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PHOTOCHEMICAL TRANSFORMATIONS—XX

A PARTIAL SYNTHESIS OF CINCHOLIC ACID*

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Abstract—A partial synthesis of cincholic acid from oleanolic acid has been completed. Photolysis of the nitrite ester of 12α-hydroxyoleanolic acid acetate lactone gave *inter alia* the expected 27-oxime which, with nitrous acid, afforded the 27-nitrimine. Since this nitrimine was resistant to normal hydrolytic conditions new methods for the conversion of nitrimine to aldehyde were developed. The 27-aldehydo-12α-hydroxyoleanolic acid acetate lactone thus obtained unexpectedly existed in the open (unmasked) form. Conversion to the diacetate and oxidation gave, after methylation, the 27-carboxylic acid methyl ester. A novel reduction procedure applied to this compound then afforded cincholic acid isolated, after methylation, as its dimethyl ester. The rather limited scope of this reductive elimination procedure has been explored in other compounds of appropriate structure.

CINCHOLIC acid (Ia) has recently been isolated from *Cinchona* bark and shown[1] to be a derivative of oleanolic acid (IIa) with a carboxylic acid function at C_{14} instead of a methyl group. The present paper describes a partial synthesis of cincholic acid from oleanolic acid and thus confirms the structural findings of Tschesche *et al.*[1]

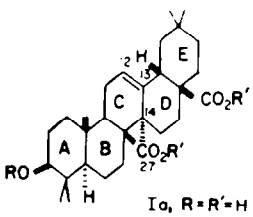
Treatment of oleanolic acid acetate (IIb) with peracetic acid afforded the known[2] 12α-hydroxyoleanolic acid acetate lactone (IIIa). In this compound the 12α-hydroxyl group is in a 1:3-diaxial relationship with the C_{14} methyl group and thus well placed for intramolecular attack. Conversion of the hydroxy-lactone to the nitrite ester (IIIb) by treatment with nitrosyl chloride in pyridine, followed by photolysis[3] in benzene solution, gave a mixture of products. Chromatography afforded the 12-ketone (IV), regenerated 12α-ol (IIIa), the expected 27-aldoxime (IIIc) and the related aldimine (IIId). The oxime showed a singlet at $\tau = 2{\cdot}44$, corresponding to the aldoxime hydrogen. Its relationship to the aldimine (IIId) is discussed further below.

The rest of the synthesis was, in principle, simple; namely, to convert the aldoxime group into a carboxyl group and to reintroduce the ethylenic linkage at $C_{12(13)}$. We consider first methods for the latter transformation. Since the Wolff-Kishner reduction of tertiary α-ketols is well known[5] to furnish the corresponding olefin we anticipated that reduction of 12-keto-oleanolic acid acetate lactone (IV), a suitable model for

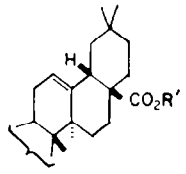
* Submitted in honour of the memory of the late Professor H. Stephen, Editor of Tetrahedron. For Part XIX see *J. Chem. Soc.* 3571 (1965).

[1] R. Tschesche, I. Duphorn and G. Snatzke, *Liebigs Ann.* **667**, 151 (1963).
[2] D. H. R. Barton and N. J. Holness, *J. Chem. Soc.* 78 (1952).
[3] D. H. R. Barton, J. M. Beaton, L. E. Geller and M. M. Pechet, *J. Amer. Chem. Soc.* **83**, 4076 (1961).
[4] Sir John Simonsen and W. C. J. Ross, *The Terpenes* Vol. V; p. 232. University Press, Cambridge (1957).
[5] See D. H. R. Barton and C. H. Robinson, *J. Chem. Soc.* 3045 (1954); and Refs. there cited.

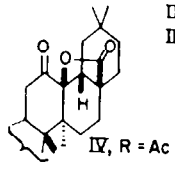
the desired transformation, would give oleanolic acid (IIa) in good yield. In the event oleananolic acid (Vb) was the only acidic product. No oleanolic acid was formed even when the reduction was carried out under nitrogen and in the presence of hydroquinone. The neutral fraction produced in the reduction contained, from mass spectrometric analysis,[6] a mixture of 3β-hydroxy-β-amyrane and 3β-hydroxy-β-amyrenes. The application of the same reducing conditions to methyl oleanolate acetate (IIc) gave oleanolic acid with no oleananolic acid. The unusual formation of oleananolic acid from the keto-lactone (IV) deserves further investigation from the mechanistic point of view.



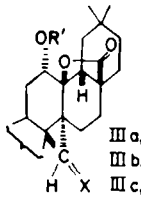
Ia, R = R' = H
Ib, R = H, R' = Me
Ic, R = Ac, R' = Me



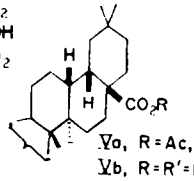
IIa, R = R' = H
IIb, R = Ac, R' = H
IIc, R = Ac, R' = Me
IId, R = H, R' = Me



IV, R = Ac

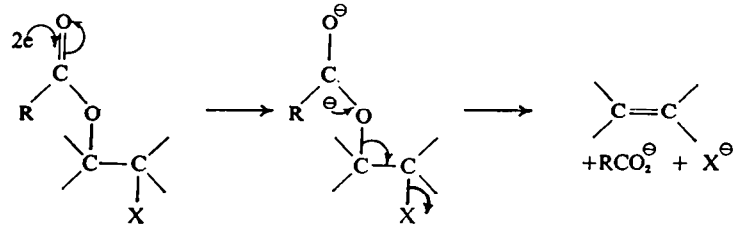


IIIa, R = Ac, R' = H, X = H_2
IIIb, R = Ac, R' = NO, X = H_2
IIIc, R = Ac, R' = H, X = NOH
IIId, R = Ac, R' = H, X = N_2O_2
IIIe, R = R' = Ac, X = H_2
IIIf, R = Ac, R' = H, X = O
IIIg, R = R' = Ac, X = O
IIIh, R = Ac, R' = $MeSO_2$
X = H_2



