Structure of Phyllostachysin A: Novel Antineoplastic Diterpenoid from *Rabdosia phyllostachys*

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A new antineoplastic diterpenoid of novel structure, phyllostachysin A, was isolated from the leaves of *Rabdosia phyllostachys* (Diels) Hara (Labiatae) and the structure has been shown to be that in the structure (**1**) from spectral and chemical evidence.

Rabdosia phyllostachys,[1] which is distributed throughout the northwest district of the Yunnan province of China, has been used as an antiphlogistic or an antibiotic agent in folk medicine. From the dried leaves of this plant a novel type of diterpenoid, phyllostachysin A (**1**), which showed an antineoplastic activity on some transplantable animal tumours,† has been isolated. This paper deals with the structure elucidation of this novel diterpenoid, which is the first diterpenoid having the 7,20-cyclo-*ent*-kaur-16-ene (**11**) as a basic skeleton, from the genus *Rabdosia*.

Phyllostachysin A (**1**), $C_{20}H_{26}O_6$, m.p. 264—265 °C, $[\alpha]_D^{20}$ $-30.5°$ (*c* 1.25, C_5H_5N) has been shown to contain a five membered ketone conjugated with an *exo*-methylene group [$\lambda_{max.}$ (EtOH) 229 nm (ε 8270); $\nu_{max.}$‡ 1725 and 1645 cm^{-1}; ^{1}H n.m.r.§ δ 6.20 and 5.41 (each 1H, br. s); ^{13}C n.m.r.§ δ 117.0 (t), 152.3 (s) (*exo*-methylene), and 204.4 (s)], a five membered ketone [$\nu_{max.}$ 1750 cm^{-1}; ^{13}C n.m.r. δ 211.6 (s)], and four hydroxy groups [$\nu_{max.}$ 3400 cm^{-1}; ^{1}H n.m.r. ([$^2H_6$]Me_2SO) δ 6.24, 5.85, 5.09, and 4.41 (disappeared on addition of D_2O)]. The signals due to three methine protons attached to carbons having a hydroxy group at δ 5.93 (1H, br.s, H_a), 5.30 (1H, m, H_b), and 4.60 (1H, br. s, H_c) in the ^{1}H n.m.r. spectrum and the four signals at δ 64.9 (d), 73.0 (d), 80.6 (d), and 90.0 (s) in the ^{13}C n.m.r. spectrum suggested that three of the four hydroxy groups are secondary and the fourth is tertiary. In addition to signals due to two t-methyl groups (δ 1.32 and 0.89), the ^{1}H n.m.r. spectrum showed signals due to two methine protons at δ 3.22 (1H, d, *J* 9 Hz, H_d) and 2.28 (1H, br.d, *J* 10 Hz, H_f), and signals due to a methylene group at δ 3.06 (1H, ddd, *J* 10, 10, and 13 Hz, H_e), and 1.90 (1H, dd, *J* 10 and 13 Hz, H_g). Spin–spin decoupling experiments revealed the presence of partial structure (A) in the structure of phyllostachysin A. On irradiation at H_d, H_a collapsed to a sharp singlet, suggesting that the dihedral angle of those protons was *ca.* 90°. When H_b was irradiated, H_e, H_g, and H_f collapsed into a double doublet (*J* 10 and 13 Hz), a doublet (*J* 13 Hz), and a singlet, respectively. On the other hand, H_b collapsed to a double doublet (*J* 10 and 10 Hz) on irradiation at H_e, and H_c changed to a sharp singlet on irradiation at H_f. These spectral data,

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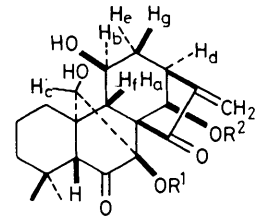
† The results will be published elsewhere.

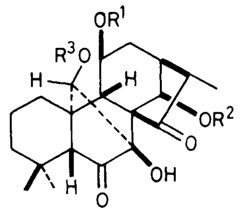
‡ All i.r. spectra were recorded for KBr discs.

§ All ^{1}H and ^{13}C n.m.r. spectra were recorded in C_5D_5N solution unless otherwise noted and the chemical shifts were expressed in δ. The ^{13}C n.m.r. spectral assignments are based on a combination of proton noise decoupling, off-resonance decoupling, single frequency selective decoupling, and comparisons with each other.



(1) $R^1 = R^2 = H$

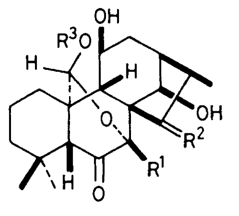
(2) $\left.\begin{matrix}R^1\\R^2\end{matrix}\right\}\!>\!\mathrm{CMe_2}$



