

## AQUATOLIDE. A NEW TYPE OF HUMULANE-RELATED SESQUITERPENE LACTONE

A. San Feliciano\*, M. Medarde, J.M. Miguel del Corral, A. Aramburu,  
M. Gordaliza and A.F. Barrero<sup>a</sup>

*Depto de Química Orgánica y Farmacéutica Facultad de Farmacia. 37007-Salamanca-Spain.*

*a)Depto de Química Orgánica. Facultad de Ciencias. Granada. Spain*

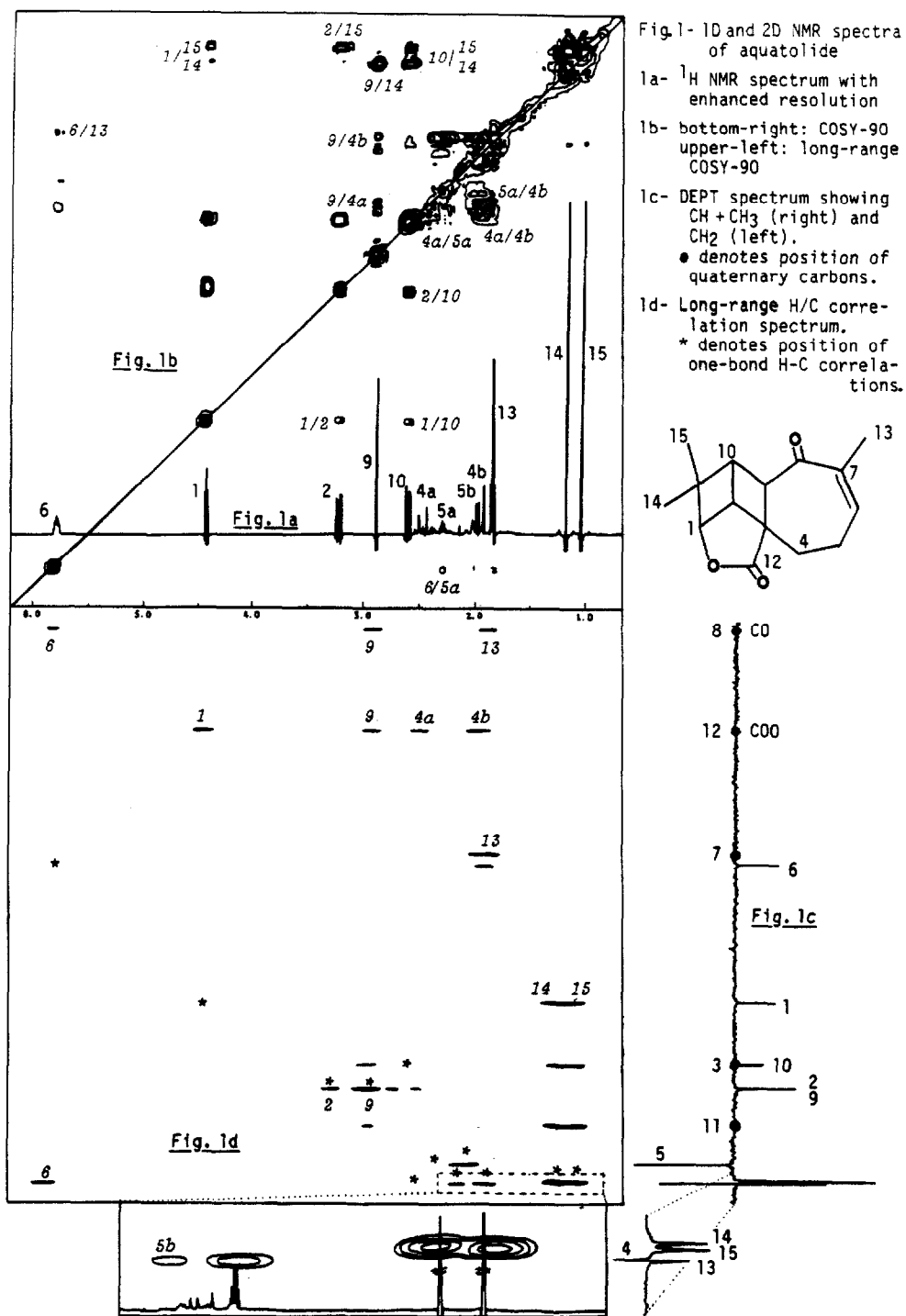
**Summary:** A new sesquiterpene lactone called aquatolide was isolated from the hexane extract of *Asteriscus aquaticus*. Its constitution and stereochemistry were deduced from spectroscopic data, mainly homonuclear and heteronuclear two-dimensional NMR correlations.

