Terpenoids. Part XXVII.[1] Structure and Stereochemistry of Ponicidin, a Diterpenoid of *Isodon japonicus*

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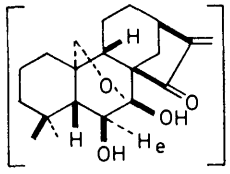
On the basis of chemical and spectroscopic (especially n.m.r.) evidence, ponicidin was assigned the structure *ent*-7β,20:14β,20-diepoxy-1β,6α,7α-trihydroxykaur-16-en-15-one (1). The results of an o.r.d. determination established its absolute configuration.

We have isolated many diterpenoids from *Isodon japonicus* Hara and elucidated their structures. They are oridonin,[2] sodoponin,[3] odonicin,[4] enmein,[5] enmein-3-acetate,[5] isodocarpin,[6] nodosin,[7] isodonal,[3,8] epinodosin,[3,9] epinodosinol,[3] isodotricin,[10] isodoacetal,[4] and nodosinin.[4] The former three are kaurene derivatives, while the latter ten are of the B-secokaurene type (enmein-type). We have also isolated ponicidin[11] from this plant source, but its structure has remained unsolved.

Ponicidin was obtained from the ethereal extract of the dried leaves as colourless crystals, m.p. 238—241°, $[\alpha]_D^{17}$ −118°. The molecular formula was determined as $C_{20}H_{26}O_6$ instead of $C_{20}H_{28}O_6$ reported previously,[11] on the basis of analytical and mass spectral data. The presence of a carbonyl group conjugated with an exocyclic methylene group in the molecule was suggested by the u.v., i.r., and n.m.r. spectra of ponicidin and its dihydro-derivative.

Acetylation of ponicidin gave a diacetate as the major product. The n.m.r. spectrum of the diacetate showed paramagnetic shifts for two proton signals (H_e and H_f) when compared with the spectrum of ponicidin, suggesting the presence of two secondary hydroxy-groups in ponicidin. Upon acetylation in the presence of boron trifluoride–ether complex, ponicidin afforded a triacetate, suggesting the presence of a tertiary hydroxy-group. The n.m.r. spectrum of the triacetate showed a large paramagnetic shift of the H_e signal when compared with the foregoing diacetate. This must be attributed to an anisotropic effect of the tertiary acetoxy-group, and suggested that the tertiary hydroxy-group and H_e were near each other in the molecule. Ponicidin was also shown to have two tertiary methyl groups by the n.m.r. spectrum. Consideration of these facts with the structures of the diterpenoids found hitherto in *Isodon japonicus* led to the assignment of a kaurene-type skeleton to ponicidin, and some functional groups were located as shown in the partial structure (A).



(A)

On the basis of an n.m.r. investigation including the decoupling experiments shown in Figure 1, H_a and H_c were assigned to the exocyclic methylene protons. The proton H_g was assigned to the C-13 proton on the basis of its chemical shift and splitting pattern.[3] The assignment of H_e shown in (A) was based on its chemical shift and its relationship to the hemiacetal hydroxy-group described above. Decoupling experiments between H_e and H_n showed that H_n must be the C-5 proton. The proton H_f on a secondary hydroxylated carbon was assigned to the C-1 or C-3 axial proton, by analogy with the chemical shift and splitting pattern of the C-1 axial proton of oridonin.[2] Thus, the partial structure (A) was extended to (B).

The absence of the signal of the C-20 methylene protons, which has been observed as an AB-type or a

