Microbiological Degradation of Bile Acids

THE PREPARATION OF SOME HYPOTHETICAL METABOLITES INVOLVED IN CHOLIC ACID DEGRADATION

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1. To identify the intermediates involved in the degradation of cholic acid, the further degradation of (4*R*)-4-[4 α -(2-carboxyethyl)-3 α -hexahydro-7 $\alpha\beta$ -methyl-5-oxoindan-1 β -yl]valeric acid (IV*a*) by *Arthrobacter simplex* was attempted. The organism could not utilize this acid but some hypothetical intermediate metabolites of compound (IV*a*) were prepared for later use as reference compounds. 2. The nor homologue (III*a*) and the dinor homologue (III*b*) of compound (IV*a*) were prepared by exposure of 3-oxo-24-nor-5 β -cholan-23-oic acid (I) and (20*S*)-3 β -hydroxy-5-pregnene-20-carboxylic acid (II) to *A. simplex* respectively. These compounds correspond to the respective metabolites produced by the shortening of the valeric acid side chain of compound (IV*a*) in a manner analogous to the conventional fatty acid α - and β -oxidation mechanisms. Their structures were confirmed by partial synthesis. 3. The following authentic samples of reduction products of the oxodicarboxylic acids (III*a*), (III*b*) and (IV*a*) were also synthesized as hypothetical metabolites: (4*R*)-4-[3 $\alpha\alpha$ -hexahydro-5 α -hydroxy-4 α -(3-hydroxypropyl)-7 $\alpha\beta$ -methylindan-1 β -yl]valeric acid (V*b*) and its nor homologue (VII*a*) and dinor homologue (IX*a*); (4*R*)-4-[3 $\alpha\alpha$ -hexahydro-5 α -hydroxy-4 α -(3-hydroxypropyl)-7 $\alpha\beta$ -methylindan-1 β -yl]-pentan-1-ol (V*c*); and their respective 5 β epimers (V*e*), (VII*c*), (IX*c*) and (V*f*). 4. In connexion with the non-utilization of compound (IV*a*) by *A. simplex*, the possibility that not all the metabolites formed from cholic acid by a certain micro-organism can be utilized by the same organism is considered.

In our previous papers we reported the isolation and identification of (4*R*)-4-[4 α -(2-carboxyethyl)-3 $\alpha\alpha$ -hexahydro-7 $\alpha\beta$ -methyl-5-oxoindan-1 β -yl]valeric acid (IV*a*) and 5-methyl-4-oxo-octane-1,8-dioic acid as the degradation products formed from 3 α ,7 α ,12 α -trihydroxy-5 β -cholan-24-oic acid (cholic acid) by *Arthrobacter simplex* (Hayakawa *et al.*, 1969*b*; Hayakawa & Fujiwara, 1969). Our continued interest in the intermediates and reaction sequence involved in the degradation of cholic acid has prompted us to search for intermediates related to a metabolic sequence which leads from compound (IV*a*) to 5-methyl-4-oxo-octane-1,8-dioic acid (C₉ acid), although a direct metabolic relationship between these acids is not established yet. We expected that the incubation of compound (IV*a*) with *A. simplex* in a manner analogous to that for cholic acid would result in the formation of further degradation products, including this C₉ acid. In this experiment, however, the organism was unable to utilize compound (IV*a*) as its carbon source, and only the starting material was recovered (Hayakawa *et al.*, 1968). Attempts to isolate the expected metabolites of compound (IV*a*)

from a mixture of the degradation products formed from cholic acid by *A. simplex* were unsuccessful except for the above C₉ acid, because of very low yields and the complexity of composition. It appeared that an alternative approach to this problem was needed, and we have designed the following two approaches: (1) involves both the preparation of some hypothetical metabolites of compound (IV*a*) and their use as reference compounds for the detection of the desired metabolites by physicochemical methods such as g.l.c.-mass spectrometry; (2) a search for other micro-organisms capable of utilizing compound (IV*a*) or cholic acid (or both) which accumulate the desired metabolites.

While these lines were being pursued in parallel, we observed that *Corynebacterium equi* was able to metabolize compound (IV*a*) to its conjugates with alanine, glutamic acid, glutamine and *O*-acetylhomoserine (Hayakawa *et al.*, 1968) and to (4*R*)-4-[2 α -(2-carboxyethyl)-3 β -(3-carboxypropionyl)-2 β -methylcyclopent-1 β -yl]valeric acid (cf. Hayakawa, 1973). It was also found that cholic acid was degraded by *Streptomyces rubescens* to the following compounds: 2,3,4,6,6 $\alpha\beta$,7,8,9,9 $\alpha\alpha$,9 $\beta\beta$ -decahydro-6 $\alpha\beta$ -methyl-7*H*-cyclopenta[*f*]quinoline-3,7-dione; (2*S*)-

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2-(2,3,4,6,6a β ,7,8,9,9a α ,9b β -decahydro-6a β -methyl-3-oxo-1H-cyclopenta[*f*]quinolin-7 β -yl)propionic acid, which is an enaminolactam derivative of (2*S*)-2-[4 α -(2-carboxyethyl)-3a α -hexahydro-7a β -methyl-5-oxoindan-1 β -yl]propionic acid (III*b*) prepared in this paper; and compound (IV*a*) and its three nitrogenous derivatives, the mono-amide (valeramide), enamino-lactam and enaminolactam valeramide (Hayakawa *et al.*, 1969*a*). Accordingly, we discontinued the first approach, although most of our hypothetical metabolites had been already prepared, but had not been used as originally planned. However, some of these compounds were available as starting materials for the partial synthesis of bile acid metabolites obtained with the above organisms, *C. equi* and *S. rubescens*.

The present paper deals with the preparation of some hypothetical metabolites involved in the microbiological degradation of compound (IV*a*), which is one of the cholic acid metabolites.

Results and Discussion

Biodegradability of metabolites by the metabolite-producing organisms

That the oxodicarboxylic acid (IV*a*) could not be utilized by *A. simplex* was in conflict with the previous observation that compound (IV*a*) was gradually metabolized in the culture broth resulting from the incubation of cholic acid with this organism (Hayakawa *et al.*, 1969*b*). During the course of our studies on the microbiological degradation of bile acids, we obtained the following evidence similar to this non-utilization of compound (IV*a*) by *A. simplex* (cf. Hayakawa, 1973): 12 α -hydroxy-3-oxocholal-1,4-dien-24-oic acid, which was produced by the incubation of cholic acid with *A. simplex*, was further metabolized with the accumulation of compound (IV*a*) on continued incubation (Hayakawa *et al.*, 1969*b*), but when the dienoic acid alone was incubated independently with this organism it could not be utilized. *A. simplex* and *S. rubescens* produced the same intermediate, 5-methyl-4-oxo-octane-1,8-dioic acid, from both cholic acid (Hayakawa & Fujiwara, 1969) and 3-(3a α -hexahydro-7a β -methyl-1,5-dioxoindan-4 α -yl)propionic acid (Hayakawa & Hashimoto, 1969), but neither of these organisms could utilize this dicarboxylic acid. Additional evidence showed that *C. equi* was able to convert compound (IV*a*) into (4*R*)-4-[2 α -(2-carboxyethyl)-3 β -(3-carboxypropionyl)-2 β -methylcyclopent-1 β -yl]valeric acid, which was not utilized by this organism. Further, one of the metabolites formed from cholic acid by *S. rubescens*, 2,3,4,6,6a β ,7,8,9,9a α ,9b β -decahydro-6a β -methyl-7H-cyclopenta[*f*]quinoline-3,7-dione, was not further metabolized at a significant rate by this organism (Hayakawa & Hashimoto, 1969). No simple explanation for such peculiar results on the

microbial biodegradability can be offered at the present time. However, they arise perhaps from a difference in catabolic enzymes or in cellular permeability (or both) between aged organisms in culture broths and freshly inoculated organisms. We believe from this evidence that when either one alone of the compounds, isolated as metabolites of cholic acid by a certain micro-organism, is exposed to the same organism, not all of them are able to be utilized.