Va, R = Ac, R' = Me
Vb, R = R' = H

We then considered the following proposition as a method for double bond formation. In principle[7–9] an ester grouping in α-relationship to a potential anion forming group should be reducible in accordance with the following scheme:



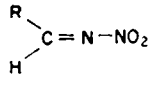
[6] Mass spectra were recorded on an A.E.I. MS9 double focussing mass spectrometer.
[7] H. Smith, *Organic Reactions in Liquid Ammonia*, Chemistry in Non-aqueous Ionizing Solvents. Vol. I, Part 2, p. 178. Interscience, New York (1963).
[8] H. Bruderer, D. Arigoni and O. Jeger, *Helv. Chim. Acta* **39**, 858 (1956).
[9] R. Howe, F. J. McQuillin and R. W. Temple, *J. Chem. Soc.* 363 (1959).

For the present objective if X were also an ester grouping ($X^{\ominus} = RCO_2^{\ominus}$) then a method for converting α-glycol diesters to the corresponding olefins would become available.

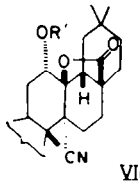
In a model experiment the lactone acetate (IIIe) was reduced with lithium or calcium in liquid ammonia to furnish oleanolic acid (IIa) in modest yield. The scope of this novel reductive process has been briefly explored and we will return to this subject later in the paper. However, the feasibility of reintroducing the $C_{12(13)}$-ethylenic linkage having been established, we can now return to the development of the synthesis.

Dehydration of the oxime (IIIc) with toluene-*p*-sulphonyl chloride in pyridine afforded the nitrile (VIa) which was resistant to both acidic and basic hydrolysis. The resistance of the nitrile group to hydrolysis must be due to extreme hindrance at the C_{27} position caused by the *cis*-fusion of rings D and E[10].

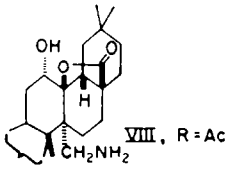
The oxime (IIIc) was also resistant to transoximation with hydrochloric acid–acetone.[11] Treatment with nitrous acid gave in good yield, not the expected aldehyde,[12] but the nitrimine (IIId), previously isolated from the photolysis of the nitrite ester (IIIb). The IR spectrum of the nitrimine showed bands at 1615, 1580 and 1380 cm^{-1} indicating, according to Freeman[14,15] a structure of type VII. Attempts to hydrolyse the nitrimine in the usual way[15] with aqueous dioxan, or urea in aqueous dioxan, were not successful. The compound also resisted hydrolysis with aqueous sulphuric acid. On pyrolysis at 240° the nitrimine was smoothly decomposed to furnish the nitrile (VIa). Reduction of the nitrimine either catalytically, or with zinc–ammonium chloride in ethanol, gave the amine (VIII) instead of the required aldehyde.



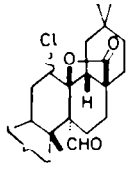
VII



VIa, R = Ac, R' = H
VIb, R = R' = Ac
VIc, R = R' = H



VIII, R = Ac



IX, R = Ac

[10] See Ref. 1 and also compare Quinovic acid, Sir John Simonsen and W. C. J. Ross, *The Terpenes* Vol. V; p. 75. University Press, Cambridge (1957).

[11] D. H. R. Barton, J. M. Beaton, L. E. Geller and M. M. Pechet, *J. Amer. Chem. Soc.* **82**, 2640 (1960).

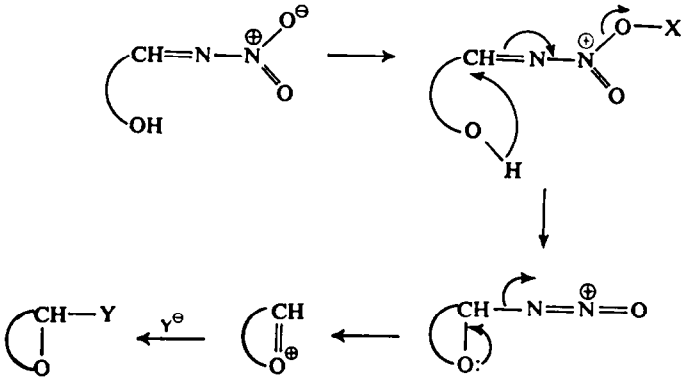
[12] D. H. R. Barton and J. M. Beaton, *J. Amer. Chem. Soc.* **82**, 2641 (1960), and **83**, 4083 (1961).

[13] See J. W. Suggitt, G. S. Myers and J. F. Wright, *J. Org. Chem.* **12**, 373 (1947) and also L. Horner, L. Hochenberger and W. Kirmse, *Chem. Ber.* **94**, 290 (1961).

[14] J. P. Freeman, *J. Org. Chem.* **26**, 4190 (1961).

[15] S. G. Brooks, R. M. Evans, G. F. H. Green, J. S. Hunt, A. G. Long, B. Mooney and L. J. Wyman, *J. Chem. Soc.* 4614 (1958).

It was clear from the above experiments that a new method for the hydrolysis of nitrimines was needed. We considered that if a nitrimine could be acylated then it would become more easily hydrolysable according to the following scheme:



In this scheme internal attack by the 12α-hydroxyl has been implicated although, in the general case, an external nucleophile would be operative. The following experiments supported our theoretical views. With methanesulphonyl chloride (X, see above = $MeSO_2$) in pyridine at 100° for 3 hr the nitrimine gave the desired hydroxy-aldehyde (IIIf) and a chlorine containing aldehyde, formulated as IX, on the basis of its IR, NMR and mass spectra. In the scheme above $Y^{\ominus}$ would be pyridine and then water in order to explain the formation of the aldehyde grouping.