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[2] E. Fujita, T. Fujita, H. Katayama, M. Shibuya, and T. Shingu, *J. Chem. Soc.* (*C*), 1970, 1674.
[3] E. Fujita, T. Fujita, M. Taoka, H. Katayama, and M. Shibuya, *Chem. and Pharm. Bull.* (*Japan*), 1973, **21**, 1357.
[4] E. Fujita, M. Taoka, Y. Nagao, and T. Fujita, *J.C.S. Perkin I*, 1973, 1760.
[5] E. Fujita, T. Fujita, and M. Shibuya, *Chem. Comm.*, 1966, 297; *J. Pharm. Soc. Japan*, 1967, **87**, 1076.
[6] E. Fujita, T. Fujita, and M. Shibuya, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 1573.
[7] E. Fujita, T. Fujita, and M. Shibuya, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 509.
[8] T. Kubota and I. Kubo, *Tetrahedron Letters*, 1967, 3781.
[9] T. Kubota and I. Kubo, *Chem. Comm.*, 1968, 763.
[10] E. Fujita, T. Fujita, Y. Okada, S. Nakamura, and M. Shibuya, *Chem. and Pharm. Bull* (*Japan*), 1972, **20**, 2377.
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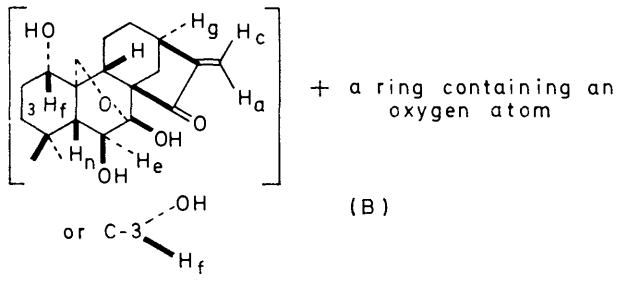
singlet in all the known kaurene-type diterpenoids found hitherto in *Isodon* species, and the observations



of a sharp singlet (H_b) at δ 5·85 and of a doublet (H_d) at δ 4·98 were characteristic of the n.m.r. spectrum of ponicidin. As shown in (B), the unsolved part of ponicidin was regarded as an oxygen-containing ring, on the basis of the molecular formula. A reasonable explanation was given by the assignments of H_b and H_d to the C-20 and the C-14 protons, respectively, assuming formation of an ether linkage between C-20 and C-14. The unusual paramagnetic shift of H_d can be attributed to the anisotropic effect of the conjugated ketone. These considerations led to a final assignment of structure (1) or its enantiomer to ponicidin. Other protons were also assigned as shown in (1) by a detailed n.m.r. investigation.

Examination of a Dreiding model indicated that the ether linkage between C-20 and C-14 compels the C-ring to form a chair-like conformation,[4] and decoupling experiments showed that the dihedral angles between H_j and H_o and between H_j and H_h were *ca.* 90° (see Figure 1 and Table 1). The conformation of the C-ring results in a distortion in the B-ring. Thus, the dihedral angle between H_e and H_n decreases to *ca.* 90° and hence, a small coupling constant (1·5 Hz) between them is to be expected. These deformations make the distance between the C-15 carbonyl and C-6 hydroxy-groups longer than that in oridonin (7). The hydrogen-bonding between them recognized in the i.r. spectrum of oridonin is not observed in ponicidin. In oridonin the C-6 hydroxy-group is not acetylated under the usual conditions,[2] whilst in poncidin it is easily acetylated.

