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THE STEREOSPECIFIC SYNTHESIS OF INOTODIOL

3β, 22R-DIHYDROXYLANOSTA-8,24-DIENE

J. P. POYSER, F. de REINACH HIRTZBACH and G. OURISSON
Laboratoire de Chimie Organique des substances Naturelles associé au C.N.R.S., Institut de chimie, Université Louis Pasteur, 1, rue Blaise Pascal, 67008-Strasbourg, France

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Abstract—Inotodiol **1a** has been stereospecifically synthesised from lanosterol in six steps and Horeau's method utilised to demonstrate the R-configuration at C-22.

Inotodiol, a triterpene diol originally isolated[1] from the birch fungus *Inonotus obliquus*, is considered to be an active component of the traditional Russian anti-cancer drug, "tchaga" featured in Solzhenitsyn's novel, "Cancer Ward".[2] We have recently shown[3] that it is 22ξ-hydroxylanosterol **1a**, an assignment which has now been confirmed by the synthesis of inotodiol. The absolute configuration at C-22 has also been determined for the first time.

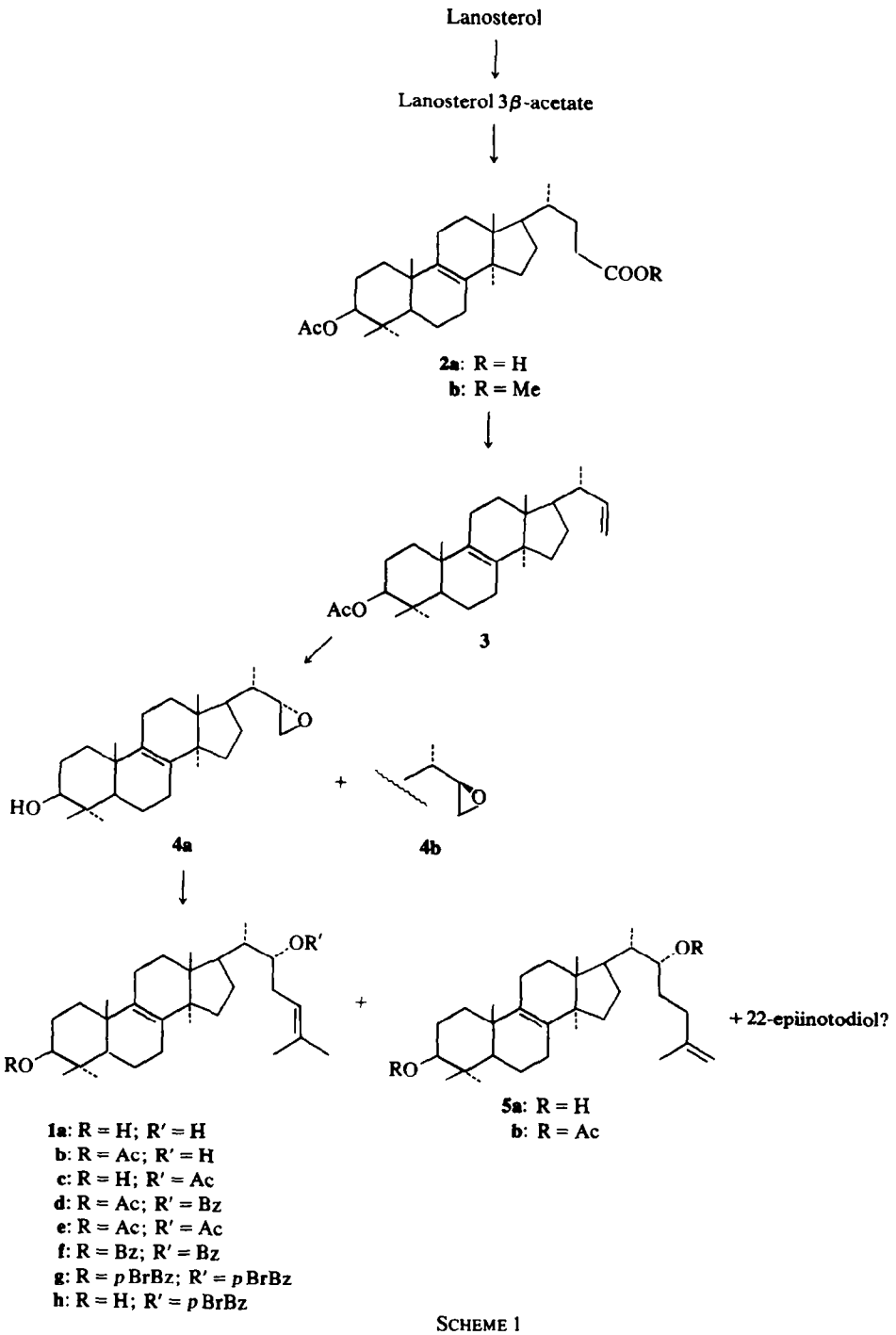
Lanosterol was converted in three steps to the tetranor-22-olefin 3 by the sequence shown in Scheme 1, analogous to that used by Sukh Dev *et al.*[4] in the cycloartenol series. The trinor-24-oic acid **2a** was identical to the compound described previously,[5] by correlation with the methyl ester **2b**. Decarboxylation using cupric acetate, lead tetracetate and pyridine in refluxing benzene gave the olefin **3** in 51% yield. This could be increased to 70% by recycling unchanged acid (contrast Ref 4).

Direct epoxidation of the side-chain double bond by peracid resulted, as expected, in a complex mixture owing to simultaneous attack on the Δ^8-bond. However, epoxidation could be effected by a two-step process. Treatment at 0° with 1·1M of N-bromosuccinimide in THF/water[6] gave the required bromohydrins. The crude mixture (three spots by TLC) was separated from polar material by filtration through silica. Cyclisation, accompanied by hydrolysis of the 3β-ester, was carried out by refluxing with sodium hydroxide in methanol. Column chromatography furnished a small amount of the acetate corresponding to **4**, followed by the epoxyalcohol **4**. Compound **4** was apparently homogeneous in our usual solvent TLC systems (ethyl acetate/hexane) even on multiple elution. In contrast, eleven elutions in toluene revealed the presence of a 5:1 mixture of two epimers (R_fs 0·12 and 0·09), the less polar being the major component. This ratio was determined by separation of the intermediate bromohydrins, and by a series of structural correlations, which are described elsewhere,[7] together with the isolation and characterisation of the pure epoxyalcohols **4** and the corresponding acetates.

One crystallisation of the epoxyalcohol mixture resulted in a product, m.p. 154–8°, containing 90% (by TLC) of the less polar epimer (m.p. 155–7° when pure).[7] Furthermore, the NMR spectra of these crystals and of the pure, major component were virtually indistinguishable. The crystals were therefore used for the final stage of the synthesis which involved stirring under argon for 60 h in THF with an excess of isobutenyl magnesium bromide. One major product was formed, the R_f of which was the same as that of authentic inotodiol (obtained by base hydrolysis of a sample of the authentic diacetate). Three elutions on TLC in ethyl acetate/hexane (1:4) revealed the presence of two minor, more polar contaminants (R_fs 0·48 and 0·45). Purification of the major product (R_f 0·51) was achieved by improved column chromatography (Experimental).

