Tetrahedron Vol 33, pp 2451 to 2456 Pergamon Press 1977 Printed in Great Britain

MICROBIOLOGICAL TRANSFORMATIONS OF 19-OXYGENATED *ENT*-KAURANES

E. L. GHISALBERTI,* P. R. JEFFERIES, M. A. SEFTON and P. N. SHEPPARD
Department of Organic Chemistry, University of Western Australia, Nedlands, W. A. 6009

(*Received in UK* 15 *April* 1977; *Accepted for publication* 24 *April* 1977)

Abstract—The microbiological transformations of three 19-oxygenated *ent*-kauranes with *Rhizopus nigricans*, *Aspergillus ochraceous* and *Calonectria decora* have been investigated. The most common transformation observed is hydroxylation at the C-1 and C-7 positions. For *ent*-kaur-16-en-19-oic acid allylic hydroxylation and hydration of the double bond also occur.

The use of microbiological transformations in modifying substrate molecules has attracted increasing attention.[1] In particular advantage has been taken of the specific hydroxylating ability of certain microorganisms to prepare synthetically or pharmacologically useful compounds.[2] This approach presents a number of advantages. The introduction of an hydroxyl at unactivated sites allows ready entry to sections of the molecule otherwise inaccessible, unutilised material from the incubation can often be recycled and in general the technique is relatively simple. The main limitation however lies in the inability to predict for any one molecule the site(s) of hydroxylation. Experiments directed towards an understanding of the factors controlling the direction of hydroxylation, i.e. the relationship between structure and hydroxylation pattern, have been undertaken for a group of steroids.[2,3] Towards similar ends we have initiated a study of microbiological hydroxylation of a number of diterpenes by *Rhizopus nigricans*, *Calonectria decora* and *Aspergillus ochraceous*. In this report we present the results obtained for three *ent*-kaurene diterpenes with oxygenation at C-19. The substrates were chosen for the initial study because of the importance of 19-oxygenated kauranes as precursors of the gibberellins.

Presentation of results. As a summary of these results formed part of a preliminary communication[4] this report is mainly concerned with a full description of the microbiological transformation and the evidence for structural assignments. Figures 1–3 illustrate the transformations of each substrate with the three microorganisms employed. The notation used refers to the position and type of modification of the substrate. The reactions required to establish the structure of a compound or for purposes of interrelations are presented in the Schemes. The arguments needed to prove the structures are trivial and therefore are not included. Accordingly description of the spectroscopic data is relegated in full to the experimental section. In those cases where identical metabolites were obtained from *R. nigricans* and *C. decora* interrelationships were carried out with material obtained from large scale fermentations of *R. nigricans*.

Nomenclature

For the systematic names of the compounds in this paper the nomenclature proposed by Rowe[5] has been used. In this system an *ent*-operator inverts the stereochemical designation of substituents. When reference is made to the configuration of substitutents in the text however we have followed the recent practice of specifying α- and β-stereochemistry according to the structural representation.[6]

Metabolism of ent-*kaur*-16-*en*-19-*oic acid* (**1**) (Fig. 1). The acid was most efficiently utilized by *C. decora* which converted it into the 7,15-dihydroxylated acid (**5**) in 30% yield. Minor quantities of the 7α-[7] and 15α[8]monohydroxylated compounds (**2** and **3**) were also isolated. Reincubation of the monohydroxylated metabolites separately yielded the dihydroxy acid (**5**), confirming the position of hydroxylation in the latter. *R. nigricans* afforded a monohydroxy compound in 25% yield which was found to be identical with the known[7] 7β-hydroxylated compound (**8**). Variable amounts of the 16,17-dihydroxy acid (**9**),[9] resulting from addition to the double bond, could also be isolated. In a separate experiment, this compound was shown to originate from the corresponding 16,17-epoxide.[10] The 16,17-dihydroxy acid (**9**) was the only metabolite which could be isolated from the incubation of **1** with *A. ochraceous*. Trial experiments showed that incubation of the sodium salt of **1** with *R. nigricans* resulted in a cleaner mixture of metabolites although the yield of the 7β-hydroxylated compound (**8**) was not significantly increased.

Metabolism of ent-16-*oxo*-17-*nor*-*kauran*-19-*oic acid* (**10**) (Fig. 2). The keto-acid was efficiently hydroxylated by *R. nigricans* and was converted into three major metabolites, **11**, **12** and **13**, resulting from monohydroxylation at the 7α-(30%), 1α-(30%) and 7β-(5%) positions respectively. Interestingly the same metabolites were produced by *C. decora* but with a marked change in the relative proportions (15%, 5% and 40% respectively). Proof for the structure of the 1α-hydroxy compound (**12**) was obtained by the sequence of reactions shown in Scheme 1. The 7α- and 7β-hydroxy compounds (**11** and **13**)[7] were interrelated as shown in Scheme 2. Hydroxylation of **10** by *A. ochraceous* occurred at C-13 with and without reduction of the 16-CO to give the known[11] compounds **14** and **15** each in 5% yield. Variations of incubation times, substrate loading and nutrient composition failed to increase significantly the yield of these potentially useful 13-hydroxy-*ent*-nor kauranes.[12]