Thus, structures (2)—(4) are assigned to the fore-

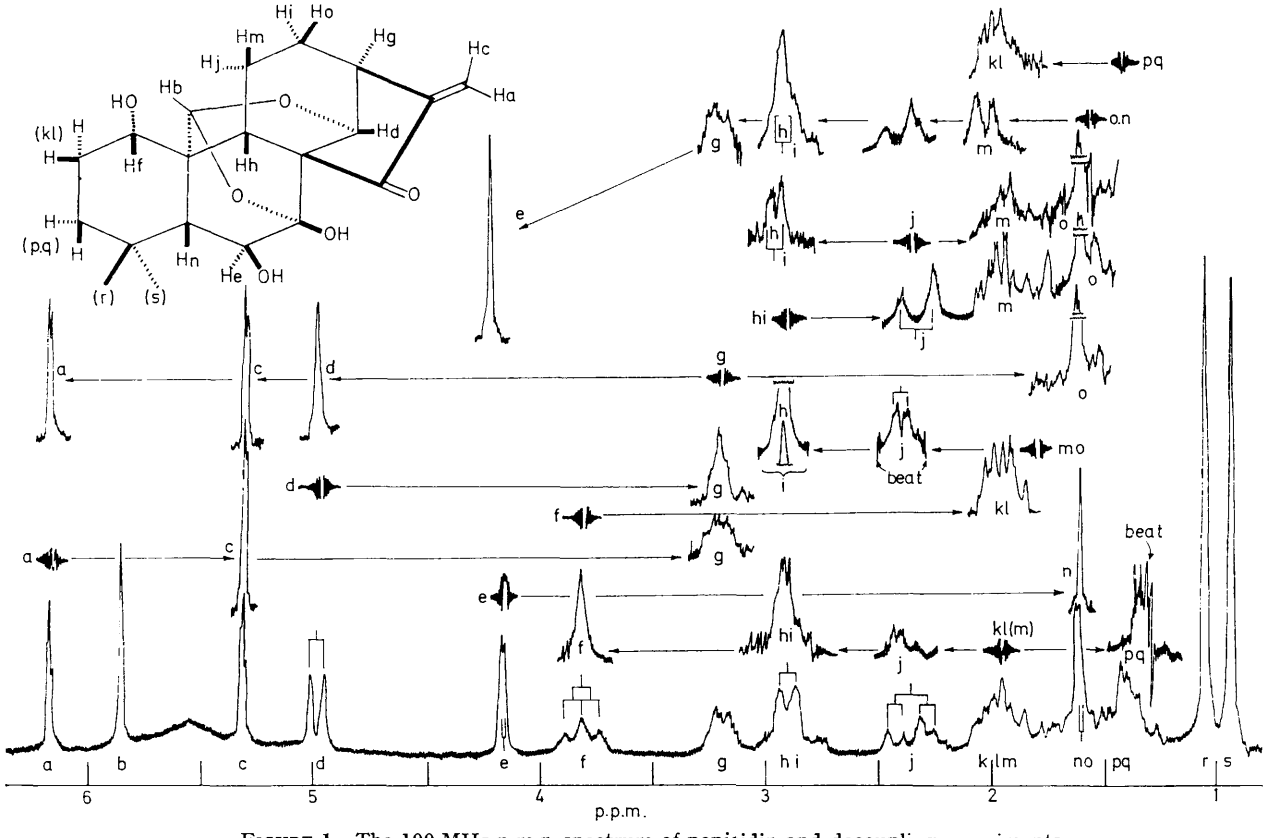


FIGURE 1 The 100 MHz n.m.r. spectrum of ponicidin and decoupling experiments

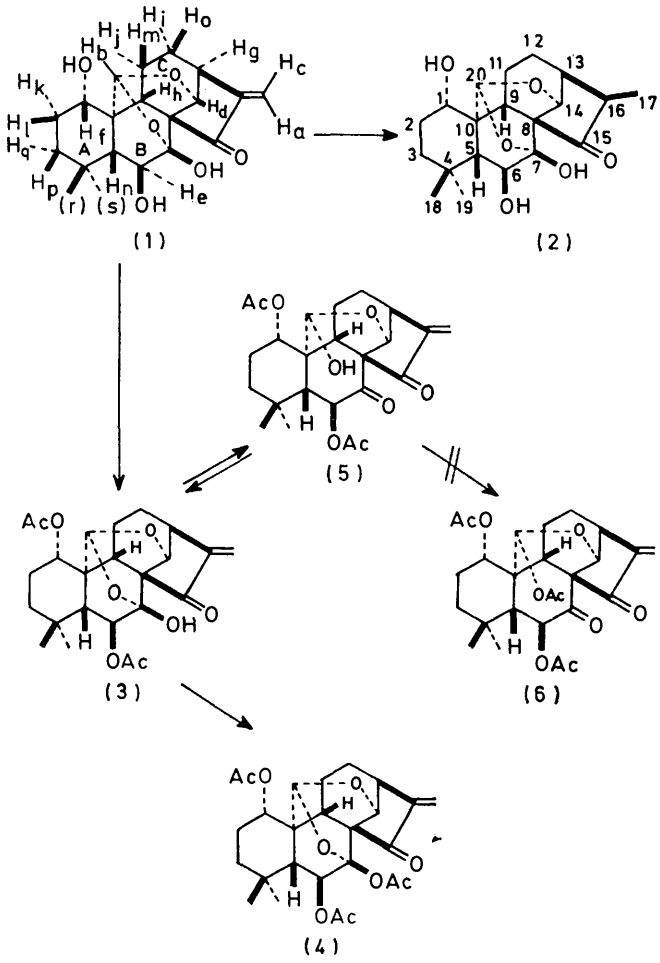
going dihydro-derivative, diacetate, and triacetate, respectively. Acetylation of ponicidin by acetic anhydride in the presence of boron trifluoride gave the triacetate (4) instead of (6) as described above. On