Slow crystallisation from MeOH gave large needles of synthetic inotodiol, m.p. 189–191·5° in an overall yield of 11% from lanosterol. A mixed m.p. with authentic material was undepressed. The synthetic sample exhibited spectral properties identical in all respects to those of naturally occurring inotodiol (Experimental). A comparison of the diacetates further supported this identity (synthetic sample m.p. 153·5–155·5°; mixed m.p. with authentic diacetate undepressed). Further corroborating evidence was obtained by preparation of the dibenzoate derivative, which possessed the required properties, though no sample of the authentic dibenzoate was available for direct comparison. The corresponding di-*p*-bromobenzoate was prepared, but could not be crystallised sufficiently well for X-ray analysis. A 22-mono-*p*-bromobenzoate was also isolated. The cytotoxic properties of inotodiol, reported earlier,[8] are now under investigation.

Attack by the Grignard reagent at C-23 (position more distant from the triterpene nucleus) appears to have been exclusive since no products have been



SCHEME 1

detected containing the —CH_2OH group, which would result from attack at C-22. The more polar of the by-products was not obtained pure. It is possibly 22-epi-inotodiol,* arising from attack at C-23 of the minor epoxide (see the attempts to oxidise inotodiol and the LAH treatment of the oxidation products described later).

The by-product of intermediate polarity has been assigned the isomeric double-bond structure **5a** on

*This was borne out by TLC comparison against synthetic 22-epi-inotodiol kindly provided by D. Arigoni.

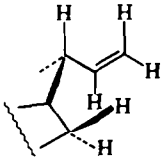
the basis of its mass spectrum and the total absence from the NMR spectrum of the 24-vinylic proton. It does present a vinylic methylene group at 5·2τ (broad singlet) and one vinylic Me only at 8·26τ (low J doublet). The diacetate **5b** of this compound had properties concordant with the assigned structure.

The problem of absolute configuration was broached by the method of Horeau.[9] Suitable substrates were elaborated by the sequence described (Experimental). Acetylation of synthetic inotodiol led predominantly to the diacetate (38%, based on inotodiol consumed), with unchanged starting material (*ca* 25%) and two other products in approximately equal proportions. They were subsequently proved to be the expected monoacetates, prepared previously[10] by hydrolysis of authentic inotodiol diacetate but assigned the erroneous 3β,12β-structures. The less polar compound **1b**, m.p. 189–191°, (26%) was recognised to be the required 3β-acetate. Its NMR spectrum closely resembled that of lanosteryl acetate (Experimental). The more polar isomer **1c** m.p. 191–193°, (28%), was the 22-acetate. It showed the necessary similarity in its NMR spectrum to lanosterol. It was identical to a sample of the monoacetate isolated as a minor product of the saponification of authentic inotodiol diacetate. A further characteristic difference between the monoacetates, **1b** and **1c**, could be seen in their mass spectra by comparison with other related compounds. It was found to be typical of those compounds bearing the free alcohol at C-22 that they exhibited a fragment ion at *m/e* 99 and a much smaller peak at *m/e* 109. This was so for inotodiol, for the by-product **5a** and for the ketone **7b**.

For 22-esters however, the favoured fragmentation resulted in the ion at *m/e* 109 with only a trace of that at *m/e* 99. Such was the case for the diacetates of inotodiol and the by-product **5a** and for inotodiol dibenzoate, the 22-benzoate **1d** and the 22-α-phenylbutyrate of **1b**. For the 3β-monoacetate **1b**, the *m/e* 99 ion was the base peak, whereas for the 22-monoacetate **1c**, it was insignificant, the *m/e* 109 ion giving rise to an intense peak. Lanosta-8,22,24-trien-3β-ol[3] also afforded the *m/e* 109 fragment in its mass spectrum. The ion is thought to have the structure **6a**. Compound **6b** may represent the *m/e* 99 fragment. The mono-*p*-bromobenzoate **1h** corresponding to **1c** also exhibited the required characteristic fragmentations.

Jones oxidation[11] of the 22-acetate **1c** gave the known ketoester **7a**[10] (wrongly assigned to be the 12β-ester). Hydrolysis of the acetate group then provided the 22-hydroxy-3-ketone **7b**, which showed Me signals in the NMR spectrum similar to lanosta - 8,24 - dien - 3 - one. Compounds **1a** and **7b** were individually subjected to esterification with racemic α-phenylbutyric anhydride according to Horeau's procedure.[9] In each case, chemical and optical yields were in the range 75–85% and 17–18%, respectively. The α-phenylbutyric acid obtained from both experiments was dextrorotatory, demonstrating that the alcohol at C-22 has the R-configuration.

The major less polar epoxyalcohol is the precursor of inotodiol and suffers no change in orientation at C-22 during the synthesis. It must therefore be the 22S-epimer **4a**. The preferential approach of electrophiles to the monosubstituted Δ^{22}-bond of the olefin **3** is, in fact, from the same side as attack on the vicinal di-substituted Δ^{22}-bond previously reported by Barton *et al.*[6] The predominance is somewhat less in the present case (5:1 compared to 13:1 for the epoxides prepared via bromohydrin formation, i.e. these are the ratios of the intermediate bromonium ions estimated from the bromohydrins). A molecular model of the tetranor-Δ^{22}-system (represented as **8**) in which $A^{1,3}$-interactions are minimised[12] indicates a slight preference for attack from the side of the 21-methyl group (e.g. of the bromonium ion to give **9**).



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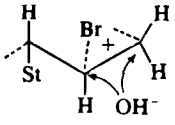

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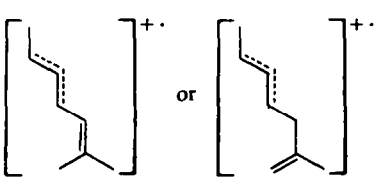
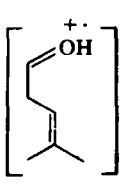
It is the 16β-hydrogen which appears to impede the approach of the electrophile from the other side. As in the case of the ergosterol-like side-chain,[6] direct epoxidation would favour the epimeric epoxide **4b**, though, as noted above, interference of the Δ^8-bond renders this method impractical.

Several observations confirmed the assignment of inotodiol as the 22R-hydroxy epimer.

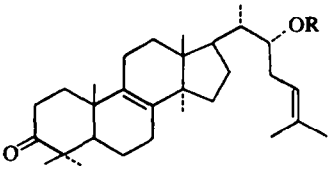
In our recent synthesis of isoinotodione[13] we reported our inability to separate the precursors



6a

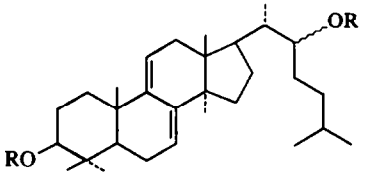


6b

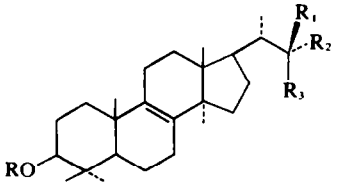


7a: R = Ac
b: R = H

either as the alcohols **10a** or acetates **10b**. The latter have now been compared by TLC (elution 4 times in toluene) with the corresponding compound derived from natural sources.[14] Both samples were found to be epimerically pure and different. The synthetic material[3] was the more polar epimer and must be 22S, since inotodiol has the 22R-configuration. There are numerous examples in the literature (see for example Ref 6) demonstrating that the 22S-epimer is the vastly predominating, *more polar* product of the reaction of alkyl magnesium halides with 20S-formyl pregnane derivatives, i.e. the "Cram product". Our findings are therefore in keeping with these observations, synthetic **10a** being the result of the reaction of isoamyl magnesium bromide with the aldehyde **11**.



