Microbiological Transformations of 17-Norkauran-16-one and 16-Norphyllocladan-16-one by *Aspergillus niger*

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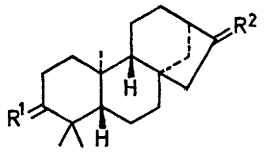
Summary Incubation of (−)-17-norkauran-16-one (**1**) with *Aspergillus niger* gives 3α-hydroxy-17-norkauran-16-one (**2**) while (+)-17-norphyllocladan-16-one (**7**) gives 3β-hydroxy-17-norphyllocladan-16-one (**8**) and the corresponding 3-ketone (**13**).

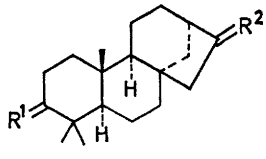
CERTAIN derivatives of (−)-kaurene, notably those believed to be precursors of the gibberellins, are known[1] to possess gibberellin-like activity. We are attempting to determine if related compounds with other tetracyclic diterpenoid skeletons have similar attributes and have investigated the possibility of effecting microbiological oxygenation of ring A in tetracyclic diterpenoids in general.

We used (−)-17-norkauran-16-one (**1**) because of its availability[2] and expected reactivity to microbial hydroxylation.‡ It was submitted to small-scale trial incubations with a range of fungal species and *Aspergillus niger* was eventually selected for large-scale investigation. Aerobic incubation of the ketone (added in DMF to a 3 day culture grown in a full nutrient solution[3]) for 5 days gave the keto-alcohol (**2**), m.p. 179—181°, [acetate (**3**), m.p. 194—196°]. The location and stereochemistry of the hydroxy-group were determined for the derived kaurenol (**4**) (required for biological testing) which was prepared from (**3**) *via* the acetate (**5**). When n.m.r. spectra of the alcohol (**4**) were recorded in the presence of steadily increasing relative amounts of $Eu(dpm)_3$, the observed shifts of the methyl resonances were in the normalised[4] ratio 10 : 9·4 : 4·2 which is consistent[5] only with a 3α-hydroxy-group. Oxidation of the alcohol (**4**) formed the ketone (**6**), m.p. 83—84°, which gave benzene-induced ^1H n.m.r. methyl shifts of the magnitudes predicted[6] for a C-3 ketone with ring A in the expected[7] flattened chair conformation. Further confirmation of these structures came from the fact that (**2**), (**3**), and (**4**) have identical physical and spectral characteristics to those prepared[8,9] from naturally occurring diterpenoids.

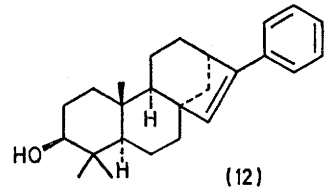
When norphyllocladanone (**7**), prepared[10] from (+)-phyllocladene, was incubated with *A. niger*, two transformation products were obtained. The major, more polar,



	R^1	R^2
(1)	H_2	O
(2)	H,α-OH	O
(3)	H,α-OAc	O
(4)	H,α-OH	CH_2
(5)	H,α-OAc	CH_2
(6)	O	CH_2



	R^1	R^2
(7)	H_2	O
(8)	H,β-OH	O
(9)	H,β-OAc	O
(10)	H,β-OAc	CH_2
(11)	H,β-OH	CH_2
(13)	O	O



(12)

product,§ m.p. 158—160°, $[\alpha]_D$ + 16°, was again a keto-alcohol (**8**). The spectral properties of this compound and its derivatives, the keto-acetate (**9**), m.p. 156—158°, $[\alpha]_D$ + 27°, the acetoxy-olefin (**10**), m.p. 121—123°, $[\alpha]_D$ + 41°, the hydroxy-olefin (**11**), m.p. 157—158°, $[\alpha]_D$ + 3°, and the hydroxy-styrene (**12**), m.p. 177—178°,

‡ These results, since they concern a fungus which is effective in steroid hydroxylation, may be of significance in the formulation of criteria for the prediction of the site of microbial hydroxylation based on the structure of the substrate.

§ Satisfactory analytical and spectral data have been obtained for all new compounds.

including $Eu(dpm)_3$ shifts of the latter two, were entirely consistent with the presence of a 3β oxygen function. The less polar transformation product (**13**), m.p. 187—189°, $[\alpha]_D$ + 13°, showed no absorption in the i.r. attributable to OH stretching but two strong peaks in the carbonyl region at 1712 and 1749 cm^{-1}. Its formulation as the diketone (**13**) was readily confirmed by its preparation from (**8**) by oxidation with Jones reagent.

In preliminary testing, although both kauren-3α-ol (**4**) and phyllocladen-3β-ol (**11**) failed to reverse phenotypic dwarfing in the "Meteor" pea bioassay (1—100 μg/plant), (**4**) showed activity (50—100 μg/plant) in the analogous dwarf maize (mutant, *d*-5) test. The two compounds, (**4**) and (**11**), showed activity comparable to that of gibberellin A_3 itself in the lettuce ("Grand Rapids") seed germination bioassay[11] which is, however, far from being specific for gibberellins. All three showed maximum activity at concentrations of 1 μg/ml [% germination: GA_3, 39 ± 5; (**4**), 40 ± 2; (**11**), 69 ± 6; control, 14 ± 2].

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[1] P. W. Brian, J. F. Grove, and P. C. Mulholland, *Phytochemistry*, 1967, 1475.
[2] H. Vorbrueggen and C. Djerassi, *J. Amer. Chem. Soc.*, 1962, **84**, 2990.
[3] J. C. Galbraith and J. E. Smith, *Brit. Mycol. Soc. Trans.*, 1969, **52**, 237.
[4] D. G. Buchley, G. H. Green, E. Ritchie, and W. C. Taylor, *Chem. and Ind.*, 1971, 298.
[5] P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *J. Amer. Chem. Soc.*, 1970, **92**, 2537.
[6] J. D. Connolly and R. McCrindle, *Chem. and Ind.*, 1965, 379; D. H. Williams and N. S. Bhacca, *Tetrahedron*, 1965, **21**, 2021.
[7] P. Witz, H. Herrmann, J.-M. Lehn, and G. Ourisson, *Bull. Soc. chim. France*, 1963, 1101.
[8] D. A. H. Taylor, *J. Chem. Soc.* (*C*), 1967, 1360.
[9] K. R. Jefferies and R. W. Retallack, *Austral. J. Chem.*, 1968, **21**, 1311.
[10] R. A. Appleton, P. A. Gunn, and R. McCrindle, *J. Chem. Soc.* (*C*), 1970, 1148.
[11] P. W. Brian, H. G. Hemmings, and D. Lowe, *Nature*, 1962, **193**, 946.