The hydroxy-aldehyde (IIIf) existed in the open (unmasked form) since it had a normal IR spectrum and showed one aldehyde proton at $\tau = -0{\cdot}13$ in its NMR spectrum. This would imply that the 12α-hydroxyl and the C_{14} aldehyde cannot be in the 1:3-diaxial relationship even though the starting nitrite must have satisfied this kind of condition. Examination of molecular models of IIIf shows that a twist-boat conformation is readily available for this compound, especially because of the lactone fusion between C_{13} and C_{17}.

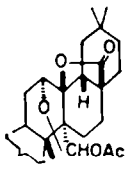
Treatment of the nitrimine (IIId) with pyridine–acetic anhydride at 100° for 3 hr gave the hemiacetal acetate (X). On selective hydrolysis over alumina this afforded the above-mentioned hydroxy-aldehyde (IIIf) there again being no evidence for the masked form of the aldehyde.[16] Acetylation of the hydroxy-aldehyde (IIIf) furnished the diacetate (IIIg) isomeric with the hemiacetal (X) described above. The formation of the hemiacetal acetate (X) implicates convincingly the 12α-hydroxyl group (in axial conformation) in the scheme for nitrimine hydrolysis outlined above. Otherwise the unmasked aldehyde diacetate would have been formed. Attempted regeneration of the oxime (IIIc) from the hydroxy-aldehyde (IIIf) showed no reaction with pyridine–hydroxylamine after 7 months at room temperature.

Oxidation of the hemi-acetal acetate (X) with chromic acid in acetone[17] gave the dilactone (XIa). Treatment of the latter with lithium and liquid ammonia afforded not cincholic acid as hoped (see above), but a trihydroxy-lactone (XIIa). On acetylation this furnished the hydroxy-diacetate (XIIb) which was resistant to attempted chromic acid oxidation. The lactone ring opened in the reduction was thus defined.

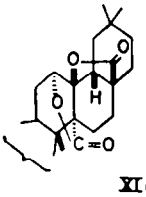
[16] See Refs. 3, 11 and 12.

[17] K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.* 39 (1946).

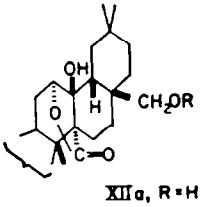
In a similar experiment the nitrile lactone (VIa) was acetylated to the diacetate (VIb) and this reduced with lithium in liquid ammonia. The product was shown to be the hemiacetal (XIIIa), oxidized by chromic acid to the corresponding 3,12-diketo-lactone. The latter was also obtained by hydrolysis of the acetate (VIa) to the corresponding diol (VIc) followed by chromic acid oxidation.



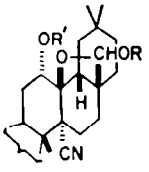
X, R = Ac



XIa; R = Ac
XIb, R = H



XIIa, R = H
XIIb, R = Ac



XIIIa, R = R' = H
XIIIb, R = Ac, R' = H

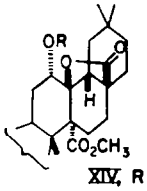
With these attempts to form the $C_{12(13)}$-ethylenic linkage defeated, another approach was adopted as follows. Prolonged treatment of the aldehyde (IIIg) with chromic acid, followed by methylation of the acidic product, afforded the ester (XIV). Reduction of this ester with lithium or calcium in liquid ammonia followed by methylation then furnished the desired cincholic acid dimethyl ester (Ib), identical with an authentic sample.[18] The identity was confirmed by acetylation to give the acetate dimethyl ester (Ic). Treatment of dimethyl cincholate (Ib) with lithium iodide in anhydrous collidine[19] gave a mixture of cincholic (Ia) and pyrocincholic (XV) acids. On titration with bromine the pyro-acid was converted to the neutral bromo-lactone[20] (XVI). Separation of the residual acidic material gave pure cincholic acid.

Dimethyl cincholate acetate (Ic) was also obtained in the following way. Chromic acid oxidation of the hydroxy-aldehyde (IIIf) gave the corresponding keto-lactone acid. Without characterization this acid was methylated with diazomethane and then reduced with zinc dust and acetic acid. The acidic product was further methylated with diazomethane to furnish the ketone dimethyl ester (XVII). Reduction with sodium borohydride gave the corresponding 12-alcohol which with methanesulphonyl chloride in pyridine afforded dimethyl cincholate acetate (Ic).

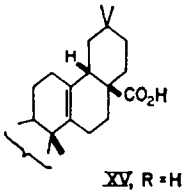
The reductive elimination of α-glycol diesters by lithium or calcium in liquid ammonia has already been referred to above. We have investigated the general scope of the reaction with results which are summarized, along with those obtained in the

[18] Kindly supplied by Professor R. Tschesche, University of Bonn, Germany.
[19] F. Elsinger, J. Schreiber and A. Eschenmoser, *Helv. Chim. Acta* **43**, 113 (1960).
[20] H. Wieland and T. Hoshino, *Liebigs Ann.* **479**, 179 (1930); H. Wieland and H. Schlenk, *Ibid.* **539**, 242 (1939).

