

## Joalin, the First Nitrogen-containing Xenicane Diterpene isolated from a Brown Seaweed collected off the Senegalese Coast

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Joalin (–)-1 isolated from a brown seaweed, *Dictyota* sp., collected off the Senegalese coast, is a rare xenicane-derived diterpene in that it contains nitrogen.

Brown seaweeds of the order Dictyotales, sea hares and alcyonaceans, have given a plethora of xenicane diterpenes containing no nitrogen,<sup>1</sup> a feature also of other cembrane-derived diterpenes,<sup>1</sup> the sole exception being a family of aminocembranolides recently discovered in a tropical alcyonacean.<sup>2</sup> Sharp contrast is thus apparent with the nitrogen-containing diterpenes of sponges belonging to the orders Axinellida<sup>3</sup> and Haplosclerida.<sup>4</sup> It is interesting, therefore, to have now found in a seaweed, collected off the Senegalese coast, the first nitrogen-bearing xenicane diterpene, joalin (–)-1, which is reported here.

The MeOH extract of the seaweed, *Dictyota* sp. (Dictyotales), was subjected to extensive chromatography to give joalin (–)-1, whose composition C<sub>21</sub>H<sub>31</sub>NO<sub>3</sub> was deduced from mass and <sup>13</sup>C NMR spectroscopy. Together with 1D (assignments from differential double irradiations) and 2D (COSY 120)<sup>5</sup> <sup>1</sup>H NMR spectra, this showed that three of the seven unsaturated positions of joalin are accounted for by a tetra substituted and two tri substituted C=C bonds. An ester or amide/lactam carbonyl group was also suggested by the signal  $\delta_C$  174.60 (s). Choice of the latter was dictated by IR absorption at  $\nu_{max}$  1702s cm<sup>-1</sup>, and the presence of one nitrogen atom was confirmed by HREIMS data. Therefore, joalin must be tricyclic. The portion from C-1 to C-4 and bonding of C-9 to C-1, was based on NMR data for the deshielded protons 4-H, 9-H and 18-H whose homo- and hetero-nuclear NMR correlations (HMQC and HMBC)<sup>6</sup> are indicated on the structural formula by double arrows. Thus, oxygen substitution at C-4 was suggested by lowfield resonances for both this carbon and 4-H. Moreover,

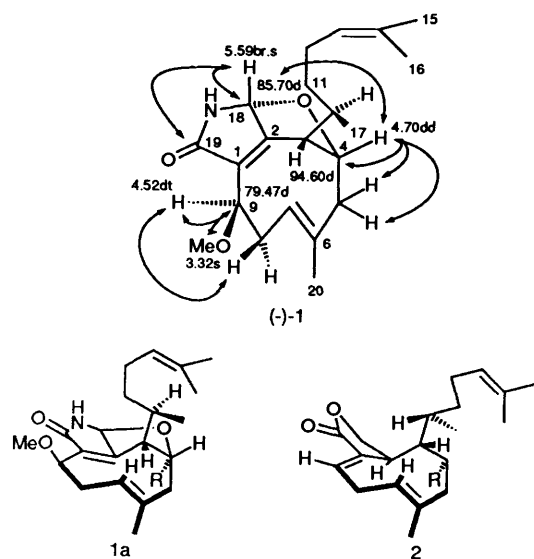
the signals  $\delta_C$  79.47 (d) and  $\delta_H$  4.52 (td) suggested C-9 to be an allylic carbon bearing a methoxy group long-range heterocoupled with 9-H. Finally, C-18 bound to both N and O was suggested by lowfield resonance for both this carbon and 18-H. Further support for the connectivity was afforded by WH(18)–H(9) coupling and long-range heterocorrelations of 3-H with C-2, C-10 and C-18, 4-H with C-18, 18-H with C-1, C-2 and C-19, and NH with C-2. The C<sub>8</sub> isoprenic side-chain was suggested by the [M – 69]<sup>+</sup> EIMS fragment ion and <sup>1</sup>H-<sup>1</sup>H COSY spectra. The missing portion C(5)–C(8) could then be constructed from the <sup>13</sup>C and <sup>1</sup>H NMR data, in particular from the coupling of 7-H with both 3 × 20-H and 8-H $\beta$ , and of the latter with 9-H. The two moieties C(9)–C(1)–C(4) and C(5)–C(8) could be joined as in the flat structure (–)-1 for joalin on the basis of long-range <sup>1</sup>H-<sup>13</sup>C heterocorrelations H(4)–C(5) and H(9)–C(1).

The  $\alpha$  position for 9-H was revealed by only small couplings with adjacent protons; 9-H $\beta$  would have required a coupling of ca. 11 Hz with 8-H $\alpha$ . Strong (differentially derived) NOE between 7-H and 3-H indicated that these two protons must be on the same face of the nine-membered ring, thus requiring that 9-H is on the opposite face. Small coupling between 3-H and 4-H, suggesting a dihedral angle of ca. 90°, allowed us to assign the  $\alpha$  position to O-4. NOE between 4-H and 3 × 17-H, and a complete conformational analysis<sup>7</sup> of the isoprenic side-chain, allowed us to establish the relative configuration and preferred conformation of joalin, as represented by **1a**. This is in line with all xenicanes so far reported,<sup>1</sup> apart from the incorporation of nitrogen and chirality at C-9 which are unique features of joalin.

