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STRUCTURE AND STEREOCHEMISTRY OF INSECTICIDAL DITERPENES FROM THE SEA PEN *Ptilosarcus gurneyi*

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Abstract - A reinvestigation of the diterpene constituents of the sea pen Ptilosarcus gurneyi was undertaken in order to clarify the stereochemistry of ptilosarcone and ptilosarcenone. In the course of reisolating those two compounds, five new briaran diterpenes were uncovered and identified. The structures and stereochemical assignments in the whole group have been established by spectroanalytical methods. Insecticidal screening of three of the compounds against the tobacco hornworm, Manduca sexta, revealed either toxicity or a reduction in weight gain.

Our investigation of the gorgonian Briareum polyanthes has resulted in the isolation and identification of five briaran diterpenes [1-5], some of which exhibit insecticidal activity [3,5]. In the course of establishing the stereochemistry of brianthein Y (1) by nmr and x-ray diffraction studies, we noted that the ^{1}H-NMR coupling constant between H-9 and H-10 was different from those reported for briarein A [6] and stylatulide [7]. Since our x-ray diffraction studies disclosed that 1 possessed the same absolute stereochemistry at C-8, C-9 and C-10 as briarein A and stylatulide, it seemed, therefore, that the coupling constant between H-9 and H-10 varied with ring conformational changes engendered by substitution at C-11 [2]. Some time ago, the Paulkner group [8] assigned structures 2 and 3 to ptilosarcone and ptilosarcenone, briaran diterpenes from the sea pen Ptilosarcus gurneyi; the relative stereochemistry at C-9 was proposed on the basis of the ^{1}H-NMR coupling constant between H-9 and H-10, which was substantially different from that reported for briarein A (5.5 Hz in 3 vs 0.5 Hz in briarein A). It occurred to us that the ketone and conjugated ketone moieties in 2 and 3 might be responsible for an alteration in ring conformation, resulting in the seemingly anomalous H_9-H_{10} coupling constants observed by Wratten et al. [8]. Prompted by our interest in the insecticidal activity in this class of compounds and the stereochemical insights gleaned from the brianthein work, we undertook a reinvestigation of the P. gurneyi diterpenes. Reported herein are the isolation and identification of five new briaran diterpenes, along with the assignment of complete stereochemistry to ptilosarcone and ptilosarcenone.

Ptilosarcus gurneyi was collected from sites near Sidney, British Columbia, and Seattle, Washington. The organic soluble extracts were partitioned following a scheme employed by Kupchan [9]; ^{1}H-NMR analysis indicated that both the CCl_4 and $CHCl_3$ solubles contained diterpenes. Gel permeation chromatography of these extracts on Bio-Beads S-X4, then Sephadex LH-20, gave fractions highly enriched in diterpenes. Separation and purification of the diterpenes was readily accomplished by HPLC on nitrile-bonded phase columns. Both the Victoria and Seattle collections yielded five diterpenes; a total of seven compounds have been isolated and identified (see Table I). In both collections, ptilosarcenone, revised structure 5, was the most abundant diterpene. Both ptilosarcone, 4, and ptilosarcenone, 5, were identified by comparison of ^{1}H-NMR and IR spectra with the data reported earlier [8]. ^{13}C-NMR data are presented for 4 and 5 in Table II.

The Sidney collection provided a new diterpene, $C_{24}H_{29}ClO_9$, which appeared closely related to 5. The key differences between the two were an additional oxygen in the molecular formula and some subtle changes in the ^{1}H-NMR spectrum (see Table III). In 5, H-10 and H-11 occur as overlapping multiplets near δ 2.75. The new compound featured a one proton doublet at δ 2.95, assigned to H-10 on the basis of coupling to H-9, and an exchangeable one proton singlet at δ 5.27. This new molecule had to be 11-hydroxyptilosarcenone, 6,since the methyl group on C-11 appeared as a singlet at δ 1.46, rather than a doublet near δ 1.30. The relative stereochemistry of 6 was determined by difference nOe experiments; the results are illustrated in structure 6. The crucial data include enhancement of H-2 and the hydroxyl group at C-11 upon irradiation of H-