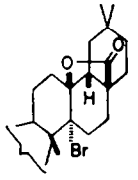
oleanane series, in the Table. From these results it is clear that for significant yields one of the esters groups should be tertiary. The yields are best when both ester groups are diaxial. The only disecondary ester affording a significant yield of olefin



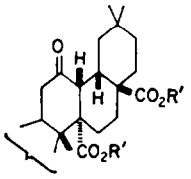
XIV, R = Ac



XV, R = H



XVI, R = H



XVII, R = Ac, R' = Me

TABLE 1

	% Olefin		% Alcohol	
	Li[a]	Ca	Li	Ca
Cholestane series				
3β,5α,6β-Triacetate[b]	72–74[c]	61	—	35[d]
3β,6β-Diacetate-5α-ol[d]	—	—	89	—
3β,5α,6α-Triacetate[e]	48	—	52[f]	—
2α,3α-Diacetate[g]	—	—	90[h]	—
2α,3β-Diacetate[g]	—	—	92[h]	—
2β,3α-Diacetate[i]	9	—	79[i]	—
Sugar series				
α-Methyl gluconate tetra-acetate	—	—	96[j]	—
α-Methyl 4,6-benzylidene-2,3-diacetyl-glucoside[k]	—	—	88[l]	—
Mannitol hexa-acetate	—	—	95[j]	—
Oleanane series				
Lactone-acetate (IIIe)	17–20	20	—	—
Lactone-mesylate (IIIh)	10	10	—	—
Lactone-acetate-27-ester (XIV)	22	22	—	—

[a] For typical reaction conditions see Experimental.
[b] R. H. Pickard and J. Yates, *J. Chem. Soc.* **93**, 1678 (1908).
[c] G. H. Alt and D. H. R. Barton, *J. Chem. Soc.* 4284 (1954).
[d] M. Davis and V. Petrow, *J. Chem. Soc.* 2536 (1949).
[e] New compound, see Experimental.
[f] A. Windaus, *Ber. Dtsch. Chem. Ges.* **41**, 611 (1908).
[g] H. B. Henbest and M. Smith, *J. Chem. Soc.* 926 (1957).
[h] C. W. Shoppee, D. N. Jones and G. H. R. Summers, *J. Chem. Soc.* 3100 (1957).
[i] R. E. Marker and L. Plambeck, *J. Amer. Chem. Soc.* **61**, 1332 (1939).
[j] Identified by reacetylation to starting material.
[k] P. S. Mathers and G. J. Robertson, *J. Chem. Soc.* 696 (1933).
[l] Identified by acetylation to α-methyl glucoside-tetra-acetate.

was the diaxial 2β,3α-diacetoxycholestane. Clearly our reductive elimination procedure is severely limited in scope, but may find useful application in certain specific cases.

EXPERIMENTAL

M.ps were determined on a Kofler block. Rotations and IR spectra were determined in $CHCl_3$ at 20–25° unless otherwise stated. UV absorption spectra were determined in EtOH solution and NMR spectra on a Varian A60 spectrometer in $CDCl_3$ solution using tetramethylsilane as an internal reference. Light petroleum refers to the fraction of b.p. 40–60°. The course of reactions and the progress of column chromatograms were followed by TLC on silica gel G.

3β-Acetoxy-13β-hydroxy-12α-nitrito-28-oleananic (28 → 13β)-lactone

By following standard procedures[8] oleanolic acid was converted into the lactone acetate (IIIa). Treatment of this (687 mg) in dry pyridine (15 ml) at −20° with excess nitrosyl chloride for 20 min followed by quenching with ice-water and ether extraction gave, after crystallization from MeOH–CH_2Cl_2, the nitrite ester (IIIb; 578 mg), m.p. 271–274°, $[\alpha]_D$ +75° (*c* 0·7).

Photolysis of the nitrite ester (IIIb)

The nitrite ester (200 mg) in dry benzene (50 ml) at 12° and under an atmosphere of dry N_2 was irradiated with a 125 watt Philips high press. Hg-arc lamp for 45 min.[8] After repeating the photolysis on further portions (2 × 200 mg) of the nitrite ester the total product was chromatographed on alumina (Grade 3; 30 gm). The least polar product, eluted with CH_2Cl_2, was IV (81 mg) obtained as needles, m.p. (from MeOH–$CHCl_3$) 284–286°, undepressed on mixed m.p. with an authentic sample. Further CH_2Cl_2-elution gave IIIa (99 mg), m.p. 292–296°. Use of MeOH–ethyl acetate (2:8) gave, initially *3β-acetoxy-12α,13β-dihydroxy-27-oximino-28-oleananic (28 → 13β)-lactone* (IIIc; 287 mg), m.p. (from MeOH) 292–295°, $[\alpha]_D$ +24·5° (*c* 0·75), ν_{max} 3550, 3300 (OH), 1770 (lactone), 1725 and 1270 (acetate), and 1635 (oxime) cm^{-1}, τ 7·92 (acetate), 6·16 (broad triplet; $C_{12}\beta$-methine) 5·48 (broad triplet; $C_3\alpha$-methine), and 2·44 (singlet; C_{27} methine). The methyl band at τ 8·85 present in the alcohol (IIIa) had disappeared. (Found: C, 70·95; H, 9·15; N, 2·6. $C_{32}H_{49}NO_6$ requires: C, 70·7; H, 9·1; N, 2·6%.) The most polar compound from the column was the *nitrimine* (IIId; 30 mg), m.p. (from $CHCl_3$–MeOH) 224° and 305–308°, $[\alpha]_D$ +3·8° (*c* 0·78), λ_{max} 206 mμ (ε 5000), ν_{max} 3450 (OH), 1770 (lactone), 1720 and 1270 (acetate) and 1615–1580 and 1305 (nitrimine) cm^{-1}. (Found: N, 5·0. $C_{32}H_{45}N_3O_7$ requires: N, 4·9%.)

Wolff-Kishner reduction[21]

Excess anhydrous hydrazine and hydroquinone (564 mg) were added to a solution of Na (415 mg) in diethylene glycol (20 ml) before adding the keto-lactone (IV, 2 = Ac; 554 mg). The mixture was heated to reflux at 180° for 16 hr. Excess hydrazine was then distilled off until the heated residual solution refluxed gently at 210°. After 24 hr the solution was cooled. Dilution with water and ether extraction gave an acidic and a neutral fraction. The acidic material was methylated to give V (160 mg), m.p. 202°, $[\alpha]_D$ +15·2° (*c* 0·39). This material gave a negative tetranitromethane test. (Found: C, 78·4; H, 11·35. $C_{31}H_{52}O_3$ requires: C, 78·75; H, 11·1%.) The neutral fraction (208 mg) had m.p. 170–180°, $[\alpha]_D$ +57° (*c* 0·9), λ_{max} 206 mμ (ε 6,720) and gave a positive tetranitromethane test. Its mass spectrum showed a parent ion peak at *M/e* 428, corresponding to β-amyranol, and also at *M/e* 426 corresponding to β-amyrin or isomer(s).