TABLE 1

Decouplings among H_h, H_i, H_j, H_m, and H_o

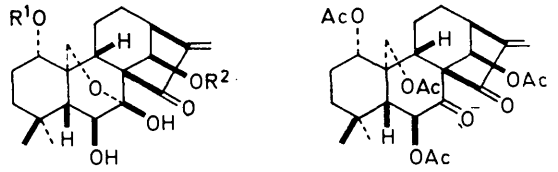
Proton before irradiation	Point of irradiation	Proton after irradiation
H_j, dd, *J* 14 and 6 Hz	H_h, H_i	d, *J* 14 Hz
	H_m, H_o	d, *J* 6 Hz
H_h, d, *J* 6 Hz	H_m	s
	H_j	sharp d

the other hand, lasiokaurin (8) [12] on acetylation under the same conditions yielded the tetra-acetate (10). Its C-20 methylene protons were observed as an AB-type



SCHEME 1

at δ 4·60 and 4·83, *i.e.* at lower field than the C-20 methylene protons, observed as a singlet at δ 4·28 in oridonin-1,14-diacetate (9);[2] the C-6 proton of lasiokaurin was observed as a doublet (*J* 13 of Hz) * at δ 6·06 indicating a chair form for the B-ring. These data supported the structure (10).



There might be an equilibrium between (3) and (5) under the conditions of acetylation of (1) in the presence of boron trifluoride. Acetylation at the C-20 hydroxy-group of (5) must be much more difficult than at the C-7 hydroxy-group of (3), because of larger steric hindrance, which may be the reason why only triacetate (4), instead of (6), is formed.

On the basis of the internuclear double resonance (INDOR) [13] spectra (Figure 2) and the decoupling experiments (Figure 1), protons with spin–spin coupling and protons in an environment in which a nuclear Overhauser effect (n.O.e.) should be observed were distinguished. The result is shown in Table 2.

TABLE 2

Results of INDOR spectra and n.O.e.

Monitor proton	Protons * which showed n.O.e. for monitor proton	Protons which showed couplings with monitor proton
b	k, s	d †, h †
d		g
e	r, s	n
f	j (6), n, p	k, l
g		c †, d, i, o
h	f, n	j, m, d †
i		g, j, m, o
j	f (6)	h, i, m, o
k	b (10), s	f, p, q
and		
l		f, p, q
n	f, h, r	e
and		
o		j, i, g
p	f	k, l
and		
q		k, l
r	e (12), n, p	
s	b (17), e (21), k, q	

* Numbers in parentheses show the increased percentage of the intensity of the proton signal. † Long range couplings.

Couplings observed gave a satisfactory explanation for structure (1). The A-ring was shown to have a chair conformation by n.O.e. [$H_b \leftrightarrow H_k$, $H_b \leftrightarrow Me_s$, $H_k \leftrightarrow Me_s$]. The INDOR spectrum showed long range couplings between H_h and H_b, H_b and H_d, and H_d and H_h, which provided further evidence for the acetal structure at C-20. The B-ring, therefore, can exist only as a boat form and the C-ring as a chair-like conformation as described above. An n.O.e. was observed between H_f and H_j, but not between H_f and Me_r. This confirms the location of the hydroxy-group at C-1.