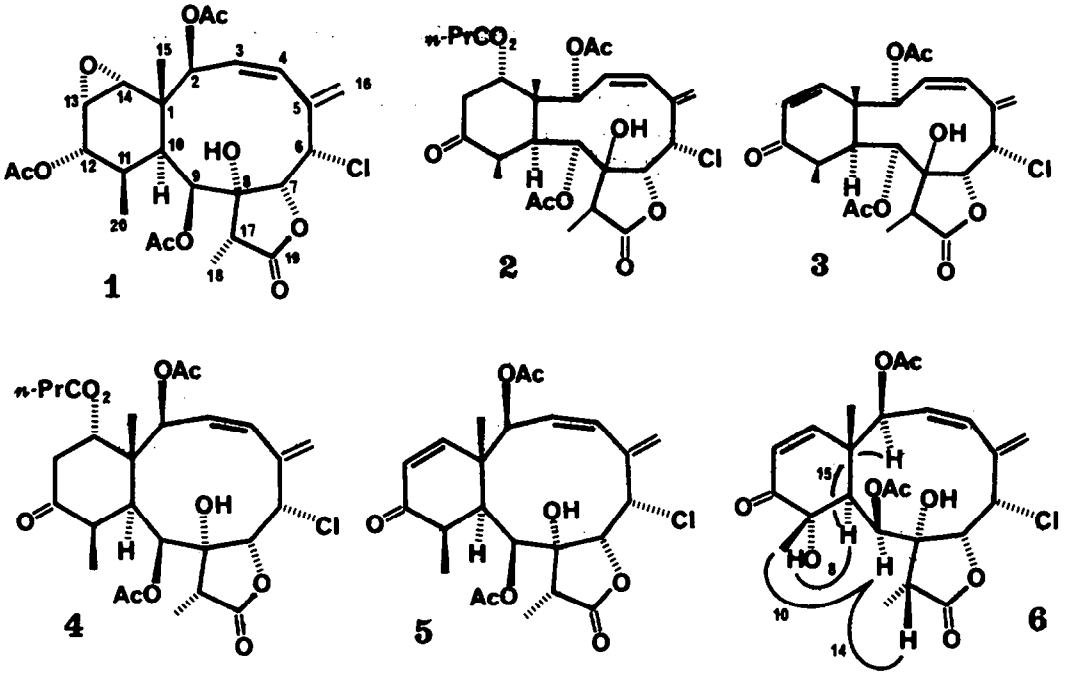


Table I

Comparison of Diterpene Content, *Ptilosarcus gurneyi*[a]

Collection Site	4	5	6	7	8	9	10
Sidney, B.C.	0.059	0.54	0.18	0	0	0.043	0.059
Seattle, WA	0.041	0.49	0	0.0082	0.0056	0.18	0

[a]% of total organic soluble extract

10, and positive nOe's between the methyl group attached to C-11 and H-9 and between H-9 and H-18. These data correlate with a Drieding model representing the relative stereochemistry shown in 6 and provide consistent stereochemical assignments in all the known briaran diterpenes.

The overlap of the signals for H-10 and H-11 in the ^{1}H-NMR of 5 complicated somewhat the nOe approach to the stereochemistry of ptilosarcenone, but irradiation of H-9 resulted in enhancement of the signals for H-18 and H-20 as in 6. Since Wratten and Faulkner had converted 4 to 5, the same relative stereochemistry could be assigned to 4-6. An x-ray diffraction study of 5 has confirmed these stereochemical assignments [10,11].

Compound 7, $C_{24}H_{31}ClO_8$, was found only in the Seattle collection and the structure was solved by ^{1}H-NMR analysis and comparison with the NMR spectrum of 5. In 7 the signal for H-11 was shifted upfield about 0.5 ppm compared to 5, as were the resonances for the H-13 and H-14 olefinic protons. These shifts and the absence of the ketone absorption in the IR, together with the appearance of a new one proton doublet of doublets at δ 3.89 in the ^{1}H-NMR, required structure 7, ptilosarcen-12-ol.

