Perulactone, a New Ergostane-type Steroid from *Physalis peruviana* (Solanaceae)

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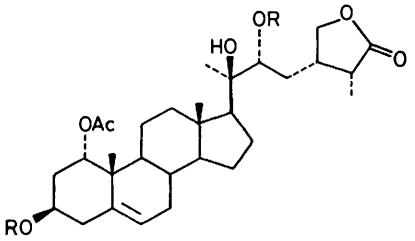
Perulactone, $C_{30}H_{46}O_7$, is the naturally occurring 1-acetate of (20*R*,22*R*,24*S*,25*R*)-1α,3β,20,22-tetrahydroxyergost-5-en-26,28-olide. It was isolated from a variety of *Physalis peruviana* growing in India (Varanasi). The carbocyclic moiety was identified by cleavage of the 20,22-bond to give 1α-acetoxy-3β-hydroxypregn-5-en-20-one. The structure assigned to the side-chain is based on chemical and spectroscopic evidence. This is the first instance when a steroid oxidised at C-28 has been isolated from a Solanaceae plant. Treatment of perulactone with base afford, after acetylation, the corresponding equilibration product (7) (γ-lactone), but no trace of the isomeric 26,22-olide (δ-lactone).

In a recent review [1] summarising the chemistry of the withanolides and related compounds, two biogenetic schemes were proposed, one for the elaboration of the substitution pattern of rings A and B, and another one for the oxidative processes taking place in the side-chain. The only missing link between the common Δ^5-3β-ol system and the AB ring systems present in the highly oxidised compounds isolated from Solanaceous plants was the product of hydroxylation at C-1; a compound possessing such a substitution pattern has recently been isolated from *Withania somnifera*.[2] In the side-chain, the only position where oxidation has never been previously encountered was C-28.

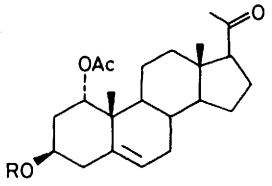
RESULTS AND DISCUSSION

Perulactone, $C_{30}H_{46}O_7$, an ergostane-type steroid related to the withanolides, which was isolated from a variety of *Physalis peruviana* growing in India (around Varanasi, Uttar Pradesh), possesses a hydroxy-group at C-1 (as the corresponding acetate), and another one at C-28 (as part of a γ-lactone). Perulactone is accompanied in the plant by physalolactone,[3] the 5β-hydroxy-6α-chloro-derivative of 4β-hydroxywithanolide E.[4]

The substitution pattern of rings A and B (1α-acetoxy-3β-hydroxy-5-ene) was deduced from the chemical shifts and multiplicity of the signals due to 1-H, 3-H, and 6-H (Table). The corresponding signals in the diacetates (1b) and (2b) are identical with those found in 1α,3β-diacetoxycholest-5-ene.[5] Furthermore, the ^{13}C chemical shifts of the steroidal ring carbons are in good agreement with the values calculated by adding the known effect of a 1α-oxy-function to the cholesterol values.[6] The only exception is the chemical shift of C-16 which is shielded by 6.2 p.p.m. relative to cholesterol; this shielding is due to the presence of a 20-hydroxy-group γ with respect to this carbon. The effect is analogous to that observed in the withanolides.[7] This functionality is confirmed in the ^{13}C spectrum by the presence of a relatively high-field non-protonated oxy-carbon signal, and in the ^1H spectrum by a singlet (δ 1.21) corresponding to the 21-methyl group. In addition to one methyl, one methylene, and two methine type carbons, the remainder of the side-chain should include a secondary alcohol ($^{13}CHOH$ at 74.9 p.p.m. and C*H*OH at δ 3.46; following acetylation, C*H*OAc is at δ 4.92) and a γ-lactone (^{13}CO at 180.9 p.p.m.; $\nu_{max.}$ 1 762 cm^{-1}); within the latter there is an oxymethylene group [$^{13}CH_2O$ at 72.6 p.p.m. and CH_2O at δ 4.12 and 4.45 (ABX type pattern)]. The



(1a) R = H
(1b) R = Ac

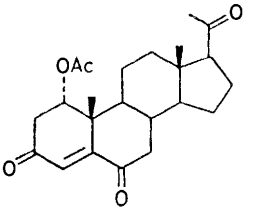


(2a) R = H
(2b) R = Ac

carbon chemical shifts and the H–H couplings are best accommodated by structure (1a).

