Microbiological Hydroxylation of 3β,7β-Dihydroxykaurenolide

By **George Ellames** and **James R. Hanson** • The School of Molecular Sciences, University of Sussex, Brighton BN1 9QJ

Hydroxylation of 3β,7β-dihydroxykaurenolide by *Rhizopus arrhizus* affords 3β,7β,11α- and 3β,7β,13-trihydroxykaurenolides in low yield.

HYDROXYLATION at C-11 [1] and C-13 [2] is a known minor substitution pattern of the kaurenolide metabolites of the fungus *Gibberella fujikuroi*. We have shown that the microbiological transformation of 7α- and 7β-hydroxykaurenolide by the fungus *Rhizopus arrhizus* affords a method of hydroxylation at both C-11 and C-13.[3] Since 3β,7β- [4] and 7β,18-dihydroxykaurenolide [5] are major metabolites of *Gibberella fujikuroi*, we considered that 3β,7β,11α-, 3β,7β,13-, 7β,11α,18-, and

[1] P. Hedden, J. MacMillan, and M. J. Grinstead, *J.C.S. Perkin I*, 1973, 2773.
[2] E. P. Serebryakov, A. V. Simolin, V. F. Kucherov, and B. V. Rosynov, *Tetrahedron*, 1970, **26**, 5215.
[3] J. R. Hanson, G. Savona, and M. Siverns, *J.C.S. Perkin I*, 1974, 2001.
[4] J. H. Bateson and B. E. Cross, *J.C.S. Perkin I*, 1972, 1117.
[5] B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J. Chem. Soc.*, 1963, 2944.

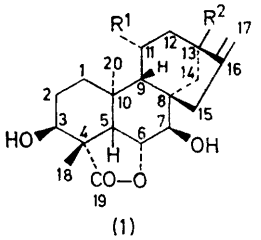
7β,13,18-trihydroxykaurenolides might occur as minor metabolites. We therefore examined the microbiological hydroxylation of the dihydroxykaurenolides by *Rhizopus arrhizus* in an effort to prepare authentic material.

3β,7β-Dihydroxykaurenolide (1; $R^1 = R^2 = H$) was incubated with shake cultures of *Rhizopus arrhizus* for 7 days. Under various conditions of culture, a substantial quantity of the starting material was recovered, together with three minor metabolites. The structures of these were readily assigned from their ^1H and ^{13}C n.m.r. spectra. Because of their low solubility, the n.m.r. spectra were determined in pentadeuteriopyridine. The ^{13}C resonances of 3β,7β- and 7β,18-dihydroxykaurenolide were readily assigned (see Table) by comparison with our previous results.[3] In particular a resonance associated with C-11 appeared at δ 17.4 and one with C-13 at δ 38.2. The first metabolite (M^+ 348), identified as 3β,7β,11α-trihydroxykaurenolide (1; R^1 = OH, R^2 = H), lacked the signal at δ 17.4 in its ^{13}C n.m.r. spectrum but had a new *C*H(OH) signal at δ 64.2. Furthermore, C-9 (δ 60.2) and to C-12 (46.7) were deshielded in comparison with the corresponding nuclei in the starting material, by a similar extent to that encountered in the 7-hydroxykaurenolide series.[3] In the ^1H n.m.r. spectrum there was a new C*H*(OH) resonance (τ 5.60) and the 20-protons (τ 8.33) were markedly deshielded in comparison with 3β,7β-dihydroxykaurenolide [τ (C_5D_5N) 9.01 (20-H_3) and 8.40 (18-H_3)]. The effect of an 11α-hydroxy-group on the 20-H_3 resonance in pentadeuteriopyridine has been noted previously.[1,3] Hence the 11α-stereochemistry was assigned to the new hydroxy-group in this transformation product.

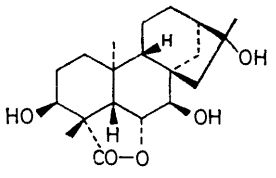
The second metabolite (M^+ 348), which retained the ^{13}C n.m.r. signals associated with rings A and B, lacked the C-13 resonance at δ 38.2 and showed a new *C*(OH) resonance at δ 77.2. In addition C-12 and -14 were deshielded in a manner consonant with 13-hydroxylation. In the ^1H n.m.r. spectrum, one of the olefinic 17-H resonances (τ 4.85 and 4.30) was deshielded—a solvent shift which has been noted in both the 13-hydroxykaurenolides and the gibberellins.[6] Consequently this hydroxylation product was identified as 3β,7β,13-trihydroxykaurenolide (1; R^1 = H, R^2 = OH). The third minor metabolite, only isolated on one occasion, was identified as 3β,7β,16ξ-trihydroxykauranolide (2). The mass spectrum (M^+ 350) agreed with the formula $C_{20}H_{30}O_5$, and the ^1H n.m.r. spectrum contained three C–CH_3 resonances, yet lacked additional C*H*·O signals and olefinic C*H* signals.