Under similar reducing conditions, but in the absence of hydroquinone and nitrogen, acetyloleanolic acid and methyl oleanolate acetate (IIc) both gave oleanolic acid, characterized as its methyl ester (IId; 75%), m.p. 198°, $[\alpha]_D$ +69° (*c* 0·7); a mixed m.p. with authentic ester was undepressed. The application of Huang-Minlon reduction conditions[22] did not affect the 12-ketone in the lactone (IV).

Metal-liquid ammonia reductions

The following is illustrative. 3β,12α-Diacetoxy-13β-hydroxy-28-oleananic (28 → 13β)-lactone (IIIe) (528 mg) in dry ether (50 ml) was added to a solution of metallic Li (435 mg) in liquid ammonia

[21] D. H. R. Barton, D. A. J. Ives and B. R. Thomas, *J. Chem. Soc.* 2056 (1955).
[22] Huang Minlon, *J. Amer. Chem. Soc.* **71**, 3301 (1949).

(50 ml) with vigorous stirring. The mixture was stirred for 30 min whilst cooling at −70° before destroying the excess Li with n-propanol (10 ml). The mixture was left at room temp until all the ammonia was evaporated and then acidified. After ether extraction the acidic product was treated with excess diazomethane. Evaporation afforded IId (17–20%), m.p. 198°, identical on comparison to an authentic sample. Repetition of the reaction using anhydrous tetrahydrofuran as solvent or with calcium metal gave similar results. The yields of olefins under various conditions and with various diacetates used are summarized in the Table.

Preparation of 3β-*acetoxy* 12α,13β-*dihydroxy*-27-*nitrile*-28-*oleananic* (28 → 13β)-*lactone and its derivatives*

The oxime (IIIc; 129 mg) in dry pyridine (5 ml) was treated at room temp for 16 hr with toluene-*p*-sulphonylchloride (280 mg). The mixture was poured into water, acidified, and extracted with CH_2Cl_2. The product crystallized from MeOH–$CHCl_3$ to give the *nitrile* (VIa; 80 mg), m.p. 305–308°, $[\alpha]_D$ +30° (*c* 0·66), ν_{max} 3500 (OH), 2260 (nitrile), 1770 (lactone), and 1720 and 1270 (acetate) cm^{-1}. (Found: C, 73·0; H, 8·8. $C_{38}H_{47}NO_5$ requires: C, 73·1; H, 9·0%.)

Treatment of the nitrile with 4N NaOH in 80% aqueous EtOH for 24 hr at reflux gave only compounds containing the nitrile function, as determined by IR spectroscopy. Similarly, treatment with diethylene glycol, in which some Na had been dissolved, at 200° under N_2 for 24 hr gave a mixture in which the nitrile group was intact.

The oxime (IIIc; 392 mg) in dry pyridine, (5 ml) and acetic anhydride (5 ml) was heated at 100° for 3 hr and then left at room temp overnight. The solution was poured into water and extracted with CH_2Cl_2 to give, on working up, the *nitrile acetate* (VIb; 341 mg), m.p. (from MeOH) 200–202°, $[\alpha]_D$ +46 (*c* 0·83), ν_{max} 2260 (nitrile), (1780 (lactone), 1745, 1725 and 1265 (acetate) cm^{-1}, τ 7·95 and 7·81 (acetates), 5·50 (broad triplet; $C_3\alpha$-methine) and 4·95 (broad triplet; $C_{12}\beta$-methine). (Found: C, 72·0; H, 8·4. $C_{34}H_{49}NO_6$ requires: C, 71·9; H, 8·7%.)

The nitrile acetate (VIb; 108 mg) in dry ether (20 ml) was added to a mixture of Li (121 mg) in liquid ammonia (20 ml). After stirring for 45 min at −70°, the excess Li was destroyed with n-propanol (10 ml), the ammonia evaporated off and the residue acidified. On working up the product gave a small amount of acidic material (3% by wt.) and a neutral fraction chromatographed over alumina (Grade III; 3 g). Elution with ethyl acetate gave 27-*nitrilo*-28-*oxo*-3β,12α,13β-*trihydroxy-oleanane* (28 → 13β)-*hemiacetal* (XIIIa; 64 mg), m.p. 275–278°, $[\alpha]_D$ +45° (*c* 0·4), ν_{max} 3600–3450 (OH), and 2250 (nitrile) cm^{-1}, τ 6·15 (broad triplet; (−12β methine) and 4·98 (singlet; C_{28} *H* hemiacetal). A similar result was obtained using Ca instead of Li. Acetylation of XIIIa at room temp, overnight, with pyridine-acetic anhydride gave 3β-*acetoxy*-12α,13β-*dihydroxy*-27-*nitrilo*-28-*oxo-oleanane* (28 → 13β)-*hemiacetal acetate* (XIIIb), m.p. (from MeOH) 292–294°, $[\alpha]_D$ +79° (*c* 0·2), ν_{max} (Nujol) 3550 (OH), 2260 (nitrile), 1725, 1250 (acetate), τ 7·96, 7·91 (acetate), 6·18 (broad triplet; $C_{12}\beta$-methine), 5·48 (broad triplet; $C_3\alpha$-methine) and 4·04 (singlet; C_{28} *H* hemiacetal acetate).

