BRIARANE DITERPENES FROM MICRONESIAN GORGONIANS

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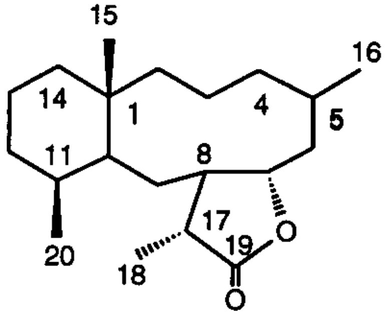
Abstract - Four new briaranes, nui-inoalides A-D, (**6-9**) are described. They were isolated from octocorals collected in Pohnpei and Ant atoll, Micronesia. The absolute stereochemistry of the known briarane juncin E (**5**) was determined. Nui-inoalide A (**6**) and the known gemmacolides A, B, and D (**2-4**) exhibit immunomodulatory activity.

Briaranes (**1**) are diterpenoid γ-lactones of a highly substituted bicyclo[8.4.0] system. From the isolation of briarane A by Ciereszko and coworkers in 1977[3] up to recent papers by Kobayashi *et al.*[4] and by He and Faulkner[5] more than 50 representatives of this class of compounds have been isolated from gorgonians, sea pens, and occasionally their predators. Faulkner's paper[5] dealing with constituents of *Junceella gemmacea* from Pohnpei, Micronesia, appeared after our work dealing with the same gorgonian from the same location had been completed. We isolated seven briaranes, four known (**2-5**) and three new (**6-8**) compounds; a fourth new briarane (**9**) was isolated from an octocoral collected at Ant atoll, Micronesia, in 1987.

The freeze-dried Pohnpei animal (0.42 kg dry) was extracted with ethyl acetate. The residue was chromatographed on a silica flash column, from which the briaranes eluted with ethyl acetate. Final separation was achieved by hplc on a C18 reversed phase column with aqueous methanol gradient elution, in the order **4,7,6,8,3,2,5**, in yields ranging from 1×10^{-4} to 3×10^{-3}% (wet wt). Briarane (**9**) was isolated by normal phase hplc (ethyl acetate/methylene chloride) and crystallized from methanol.

Spectral data (^{1}H and ^{13}C nmr, ir, uv, ms, $[\alpha]_D$) of briaranes (**2-4**) were identical with those reported by He and Faulkner[5] for gemmacolide A (**2**), B (**3**), and D (**4**), while the data for briarane (**5**) matched those reported by Kashman and coworkers[6] from a Red Sea gorgonian (*Junceella juncea*) constituent juncin E.



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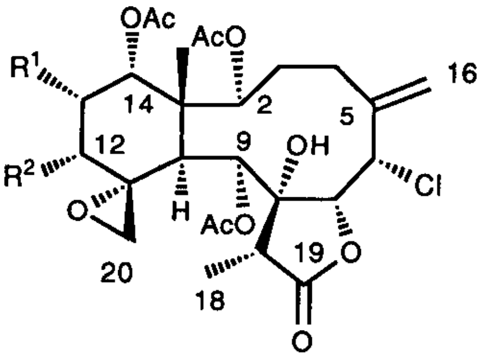
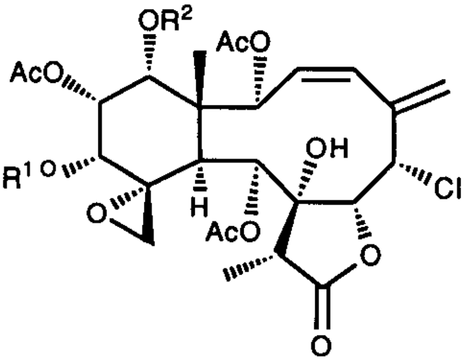




2 $R^1 = OAc$ $R^2 = OAc$

3 $R^1 = OCOCH_2CH[CH_3]_2$ $R^2 = OAc$

6 $R^1 = OAc$ $R^2 = H$

4 $R^1 = H$ $R^2 = Ac$

5 $R^1 = R^2 = Ac$

Diffusion of methanol into a chloroform solution of **5** produced colorless block crystals, which allowed us to determine the absolute stereochemistry of **5**, for which Kashman[6] had deduced the relative stereochemistry by spectral methods (Figure 1).

