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AGROSTISTACHIN, A NOVEL CYTOTOXIC MACROCYCLIC DITERPENE FROM AGROSTISTACHYS HOOKERI

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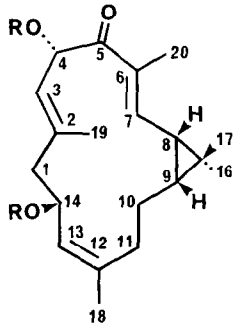
Summary: Agrostistachin, a cytotoxic constituent of Agrostistachys hookeri, was characterized as a new member of the rare casbane class of diterpenoids. The structure and stereochemistry of this compound were established by analysis of its spectral and X-ray crystallographic parameters.

In the course of our continuing search for plant-derived antineoplastic agents[1], it was found that a chloroform extract of the twigs of Agrostistachys hookeri Benth. & Hook. f. (Euphorbiaceae) displayed significant in vitro activity against the P-388 lymphocytic leukemia test system, when tested according to standard protocols[2]. Bioactivity-guided fractionation of this extract has led to the isolation of a new crystalline (mp 155-157°, from ethyl acetate) and optically active ($[\alpha]_D$ +248°, c 0.18, chloroform) constituent, agrostistachin (**1**), with demonstrated cytotoxicity (ED_{50}, 1.4 μg/ml).



1 R = H

2 R = $COCH_3$

By high-resolution mass spectrometry, the elemental formula of the molecular ion of **1** was determined as $C_{20}H_{30}O_3$[3]. Resonances in the IR spectrum at 3460, 3396, and 1660 cm^{-1} suggested that the oxygen-containing functionalities of this molecule were, respectively, a non-hydrogen-bonded hydroxy group, a hydrogen-bonded OH, and an αβ-unsaturated carbonyl. One olefinic proton appeared as a doublet at δ 6.25 (J = 10 Hz) in the ^{1}H-NMR spectrum of **1**[4], and was assigned as a proton β to a carbonyl group[5]. A maximum in the UV spectra of **1** occurred at 272.5 nm (EtOH, log ε 4.10), a value consistent with this enone moiety being affixed to a cyclopropyl ring[5,6]. Evidence for the presence of gem-dimethyl functionality

in the cis-substituted cyclopropyl ring of 1 was obtained by the observation of signals at δ 1.14 and 0.96 in the ^{1}H-NMR spectrum, and by the chemical shifts at δ 28.99 and 16.03[6,7] in its ^{13}C-NMR spectrum[8]. The other three methyl groups in the molecule of **1** were vinylic [^{1}H-NMR, δ 1.87, 1.76, 1.70; ^{13}C-NMR (SFORD), singlets at δ 133.41, 137.92, 137.31]. Of the remaining downfield signals in the ^{1}H-NMR spectrum of **1**, two protons that resonated at δ 5.11 were olefinic, while the methine protons at δ 5.22 and 4.18 adjacent to hydroxyl groups were each shifted downfield by about 1 ppm (to δ 6.24 and 5.29, respectively) in the diacetate, **2**[9], produced from **1** by treatment with equal parts of acetic anhydride and pyridine at room temperature.

Compound **1** was tentatively assigned a casbane carbon skeleton[10] by a consideration of the isoprene rule and the allylic coupling evident in its homonuclear ^{1}H-^{1}H- COSY 2D-NMR spectrum. An unambiguous assignment of the ^{1}H- and ^{13}C-NMR spectra of **1** was provided after the performance of a heteronuclear ^{1}H-^{13}C chemical shift-correlated 2D-NMR spectral study. The geometry of the three double bonds in the molecule of **1** between position 2,3, position 6,7, and position 12,13, was postulated as E, E, and Z, respectively, on analysis of the ^{13}C-NMR chemical shifts of the 20- (δ 11.57), 19- (δ 17.83), and 18- (δ 22.89) methyl carbons, as well as the 11-methylene carbon (δ 31.30)[8,11,12].

