CH_2OH), 1.06 (3 H, s, Me), 1.00 (9 H, s, 3 × Me), 0.86 (6 H, s, 2 × Me), and 0.77 (3 H, s, Me); *m/e* 444 (M^+, 4%), 426(9), 413(24), 408(18), 365(28), 247(17), 231(9), 229(40), 218(10), 217(15), 207(45), 205(25), 203(35), 191(75), 189(100), 177(39), 175(34), 161(40), 149(36), 147(40), 135(86), 123(40), 121(70), and 123 (41).

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