Oxidation of (1a) with either periodic acid or activated manganese dioxide resulted in cleavage of the 20,22-glycol system, leading to the formation of 1α-acetoxy-3β-hydroxypregn-5-en-20-one (2). The circular dichroism (c.d.) curve of this compound is identical to that of pregnenolone, hence the C-17 side-chain is β-oriented. This finding is important in view of the fact that physalolactone,[5] which co-exists in the plant with perulactone, has the C-17 side-chain α-oriented. Alternatively, chromium trioxide oxidation of (1a) (Jones conditions)

afforded in one experiment (Varanasi) 1α-acetoxypregn-4-ene-3,6,20-trione (3) and the γ-lactonocarboxylic acid (8a) resulting from the side-chain of (1a). The latter was characterised as the corresponding methyl ester (8b). The ^{1}H n.m.r. assignments as shown on formula (8a) were confirmed by all possible decouplings. Irradiation of 3-H * [numbering is as given in (8b)] results in de-



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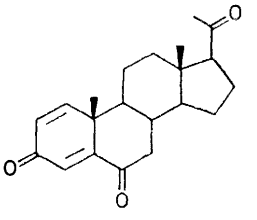

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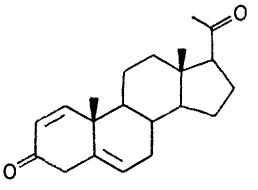

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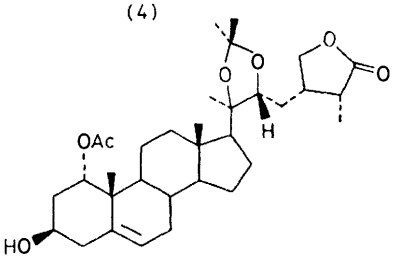

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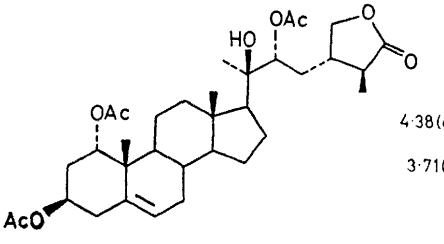
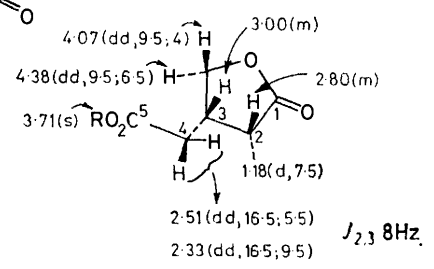


(7)

equilibration product



(8a) R = H

(8b) R = Me

coupling of the signals of both neighbouring methylene groups: the C-4 methylene gives an AB system δ 2.51 and 2.33 (J 16.5 Hz), while the C-3′ methylene gives an AB system δ 4.38 and 4.07 (J 9.5 Hz). Following irradiation of 2-Me, 2-H becomes a doublet δ 2.80 (J 8 Hz), pointing to the *cis* relationship between 2-H and 3-H.

A similar oxidation (Rehovot) afforded (3), the cross-conjugated pregna-1,4-diene-3,6,20-trione (4), and small amounts of pregna-1,5-diene-3,20-dione (5). Elimination of the 1α-acetoxy-group in (3) took place when the chloroform solution of the latter was left aside for *ca.* 30 min (see Experimental section).

The stereochemistry of the 20,22-glycol was determined by ^{1}H n.m.r. measurements of the 20,22-*O*-isopropylidene derivative (6). The *threo*-arrangement was confirmed by the procedure developed by Nakanishi and co-workers.[8] Irradiation at the resonance frequency of 22-H (δ 3.65) resulted in cancellation of the long range *W* coupling with the 21-methyl protons (δ 1.13). The height of the signal of the latter increased by 10% without any observable NOE. The same procedure was previously applied to the cyclic sulphite derivative of ixocarpalactone A.[9] The chemical shifts of 21-Me and 22-H in (6) are the same as in the 20,22-*O*-isopropylidene derivative of ponasterone A.[10]

The configuration of the two asymmetric centres (24 and 25) of the γ-lactone system is the same as in isocyasterone.[11a] Upon irradiation of the 27-methyl protons (δ 1.22, d, J 7.5 Hz) in the triacetate (1b), the multiplet due to 25-H (δ 2.68) collapsed to a doublet, $J_{24,25}$ 8 Hz, thus pointing to a *trans*-diequatorial or to a

