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MICROBIAL METABOLISM OF TETRA- AND HEXAHYDROINDANPROPIONIC ACID DERIVATIVES

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SUMMARY

Both microorganims, *Nocardia restrictus* and *Nocardia opaca* metabolized 7aβ-methyl-1,5-dioxo-$\Delta^{6(7)}$-tetrahydro-4-indanpropionic acid and 7aβ-methyl-1β- acetoxy-5-oxo-3aα-hexahydro-4-indanpropionic acid to form 7aβ-methyl-1,5-dioxo-7α-hydroxy-3aα-hexahydro-4-indanpropionic acid and 7αβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionyl-7aβ-methyl-5-oxo-1β-hydroxy-3aα-hexahydro-4-indanpropionic acid respectively. The structures of these metabolites were established by converting them to common intermediates, derived from unambiguous chemical synthesis. The following compounds were not metabolized: 1β-Acetoxy-2-methyl-2-(propionic acid)-3-(α-hydroxy-β-carboxyethyl)cyclopentane-ε-lactone; 2-methyl-cyclopentane-1,3-dione; 2-methyl-2-(β-carboxyethyl)cyclopentane-1,3-dione; (±)-7,7a-dihydro-7aβ-methyl-1,5(6H)-indandione; (±)-3,4,8,8a-tetrahydro-8aβ-methyl-1,6-(2H,7H)-naphthalenedione and 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanacetic acid.

INTRODUCTION

The pathway for the degradation of androst-4-ene-3,17-dione into 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionic acid (I) by microorganisms is now reasonably well understood[1–4].

In contrast, relatively little progress has been made over the years relative to defining the intermediates and reaction sequence in the microbial metabolism of I. The difficulty has been the recalcitrant microbes to accumulate some desired degradative intermediates. However, investigations[5] using deoxo-analogs of I revealed that microorganisms removed two carbon atoms from the propionic acid side chain of I, corresponding to carbons 5 and 6 of the steroid skeleton. Since this approach to the problem appeared more fruitful, we continued our studies along this line by synthe-

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sizing various derivatives of I and exposing them to microorganisms with a view to obtaining some information concerning the mechanism of degradation. The present paper deals with the details and structure proofs of some new unexpected metabolites derived from analog studies.

MATERIALS AND METHODS

2-Methyl-2-(β-carboxyethyl)-cyclopentane-1,3-dione was a gift from Dr. R. E. BROWN of Warner-Lambert Research Institute. 2-Methylcyclopentane-1,3-dione was provided by Dr. H. SMITH of Wyeth Labs. ($\pm$)-7,7a-Dihydro-7aβ-methyl-1,5(6H)-indandione was obtained from Dr. Z. G. HAJOS of Hoffmann–La Roche. 7aβ-Methyl-1,5-dioxo-3aα-hexahydro-4-indanacetic acid and 7aβ-methyl-1β-hydroxy-5-oxo-3aα-hexahydro-4-indanpropionic acid were gifts from Dr. G. NOMINE of Roussel Uclaf. 3,4,8,8a-Tetrahydro-8a-methyl-1,6(2H,7H)-naphthalenedione was purchased from the Aldrich Chemical.

Nocardia restrictus (ATCC 14887) and *Nocardia opaca* were kindly supplied by Dr. R. E. KALLIO, University of Illinois, Urbana, Ill. The cultures were maintained on nutrient agar slants, supplemented with 1% yeast extract and 1% glucose. All solvents and inorganic chemicals were of reagent grade. Light petroleum refers to the fraction with a boiling point of 40–70°. Silicic acid (Mallinckrodt 2847) was used for column chromatography and silica gel HF (Merck 7741) was used for thin-layer chromatography.

Melting points, determined on a Thomas-Hoover melting point apparatus are corrected. Ultraviolet absorption spectra were determined on a Cary Model 11 MS recording spectrophotometer and 95% ethanol was used as solvent. Infrared spectra were recorded on a Beckman IR 5A double-beam infrared recording spectrophotometer. Nuclear Magnetic Resonance (NMR) spectra were determined on a Varian Associates recording spectrometer (A60 A) at 60 Mcycles in either deuterated dimethylsulfoxide or chloroform with tetramethylsilane as an internal standard. Chemical shifts are reported in τ values (ppm) (ref. 6). Mass spectra were taken by Morgan–Schaffer, Montreal, Quebec, Canada, on an Atlas CH 4 mass spectrometer, operating at an ionization of 70 V and employing a heated glass inlet system at 250°. Values of $[\alpha]_D$ have been approximated to the nearest degree. The growth conditions for the microorganisms have been described previously[7].