Structures of the three new briaranes, nui-inoalides[7] A (**6**, 10 mg), B (**7**, 4 mg), and C (**8**, 1 mg) were determined by spectral analyses. High resolution EI mass spectra furnished the molecular formulas, though all had weak signals for their molecular ions. Loss of acetate, hydroxyl, and chloride could be seen readily. The nmr carbon signals arising from some of the carbons of the 10-membered ring, on the other hand, were so broad as to be nearly undetectable. ^{13}C chemical shifts could be assigned from the HMQC [^1H-^{13}C (1 bond) heteronuclear multiple quantum coherence spectroscopy] experiments. Relative stereochemistry was assigned on the basis of ROESY [rotating frame nuclear Overhauser spectroscopy]. Nui-inoalide B (**7**) might have arisen from an allylic rearrangement of the corresponding 6-chlorobriarane (**4**).

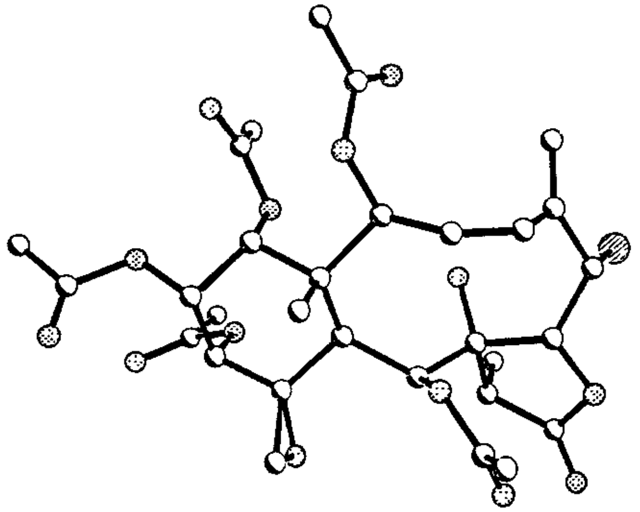
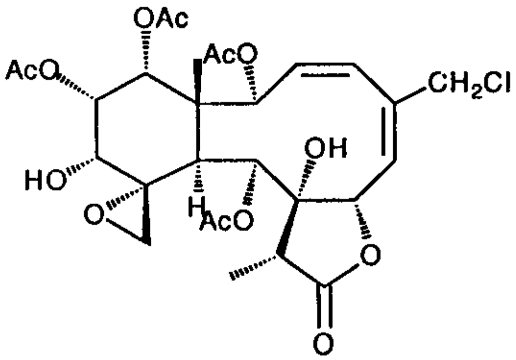


Figure 1. A computer-generated perspective drawing of briarane (**5**). The absolute stereochemistry was determined by refinement of the Roger's η parameter. (Hydrogens are omitted for clarity).



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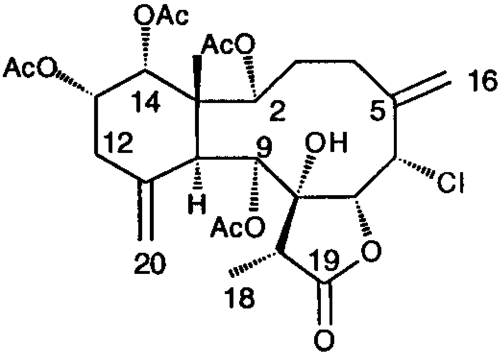

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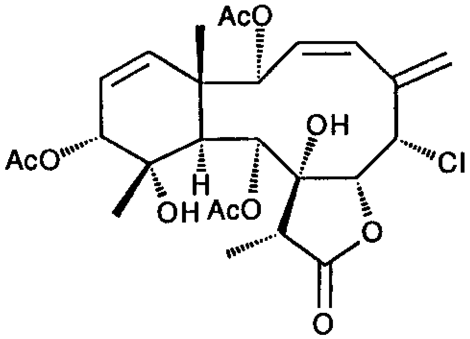

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Nui-inoalide D (**9**), composition $C_{26}H_{33}ClO_{10}$ obtained by FAB mass spectroscopy, crystallized as colorless prisms from methanol. The structure of **9** was determined by extensive nmr experimentation, including double quantum filtered COSY (^1H-^1H correlation spectroscopy), RCT (relay coherence transfer), DEPT (distortionless enhancement through polarization transfer), and CSCM (chemical shift correlation mapping) experiments.

None of the briaranes (**2**-**8**) (**9** was not assayed) exhibited antimicrobial, antiviral, or cytotoxic activities. However compounds (**2**, **3**, **4**, and **6**) showed selective immunomodulatory activity with MLR (mixed lymphocyte reaction) to LcV (lymphocyte viability) in ratios of 23, 23, 11 and 15. These ratios indicate immunosuppressive activity at concentrations significantly lower than the cytotoxicity levels.

