Resolution of 2,3-Dihydrosqualene-2,3-diol, 10,11-Dihydro-10,11-dihydroxyfarnesyl Benzoate, and 6,7-Dihydro-6,7-dihydroxygeranyl Benzoate. Synthesis of (3*R*)- and (3*S*)-2,3-Epoxy-2,3-dihydrosqualene

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The title compounds are conveniently resolved by reaction with 3β-acetoxy-17β-chloroformylandrost-5-ene and chromatographic separation of the resulting diastereoisomeric esters. The absolute configurations of the enantiomers have been established. The important biosynthetic precursors, (+)-(3*R*)- and (−)-(3*S*)-2,3-epoxy-2,3-dihydrosqualene are readily prepared from (−)-(3*S*)- and (+)-(3*R*)-2,3-dihydrosqualene-2,3-diol by treatment with toluene-*p*-sulphonyl chloride in pyridine, followed by base.

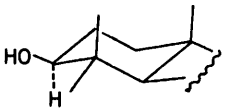
2,3-EPOXY-2,3-DIHYDROSQUALENE (squalene epoxide) [1] is a key intermediate in the pathways leading to the biosynthesis of 3-oxygenated triterpenes (and derived sterols) in mammalian tissue,[2] micro-organisms,[3] and plants.[4] The apparently concerted nature of the mechanism [5] for the cyclisation of squalene epoxide led to the reasonable assumption that (3*S*)-squalene epoxide (1a) acts as a specific precursor of 3β-oxygenated triterpenes [as (2)]. Prior to our work,[6,7] this assumption had received only indirect experimental support.[8] We now report details of the resolution of the title compounds, and the further synthesis of the optically pure enantiomers of squalene epoxide (1a).

During the acylation of 2,3-dihydrosqualene-2,3-diol [1] (3a) with (±)-α-phenylbutyric anhydride we observed

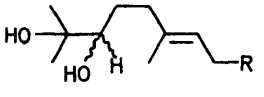
[1] E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, 1962, 121.
[2] J. D. Willett, K. B. Sharpless, K. E. Lord, E. E. van Tamelen, and R. B. Clayton, *J. Biol. Chem.*, 1967, **242**, 4182.
[3] D. H. R. Barton, G. Mellows, D. A. Widdowson, and J. J. Wright, *J. Chem. Soc.* (*C*), 1971, 1142.
[4] R. Heintz and P. Benveniste, *Phytochemistry*, 1970, **9**, 1499.
[5] P. D. G. Dean, *Steroidologia*, 1971, **2**, 143.
[6] Preliminary communications, R. B. Boar and K. Damps, *Tetrahedron Letters*, 1974, 3731; D. H. R. Barton, T. R. Jarman, K. C. Watson, D. A. Widdowson, R. B. Boar, and K. Damps, *J.C.S. Chem. Comm.*, 1974, 861.
[7] D. H. R. Barton, T. R. Jarman, K. C. Watson, D. A. Widdowson, R. B. Boar, and K. Damps, *J.C.S. Perkin I*, 1975, 1134.
[8] T. Shishibori, T. Fukui, and T. Suga, *Chem. Letters*, 1973, 1137.

the formation of a product which, although having the expected spectroscopic properties, separated on t.l.c. into two components. That these two components were diastereoisomerically related to one another, each being

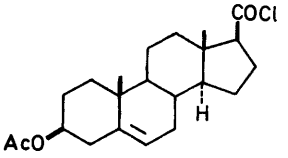
(1)



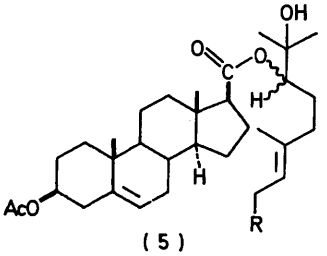
(2)