The above viewpoint is in disagreement with that of Kondo *et al.* (1969), who examined the microbial biodegradability of possible synthetic intermediates involved in the degradation of 3-(3a α -hexahydro-7a β -methyl-1,5-dioxoindan-4 α -yl)propionic acid by *Nocardia restrictus* or *Nocardia opaca* and concluded that the synthetic specimens, which were not utilized by the organisms, were not intermediates in the degradative pathway(s) of this hexahydroindane derivative. The discrepancy needs to be investigated further. However, as it is well known that several micro-organisms are able to degrade a wide variety of steroidal compounds to the corresponding hexahydroindane derivatives such as compound (IV*a*) (cf. Sih *et al.*, 1968; Schubert *et al.*, 1970; Smith, 1974), there seems no doubt that compound (IV*a*) is one of the actual intermediates involved in the degradation of cholic acid by *A. simplex*.

Origin of structures of hypothetical metabolites

It has been known that the C₂₄ bile acids are degraded by micro-organisms to the C₂₃ and C₂₂ bile acid derivatives, which correspond to the respective metabolites produced by the shortening of their valeric acid side chain in a manner analogous to the conventional fatty acid α - and β -oxidation mechanisms (cf. Hayakawa, 1973). As hypothetical metabolites of compound (IV*a*), therefore, the nor and dinor homologues of compound (IV*a*), namely (3*R*)-3-[4 α -(2-carboxyethyl)-3a α -hexahydro-7a β -methyl-5-oxoindan-1 β -yl]butyric acid (III*a*) and (2*S*)-2-[4 α -(2-carboxyethyl)-3a α -hexahydro-7a β -methyl-5-oxoindan-1 β -yl]propionic acid (III*b*), were first prepared. Then the oxodicarboxylic acids, (III*a*), (III*b*) and (IV*a*), were converted into the corresponding reduction products, dihydroxymonocarboxylic acids or triols (or both). The reason for this is that such types of microbiological transformations have been known in the literature (cf. Hörhold *et al.*, 1969), although Lee & Sih (1967) have considered that such reduction products are probably side metabolites rather than degradative intermediates.

*Preparation of the noroxodicarboxylic acid (III*a*) and the dinoroxodicarboxylic acid (III*b*)*

*Microbiological degradation of 3-oxo-24-nor-5 β -cholan-23-oic acid (I) and (20*S*)-3 β -hydroxy-5-pregnene-20-carboxylic acid (II).* Since both cholic

acid and 3 α -hydroxy-5 β -cholan-24-oic acid (lithocholic acid) have been easily converted into compound (IVa) by *A. simplex* (Hayakawa *et al.*, 1969b), one can expect that the desired compounds (IIIa) and (IIIb) would be obtained by exposure of the appropriate derivatives of both 24-nor-5 β -cholan-23-oic acid and pregnane-20-carboxylic acid to this organism. However, 3 α -hydroxy-24-nor-5 β -cholan-23-oic acid, which is the 24-nor homologue of lithocholic acid, was metabolized at an exceedingly low rate, whereas the incubation of its oxidation product, 3-oxo-24-nor-5 β -cholan-23-oic acid (I), with *A. simplex* resulted in the accumulation of a new metabolite, which was isolated as crystals with m.p. 141–142°C, with yield approx. 70% (Scheme 1). The elementary analysis was consistent with C₁₇H₂₆O₅. The i.r. spectrum showed the presence of a carboxyl group at approx. 2600–3200 and 1700 cm⁻¹. The analysis of the methyl ester was consistent with C₁₉H₃₀O₅, and its i.r. spectrum showed the presence of both six-membered ring carbonyl and ester carbonyl groups at 1708 (shoulder) and 1735 cm⁻¹ respectively. The n.m.r. spectrum showed bands at δ 0.99 (superimposable doublet; 3H; one secondary methyl group), 1.02 (singlet; 3H; one tertiary methyl group), 3.63 (singlet; 3H; one ester methyl group) and 3.65 (singlet; 3H; one ester methyl group) p.p.m. On the basis of its source and these physical data, the structure of this metabolite was assigned as (3R)-3-[4 α -(2-carboxyethyl)-3 $\alpha\alpha$ -hexahydro-7 $\alpha\beta$ -methyl-5-oxoindan-1 β -yl]butyric acid (IIIa). A more definitive proof of the structure was established by partial synthesis.

We chose commercially available (20S)-3 β -hydroxy-5-pregnene-20-carboxylic acid (II) as one of the derivatives of pregnane-20-carboxylic acid, incubated it with *A. simplex* as described above and obtained a new degradation product as a major metabolite, with yield approx. 50%. The structure of this metabolite was assigned as (2S)-2-[4 α -(2-carboxyethyl)-3 $\alpha\alpha$ -hexahydro-7 $\alpha\beta$ -methyl-5-oxoindan-1 β -yl]propionic acid (IIIb) by a comparison of the physical data of this metabolite and its dimethyl ester with those of both compounds (IIIa) and (IVa) and their dimethyl esters. The exact proof of the structure was given by its partial synthesis (Scheme 2).

In the above experiments, the course of the degradation of both the bile acid homologues (I) and (II) was followed by t.l.c. as described for the cholic acid degradation (Hayakawa *et al.*, 1969b). The t.l.c. results showed that both the acids were converted by *A. simplex* into hexahydroindane derivatives (IIIa) and (IIIb) respectively via the corresponding steroidal intermediates containing the Δ^4 -3-oxo and $\Delta^{1,4}$ -3-oxo structures, as seen in the degradation of cholic acid by *A. simplex*, although such intermediates were not isolated for their definitive identification. This result provides support for the presence of the general degradative pathway for bile acids proposed by Hayakawa *et al.* (1969b).