The structures for 8, $C_{26}H_{33}ClO_9$, and 9, $C_{27}H_{35}ClO_9$, followed directly from the identification of 7. Both compounds have ^{1}H-NMR spectra very similar to that of 7; in each case the multiplet for H-12 is shifted downfield near δ 4.9. The spectrum for 8 features an additional methyl singlet at δ 2.01, while that of 9 has instead an isolated ethyl group, indicated by a two proton quartet at δ 2.26 and a methyl triplet at δ 1.10. These compounds had to be the acetate and propionate of 7, respectively. The stereochemistry of 7, 8 and 9 was deduced from the small coupling constant (2.5 Hz) between H_{11} and H_{12} and nOe's between H_{12} and H_{13} (4.7%) and H-11 and H-10 (5.2%) in 9. These data can be accommodated only by the configuration illustrated.

Table II

^{13}C NMR Assignments of More Abundant *P. gurneyi* Diterpenes[a]

Carbon #	4[b]	5	6[c]	9d
1	44.72 s	43.44 s	43.12	42.50 s
2	76.01 d	76.58 d	76.16	77.75 d
3	131.06 d	129.60 d	130.30	130.97 d
4	128.02 d	128.63 d	127.25	127.84 d
5	136.56 s	136.47 s	136.50	137.24 s
6	62.91 d	62.07 d	60.88	62.47 d
7	72.16 d	68.85 d	69.22	69.18 d
8	82.85 s	83.98 s	81.55	84.92 s
9	77.92 d	77.72 d	78.87	77.85 d
10	38.86 d	38.89 d	30.89	36.20 d
11	48.62 d	45.80 d	76.10	36.31 d
12	209.72 s	202.16 s	199.15	70.42 d
13	37.77 t	124.06 d	121.77	120.72 d
14	72.52 d	154.13 d	156.72	141.87 d
15	13.64 q	14.67 q	13.71	12.92 q
16	117.79 t	118.75 t	117.33	118.35 t
17	46.28 d	45.07 d	45.76	44.83 d
18	7.07 q	6.23 q	6.74	6.27 q
19	174.50 s	174.25 s	174.81	174.54 s
20	15.49 q	14.89 q	25.17	15.65 q
CH_3COO-	169.82 (2C, s)	169.98 s, 169.72 s	169.99, 169.33	170.11 s, 170.04s
$\underline{CH_3}COO$-	21.69 q, 21.08 q	21.81 q, 21.00	21.86,21.09	21.99 q, 21.07q

[a] reported as chemical shift, multiplicity; recorded in $CDCl_3$
[b] n-PrCOO-: δ 172.35 s, 36.11 t, 17.95 t, 15.58 q
[c] neither off resonance nor gated spectrum was obtained; assignments based on comparison to 5
[d] EtCOO-: δ173.68 s, 27.58 t, 9.18 q

The seventh diterpene, $C_{29}H_{39}ClO_{10}$, was quite different from 5-9, but did resemble 4 somewhat. The key differences, as in 7, resided in the absence of the ketone absorption in the IR, the appearance of a one proton multiplet near δ 3.7 and an upfield shift of the signals corresponding to H-11 and H-13. Decoupling experiments warranted structure 10, ptilosarcol, for this compound.

The effect of the three more abundant compounds, 4, 5, and 9, on larvae of the tobacco hornworm, Manduca sexta, was studied. Ptilosarcenone, 5, was toxic to the larvae at 250 ppm, inducing 40% mortality in three days and 90% mortality after 6 days. Surviving insects grew to only 20-35% of the weight of controls during the test. This level of activity is similar to that exhibited by brianthein Y, 1 [5].