If the signs of the Cotton effects for joalin ( $\Delta\epsilon_{259} + 5.8$  and  $\Delta\epsilon_{217.5} - 39.6$ , CDCl<sub>3</sub>) are interpreted as for  $\gamma$ -butenolides, according to assumptions for related cases,<sup>8</sup> joalin may be assigned the configuration 3*S*,4*R*,9*R*,10*R*,18*R*. This accords with all other xenicanes of algal origin so far reported.<sup>1</sup> With the caveat about this extrapolation, based on chiroptical data for very limited models,<sup>8</sup> (–)-1 also represents the absolute configuration for joalin.

Apart from the unique feature of nitrogen incorporation, joalin differs notably from previously known xenicanes<sup>1</sup> in showing high  $J_{3,10}$  <sup>1</sup>H-<sup>1</sup>H coupling, 10.4 Hz. Typically, dictyolactone shows  $J_{3,10} \approx 0$ ,<sup>9</sup> which, in accordance with the structure in the crystal,<sup>9</sup> indicates the conformation of the C-3 chain represented in **2**, with 17-Me pointing toward the side of the lactone ring. For joalin, the large  $J_{3,10}$  coupling suggests, in agreement with the results from molecular mechanics calculations,<sup>7</sup> that 17-Me points toward the opposite side of the lactam ring **1a**.

The only precedent for an oxygen bridge between C-4 and C-18 in xenicanes is given by dictyotalide, isolated from an Okinawan collection of *Dictyota dichotoma*.<sup>10</sup> Bearing a lactone carbonyl at C-18 and a free aldehyde group at C-19,<sup>10</sup>



dictyotalide may be thought to originate from a xenicane bearing a 4-hydroxy and C-18, C-19 aldehyde groups, followed by oxidation at C-18. A precursor of the same type, with C-19 oxidized to the carboxy group, may be imagined on the route to joalin by way of C-19 amide formation, enzyme-driven amide nitrogen attack at the C-18 aldehyde and aminoketalization by 4-OH.

### Experimental

The brown seaweed *Dictyota* sp., likely *Dictyota ciliolata* Sonder (ex. Kützing)<sup>11</sup> according to Prof. A. Meinesz and Mr. R. Lemée, who retain a voucher specimen, collected at low tide in September 1991 at the Pointe de Senti à Joal, south of Dakar on the Senegal coast, was immersed in MeOH and homogenized. Solvent evaporation, hexane extraction (1.2 g residue), and flash-chromatography with hexane/EtOAc gradient elution, was followed by evaporation of fractions 33–37 (40 cm<sup>3</sup> each) and HPLC of the residue on Merck Lichrosorb Si60 (25 × 1 cm) with hexane/isopropyl alcohol 97:3, 5 cm<sup>3</sup> min<sup>-1</sup>, to give joalin (–)-1, *t*<sub>r</sub> 10 min, as colourless, mildly cytotoxic on L1210 cell lines, semicrystals (4.5 mg, 0.02% based on dried seaweed residue);  $[\alpha]_{\text{D}}^{29} - 59.0$ ,  $[\alpha]_{\text{D}}^{20} - 102$  (CHCl<sub>3</sub>, *c* 0.01); †  $\lambda_{\text{max}}$ (MeOH)/nm 205 (12 300) and 245sh;  $\delta_{\text{C(TMS=0 ppm)}}(\text{CDCl}_3)$ , 75.43 MHz 162.60 (s, C-1), 131.91 (s, C-2), 49.72 (d, C-3), 94.60 (d, *J* 154, C-4), 44.82 (t, C-5), 140.05 (s, C-6), 120.92 (d, C-7), 32.06 (t, C-8), 79.47 (d, *J* 148, C-9), 34.78 (d, C-10), 35.43 (t, C-11), 25.71 (t, C-12), 123.96 (d, C-13), 131.58 (s, C-14), 25.53 (q, C-15), 17.69 (q, C-16), 18.08 (q, C-17), 85.70 (d, *J* 172, C-18), 174.60 (s, C-19) and 19.80 (q, C-20);  $\delta_{\text{H(TMS=0 ppm)}}(\text{CDCl}_3)$ , 299.94 MHz, *J* in Hz 2.77 (d, *J*<sub>3,10</sub> 10.4, 3-H), 4.70 (dd, *J*<sub>4,5 $\alpha$</sub>  3.6, *J*<sub>4,5 $\beta$</sub>  2.2, 4-H), 2.41 (dd, *J*<sub>gem</sub> 13.4, *J*<sub>5 $\alpha$ ,4</sub> 3.6, 5-H $\alpha$ ), 2.10 (dd, *J*<sub>gem</sub> 13.4, *J*<sub>5 $\beta$ ,4</sub> 2.2, 5-H $\beta$ ), 5.31 (br dd, *J*<sub>7,8 $\alpha$</sub>  12.7, *J*<sub>7,8 $\beta$</sub>  3.9, 7-H), 2.31 (ddd, *J*<sub>gem</sub> 13.5, *J*<sub>8 $\alpha$ ,7</sub> 12.7, *J*<sub>8 $\alpha$ ,9</sub> 3.5, 8-H $\alpha$ ), 2.56 (ddd, *J*<sub>gem</sub> 13.5, *J*<sub>8 $\beta$ ,7</sub> 3.9, *J*<sub>8 $\beta$ ,9</sub> 3.1, 8-H $\beta$ ), 4.52 (td, *J*<sub>9,8 $\beta$</sub>   $\approx$  *J*<sub>9,8 $\alpha$</sub>  = 3.1, *J*<sub>9,18</sub> 1.3, 9-H), 3.32 (s, 9-MeO), 1.75 (d sext, *J*<sub>10,3</sub> 10.4, *J*<sub>10,17</sub>  $\approx$  *J*<sub>10,11</sub> 6.5, 10-H), 1.15 (m, 2 × 11-H), 1.85 (m, 2 × 12-H), 5.00 (t sept, *J*<sub>13,12</sub> 6.7, 1.5, 13-H), 1.66 (br s, 3 × 15-H), 1.57 (br s, 3 × 16-H), 1.01 (d, *J*<sub>17,10</sub> 6.5, 3 × 17-H), 5.59 (br s, 3 × 18-H), 1.58 (d, *J*<sub>20,7</sub> 1.5, 3 × 20-H) and 5.93 (s, NH); *m/z* (EIMS) (%) 345 (7, M<sup>+</sup>), 313 (4 [M – MeOH]<sup>+</sup>), 277 (28, M<sup>+</sup> – 69 – H), 276 (19 [M – 69]<sup>+</sup>), 166 (50), 109 (39), 69 (66) and 41 (100); *m/z*