Chemical proof of structures of the degradation products (IIIa) and (IIIb). Methyl (4R)-4-[3 $\alpha\alpha$ -hexahydro-5 β -hydroxy-4 α -(3-hydroxypropyl)-7 $\alpha\beta$ -methylindan-1 β -yl]valerate (Vd) was subjected to Barbier–Wieland degradation (cf. Riegel *et al.*,

Scheme 1. Microbiological degradation of 3-oxo-24-nor-5 β -cholan-23-oic acid (I) and (20S)-3 β -hydroxy-5-pregnene-20-carboxylic acid (II) to form the noroxodicarboxylic acid (IIIa) and the dinoroxodicarboxylic acid (IIIb)

Scheme 2. Partial syntheses of (2S)-2-[4 α -(2-carboxyethyl)-3 α -hexahydro-7 β -methyl-5-oxoindan-1 β -yl]propionic acid

1944): treatment with phenylmagnesium bromide furnished the diphenylcarbinol derivative (VI), which was further converted into the corresponding diacetoxypiphenylethylene derivative. It was oxidized with chromic anhydride, subsequently hydrolysed

with alkali and yielded the corresponding nor-5 β -dihydroxy acid, (3R)-3-[3 α -hexahydro-5 β -hydroxy-4 α -(3-hydroxypropyl)-7 α -methylindan-1 β -yl]butyric acid (VIIc). Oxidation of its methyl ester (VIId) with Jones reagent (Curtis *et al.*, 1953) and hydrolysis

with alkali gave the noroxodicarboxylic acid (IIIa). Identity of compound (IIIa) with a sample obtained from growing cultures was established by mixed melting point and by comparison of the optical rotation and the i.r. spectrum.

A mixture, which mainly consists of the dihydroxy esters (Va) and (Vd) and which is inseparable into its component parts without chromatography, was submitted to Barbier–Wieland degradation as described above and yielded a mixture of the corresponding nordihydroxy acids. Esterification and subsequent chromatography separated it into two main components. Since one of them was identical with the nor-5 β -dihydroxy ester (VIIa) obtained above, another one was assigned as its 5 α epimer (VIIb) and converted by alkali into the corresponding free acid (VIIa).

The above degradative sequence, which had led from the 5 β -dihydroxy ester (Vd) to the noroxodicarboxylic acid (IIIa), was then repeated with the nor-5 β -dihydroxy ester (VIIa), and yielded successively the diphenylcarbinol derivative (VIII), the dinor-5 β -dihydroxy acid (IXc), the dinor-5 β -dihydroxy ester (IXd) and the dinoroxodicarboxylic acid (IIIb). Identity with the product (IIIb) obtained from growing cultures was established as described above in the identification of compound (IIIa). The sequence of reactions described above conclusively established the structure of both the products (IIIa) and (IIIb), including their stereochemistry. The results show that, in the degradative sequence, the original stereochemistry of both molecules of the bile acid homologues (I) and (II) remains undisturbed, as observed in the degradation of cholic acid by *A. simplex* (Hayakawa *et al.*, 1969b).

Preparation of reduction products of the oxodicarboxylic acids (IIIa), (IIIb) and (IVa)

Atwater (1961) has obtained complex mixtures of lactones, hemiacetals and in some cases diols from the borohydride reduction of δ -oxo esters derived from the chemical cleavage of ring A or ring B of certain steroids. Pesterfield & Wheeler (1965) have also proposed the mechanism of borohydride reduction of the ester groups in such δ -oxo esters to primary alcohols. Since the esters of the oxodicarboxylic acids (IIIa), (IIIb) and (IVa) resembled the δ -oxo esters used by these investigators in the relative locations of two functional groups, we surmised that they would be reduced by NaBH₄ to the corresponding dihydroxy esters.

Treatment of the dimethyl ester (IVb) of compound (IVa) with a large excess of NaBH₄ in methanol yielded a mixture of the expected products, namely methyl (4R)-4-[3 α -hexahydro-5 α -hydroxy-4 α -(3-hydroxypropyl)-7 α β -methylindan-1 β -yl]valerate (Va) and its 5 β epimer (Vd), in a ratio of approx. 1:2. The configuration of the substituents at C-4 and C-5 in

these compounds was assigned on the following bases. (a) The propionic acid side chain at C-4 would be expected to retain its equatorial conformation, since in the β -configuration it would lie 1,3-diaxially to the 7 α β -methyl group. (b) The metal hydride reduction of the unhindered carbonyl group at C-5 should lead predominantly to the thermodynamically more stable equatorial 5 β -hydroxy compound (Dauben *et al.*, 1956), and thus the yield of the 5 β -dihydroxy ester (Vd) was approximately twice that of its 5 α epimer (Va). That the above stereochemical conclusions were correct was ascertained by an examination of the n.m.r. spectra of these compounds and their diacetates. Thus the n.m.r. spectrum of the 5 α epimer (Va) showed an unresolved multiplet at δ 3.94 [5 β -H, full width at half-height (WH) 5.0 Hz] p.p.m. The position of absorption and the relatively small coupling with adjacent protons indicated that the C-5 proton was equatorial (Williamson & Johnson, 1961; Hassner & Heathcock, 1964). The n.m.r. spectrum of the 5 β epimer (Vd), on the other hand, showed a broad multiplet centred at δ 3.27 (5 α -H, WH approx. 20 Hz) p.p.m. The n.m.r. spectra of the diacetates of both the 5 α and 5 β epimers exhibited a multiplet at δ 5.07 (5 β -H, WH 5.0 Hz) and 4.50 (5 α -H, WH approx. 20 Hz) p.p.m. respectively. Thus the C-5 proton signals were displaced to lower field by approx. 1.1 p.p.m. compared with those of the corresponding parent hydroxy esters. These n.m.r. data were in good agreement with the assigned structures. These dihydroxy esters were hydrolysed with alkali to the corresponding free acids (Vb) and (Ve) respectively.

As well as the above dihydroxy esters, a small amount of two other reduction products of compound (IVb) was obtained. One of them was isolated as crystals with m.p. 126.5–127°C, and its elementary analysis was consistent with C₁₈H₃₄O₃, but the other product could not be induced to crystallize. The i.r. spectra of these products showed the presence of a hydroxyl group only near 3300–3400 cm⁻¹. From these physical data and the report by Brown & Rapoport (1963), who demonstrated the reduction of isolated ester groups to primary alcohols by a large excess of NaBH₄ in methanol, the structures of these products were assigned as (4R)-4-[3 α -hexahydro-5 α -hydroxy-4 α -(3-hydroxypropyl)-7 α β -methylindan-1 β -yl]pentan-1-ol (Vc) and its 5 β epimer (Vf) respectively. This assignment was confirmed by a direct comparison with authentic samples of the respective triols, which were prepared independently by the LiAlH₄ reduction of the α -dihydroxy ester (Va) and its 5 β epimer (Vd).

The dimethyl ester of the dinoroxodicarboxylic acid (IIIb) was converted into the corresponding dihydroxy esters and triols as described above for the oxo ester (IVb). The free acid resulting from alkaline hydrolysis of one of the esters was identical with the dinor-5 β -dihydroxy acid (IXc) described above.

Therefore the other one was assigned as its 5 α epimer (IXb) and hydrolysed with alkali to yield the corresponding free acid (IXa). The triols were not further treated.