Transformation of 7aβ-methyl-1,5-dioxo-$\Delta^{6(7)}$-tetrahydro-4-indanpropionic acid (II)

N. opaca was grown in 4.4 l of Difco nutrient broth (eleven 2-l erlenmeyer flasks). After 20 h 3.52 g of II in 25 ml of dimethylformamide was distributed equally among the flasks. After an additional 30 h, the fermentation was terminated by the addition of glacial acetic acid (4 ml) to each flask, and the cell-debris was removed by filtration. The combined filtrate was extracted three times with ethyl acetate. The combined ethyl acetate extract (15 l) was washed with water, dried over Na_2SO_4 and evaporated to dryness to yield 1.32 g of residue. The residue was chromatographed over a silicic acid (150 g) column. The column was eluted with chloroform–methanol–acetic acid (98:2:0.1, by vol.) and 20-ml fractions were collected. Fractions 216–255 were pooled and evaporated to give 185 mg of 7aβ-methyl-1,5-dioxo-7α-hydroxy-3aα-hexahydro-4-indanpropionic acid (III). Recrystallization from acetone–ether afforded

164 mg of an analytical specimen, m.p. 138–140°; λ_{max}^{Nujol}, 2.89, 3.13 and 5.82 μ; $[\alpha]_D^{25}$, +133° (*c*, 0.9 in chloroform).

Analysis. Calculated for $C_{13}H_{18}O_5$ (254.27): C, 61.40; H, 7.14. Found: C, 61.35; H, 7.26.

Dehydration of 7aβ-methyl-1,5-dioxo-7α-hydroxy-3aα-hexahydro-4-indanpropionic acid (III)

To 32 mg of III, dissolved in 5 ml of acetone was added 2 drops of concentrated HCl and the mixture was refluxed for 15 min. After extraction of the reaction mixture with chloroform, the chloroform was washed with water, dried over Na_2SO_4 and evaporated to dryness to yield 4.2 mg of crystals, m.p. 120–122°. This sample was found to be identical to an authentic sample of II, with respect to chromatographic behavior, infrared spectrum and mixed melting point.

7aβ-Methyl-1,5-dioxo-$\Delta^{6(7)}$-3aα-tetrahydro-4-indanpropionic acid (II)

To 7 g of 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionic acid (I), dissolved in 350 ml of acetic acid was added dropwise a mixture containing 4.94 g of Br_2, 15 drops of 48% aqueous HBr in 160 ml of acetic acid at room temperature over a 30-min period. The reaction mixture was then diluted with water and extracted with chloroform three times. The combined chloroform extract was washed with water, dried over Na_2SO_4 and evaporated to dryness to yield an oily residue (10.2 g). The bromoketone was dissolved in 120 ml of dimethylformamide. After the addition of $CaCO_3$ (7 g), the mixture was refluxed for 35 min. The reaction was terminated by dilution with water, acidified with 2 M HCl, and extracted with chloroform. After evaporation of chloroform, the residue was dissolved in 200 ml of 5% $NaHCO_3$. The bicarbonate layer was extracted with chloroform and the chloroform layer was discarded. The aqueous layer was acidified with 2 M HCl and was then extracted with ethyl acetate. The combined ethyl acetate layer was washed with water, dried over Na_2SO_4 and evaporated to dryness. The oily residue obtained was dissolved in chloroform and chromatographed over a silicic acid (400 g) column (5 cm × 32 cm). After elution of the column with 2 l of chloroform, the solvent was changed to chloroform–methanol–acetic acid (99.5:0.5:0.1, by vol.) and 20-ml fractions were collected. Fractions 91–291 were pooled and afforded 2.8 g of crystals. Two recrystallizations from acetone–light petroleum afforded an analytical sample, m.p. 123–125°; λ_{max}^{Nujol}, 3.02, 5.79, 5.99 and 6.19 μ; $[\alpha]_D^{25}$, +46° (*c*, 0.9 in chloroform); $\lambda_{max}^{ethanol}$, 223 mμ (ε, 9000). NMR, τ 8.85 (3H, singlet, CH_3, at C-7a), τ 4.03 and 2.69 (2H, two perturbed doublets, J = 10 cycles/sec, vinylic proteins), and τ −1.1 (1H, singlet, COOH).

Analysis. Calculated for $C_{13}H_{16}O_4$ (236.26): C, 66.08; H, 6.83. Found: C, 66.47; H, 6.89.