Confirmation of the structure of agrostistachin as **1**[13] was obtained by the application of X-ray crystallography (Fig. 1). Suitable crystals were obtained from diisopropyl ether as transparent colorless needles (0.2 X 0.2 X 1.0 mm), and gave the following crystal data: a = 10.885 Å, b = 12.009 Å, c = 15.219 Å, orthorhombic space group $P2_12_12_1$, Z = 4, $D_{calc.}$ = 1.06 gcm^{-3}, $D_{meas.}$ = 1.07 gcm^{-3}. A total of 1566 unique reflections [(1453 observed I ≥ 3θ (I)] were measured on a Nicolet T3m diffractometer with Ni-filtered CuK_α-radiation. The structure was solved by direct methods using SHELXTL[14]. The first attempt showed all nonhydrogen atoms in an E-map, while the refinement with unit weights converged at R = 9.7% (without hydrogen). Atomic coordinates and bond lengths are deposited at the Cambridge Crystallographic Data Centre.

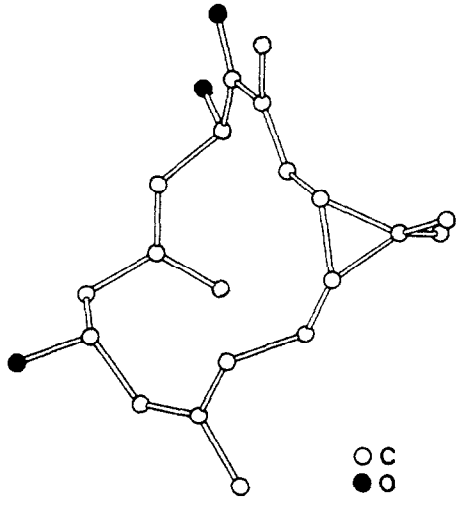


Figure 1. Crystal structure of agrostistachin (**1**).

Agrostistachin (**1**) represents the first casbane-type diterpenoid to exhibit cytotoxic activity. The parent compound, (-)-casbene, which has a 2,6,12-all-E-triene system, is regarded as an important biogenetic precursor of biologically active cyclopropanoid diterpenes of the phorbol ester type, some of which are widely used in chemical carcinogenesis experiments[6,15]. While agrostistachin (**1**) and the monoolefin, crotonitenone[5,16], are the only oxygenated casbane derivatives so far known, **1** may be regarded as being more biogenetically primitive than the latter compound, in having three double bonds in the same positions as (-)-casbene. However, agrostistachin (**1**) is the first casbane diterpene with Δ^{12}-cis-stereochemistry, and the C-14 hydroxy group represents an unusual oxidation pattern of potential importance in the study of the biosynthesis of macrocyclic diterpenoids of the Euphorbiaceae.

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References and Notes:

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2. Arisawa, M.; Pezzuto, J.M.; Bevelle, C.; Cordell, G.A. J. Nat. Prod. **1984**, 47, 453.
3. MS of **1**: Mass measurement, M^+, m/z 318.2198, calc. for $C_{20}H_{30}O_3$, 318.2195; CI (CH_4), m/z 319 ($M^+ + H$, 30%), 301 ($M^+ - OH$, 100).
4. ^{1}H-NMR of **1**: δ (360 MHz, $CDCl_3$) 6.25 (1H, d, $J_{7,8}$ = 10 Hz, 7-H), 5.22 (1H, d, $J_{3,4}$ = 10 Hz, 4-H), 5.11 (2H, br. d, 3-H, 13-H), 4.18 (1H, m, 14-H), 2.44 (1H, dd, J_{gem} = 12 Hz, $J_{1,14}$ = 2.5 Hz, 1-H), 2.25 (1H, m, 11-H), 2.01 (2H, m, 10-H, 1-H), 1.87 (3H, d, $J_{7,20}$ = 0.7 Hz, 20-CH_3), 1.78 (1H, m, 11-H), 1.76 (3H, d, $J_{3,19}$ = 1.5 Hz, 19-CH_3), 1.70 (3H, br. s, 18-CH_3), 1.51 (1H, dd, $J_{7,8}$ = 10 Hz, $J_{8,9}$ = 8 Hz, 8-H), 1.14 (3H, s, 17-CH_3), 1.11 (1H, m, 9-H), 0.96 (3H, s, 16-CH_3), 0.83 (1H, m, 10-H).
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8. ^{13}C-NMR spectrum of **1**: δ (90.8 MHz, $CDCl_3$) 199.02 (s, 5-C=O), 145.06 (d, 7-C), 137.92 (s, 2-C), 137.31 (s, 12-C), 133.41 (s, 6-C), 128.67 (d, 3-C), 127.08 (d, 13-C), 70.21 (d, 14-C), 68.32 (d, 4-C), 47.56 (t, 1-C), 34.57 (d, 9-C), 31.70 (t, 11-C), 28.99 (q, 17-C), 27.47 (d, 8-C), 27.31 (s, 15-C), 22.89 (t, 10-C), 22.83 (q, 18-C), 17.83 (q, 19-C), 16.03 (q, 16-C), 11.57 (q, 20-C).