* A referee made the observation that the chemical shift of 3-H (δ 3.00) is too low in view of its position in the molecule. A reasonable explanation of this apparent discrepancy is that in the preferred conformation of (8b) this hydrogen enters into the deshielding cone of the ester carbonyl. In compound (1a) where such a deshielding does not exist, 24-H [the equivalent of 3-H in (8b)] resonates at much higher field, together with other methine and methylene protons.

cis relationship between 24-H and 25-H; this is in contrast to the 12 Hz coupling observed in ixocarpalactone A,[9] a related steroidal γ-lactone in which the substituents are *trans* and the protons diaxial. Only a *cis* relationship of the substituents in perulactone is consistent with the high-field absorption of C-27 (10.6 p.p.m.), as compared to the equivalent carbon in ixocarpalactone A (14.4 p.p.m.); this is due to a stronger γ-interaction with a *cis* rather than with a *trans* adjacent substituent in a five-membered ring.[12]

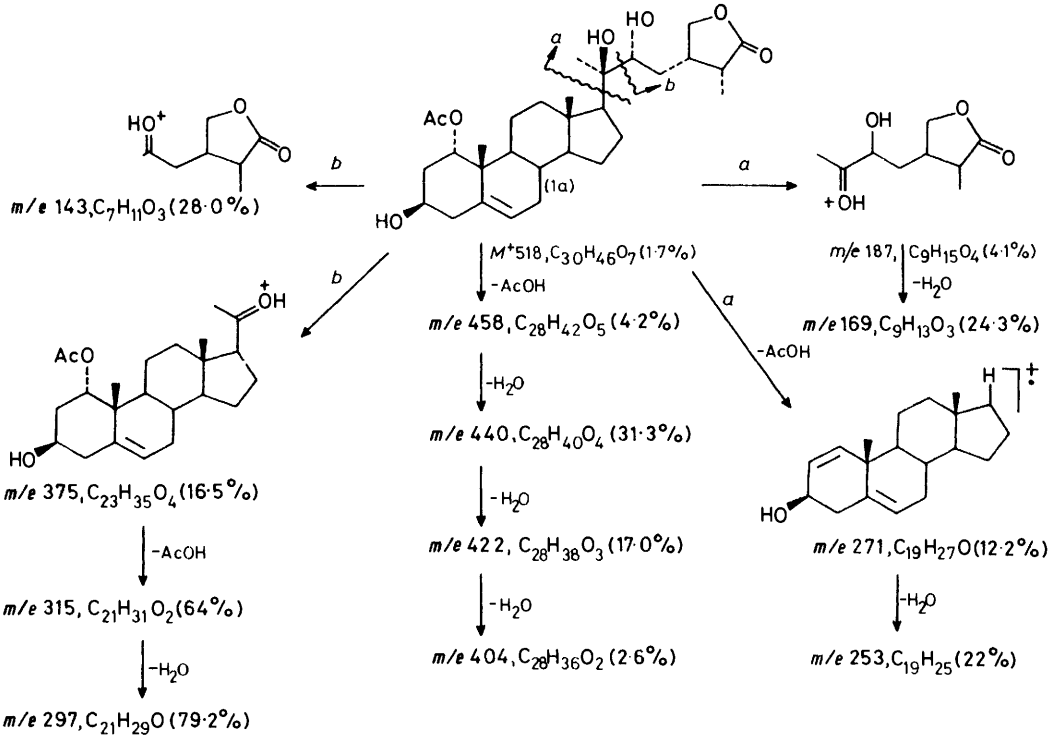
In order to confirm the *quasi*-axial orientation of the 27-Me group, compound (1a) was submitted to equilibrating conditions under basic catalysis. Following reacetylation, the 1,3,22-triacetate of 25-*epi*-perulactone (7) was obtained, in which the secondary 27-Me group resonates at slightly lower field (δ 1.26). Irradiation of this group resulted in decoupling of 25-H which appeared as a doublet, $J_{24.25}$ 12.5 Hz, thus pointing to a diaxial relationship, and consequently to the *quasi*-equatorial orientation of the methyl group. The same stereochemistry was previously assigned to sengosterone [11b] and to cyasterone.[11c]

TABLE

^{1}H N.m.r. data [a,b]