EXPERIMENTAL PART

Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Infrared spectra were recorded on a Nicolet MX-5 FTIR spectrophotometer. Mass spectra were measured on a VG-70SE magnetic sector mass spectrometer. Nmr spectra were measured on a General Electric QE-300 or a GN OMEGA 500 instrument. ^1H nmr chemical shifts are reported in ppm with the chemical shift of the residual protons of the solvent used as internal standard. ^{13}C nmr chemical shifts are reported in ppm by using the natural abundance ^{13}C of the solvent as an internal standard. Ultraviolet spectra were recorded on a Hewlett-Packard Model 8452A diode array spectrophotometer. All solvents were distilled from glass before use.

Isolation

A red gorgonian (1.22 kg) collected at Palikir pass, Pohnpei, June 1990 (-20 m) was extracted three times (12 h each) at room temperature with EtOAc (1.5 l). The extracts were chromatographed using silica gel flash chromatography (hexane, CH_2Cl_2, EtOAc, and MeOH with 1% HOAc 0.75 l each). The briaranes were found in the EtOAc fraction. Repeated hplc on RP 18 using MeOH/H_2O (1:1) - MeOH/H_2O (7:3) yielded 7 briaranes in the order of elution: **4** (10 mg, 0.001%), **7** (4 mg, 0.0003%), **6** (10 mg, 0.001%), **8** (1 mg, 0.0001%), **3** (25 mg, 0.002%), **2** (40 mg, 0.003%), and **5** (30 mg, 0.002%).

An orange-yellow octocoral, which grows as solitary small fingers on the underside of rocks or on the roof of caves, was collected at -30 m off the southwest edge of the reef at Ant Atoll, Micronesia, in October, 1987. The coral was blended in ethanol, filtered and the residue blended with chloroform and filtered. The ethanol extract was evaporated to half volume and extracted repeatedly with chloroform. The chloroform extracts were combined and the solvent removed under vacuum. Partial fractionation was accomplished by vacuum liquid chromatography on silica using a solvent gradient, hexane-ethyl acetate-methanol. Further purification of these fractions was accomplished by three successive normal phase hplc steps: (1) EtOAc/CH$_2$Cl$_2$ (14:86); (2) EtOAc/hexane (30:70); (3) EtOAc/CH$_2$Cl$_2$ (25:75). The resulting pure fraction was crystallized from MeOH.

Nui-inoalide A (6) - Isolated as a white colored solid. $[\alpha]_D$ -15° (*c* 1.20, CHCl$_3$). ^{13}C Nmr (125 MHz, CDCl$_3$): 174.70 [C-19], 171.29, 170.41, 170.35, 169.52, [4 Ac], 146.00 br [C-5], 55.48 [C-11], 120.90 [C-16], 51.05 [C-20], 81.13 [C-7, 8], 72.75 [C-2, 9, 14], 67.49 [C-13], 55.48 [C-6], 51.05 [C-17], 47.50 [C-1], 40.03 [C-10], 35.41 [C-12], 33.00 [C-4], 28.47 [C-3], 21.60, 21.20, 20.90, 20.86, [4 Ac], 14.30 [C-15], 6.60 br [C-18] ppm; ^{1}H nmr (500 MHz, CDCl$_3$): δ 5.76 [m, H-2], 5.74 [s, H-16], 5.63 [br s, H-9], 5.45 [s, H-16], 5.28 [d, *J* = 2.1 Hz, H-14],5.19 [ddd, *J* = 12.7, 5.5, 3.1 Hz, H-13], 4.65 [s, H-6], 4.50 [br s, H-7], 3.33 [s, OH-8], 3.18 [s, H-10], 3.00 [m, H-17], 2.72 [dd, *J* = 3.6, 0.8 Hz, H-20], 2.65 [m, H-3], 2.42 [m, 2H-4], 2.35 [t, *J* = 11.4 Hz, H-12], 2.25 [m, H-20], 2.21, 2.03, 2.01, 1.96 [4 s, 3H-Ac], 1.62 [m, H-3], 1.45 [ddd, *J* = 12.0, 5.5, 1.0 Hz, H-12], 1.26 [d, *J* = 7.2 Hz, 3H-18], 1.22 [s, 3H-15]; ir neat (NaCl): 3476 (m, br), 2940 (m, br), 1798 (s), 1742 (s), 1371(m), 1251 (s), 1042 (m) cm^{-1}; ms [EI], *m/z* [fragment, %]: [calcd for C$_{28}$H$_{37}$ClO$_{12}$: 601.02], 565.2299 [M$^+$ - Cl, 0.2][calcd for C$_{28}$H$_{37}$O$_{12}$: 565.2285], 540.18 [M$^+$ - HOAc, 3], 505.21 [M$^+$ - HOAc, Cl, 4],121 [100]; uv (MeOH): λ_{max} 204 (5,545)nm.