(HREIMS) 345.2303 (C<sub>21</sub>H<sub>31</sub>NO<sub>3</sub> requires 345.2304), 313.2031 (C<sub>20</sub>H<sub>27</sub>NO<sub>2</sub> requires 313.2042), 277.1672 (C<sub>16</sub>H<sub>23</sub>NO<sub>3</sub> requires 277.1678), 276.1602 (C<sub>16</sub>H<sub>22</sub>NO<sub>3</sub> requires 276.1600).

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### References

- 1 D. J. Faulkner, *Natural Products Reports*, 1984, 251 and 551; 1986, 1, 539; 1988, 613; 1990, 269, 97; 1992, 323.
- 2 K. Iguchi, K. Nishimura, K. Yamazaki, M. Iwashima and Y. Yamada, *Chem. Lett*, 1992, 127.
- 3 K. Ishida, M. Ishibashi, H. Shigemori, T. Sasaki and J. Kobayashi, *Chem. Pharm. Bull.*, 1992, 40, 766; S. A. Fedoreyev, S. G. Ilyin, N. K. Utkina, O. B. Maximov and M. V. Reshetkyak, *Tetrahedron*, 1989, 45, 3487; L. V. Manes, S. Naylor, P. Crews and G. J. Bakus, *J. Org. Chem.*, 1985, 50, 284; L. V. Manes, P. Crews, M. R. Kernan, D. J. Faulkner, F. R. Fronczek and R. D. Gandour, *J. Org. Chem.*, 1988, 53, 570; P. J. Scheuer, *Acc. Chem. Res.*, 1992, 25, 433.
- 4 H. A. Sharma, J. Tanaka, T. Higa, A. Lithgow, G. Bernardinelli and C. W. Jefford, *Tetrahedron Lett.*, 1992, 33, 1593.
- 5 V. Piantini, O. W. Sorensen and R. R. Ernst, *J. Am. Chem. Soc.*, 1982, 104, 6800; A. Bax and R. Freeman, *J. Magn. Reson.*, 1981, 44, 542.
- 6 L. Müller, *J. Am. Chem. Soc.*, 1979, 55, 301; G. Gray, *Magn. Moments*, 1987, III, 6.
- 7 'PCMODEL 4.0' Serena Software, Bloomington, Indiana.
- 8 I. Uchida and K. Kuriyama, *Tetrahedron Lett.*, 1974, 3761; M. Masuko, K. Miyamoto, K. Sakurai, M. Iino, Y. Takeuchi and T. Hashimoto, *Phytochemistry*, 1983, 22, 1278; H. Shimomura, Y. Sashida, Y. Mimaki and Y. Minegishi, *Phytochemistry*, 1987, 26, 582.
- 9 J. Finer, J. Clardy, W. Fenical, L. Minale, R. Riccio, J. Battaile, M. Kirkup and R. Moore, *J. Org. Chem.*, 1979, 44, 2044.
- 10 M. O. Ishitsuka, T. Kusumi and H. Kakisawa, *J. Org. Chem.*, 1988, 53, 5010.
- 11 G. W. Lawson and D. M. John, *Beihfte zur Nova Hedwigia*, 1982, 70, 140.

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†  $[\alpha]_{\text{D}}$  Values recorded in units of 10<sup>-1</sup> deg cm<sup>2</sup> g<sup>-1</sup>.