Metabolism of ent-19-*hydroxy*-16-*oxo*-17-*nor*-*kaurane* (**16**) (Fig. 3). The ketol on incubation with *R. nigricans* was converted into the 7α-(**17**) and 1α-(**18**) hydroxylated compound each in 20% yield. Somewhat lower yields of the same compounds were obtained with *C. decora* although a greater percentage of the mixture consisted of more polar metabolites. The structure of the 1α-hydroxy compound (**18**) was confirmed by the interrelation shown in Scheme 3. In the preliminary communication[4] we

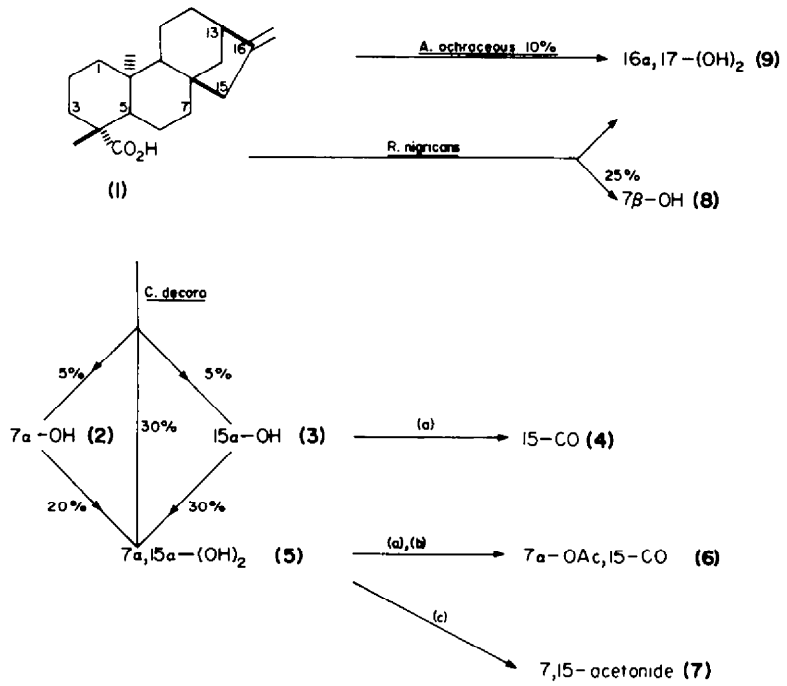


Fig. 1. Microbiological transformations of *ent*-kaur-16-en-19-oic acid (1) and chemical modifications carried out to determine the structures of new compounds. Reagents: (a) CrO_3/pyr., (b) Ac_2O/pyr., (c) $Me_2CO/CuSO_4$.

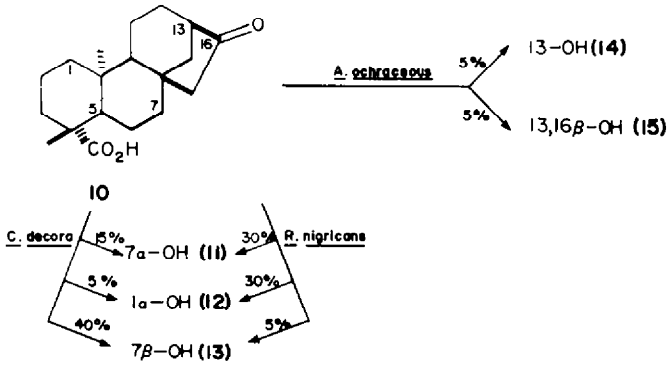


Fig. 2. Microbiological transformations of *ent*-16-oxo-17-nor-kauran-19-oic acid (**10**).