10a: R = H
b: R = Ac



	R	R_1	R_2	R_3
11:	Ac	O		H
12:	Ac	O		Me
13a:	H	OH	H	Me
13b:	H	H	OH	Me
14:	Ac	H	OH	CH_2Br
18a:	Ac	OH	H	$C(CH_3)_2CH{=}CH_2$
18b:	Ac	OBz	H	$C(CH_3)_2CH{=}CH_2$

Secondly, the LAH reduction of the tetranor-22-keto-3β-acetate **12** affords a 3β,22-diol mixture **13**, in which the more polar epimer **13a** predominates (4:1). It is the less polar epimer **13b**, which is identical to the diol derivable from the least polar bromohydrin **14**, a precursor of inotodiol. There is precedent (Ref 5 and refs cited) for the preferred formation from steroidal 22-ketones of the 22S-alcohol on treatment with LAH (the "anti Cram" product). The preparation of compounds **12**, **13**, and **14** and the above transformations are fully described elsewhere.[7]

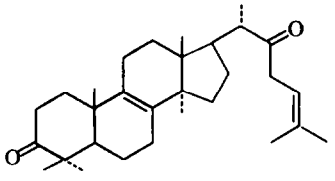
Finally it is an observed empirical rule[6,15] (so far without exception) that for epimeric pairs of 22-alcohols, the following relationship holds for the difference of molecular rotations:

$$\Delta_{R-S} = [M]_{22R} - [M]_{22S} \gg 0.$$

This is in accord with the assignments for the 3β,22ξ-diols **13**

$$[M]_{13b} = +194°;\ [M]_{13a} = +123°;\ \Delta_{R-S} = +71°.$$

In order to test this relationship for the 22ξ-hydroxylanosterols, it became incumbent to prepare 22S-hydroxylanosterol. The cytotoxicity of this compound would also merit investigation. Our efforts to isolate this epimer in a pure state from the synthesis of inotodiol have proved unsuccessful. Its preparation by alternative routes has therefore been attempted. The LAH reduction of inotodione **15** would be expected to furnish mainly the required 22S-epimer* (*cf* reduction of **12**, **16a**[6] and **16b**[6] and other examples[16]).



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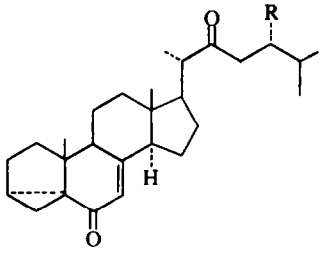
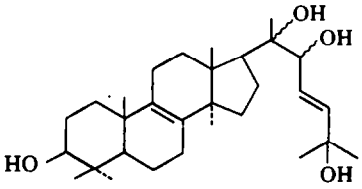


16a: R = H
b: R = Me

Inotodione has been described by one group of workers[17] as the product of the action of chromium trioxide on inotodiol, but other reports[18] have indicated the failure to isolate the pure dione by either Oppenhauer or chromic acid/acetic acid oxidations. An oxidant, silver carbonate on celite,[19] not previously utilised was therefore chosen. However after 24 h in refluxing benzene, inotodiol was recovered unchanged from the reaction medium. This is perhaps not surprising, since in the allylic oxidation of the alcohol **17** under similar conditions, the 3β-OH group did not react.[20] The 3α-hydrogen, together with the 22S-hydrogen in the case of inotodiol, must be inaccessible to the reagent. Collins' oxidation[21] of **1a** or **1b**, followed by LAH re-

*Hydride reduction of 22 - oxolanosta - 8,24 - dien - 3β - ol (obtained from the pentanor-22-oic acid) gives mainly 22S-epi-inotodiol (2:1). Private communication, D. Arigoni.

duction in ether at 0°, gave a mixture of two compounds, in which the more polar predominated by 6:4 (22S:22R). Each component was correspondingly slightly more polar than inotodiol and its more polar impurity, and appeared to be the related Δ^{23}-allylic alcohols. LAH reduction of inotodione has previously been reported[10] to be difficult, no isolable products being obtained.



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Whilst these efforts were underway, the 22-aldehyde **11**,[23,24] formed in high yield by the ozonolysis of **3** using the usual conditions[25] and a reductive work-up, was treated with the magnesium Grignard reagent derived from γ,γ-dimethylallyl chloride. As expected, the almost exclusive product was not a hydroxylanosterol, but **18a**, resulting from isomerization of the Grignard reagent and assigned the 22R($22\alpha_F$)-configuration for reasons of precedent mentioned previously. A small amount of a less polar compound, presumably the 22S($22\beta_F$)-epimer, was also formed, but not investigated further. **18a** was subjected to the normal Horeau conditions.[9] The resulting α-phenylbutyric acid was however optically inactive, perhaps due to the high steric impedance and low chemical yield. If for the purposes of molecular rotation differences, the groups $-CH_2CH{=}CMe_2$ and $-CMe_2CH{=}CH_2$ are considered to behave similarly, it is satisfactory to note that inotodiol 3β-acetate **1b** has a molecular rotation (+260°) considerably higher than the isomeric **18a** (+211°) ($\Delta_{R-'S''} = +49°$). Similarly, the difference (+67·5°) between the molecular rotations of the benzoate **1d** (+327·5°) and the alcohol **1b**, and the difference (+109°) between those of the benzoate **18b** (+318°) and the alcohol **18a** are also in accord with results found previously for epimeric alcohols and their benzoates.[6]

Naturally-occurring steroids and triterpenes of unequivocally determined absolute stereochemistry, oxygenated at the 22-position, exhibit in the vast majority of cases the β_F-configuration, e.g. 22R-hydroxycholesterol (Liliaceae),[26,16] carpesterol (Solanaceae),[27] ecdysone[28] and its numerous analogues, withaferin A (22R),[32] withacnistin (22R),[32] jaborosolactone A (22R),[33] 23-deoxyantheridiol (22R),[34] antheridiol (22S, 23R) (from the water fungus *Achlya bisexualis*[34]). Senexonol,22ξ - hydroxy - 4β - norlanosta - 8,24 - dien - 3 - one, (from *Fomes senex*[29]) would also appear to be closely related to inotodiol. In contrast, echinodol and related compounds[30,31] are fungal lanosterol derivatives, oxygenated at C-22, C-23, and C-16, assigned the $22\alpha_F$ (22R)-configuration on the basis of NMR. *Echinodontium tinctorium* (Polyporaceae), the source of echinodol, is, like *Inonotus obliquus*, reputed to possess tumour-inhibitory properties. If an echinodol derivative is the active principle, the difference of configuration from inotodiol would be of interest from the point of view of structure/activity relationships.

Oxidized lanosterol derivatives seem to occur generally in the Polyporaceae (Basidiomycetes). 23S ($23\beta_F$)-hydroxylanosterol (from *Scleroderma aurantium*,[22] is the only 23-alcohol to be found so far. In higher plants, γ-lactones of opposite configuration are known (abieslactone[35]), and both epimers have been obtained in the case of cyclograndisolide (23R(α_F)) and epicyclograndisolide (23S(β_F)).[36]

The synthetic method described above, employing the major, bromohydrin-derived, 22,23-epoxide analogues of **4a**, provides an attractive highly stereoselective route to 22R ($22\beta_F$)-alcohols (in the absence of adjacent, polar groups). Should the cytotoxic properties of inotodiol be verified on closer examination, the synthesis of 22R-alcohols in other series is envisaged.