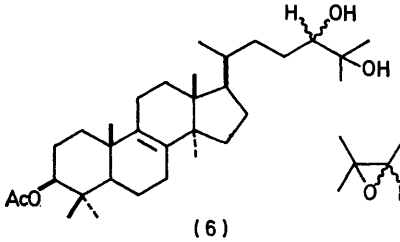
(3)



(4)



(5)



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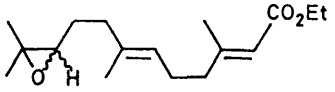
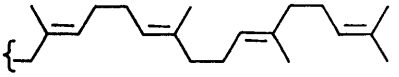
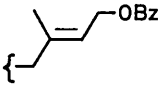


(7)

a; R = 

b; R = OBz

c; R = OH

d; R = 

a pair of enantiomers, was confirmed by repeating the above experiment, but using (+)-α-phenylbutyroyl chloride.[9] Chromatographic separation of the two components and reduction of each with lithium aluminium hydride then afforded optically active 2,3-dihydrosqualene-2,3-diols. Unfortunately, under the reaction conditions necessary to effect acylation, both α-phenylbutyric anhydride and α-phenylbutyroyl chloride undergo rapid racemisation.[9] They were therefore unsuitable for the production of the optically *pure* diols (3a). Of other optically active acid chlorides investigated, by far the most convenient proved to be the readily available 3β-acetoxy-17β-chloroformylandrost-5-ene (4).[10]

Treatment of 2,3-dihydrosqualene-2,3-diol with the freshly prepared acid chloride (4) in *dry* pyridine readily afforded the oily esters (5a). The diastereoisomers of (5a) could be separated on t.l.c. by a single elution, but for preparative-scale work it was expedient to elute the plates several times. The pure diastereoisomers thus obtained yielded essentially identical spectral data which were in full accord with the assigned structure. Reduction of the individual isomers of (5a) with lithium aluminium hydride gave the enantiomeric diols (3a), $[\alpha]_D \pm 10.7°$. The optical purity of these diols follows from the chromatographic purity of the precursor esters. Application of Horeau's procedure [11] to the (+)- and (−)-diols (3a) showed them to have the 3*R*- and 3*S*-absolute configurations, respectively. This is in accord with molecular rotation requirements [12] and with the results from c.d. studies.[8] The (+)-(3*R*)- and (−)-(3*S*)-diols (3a) were cleanly converted, with inversion, into (−)-(3*S*)- and (+)-(3*R*)-squalene epoxide (1a), respectively, by reaction with toluene-*p*-sulphonyl chloride in pyridine, followed by treatment of the resulting monotosylate with ethanolic potassium hydroxide. The specific rotation of the epoxides (1a) in chloroform was very low. A more measureable quantity, but still not one that could be used realistically to assess optical purity, was obtained by use of methanol as solvent. That we indeed had optically pure material was established as follows. First, on acid-catalysed hydrolysis the epoxides (1a) gave diols (3a) which were enantiomerically pure as judged by their specific rotations. Secondly, model experiments involving the interconversion of the 3β-acetoxy-5α-lanost-8-ene-24,25-diols (6) and the corresponding 24,25-epoxides [12] indicated that within the limits of t.l.c. detection and of specific rotation measurement no racemisation was occurring at C-24.

In experiments in collaboration with Professor D. H. R. Barton and Dr. D. A. Widdowson and their co-workers, radiochemically labelled samples of the (3*R*)- and (3*S*)-squalene epoxides (1a) were tested as potential precursors of a representative range of 3β-hydroxy-triterpenes.[7] In all cases the (3*S*)-isomer was shown to act as an essentially exclusive precursor. These biochemical experiments also serve to confirm the optical purity of our synthetic compounds.

The origin of the relatively small number of naturally occurring 3α-hydroxy-triterpenes remains unclarified. Although some may arise directly by cyclisation of (3*R*)-squalene epoxide, it is likely that others, particularly those that co-occur with related 3β-hydroxy- or 3-oxo-compounds, are formed *via* cyclisation of the (3*S*)-epoxide and thence the corresponding 3β-hydroxy- and

[9] R. Weidmann and A. Horeau, *Bull. Soc. chim. France*, 1967, 117.