Fractions 324–460 were evaporated to dryness and afforded 537 mg of 7aβ-methyl-1,5-dioxo-$\Delta^{4(3a)}$-tetrahydro-4-indanpropionic acid, m.p. 142–144°, identical to an authentic specimen with respect to infrared spectrum and mixed melting point.

7aβ-Methyl-1,5-dioxo-6α,7α-oxido-3aα-hexahydro-4-indanpropionic acid (IV)

To 1.6 g of II, dissolved in 80 ml of dioxane was added dropwise with stirring 5 ml of 10% NaOH and 2.5 ml of 30% H_2O_2 at 10–15°. After 1 h, the reaction mixture was diluted with water (900 ml), acidified with glacial acetic acid (2.5 ml) and was

extracted with chloroform 3 times. The combined chloroform layers were washed twice with water, dried over Na_2SO_4 and concentrated to yield 1.176 g of crystalline residue. Two crystallizations from ether–light petroleum afforded 0.66 g of IV, m.p. 131–133°; λ_{max}^{Nujol}, 3.13, 5.78, 5.81 and 5.86 μ; $[\alpha]_D^{25}$, +163° (*c*, 0.97 in chloroform). NMR, τ 8.97 (3H, singlet, CH_3 at C-7a), τ 6.63 and 6.18 (2H, two perturbed doublets, $J = 5$ cycles/sec, proteins at C-6 and C-7), τ −1.0 (1H, singlet, COOH).

Analysis. Calculated for $C_{13}H_{16}O_5$ (252.26): C, 61.89; H, 6.39. Found: C, 62.09; H, 6.69.

7aβ-Methyl-1β,5β,7α-trihydroxy-3aα-hexahydro-4-indanpropanol (V)

(A) From reaction of IV with LiAlH₄. To 600 mg of the epoxide (IV) in 23 ml of dry tetrahydrofuran, was added 1.2 g of $LiAlH_4$ and the mixture was refluxed for 3 h. Excess $LiAlH_4$ was destroyed by the addition of water. After acidification with acetic acid, the mixture was extracted with ethyl acetate. The combined ethyl acetate extracts were washed with water, dried over Na_2SO_4 and evaporated to dryness to give 280 mg of an oily residue. The residue was chromatographed over a celite (15 g) column using glycerol (4 ml)–methanol (12 ml) as the stationary phase. Benzene was used as the mobile phase and 15-ml fractions were collected. Fractions 22–49 were pooled and evaporated to yield 36 mg of V after recrystallizations from acetone–ether, m.p. 125–127°; λ_{max}^{Nujol}, 3.02 μ.

(B) From reduction of III with LiAlH₄. To 240 mg of $LiAlH_4$, suspended in 10 ml of tetrahydrofuran, was added 120 mg of III and the mixture was refluxed for 2.5 h. Using the same work-up procedure as above, 41 mg of an oily product was obtained. It was chromatographed over 3 g of celite using the same stationary phase. Elution of the column with benzene afforded 27 mg of V, m.p. 125–127°, identical in all respects (mixed melting point and infrared spectrum) with a sample of V, obtained from A above.

1β-Acetoxy-2-methyl-2-(propionic acid)-3-(α-hydroxy-γ-carboxypropyl)-cyclopentane-ε-lactone (VI)

1 g of the acetate 7aβ-methyl-1β-acetoxy-5-oxo-3aα-hexahydro-4-indanpropionic acid (VII) was dissolved in 20 ml of acetic acid. To this solution was added dropwise 3 g of *m*-chloroperbenzoic acid, dissolved in 30 ml of acetic acid. After the addition of 20 mg of *p*-toluenesulfonic acid, the reaction mixture was allowed to stand at room temperature for 15 h. The content was then diluted with water and filtered to remove the *m*-chlorobenzoic acid. The filtrate was extracted with chloroform three times and the combined chloroform layers were washed with water and evaporated to dryness. The residue was chromatographed over 120 g of silicic acid column (3.3 cm × 33 cm). After washing the column with 500 ml of chloroform, the eluent was changed to chloroform–methanol–acetic acid (99.5:0.5:0.1, by vol.) and 15-ml fractions were collected. Fractions 110–170 were pooled and evaporated to dryness to give 699 mg of VI, m.p. 151.5–153°; $[\alpha]_D^{25}$, +41° (*c*, 1.02 in $CHCl_3$); λ_{max}^{Nujol}, 5.65, 5.73 and 5.90 μ. NMR, τ 9.00 (3H, singlet, CH_3 at C-7a), τ 7.95 (3H, singlet $CH_3-\overset{O}{\overset{\|}{C}}-O-$), τ 5.17 (1H, triplet, $J = 7$ cycles/sec, proton at C-1), and τ −0.40 (1H, COOH).