Experimental

General

The methods of chromatography and physical measurements and the micro-organism *A. simplex* were as described by Hayakawa *et al.* (1969b). Unless otherwise stated, optical rotations and i.r.-absorption spectra were determined in chloroform solution. The acidic and neutral solvent systems for t.l.c. were cyclohexane/ethyl acetate/acetic acid (5:5:1, by vol.) and cyclohexane/ethyl acetate (1:8, v/v) saturated with water respectively.

3-Oxo-24-nor-5 β -cholan-23-oic acid (I), with m.p. 176°C, was prepared from a commercial product (Mann Research Laboratories, New York, NY, U.S.A.) of lithocholic acid by the method of Sarel *et al.* (1967). They give m.p. 180–181°C for this compound. A commercial preparation (Mann) of (20S)-3 β -hydroxy-5-pregnene-20-carboxylic acid (II) was subjected to microbiological degradation without further purification. (4R)-4-[4 α -(2-Carboxyethyl)-3 $\alpha\alpha$ -hexahydro-7 $\alpha\beta$ -methyl-5-oxoindan-1 β -yl]valeric acid (IVa) and its dimethyl ester (IVb) were prepared as described by Hayakawa & Fujiwara (1969). Other materials were purchased from commercial sources and where necessary recrystallized or distilled before use. Light petroleum refers to the fraction of b.p. 40–60°C.

Preparation of the noroxodicarboxylic acid (IIIa) and the dinoroxodicarboxylic acid (IIIb)

Microbiological degradation of 3-oxo-24-nor-5 β -cholan-23-oic acid (I). The incubation of the acid with *A. simplex* was carried out in a manner similar to that described by Hayakawa *et al.* (1969b), except that the acid concentration in the medium and the incubation period were 0.08% and 5 days respectively. By using 100 flasks each containing medium (100 ml), compound (I) (8 g) yielded a mixture of degradation products (6.2 g), which was chromatographed on silicic acid (180 g). Elution with chloroform/acetone (9:1, v/v) and chloroform/acetone (4:1, v/v) gave (3R)-3-[4 α -(2-carboxyethyl)-3 $\alpha\alpha$ -hexahydro-7 $\alpha\beta$ -methyl-5-oxoindan-1 β -yl]butyric acid (IIIa), which crystallized from acetic acid/water in prisms (5.06 g), m.p. 141–142°C, $[\alpha]_D^{24} +19.1 \pm 1.4^\circ$ (c 0.424 in ethanol) and i.r. max. (in Nujol) at approx. 2600–3200 (OH of CO₂H) and 1700 (C=O and C=O of CO₂H) cm⁻¹ (Found: C, 65.8; H, 8.4; C₁₇H₂₆O₅ requires C, 65.8; H, 8.4%).

The dimethyl ester, prepared with ethereal diazomethane, crystallized from ether/light petroleum to

yield methyl (3R)-3-[3 $\alpha\alpha$ -hexahydro-4 α -(2-methoxycarbonyl)ethyl)-7 $\alpha\beta$ -methyl-5-oxoindan-1 β -yl]butyrate in plates, m.p. 70.5–71°C, $[\alpha]_D^{25.8} +18.0 \pm 0.7^\circ$ (c 0.830 in ethanol), i.r. max. at 1735 (ester) and 1708 (shoulder) (C=O) cm⁻¹ and n.m.r. absorptions at 0.99 (3H; superimposable doublet; *J* approx. 6 Hz; Me in the side chain), 1.02 (3H; singlet; 7 $\alpha\beta$ -Me), 3.63 (3H; singlet; CO₂Me) and 3.65 (3H; singlet; CO₂Me) p.p.m. (Found: C, 67.6; H, 9.1; C₁₉H₃₀O₅ requires C, 67.4; H, 8.9%).

Microbiological degradation of (20S)-3 β -hydroxy-5-pregnene-20-carboxylic acid (II). The incubation of the acid with *A. simplex* was on a flask scale, exactly as described in the cholic acid degradation by Hayakawa *et al.* (1969b). A mixture of degradation products (560 mg) obtained from compound (II) (1.3 g), on crystallization from acetone, gave (2S)-2-[4 α -(2-carboxyethyl)-3 $\alpha\alpha$ -hexahydro-7 $\alpha\beta$ -methyl-5-oxoindan-1 β -yl]propionic acid (IIIb) (408 mg). An analytical sample, further recrystallized from acetone, had m.p. 158.5–159.5°C, $[\alpha]_D^{24} +1.2 \pm 0.04$ (c 9.96 in ethanol) and i.r. max. (in Nujol) at approx. 2500–3200 (OH of CO₂H) and 1742, 1712 and 1682 (C=O and C=O of CO₂H) cm⁻¹ (Found: C, 64.9; H, 8.1; C₁₆H₂₄O₅ requires C, 64.8; H, 8.2%). A large-scale incubation was carried out with the use of jar fermentors in a manner similar to that described by Hayakawa & Fujiwara (1969) and yielded a mixture of crystalline products (16.4 g) from compound (II) (20 g). Repeated recrystallization from acetone gave compound (IIIb) (8.61 g).

The dimethyl ester, prepared with ethereal diazomethane, gave an oil and showed i.r. max. at 1732 (ester) and 1707 (shoulder) (C=O) cm⁻¹ and n.m.r. absorptions at 1.00 (3H; singlet; 7 $\alpha\beta$ -Me), 1.20 (3H; doublet; *J* 6.6 Hz; Me in the side chain) and 3.65 (6H; singlet; 2 CO₂Me) p.p.m.

Chemical proof of structures of the degradation products (IIIa) and (IIIb)

Partial synthesis of compound (IIIa). (a) Barbier-Wieland degradation of the 5 β -dihydroxy ester (Vd) to the nor-5 β -dihydroxy acid (VIIc). A solution of the ester (3.05 g), prepared from the oxodicarboxylic acid (IVa) as described below, in anhydrous benzene (40 ml) was added to a Grignard complex, prepared from Mg (3.48 g), bromobenzene (15.7 ml), ether (59 ml) and trace amounts of I₂, over a period of 10 min, with stirring and in an atmosphere of N₂. The mixture was heated for 3 h under reflux, cooled in ice and poured into a mixture of ice (approx. 150 g) and conc. HCl (25 ml). The organic layer was separated and the aqueous layer extracted with ether. The combined organic layer was successively washed with aq. 5% (w/v) Na₂S₂O₃, aq. 5% (w/v) NaHCO₃ and water, dried over anhydrous Na₂SO₄ and then evaporated *in vacuo* to yield an oily mass (6.22 g),