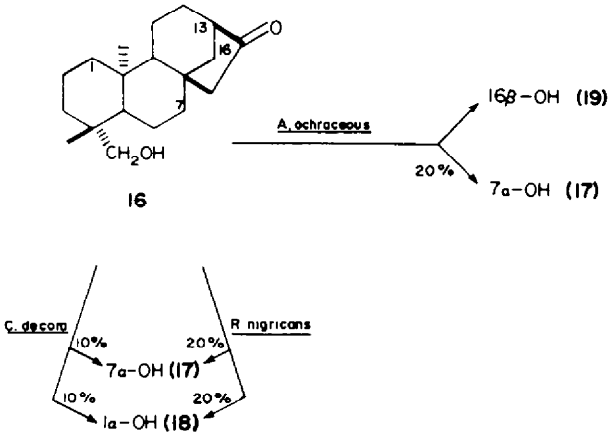
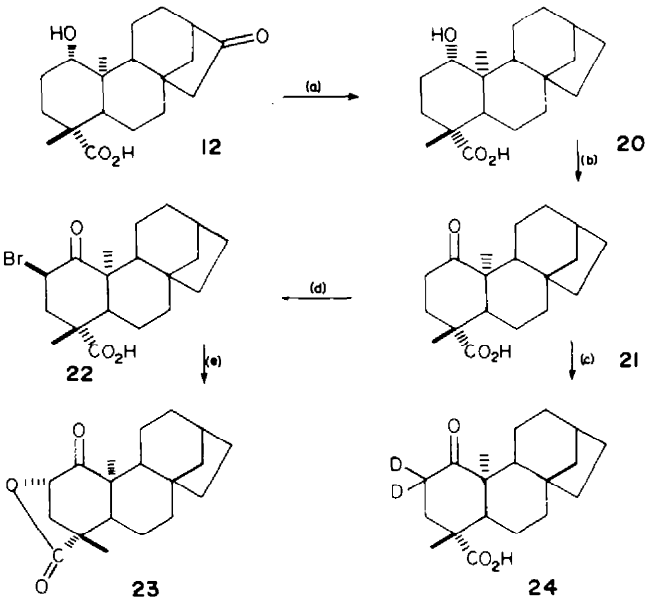
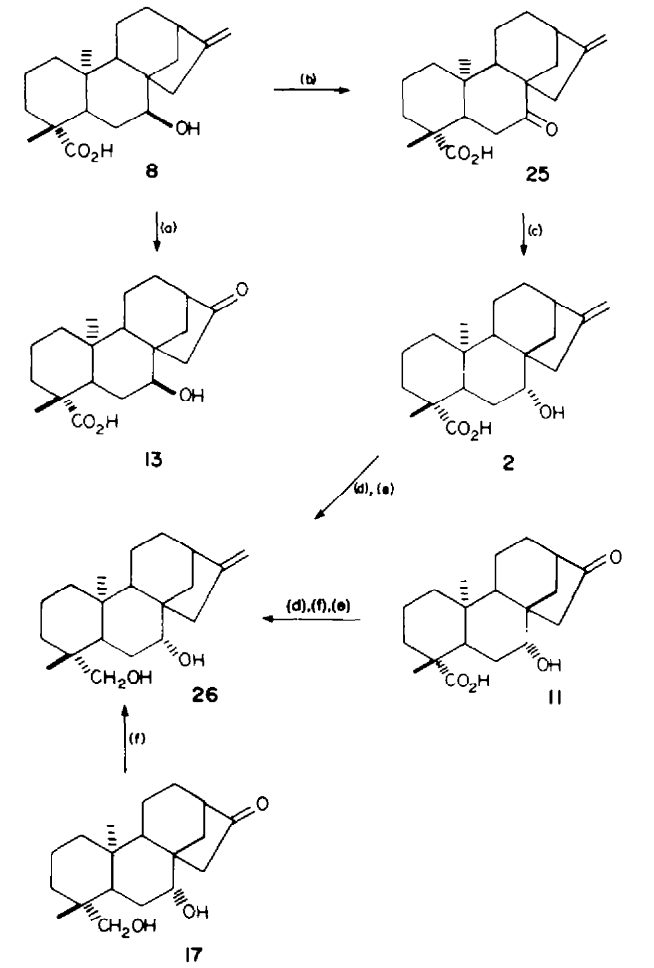


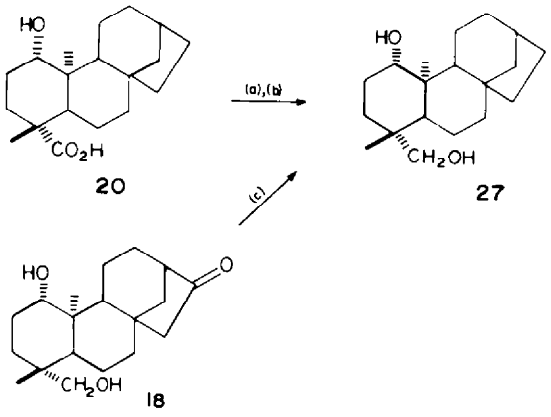
Fig. 3. Microbiological transformations of *ent*-19-hydroxy-16-oxo-17-nor-kaurane (**16**).



Scheme 1. Proof for the structure of 12 from *R. nigricans*. Reagent: (a) N_2H_4/KOH, (b) H_2CrO_4-Me_2CO, (c) DCl/AcOD, (d) HBr/Br_2, (e) C_5H_5N/Δ.



Scheme 2. Interrelationship of 7-hydroxy kauranes. Reagents: (a) OsO_4/$NaIO_4$, (b) H_2CrO_4-Me_2CO, (c) $NaBH_4$, (d) CH_2N_2, (e) $LiAlH_4$, (f) $(Ph)_3PCH_3I$.



Scheme 3. Interrelationship of 1-hydroxy-*ent*-kauranes. Reagents: (a) CH_2N_2, (b) LAH, (c) N_2H_4/KOH.

reported that the only product identified from the incubation of the ketol with *A. ochraceous* was the 16β-hydroxy compound (**19**) resulting from microbiological reduction of the 16-carbonyl. Repetition of this incubation on two other occasions yielded a mixture of compound in which **19** was present only in minor amounts. The major metabolite (20%) isolated was identified as the 7α-hydroxylated compound (**17**).