EXPERIMENTAL

M.ps were determined on a Reichert hot-stage microscope and are uncorrected. IR spectra were taken for Nujol mulls on a Beckman IR-8 spectrometer, and optical rotations for chloroform solns on a Perkin-Elmer 141 polarimeter, unless otherwise stated. UV spectra were recorded on a Beckman DK2 or a Cary 118 apparatus. ^{1}H NMR spectra were run on a Perkin-Elmer R12B or on a Varian A60 spectrometer, with deuteriochloroform as solvent and with TMS as internal reference. Mass spectra were determined on a Thomson-Houston THN 208 or on a LKB 9000 mass spectrometer at 70 eV. Microanalyses were performed by the Strasbourg Division of the Service Central de Microanalyses of the C.N.R.S.

Column chromatography was normally effected with Merck silica gel (0·05–0·2 mm). PLC was carried out on 20 × 20 cm plates, 0·2 mm thick, using pre-coated Merck silica gel F_{254}, and TLC on 20 × 20 cm plates, 0·25 mm thick, using pre-coated Merck silica gel 60 F_{254}. The latter were also used for general analytical purposes.

Organic solvent extracts of aqueous solns were dried using $MgSO_4$. "The usual work-up" refers to dilution with water, extraction with EtOAc, washing to neutrality, drying, filtration and evaporation under vacuum. Ether refers to diethyl ether, THF to tetrahydrofuran, and LAH to lithium aluminium hydride.

Unless otherwise stated, all crystalline compounds were crystallised to constant m.p. from EtOAc/MeOH.

The following abbreviations apply to NMR data: b = broad; s = singlet; d = doublet; dd = double doublet; t = triplet; q = quartet; m = multiplet.

3β-Acetoxy-24,25,26,27-tetranorlanosta-8,22-diene **3**. A mixture of lanosterol and lanostenol (6:4; 106 g) was refluxed for 30 min in Ac_2O (800 ml). On cooling, the white ppt was filtered off at the pump, washed well with

water/MeOH (3:1), and vacuum-dried. The crystalline product was used as such for the next stage.

The acetate mixture (32 g) in CH_2Cl_2 (1400 ml) was cooled to −70° and stirred mechanically during the passage of ozonised O_2 (3 l/min). The reaction was followed to completion by GLC on an OV17 2% column at 265°. The solvent was largely removed under vacuum below 30°, replaced by acetone (800 ml), the soln cooled to 0° and Jones' reagent[11] added slowly with mechanical stirring until the mixture remained orange-brown in colour (*ca* 45 ml). MeOH (200 ml) was used to consume excess oxidant. Evaporation of the solvents at the pump, followed by the usual work-up, gave a crude product which was chromatographed on silica (750 g). Elution with chloroform afforded unchanged lanost-8-en-3β-yl acetate. $CHCl_3$/EtOAc (1:1) as eluent provided **2** (18 g; 94% with respect to the lanosteryl 3β-acetate of the starting material) as a white solid. It was characterised as the methyl ester (prepared by refluxing the acid (200 mg) in MeOH (50 ml) in the presence of one drop of BF_3-etherate, concentrating the soln and crystallising directly from the mixture) m.p. 171–2°, $[\alpha]_D$ +61° (*c* 1·41) (Lit.[5] m.p. 174–6°, $[\alpha]_D$ +58°). The IR and NMR spectra were as described previously.

The acid **2** (15·6 g) was treated according to the reported procedure[4] for 4 h under argon to effect the required decarboxylation. The usual work-up, using ether for extraction purposes and with the addition of dil HNO_3 to render soluble the inorganic material, gave a yellowish solid. Chromatography on silica (450 g) using EtOAc/hexane (5:95) yielded the *olefin* **3** (7·2 g; 51%) m.p. (MeOH) 147–8°, $[\alpha]_D$ +49° (*c* 0·8), γ_{max} 1734, 1640, 1246, 980 and 910 cm^{-1}, τ 4·05–4·7 (1H, m, 22-H), 5·03 (1H, dd, J 4 and 15 Hz), 5·25 (1H, d, J 4 Hz), 5·43 (1H, t, 3α-H), 7·97 (3H, s), 8·94, 9·00, 9·04, 9·12, and 9·29 (3H, s), *m/e* 412 (M^+), 397, 337 (M^+—Me—AcOH), 215, 81, 69, and 43 (base peak). (Found: C, 81·3; H, 10·9. $C_{28}H_{44}O_2$ requires C, 81·5; H, 10·8%).

Elution with chloroform/ethyl acetate (4:1) allowed the recovery of unreacted acid (4·1 g). With respect to acid consumed, the yield of olefin was 70%.

22ξ,23-Epoxy-24,25,26,27-tetranorlanost-8-en-3β-ols **4** and the *3β-yl acetates*

(a) Stirring of the olefin **3** (40 mg) in Na-dried ether (5 ml) at 0° with *p*-nitroperbenzoic acid (19 mg) gave a complex mixture of products.[37] This method was not investigated further.

(b) By the method described previously,[6] **3** (6·18 g; 15 mM) in THF/water (4:1; 130 ml) was treated with N-bromosuccinimide (2·84 g; 16·5 mM). After removal of solvent under reduced pressure below 40°, the usual work-up provided a crude product containing three bromohydrins (described in full elsewhere[7]). Chromatography on silica (210 g), eluting with benzene and then benzene/EtOAc (19:1), gave the bromohydrins as a mixture (4·8 g), free of polar material.

This mixture was treated in MeOH (210 ml) with NaOH (1·2 g) under reflux for 4 h. Evaporation of the solvent and the usual work-up, followed by chromatography on silica (120 g), using hexane/EtOAc (19:1 to 9:1), afforded first a small amount of the *acetate* esters corresponding to **4** (30 mg; 0·5%) as a 5:1 mixture in which the less polar epimer predominated (TLC eluted 5 times in toluene, R_fs 0·27 and 0·21), m.p. 153–6° (needles), $[\alpha]_D^{20}$ +44° (*c* 0·9), γ_{max} 1745, 1262, 1040, 917, and 841 cm^{-1}, τ 5·47 (1H, dd, J 5 and 9 Hz, 3α-H), 7·32 (2H, m), 7·6 (1H, m), 7·96 (3H, s), 8·99, 9·02, 9·12, and 9·31 (3H, s), *m/e* 428 (M^+), 413 (base peak), 353 (M^+—Me—AcOH), 281, 187, 135, 121, 119, and 107. (Found: C, 78·7; H, 10·5. $C_{28}H_{44}O_3$ requires: C, 78·5; H, 10·4%). The preparation, separation and characterisation of these epimers is described elsewhere.[7]

Further elution yielded the *epoxyalcohols* **4** as a 5:1 mixture in favour of the less polar epimer **4a**, shown by elution 11 times in toluene on TLC). Crystallisation gave a product containing 90% **4a** (2·88 g; 49·7%), m.p. 154–158°, $[\alpha]_D^{20}$ +43° (*c* 0·2), γ_{max} 3280, 1035, 904, and 834 cm^{-1}, τ 6·79 (1H, b, 3α-H), 7·3 (2H, m), 7·6 (1H, m), 9·01, 9·08 (3H, s), 9·19 (3H, s), and 9·32 (3H, s), *m/e* 386 (M^+), 371 (base peak), 353 (M^+—Me—H_2O), 107, and 95. (Found: C, 80·5; H, 11·2. $C_{26}H_{42}O_2$ requires: C, 80·8; H, 11·0%). The separation and characterisation of these epimers is described elsewhere.[7]

1-Bromo-2-methylprop-1-ene (*isobutenyl bromide*). The method used for the preparation was that of Braude and Timmons,[38] starting from 3,3-dimethylacryllic acid. In place of steam distillation, a Dean–Stark apparatus was employed for the isolation of the product.