Preparation of 3,12-*dioxo*-13β-*hydroxy*-27-*nitrilo*-28-*oleananic* (28 → 13β)-*lactone*

The nitrile acetate (VIa; 160 mg) was dissolved in 2N ethanolic HCl (45 ml) and heated to reflux for 2 hr. The mixture was poured into water and extracted with CH_2Cl_2 to give 27-*nitrilo*-3β,12α,13β-*trihydroxy*-28-*oleananic* (28 → 13β)-*lactone* (VIc; 120 mg) m.p. (from benzene) 304–309°, $[\alpha]_D$ +23° (*c* 1·2), ν_{max} 3600–3470 (OH), 2260 (nitrile), and 1768 (lactone) cm^{-1}. (Found: C, 74·7; H, 9·2. $C_{30}H_{45}NO_4$ requires: C, 74·5; H, 9·4%.) Oxidation of this compound in acetone with 8N CrO_3 in 2N H_2SO_4 gave, after extraction with CH_2Cl_2 and crystallization from MeOH, 3,12-*dioxo*-13β-*hydroxy*-27-*nitrilo*-28-*oleananic* (28 → 13β)-*lactone*, m.p. 285–288°, $[\alpha]_D$ +21° (*c* 0·81), ν_{max} 2260 (nitrile), 1780 (lactone), and 1715–1700 (ketones) cm^{-1}. (Found: C, 75·05; H, 8·8. $C_{30}H_{41}NO_4$ requires: C, 75·1; H, 8·6%.) This compound was also prepared by oxidation, with chromic–sulphuric acid, of the hemiacetal (XIIIa).

Preparation of 3β-*acetoxy*-12α,13β-*dihydroxy*-27-*nitrimino*-28-*oleananic* (28 → 13β)-*lactone and attempted hydrolyses*

The oxime (IIIc; 120 mg) in dioxan (3 ml) and acetic acid (8 ml) was treated with a 5% solution of $NaNO_2$ aq (6 ml) at room temp with stirring for 24 hr. The precipitate was collected, washed with water, dried and recrystallized from $CHCl_3$–MeOH to give the *nitrimine* (IIId; 106 mg), m.p. 222–224° and 305–308, $[\alpha]_D$ +4 (*c* 1·0). This material was shown to be identical to the nitrimine obtained

from the photolysis (*vide supra*) by TLC and IR spectroscopy. On heating the nitrimine in aqueous dioxan at 100° for 5 hr only starting material was recovered. Similarly, treatment of the nitrimine with either urea in aqueous dioxan at reflux for 51 hr or with 10% conc H_2SO_4 in aqueous dioxan at reflux for 22 hr gave back unchanged nitrimine.

Reduction of the nitrimine

The nitrimine (IIId; 109 mg) in ethyl acetate (7 ml) was hydrogenated over PtO_2 (126 mg) until H_2 uptake (4 equivs) was complete. The product isolated was characterized as 3*β-acetoxy*-27-*amino*-12*α*,13*β-dihydroxy*-28-*oleananic* (28 → 13*β*)-*lactone* (VIII; 80 mg), m.p. (from $CHCl_3$–MeOH) 267–270° $[\alpha]_D$ +37° (*c* 0·71), ν_{max} 3500 (OH), 1760 (lactone), 1720, 1265 (acetate), 1635 (amine) cm^{-1}, τ 7·98 (acetate), 6·23 (broad triplet; $C_{13}\beta$-methine), 5·50 (broad triplet; $C_3\alpha$-methine). (Found: C, 72·8; H, 9·3. $C_{32}H_{51}NO_5$ requires: C, 72·55; H, 9·7%.) This compound was insoluble in dil HCl at room temp.

Partial reduction of IIId using Pd–C, and stopping the reduction after uptake of 1, 2 and 3 equivs of H_2, gave, in every case, only starting material and the amine. Reduction of the nitrimine with zinc dust–ammonium chloride in EtOH at reflux also gave the amine.

Acylation of the nitrimine

(a) A solution of IIId (889 mg) in dry pyridine (13·3 ml) containing methanesulphonyl chloride (3·35 ml) was heated at 100° for 3 hr. The resulting solution was poured into water and extracted with CH_2Cl_2. Chromatography of the extract through alumina (Grade III: 40 g) gave, with benzene elution, 3*β-acetoxy*-12*ξ-chloro*-13*β-hydroxy*-27-*oxo*-28-*oleananic* (28 → 13*β*)-*lactone* (IX; 160 mg), m.p. (from $CHCl_3$–MeOH) 305–307°, $[\alpha]_D$ +27° (*c* 1·4), ν_{max} (Nujol) 2750 (aldehyde), 1775 (lactone), 1720 (acetate and aldehyde) and 1245 acetate cm^{-1}, ν_{max} ($CHCl_3$) 2750 aldehyde, 1770 (lactone), 1720 (acetate and aldehyde) cm^{-1}, τ 7·93 (acetate), 5·55 (broad triplet, $C_3\alpha$-methine) and −0·13 (aldehyde). The mass spectrum showed its parent ion at *M/e* 546. (Found: C, 70·3; H, 8·7; Cl, 6·6. $C_{32}H_{47}ClO_5$ requires: C, 70·2; H, 8·65; Cl, 6·5%.) Elution of the column with benzene: ethyl acetate (95:5) afforded needles of 3*β-acetoxy*-12*α*,13*β-dihydroxy*-27-*oxo*-28-*oleananic* (28 → 13*β*)-*lactone* (IIIf), m.p. 264–269°, $[\alpha]_D$ +31° (*c* 0·8), ν_{max} 3500 (OH), 2750 (aldehyde), 1765 (lactone), 1720 (acetate, aldehyde) and 1265 (acetate) cm^{-1}, τ 7·95 (acetate), 6·03 (broad triplet; $C_{13}\beta$-methine), 5·56 (broad triplet; $C_3\alpha$-methine) and −0·13 (aldehyde). (Found: C, 72·6; H, 9·1. $C_{32}H_{48}O_6$ requires: C, 72·7; H, 9·15%.) Treatment of this compound with hydroxylamine hydrochloride in pyridine at room temp for 7 months gave back only starting material, as did treatment of the compound with hydroxylamine hydrochloride in refluxing EtOH in the presence of sodium acetate.