EXPERIMENTAL

General details are as previously given.[13]

General fermentation conditions

(a) *Storage.* Cultures of *Aspergillus ochraceous*,† *Calonectria decora*† and *Rhizopus nigricans*† were kept on slopes prepared from potato dextrose agar and new slopes and fermentation cultures started using spores taken from such a culture.

(b) *Growth.* Moulds were grown in glucose-malt medium (400 ml in 1 litre conical flasks) in shaken culture until a sufficient mass of mycelium had developed (3–4 days). One litre of this medium contained 2 g each of beef, malt and yeast extract; 2 ml of corn steep liquor; 2 g of sucrose and 5 g of glucose.

(c) *Shaking apparatus.* The shaker used during these experiments rotated horizontally at 150 rpm and had a throw of 2 cm.

(d) *Feeding of substrates.* Substrates (100–200 mg/400 ml culture) were dissolved in sufficient EtOH so that the final concentration of EtOH in medium was about 2% (8–10 ml /400 ml culture). This soln was added in two or three equal portions about 12 hr apart.

(e) *Isolation procedure.* At harvest, the mycelium was removed by filtration, the filtrate and washings were acidified with 2 M HCl and extracted with EtOAc (2 × 0.5 the volume of the aqueous layer). The combined EtOAc extracts were washed with water, dried (Na_2SO_4) and evaporated to give the "crude metabolites".

Metabolism of ent-*kaur*-16-*en*-19-*oic acid* (**1**)

1. *By* C. decora. The acid **1** (400 mg) in EtOH (20 ml) was incubated with *C. decora* (2 flasks) for 4 days. The residue obtained after work-up crystallized from EtOAc as plates of ent-7β,15β-*dihydroxy*-*kaur*-16-*en*-19-*oic acid* (**5**, 123 mg), m.p. 283–288°, $[\alpha]_D^{EtOH}$ – 168° (*c*, 0.2) (Found: C, 72.0: H, 9.1. $C_{20}H_{30}O_4$ requires: C, 71.8; H, 9.0%). NMR (C_5H_5N) δ: 1.17 (s, 20-H_3), 1.34 (s, 18-H_3), 4.14 (m, $W_{1/2}$ 18 Hz, 7-H) 4.38 (br, s, $W_{1/2}$ 4 Hz, 15-H), 5.16, 5.38 (s, 17-H_2). MS: *m/e* (%), 334 (M^-, 30), 316 (35), 301 (10), 298 (11), 276 (35), 122 (45), 121 (60), 120 (45), 119, (55), 109 (65), 107 (55), 105 (65), 95 (45), 93 (65), 91 (100).

†*A. ochraceous* (CMI 130970); *C. decora* (CBS 132.35); *R. nigricans* wild strain.

Preparative tlc of the mother liquors afforded a further quantity of **5** (21 g) and **2** (9 mg) and **3** (6 mg). The 7α-hydroxy acid (**2**) cyrstallized from EtOAc as prisms, m.p. 248–251°, undepressed on admixture with an authentic sample. The 15α-hydroxy acid (**3**) crystallized from benzene as plates, m.p. 204–208° (lit.[8] 214–216°). NMR (C_5H_5N) δ: 1.18 (s, 20-H_3), 1.34 (s, 18-H_3), 4.13 (br, s, $W_{1/2}$ 4 Hz, 15-H), 5.17, 5.47 (s, 17-H_2). MS: *m/e* (%), 318 (M^+, 50), 303 (25), 300 (50), 285 (50), 273 (12), 272 (18), 260 (100), 257 (35), 245 (25), 243 (25), 239 (25), 235 (20), 189 (35). The hydroxy acid **3**, (20 mg) was oxidized by the method of Ratcliffe and Rodehorst[14] to give the keto acid **4**, (18 mg) which crystallized from MeOH as plates, m.p. 185–190°, undepressed on admixture with an authentic sample.[15] The two hydroxy-acids (**2** and **3**) on incubation with *C. decora* for 6 days were converted to the dihydroxy-acid (**5**) in 20% and 30% yields respectively.