Analysis. Calculated for $C_{15}H_{22}O_6$ (298.33): C, 60.39; H, 7.43. Found: C, 61.17; H, 7.75.

Fermentation of 7aβ-methyl-1β-acetoxy-5-oxo-3aα-hexahydro-4-indanpropionic acid (VII)

N. restrictus was grown in 4 l of Difco nutrient broth (ten 2-l erlenmeyer flasks). After 24 h, 2 g of VII, dissolved in 25 ml of dimethylformamide was distributed equally among the flasks and the incubation was then continued for an additional 18 h. The broth was acidified with glacial acetic acid (40 ml) and extracted with chloroform. The combined chloroform extract was dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed over a silicic acid (150 g) column. The column was eluted with chloroform–methanol–acetic acid (99.5:0.5:0.1, by vol.) and 15-ml fractions were collected. After pooling together Fractions 169–230, 482 mg of XIV was obtained which resisted crystallization. Molecular weight 460 (mass spectrum; (Fig. 1); λ_{max}^{Nujol}, 3.07, 5.72, 5.78 and 5.83 μ. NMR spectrum (see Fig. 2).

7aβ-Methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionyl-7aβ-methyl-5-oxo-1β-hydroxy-3aα-hexahydro-4-indanpropionic acid methyl ester (XVI)

(A) To 1.36 g of I, suspended in 10 ml of dry benzene, was added 3.5 ml of

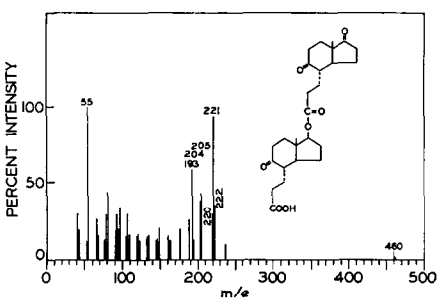


Fig. 1. The mass spectrum of 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionyl-7aβ-methyl-5-oxo-1β-hydroxy-3aα-hexahydro-4-indanpropionic acid (XIV).

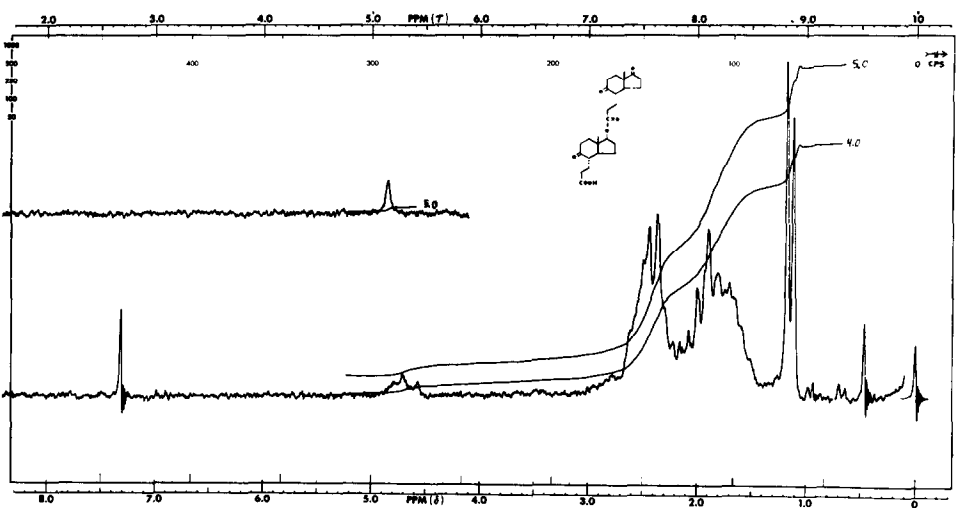


Fig. 2. The NMR spectrum of 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionyl-7aβ-methyl-5-oxo-1β-hydroxy-3aα-hexahydro-4-indanpropionic acid (XIV) in deuterated chloroform.

oxalyl chloride and 3 drops of dimethylformamide. Upon the addition of oxalyl chloride, there was an immediate evolution of gases (CO, CO_2, HCl) and when the dimethylformamide was added, there was an additional evolution of gas. After stirring for 1 h, the solvent and excess oxalyl chloride were evaporated under vacuum, yielding the acid chloride. Methyl-7aβ-methyl-5-oxo-1β-hydroxy-3aα-hexahydro-4-indanpropionic acid (1 ml) (XV), dissolved in 5 ml of benzene was added dropwise to the acid chloride. The reaction mixture was allowed to stir overnight. The mixture was evaporated to dryness and chromatographed over 250 g of silica gel (Merck). Elution of the column with acetone–chloroform (10:90, by vol.) afforded 0.5 ml of XVI, λ_{max}^{Nujol}, 5.82, 5.87, 5.93 and 8.48 μ. Molecular weight 474 (mass spectrum; Fig. 4); NMR spectrum is shown in Fig. 3.