^{13}C N.m.r. spectra of some kaurenolides (in [$^2H_5$]pyridine; δ in p.p.m. from Me_4Si)

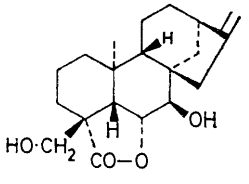
Compound	Carbon atom 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(1; $R^1 = R^2 = H$)	36.6	27.5	65.6	47.2	52.2	84.5	70.9	46.1	56.4	34.3	17.4	32.9	38.2	34.2	42.4	160.5	106.9	18.4	182.6	22.4
(3)	36.9	17.4	23.5	49.1	48.4	85.7	70.9	45.9	55.8	34.3	17.4	32.9	38.2	34.3	42.5	160.5	106.8	68.3	182.0	21.2
(1; R^1 = OH, R^2 = H)	37.0	27.7	65.8	47.1	52.7	84.9	71.0	45.9	60.2	35.6	64.2	46.7	38.8	34.6	43.1	160.5	106.7	18.6	182.1	25.0
(1; R^1 = H, R^2 = OH)	36.6	27.5	65.6	47.3	52.5	84.3	70.9	43.7	56.2	34.2	19.1	40.9	77.2	41.3	42.7	161.9	106.4	18.4	182.7	22.4



(1)



(2)



(3)

The yields obtained in the transformations by this culture were low; hence this did not afford a means of preparing sufficient quantities of these metabolites for further transformation, for example chemical conversion into the gibberellins. Furthermore, despite several attempts, we were unable to achieve transformation of 7,18-dihydroxykaurenolide.

EXPERIMENTAL

General details, including the culture conditions for *Rhizopus arrhizus*, have been described previously.[3]

Microbiological Hydroxylation of 3β,7β-*Dihydroxykaurenolide*.—The kaurenolide (2.0 g) in ethanol (40 ml) was distributed between 40 flasks (100 ml medium [3] in 250 ml flasks) of *Rhizopus arrhizus* and incubated for 7 days. The metabolites were recovered in ethyl acetate. Unchanged 3β,7β-dihydroxykaurenolide (1.3 g) was recovered from the mycelium. The broth extract was purified by chromatography on silica gel. Elution with ethyl acetate gave 3β,7β-dihydroxykaurenolide (170 mg). Elution with 99 : 1 ethyl acetate–methanol gave a mixture of 3β,7β,11α- and 3β,7β,13-trihydroxykaurenolides (72 mg), which were separated by preparative layer chromatography on silica with 95 : 5 di-isopropyl ether–acetic acid as the mobile phase. The faster moving band gave 3β,7β,11α-*trihydroxykaurenolide* (17 mg), which crystallized from acetone–light petroleum as prisms, m.p. 269—273° (Found: C, 67.1; H, 8.6. $C_{20}H_{28}O_5$,0.5H_2O requires C, 67.2; H, 8.1%), $\nu_{max.}$ 3 570, 3 520, 3 490, 1 742, 1 650, and 897 cm^{-1}, τ (C_5D_5N) 8.33 (6 H, s, 18- and 20-H_3), 5.20 (1 H, m, 7-H), 4.90 (4 H, m, 3-H, 6-H, and 17-H_2), and 4.22, 3.45, and 2.62 (each d, *J* 4 Hz, exchanged by D_2O). The slower moving band gave 3β,7β,13-*trihydroxykaurenolide* (32 mg), which crystallized

[6] J. R. Hanson, *J. Chem. Soc.*, 1965, 5036.

from acetone–light petroleum as prisms, m.p. 260—265° (Found: C, 67.0; H, 8.1. $C_{20}H_{28}O_5,0.5H_2O$ requires C, 67.2; H, 8.1%), $\nu_{max.}$ 3 562, 3 520, 3 492, 1 740, 1 650, and 893 cm^{-1}, τ (C_5D_5N) 9.01 (3 H, s, 20-H_3), 8.38 (3 H, s, 18-H_3), 5.20 (m, 7-H, part obscured by H_2O signal), 4.90 (3 H, m, 3-H, 6-H, and 17-H), and 4.30 (1 H, m, 17-H). On one occasion an incubation with 3β,7β-dihydroxykaurenolide (1.5 g) gave, after purification as above, 3β,7β,16ξ-*trihydroxykauranolide* (28 mg), m.p. 265—267° (Found: C, 64.5; H, 8.4. $C_{20}H_{30}O_5,H_2O$ requires C, 65.2; H, 8.7%), $\nu_{max.}$ 3 390, 3 290, and 1 777 cm^{-1}, τ (C_5D_5N) 9.01 (3 H, s, 20-H_3), 8.42 (3 H, s, 17-H), 8.38 (3 H, s, 18-H), 5.20 (1 H, m, 7-H), and 5.10 (2 H, m, 3- and 6-H).

We thank Mrs. A. Ward for growing the fermentations.

[6/416 *Received*, 1*st March*, 1976]