Transformations of ent-7β,15β-*dihydroxykaur*-16-*en*-19-*oic acid* (**5**). The dihydroxy-acid **5**, (90 mg) in pyridine (2 ml) was treated with CrO_3/pyridine (100 mg in 1 ml) for 1 hr. Recovery of the product afforded a pale yellow gum which was acetylated to give the *acetoxy-keto-acid* **6**, (55 mg) which crystallized from ether-n-pentane as needles, m.p. 166–168°, $[\alpha]_D^{EtOH}$ + 180°(*c*, 0.7) (Found: C, 70.6; H, 8.5. $C_{22}H_{30}O_5$ requires: C, 70.6; H, 8.1%). IR: $\nu_{max}^{CHCl_3}$3500 (OH), 1735 (15-CO and acetate), 1690 cm^{-1} (carboxylic acid), λ_{max} 236 nm (ϵ 6900). NMR ($CHCl_3$) δ: 1.05 (s, 20-H_3), 1.30 (s, 18-H_3), 1.95 (s, acetoxymethyl), 3.12 (m, $W_{1/2}$ 10 Hz, 13-H), 5.06 (m, $W_{1/2}$ 19 Hz, 7-H), 5.30, 5.98 (s, 17-H_2). MS: *m/e* (%), 374 (M^+, 3), 332 (15), 314 (100), 304 (15), 286 (15), 268 (25), 253 (15), 160 (40). The dihydroxy-acid **5**, (100 mg) in acetone (20 ml) was treated with $CuSO_4$ (10 g) for 24 hr. The product recovered (100 mg) crystallized from acetone as plates of the *acetonide* (**7**), m.p. 266–268°, $[\alpha]_D^{EtOH}$ – 142° (*c*, 0.2). (Found: C, 73.9; H, 9.2. $C_{23}H_{34}O_4$ requires: C, 73.8; H, 9.2%). IR: $\nu_{max}^{CHCl_3}$ 3500 (OH), 1680 (carboxylic acid), 895, 875 cm^{-1}. NMR ($CHCl_3$) δ: 1.02, 1.26, 1.45, 1.51 (s, tertiary methyl protons), 2.83 (m, $W_{1/2}$ 10 Hz, 13-H), 3.72 (m, $W_{1/2}$ 20 Hz, 7-H), 4.05 (br s, $W_{1/2}$ 4 Hz, 15-H), 5.04, 5.16 (s, 17-H_2).

2. *By* R. nigricans. The acid **1** (600 mg) in EtOH (16 ml) was incubated with *R. nigricans* (4 flasks) for 3 days. The products obtained were separated by preparative tlc to give (a) **8** (170 mg) which crystallized from MeOH as cubes, m.p. 255–263°, undepressed on admixture with an authentic sample,[7] $[\alpha]_D^{CHCl_3}$ – 46° (*c*, 0.2) and (b) **9** (60 mg) which was purified by conversion to the methyl ester. This was shown to be identical with an authentic sample.[9]

3. *By* A. ochraceous. The acid **1** (400 mg) in EtOH (8 ml) was incubated with *A. ochraceous* (2 flasks for 4 days). The product obtained was recrystallized from MeOH to give **9** (97 mg) identical with an authentic sample.[9]

Metabolism of ent-16-*oxo*-17-*nor*-*kauran*-19-*oic acid* (**10**)

1. *By* R. nigricans. The acid **10** (5.2 g) in EtOH (100 ml) was added in three equal portions (12 hr apart) to cultures of *R. nigricans* cultivated in a 10 litre Biotec fermentor. After 4 days the fermentation was terminated and the metabolites (5.1 g) recovered in the unusual way. Fractional crystallization of the mixture from EtOAc gave **11** (1.3 g) as prisms, m.p. 286–9°. (lit.[7] 296–9°). Recrystallization of the mother liquor residues from MeOH afforded ent - 1β - *hydroxy* - 16 - *oxo* - 17 - *nor* - *kauran* - 19 - *oic acid* (**12**, 1.4 g) as prisms, m.p. 276–9°, $[\alpha]_D^{MeOH}$ -46° (*c*, 0.4) (Found: C, 70.9; H, 8.8. $C_{19}H_{28}O_4$ requires: C, 71.2; H, 8.8%). IR: ν_{max}^{Nujol} 3470 (OH), 1720 (CO) 1680 cm^{-1} (carboxylic acid). NMR (C_5H_5N) δ: 1.35, 1.48 (s, 18-, 20-H_3), 3.65 (m, $W_{1/2}$ 25 Hz, 1-H). MS: *m/e* (%), 320 (M^+, 30), 305 (5), 302 (25), 274 (20), 130 (100), 123 (100). Preparative tlc of the residues afforded a further amount of **12** (270 mg) and **13** (100 mg) which crystallized from EtOAc–n-pentane as plates, m.p. and mixed m.p. with an authentic sample 245°, (lit.[7] 239–41°), $[\alpha]_D^{MeOH}$ – 25°, (*c*, 0.1). The authentic sample was prepared by OsO_4/$NaIO_4$ oxidation of an authentic sample of **8**.

2. *By* C. decora. The keto-acid **10** (800 mg) in EtOH (40 ml) was incubated with *C. decora* (4 flasks) for 5 days. Preparative tlc of the residue (890 mg) yielded fractions consisting of (a) **13** (280 mg) which crystallized from acetone–n-pentane as plates, m.p. 247–9° undepressed on admixture with an authentic sample,

(b) the metabolite **11** (140 mg) which crystallized from acetone–n-pentane as prisms, m.p. and mixed m.p. 288–289° and (c) **12** (50 mg) which crystallized from MeOH as prisms, m.p. and mixed m.p. 272–276°.