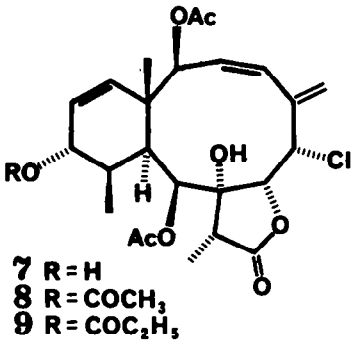
Compounds 4 and 9 were tested at 125 ppm. Neither exhibited significant toxicity, but ptilosarcone produced the same deleterious effect on weight gain that 5 did; at the end of the six day test period, the larvae were only 20-25% the size of controls. The case of compound 9 was interesting in that reduced weight gain (~ 25% of controls) was observed after three days, but by day six, the larvae were about 90% the weight of the control larvae. A possible explanation is that gradual weight gain reduced the mg/kg dose below the active level of 9; this would suggest that 4 is more potent than 9. The availability of a dozen briaran diterpenes from this work and the B. polyanthes investigation [2-5] should lead to some interesting structure-activity data from dose-response studies.

Table III

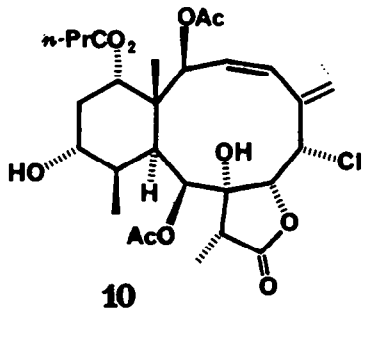
^{1}H-NMR Data of New *P. gurneyi* Diterpenes[a]

	6	7	8	9	10
H_2	5.89 (d,9.0)	5.78 (d,8.9)	5.76 (d,8.9)	5.74 (d,9.6)	6.17 (d,9.7)
H_3	5.54 (dd,11.7,9.0)	5.56 (dd,8.9,11.7)	5.57 (dd,11.6,8.9)	5.56 (dd,11.8,9.6)	5.64 (dd,9.7,10.4)
H_4	5.98(d,11.7)	5.89(dd,11.7,1)	5.91 (d,11.6)	5.90 (d,11.8)	5.90 (d,10.4)
H_6	4.96 (br d,3.2)	5.22 (m,2.6,2,3.7)	5.26 (br dd,3.4,2.1)	5.25 (br d, 3.5)	5.16 (br s)
H_7	5.02 (br s)	4.97 (d,3.7)	4.78 (d,3.4)	4.97 (d,3.5)	4.86 (d,3.7)
C_8-OH	4.88 (s)	3.21 (s)	3.17 (s)	3.13 (s)	3.29 (s)
H_9	5.77 (d,5.6)	5.40 (d,8.4)	5.38 (d,8.4)	5.37 (d,8.3)	5.34 (d,4.1)
H_{10}	2.92 (d,5.6)	2.84 (dd,3.4,8.4)	2.76 (dd,2.3,8.4)	2.74 (dd,3.4,8.3)	3.06 (dd,4.1,4.5)
H_{11}	5.27 (s, OH)	2.32 (ddq,2.5,3.4,7.1)	2.45 (ddq,2.3,2.3,7.0)	2.39 (ddq,3.4,4.1,7.1)	2.10 (m)[b]
H_{12}	—	3.89 (dd,2.5,5.4)	4.85 (dd,2.3,5.1)	4.88 (dd,2.5,4.1)	3.73 (br m)[c]
H_{13}	6.08 (d,10.5)	5.71 (dd,5.4,10.3)	5.72 (dd,5.1,10.3)	5.73 (dd,9.0,2.5)	2.10 (m)[b] 1.85 (m)
H_{14}	6.70 (d,10.5)	5.66 (d,10.3)	5.74 (d,10.3)	5.75 (d,9.0)	5.05 (br t)
H_{15}	1.23 (s)	0.98 (s)	0.99 (s)	0.99 (s)	1.09 (s)
H_{16}	6.00 (br s)	5.95 (d,2.6)	6.16 (br d,2.1)	6.14 (br s)	5.87 (br s)
H_{16}	5.88 (br s)	6.10 (dd,1,2)	5.93 (dd,1,1)	5.92 (br s)	6.11 (br s)
H_{17}	2.35 (q,7.1)	2.37 (q,7.0)	2.35 (q,7.1)	2.34 (q,7.0)	2.69 (q,7.1)
H_{18}	1.21 (d,7.1)	1.18 (d,7.0)	1.17 (d,7.1)	1.15 (d,7.0)	1.20 (d,7.1)
H_{20}	1.46 (s)	0.95 (d,7.1)	1.00 (d,7.0)	1.01 (d,7.1)	1.11 (d,7.3)
CH_3COO-	2.12 (s) 2.20 (s)	2.08 (s) 2.13 (s)	2.14 (s) 2.09 (s),2.01 (s)	2.13 (s) 2.08 (s)	2.17 (s) 1.96 (s)
C_3H_7COO-					2.26 (t), 1.62 (m),0.95 (t)
C_2H_5COO-				2.26(q),1.10(t)	