(b) *Acetic anhydride.* The nitrimine (526 mg) in dry pyridine (5 ml) and acetic anhydride (10 ml) was heated at 100° for 3 hr before pouring into water and extracting with CH_2Cl_2. Crystallization of the product from $CHCl_3$–MeOH gave 3*β-acetoxy*-12*α*,13*β-dihydroxy*-27-*oxo*-28-*oleananic* (27 → 12*α*) *hemiacetal acetate* (28 → 13*β*)-*lactone* (X; 41 mg), m.p. 306–308°, $[\alpha]_D$ −14° (*c* 1·2), ν_{max} (Nujol) 1775 (lactone), 1748 (hemiacetal acetate), 1730, 1245 (acetate) cm^{-1}, τ 7·95, 7·87 (acetate), 5·78 (broad triplet; $C_{13}\beta$-methine), 5·45 (broad triplet; $C_3\alpha$-methine) and 3·68 (singlet; $C_{27}H$-hemiacetal acetate). (Found: C, 71·15; H, 9·2. $C_{34}H_{50}O_7$ requires: C, 71·55; H, 8·8%.) The nitrimine was unchanged by refluxing in pyridine alone.

When the hemiacetal acetate (1·00 g) in benzene–CH_2Cl_2 (9:1) was left on a column of alumina (Grade III; 50 g) for 16 hr elution with benzene–ethyl acetate gave IIIf identical in its physical properties to the material isolated from treatment of the nitrimine with methanesulphonyl chloride in pyridine. Acetylation of this hydroxy aldehyde with acetic-anhydride in pyridine for 3 hr on the steam bath did not regenerate the hemiacetal acetate but instead afforded 3*β*,12*α-diacetoxy*-13*β-hydroxy*-27-*oxo*-28-*oleananic* (28 → 13*β*)-*lactone* (IIIg), m.p. (from MeOH) 271–274°, $[\alpha]_D$ +36° (*c* 0·9), ν_{max} (Nujol) 1780 (lactone), 1740, 1260–1240 (acetate) and 1707 (aldehyde) cm^{-1}, τ 7·95, 7·85 (acetate), 5·50 (broad triplet; $C_3\alpha$-methine), 4·75 (broad triplet; $C_{13}\beta$-methine) and −0·22 (aldehyde). (Found: C, 71·90; H, 8·9. $C_{34}H_{50}O_7$ requires: C, 71·55; H, 8·8%.)

Preparation of 3*β-acetoxy*-12*α*,13*β-dihydroxyoleanane*-27,28-*dioic* (27 → 12*α*), (28 → 13*β*)-*dilactone and its derivatives*

The hemiacetal acetate (X; 1·03 g) in acetone (400 ml) was stirred with 8N CrO_3 in 2N H_2SO_4 (40 ml) at room temp for 15 hr. Excess aqueous MeOH was added and then the mixture extracted

with CH_2Cl_2. Chromatography over alumina (Grade III; 40 g) gave, with benzene elution, the *dilactone acetate* (XIa; 381 mg) m.p. $>345°$, $[\alpha]_D$ $+5°$ (*c* 0·96), ν_{max} 1780 (lactone), 1720 and 1275 (acetate) cm^{-1}, τ 7·98 (acetate), 5·56 (broad triplet; $C_3\alpha$-methine), 5·41 (broad triplet ($C_{12}\beta$-methine). (Found: C, 72·9; H, 8·4. $C_{32}H_{46}O_6$ requires: C, 73·0; H, 8·8%.) Hydrolysis of the dilactone acetate with 2N ethanolic HCl at reflux for 3 hr gave XIb m.p. $>345°$, $[\alpha]_D$ $-1°$ (*c* 0·77), ν_{max} 3500 (OH), 1770 (lactone cm^{-1}). This compound, on acetylation with pyridine–acetic anhydride gave back the dilactone acetate.

Reduction of the dilactone acetate (95 mg) in dry ether (40 ml) with Li (123 mg) in liquid ammonia (40 ml) for 40 min and working up in the usual manner, gave 3β,12α,13β,28-*tetrahydroxy*-27-*oleananic* (27 → 12α)-*lactone* (XIIa; 63·4 mg), m.p. (from MeOH) 325–327°, $[\alpha]_D$ $+3°$ (*c* 0·6), ν_{max} 3500 (OH) and 1760 (lactone) cm^{-1}. (Found: C, 73·8; H, 10·1. $C_{30}H_{48}O_5$ requires: C, 73·7; H, 9·9%.) Acetylation of this triol with pyridine and acetic anhydride at 100° for 3 hr gave the *diacetate* (XIIb), m.p. 314–318°, $[\alpha]_D$ $+1·6°$ (*c* 0·61), ν_{max} (Nujol) 3500 (OH), 1760 (lactone), 1718, 1265 and 1245 (acetate) cm^{-1}, τ 7·98, 7·92 (acetates), 5·78 (singlet; C_{28} methylene), 5·70 (broad triplet; $C_{12}\beta$-methine) and 5·56 (broad triplet; $C_3\alpha$-methine). (Found: C, 71·1; H, 9·0. $C_{34}H_{52}O_7$ requires: C, 71·3; H, 9·15%.) This diacetate was recovered unchanged after oxidation in acetone with 8N CrO_3 in 2N H_2SO_4 at room temp for 15 min.