3. *By* A. ochraceous. The acid **10** (1.0 g) in EtOH (80 ml) was incubated with *A. ochraceous* (10 flasks) for 3 days. The products recovered (900 mg) were methylated with CH_2N_2 and the mixture separated by column chromatography, followed by preparative tlc. The three major factions isolated were (a) starting material (600 mg), m.p. 228-231° alone or on admixture with an authentic sample,[11] and (c) **15** (55 mg), m.p. 182–185°, alone or an admixture with an authentic sample.[11]

Metabolism of ent-19-*hydroxy*-16-*oxo*-17-*nor-kaurane* (**16**)

1. *With* R. nigricans. The ketol **16** (1.0 g) in EtOH (48 ml) was incubated with *R. nigricans* (8 flasks) for 4 days. The products recovered (950 mg) were separated by preparative tlc to give three major fractions: (a) starting material (100 mg), (b) **17** (210 mg), which crystallized from acetone–n-pentane as plates, m.p. 190–191°, $[\alpha]_D^{CHCl_3} - 36°$ (*c*, 0.3). (Found: C, 74.3; H, 10.0. $C_{19}H_{30}O_3$ requires: C, 74.5; H, 9.9%). IR: ν_{max}^{Nujol} 3450 (OH), 1720 cm^{-1} (carbonyl). NMR ($CDCl_3$) δ: 1.01, 1.12 (s, 18-, 20-H_3), 3.55, 3.75 (AB system, J 12 Hz, 19-H_2), 3.43 (m, $W_{1/2} > 15$ Hz, 7-H). MS: *m/e* (%), 306 (M^+, 25), 275 (30), 257 (35), 154 (10), 123 (100), metastables 77.5 (306→154), 98.2 (154→123), 240.2 (275→257), 247.0 (306→275), and (c) **18** (205 mg) was recrystallized from EtOAc–n-pentane as rods, m.p. 159–162°, $[\alpha]_D^{CHCl_3} - 24°$ (*c*, 0.2) (Found: C, 74.7; H, 9.9. $C_{19}H_{30}O_3$ requires: C, 74.5; H, 9.9%). IR: ν_{max}^{Nujol} 3520, 3250 (OH), 1730 cm^{-1} (CO). NMR (C_5H_5N) δ: 1.17 (s, 18-H_3), 1.38 (s, 20-H_3), 3.69, 4.05 (AB system, J 12 Hz, 19-H_2), 3.58 (app. Triplet, J ~ 9 Hz, 1-H). MS: *m/e* (%) 306 (M^+, 10), 288 (15), 275 (10), 257 (100), 190 (50).

2. *By* C. decora. The ketol **16** (400 mg) in EtOH (20 ml) was incubated with *C. decora* (2 flasks) for 36 hr. The residue (240 mg) recovered was adsorbed onto a column of alumina (Act V, neutral, 5 g). Elution with $CHCl_3$ gave a fraction (100 mg) containing **17** and **18**. Preparative tlc of this fraction gave **17** (42 mg) which crystallized from acetone–n-pentane as plates m.p. and m.p. with authentic material 190–191°, and **18** (31 mg) which was recrystallized from acetone–n-pentane as rods, m.p. and mixed m.p. with authentic material, 159–162°.

3. *By* A. ochraceous. The ketol **16** (850 mg) in EtOH (40 ml) was incubated with *A. ochraceous* (4 flasks) for 4.5 days. The residue (700 mg) recovered was adsorbed onto a column of alumina (Act III, neutral, 45 g). Elution with $CHCl_3$–light petroleum (9:1) and $CHCl_3$ gave fractions (310 mg) which on fractional crystallization yielded **17**, m.p. and mixed m.p. with an authentic sample 186–189°.

Transformations of ent - 1β - *hydroxy* - 16 - *oxo* - 17 - *nor* - *kauran* - 19 - *oic acid* (**12**) (Scheme 1)