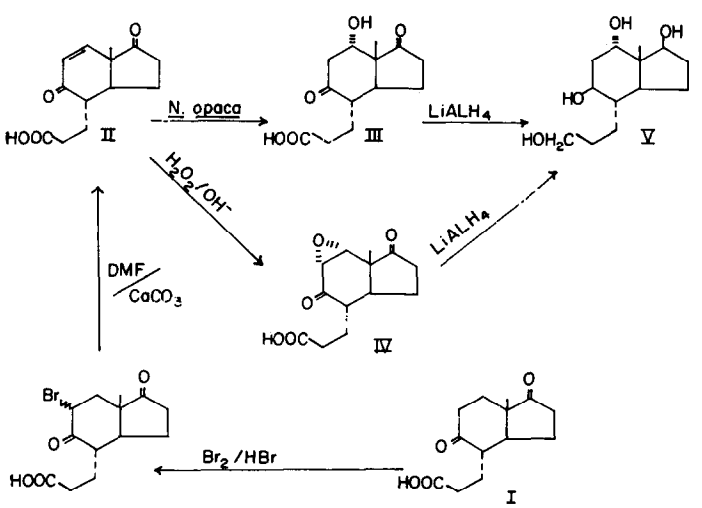
Analysis. Calculated for $C_{27}H_{38}O_7$ (474.57): C, 68.33; H, 8.07. Found: C, 68.14; H, 7.84.

(B) Diazomethane was prepared in the usual manner from Diazald, NaOH and the dimeric acid XIV in ether. Distillation was continued until the solution remained yellow. It was allowed to stand overnight. After evaporation of the solvent, the methyl ester was recovered in the usual way. On thin-layer chromatography (acetone–chloroform (2:8, by vol.)), a single spot (R_F = 0.66) less polar than the starting material was obtained. Its infrared spectrum, chromatographic behavior and mass spectrum was identical to the sample of methyl ester obtained by A above.

RESULTS AND DISCUSSION

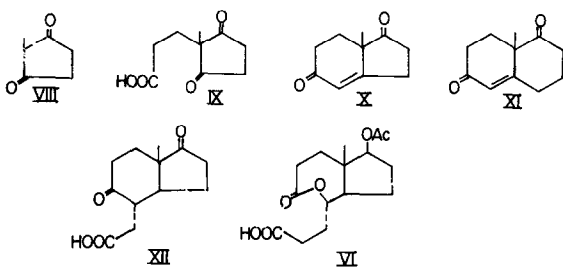
Previous work from our laboratory[5] showed that the first site of attack of 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionic acid (I) by *N. restrictus* probably involves the β-oxidation of the propionic acid side chain, in a manner analogous to the conventional fatty acid oxidation mechanism, resulting in the loss of a C-2 unit. It occurred to us initially that the compound, 7aβ-methyl-1,5-dioxo-$\Delta^{6(7)}$-3aα-tetrahydro-4-indanpropionic acid (II) would be a desirable analog since one could easily follow its metabolic fate by virtue of its ultraviolet-absorption property. Thus, II was synthesized from I by the conventional bromination–debromination procedure[8] and was exposed to *N. restrictus* and *N. opaca*. Both micro organisms metabolized II to yield a more polar product as evidenced on thin-layer chromatograms, developed with chloroform–acetone–acetic acid (80:20:1, by vol.). A large-scale fermentation of II was conducted using *N. opaca*, since this microorganisms appeared to give a better yield of the product. On the basis of the following physical and chemical data, this product was assigned the structural formula, 7aβ-methyl-1,5-dioxo-7α-hydroxy-3aα-hexahydro-4-indanpropionic acid (III). Carbon hydrogen analysis was in good agreement with $C_{13}H_{18}O_5$; its infrared spectrum showed bands at 2.89 (hydroxyl), 3.13 (OH of carboxyl), 5.77 and 5.83 μ (carbonyls); its NMR spectrum in deuterated dimethylsulfoxide showed peaks ar τ 8.97 singlet (3H, tertiary CH_3), τ 5.92 triplet (1H, J = 2.5 cycles/sec), which strongly suggest that a hydroxyl group was introduced. Since the coupling constant (J) of the proton on the carbon bearing the hydroxyl group with adjacent protons is only 2.5 cycles/sec, this suggests that the hydroxyl group is axially-oriented. Treatment of III with acetic anhydride and pyridine, in an attempt to prepare its acetyl derivative, resulted in dehydration of the product back to II. Similarly, III was readily dehydrated to II by acid treatment.

