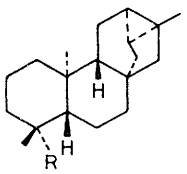
The Metabolism of the Diterpenoid Hydrocarbon, *ent*-Trachylobane, by *Gibberella fujikuroi* and the *X*-Ray Structure Determination of the Methyl Ester of Trachylobagibberellin A_{40}

Braulio M. Fraga,[a] Antonio G. Gonzalez,[a] Melchor G. Hernandez,[a] James R. Hanson,[b] and Peter B. Hitchcock[b]

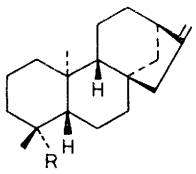
[a] *Instituto de Productos Naturales del C.S.I.C., Carretera La Esperanza 2, La Laguna, Tenerife, Spain*
[b] *School of Molecular Sciences, University of Sussex, Brighton, Sussex, BN1 9QJ, U.K.*

The diterpenoid pentacyclic hydrocarbon, *ent*-trachylobane, has been shown to be converted by *Gibberella fujikuroi* to trachylobagibberellin A_{40} using spectroscopic and *X*-ray methods.

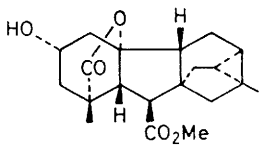
A number of pentacyclic diterpenoids are known[1–3] which possess the *ent*-trachylobane carbon skeleton (**1**). *ent*-Trachylobane (**1**) bears a considerable structural resemblance to the tetracyclic diterpenoid, *ent*-kaur-16-ene (**2**) which is the parent biosynthetic hydrocarbon of the gibberellin plant hormones.[4] Despite a lack of substrate specificity in the later stages of gibberellin biosynthesis in *Gibberella fujikuroi*, the enzyme systems responsible for the immediate oxidation of *ent*-kaurene show some features of structure specificity. Thus *ent*-kaur-15-ene (isokaurene) accumulates[5] in the gibberellin deficient d-5 mutant of maize and is not readily further metabolized whilst 16,17-epoxy-*ent*-kaurane displaces *ent*-kaur-16-ene and inhibits its oxidative modification by *Gibberella fujikuroi*.[6] The formation of *ent*-kaur-16-ene has been suggested to be a limiting step in gibberellin plant hormone biosynthesis. Despite the occurrence of several structurally similar series of polycyclic diterpenoids related to *ent*-kaur-16-ene, nevertheless all (more than 60) the naturally occurring gibberellin plant hormones isolated so far belong to the *ent*-kaurene series. Consequently it was of interest to examine the metabolism of the diterpenoid hydrocarbons of the same enantiomeric series such as *ent*-trachylobane by *G. fujikuroi*. The conversion of *ent*-trachylobanic acid (**3**) into pentacyclic analogues of the gibberellins by a mutant (B1–41a) of *G. fujikuroi* which is blocked for gibberellin biosynthesis between *ent*-kaurenal (**4**) and *ent*-kaurenoic acid (**5**) has been reported[7] and reveals the lack of structure specificity in the formation of the gibberellins once the 19-carboxylic acid is formed.



(1) R = Me
(3) R = CO_2H
(7) R = CH_2OH



(2) R = Me
(4) R = CHO
(5) R = CO_2H



(6)

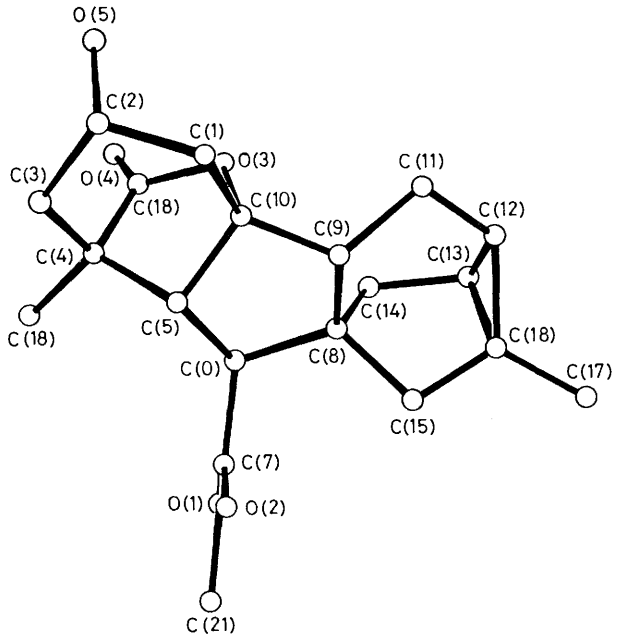


Figure 1. Molecular structure of trachylobagibberellin A_{40} methyl ester (**6**).

The transformation of *ent*-trachylobane (**1**) by the 'wild-type' *Gibberella fujikuroi* (ACC 917) was examined in shake culture in the presence of AMO-1618 which inhibits[8] kaurene synthetase and hence the formation of endogenous kaurenoid metabolites. The yield of trachylobane transformation products was low. The acidic metabolites were separated as their methyl esters. The ^{1}H n.m.r. spectrum of the major component, $C_{20}H_{26}O_5$ (Found: 346.1752; calc. 346.1724), m.p. 227—229 °C, showed a C*H*(OH) signal as a triplet, δ 4.26 (J 4 Hz). The 5-H and 6-H signals were co-incident (δ 2.49) whilst the cyclopropane signals (H-12 and H-13, δ 0.6 and 0.9) showed little perturbation[9] which might be assigned to adjacent hydroxylation thus suggesting that the hydroxyl group was located on the α-face of ring A. The complete structure (Figure 1) was determined by *X*-ray analysis of the methyl ester (**6**). The metabolite was thus trachylobagibberellin A_{40}.[10]

Since this metabolite, which represents a relatively unusual hydroxylation pattern, was not reported[7] in the study of *ent*-trachylobanic acid, the metabolism of *ent*-trachyloban-19-ol (**7**) by the 'wild-type' strain of *G. fujikuroi* was also examined. The acidic metabolites were again separated by chromatography of their methyl esters. This afforded a mixture of the esters of trachylobagibberellins A_9, A_{25}, A_{13}, A_7, A_{40}, and possibly A_{47}. These were identified partly by their ^{1}H n.m.r. spectra and by comparison of their mass spectra with published data[10] for the natural gibberellins.

Thus the fungal gibberellin pathway is able to handle, albeit in low yield, abnormal but naturally-occurring diterpenoid hydrocarbons. However, it is interesting to note that the dominant 2α-hydroxylation differs from the more common 3β-hydroxylation found in the natural gibberellins of *G. fujikuroi*.

Crystal data: $C_{20}H_{26}O_5$, M = 346.4, orthorhombic, space group $P2_12_12_1$, a = 8.298(2), b = 10.612(5), c = 20.152(9) Å, U = 1774.5 Å^3, Z = 4, D_c = 1.30 g cm^{-3}, $F(000)$ = 744, Mo-K_α radiation, λ = 0.71069 Å, μ = 1.0 cm^{-1}. The data were measured on an Enraf–Nonius CAD-4 diffractometer whilst the structure solution and refinement were carried out on a PDP 11/34 computer using the Enraf–Nonius structure determination package. The structure was solved from 1006 reflections and refined to R = 0.063 and R' = 0.069.†

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† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW. Any request should be accompanied by the full literature citation for this communication. The structure factor table is available as Supplementary Publication No. SUP 23347 (6 pp) from the British Library Lending Division. For details of how to obtain this material, see Notice to Authors No. 7, *J. Chem. Soc., Dalton* or *Perkin Trans.*, Index Issues.