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# MICROBIOLOGICAL TRANSFORMATIONS OF TETRACYCLIC DITERPENES

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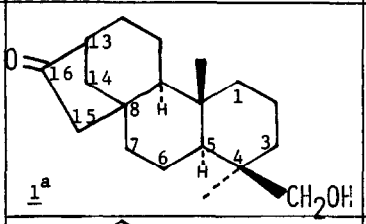
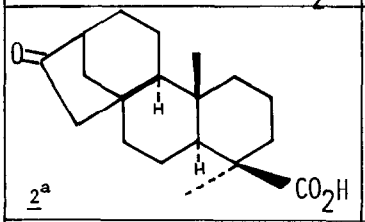
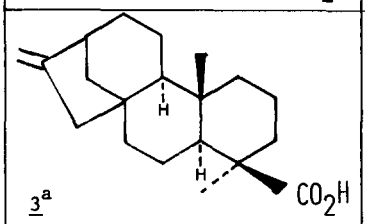
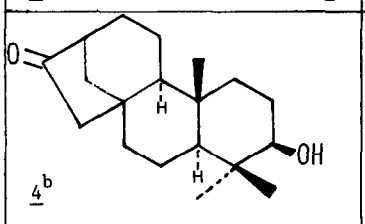
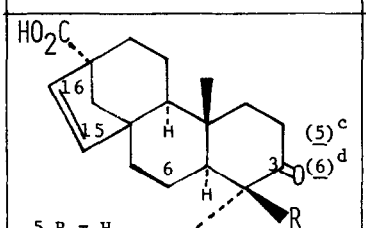
Hydroxylation of steroid substances by micro-organisms has proved an efficient preparative method in a number of cases[1]. An understanding of the factors which determine the specific position of hydroxylation should extend the scope of this method.[2] With these two points in mind we have undertaken a study of the microbiological transformation of some tetracyclic diterpenes. A recent communication[3] on the hydroxylation of ent-17-norkauran-16-one and 17-nor-13β-kauran-16-one by Aspergillus niger encourages us to report a summary of our results.

For our investigation we selected four substrates (1-4) containing the ent-kaurane skeleton and two (5,6) with the ent-beyerene skeleton as shown in Table I[4]. The transformations of these with Aspergillus ochraceous, Calonectria decora and Rhizopus nigricans, micro-organisms extensively studied in steroid hydroxylations, was examined[5]. The major products obtained from each substrate and the percentage conversions are shown in Table I. A number of metabolites obtained were known compounds and these were identified by direct comparison or by comparison of their physical and spectral properties with those in the literature. Where no reference is given, proof for the structure of the metabolite was obtained by a combination of spectroscopic and chemical methods[6]. Identical hydroxylations for 1 and 2 were confirmed by an interrelation involving the conversion to the 16-ene compounds for the 19-hydroxylated metabolites and methylation, Wittig reaction, and $LiAlH_4$ reduction for the 19-acids. Description of the chemical transformations involved will be given in a full paper.

Without drawing any conclusion about the probable factors influencing and directing hydroxylation of the tetracyclic diterpene skeleton a number of other interesting points can be made. The transformation of ent-kaurenoic acid (3) to ent-7α-hydroxy-kauren-19-oic acid is particularly important in view of the role of the latter as an intermediate in

TABLE I MIRCROBIOLOGICAL TRANSFORMATION OF SOME TETRACYCLIC DITERPENES

| SUBSTRATE | SUBSTRATE MODIFICATION IN MAJOR PRODUCTS OBTAINED (%) | | |
|---|---|---|---|
| | Aspergillus ochraceous | Calonectria decora | Rhizopus nigricans |
|  1[a] | 16α-OH (10%)[7] | 1β-OH (10)<br>7β-OH (10) | 1β-OH (20)<br>7β-OH (20) |
|  2[a] | 13-OH (5)[8]<br>13,16α-OH (5)[8] | 1β-OH (5)<br>7β-OH (15)[9]<br>7α-OH (40)[9] | 1β-OH (30)<br>7β-OH (30)[9]<br>7α-OH (5)[9] |
|  3[a] | 16β,17-OH (20)[10] | 15β-,7β-OH (30)<br>15β-OH (5)[11]<br>7β-OH (5)[9] | 7α-OH (25)[9,12] |
|  4[b] | 6α-OH (30)<br>7β-OH (25) | 7β-OH (40) | 1β-OH (25)<br>7β-OH (35) |
|  5 R = H<br>6 R = $CH_3$ | 3α-OH<br>— | 6α-OH (50)<br>6α-OH (50)[13] | ——<br>—— |

[a] C.A. Henrick and P.R. Jefferies, Aust. J. Chem., 17, 915 (1964); [b] P.R. Jefferies and R.W. Retallack, Aust. J. Chem., 21, 1311 (1968); [c] D.E. White, P.R. Jefferies, R.S. Rosich, and M.C. Woods, Aust. J. Chem., 15, 521 (1962; [d] Ref. 13.

gibberellin biosynthesis[12]. In general C. decora and R. nigricans show a predilection for the introduction of hydroxyl groups at C-1 and C-7 on the ent-kaurane skeleton. The hydroxylation of ent-16-norkauran-19-oic acid (2) at C-13 by A. ochraceous provides intermediates which can readily be converted[8] to steviol. Chemical methods for the construction of the bicyclo[3,2,1]octane system with a bridgehead hydroxyl have been developed[8,14] for the synthesis of steviol but these involve long and complicated sequences. The microbiological method for C-13 hydroxylation, although at this stage giving poor conversion, has the advantage of being a one step reaction in which the unreacted material (75%) can be recycled. Finally, the introduction of a 15β-OH in (3) and of a 6α-OH in (6) by C. decora parallels the hydroxylation of these or closely related compounds in plants[11,13].

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