Compound	1-H	2-H	3-H	4-H	6-H	22-H	28-H$_2$	Me groups: 18-Me	19-Me	21-Me	27-Me	Other
(1a)	5.05 (t, 2.5)		3.87 (m)		5.52 (d, 5.5)	3.46 (dd, 10.5; 1.5)	4.45 (dd, 9; 7) 4.12 (dd, 9; 7.5)	0.88 (s) [1.11]	1.08 (s) [1.16]	1.22 (s) [1.49]	1.20 (d, 7) [1.26]	2.05 (s) (OAc)
(1b) [c]	5.07 (t, 2.5)		4.92 [c]		5.54 (d, 5.5)	4.92 [c]	4.28 (dd, 9; 7) 3.97 (dd, 9; 7.5)	0.86 (s)	1.08 (s)	1.25 (s)	1.22 (d, 7)	2.12 (s) (OAc) 2.06 (s) (OAc) 2.03 (s) (OAc)
(2a)	5.06 (t, 2.5)		3.87 (m)		5.51 (d,			0.62 (s)	1.08 (s)	2.11 (s)		2.05 (OAc)
(2b) [c]	5.08 (t, 2.5)		4.93 (m)		5.54 (d, 5.5)			0.62 (s)	1.09 (s)	2.11 (s)		2.07 (s) (OAc) 2.03 (s) (OAc)
(3)	5.37 (t, 4)			6.37 (d, 1)				0.68 (s)	1.26 (s)	2.13 (s)		2.06 (s) (OAc)
(4)	7.09 (d, 10.5)	6.32 (dd, (10.5; 2)		6.39 (d, 2)				0.72 (s)	1.21 (s)	2.14 (s)		
(5)	6.97 (d, 10)	5.89 (dd, (10; 1)			5.44 (m)			0.69 (s)	1.22 (s)	2.13 (s)		
(6)	5.05 (t, 2.5)		3.86 (m)		5.51 (d, 5.5)	3.65 (dd, 9.0; 3.5)	4.36 (dd, 9.5; 6.5) 4.18 (dd, 9.5; 6.5)	0.80 (s)	1.08 (s)	1.13 (s)	1.19 (d, 7)	2.03 (s) (OAc) 1.39 (s) } acetonide- 1.28 (s) } Me groups
(7)	5.07 (t, 2.5)		4.92 [c]		5.53 (d, 5.5)	4.92 [c]	4.33 (dd, 9; 7.5) 3.83 (t, 9)	0.87 (s)	1.08 (s)	1.25 (s)	1.26 (d, 8)	2.11 (s) (OAc) 2.09 (s) (OAc) 2.05 (s) (OAc)

[a] Recorded at 270 MHz; solvent $CDCl_3$; δ values; multiplicities and coupling constants (Hz) in parentheses; data for solutions in C_5D_5N in square brackets. [b] Values found (270 MHz) in 1α,3β-dihydroxycholest-5-ene; 1-H, δ 3.84 (t); 3-H δ 3.98 (m); 5 H δ 5.59 (d); 19 H δ 1.03 (s) (gift from Professor N. Ikekawa [5]) and in 1α,3β-diacetoxycholest-5-ene: 1-H δ 5.06 (t); 3-H δ 4.92 (m); 5-H δ 5.53 (dd); 19-H 1.08 (s) (gift from Professor Y. Mazur [6]). [c] Partial overlap.



SCHEME Fragmention under electron impact of perulactone (1a)

According to the data presented above, perulactone is (20*R*,22*R*,24*S*,25*R*)-1α-acetoxy-3β,20,22-trihydroxyergost-5-en-26,28-olide.

Supporting evidence was obtained by the fragmentation under electron impact (high resolution) of perulactone, as shown in the Scheme.

EXPERIMENTAL

M.p.s were taken with a Fisher-Johns apparatus. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter and refer to solutions in chloroform, unless otherwise stated. C.d. measurements were performed with a Cary 60 instrument for solutions in acetonitrile. I.r. spectra were recorded on a Perkin-Elmer Infracord 137 spectrophotometer and refer to chloroform solutions; u.v. spectra were recorded on a Cary 14 instrument for solutions in ethanol; ^{1}H n.m.r. spectra were recorded at 270 MHz on a Bruker WH instrument, and ^{13}C n.m.r. spectra were recorded 22.63 MHz on a Bruker WH-90 instrument. The multiplicities of the signals were determined by a single-frequency off-resonance decoupled (SFORD) spectrum, and were confirmed by a partially-relaxed Fourier-transform experiment. The resonances of the methyl as well as of the CH-O and CH_2-O groups were unambigously correlated with the ^{1}H n.m.r. absorptions of the directly bound protons, by measuring the residual couplings in the SFORD spectrum.