which was chromatographed on alumina (180g; grade III). Elution with ethyl acetate/benzene (1:4, v/v) and ethyl acetate/benzene (2:3, v/v) gave a gum (3.14g). A solution of the gum in methanolic 5% (w/v) KOH (80 ml) was heated for 1 h under reflux to hydrolyse the unchanged ester, and a neutral fraction was taken up in ether. The ethereal solution was washed with water, dried over anhydrous Na_2SO_4 and then evaporated *in vacuo* to yield a residue (3.025g), which was triturated with ether/light petroleum to give a microcrystalline powder (1.089g) and a gum (1.936g). A small sample of the powder was recrystallized from ether and then from ether/light petroleum to afford an analytical sample of (4*R*)-4-[3*αα*-hexahydro-5*β*-hydroxy-4*α*-(3-hydroxypropyl)-7*αβ*-methylindan-1*β*-yl]-1,1-diphenylpentan-1-ol (VI) as prisms, m.p. 124–125°C, $[\alpha]_D^{25} +29.3 \pm 2^\circ$ (c 1.022) and i.r. max. at 3600 and 3430 (OH) and 1604 and 1495 (C_6H_5) cm^{-1} (Found: C, 79.5; H, 9.9; $\text{C}_{30}\text{H}_{42}\text{O}_3$ requires C, 80.0; H, 9.4%). The sample gave no satisfactory analytical data, but it ran as one spot (R_F 9.55) on t.l.c. in the acidic solvent system. The remaining powder was combined with the gum and used for the next step without further purification. A solution of the crude diphenylcarbinol derivative (VI) (2.79g) in a mixture of acetic acid (26 ml) and acetic anhydride (13 ml) was heated for 1 h under reflux and the solvents were evaporated *in vacuo* to dryness. The residue was dissolved in ether, successively washed with dilute HCl, water, aq. 5% (w/v) NaHCO_3 and water, and dried over anhydrous Na_2SO_4 . Evaporation of the solvent gave a yellowish oil, which is believed to be mainly (4*R*)-4-[5*β*-acetoxy-4*α*-(3-acetoxypentyl)-3*αα*-hexahydro-7*αβ*-methylindan-1*β*-yl]-1,1-diphenylpent-1-ene. It showed i.r. bands (in film) at 1725 ($\text{C}=\text{O}$ of acetate) and 1600 and 1500 (C_6H_5) cm^{-1} and ran as one spot (R_F 0.83) on t.l.c. in the acidic solvent system, but could not be induced to crystallize. The crude oily diphenylethylene derivative was used for the next step without further purification. A solution of the oil (3.197g) in acetic acid (36 ml) was treated with a solution of CrO_3 (2.359g) in 80% (v/v) acetic acid (16 ml) at approx. 52°C over a period of 30 min. Methanol was added to the mixture to destroy the excess of CrO_3 and the mixture was concentrated *in vacuo*, diluted with water and extracted with ether. The ethereal solution was extracted several times with aq. 5% (w/v) NaHCO_3 . After the combined aqueous layer was washed with ether to remove contaminated neutral materials, it was acidified with dilute HCl and extracted with ether to recover an acidic fraction (1.502g). The combined ethereal solution, after removal of the acidic fraction, was washed with water, dried over anhydrous Na_2SO_4 and then evaporated *in vacuo* to yield a residue (1.432g), which mainly consisted of starting material, as indicated by t.l.c. in the acidic solvent system. The acidic fraction was dissolved in methanolic 5% (w/v) KOH (60 ml), and

heated for 1.5 h under reflux. The mixture was neutralized with dilute HCl to pH approx. 8 and as much solvent as possible was evaporated *in vacuo*. The concentrate was diluted with water, acidified with dilute HCl and then extracted with ether/chloroform (4:1, v/v). The extract was washed with aq. saturated NaCl, dried over anhydrous Na_2SO_4 and then evaporated *in vacuo* to yield a semicrystalline solid (966 mg), which was recrystallized from acetone to yield (3*R*)-3-[3*αα*-hexahydro-5*β*-hydroxy-4*α*-(3-hydroxypropyl)-7*αβ*-methylindan-1*β*-yl]butyric acid (VIIc) (838 mg, m.p. 145–148°C) as plates. Further recrystallization from acetone led to an analytical sample, m.p. 152.5–153.5°C, $[\alpha]_D^{24} +33.4 \pm 2^\circ$ (c 1.016 in ethanol) and i.r. max. (in Nujol) at 3440 and 3235 (OH), approx. 2500–2800 (OH of CO_2H) and 1691 ($\text{C}=\text{O}$ of CO_2H) cm^{-1} (Found: C, 68.5; H, 10.2; $\text{C}_{17}\text{H}_{30}\text{O}_4$ requires C, 68.4; H, 10.1%).

The methyl ester, prepared with ethereal diazomethane, crystallized from ether/light petroleum to yield methyl (3*R*)-3-[3*αα*-hexahydro-5*β*-hydroxy-4*α*-(3-hydroxypropyl)-7*αβ*-methylindan-1*β*-yl]butyrate (VIIId) in rods, m.p. 56–57.5°C, $[\alpha]_D^{23} +31.7 \pm 2^\circ$ (c 1.049) and i.r. max. at 3600 and 3440 (OH) and 1733 (ester) cm^{-1} (Found: C, 69.2; H, 10.5; $\text{C}_{18}\text{H}_{32}\text{O}_4$ requires C, 69.2; H, 10.3%).

(b) Oxidation of the nor-5*β*-dihydroxy ester (VIIId). A solution of the ester (400 mg) in acetone (10 ml) was treated with Jones reagent (0.98 ml) at 0°C for 1 h, which was prepared by dissolving CrO_3 (26.7 g) in water (50 ml) and conc. H_2SO_4 (23 ml) and by diluting with water to a volume of 100 ml. After the mixture had been kept overnight at approx. 20°C, it was diluted with water, saturated with NaCl and then extracted with ether. The extract was washed with aq. saturated NaCl, dried over anhydrous Na_2SO_4 and then evaporated *in vacuo* to yield a yellowish oil (357 mg), which was chromatographed on silicic acid (11 g). Elution with dichloromethane and acetone/dichloromethane (1:99, v/v) gave a crude sample of methyl (3*R*)-3-[4*α*-(2-carboxyethyl)-3*αα*-hexahydro-7*αβ*-methyl-5-oxoindan-1*β*-yl]butyrate as an oil (241 mg), which showed i.r. bands (in film) at approx. 2600–3200 (OH of CO_2H), 1727 (ester) and 1708 ($\text{C}=\text{O}$ and $\text{C}=\text{O}$ of CO_2H) cm^{-1} . The oily half-ester was hydrolysed with aq. 1*M*-KOH (2 ml) at approx. 80°C for 2 h without further purification. The mixture was acidified with dilute HCl, saturated with NaCl and then extracted with ether. The extract was washed with aq. saturated NaCl, dried over anhydrous Na_2SO_4 and then evaporated *in vacuo* to yield a pale-yellowish oil (232 mg), which was chromatographed on silicic acid (7 g). Elution with acetone/dichloromethane (1:19, v/v) gave (3*R*)-3-[4*α*-(2-carboxyethyl)-3*αα*-hexahydro-7*αβ*-methyl-5-oxoindan-1*β*-yl]butyric acid (IIIa), which crystallized from acetone in needles, m.p. 139–140°C and $[\alpha]_D^{26.5} +20.0 \pm 1.5^\circ$ (c 0.406 in ethanol) (Found: C, 66.0; H, 8.2; $\text{C}_{17}\text{H}_{26}\text{O}_5$ requires

C, 65.8; H, 8.4%). Identity with the product obtained from growing cultures was established by mixed m.p. and by comparison of the $[\alpha]_D$ and the i.r. spectrum.