(3) $R^1 = R^2 = R^3 = H$

(4) $R^1 = R^2 = COMe$; $R^3 = H$

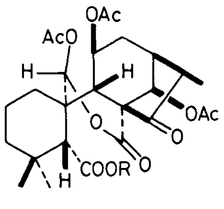
(5) $R^1 = R^2 = R^3 = COMe$



(6) $R^1 = OH$, $R^2 = O$, $R^3 = H$

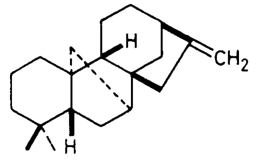
(7) $R^1 = OH$, $R^2 = O$, $R^3 = Me$

(8) $R^1 = H$, $R^2 = \beta\text{-OH},\alpha\text{-H}$, $R^3 = H$

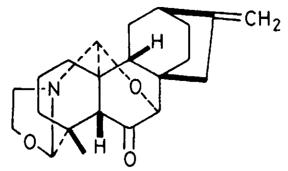


(9) R = H

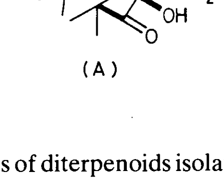
(10) R = Me



(11)



(12)



(A)

coupled with structures of diterpenoids isolated so far from the genus *Rabdosia*,[2] lead us to assume that phyllostachysin A has an *ent*-kaurene structure as its basic skeleton. The structure (**1**) was presumed for phyllostachysin A from the following evidence. The compound is pentacyclic from the degree of unsaturation. Comparison of ^{13}C n.m.r. spectrum (Figure 1) with those of kaurene derivatives suggested that no hydroxy groups are present on the A ring. The chemical shift of methine carbon at δ 63.5 (C-5) suggested that this carbon should be located adjacent to a ketonic function. The signals of H_f (9-H) and H_c (20-H) are coupled by long-range interaction *via* W letter theory.[3]

The presumed structure was supported by the chemical reactions described below. Treatment of (**1**) with 2,2-dimethoxypropane in $HCON(Me)_2$ in the presence of *p*-toluenesulphonic acid gave the acetonide (**2**) [$C_{23}H_{30}O_6$, m.p. 240—242 °C, δ_H 1.32 and 1.95 (each 3H, s, acetal Me_2); δ_C 87.8 (C-7) and 69.5 (C-14)] confirming the presence of the 7β-hydroxy group which has a *cis* relationship to the 14β-hydroxy group. The dihydro-compound (**3**) [$C_{20}H_{28}O_6$, m.p. 230—233 °C], obtained by catalytic hydrogenation, gave the hemiacetal (**6**) [$C_{20}H_{28}O_7$, m.p. 190—193 °C, $\nu_{max.}$ 1717 cm^{-1} (six membered ketone); δ_H 6.31 (1H, br.s, 20-H); δ_C 95.9 (d, C-20) and 96.4 (s, C-7)] by treatment with $NaIO_4$, showing the presence of a secondary hydroxy group at C-20. Compound (**6**) gave methyl ether (**7**) [$C_{21}H_{30}O_7$, m.p. 228—231 °C; δ_H 3.54 (3H, s, OMe) and 5.62 (1H, br.s, 20-H)] on treatment with methanol in the presence of BF_3–ether adduct and a reduction product (**8**) [$C_{20}H_{30}O_6$, m.p. 292—294 °C, δ_H 6.71 (1H, d, *J* 6 Hz, 15-OH), 4.75 (1H, dd, *J* 6 and 10 Hz, 15-H), and 4.73 (1H, s, 7-H)] by reduction with sodium borohydride. The stereochemistry at C-20 in (**1**) was assigned as *R* from the fact that, in the ^{1}H n.m.r. spectrum of (**1**), a nuclear Overhauser enhancement (n.O.e.) (11%) was observed for H_c when irradiated at δ 0.89 (C-4α Me). On acetylation (acetic anhydride–C_5H_5N), (**3**) yielded triacetate (**5**) [$C_{26}H_{34}O_9$, m.p. 181—183 °C, δ_H 2.50, 2.16, and 2.01 (each 3H, s)] and diacetate (**4**) [$C_{24}H_{32}O_8$, m.p. 198—201 °C, δ_H 2.04 and 1.97 (each 3H, s)]. The triacetate (**5**) was oxidised with lead tetra-acetate to furnish a lactone-carboxylic acid (**9**) [$C_{26}H_{34}O_{10}$, m.p. 208—210 °C, $\nu_{max.}$ 1770, 1735, and 1700 cm^{-1}; δ_C 175.0 (C-6) and 206.2 (C-7)] which was further converted, on treatment with ethereal diazomethane, into the methyl ester (**10**) [$C_{27}H_{36}O_{10}$, m.p. 191—193 °C; δ_H 3.70 (3H, s, COOMe)]. The results showed the presence of a ketone group at C-6. Finally, the absolute stereochemistry around the B-ring was deduced from the fact that compounds (**8**) ($\Delta\varepsilon_{320}$ −0.66) and (**12**)[4] ($\Delta\varepsilon_{318}$ −3.33) showed a Cotton effect of the same sign in the c.d. spectra. On the basis of the above evidence, the structure (**1**) was elucidated for phyllostachysin A. This carbon skeleton is found in diterpene alkaloids such as napelline and sangorine.[5]

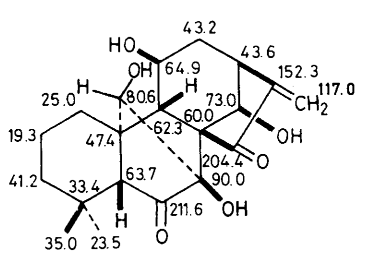


Figure 1. ^{13}C N.m.r. data in [$^2H_5$]pyridine solution.

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