Preparation and reduction of methyl 3β,12α-*diacetoxy*-13β-*hydroxy*-27-*oleananate*-28-*oic* (28 → 13β)-*lactone*

3β,12α-Diacetoxy-13β-hydroxy-27-oxo-28-oleananic (28 → 13β)-lactone (IIIg; 107 mg) in acetone (20 ml) was treated with 8N CrO_3 in 2N H_2SO_4 (4 ml) at room temp, with stirring, for 5 hr. After working up in the usual manner the mixture was methylated with excess diazomethane and then chromatographed over alumina (Grade III; 25 g). Elution with benzene–ethyl acetate (98:2) gave the starting material and the *lactone ester* (XIV; 35 mg), m.p. (from MeOH) 331–333°, $[\alpha]_D$ $+33°$ (*c* 1·17), ν_{max} 1770 (lactone), 1720 (ester and acetate) and 1275 (acetate) cm^{-1}, τ 7·95, 788, (acetate), 6·37 (methyl ester), 5·56 (broad triplet; $C_3\alpha$-methine), 4·90 (broad triplet; $C_{12}\beta$-methine). (Found: C, 69·65; H, 8·9. $C_{35}H_{52}O_8$ requires: C, 70·0; H, 8·7%.)

The lactone ester (180 mg) in dry tetrahydrofuran (40 ml) was added to a mixture of Li (295 mg) in liquid ammonia (40 ml) at $-60°$ and then stirred for 30 min. After the normal workup the product was treated with excess diazomethane before chromatographing over alumina (Grade III; 15 g). Elution with benzene–ethyl acetate (97:3) gave Ib (46 mg). Crystallization from MeOH afforded needles, m.p. 214–216° $[\alpha]_D$ $+115°$ (*c* 1·2), ν_{max} 3550 (OH) and 1710 (ester) cm^{-1}, τ 6·35, 6·31 (methyl esters), and 4·25 (broad triplet; C_{12} vinyl proton). The mixed m.p. with an authentic specimen showed no depression. The same yield of dimethyl cincholate was obtained using calcium, instead of Li, in the reduction.

Acetylation of the synthetic dimethyl cincholate with pyridine–acetic anhydride at room temp, gave Ic, m.p. (from MeOH) 249–254°, $[\alpha]_D$ $+110°$ (*c* 1·05), $[\alpha]_D$ $+96°$ (pyridine, *c* 0·9), ν_{max} 1710, 1270 (ester and acetate) cm^{-1}, τ 7·95 (acetate), 6·35, 6·31 (methyl esters), 5·50 (broad triplet, $C_3\alpha$-methine) and 4·27 (C_{12} vinyl proton). The mixed m.p. with an authentic sample was undepressed and the mass spectrum of this compound was identical to that obtained from the natural material.

Preparation of cincholic acid

Dimethyl cincholate (Ib; 46 mg) in dry, freshly distilled collidine (50 ml) was refluxed for 2 hr in the presence of anhydrous LiI (1 g).[19] The resulting product, shown by TLC to be principally a mixture of cincholic and pyrocincholic acids, was dissolved in MeOH and treated dropwise with a solution of Br_2 in CCl_4 until the Br-colour just persisted. After extraction the acidic fraction was identical to authentic cincholic acid (Ia) by TLC. Methylation of the acid with diazomethane regenerated the methyl ester, characterized by mixed m.p. and comparison of the IR spectra.

Alternative route to dimethyl cincholate acetate

3β-Acetoxy-12α,13β-dihydroxy-27-oxo-28-oleananic (28 → 13β)-lactone (IIIf; 834 mg) in acetone (100 ml) was oxidized with 8N CrO_3 in 2N H_2SO_4 (10 ml) for 24 hr. Isolation of the products in the normal manner was followed by methylation with excess diazomethane and then treatment with Zn dust (2 g) in refluxing acetic acid (40 ml) for 30 hr.

The product was remethylated with diazomethane and then chromatographed over alumina (Grade III; 60 g) to give, on benzene elution, *dimethyl 3β-acetoxy-12-oxo-oleanan-27,28-dioate* (XVII; 364 mg), m.p. (from MeOH) 235–237°, $[\alpha]_D$ −21° (*c* 0·8), ν_{max} 1718 (acetate, ester and ketone) and 1275 (acetate) cm^{-1}, τ 7·98 (acetate), 6·34, 6·32 (methyl esters), 5·56 (triplet; $C_3\alpha$-methine). (Found: C, 71·3; H, 9·1. $C_{34}H_{52}O_7$ requires: C, 71·3; H, 9·15%.) The latter (90 mg) was reduced with $NaBH_4$ (100 mg) in EtOH at room temp for 28 hr. Heating of the product with methanesulphonyl chloride (1 ml) in pyridine (25 ml) at 100° for 1 hr afforded, after acidification, CH_2Cl_2 extractions and chromatography over alumina (Grade III; 6 g) and benzene elution, dimethyl cincholate acetate (Ic; 12 mg), m.p. (from MeOH) 248–252°, $[\alpha]_D$ +95° (pyridine, *c* 0·8), undepressed in m.p. on admixture with an authentic specimen.

Preparation of 3β,5α,6α-triacetoxycholestane

3β,5α,6α-Trihydroxycholestane[23] m.p. 235–238°, $[\alpha]_D$ +26·5° (dioxan, *c* 0·64) was acetylated with pyridine–acetic anhydride to give 3β,6α-diacetoxycholestan-5α-ol,[23] m.p. 188–189°, $[\alpha]_D$ +20° (*c* 0·61). This diacetate (338 mg) was further acetylated with acetic anhydride (37 ml) and toluene-*p*-sulphonic acid (130 mg) at 100° for 105 min. Ether extraction of the product, followed by chromatography on alumina (Grade III; 6 g) gave, by elution with light petroleum 3β,5α,6α-triacetoxycholestane (90 mg), m.p. (from light petroleum at −60°) 112–114°, $[\alpha]_D$ +14 (*c* 0·7), τ 8·0, 7·95, 7·91 (acetates), 5·30 (broad; $C_3\alpha$-methine) and 4·94 (broad triplet; $C_6\beta$-methine). (Found: C, 72·5; H, 9·8. $C_{33}H_{54}O_6$ requires: C, 72·5; H, 10·0%.)

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[23] A. Windaus, *Ber. Dtsch. Chem. Ges.* **41**, 611 (1908); V. Prelog and E. Tagmann, *Helv. Chim. Acta* **27**, 1867 (1944).