Partial synthesis of compound (IIIb). (a) Barbier–Wieland degradation of the nor-5 β -dihydroxy ester (VIIId) to the dinor-5 β -dihydroxy acid (IXc). Exactly as described above in the Barbier–Wieland degradation of the 5 β -dihydroxy ester (Vd), the ester (320 mg) was converted into the corresponding crude diphenylcarbinol derivative (970 mg), which was chromatographed on alumina (28 g; grade III). Elution with ethyl acetate/benzene (1:4, v/v) yielded (3*R*)-3-[3 α -hexahydro-5 β -hydroxy-4 α -(3-hydroxypropyl)-7 β -methylindan-1 β -yl]-1,1-diphenylbutan-1-ol (VIII), which after trituration with ether/light petroleum formed an amorphous powder, m.p. 130.5°C, $[\alpha]_D^{22} +29.5 \pm 4^\circ$ (c 0.552 in ethanol) and i.r. max. at 3587 and 3426 (OH) and 1603 and 1495 (C₆H₅) cm⁻¹ (Found: C, 79.8; H, 9.3; C₂₅H₄₀O₃ requires C, 79.8; H, 9.2%).

The crude diphenylcarbinol derivative (VIII) (354 mg, m.p. 125–128°C) was degraded to the corresponding carboxylic acid via its diphenylethylene derivative as described for compound (VI). The product, on crystallization from acetone, afforded (2*S*)-2-[3 α -hexahydro-5 β -hydroxy-4 α -(3-hydroxypropyl)-7 β -methylindan-1 β -yl]propionic acid (IXc), m.p. 157–158°C, $[\alpha]_D^{23} +17.1 \pm 2^\circ$ (c 0.820 in ethanol) and i.r. max. (in Nujol) at 3490 and 3230 (OH), approx. 2500–2800 (OH of CO₂H) and 1715 (C=O of CO₂H) cm⁻¹ (Found: C, 67.7; H, 9.9; C₁₆H₂₈O₄ requires C, 67.6; H, 9.9%).

The *methyl ester*, prepared with ethereal diazomethane, crystallized from ether/light petroleum to yield *methyl* (2*S*)-2-[3 α -hexahydro-5 β -hydroxy-4 α -(3-hydroxypropyl)-7 β -methylindan-1 β -yl]propionate (IXd) in prisms, m.p. 60–62°C, $[\alpha]_D^{23} +23.4 \pm 4^\circ$ (c 0.590 in ethanol) and i.r. max. at 3600 and 3435 (OH) and 1732 (ester) cm⁻¹ (Found: C, 68.2; H, 10.1; C₁₇H₃₀O₄ requires C, 68.4; H, 10.1%).

(b) Oxidation of the dinor-5 β -dihydroxy ester (IXd). As described above for the nor-5 β -dihydroxy ester (VIIId), the ester (277 mg) was oxidized with Jones reagent to yield a mixture of products (187 mg), which was chromatographed on silicic acid (6 g). Elution with dichloromethane gave *methyl* (2*S*)-2-[4 α -(2-carboxyethyl)-3 α -hexahydro-7 β -methyl-5-oxoindan-1 β -yl]propionate, which crystallized from ether/light petroleum in prisms, m.p. 93–94°C, $[\alpha]_D^{22} +10.7 \pm 4^\circ$ (c 0.516 in ethanol) and i.r. max. (in Nujol) at 3200 (broad) (OH of CO₂H), 1739 (ester) and 1702 (C=O and C=O of CO₂H) cm⁻¹ (Found: C, 65.9; H, 8.5; C₁₇H₂₆O₅ requires C, 65.8; H, 8.4%). The half-ester was hydrolysed with aq. 1*M*-KOH to yield (2*S*)-2-[4 α -(2-carboxyethyl)-3 α -hexahydro-7 β -methyl-5-oxoindan-1 β -yl]propionic acid (IIIb), which crystallized from acetone in prisms, m.p. 156–157°C and $[\alpha]_D^{22} +2.0 \pm 4^\circ$ (c 0.642 in ethanol) (Found: C,

65.1; H, 8.1; C₁₆H₂₄O₅ requires C, 64.8; H, 8.2%). Identity with the product obtained from growing cultures was established by mixed m.p. and by comparison of the $[\alpha]_D$ and the i.r. spectrum.

Preparation of reduction products of the oxodicarboxylic acids (IIIa), (IIIb) and (IVa)

Preparation of the dihydroxy acids (Vb) and (Ve) and the triols (Vc) and (Vf). NaBH₄ (6.25 g) in limited amounts was added to a stirred solution of the oxo ester (IVb) (10 g) in methanol (125 ml) during a period of 15 min, under cooling at approx. 0°C. After stirring for a further 1 h at room temperature, the mixture was treated with dilute acetic acid to destroy the excess of NaBH₄, and then most of the methanol was evaporated *in vacuo*. The concentrate was diluted with water, saturated with NaCl and then extracted with ether. The extract was washed with aq. 5% (w/v) NaHCO₃ and then with water, dried over anhydrous Na₂SO₄ and evaporated *in vacuo* to afford a mixture of products, which was chromatographed on alumina (300 g; grade III). Elution with various solvent systems gave the respective methyl esters of the dihydroxy acids (Vb) and (Ve) and the triols (Vc) and (Vf) as follows.

(a) Isolation of the 5 α -dihydroxy ester (Va). Elution with benzene and ethyl acetate/benzene (1:19, v/v) gave *methyl* (4*R*)-4-[3 α -hexahydro-5 α -hydroxy-4 α -(3-hydroxypropyl)-7 β -methylindan-1 β -yl]valerate (Va), which crystallized from ether/light petroleum in needles (1.83 g), m.p. 115–116.5°C, $[\alpha]_D^{28} -4.3 \pm 0.6^\circ$ (c 0.767 in ethanol), i.r. max. at 3600 and 3430 (OH) and 1732 (ester) cm⁻¹ and n.m.r. absorptions at 0.68 (3H; singlet; 7 β -Me), 0.94 (3H; doublet; *J* 5.2 Hz; Me in the side chain), 1.86 (2H; singlet; 2OH), approx. 3.60 (2H; superimposable triplet; CH₂OH), 3.67 (3H; singlet; CO₂Me) and 3.94 (1H; multiplet; *WH* 5.0 Hz; 5 β -H) p.p.m. (Found: C, 69.8; H, 10.6; C₁₉H₃₄O₄ requires C, 69.9; H, 10.5%).

Its *diacetate*, prepared with acetic anhydride/pyridine (16 h at room temperature), could not be induced to crystallize. It showed i.r. band at 1730 (C=O of ester and acetate) cm⁻¹ and n.m.r. absorptions at 0.69 (3H; singlet; 7 β -Me), 0.93 (3H; doublet; *J* 5.0 Hz; Me in the side chain), 2.01 (3H; singlet; OAc), 2.03 (3H; singlet; OAc), 3.66 (3H; singlet; CO₂Me), 4.01 (2H; triplet; *J* 6.4 Hz; CH₂OAc) and 5.07 (1H; multiplet; *WH* 5.0 Hz; 5 β -H) p.p.m.