[10] J. Staunton and E. J. Eisenbraun, *Org. Synth.*, 1962, **42**, 4.

[11] A. Horeau, *Tetrahedron Letters*, 1961, 506; 1962, 965.

[12] R. B. Boar, D. A. Lewis, and J. F. McGhie, *J.C.S. Perkin I*, 1972, 2231.

3-oxo-triterpenes. The latter pathway has already been established for 3-epimaslinic acid.[13] Recently, Ourisson and his co-workers [14] have shown that a cell-free system derived from *Acetobacter rancens*, a micro-organism which can itself synthesise squalene but not squalene epoxide, cyclises exogenous (3*R*)- and (3*S*)-squalene epoxides to yield 3α- and 3β-hydroxyhopanes, respectively.

A particular advantage of the above chromatographic method of resolution is that the degree of optical purity achieved can, within the limits of t.l.c. detection, be easily assessed. This contrasts with the classical method involving the crystallisation of diastereoisomeric salts. There one is obliged to keep repeating the process until a steady state is reached, and then to assume that this corresponds to 100% separation.

To test the generality of our particular system we next considered the resolution of 6,7-dihydro-6,7-dihydroxygeranyl benzoate [15] (3b). The inclusion of the u.v.-active benzoate grouping facilitates the detection of the compounds during p.l.c. The formation and chromatographic separation of the diastereoisomeric esters (5b) proceeded exactly as in the case of the analogous squalene compounds. The less and more polar esters, $[\alpha]_D$ −29.5 and −6.3°, respectively, afforded on reduction with lithium aluminium hydride the enantiomeric (6*S*)- and (6*R*)-triols (3c), $[\alpha]_D$ −18.4 and +17.4°, respectively. The triols (3c), which have some potential as synthetic building blocks, are thus now readily available in optically pure form.

Finally, we demonstrated that 10,11-dihydro-10,11-dihydroxyfarnesyl benzoate [16] (3d), derived from commercial farnesol, formed the mixed esters (5d), $[\alpha]_D$ −13.2°, which were readily separated into the pure diastereoisomers $[\alpha]_D$ −19.5 and −7.3°. 10,11-Epoxy-10,11-dihydrofarnesol has been partially resolved previously by preferential fungal metabolism.[17]

We believe that the resolutions reported above are a more convenient route to the enantiomerically pure title compounds than the recently reported total syntheses.[18] Our method has already been reported to be the best available route for the preparation of optically pure samples of the juvenile hormone (7).[19]

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. N.m.r. data refer to solutions in deuteriochloroform with tetramethylsilane as internal standard, and were recorded at 90 MHz. Unless otherwise stated rotations are for solutions in chloroform. Mass spectra were obtained with an A.E.I. MS9 double-focusing spectrometer; samples were introduced by direct probe insertion with an ionisation potential of 70 eV. T.l.c. and p.l.c. plates were prepared by using Merck silica gel GF_{254}. Light petroleum refers to the fraction of b.p. 60—80 °C.