All of these physical and chemical data are consistent with the presence of a β-hydroxy ketone, which is known to undergo dehydration easily. The absence of a conjugated carbonyl chromophore both in the infrared and ultraviolet spectra suggests that the hydroxyl group may reside at position C-7. The following series of reactions were used to establish the location of the hydroxyl function. Reaction of II with alkaline H_2O_2 afforded 7aβ-methyl-1,5-dioxo-6α,7α-oxido-3aα-hexahydro-4-indanpropionic acid (IV), which was reduced with $LiAlH_4$ to yield 7aβ-methyl-1β,5β-7α-trihydroxy-3aα-hexahydro-4-indanpropanol (V). The stereochemistry of the hydroxyl groups were deduced from the NMR spectrum which showed the following bands: τ 9.44 (3H, singlet tertiary CH_3 at C-7a); τ 6.25 (1H, triplet, $J = 7$ cycles/sec, proton at C-1); τ 5.92 (1H, triplet, $J = 4.0$ and 2.5 cycles/sec, proton at C-7); τ 5.52 (1H, multiplet, $J =$ at least 14 cycles/sec, proton at C-5) and τ 6.65 (2H, triplet, $J = 5$ cycles/sec, CH_2OH). The stereochemistry of the hydroxyl groups at C-1 and C-7 were assigned β and α respectively since hydride reductions of these functional groups at these positions have been well established, and the NMR data are in good agreement with these assignments. It is known also that the reduction of the ketone at C-5 yields predominantly the thermodynamically more stable form[9], which is consistent with the NMR results. Similarly when III was reduced with $LiAlH_4$, a tetrol whose infrared spectrum, mixed melting point and chromatographic behavior identical to V was obtained. Based on this series of reactions, we can conclusively assign the structure of the metabolite as 7aβ-methyl-1,5-dioxo-7α-hydroxy-3aα-hexahydro-4-indanpropionic acid (III), which is presumably formed *via* microbial hydration of the C-6–C-7 double bond in II in a manner analogous to the hydration reaction in fatty acid oxidation[10]. It is interesting to note that the reverse reaction-dehydration of the 7α-hydroxyl group by microorganisms of the genus Arthrobacter has been reported[11]. In a previous publication we proposed that an alternate pathway of degradation of the hexahydroindanpropionic acid (I) molecule may involve cleavage of the six-membered ring *via* oxygenation of the Baeyer–Villiger type, resulting in the formation of ε-lactones. Since this type of biological oxygenation is prevalent in nature[12], 1β-



DMF = dimethylformamide

acetoxy-2-methyl-2-(propionic acid)-3-(α-hydroxy-γ-carboxypropyl)-cyclopentane-ε-lactone (VI) was synthesized by reacting 7aβ-methyl-1β-acetoxy-5-oxo-3aα-hexahydro-4-indanpropionic acid (VII) with *m*-chloroperbenzoic acid. However, when the ε-lactone was exposed to *N. restrictus* or *N. opaca*, no apparent degradative intermediates were detected and only starting material were recovered. In fact, a number of other analogs were evaluated as possible degradative intermediates but none of them were metabolized. These include: 2-Methylcyclopentane-1,3-dione (VIII), 2-methyl-2-(β-carboxyethyl)-cyclopentane-1,3-dione (IX), (±)-7,7α-dihydro-7aβ-methyl-1,5(6H)-indandione (X), (±)-3,4,8,8aβ-tetrahydro-8a-methyl-1,6(2H,7H)-naphthalenedione (XI) and 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanacetic acid (XII). Although negative in nature, these results do tend to eliminate certain degradative pathways, such as the one proposed by SCHUBERT, BOHME AND HORHOLD[13], involving bicyclic derivatives such as X as key intermediates.