A solution of the ester (Va) (120 mg) in methanolic 5% (w/v) KOH (2 ml) was heated for 2 h under reflux. The mixture was neutralized with dilute HCl to pH approx. 8, the solvents were evaporated *in vacuo*, the residue was dissolved in water and the solution was acidified with dilute HCl. Recovery in ether gave (4*R*)-4-[3 α -hexahydro-5 α -hydroxy-4 α -(3-hydroxypropyl)-7 β -methylindan-1 β -yl]valeric acid (Vb), which crystallized from acetone in prisms, m.p. 106–

107°C, $[\alpha]_D^{25} -4.5 \pm 0.9^\circ$ (c 0.485 in ethanol) and i.r. max. (in Nujol) at 3290 (broad) (OH), approx. 2500–2800 (OH of CO₂H) and 1740 and 1694 (C=O of CO₂H) cm⁻¹ (Found: C, 69.3; H, 10.4; C₁₈H₃₂O₄ requires C, 69.2; H, 10.3%).

(b) Isolation of the 5 β -dihydroxy ester (Vd). Elution with ethyl acetate/benzene (from 1:9, v/v, to 1:4, v/v), ethyl acetate and acetone yielded methyl (4R)-4-[3 α -hexahydro-5 β -hydroxy-4 α -(3-hydroxypropyl)-7 β -methylindan-1 β -yl]valerate (Vd), which crystallized from ether/light petroleum in plates (2.357 g), m.p. 64.5–65°C, $[\alpha]_D^{26.9} +36.9 \pm 0.9^\circ$ (c 0.895 in ethanol), i.r. max. at 3595 and 3435 (OH) and 1731 (ester) cm⁻¹ and n.m.r. absorptions at 0.71 (3H; singlet; 7 α -Me), 0.88 (3H; doublet; J 5.1 Hz; Me in the side chain), 1.95 (2H; singlet; 2OH), 3.27 (1H; multiplet; WH approx. 20 Hz; 5 α -H), approx. 3.60 (2H; superimposable triplet; CH₂OH) and 3.63 (3H; singlet; CO₂Me) p.p.m. (Found: C, 69.9; H, 10.5; C₁₉H₃₄O₄ requires C, 69.9; H, 10.5%).

The mother liquors, after removal of both the esters (Va) and (Vd), were combined and evaporated *in vacuo* to yield a semicrystalline residue (3.405 g) that consisted mainly of compounds (Va) and (Vd). The residue was rechromatographed on alumina (100g; grade III) and yielded compound (Va) (447 mg), compound (Vd) (1.448 g) and a mixture of these compounds (566 mg).

The oily diacetate of compound (Vd), prepared with acetic anhydride/pyridine (16 h at room temperature), showed i.r. band at 1730 (C=O of ester and acetate) cm⁻¹ and n.m.r. absorptions at 0.76 (3H; singlet; 7 α -Me), 0.91 (3H; doublet; J 5.3 Hz; Me in the side chain), 2.02 (6H; singlet; 2 OAc), 3.65 (3H; singlet; CO₂Me), 4.00 (2H; triplet; J 6.2 Hz; CH₂OAc) and 4.50 (1H; multiplet; WH approx. 20 Hz; 5 α -H) p.p.m.

The ester (Vd) was treated with alkali as described above for the ester (Va), and the resulting free acid was recrystallized from ether/benzene to yield (4R)-4-[3 α -hexahydro-5 β -hydroxy-4 α -(3-hydroxypropyl)-7 β -methylindan-1 β -yl]valeric acid (Ve) as prisms, m.p. 133–134°C, $[\alpha]_D^{24} +37.0 \pm 0.8^\circ$ (c 0.894 in ethanol) and i.r. bands (in Nujol) at 3440 and 3200 (OH), approx. 2500–2700 (OH of CO₂H) and 1704 (C=O of CO₂H) cm⁻¹ (Found: C, 69.5; H, 10.3; C₁₈H₃₂O₄ requires C, 69.2; H, 10.3%).

(c) Isolation of the 5 α -triol (Vc) and its 5 β epimer (Vf). Elution with methanol gave an intractable gum (1.78 g), which was heated with methanolic 5% (w/v) KOH (15 ml) for 1 h under reflux. The mixture was neutralized with dilute HCl to pH approx. 8 and concentrated *in vacuo*. The concentrate was diluted with water and extracted with ether. The extract was washed with aq. saturated NaCl, dried over anhydrous Na₂SO₄ and then evaporated *in vacuo* to yield a neutral fraction (637 mg). The aqueous layer, after removal of the neutral materials, was acidified with

dilute HCl and extracted with ether. The extract, on crystallization from acetone, yielded also the 5 β -dihydroxy acid (Ve) (570 mg). The neutral fraction, on crystallization from acetone, gave (4R)-4-[3 α -hexahydro-5 β -hydroxy-4 α -(3-hydroxypropyl)-7 β -methylindan-1 β -yl]pentan-1-ol (Vf) (225 mg) with m.p. 123–126.5°C. Further recrystallization from acetone gave an analytical sample, m.p. 126.5–127°C, $[\alpha]_D^{28.8} +40.7 \pm 1.1^\circ$ (c 0.765 in ethanol) and i.r. max. (in Nujol) at 3280 (broad) (OH) cm⁻¹ (Found: C, 72.6; H, 11.4; C₁₈H₃₄O₃ requires C, 72.4; H, 11.5%). Identity with an authentic sample, obtained from the LiAlH₄ reduction of the 5 β -dihydroxy ester (Vd) as described below, was established by mixed m.p. and by comparison of the $[\alpha]_D$ and the i.r. spectrum.

The mother liquor, after removal of the 5 β -triol (Vf), was subjected to preparative t.l.c. with ten plates (20 cm $\times$ 20 cm, 0.75 mm thick) of silica gel GF₂₅₄, and two bands (R_F 0.19 and 0.23), as revealed by a water spray, were separated by developing with the neutral solvent system. The faster-moving band was scraped from the plates and extracted with chloroform/methanol (1:1, v/v). Evaporation of the solvents gave (4R)-4-[3 α -hexahydro-5 α -hydroxy-4 α -(3-hydroxypropyl)-7 β -methylindan-1 β -yl]pentan-1-ol (Vc) as a gum (150 mg), which could not be crystallized. Its i.r. absorptions showed bands at 3600 and 3440 (OH) cm⁻¹, and identity with an authentic sample, obtained from the LiAlH₄ reduction of the 5 α -dihydroxy ester (Va) as described just below, was determined by i.r. and t.l.c. comparisons. The slower-moving band was treated in a manner similar to that described above for compound (Vc) and the 5 β -triol (Vf) (130 mg) recovered.