3β,22R-Dihydroxylanosta-8,24-diene (*inotodiol*) **1a**. Mg turnings (1·276 g) were stirred magnetically in anhyd THF (33 ml) under argon, and isobutenyl bromide (6·6 ml, freshly distilled from Na) added from a syringe via a serum-cap. No reaction was observed. In a separate vessel, Mg (120 mg) and dibromoethane (200 μl) in anhyd THF (2 ml) were warmed gently until a vigorous exothermic reaction had begun, and then added to the first vessel. After 30 min, the soln became yellow-brown in colour, and after 90 min was refluxing spontaneously. All Mg had been consumed after 16 h, forming a brown-yellow ppt in a dark brown soln. The suspension was cooled to −15°, and recrystallised epoxyalcohol (1·58 g) in anhyd THF (30 ml) added dropwise. After 24 h at room temp, the mixture was cooled to −10°, and a further quantity (0·44 g) of epoxyalcohol in THF (10 ml) introduced as before. The reaction was terminated after 48 h at room temp by hydrolysis using satd NH_4Cl aq, followed by dil H_2SO_4 and the usual work-up. Initial chromatography on silica (100 g) allowed the separation of the material of R_f similar to that of authentic inotodiol (2·2 g). GLC of a silylated sample (pyridine, BSA) showed the presence of a major peak (80–90%), a minor peak (10%) and several trace impurities. TLC in EtOAc/hexane (1:4) 3 times revealed one major and two minor components (R_fs 0·51, 0·48, and 0·45, respectively). Initial estimate of the yield before final chromatography was in excess of 75%. The mixture (1 g) was chromatographed on silica G_{254} (150 g) with an applied pressure of 700 g/cm^2, eluting with EtOAc/hexane (1:4). 25 ml fractions were collected at a flow rate of 2 drops/sec. Fractions 32–35 contained pure inotodiol (257 mg), m.p. 189–191·5° (needles from MeOH), $[\alpha]_D^{20}$ + 59° (*c* 0·8), (Lit.,[1] 191–3°, $[\alpha]_D^{17\cdot5}$ + 56°), γ_{max} 3364, 1650, 1412, 1050, 1032, 890, and 865 cm^{-1}, τ 4·80 (1H, bdd, 24-H), 6·32 (1H, m, 22-H), 6·52 (s, MeOH of crystallisation), 6·76 (1H, m, 3α-H), 8·26, 8·34 (6H, bs, 26- and 27-Me), 9·01, 9·13, 9·19, and 9·28 (3H, s), *m/e* 442 (M^+), 427, 409 (M^+—Me—H_2O), 391 (409-H_2O), 372 (M^+-side-chain from C-22), 357, 339, 109, 99 (base peak), and 69. (Found: C, 79·1; H, 11·6. $C_{30}H_{50}O_2$. 0·5 MeOH requires: C, 79·9; H, 11·4%), m.p. 177–9° (from EtOAc/hexane). (Found: C, 81·2; H, 11·5. $C_{30}H_{50}O_2$ requires: C, 81·4; H, 11·4%), yield, 24·4% directly. After repurification of slightly impure fractions, the yield was 54·5%. From lanosterol, the overall yield was 11%.

An authentic sample of inotodiol was obtained by hyd-

rolysis of the authentic diacetate (21 mg) in MeOH/water (9:1; 5 ml) containing K_2CO_3 (35 mg; 0·6%) by heating under reflux for 4 h. The crude product from the usual work-up was chromatographed on silica (5 g), using EtOAc/hexane (1:9 and then 1:4). The first compound to be eluted was a small amount of the known 22-monoacetate.[10] Its NMR and mass spectra have been recorded for the first time: τ 4·95 (1H, m, 24-H), 5·10 (1H, m, 22-H), 6·75 (1H, bm, 3α-H), 8·00 (3H, s), 8·35 (6H, bs), 9·01, 9·13, 9·19, and 9·31 (3H, s), *m/e* 484 (M^+), 469, 424, 409 (M^+—Me—AcOH; base peak), 391 (409-H_2O), 327, 109, and 69. Further elution gave authentic inotodiol (13 mg; 73%) as large needles, m.p. 181–6° (from MeOH), admixture with synthetic inotodiol m.p. 183–7°, γ_{max} 3358, 1650, 1414, 1052, 1031, 890, and 866 cm^{-1}. Its NMR spectrum differed from that recorded above for the synthetic material only in that MeOH of crystallisation was not observed. Its mass spectrum was identical to that of synthetic inotodiol in all respects. The R_fs of both samples were identical in a variety of solvent systems.

3β,22R-*Dihydroxylanosta*-8,25-*diene* **5a**. From the purification of synthetic inotodiol on silica under pressure, several fractions were obtained containing a more polar contaminant only. Evaporation gave the *diene*, m.p. 170–173·5° (as needles from MeOH), $[\alpha]_D^{17}$ + 49° (*c* 0·7), γ_{max} 3320, 1648, 1029, 888, and 618 cm^{-1}, τ 5·26 (2H, bm), 6·35 (1H, m, 22-H), 6·53 (s, MeOH of crystallisation), 6·78 (1H, m, 3α-H), 8·26 (3H, b), 9·01, 9·12, 9·19, and 9·28, *m/e* 442 (M^+), other fragments as for inotodiol except that 372, 357, and 339 peaks were of diminished intensity. (Found: C, 79·9; H, 11·3. $C_{30}H_{50}O_2$. 0·5 MeOH requires: C, 79·9; H, 11·4%. Found: 442·380. $C_{30}H_{50}O_2$ requires: 442·381).

3β,22R-*Diacetoxylanosta*-8,25-*diene* **5b**. Acetylation of the diol **5a** (10 mg) in pyridine/Ac_2O (2:1; 1 ml) overnight at room temp gave, after azeotropic removal of solvents with toluene, a *diacetate* (10 mg), m.p. 148–150° (as needles from MeOH), $[\alpha]_D^{20}$ + 47·5° (*c* 0·16), γ_{max} 1728–1723, 1645, 1255, 1019, 975, and 888 cm^{-1}. τ 5·13 (1H, m, 22-H), 5·32 (2H, bm), 5·49 (1H, m, 3α-H), 7·97 (6H, s), 8·28 (3H, b), 8·99, 9·12, and 9·30 (3H, s), *m/e* 526 (M^+), 511, 466, 451 (M^+—Me—AcOH), 391, 109, 81, and 69. (Found: C, 77·4; H, 10·5. $C_{34}H_{54}O_4$ requires: C, 77·5; H, 10·3%).

The more polar by-product of the inotodiol synthesis could not be obtained free of **5a**, but its spectral properties indicated that it may be the 22S-epiinotodiol, γ_{max} 3420, and 1030 cm^{-1}, τ 4·8 (0·5H, b, 24-H), 5·26 (1H, bm, from **5a**), 6·36 (1H, m, 22-H), 6·77 (1H, m, 3α-H), 8·26 (from **5a**), 8·35 (bs), 9·01, 9·05, 9·12, 9·18, and 9·28, *m/e* as for inotodiol, but with 353 of greater intensity (arising from **5a**).