[a] reported as chemical shift (multiplicity, coupling constants)
[b] unresolved overlapping multiplets
[c] three small, unresolved coupling constants



7 R = H
8 R = $COCH_3$
9 R = COC_2H_5



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EXPERIMENTAL

NMR spectra were obtained with a Bruker WM-250 Fourier Transform spectrometer with $CDCl_3$ as solvent and internal standard. Mass spectral analyses were performed on VG Instruments MM16F and 7070 EHF mass spectrometers. Electron impact mass spectra were obtained using an ionization potential of 70 eV. Negative chemical ionization mass spectra were obtained using methane as the reagent gas. IR spectra were recorded with a Nicolet 5DX spectrophotometer. UV spectra were determined with a Varian G34 spectrophotometer. Optical rotations were determined on a Perkin-Elmer 241MC polarimeter. Melting points were determined on a Fischer-Johns apparatus and are uncorrected.

Collection and Extraction of Ptilosarcus gurneyi

Ptilosarcus gurneyi was collected at depths of 5-7 meters in March, 1985, near Sidney, British Columbia. The specimens were stored in acetone for three days prior to extraction. The acetone was decanted, and the sea pens were macerated in a Waring blender with methanol. The combined, filtered extracts were reduced to an aqueous suspension. The marc was then extracted twice with CH_2Cl_2 (12 hours each), after which the CH_2Cl_2 extracts and the aqueous suspension were partitioned. In vacuo evaporation of the CH_2Cl_2 phase gave 8.78 g of a thick orange oil (from 114.7 g dry weight).

A second collection was made in December 1985 near Seattle, Washington. Treatment similar to that described above yielded 66.0 g of a thick orange oil (from 690.3 g of dry weight).

Partitioning and Fractionation of the Crude Extract

The crude extract was dissolved in 10% aqueous MeOH and extracted twice with 300 ml of hexanes. The polar phase was increased to 20% water and extracted twice with 300 ml of CCl_4. Finally, the upper phase was increased to 40% water and extracted twice with 300 ml of $CHCl_3$. Evaporation of the hexane phase gave 5.22 g of extract, the CCl_4 phase gave 1.21 g and the $CHCl_3$ phase gave 0.74 g. Similar treatment of the crude from the Seattle collection yielded 48.5 g of hexane extract, 6.8 g of CCl_4 extract and 4.1 g of $CHCl_3$ extract.

The $CHCl_3$ extract was permeated through a 4.0 x 90 cm column of BioBeads S-X4 with 4:3:1 hexanes-CH_2Cl_2-EtOAc. The Sidney crude yielded 9 fractions, while the Seattle crude gave 7 fractions.