Finally, the absolute configuration is confirmed as that shown in (1) on the basis of a negative Cotton effect [14] in the o.r.d. spectrum of the dihydro-derivative (2).

EXPERIMENTAL

M.p.s were taken on a micro hot-stage. Unless otherwise stated, i.r. spectra were recorded in KBr discs on a Hitachi model EPI-S2 spectrometer and n.m.r. spectra with Varian A-60, T-60, or HA-100 spectrometers in deuteriochloroform; signals are reported in p.p.m. from

* That the dihedral angle between C(5)-H and C(6)-H is *ca.* 180° is suggested.

[12] E. Fujita and M. Taoka, *Chem. and Pharm. Bull (Japan)*, 1972, **20**, 1752.

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tetramethylsilane as internal standard. The mass spectra were determined on a JMS-OISG double-focusing mass spectrometer. Optical rotations were measured on a JASCO DIP-180 automatic polarimeter. The o.r.d. spectrum was taken on a Yanagimoto model ORD-185A recording spectropolarimeter.

Ponicidin (1).—The ethereal extract of the leaves of the plant, after removal of the acidic substances and almost all the enmein, was chromatographed (methylene dichloride–acetone, 9 : 1) on a silica gel column to give crude ponicidin contaminated by enmein. Repeated chromatography followed by recrystallization from methanol yielded pure *ponicidin*,* m.p. 238—241°, $[\alpha]_D^{17}$ −118° (*c* 0·1 in pyridine) (Found: C, 66·2; H, 7·7%; M^+, 362. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2%; *M*, 362), $\lambda_{max.}$ (EtOH) 230 nm (ε 7400), $\nu_{max.}$ 3350, 1725, and 1630 cm^{-1}, δ (C_5D_5N; HA-100) 6·17 (1H, t, *J* 1·0 Hz, H_a), 5·54br (3H, s, OH), 5·31 (1H, t, *J* 1·0 Hz, H_c), 4·98 (1H, d, *J* 6 Hz, H_d), 4·16 (1H, d, *J* 1·3 Hz, H_e), 3·82 (1H, t, *J* 8 Hz, H_f), 3·3—3·1 (1H, m, H_g), 2·90 (1H, d, *J* 6·5 Hz, H_h), 3·05—2·7 (1H, m, H_i), 2·5—2·2 (1H, dd, *J* 6·5 and 14 Hz, H_j), 1·61 (1H, *J* 1·2 Hz, H_n), and 1·04 and 0·92 (each 3H, s, Me_r and Me_s).

* See ref. 3 for details of the yield of ponicidin.

Dihydro-derivative (2) *of Ponicidin*.—Catalytic hydrogenation [platinum oxide (10 mg), methanol (10 ml)] of crude ponicidin (18 mg) afforded the *dihydro-derivative* (2) (8 mg) as crystals (from methanol–ethyl acetate), m.p. 214—218° (Found: M^+, 364·184. $C_{26}H_{28}O_6$ requires *M*, 364·188), $\nu_{max.}$ 3350, 1735, and 1075 cm^{-1}, δ (C_5D_5N; T-60) 5·89 (1H, s, 20-H), 5·05 (1H, d, *J* 5 Hz, 14-H), 4·16 (1H, d, *J* 1·5 Hz, 6-H), 3·85 (1H, t, *J* 8 Hz, 1-H), 1·60 (1H, d, *J* 1·5 Hz, 5-H), 1·08 (3H, d, *J* 7 Hz, 16-Me), and 0·96 and 1·02 (each 3H, s, 2 × Me), o.r.d. (*c* 0·93 × 10^{-3} in methanol) $[\phi]_{325}$ − 2301, $[\phi]_{295}$ −273, $[\phi]_{280}$ −1170, and $[\phi]_{270}$ −1740, *a* −20·3.