Nui-inoalide B (7) - Isolated as a white colored solid. $[\alpha]_D$ -9° (*c* 0.52, CHCl$_3$). ^{13}C Nmr (125 MHz, CDCl$_3$): 174.98 [C-19], 170.11, 2 [169.81], 169.65 [4 Ac], 139.94 [C-5], 131.55 [C-3], 128.45 [C-4], 126.62 [C-6], 81.23 [C-8], 78.64 [C-7], 75.61 [C-14], 75.05 [C-12], 73.98 [C-2], 67.37 [C-13], 63.95 [C-9], 60.17 [C-11], 48.39 [C-20], 46.52 [C-1], 44.87 [C-16], 44.17 [C-17], 31.58 [C-10], 21.51, 21.22, 20.92, 20.59 [4 Ac], 14.23 [C-15], 6.30 [C-18] ppm; ^{1}H nmr (500 MHz, CDCl$_3$): δ 6.37 [d, *J* = 10.2 Hz, H-4], 6.02 [d, *J* = 8.1 Hz, H-6], 5.61 [t, *J* = 9.9 Hz, H-3], 5.45 [d, *J* = 9.5 Hz, H-2], 5.30 [dd, *J* = 3.0, 1.2 Hz, H-14], 4.98 [t, *J* = 3.2 Hz, H-13], 4.95 [d, *J* = 8.7 Hz, H-7], 4.80 [d, *J* = 4.7 Hz, H-9], 4.59 [d, *J* = 12.9 Hz, H-16], 4.54 [d, *J* = 12.9 Hz, H-16], 3.56 [d, *J* = 4.6 Hz, H-10], 3.54 [d, *J* = 2.6 Hz, H-20], 3.49 [br s, H-12], 2.88 [br s, OH-12], 2.76 [d, *J* = 2.2 Hz, H-20], 2.48 [br s, OH- 8], 2.31 [q, *J* = 7.1 Hz, H-17], 2.18, 2.11, 2.02, 1.95 [4 s, 3H-Ac], 1.16 [d, *J* = 6.9 Hz, 3H-18], 1.13 [s, 3H-15]; ir neat (NaCl): 3510 (m, br), 2943 (m, br), 1780 (s), 1739 (s), 1371(m), 1223 (s), 1053 (m) cm^{-1}; ms [EI], *m/z* [fragment, %]: 614.1769 [M$^+$, 1] [calcd for C$_{28}$H$_{35}$ClO$_{13}$: 614.1767], 596.13 [M$^+$ - H$_2$O, 0.3], 579.17 [M$^+$ - Cl, 2], 554.13 [M$^+$ - HOAc, 4], 95.07 [100]; uv (MeOH): λ_{max} 204 (8,090)nm.

Nui-inoalide C (8) - Isolated as a white colored solid. $[\alpha]_D$ +29° (*c* 0.4, CHCl$_3$). ^{13}C Nmr (125 MHz, CDCl$_3$): 174.00 [C-19], 171.34, 170.37, 170.18, 169.86, [4 Ac], too broad to detect [C-5], 146.08 [C-11], 120.95 [C-16], 112.97 [C-20], 81.38 [C-8], 81.38 [C-7], 79.50 [C-9], 73.14 [C-14], 72.72 [C-2], 69.33 [C-

13], too broad to detect [C-6], 51.32 [C-17], 47.94 [C-1], 43.44 [C-10], 38.30 [C-12], 33.12 [C-4], 28.85 [C-3], 21.53, 21.09, 2[20.85], [4 Ac], 14.14 [C-15], 7.00 br [C-18] ppm; ^{1}H nmr (500 MHz, $CDCl_3$): δ 5.89 [s, H-9], 5.80 [m, H-2], 5.77 [s, H-16], 5.47 [s, H-16], 5.21 [d, *J* = 2.6 Hz, H-14], 5.11 [s, H-20], 5.03 [ddd, *J* = 11.5, 6.6, 3.0 Hz, H-13], 4.70 [br s, H-6], 4.62 [s, H-20], 4.60 [br s, H-7], 3.45 [s, H-10], 3.33 [s, OH-8], 3.01 [q, *J* = 6.8 Hz, H-17], 2.45 [m, 2H-12], 2.44 [m, 2H-4], 2.23, 2.02, 2.00, 1.94 [4 s, 3H-Ac], 1.62 [m, 2H-3], 1.20 [d, *J* = 7.0 Hz, 3H-18], 1.11 [s, 3H-15]; ir neat (NaCl): 3474 (m, br), 2935 (m, br), 1794 (s), 1743 (s), 1370(m), 1252 (s), 1041 (m) cm^{-1}; ms [EI], *m/z* [fragment, %]: 584 [M^+, 0.04] [calcd for $C_{28}H_{37}ClO_{11}$: 584.2024], 549.2347 [M^+ - Cl, 0.2][calcd for $C_{28}H_{37}O_{11}$: 549.2336], 524.33 [M^+ - HOAc, 3], 489.35 [M^+ - HOAc, Cl, 4],91.07 [100]; uv (MeOH): λ_{max} 204 (12,140)nm.