The hydroxyketo acid **12** (800 mg), KOH (300 mg) and $H_2NNH_2 \cdot H_2O$ (0.3 ml) in diethylene glycol (2 ml) were heated at 130° under N_2 for 1 hr. The temp. was then raised to 195–200° and maintained for 2 hr. The mixture was cooled, poured into 2M HCl and extracted with ether. Removal of the solvent and crystallization of the residue from acetone–n-pentane gave ent - 1β - *hydroxy* - 17 - *nor* - *kauran* - 19 - *oic acid* **20** (660 mg) as prisms, m.p. 189–190° $[\alpha]_D^{CHCl_3} - 52°$ (*c*, 1.0). (Found: C, 74.8; H, 10.2. $C_{19}H_{30}O_3$ requires: C, 74.5; H, 9.9%). IR: ν_{max}^{Nujol} 3270 (OH), 1690 cm^{-1} (carbonyl). NMR (C_5H_5N) δ: 1.31 and 1.44 (s, 18-, 20-H_3), 3.61 (d of d, J5, 12 Hz, 1-H). MS: *m/e* (%), 306 (M^+, 10), 288 (70), 273 (10), 260 (15), 243 (50), 175 (85), 130 (100). The hydroxy-acid **20** (560 mg) in acetone (60 ml) was treated with Jones reagent. The product isolated with ether was purified by preparative tlc. Recrystallization of the fraction obtained from acetone–n-pentane afforded ent - 1 - *oxo* - 17 - *nor* - *kauran* - 19 - *oic acid* **21** (460 mg) as rods m.p. 165–169°, $[\alpha]_D^{CHCl_3} - 133°$ (*c*, 0.8). (found: C, 74.6; H, 9.6. $C_{19}H_{28}O_3$ requires: C, 74.9; H, 9.3%. IR: ν_{max}^{Nujol} 1715 (CO), 1685 cm^{-1} (carboxylic acid). NMR ($CDCl_3$) δ: 1.30, 1.37 (s, 18-, 20-H_3), 3.25 (d of t, J_d 5 Hz, J_t 13.5 Hz, 2-H). MS: *m/e* (%) 304 (M^+, 60), 289 (5), 286 (5), 258 (10), 218 (20), 182 (30), 169 (100). The keto-acid **21** (30 mg) in dioxan (5 ml) was added to a DCl/AcOD soln, prepared by adding AcCl (1.5 ml) to dioxan (5 ml) and D_2O (0.5 ml), and left for 24 hr. The product (**24**) recovered with ether showed M^+ at *m/e* 306 (95% d_2). The signals assignable to the C-2 protons in the NMR spectrum of **21** were absent in that of **24**. The keto-acid **21** (230 mg) in HOAc (10 ml, saturated with HBr) was treated by dropwise addition with Br_2 (1.1 equivs) in HOAc (5 ml) during 3 hr. The bromoketo-acid **22** (250 mg) recovered could not be recrystallized but was shown to be homogeneous and had the following spectral characteristics: NMR ($CDCl_3$, 90 MHz) δ: 1.33, 1.38 (s, 18-, 20-H_3), 3.08 (d of d, J 13.2, 6.1, 3-H), 5.60 (d of d, J 13.2, 6.1 Hz, 2-H). The presence of an isolated AMX system was confirmed by INDOR[16] experiments which also allowed the observation of the third component of the spin system at δ 1.83. MS: *m/e* (%) 384, 382 (M^+, 5), 302 (100), 147 (95). The bromoketo-acid (**22**, 170 mg) in C_5H_5N (20 ml) was heated under reflux for 2.5 hr. The product recovered crystallized from acetone–n-pentane as prisms of **23** (140 mg), m.p. 183–184°, $[\alpha]_D^{CHCl_3} - 72°$ (*c*, 0.9). (Found: C, 75.0; H, 8.4. $C_{19}H_{26}O_3$ requires: C, 75.4; H, 8.6%). IR: ν_{max}^{Nujol} 1775 (γ-lactone CO), 1720 cm^{-1} (CO). NMR ($CDCl_3$) δ: 1.22, 1.50 (s, 18-, 20-H_3), 2.04 (d, J 12 Hz, 3-H_{ax}), 2.61 (d of d, J 6, 12 Hz, 3-H_{eq}), 4.63 (d, J 6 Hz, 2-H). MS: *m/e* (%), 302 (M^+, 40), 274 (30), 177 (85), 176 (90), 175 (85), 147 (100).

Interrelation between **8** *and* **11** [Scheme 2)

(a) *Preparation of* ent - 7β,19 - *dihydroxy* - *kaur* - 16 - *ene* (**26**) *from* **8**. The hydroxy-acid **8** (50 mg) in acetone (20 ml) was treated with excess Jones reagent for 10 min. The product isolated crystallized from acetone–n-pentane to give needles of **25** (33 mg), m.p. 203–207° (lit.[17] 208–210°). IR: $\nu_{max}^{CHCl_3}$ 1705 (CO), 1695 cm^{-1} (carboxylic acid). NMR ($CHCl_3$) δ: 1.18, 1.27 (s, 18-, 20-H_3), 4.85 (br. s, $W_{1/2}$ 5 Hz, 17-H_2), MS: *m/e* (%), 316 (M^+, 90), 270 (80), 147 (100). The keto-acid **25** (30 mg) in MeOH (30 ml) was treated with $NaBH_4$ (100 mg) for 4 hr. The product recovered with ether was recrystallized from EtOAc as needles of **2** (30 mg), m.p. 250–252° (lit.[7] 248-250°). NMR ($CHCl_3$) δ: 1.07 (s, 18-H_3), 1.36 (s, 20-H_3), 3.75 (m, $W_{1/2}$ 20 Hz, 7-H), 4.87 (br s, $W_{1/2}$ 6 Hz, 17-H_2). MS: *m/e* (%), 318 (100), 303 (20), 300 (30), 285 (5), 273 (35), 272 (40). The hydroxy-acid **2** (28 mg) was methylated with CH_2N_2 and the resulting methyl ester was reduced with LAH in the usual way. Preparative tlc of the recovered material gave **26** (12 mg) which was recrystallized from acetone–n-pentane as rosettes, m.p. 206–207°. (Found: M^+ 304.2391. $C_{20}H_{32}O_2$ requires: M^+ 304.2402). IR: 3400 (OH), 3070 (vinyl group). NMR ($CDCl_3$, 90 Mhz) δ: 0.98, 1.03 (s, 18-, 20-H_3), 3.45, 3.73 (AB system, J 12 Hz, 19-H_2), 3.42 (d of d, J 4, 10.5 Hz, 7-H), 4.80 (br s, $W_{1/2}$ 6 Hz, 17-H_2). MS: *m/e* (%), 304 (100), 286 (60), 255 (50), 206 (20).