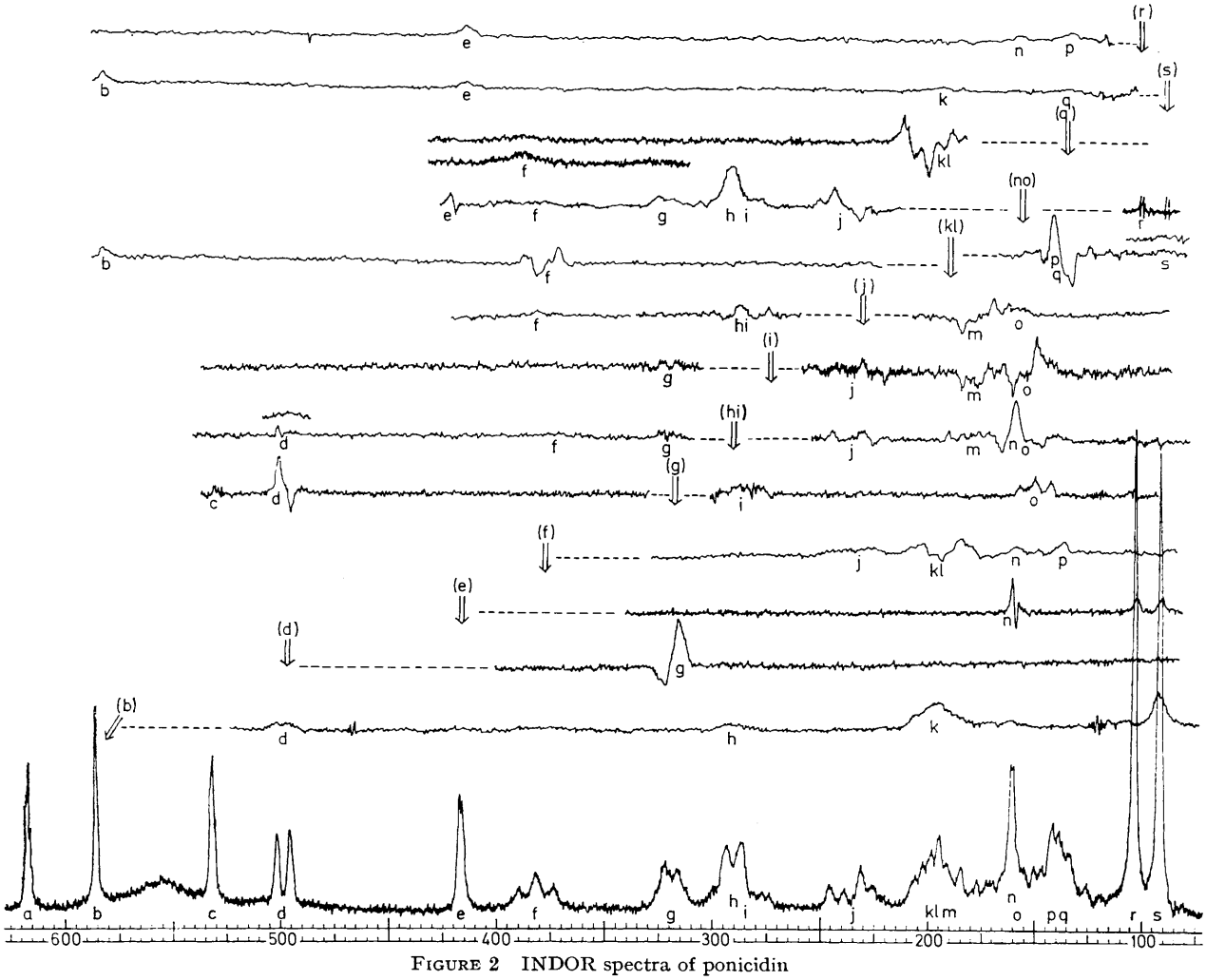

FIGURE 2 INDOR spectra of ponicidin

Acetylation of Ponicidin.—A solution of ponicidin (9 mg) in a mixture of acetic anhydride and pyridine (1 : 1) was allowed to stand at room temperature overnight. Methanol was added to decompose excess of the reagent, and the solvent was evaporated off to leave a residue (11 mg), which was chromatographed (methylene dichloride–acetone, 19 : 1) on a silica gel column to separate ponicidin 1,6,7-triacetate (4) (2 mg) and 1,6-diacetate (3) (8 mg). The 1,6,7-triacetate (4), m.p. 236—238°, was obtained as prisms (from methanol), $\nu_{max.}$ 1770, 1740, 1730, 1640, and 1230—1240 cm^{-1}, which were identical with the triacetate

prepared using acetic anhydride in the presence of boron trifluoride as described later. *Ponicidin* 1,6-*diacetate* was obtained as an oil, (Found: M^+ — AcOH, 386·170. $C_{22}H_{26}O_6$ requires 386·172), $\nu_{max.}$ 3400, 1730, 1640, and 1240 cm^{-1}, δ (T-60) 6·13 (1H, s, 17-H), 5·44 (2H, s, 20-H and 17-H), 4·87 (1H, d, *J* 1·5 Hz, 6-H), 4·83 (1H, dd, *J* 5 and 10 Hz, 1-H), 4·52 (1H, d, *J* 6 Hz, 14-H), 2·10 and 2·27 (each 3H, s, 2 × OAc), and 0·90 and 0·95 (each 3H, s, 2 × Me).

Acetylation of Poncidin with Acetic Anhydride in the Presence of Boron Trifluoride.—To a solution of crude ponicidin (87 mg) in acetic anhydride (6 ml) was added boron trifluoride–ether complex (4 drops), and the mixture was allowed to stand at room temperature for 8 h. Methanol was added to decompose excess of acetic