Predicated on our earlier finding[5] that the propionic acid side chain is first degraded *via* fatty acid oxidation, the acetate, propionate, and benzoate esters of 7aβ-methyl-1β-hydroxy-5-oxo-3aα-hexahydro-4-indanpropionic acid (XIII) were prepared. If the microorganisms should then proceed to oxidize the six-membered ring, the blocking of the oxygen function at C-1 may well result in the accumulation of key degradative intermediates. Although exposure of the propionate and benzoate esters to *N. restrictus* and *N. opaca* did not yield significant quantities of any apparent metabolic products, surprisingly, *N. restrictus* and *N. opaca* metabolized the acetoxy ester VII to yield a product which has a molecular weight of 460 based on mass spectral analysis. The (P+1) and (P+2) peaks are compatible with the molecular formula $C_{26}H_{36}O_7$ (Fig. 1). The peak at *m/e* 240 may be due to $C_{13}H_{20}O_4$ and there is very little fragmentation in the higher mass region. This suggests that the *m/e* 460 probably represents a dimer minus (H_2O+H_2) of an ion of mass 240. This theory is further supported by the NMR spectrum (Fig. 2), which showed the presence of two tertiary methyl peaks at τ 8.89 and 8.82. The peak at τ 5.29 corresponds to proton on carbon bearing acyloxy function.

On the basis of these physical data, the chemical structure of this metabolite appears to be consistent with 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionyl-7aβ-methyl-5-oxo-1β-hydroxy-3aα-hexahydro-4-indanpropionic acid (XIV). The structure of this metabolite was conclusively established by chemical synthesis. Reaction of I with 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionic acid methyl ester (XV), in the presence of oxalyl chloride afforded the dimeric methyl ester (XVI). Its infrared spectrum, NMR spectrum (Fig. 3) and mass spectrum

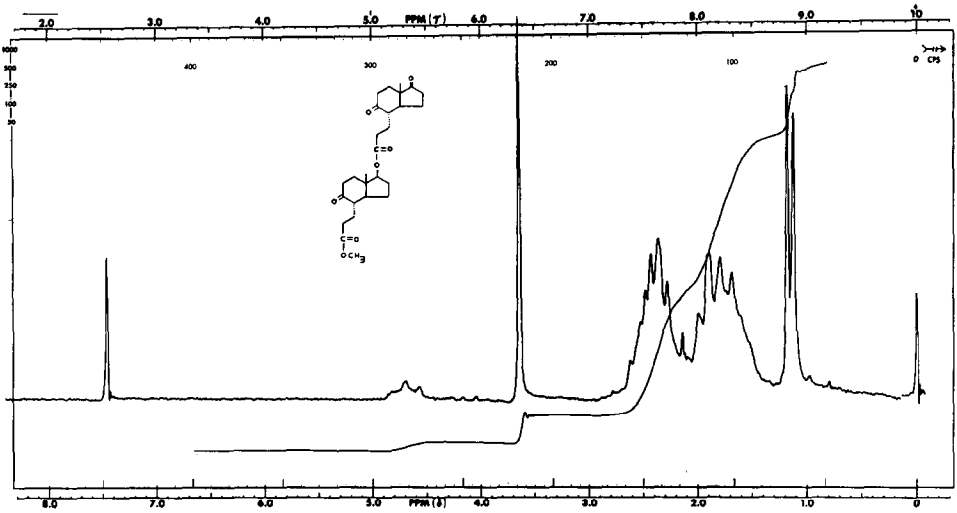


Fig. 3. The NMR spectrum of 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionyl-7aβ-methyl-5-oxo-1β-hydroxy-3aα-hexahydro-4-indanpropionic acid methyl ester (XVI) in deuterated chloroform.

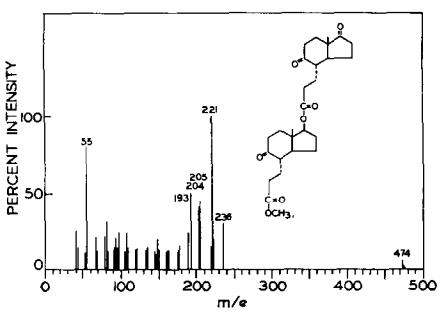


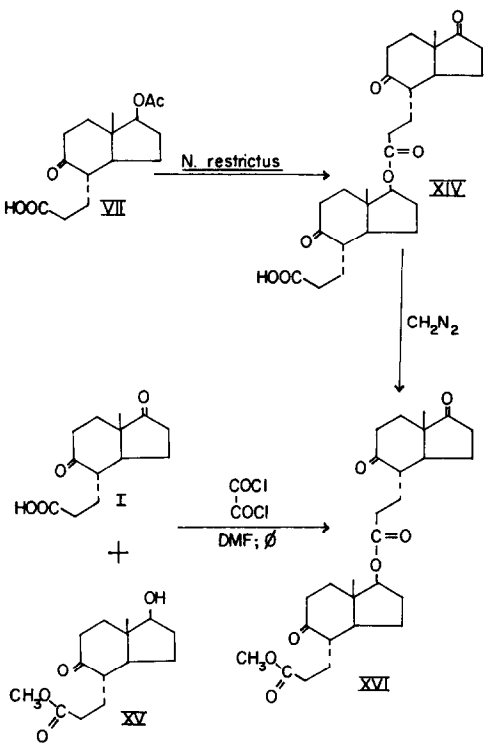
Fig. 4. The mass spectrum of 7aβ-methyl-1,5-dioxo-3aα-hexahydro-4-indanpropionyl-7aβ-methyl-5-oxo-1β-hydroxy-3aα-hexahydro-4-indanpropionic acid methyl ester (XVI).