Controlled acetylation of inotodiol **1a**. Pure synthetic inotodiol (400 mg) in pyridine (5 ml) containing Ac_2O (323 mg) was left at 0° for 14 hr. Azeotropic distillation with toluene gave a crude mixture of 4 compounds, which were separated by chromatography on silica (25 g), using EtOAc/hexane (1:19). Unreacted inotodiol (100 mg) was recovered. The first compound to be eluted was inotodiol diacetate **1e** (135 mg; 38%), obtained as platelets, m.p. 153·5–155·5°, $[\alpha]_D^{17}$ +45° (*c* 1·1) (Lit.,[1] m.p. 157·5–158·5°, $[\alpha]_D$ + 47°), γ_{max} 1737, 1730, 1245, 1030, and 980 cm^{-1}, τ 4·97 (2H, m, 24- and 22-H), 5·48 (1H, dd, 3α-H), 7·97 (3H, s, 3β-OAc), 8·00 (3H, s, 22-OAc), 8·34 (6H, b), 9·00, 9·12, and 9·31 (3H, s), *m/e* 526 (M^+), 511, 466, 451 (M^+—Me—AcOH, base peak), 391, 369, 309, 109, and 69. (Found: C, 77·5; H, 10·5. $C_{34}H_{54}O_4$ requires C, 77·5; H, 10·3%). An admixture with an authentic specimen (m.p. 150–155°) had m.p. 150–153°. The NMR and mass spectra of the authentic specimen were identical to those of the synthetic material in all respects. TLC properties of both samples were the same.

Further elution of the column gave **1b** (85 mg; 26%), m.p. 189–191° (needles), $[\alpha]_D^{17}$ + 54° (*c* 1·3), γ_{max} 3565, 1734, 1253, 1050 and 1027 cm^{-1}, (Lit.,[10] m.p. 198–201°, $[\alpha]_D^{20}$ + 57°, γ_{max} 3565 and 1730 cm^{-1}), τ 4·8 (1H, m, 24-H), 5·5 (1H, m, 3α-H), 6·35 (1H, bm, 22-H), 7·96 (3H, s), 8·27, 8·36 (6H, bs), 9·01, 9·04, 9·12, 9·22, and 9·28, *m/e* 484 (M^+), 469, 451, 414 (M^+-side-chain from C-22), 409, 399, 391, 339, 109, 99 (base peak), and 69. (Found: C, 79·5; H, 10·8. $C_{32}H_{52}O_3$ requires: C, 79·3; H, 10·8%).

The third compound to be obtained was **1c** (92 mg; 28%), m.p. 191–193° (needles), $[\alpha]_D^{17}$ + 44° (*c* 0·4), γ_{max} 3562, 1730–1723, 1253, 1037, and 1027 cm^{-1}, (Lit.,[10] m.p. 191–193°, $[\alpha]_D$ + 45°, γ_{max} 3565, 1730, and 1250 cm^{-1}), τ 4·9 (1H, m, 24-H), 5·07 (1H, m, 22-H), 6·79 (1H, bm, 3α-H), 8·00 (3H, s), 8·35 (6H, bs), 9·01, 9·13, 9·19, and 9·31, *m/e* 484 (M^+), 469, 451, 424, 409 (M^+—Me—AcOH, base peak), 391, 327, 109, and 69. (Found: C, 79·3, H, 10·9. $C_{32}H_{52}O_3$ requires: C, 79·3; H, 10·8%). Its NMR and mass spectra and TLC properties were identical to those of the authentic sample described above.

The NMR and mass spectra of these three compounds are detailed for the first time.

3β,22R-*Dibenzoyloxylanosta*-8,24-*diene* **1f**. Inotodiol **1a** (18 mg, not purified on silica G_{254}) was treated with benzoyl chloride (4 drops) in pyridine (1 ml). After standing at room temp overnight, water (2 ml) was added, the mixture stirred for several hours, and the solvents removed under vacuum. Chromatography on silica (8 g), using EtOAc/hexane (1:9), gave the dibenzoate, (21 mg; 81%), m.p. 173·5–174° (from CH_2Cl_2/MeOH), $[\alpha]_D$ + 71° (*c* 0·4), (Lit.,[1] m.p. 177·5–179·5°, $[\alpha]_D$ + 76°), γ_{max}^{KBr} 1710, 1275–1260, 1110, 969, and 710 cm^{-1}, $\lambda_{max}^{Cyclohexane}$ 228 nm (ε 30,600), τ 1·9 (4H, m), 2·5 (6H, m), 4·63–5·01 (2H, m, 24- and 22-H), 5·26 (1H, m, 3α-H), 8·24, 8·36 (6H, bs), 8·95, 9·03, 9·08, and 9·28 (3H, s), *m/e* 650 (M^+), 635, 528, 513, 391 (M^+—Me—2$C_6H_5CO_2H$), 297, 255, 109, 105 ($C_6H_5CO^+$, base peak), and 69. (Found: C, 81·1; H, 9·2. $C_{44}H_{58}O_4$ requires: C, 81·2; H, 9·1%. Found: 650·434, 528·399 and 513·373. $C_{44}H_{58}O_4$, $C_{37}H_{52}O_2$ and $C_{36}H_{49}O_2$ require: 650·433, 528·396, and 513·373, respectively). By TLC, the product was homogeneous in various systems (hexane/benzene, EtOAc/hexane). An NMR spectrum of authentic inotodiol dibenzoate measured in 1962 showed the same peaks with the same multiplicities, but with chemical shifts slightly different for the low-field protons, τ 2·05 (4H, m), 2·63 (6H, m), 4·74–5·05 (2H, m), 5·3 (1H, m), 8·23, 8·36 (6H, bs), 8·95, 9·03, 9·09, and 9·28 (3H, s).

Controlled reaction of inotodiol **1a** *with p-bromobenzoyl chloride*. Pure, synthetic inotodiol (396 mg; 0·9 mM) in pyridine (3 ml) containing *p*-bromobenzoyl chloride (394 mg; 1·8 mM) was kept at 0° for 24 h. After azeotropic distillation with toluene, a few drops of water were added, and the mixture left for 48 h. Azeotropic distillation with benzene gave a crude product which was chromatographed on silica (30 g), eluting with hexane/EtOAc mixtures. Five components could be detected on TLC using UV light, of which two were obtained pure by further PLC or TLC. Unchanged inotodiol (173 mg) was recovered.

The least polar component proved to be 3β,22R-*di*-*p-bromobenzoyloxylanosta*-8,24-*diene* **1g** (161 mg; 40%), obtained as platelets, m.p. 159–162°, $[\alpha]_D^{17}$ + 59° (*c* 0·7), γ_{max} 1719, 1597, 1292, 1284, 1179, 1123, 1016, 859, 852, and

763 cm^{-1}, $\lambda_{max}^{Dioxane}$ 244 nm (ε 48,100), τ 2·09 (4H, d, J 8 Hz, 2'-, 2''-, 6'- and 6''-H), 2·45 (4H, d, J 8 Hz, 3'-, 3''-, 5'- and 5''-H), 4·87 (2H, bm, 24- and 22-H), 5·28 (1H, bm, 3α-H), 8·23, 8·36 (6H, bs), 8·95, 9·05, 9·08, and 9·28 (3H, s), *m/e* 810, 808, 806 (M^+), 795, 793, 791, 608, 606, 593, 591, 391 (M^+—Me—$2BrC_6H_4CO_2H$), 297, 202, 200, 185, 183 ($BrC_6H_4CO^+$, base peak), 109, and 69. (Found: C, 65·5; H, 7·1; Br, 19·8. $C_{44}H_{56}Br_2O_4$ requires: C, 65·3; H, 7·0; Br, 19·8%).