Mass spectra were determined with a Varian MAT 731 high resolution mass spectrometer, under the supervision of Dr. Z.V. Zaretskii. Analyses were performed in the Microanalytical Laboratory of the Weizmann Institute, under the direction of Mr. R. Heller.

Plant Material.—The leaves of *Physalis peruviana* were collected in the month of April from the fields in the suburbs of Varanasi city, where it is cultivated for its edible berries.

Isolation of Perulactone (1a).—The air-dried leaves (5 kg) were crushed and extracted with 95% ethanol by cold percolation. The extract was concentrated under reduced pressure to a small volume (3l) and was then diluted with an equal volume of water. The solution was extracted successively with light petroleum (b.p. 60—80 °C) and with ether. The residue from the ether extract was chromatographed over silica gel (B.D.H.). Elution with benzene–ethyl acetate (7 : 3) furnished an almost homogeneous fraction which was purified by filtration through a short bed of neutral alumina (Sarabhai M.), eluting with ethyl acetate. The residue from this eluate crystallised from methanol (0.02% from the dry leaves) as needles, m.p. 239—240 °C (214—215 °C from ethyl acetate); $[\alpha]_D$ − 3.2° (CH_3CN solution; *c*, 0.20); $\nu_{max.}$ 1 762, 1 732, and 1 260 cm^{-1} (Found: M^+ 518.322 2. $C_{30}H_{46}O_7$ requires *M*, 518.323 1).

^{13}C N.m.r. ($CDCl_3$ solution to which a few drops of CH_3OH were added to improve solubility; data are in p.p.m. downfield from internal $SiMe_4$): 180.9 (C-26), 107.7 ($MeCO_2$), 137.3 (C-5), 124.1 (C-6), 77.2 (C-20), 75.3 (C-1), 74.9 (C-22), 72.6 (C-28), 66.4 (C-3), 56.7 (C-14), 54.8 (C-17), 43.3 (C-13), 42.1 (C-9), 41.3 (C-4), 40.3 (C-10), 40.1 (C-12), 38.0 and 37.9 (C-24 and C-25), 35.4 (C-2), 31.5 (C-7), 31.1 (C-8), 29.0 (C-23), 24.0 (C-15), 22.1 (C-16), 21.2 (CH_3CO_2), 20.3 (C-11), 20.2 (C-21), 19.5 (C-19), 13.5 (C-18), and 10.6 (C-27).

MnO_2 *Oxidation of Perulactone.*—A solution of (1a) (200 mg) in dry acetone (50 ml) was stirred with active MnO_2 (Merck) for 40 h. After filtration, the solution was chromatographed over silica gel. Elution with light petroleum–ethyl acetate (7 : 3) afforded 1α-acetoxy-3β-hydroxypregn-5-en-20-one (2a), (110 mg), rosettes from light petroleum–ether, m.p. 154—155 °C; $[\alpha]_D$ +56.3 (*c*, 0.3); c.d. $\Delta\varepsilon_{286\cdot8}$ +3.50; $\nu_{max.}$ 1 737, 1 700, and 1 210 cm^{-1} (Found: M^+ 374.242 3. $C_{23}H_{34}O_4$ requires *M*, 374.244 8).

HIO_4 *Oxidation of Perulactone.*—A solution of (1a) (50 mg) in ethanol (3 ml) was added to a solution of $NaIO_4$ (30 mg) in 1N H_2SO_4 (3 ml) and the mixture was warmed for 15 min at 40 °C. After dilution with water the product was extracted with ether. The residue crystallised from light petroleum–ether to give compound (2a), identical with that obtained above.

Acetylation of (2a).—This was performed with acetic anhydride–pyridine, overnight at room temperature. The product (2b) had m.p. 141—142 °C (from abs. ethanol); $\nu_{max.}$ 1 730, 1 700, 1 250, and 1 210 cm^{-1} (Found: M^+ 416.254 9. $C_{25}H_{36}O_5$ requires *M*, 416.255 3).