Resolution of 2,3-*Dihydrosqualene*-2,3-*diol* (3a).—Freshly prepared 3β-acetoxy-17β-chloroformylandrost-5-ene [10] (500 mg) was added in portions over 1 h to 2,3-dihydrosqualene-2,3-diol [1] (136 mg) in dry pyridine (5 ml). The mixture was shaken periodically. After 2 h at room temperature, t.l.c. indicated complete conversion of the diol into two diastereoisomeric esters. Water (5 ml) was added, and after 5 min the mixture was poured into water and extracted with chloroform. The combined extracts were washed with 2N-hydrochloric acid, then water, dried, and evaporated. The residue was treated with hot benzene and the mixture was then filtered, thus removing the bulk of the 3β-acetoxyandrost-5-ene-17β-carboxylic acid. The filtrate was evaporated and the residue was purified by preparative layer chromatography (p.l.c.) (1 elution with 25% ethyl acetate in light petroleum) to yield the mixed, diastereoisomeric esters (5a) as a gum (204 mg, 85%), $[\alpha]_D$ −14.9° (*c* 3.3). The separate diastereoisomers were then obtained by p.l.c. (6 elutions with 10% ethyl acetate in light petroleum). The *less polar ester* was obtained as a gum, $[\alpha]_D$ −23.5° (*c* 2.8) τ 4.62 (1 H, d, 6-H), 4.87br (5 H, s), 5.19 (1 H, dd, 3′-H), 5.35 (1 H, m, 3α-H), and 8.98 and 9.23 (each 3 H, s, 19- and 18-H_3, respectively) (Found: M^+, 786.619 4. $C_{52}H_{82}O_5$ requires *M*, 786.616 2). The *more polar ester* had $[\alpha]_D$ −6.5° (*c* 3.1) and n.m.r. and mass spectra identical with those of the less polar ester.

The less polar ester (80 mg) in dry ether (20 ml) was stirred at room temperature with lithium aluminium hydride (150 mg) for 24 h. Work-up in the usual fashion then afforded (−)-2,3-*dihydrosqualene*-2,3-*diol* (32 mg, 70%), $[\alpha]_D$ −10.7° (*c* 1.5) (Found: M^+, 444.396 1. $C_{30}H_{52}O_2$ requires *M*, 444.396 7). The more polar ester similarly gave (+)-2,3-*dihydrosqualene*-2,3-*diol*, $[\alpha]_D$ +10.7° (*c* 1.6) (Found: M^+, 444.396 1).

Absolute Configurations of the Resolved 2,3-*Dihydrosqualene*-2,3-*diols*.—(+)-2,3-Dihydrosqualene-2,3-diol (50 mg) in dry pyridine (1 ml) was treated with (±)-α-phenylbutyric anhydride (180 mg) at room temperature for 2 days. Work-up exactly as previously described [12] then gave α-phenylbutyric acid, $[\alpha]_D$ +1.73° (*c* 6.5 in benzene), optical yield 19%. The (+)-diol therefore has the 3*R* absolute configuration. Similarly, (−)-2,3-dihydrosqualene-2,3-diol afforded α-phenylbutyric acid, $[\alpha]_D$ −1.84° (*c* 5.2 in benzene), optical yield 18%, indicating 3*S* stereochemistry.

(3R)- *and* (3S)-2,3-*Epoxy*-2,3-*dihydrosqualene* (1a).—(−)-(3*S*)- and (+)-(3*R*)-2,3-Dihydrosqualene-2,3-diols were separately converted by the previously described [7] method into (+)-(3R)- and (−)-(3S)-2,3-*epoxy*-2,3-*dihydrosqualene*, $[\alpha]_D$ +2.0° (*c* 1.8 in MeOH) and −1.8° (*c* 2.0 in MeOH), respectively (Found: M^+, 426.386 6. $C_{30}H_{50}O$ requires *M*, 426.386 1). When treated with 3% perchloric acid in 1,2-dimethoxyethane,[1,7] these epoxides afforded the corresponding diols, which, within the limits of experimental error, were optically pure as judged by their optical rotations.

(24R)- *and* (24S)-24,25-*Dihydroxy*-5α-*lanost*-8-*en*-3β-*yl*

[13] Y. Tomita and S. Seo, *J.C.S. Chem. Comm.*, 1973, 707.
[14] C. Anding, M. Rohmer, and G. Ourisson, *J. Amer. Chem. Soc.*, 1976, **98**, 1274.
[15] L. Lizzani and R. Luft, *Bull. Soc. chim. France*, 1971, 198; L. Canonica, B. Rindone, E. Santaniello, and C. Scolastico, *Tetrahedron*, 1972, **28**, 4395.
[16] See, E. E. van Tamelen, A. Storni, E. J. Hessler, and M. Schwartz, *J. Amer. Chem. Soc.*, 1963, **85**, 3295.
[17] Y. Suzuki, K. Imai, and S. Marumo, *J. Amer. Chem. Soc.*, 1974, **96**, 3703.
[18] S. Yamada, N. Oh-hashi, and K. Achiwa, *Tetrahedron Letters*, 1976, 2557 and 2561.
[19] K. Imai, S. Marumo, and T. Ohtaki, *Tetrahedron Letters*, 1976, 1211.