The 22R-p-*bromobenzoyloxy*-3β-*hydroxylanosta*-8,24-*diene* **1h** was obtained as needles (75 mg; 23%), m.p. 166–168·5°, $[\alpha]_D^{20}$ + 51° (*c* 0·8), γ_{max} 3410, 1714, 1590, 1276, 1176, 1115, 1103, 1012, 845, and 760 cm^{-1}, $\lambda_{max}^{Dioxane}$ 244 nm (ε 21, 100), τ 2·09 (2H, d, J 8 Hz), 2·43 (2H, d, J 8 Hz), 4·87 (2H, m, 24- and 22-H), 6·78 (1H, m, 3α-H), 8·36 (6H, bs), 8·73, 8·99, 9·10, 9·18 (3H, s), and 9·31 (3H, s), *m/e* 626, 624 (M^+), 611, 609, 593, 591, 424, 409 (M^+—Me—$BrC_6H_4CO_2H$, base peak), 391 (409–H_2O), 342, 327, 315, 185, 183 ($BrC_6H_4CO^+$), 109, and 69. (Found: C, 70·2; H, 8·7. $C_{37}H_{53}BrO_3$. 1/2 MeOH requires: C, 70·2; H, 8·6%).

22R-*Acetoxylanosta*-8,24-*dien*-3-*one* **7a**. The 22R-monoacetate **1c** (65 mg) in acetone (10 ml) at 0° was treated with Jones' reagent[11] dropwise. The crystalline residue (after work-up as described earlier) was chromatographed on silica (5 g), eluting with hexane/EtOAc (9:1), giving the ketone (49 mg; 76%) as needles, m.p. 142–4°, $[\alpha]_D^{17}$ + 60° (*c* 2·0), γ_{max} 1731, 1708, 1250, 1116, and 1024 cm^{-1}, (Lit.,[10] m.p. 141–3°, $[\alpha]_D^{23}$ + 54° (*c* 0·7), γ_{max} 1735, 1710, and 1250 cm^{-1}), τ 4·92 (1H, m, 24-H), 5·07 (1H, m, 22-H), 7·54 (2H, m, 2α- and 2β-H), 7·99 (3H, s), 8·32, 8·37, 8·89, 8·92, 8·94, 9·02, 9·12 (3H, s), and 9·28 (3H, s), *m/e* 482 (M^+), 467, 422, 407 (M^+—Me—AcOH, base peak), 340, 325, 313 (422–side-chain from C-22), 271, 255, 163, 109, 69, and 43. (Found: C, 79·6; H, 10·5. $C_{32}H_{50}O_3$ requires: C, 79·6; H, 10·4%). The NMR and mass spectra of this compound have not been previously described.

22R-*Hydroxylanosta*-8,24-*dien*-3-*one* **7b**. The acetate **7a** (40 mg) was heated for 8 h under argon in refluxing aqueous MeOH (1:9; 10 ml) containing K_2CO_3 (30 mg). After the usual work-up, the residue was chromatographed on silica (3g). Elution with EtOAc/hexane (1:9) afforded the *ketone* (23 mg; 62%) as needles, m.p. 124·5–127°, $[\alpha]_D^{20}$ + 69° (*c* 0·8), γ_{max} 3557, 1702, 1056, 1042, 1024, and 1016 cm^{-1}, τ 4·8 (1H, m, 24-H), 6·33 (1H, m, 22-H), 7·55 (2H, m), 8·26, 8·34 (6H, bs), 8·88, 8·92, 8·94, 9·02, 9·12 (3H, s), and 9·24 (3H, s), *m/e* 440 (M^+), 425, 407, 370, 355 (M^+—Me-side-chain from C-22, base peak), 337 (355-H_2O), 327, 325, 312, 297, 271, 255, 109, 99, 81, and 69. (Found: C, 82·0; H, 10·8. $C_{30}H_{48}O_2$ requires: C, 81·8; H, 11·0%).

Determination of the configuration at C-22 *of inotodiol by the method of Horeau*[9]

(a) *Applied to the* 3β-*monoacetate* **1b**. The acetate **1b** (24·2 mg) was dissolved in a pyridine soln of racemic α-phenylbutyric anhydride (0·219 mg of a soln of anhydride (653 mg) in pyridine (5 ml), total weight 4·981 g), and left for 16 h. TLC showed ester formation to be complete. A drop of water was then added and the soln heated at 90° for 30 min. Benzene (2 ml) was added, and α-phenylbutyric acid titrated with N/10 NaOH (1·33 ml) in the presence of phenolphthalein. The aqueous soln was separated, acidified, and extracted with benzene. After drying and concentrating to a volume of *ca* 1 ml, the benzene soln was weighed and its optical rotation taken.

$[\alpha]_{observed}$ + 0·064° $[\alpha]_D$ + 6·6° (*c* 0·98, benzene)
$[\alpha]_D$ theoretical ± 36° Optical yield 18%.

Evaporation of the initial benzene phase furnished the *ester* (27 mg; 86%) as an amorphous solid, γ_{max} 1730, 1722, 1600, 1256, 1168, 1023, 975, and 693 cm^{-1}, λ_{max} 218 (ε 8,900), 245 (ε 3,400) and 251 (ε 2,850), τ 2·74 (5H+, s), 4·92 (1H, m), 5·07 (1H, m), 5·47 (1H, m), 6·61 (1H, t, J 7 Hz, 2'-H), 7·98 (3H, s), 8·38, 8·52, 9·01, 9·12, 9·15, 9·33, and 9·39, *m/e* 630 (M^+), 466, 451, 391 (M^+—Me—α-φ butyric acid-AcOH), 357 (M^+–side-chain from C-17), 297, 119 ($C_6H_5C(Et)H$, base peak), 109·91 (119-C_2H_4), 69 and 43. (Found: 630·461. $C_{42}H_{62}O_4$ requires: 630·464).

(b) *Applied to the* 3-*ketone* **7b**. As above, using the ketone (9·9 mg) in pyridine/α-phenylbutyric anhydride from the same standard soln (150 mg). The titre was 0·985 ml N/10 NaOH.

$[\alpha]_{observed}$ + 0·028° $[\alpha]_D$ + 3·6° (*c* 0·78, benzene)
$[\alpha]_D$ theoretical ± 21° Optical yield 17·4%.

The first benzene phase yielded an amorphous solid (13 mg; 75%), γ_{max} 1721, 1700, 1171, 970, and 692 cm^{-1}, τ 2·73 (5H+, s), 4·9–5·3 (2H, m), 6·6 (1H, t, J 7 Hz), 7·56 (2H, m), 8·4, 8·53, 8·75, 8·92, 9·13, 9·24, 9·31, and 9·37.

Attempted oxidations of inotodiol **1a** *and of the* 3β-*monoacetate* **1b**

(a) Using silver carbonate on celite according to the procedure of Fétizon *et al.*[19] inotodiol was recovered unchanged after heating under reflux in benzene for 24 h. This method was not investigated further.