9. The diacetate, **2**, exhibited the following data: mp 115-117 °C; $[\alpha]_D$ + 203° (c 0.12, chloroform); UV, (EtOH) λ_{max} 272.5 nm (log ε 4.06); IR, ν_{max} 1733, 1677, 1625 cm^{-1}; ^{1}H-NMR, δ (360 MHz, $CDCl_3$), 6.24 (2H, br. d, 4-H, 7-H), 5.40 (1H, d, $J_{3,4}$ = 10 Hz, 3-H), 5.29 (1H, m, 14-H), 5.08 (1H, d, $J_{13,14}$ = 10 Hz, 13-H), 2.46 (1H, dd, J_{gem} = 12 Hz, $J_{1,14}$ = 3 Hz, 1-H), 2.44 (1H, m, 11-H), 2.15 (1H, m, 1-H), 2.14 (3H, s, -OAc), 2.10 (1H, m, 10-H), 1.99 (3H, s, -OAc), 1.90 (3H, br. s, 20-CH_3), 1.83 (1H, m, 11-H), 1.78 (3H, d, $J_{3,19}$ = 1.3 Hz, 19-CH_3), 1.76 (3H, br. s, 18-CH_3), 1.54 (1H, dd, $J_{7,8}$ = 10 Hz, $J_{8,9}$ = 8 Hz, 8-H), 1.18 (3H, s, 17-CH_3), 1.13 (1H, m, 9-H), 1.04 (3H, s, 16-CH_3), 0.91 (1H, m, 10-H); ^{13}C-NMR, δ (90.8 MHz, $CDCl_3$) 199.21 (s, 5-C), 170.22 (s, $\underline{C}OCH_3$), 169.76 (s, -$COCH_3$), 142.73 (d, 7-C), 140.89 (s, 2-C), 140.35 (s, 12-C), 134.89 (s, 6-C), 124.02 (d, 3-C), 123.34 (d, 13-C), 72.53 (d, 14-C), 71.39 (d, 4-C), 44.36 (t, 1-C), 34.02 (d, 9-C), 31.78 (t, 11-C), 29.05 (q, 16-C), 27.43 (d, 8-C), 26.70 (s, 15-C), 22.96 (t, 10-C), 22.81 (q, 18-C), 21.26 (q, -CO$\underline{C}H_3$), 20.72 (q, -CO$\underline{C}H_3$), 18.58 (q, 17-C), 16.14 (q, 17-C), 11.61 (q, 20-CH_3); MS, CI (CH_4) m/z 403 (M^+ + 1, 4%), 343 (M^+ + H - OAc, 72.7), 283 (M^+ + H - 2 x OAc, 100).

10. The numbering system adopted for agrostistachin (**1**) and its acetate (**2**) is that published for the oxygenated casbane derivative, crotonitenone (see ref. 5).

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13. The systematic nomenclature for **1** based on the Chemical Abstracts system is therefore {(E,E,Z)-(1S*,5R*,9R*,14R*)-5,9-dihydroxy-3,7,11,15,15-pentamethylbicyclo[12.1.0]pentadec-2,6,10-trien-4-one}-(+).

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