Acetates.—A mixture of (24*R*)-24,25-epoxy-5α-lanost-8-en-3β-yl acetate [12] (1.2 g), 1,2-dimethoxyethane (150 ml), water (25 ml), and perchloric acid (70%; 3 drops) was stirred rapidly at room temperature overnight. 2N-Sodium carbonate (50 ml) was added. Extraction with ether and crystallisation from acetone–light petroleum then gave the (24*R*)-24,25-diol (1.0 g, 80%), m.p. 190—193°, $[\alpha]_D$ +60° (*c* 1.52), τ 5.50 (1 H, m, 3α-H), 6.68 (1 H, m, 24-H), 7.96 (3 H, s, OAc), 8.78 and 8.83 (each 3 H, s, 26- and 27-H_3), 8.98 and 9.29 (each 3 H, s), and 9.11 (9 H, s). The (24*S*)-24,25-epoxide [12] similarly afforded the (24*S*)-24,25-diol, m.p. 181—184°, $[\alpha]_D$ +45° (*c* 1.5). Treatment [7] of either 24,25-diol with toluene-*p*-sulphonyl chloride in pyridine, followed by ethanolic potassium hydroxide, gave a 24,25-epoxide which had, within the limits of t.l.c. detection,[12] undergone inversion at C-24 with no racemisation.

Resolution of 6,7-*Dihydro*-6,7-*dihydroxygeranyl Benzoate* [15] (3b).—This was performed exactly as described above for 2,3-dihydrosqualene-2,3-diol. The mixed diastereoisomeric esters (5b) were obtained as a gum, $[\alpha]_D$ −18.5° (*c* 1.5). The separate diastereoisomers were then obtained by p.l.c. (7 elutions with 10% ethyl acetate in light petroleum). The *less polar ester* was a low melting crystalline solid, $[\alpha]_D$ −29.5° (*c* 1.6), τ 1.95 and 2.50 (5 H, m, Bz), 4.4—4.7 (2 H, m, 6- and 2′-H), 5.0—5.6 (4 H, m, 3α-H, 1′-H_2, and 6′-H), 7.98 (3 H, s, OAc), 8.78 and 8.79 (each 3 H, s, 8′-H_3 and 7′-CH_3), and 8.99 and 9.24 (each 3 H, s, 19- and 18-H_3) (Found: C, 73.8; H, 8.7. $C_{39}H_{54}O_7$ requires C, 73.8; H, 8.6%). The *more polar ester* had $[\alpha]_D$ −6.3° (*c* 1.4).

The less polar ester (5b) (95 mg) in dry tetrahydrofuran (40 ml) was treated with lithium aluminium hydride (100 mg) under reflux overnight. Work-up in the usual fashion gave (−)-(6*S*)-6,7-dihydro-6,7-dihydroxygeraniol (3c) (23 mg, 80%) as an oil, $[\alpha]_D$ −18.4° (*c* 1.5). (+)-(6*R*)-6,7-Dihydro-6,7-dihydroxygeraniol, $[\alpha]_D$ +17.4° (*c* 1.4) was similarly obtained from the more polar ester (5b).

Similarly, 10,11-dihydro-10,11-dihydroxyfarnesyl benzoate [1,16] (3d) afforded the mixed diastereoisomeric esters (5d), $[\alpha]_D$ −13.2° (*c* 1.9), which were separated by p.l.c. into the less and more polar diastereoisomers, $[\alpha]_D$ −19.5 (*c* 1.3) and −7.3° (*c* 1.5), respectively. The further transformation of these compounds was not pursued.

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