(b) *Preparation of* **26** *from* **11**. The keto-hydroxy-acid **11** (83 mg) was methylated with CH_2N_2 and the methyl ester dissolved in ether (30 ml) was added to a soln prepared by stirring triphenylphosphonium methyliodide (1.3 g) and KOBut (350 mg) in dry ether (30 ml) under N_2. The mixture was stirred for 12 hr under N_2 and then heated under reflux for 3 hr. The product (60 mg) recover was treated with excess LAH to give a compound which, after preparative tlc, crystallized from acetone–n-pentane as rosettes of **26** (35 mg), m.p. and mixed m.p. with the sample from (a) 206–207°.

Interrelation of **20** *and* **18** (Scheme 3). The hydroxy-acid **20** (30 mg) was methylated with CH_2N_2 and the methyl ester was treated with LAH in ether. The product recovered was recrystallized from acetone–n-pentane as needles of **27** (15 mg), m.p. 164–165°, $[\alpha]_D^{CHCl_3} - 47°$ (*c*, 0.3). (Found: M^+, 292.24041. $C_{19}H_{32}O_2$ requires: M^+, 292.24040). IR: ν_{max}^{Nujol} 3260 cm^{-1} (OH). NMR ($CDCl_3$) δ: 0.93, 1.10 (s, 18-, 20-H_3), 3.35 (m, $W_{1/2}$ 15 Hz, 1-H), 3.40, 3.72 (AB system, J 12 Hz, 19-H_2). MS: *m/e* (%), 292 (M^+, 1) 274 (15), 261 (5), 243 (100). The keto-diol (**18**, 40 mg), KOH (20 mg) and $H_2NNH_2 \cdot H_2O$ (0.02 ml) in diethylene glycol (0.2 ml) were heated at 120° under N_2 for 1 hr. The temp. was then raised to and maintained at 200° for 3 hr. The product recovered was purified by preparative tlc to give **27** (10 mg) which was crystallized from acetone–n-pentane and proved identical with the sample prepared above.

Acknowledgements—We are indebted to Dr. J. Pinhey, Department of Chemistry, University of Sydney, for supplying us with

the strain of *C. decora*, to Mr. R. Hilton, Department of Botany, University of W. A., for the strain of *A. ochraceous* and to Associate Prof. J. R. Cannon of this Department for *R. nigricans*.

One of us (P.R.J.) obtained valuable experience in microbial methods as a guest of the Dyson Perrins Laboratory, Oxford.

REFERENCES

[1]G. S. Fonken and R. A. Johnson, *Chemical Oxidations with Micro-organisms*. Marcel Dekker, New York (1972).
[2]E. R. H. Jones, *Pure and Applied Chem.* **33**, 39 (1973).
[3]A. M. Bell, V. E. M. Chambers, E. R. H. Jones, G. D. Meakins, W. E. Muller and J. Pragnell, *J. Chem. Soc.* Perkin I, 312 (1974); and other papers in this series.
[4]J. P. Beilby, E. L. Ghisalberti, P. R. Jefferies, M. A. Sefton and P. N. Sheppard, *Tetrahedron Letters*. 2589 (1973).
[5]J. W. Rowe, *The Common and Systematic Nomenclature of Cyclic Diterpenes*. Third Revision (1968).
[6]D. M. Harrison and J. MacMillan, *J. Chem. Soc.* (C), 631 (1971); P. R. Jefferies, J. R. Knox and T. Ratajczak, *Phytochemistry* **13**, 1423 (1974).
[7]J. R. Hanson and A. F. White, *Tetrahedron* **25**, 2743 (1969).
[8]C. H. Brieskorn and E. Pohlmann, *Chem. Ber.* **102**, 2621 (1969).
[9]C. A. Henrick and P. R. Jefferies, *Austral. J. Chem.* **17**, 915 (1964).
[10]K. D. Croft, unpublished results.
[11]I. F. Cook and J. R. Knox, *Tetrahedron* **32**, 369 (1976).
[12]I. F. Cook and J. R. Knox, *Ibid.* **32**, 363 (1976).
[13]E. L. Ghisalberti, P. R. Jefferies, T. G. Payne and G. K. Worth, *Ibid.* **29**, 403 (1973).
[14]R. Ratcliffe and R. Rodehorst, *J. Org. Chem.* **35**, 400 (1970).
[15]J. R. Cannon, P. W. Chow, P. R. Jefferies and G. E. Meehan, *Austral. J. Chem.* **19**, 861 (1966).
[16]W. von Philipsborn, *Angew. Chem.* Internat. Edit. **10**, 472 (1971).
[17]R. H. B. Galt and J. R. Hanson, *Tetrahedron* **22**, 3185 (1966).