Isolation of Ptilosarcenone and 11-Hydroxyptilosarcenone

Fraction six from the S-X4 separation of the Sidney crude (123 mg) was permeated through Sephadex LH-20 (column 2.7 x 181 cm) with CH_2Cl_2-MeOH (1:1). The third (94 mg) of seven fractions was subjected to HPLC on an Ultrasphere-Cyano column (1.0 x 25 cm); elution with hexane-i-PrOH (2:1) gave five fractions. The fourth was ptilosarcenone (**5**, 47.2 mg), and the fifth was 11-hydroxyptilosarcenone (**6**, 15.8 mg).

Isolation of Ptilosarcol and Ptilosarcone

Fraction two from the above LH-20 separation was subjected to the same HPLC conditions to yield four fractions. Fraction two was ptilosarcol (**10**, 5.2 mg), while fraction three was ptilosarcone (**4**, 5.2 mg).

Isolation of Ptilosarcen-12-propionate

Fraction four of the S-X4 separation of the Seattle crude (555 mg) was permeated through Sephadex LH-20 (2.7 x 181 cm) with CH_2Cl_2-MeOH (1:1) to give four fractions. Fraction three was subjected to HPLC on an Ultrasphere-Cyano column (1.0 x 25 cm); elution with hexane-i-PrOH (4:1) gave four fractions. Fraction three was ptilosarcen-12-propionate (**9**, 21.0 mg).

Isolation of Ptilosarcen-12-acetate and Ptilosarcen-12-ol

Fraction five from the S-X4 chromatography of the Seattle crude (549 mg) was subjected to Sephadex LH-20 (2.7 x 181 cm) with CH_2Cl_2-MeOH (1:1) to give six fractions. Fraction three (517 mg) was subjected to preparative HPLC on an Ultrasphere-Cyano column (1.0 x 25.0 cm) using 20-30 mg injections; elution with hexane-i-PrOH (2:1) yielded 5 fractions. Fraction two (50.6 mg) was subjected to HPLC on a Chemcopak-Cyano column (1.0 x 25.0 cm); elution with hexane-CH_2Cl_2 (7:5) yielded six fractions. Fraction five was ptilosarcen-12-acetate (**8**, 3.7 mg). Fraction three (22.2 mg) of the prep-LC was subjected to HPLC on a Chemcopak-Cyano column (1.0 x 25 cm); elution with hexane-CH_2Cl_2 (9:11) yielded three fractions. Fraction one was ptilosarcen-12-ol (**7**,5.4 mg).

Characterization of Diterpenes

Ptilosarcone (**4**): λ_{max} (EtOH) 218 nm (ε = 5100); $[\alpha]_D^{26}$ -76.7° (c 0.64, CH_2Cl_2); ν_{max} (CCl_4) 3549, 2963, 1797, 1745, 1712 (sh), 1381, 1369, cm^{-1}; MS: m/z (relative intensity) 568/570 (<1), 526 (2), 438 (5), 402 (5), 378 (5), 360 (5),343 (6), 307 (3), 123 (33), 71 (45), 43 (100); HRMS: m/z 568.2089 ($C_{28}H_{37}ClO_{10}$ requires 568.2075), 526.1971 ($C_{26}H_{35}ClO_9$ requires 526.1969), 438.1444 ($C_{22}H_{27}ClO_7$ requires 438.1445).

Ptilosarcenone (5): mp 153-155°; λ_{max} (EtOH) 224 nm (ε =12600); $[\alpha]_D^{26}$-72.4° (c 1.01, CH_2Cl_2); ν_{max} (CCl_4) 3542, 2980, 1798, 1750, 1694, 1381, 1369 cm^{-1}; MS: m/z (relative intensity) 480/482 (<1), 438/440 (2,1), 342/344 (2,1), 231 (4), 191 (6), 123 (45), 107 (8), 79 (8), 43 (100); HRMS: m/z 480.1551 ($C_{24}H_{29}ClO_8$ requires 480.1550), 438.1449 ($C_{22}H_{27}ClO_7$ requires 438.1445).