CrO_3 *Oxidation of Perulactone* (*Varanasi*).—A solution of (1a) (100 mg) in acetone (25 ml) was titrated with Jones reagent (0.25 ml) at room temperature (20 °C). The reaction mixture was left for 30 min, when the green chromium salt settled. The supernatant liquid was decanted off, mixed with the acetone washings, and evaporated to dryness at room temperature. The residue was chromatographed over silica gel. Elution with benzene–ethyl acetate (9 : 1) gave 1α-acetoxypregn-4-ene-3,6,20-trione (3) (45 mg), m.p. 193—194 °C (from benzene); $[\alpha]_D$ +35.6° (*c*, 0.2); $\nu_{max.}$ 1 735, 1 702s, 1 695, 1 686, 1 604, 1 240, and 1 210 cm^{-1}; $\lambda_{max.}$ 249 nm (ε 11 000). The same product was obtained by Jones oxidation of compound (2a) (Found: C, 71.4; H, 7.85%; M^+ 386.3. $C_{23}H_{30}O_5$ requires C, 71.5; H, 7.8%). Further elution with a more polar mixture afforded the liquid carboxylic acid (8a), (3*S*, 4*R*)-3-hydroxymethyl-4-methylpentane-1,5-dioic acid 5,3′-lactone which was converted with diazomethane into the liquid methyl ester (8b); $\nu_{max.}$ 1 774, 1 735, and 1 173 cm^{-1}; n.m.r. data are as indicated in formula (8b). The assignments were confirmed by all possible decouplings (Found: M^+ 172.2 $C_8H_{12}O_4$ requires *M*, 172.18).

A similar oxidation of (1a) (70 mg) (Rehovot) but for 2 min at 0 °C afforded a crude product (62 mg) which was separated on a preparative plate (silica gel PF_{254}) developed in hexane–ethyl acetate (2 : 3). The upper band (13 mg) was identified as pregna-1,4-diene-3,6,20-trione (4), $\nu_{max.}$ 1 703, 1 695, 1 684, and 1 660 cm^{-1}; $\lambda_{max.}$ 248 nm (ε 12 400). Found: M^+ 326.189 3 $C_{21}H_{26}O_3$ requires 326.187 5). The middle band was identified as (3) (27 mg). On standing in chloroform solution (spectrograde, Fluka) compound (3) decomposed to give (4). No elimination occurred when the solvent was previously filtered through basic alumina (Woelm). The lower band (8 mg) was identified as pregna-1,5-diene-3,20-dione (5); $\nu_{max.}$ 1 705 and 1 660 cm^{-1}; $\lambda_{max.}$ 234 nm (ε 11 200) (Found: C, 80.7; H, 9.0%; M^+ 312.3. $C_{21}H_{28}O_2$ requires C, 80.7; H, 9.0%).

Preparation of the 20,22-*Acetonide* (6).—Compound (1a) (60 mg) in dry acetone (50 ml) containing toluene-*p*-sulphonic acid (2 mg) was heated to reflux for 4 h. Chromatography of the crude product (57 mg) over silica gel [elution with benzene–ether (1 : 1)] gave the 20,22-*O*-isopropylidenedioxy-derivative (6), m.p. 186—187 °C (from methanol); $[\alpha]_D$ −13.6° (*c* 0.5); $\nu_{max.}$ 1 763, 1 730, and 1 260 cm^{-1} (Found: C, 71.0; H, 9.2%; M^+ − Me, 543.3, M^+ −AcOH, 498.338 2. $C_{33}H_{50}O_7$ requires C, 70.9, H, 9.0%).

Acetylation of (1a).—This was performed as above. The

product (1b) had m.p. 190—191 °C (from absolute ethanol); $\nu_{max.}$ 1 760, 1 735, 1 730, 1 260, and 1 245 cm^{-1} (Found: C, 67.6; H, 8.5%; M^+ 602.3. $C_{34}H_{50}O_9$ requires C, 67.7; H, 84%).

Alkaline Treatment of (1a).—To a solution of (1a) (20 mg) in methanol (6 ml), a methanolic solution of sodium methoxide (10 mg Na in 2 ml MeOH) was added, and the solution was heated to reflux for 6 h under N_2. The pH was then adjusted to *ca.* 5 and the product was isolated with chloroform. After acetylation, the crude product (18 mg) was chromatographed over silica gel. Elution with benzene–ether gave the acetate of 25-*epi*-perulactone (7), m.p. 196—197 °C (from absolute ethanol); $\nu_{max.}$ 1 770, 1 735, 1 730 1 260, and 1 205 cm^{-1} (Found: M^+, 602.3; M^+ —AcOH 542.324 0).

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