(b) Inotodiol 3β-monoacetate **1b** (30 mg) was treated with the CrO_3/pyridine complex, generated *in situ* from anhyd CrO_3 (1 g) and pyridine (1·67 ml; distilled from CaH_2) in CH_2Cl_2 (17 ml; distilled from CaH_2) by the modified procedure of Ratcliffe and Rodehurst.[21] Reaction was complete within 15 min. The mixture was filtered through silica (13 g) and eluted with ether. The first two fractions contained the non-polar material (20 mg). This was chromatographed on silica (6 g), and eluted with ethyl acetate/hexane (1:19) to give a slightly yellow, crystalline product (10 mg), visible on TLC under UV light, and apparently therefore the conjugated 23-en-22-one, γ_{max}^{Chl} 1720, 1700, and 1250 cm^{-1}.

(c) Inotodiol **1a** (50 mg) was similarly treated with the complex prepared from CrO_3 (3 g) and pyridine (5 ml) in CH_2Cl_2 (50 ml). The crude yellowish product (29 mg), $\gamma_{max}^{CHCl_3}$ 1708, and 1680–1660 cm^{-1}, again appeared to be the conjugated ketone (UV light). Chromatography on silica (7·5 g), eluting with EtOAc/hexane (1:19) gave a ketonic product, impure and in low yield.

Hydride reduction of the ketonic products of (*b*) *and* (*c*). Treatment of the crudely purified products of (b) and (c) with LAH in ether at 0° gave reaction mixtures containing the same two products (by TLC), in a 6:4 ratio, favouring the more polar component. Both components were visible under UV light. Neither corresponded to inotodiol nor to the more polar by-product of the inotodiol synthesis. The less polar component was slightly more polar than the former; the second component was slightly more polar than the latter, an indication that the more polar by-product of the inotodiol synthesis may be 22S-epiinotodiol.

3β - *Acetoxy* - 20S - *formyl* - 4,4,14α - *trimethyl* - 5α - *pregn* - 8 - *ene* **11**. The known aldehyde[23,24] was prepared by ozonolysis at −70° of the olefin **3** (1·9 g) in CH_2Cl_2

(90 ml) containing pyridine (0·4 ml), the reaction being followed by GLC. Despite the application of the above conditions,[23] two products of similar polarity were observed by TLC. Reduction with Zn dust (2·25 g) and AcOH (5 ml) at −70°, followed by stirring at room temp for 2 h, gave, after filtration, removal of AcOH by washing with water, and drying, the crude crystalline aldehyde (1·5 g; 78%). It had properties identical to those described.[3]

3β - *Acetoxy* - 22R - *hydroxy* - 23,23 - *dimethyl* - 26,27 - *dinorlanosta* - 8,24 - *diene* **17a**. Mg turnings (560 mg) were stirred under argon during the careful addition of a soln of 4 - chloro - 2 - methylbut - 2 - ene (1·8 ml; freshly distilled from K_2CO_3) and 1,2-dibromoethane (1 ml) in anhyd THF (20 ml). After 30 min, the aldehyde **11** (500 mg) in THF (40 ml) was added, and the mixture left to react for 12 h. The reaction was terminated as described for inotodiol, affording an oil (2·3 g). Chromatography on silica (40 g) gave, after several crystallisations from $CHCl_3$/MeOH, the homogeneous 3β - *acetoxy* - 22R(22α_P) - *alcohol* (500 mg; 85%), m.p. 235–6°, $[\alpha]_D^{20}$ + 44° (*c* 1·2), $\gamma_{max}^{CHCl_3}$ 3500, 1725, 1640, and 1260 cm^{-1}, τ 4·03 (1H, dd, J 9·5 and 19 Hz, 24–H), 4·94 (1H, dd, J 2 and 19 Hz), *trans*-25-H), 4·97 (1H, dd, J 2 and 9·5 Hz, *cis*-25-H), 5·48 (1H, m, 3α-H), 6·58 (2H, m, 22-H and -OH), 7·94 (3H, s), 8·93, 8·97, 9·12, and 9·28 (3H, s), *m/e* 484 (M^+), 469, 451, 414 (M^+–side-chain from C-22), 399, 339, 311, and 43 (base peak). (Found: C, 79·3; H, 10·9. $C_{32}H_{52}O_3$ requires: C, 79·3; H, 10·8%). The method of Horeau[9] was applied to this alcohol (50 mg) as described for **1b** and for **7b** above. TLC indicated that the reaction was approximately 50% complete after 24 hr. However, the resulting α-phenylbutyric acid had $[\alpha]_D$ 0°.

3β - 22R - *benzoyloxy* - 23,23 - *dimethyl* - 26,27 - *dinorlanosta* - 8,24 - *diene* **18b**. The alcohol **18a** (96 mg) in pyridine (2 ml) containing benzoyl chloride (0·2 ml) for 4 h, yielded, after work-up as described for **1f**, the crude benzoate ester. Chromatography on silica (10 g), eluting with EtOAc/hexane (1 : 1), and then on alumina, furnished the *benzoate* (96 mg; 83%), m.p. 215–217° ($CHCl_3$/MeOH), $[\alpha]_D^{20}$ + 54° (*c* 0·9), γ_{max} 1736, 1712, 1280, 1251, and 712 cm^{-1}, $\lambda_{max}^{Dioxane}$ 230 nm (ε 20,700), τ 1·9 (2H, m), 2·46 (3H, m), 3·93 (1H, dd, J 10 and 18 Hz), 4·85 (1H, m, 22-H), 4·95 (1H, dd, J 2 and 10 Hz), 4·98 (1H, dd, J 2 and 18 Hz), 5·51 (1H, m, 3α-H), 7·98 (3H, s), 8·47, 8·92, 9·02, 9·13, 9·18, and 9·29 (3H, s), *m/e* 588 (M^+), 573, 528, 513, 451 (573-C_6H_5COOH), 391 (451-AcOH), 369 (573–side-chain from C-20), 309, 105 (base peak; C_6H_5CO), and 69 (Me_2CCHCH_2). (Found: C, 79·4; H, 9·8. $C_{39}H_{56}O_4$ requires: C, 79·55; H, 9·6%).

3β - *Acetoxy* - 22R - *benzoyloxylanosta* - 8,24 - *diene* **1d**. The 3β-acetate **1b** (20 mg) was esterified using pyridine (1 ml) containing benzoyl chloride (35 mg), for 12 h. Work-up as above, followed by PLC in EtOAc/hexane (1 : 19), gave the *benzoate* (15 mg; 60%), m.p. 150–3° (MeOH), $[\alpha]_D^{17}$ + 56° (*c* 0·5), γ_{max} 1735, 1719, 1602, 1318, 1279, 1252, 1111, 1026, and 711 cm^{-1}, $\lambda_{max}^{Dioxane}$ 229 nm (ε 13,800), τ 1·96 (2H, m), 2·52 (3H, m), 4·83 (2H, m, 24- and 22-H), 5·48 (1H, m, 3α-H), 7·97 (3H, s), 8·37 (6H, bs), 9·00, 9·12, and 9·31 (3H, s), *m/e* 588 (M^+), 573, 528, 466, 451, 391 (M^+—Me—$C_6H_5CO_2H$—AcOH), 357, 297, 109, 105 ($C_6H_5CO^+$, base peak), 77 ($C_6H_5^+$), 69, and 43. (Found: C, 79·5; H, 9·6. $C_{39}H_{56}O_4$ requires: C, 79·55; H, 9·6%).

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