(Fig. 4) were found to be identical to a sample of the metabolite, which had been treated with diazomethane.

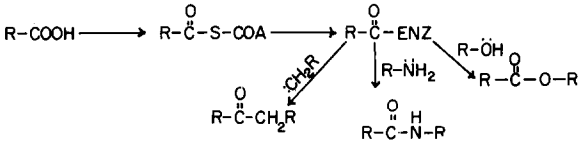
It is noteworthy that exposure of I or 7aβ-methyl-1β-hydroxy-5-oxo-3aα-hexahydro-4-indanpropionic acid (XIII) to *N. restrictus* did not result in the formation of this dimeric ester. A plausible explanation may be that XIII is a good inducer of the hydroxydehydrogenase which readily oxidize the 1β-hydroxyl group to a carbonyl function, thus rendering the molecule unavailable for esterification. On the other hand, VII is a poor inducer of the hydroxydehydrogenase, thereby the alcohol accumulates to favor esterification.

The formation of the dimeric ester, appears to resemble the synthesis of phosphatidic acid from L-glycerophosphate and fatty acyl-CoA[14]. One may visualize the formation of the dimeric ester as follows:

The activation of the carboxyl group to form acyl-CoA; the formation of the acyl–enzyme complex, which is susceptible to attack by a variety of nucleophilic agents.



DMF = dimethylformanide



In the present case, the nucleophile or acceptor is oxygen to yield the dimeric ester. The formation of benzylpenicillin from 6-aminopenicillanic acid and phenylacetyl-CoA is an example where the nucleophile is nitrogen[15]. Alternatively, the acceptor may be a carbanion as in the case of fatty acid synthesis[16].

It is evident from these results that it is virtually impossible to predict the nature of metabolic products of microbial reactions since a small modification in chemical structure of substrates may drastically alter the course of metabolism to yield different metabolites.

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REFERENCES

1 R. M. DODSON AND R. D. MUIR, *J. Am. Chem. Soc.*, 83 (1961) 4631.
2 K. SCHUBERT, R. H. BOHME AND C. HORHOLD, *Z. Physiol. Chem.*, 325 (1961) 260.
3 D. T. GIBSON, K. C. WANG, C. J. SIH AND H. WHITLOCK, *J. Biol. Chem.*, 241 (1966) 551.
4 D. A. SHAW, L. F. BORKENHAGEN AND P. TALALAY, *Proc. Natl. Acad. Sci. U.S.*, 54 (1965) 837.
5 S. S. LEE AND C. J. SIH, *Biochemistry*, 6 (1967) 1395.
6 G. V. D. TIERS, *J. Phys. Chem.*, 62 (1958) 1151.
7 C. J. SIH, H. H. TAI, Y. Y. TSONG, S. S. LEE AND R. G. COOMBE, *Biochemistry*, 7 (1968) 808.
8 C. DJERASSI, G. ROSENKRANZ, J. ROME, S. KAUFMAN AND J. PATAKI, *J. Am. Chem. Soc.*, 72 (1950) 4535.
9 S. MAHAPATRA AND R. M. DODSON, *J. Med. Chem.*, 9 (1966) 147.
10 H. R. MAHLER, in K. S. MARKLEY, *Fatty Acids*, Part 3, Interscience, New York, 2nd ed., 1964, p. 1487.
11 S. HAYAKAWA, Y. KENEMATSU AND T. FUJIWARA, *Nature*, 214 (1967) 520.
12 A. LASKIN, P. GRABOWICH, C. D. MEYERS AND J. FRIED, *J. Med. Chem.*, 7 (1964) 406.
13 K. SCHUBERT, H. BOHME AND C. HORHOLD, *Acta Biol. Med. Ger.*, 18 (1967) 295.
14 S. B. WEISS AND E. P. KENNEDY, *J. Am. Chem. Soc.*, 78 (1956) 3550.
15 B. SPENCER, *Biochem. Biophys. Res. Commun.* 31 (1968) 170.
16 F. LYNEN, *Federation Proc.*, 20 (1961) 941.