Nui-inoalide D (9) - Crystallized from methanol as small colorless prisms. ^{13}C Nmr (75 MHz, $CDCl_3$): 174.9 [C-19], 172.0, 170.0, 169.7, [3 Ac], 142.4 [C-14], 137.1 [C-5], 129.2 [C-3], 129.0 [C-4], 121.2 [C-13], 116.7 [C-16], 83.1 [C-8], 78.8 [C-7], 77.5 [C-2], 75.5 [C-11], 72.8 [C-12], 69.1 [C-9], 62.0 [C-6], 45.8 [C-1], 45.5 [C-17], 39.2 [C-10], 23.5 [C-20], 22.0, 21.1, 20.9, [3 Ac], 14.8 [C-15], 7.0 [C-18] ppm; ^{1}H nmr (300 MHz, $CDCl_3$): δ 6.19 [s, H-16], 5.98 [d, *J* = 9.6 Hz, H-2], 5.94 [d, *J* = 1.9 Hz, H-16], 5.94 [d, *J* = 11 Hz, H-4], 5.78 [d, *J* = 3.0 Hz, H-14], 5.78 [d, *J* = 3 Hz, H-13], 5.76 [d, *J* = 6 Hz, H-9], 5.58 [dd, *J* = 11, 9.6 Hz, H-3], 5.22 [s, OH-11], 5.12 [m, H-6], 5.07 [d, *J* = 4 Hz, H-7], 4.94 [t, *J* = 3 Hz, H-12], 3.58 [s, OH- 8], 3.18 [d, *J* = 6 Hz, H-10], 2.39 [q, *J* = 7.7 Hz, H-17], 2.18, 2.13, 2.12, [3 s, 3H-Ac], 1.39 [s, 3H-20], 1.16 [d, *J* = 7.7 Hz, 3H-18], 1.12 [s, 3H-15]; ms [EI], *m/z* [fragment]: 498.1666 [calcd for $C_{24}H_{31}ClO_9$: 498.1657]; ms [FAB], *m/z* [fragment]: 541 [calcd for $C_{26}H_{33}ClO_{10}$ + H: 541]; 579 [calcd for $C_{26}H_{33}ClO_{10}$ + K: 579].

Juncin E (5) - (C. A. Registry No. 129602-22-8) Crystallized from chloroform by diffusion with methanol as colorless prisms. $[\alpha]_D$ -108° (*c* 4.93, $CHCl_3$). Literature value $[\alpha]^{25}_D$ -66° (*c* 0.02, $CHCl_3$).[6]

Crystallography of briarane (5). Crystals of **5** are orthorhombic, a = 9.646 (2) Å, b = 15.134 (3) Å, c = 21.247 (4) Å, space group $P2_1\,2_1\,2_1$, Z = 4, ρ (calcd) = 1.407 g/cm^3 for $C_{30}H_{37}ClO_{14}$. A total of 4802 reflections, including Friedel pairs, were measured with graphite-monochromated MoKα radiation at 25° C on a Nicolet R 3m diffractometer to a maximum 2θ of 50°. The structure was solved without difficulty by using direct and heavy-atom methods. Full-matrix least-squares refinement with 4302 observed reflections [$|F_o| \geq 3\sigma(F_o|)$] converged to crystallographic residuals of R = 4.84%, R_w = 7.08% with η = 1.1(2) for the enantiomer shown. The primary programs used were SHELXTL, by G. Sheldrick, University of Gottingen, FRG.

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6. S. Isaacs, S. Carmely, and Y. Kashman, *J. Nat. Prod.*, 1990 **53**, 596.
7. Nui-inoalide means "many names" in Hawaiian; it is coined in recognition of the fact that there exist in the literature nearly as many trivial names as there are briaranes.

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