

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*.

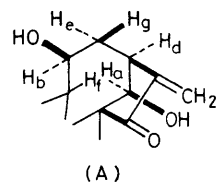
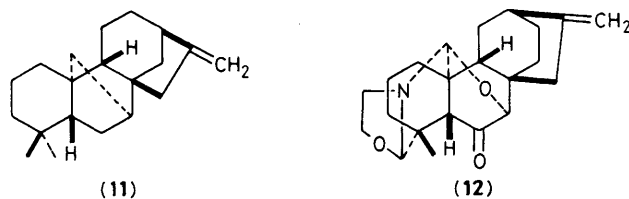
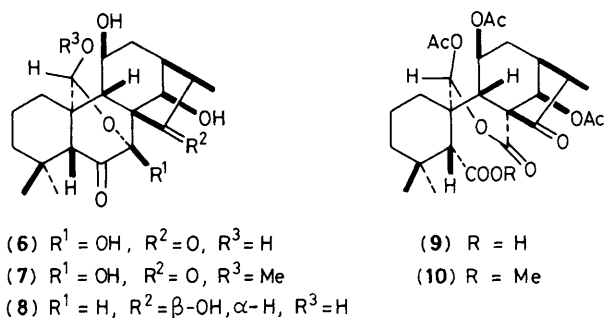
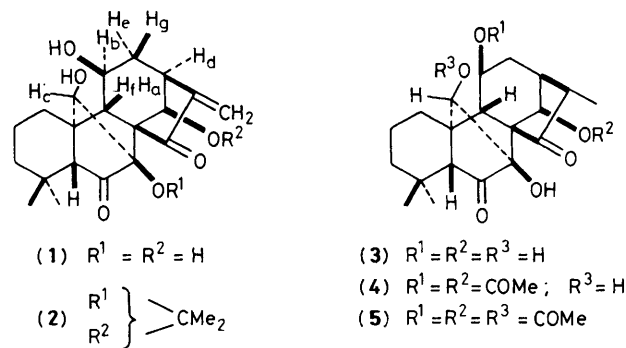
Phyllostachysin A (1), C₂₀H₂₆O₆, m.p. 264–265 °C, [α]_D²⁰ –30.5° (*c* 1.25, C₅H₅N) has been shown to contain a five membered ketone conjugated with an *exo*-methylene group [λ_{max} (EtOH) 229 nm (ε 8270); ν_{max} ‡ 1725 and 1645 cm⁻¹; ¹H n.m.r. § δ 6.20 and 5.41 (each 1H, br. s); ¹³C n.m.r. § δ 117.0

(t), 152.3 (s) (*exo*-methylene), and 204.4 (s)], a five membered ketone [ν_{max} 1750 cm⁻¹; ¹³C n.m.r. δ 211.6 (s)], and four hydroxy groups [ν_{max} 3400 cm⁻¹; ¹H n.m.r. ([²H₆]Me₂SO) δ 6.24, 5.85, 5.09, and 4.41 (disappeared on addition of D₂O)]. 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 ¹H 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 ¹³C 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 ¹H 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,

† The results will be published elsewhere.

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

§ All ¹H and ¹³C n.m.r. spectra were recorded in C₅D₅N solution unless otherwise noted and the chemical shifts were expressed in δ. The ¹³C 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.



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 ¹³C 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) [C₂₃H₃₀O₆, m.p. 240–242 °C, δ_H 1.32 and 1.95 (each 3H, s, acetal Me₂); δ_C

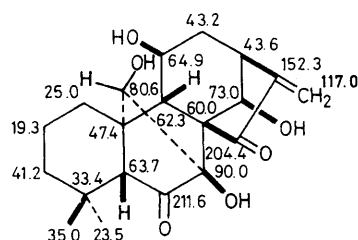


Figure 1. ¹³C N.m.r. data in [2H₅]pyridine solution.

87.8 (C-7) and 69.5 (C-14)] confirming the presence of the 7 β -hydroxy group which has a *cis* relationship to the 14 β -hydroxy group. The dihydro-compound (3) [C₂₀H₂₈O₆, m.p. 230–233 °C], obtained by catalytic hydrogenation, gave the hemiacetal (6) [C₂₀H₂₈O₇, m.p. 190–193 °C, ν_{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 NaIO₄, showing the presence of a secondary hydroxy group at C-20. Compound (6) gave methyl ether (7) [C₂₁H₃₀O₇, 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 BF₃-ether adduct and a reduction product (8) [C₂₀H₃₀O₆, 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 ¹H 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-4 α Me). On acetylation (acetic anhydride–C₅H₅N), (3) yielded triacetate (5) [C₂₆H₃₄O₉, m.p. 181–183 °C, δ_H 2.50, 2.16, and 2.01 (each 3H, s)] and diacetate (4) [C₂₄H₃₂O₈, 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) [C₂₆H₃₄O₁₀, m.p. 208–210 °C, ν_{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) [C₂₇H₃₆O₁₀, 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) ($\Delta_{\epsilon_{320}} -0.66$) and (12)⁴ ($\Delta_{\epsilon_{318}} -3.33$) 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

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