11-Hydroxyptilosarcenone (6): λ_{max} (EtOH) 219 nm (ε =11700); $[\alpha]_D^{26}$-62.5° (c 0.74, CH_2Cl_2); ν_{max} (CCl_4) 3347, 2997, 1795, 1754, 1697, 1379, 1369 cm^{-1}; MS: m/z (relative intensity) 496/498 (<1), 478/480 (<1), 453/455 (2,1), 235 (3), 151 (7), 107 (9), 43 (100); HRMS: m/z 496.1497 ($C_{24}H_{29}ClO_9$ requires 496.1500); 478.1434 ($C_{24}H_{27}ClO_8$ requires 478.1394), 453.1310 ($C_{22}H_{26}ClO_8$ requires 453.1316).

Ptilosarcen-12-ol (7): λ_{max} (EtOH) 218 nm (ε = 4900); $[\alpha]_D^{26}$-62.9° (c 0.56, CH_2Cl_2); ν_{max} (CCl_4) 3555, 3542, 2982, 1798, 1765, 1744, 1382, 1369 cm^{-1}; MS: m/z (relative intensity) 440/442 (2,1), 380 (2), 362 (1), 344 (2), 327 (2), 309 (2), 107 (40), 43 (100); NCIMS (CH_4): m/z 482 (100), 386 (58), 326 (56); HRMS: m/z 440.1605 ($C_{22}H_{29}ClO_7$ requires 440.1601), 422.1499 ($C_{22}H_{27}ClO_6$ requires 422.1496).

Ptilosarcen-12-acetate (8): λ_{max} (EtOH) 218 nm (ε =4900); $[\alpha]_D^{26}$-82.4° (C 0.40, CH_2Cl_2); ν_{max} (CCl_4) 3555, 2983, 1798, 1744, 1382, 1370 cm^{-1}; MS: m/z (relative intensity) 482/484 (<1), 422/424 (5,2), 380 (3), 309 (3), 135 (23), 107 (48), 43 (100); NCIMS (CH_4): m/z 524 (100), 428 (69), 368 (39); HRMS: m/z 422.1498 ($C_{22}H_{27}ClO_6$ requires 422.1496).

Ptilosarcen-12-propionate (9): λ_{max} (EtOH) 218 nm (ε =5500); $[\alpha]_D^{26}$-117.0° (c 0.90, CH_2Cl_2); ν_{max} (CCl_4) 3555, 2985, 1797, 1744, 1381, 1369; MS:m/z (relative intensity) 538/540 (<1), 496/498 (<1), 422,424 (10,4), 380/382 (7,3), 362 (2), 344 (2), 327 (2), 309 (4), 253 (5), 135 (37), 107 (67), 57 (36) 43 (100); NCIMS (CH_4): m/z 538 (100), 442 (72), 428 (25), 382 (29), 368 (24); HRMS: m/z 496.1864 ($C_{25}H_{33}ClO_8$ requires 496.1864); 422.1495 ($C_{22}H_{27}ClO_6$ requires 422.1496).

Ptilosarcol (10): λ_{max} (EtOH) 218 nm (ε =5100); $[\alpha]_D^{26}$-55.8° (c 1.46, CH_2Cl_2); ν_{max} (CCl_4) 3594, 3551, 2964, 1796, 1745, 1368cm^{-1}; MS: m/z (relative intensity) 570/572 (<1), 528/530 (<1), 492/494 (<1), 475 (1), 440 (2), 344 (3), 327 (3), 309 (3), 107 (19), 71 (56), 43 (100); HRMS: m/z 570.2240 ($C_{28}H_{39}ClO_{10}$ requires 570.2231), 528.2123 ($C_{26}H_{37}ClO_9$ requires 528.2126), 422.1495 ($C_{22}H_{27}ClO_6$ requires 422.1496).

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11. Added Note: After submission of this article, another report addressing this stereochemical question in other briaran diterpenes was published: M.B. Ksebati and F.J. Schmitz, Bull. Soc. Chim. Belg., 95, 835 (1986).

12. Results of in vitro and in vivo testing of 4, 5, and 9 will be reported elsewhere.