anhydride, and the mixture was extracted with chloroform. The usual treatment of the chloroform layer gave *ponicidin* 1,6,7-*triacetate* (4), after column chromatography (silica gel; methylene dichloride), as prisms, m.p. 236—238° (from methanol) (Found: C, 63·9; H, 6·7. $C_{26}H_{32}O_9$ requires C, 63·9; H, 6·6%), $\nu_{max.}$ 1770, 1740, 1730, 1640, and 1230—1240 cm^{-1}, δ (C_5D_5N, A-60) 6·40 (1H, d, *J* 1·5 Hz, 6-H), 6·20, 5·39 (each 1H, m, 17-H_2), 5·01 (1H, dd, *J* 6 and 9 Hz, 1-H), 4·70 (1H, d, *J* 6·5 Hz, 14-H), 3·20 (1H, m, 13-H), 2·35, 2·14, and 2·03 (each 3H, s, 3 × OAc), and 1·16 and 0·87 (each 3H, s, 2 × Me).

Lasiokaurin Triacetate (10).—To a solution of lasiokaurin [12] (8) (134 mg) in acetic anhydride (3 ml) was added boron trifluoride–ether complex (47%; 2 drops), and the mixture left at room temperature overnight. Usual treatment and chromatography (methylene dichloride) of the crude product on a silica gel (1 g) column gave ent-1β,6α,14α,20-*tetrahydroxykaur*-16-*ene*-7,15-*dione* 1,6,14,-20-*tetra-acetate* (10) as prisms (133 mg), m.p. 221—222° (from methanol) (Found: C, 63·1; H, 6·9. $C_{28}H_{36}O_{10}$ requires C, 63·1; H, 6·8%), $\nu_{max.}$ 1735, 1645, and 1240—1230 cm^{-1}, δ (A-60) 6·20, 5·45 (each 1H, br s, 17-H_2), 5·93 (1H, s, 14-H), 4·83, 4·60 (each 1H, AB, *J* 14 Hz, 20-H_2), 4·9—4·6 (1H, m, 1-H), *ca.* 3·14 (1H, m, 13-H), 2·23, 2·17, 2·04, and 1·97 (each 3H, 4 × OAc), and 1·12 and 0·98 (each 3H, s, 2 × Me).

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