Alternative preparation of the 5 α -triol (Vc) and its 5 β epimer (Vf). A solution of the 5 α -dihydroxy ester (Va) (778 mg) in anhydrous ether (40 ml) was added dropwise to a solution of LiAlH₄ (900 mg) in anhydrous ether (20 ml), with stirring. The mixture was heated for 3 h under reflux, cooled to room temperature and carefully treated with ethyl acetate (10 ml) and then with dilute HCl (10 ml). The organic layer was separated, and the aqueous layer saturated with NaCl and extracted with ether. The combined organic layer was washed with aq. 5% (w/v) NaHCO₃ and then with water, dried over anhydrous Na₂SO₄ and evaporated *in vacuo* to yield a mixture of products, which was heated with methanolic 5% (w/v) KOH (10 ml) for 1 h under reflux. The mixture was neutralized with dilute acetic acid, concentrated *in vacuo* and then extracted with ether. The extract was washed with aq. 5% (w/v) NaHCO₃ and then with aq. saturated NaCl, dried over anhydrous Na₂SO₄ and evaporated *in vacuo* to yield the 5 α -triol (Vc) as a gum (555 mg). It could not be induced to crystallize, but ran as one spot (R_F 0.21) on t.l.c. in the neutral solvent system.

The 5 β -dihydroxy ester (Vd) (389 mg) in ether was treated with LiAlH₄, exactly as described above for the 5 α -dihydroxy ester (Va). The product was recrystallized from acetone to yield the 5 β -triol (Vf) as prisms (209 mg), m.p. 126.5–127.5°C and $[\alpha]_D^{23} +41.1 \pm 0.2$ (c 0.609 in ethanol) (Found: C, 72.1; H, 11.3; C₁₈H₃₄O₃ requires C, 72.4; H, 11.5%).

Preparation of the nor-5 α -dihydroxy acid (VIIa) and its 5 β epimer (VIIc). The 5 β epimer was already prepared by the Barbier–Wieland degradation of the 5 β -dihydroxy ester (Vd) [see under ‘Partial synthesis of compound (IIIa), (a)’ above]. The preparation of the 5 α epimer was as follows. A mixture (6.2 g), resulting from the NaBH₄ reduction of compound (IVb) and consisting mainly of the 5 α -dihydroxy ester (Va) and its 5 β epimer (Vd), was converted by the Barbier–Wieland method into a mixture (1.399 g) of the corresponding nordihydroxy acids (VIIa) and (VIIc). It was esterified with ethereal diazomethane and chromatographed on alumina (40 g; grade IV). Elution with light petroleum/benzene (3:2, v/v) gave methyl (3R)-3-[3 α -hexahydro-5 α -hydroxy-4 α -(3-hydroxypropyl)-7 $\alpha\beta$ -methylindan-1 β -yl]butyrate (VIIb), which crystallized from ether in needles (192 mg, m.p. 114–118°C). Further recrystallization from ether gave an analytical sample, m.p. 118–119°C, $[\alpha]_D^{22} -10.1 \pm 4^\circ$ (c 0.483 in ethanol) and i.r. max. at 3615 and 3445 (OH) and 1734 (ester) cm⁻¹ (Found: C, 68.9; H, 10.4; C₁₈H₃₂O₄ requires C, 69.2; H, 10.3%).

Alkaline hydrolysis of the ester (VIIb) in the usual way, described above for the 5 α -dihydroxy ester (Va), gave (3R)-3-[3 α -hexahydro-5 α -hydroxy-4 α -(3-hydroxypropyl)-7 $\alpha\beta$ -methylindan-1 β -yl]butyric acid (VIIa), which crystallized from acetone/light petroleum in prisms, m.p. 138.5–140°C, $[\alpha]_D^{22} -11.6 \pm 4^\circ$ (c 0.533 in ethanol) and i.r. max. (in Nujol) at 3325 (broad) and 3165 (shoulder) (OH), approx. 2500–2800 (OH of CO₂H) and 1724 and 1660 (C=O of CO₂H) cm⁻¹ (Found: C, 68.2; H, 10.1; C₁₇H₃₀O₄ requires C, 68.4; H, 10.1%).

Further elution with light petroleum/benzene (1:4, v/v), benzene and ethyl acetate gave a gum. It was further purified by rechromatography on alumina (grade IV) to yield the nor-5 β -dihydroxy ester (VIIc) with m.p. 56–57.5°C. Identity with the product, prepared by the Barbier–Wieland degradation of the 5 β -dihydroxy ester (Vd), was established by mixed m.p. and by comparison of the i.r. spectrum.

Preparation of the dinor-5 α -dihydroxy acid (IXa) and its 5 β epimer (IXc). Exactly as described above for the oxo ester (IVb), the dimethyl ester (10 g) of the dinoroxodicarboxylic acid (IIIb), obtained from growing cultures, was treated with NaBH₄ in methanol to afford a mixture of products, which was chromatographed on alumina (300 g; grade IV). Elution with light petroleum/benzene (1:4, v/v) and benzene gave methyl (2S)-2-[3 α -hexahydro-5 α -

hydroxy-4 α -(3-hydroxypropyl)-7 $\alpha\beta$ -methylindan-1 β -yl]propionate (IXb), which crystallized from acetone/light petroleum in plates (890 mg), m.p. 120–121°C, $[\alpha]_D^{22} -21.9 \pm 4^\circ$ (c 0.547 in ethanol) and i.r. max. at 3610 and 3435 (OH) and 1732 (ester) cm⁻¹ (Found: C, 68.1; H, 10.1; C₁₇H₃₀O₄ requires C, 68.4; H, 10.1%).

Alkaline hydrolysis of the ester (IXb) in the usual way yielded (2S)-2-[3 α -hexahydro-5 α -hydroxy-4 α -(3-hydroxypropyl)-7 $\alpha\beta$ -methylindan-1 β -yl]propionic acid (IXa), which crystallized from acetone in plates, m.p. 165°C, $[\alpha]_D^{22} -34.4 \pm 3^\circ$ (c 0.720 in ethanol) and i.r. max. (in Nujol) at 3390 (OH), approx. 2600–2800 (OH of CO₂H) and 1704 (C=O of CO₂H) cm⁻¹ (Found: C, 67.7; H, 9.9; C₁₆H₂₈O₄ requires C, 67.6; H, 9.9%).

Elution with ethyl acetate/benzene (from 1:19, v/v, to 1:1, v/v) and ethyl acetate gave the dinor-5 β -dihydroxy ester (IXd), which after hydrolysis with alkali, formed the free acid (IXc), m.p. 154–157°C and $[\alpha]_D^{23} +16.9 \pm 0.6$ (c 0.692 in ethanol) (Found: C, 67.9; H, 10.0; C₁₆H₂₈O₄ requires C, 67.6; H, 9.9%). The behaviour on t.l.c. and the i.r., m.p. and $[\alpha]_D$ of this acid were identical with those of the product obtained by the duplicated Barbier–Wieland degradation of the 5 β -dihydroxy ester (Vd) [see under ‘Partial synthesis of compound (IIIb), (a)’ above].

Elution with methanol gave a gummy material (1.86 g), which probably consists of a mixture of the C₁₆ triols corresponding to the C₁₈ triols (Vc) and (Vf), but it was not further treated. The mother liquors, after removal of both the esters (IXb) and (IXd), were combined and evaporated *in vacuo* to afford a residue, which was rechromatographed on alumina as described above yielding compound (IXb) (1.53 g) and compound (IXd) (3.41 g).

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