From the neutral part of a methanol defatted hexane extract of *Asteriscus aquaticus* L. (family, Compositae; Tribe, Inuleae), by repeated chromatography and crystallization, a small amount of a new substance **1** was isolated<sup>1</sup>. Its origin and the preliminary analysis of its spectroscopic properties, readily suggested the structure of a sesquiterpene lactone for the compound.

Its MS displayed the M<sup>+</sup> at  $m/z = 246^2$  in the EI mode and showed peaks at  $m/z = 281$  (M<sup>+</sup> + 35) and  $m/z = 264$  (M<sup>+</sup> + 18) in the CI mode (NH<sub>3</sub>). These data, together with <sup>13</sup>C NMR data obtained from its BB decoupled and DEPT spectra (Table I), established a molecular formula of C<sub>15</sub>H<sub>18</sub>O<sub>3</sub> for aquatolide. In its IR spectrum prominent bands of saturated  $\gamma$ -lactone (1770 cm<sup>-1</sup>) and  $\alpha,\beta$ -unsaturated ketone (1680 cm<sup>-1</sup>) accounted for all the oxygenated functions in the molecule. Conjugation of the carbonyl group was confirmed by an absorption maximum at 240 nm in the UV spectrum and the presence of only one double bond, as was deduced from the signals at 130.9 and 135.0 ppm in the <sup>13</sup>C NMR spectrum, permitted us to deduce the existence of four rings in the molecule.

The <sup>1</sup>H-NMR spectrum of aquatolide (fig 1a, Table I) showed two methyl singlets at 1.04 and 1.18 ppm, as well as another three protons signal (1.86 ppm), assigned to a methyl attached to an olefinic carbon. Furthermore, signals of a deshielded olefinic proton (5.84 ppm) and of a proton geminal to the lactone oxygen (4.48 ppm), which formed an AMX system with another two protons absorbing at 3.26 and 2.64 ppm were also observed. A singlet of one proton at 2.92 ppm and some overlapping multiplets in the range between 1.8 and 2.6 ppm, corresponding to four protons, completed the spectrum. In agreement with these data, apart from the signals of two carbonyls, two olefinic carbons and one oxygenated methine, the <sup>13</sup>C NMR spectrum of aquatolide (fig. 1c) showed signals for three methyls, two methylenes, three methines and two sp<sup>3</sup> quaternary carbons.

With these data, also taking into consideration the large deshielding of the methines (54.3, 54.4 and 62.5 ppm), which were similar to those of photoisabelin<sup>3</sup>, as well as the structure of Asteriscunolides A - D and Asteriscanolide, described by us previously as metabolites of *A. aquaticus*<sup>4,5</sup>, we were able to propose the structure **1** for aquatolide.



To confirm our proposal and to perform the unequivocal assignment of the spectra, several 2D-NMR experiments were carried out. The connectivities detected in the COSY-90 spectrum<sup>6</sup> (fig. 1b) are all in agreement with the proposed structure and by the long-range connectivities observed for the singlet at 2.92 ppm; namely, H<sub>4a</sub>, H<sub>4b</sub> and H<sub>14</sub>, the proton environment ( four or five bonds apart) of the "uncoupled " H<sub>9</sub> was confirmed. Similarly, protons H<sub>1</sub> and H<sub>10</sub> were long-range connected to H<sub>14</sub> and H<sub>15</sub>, whereas H<sub>2</sub> showed long-range coupling with H<sub>15</sub> only. The <sup>1</sup>H/<sup>13</sup>C correlations (fig. 1d)<sup>7</sup> also served to confirm the constitution of aquatolide through the numerous connectivities observed. In particular, we were able to discover the environment of non-protonated carbons . Thus, the ketone carbonyl must be located near (two or three bonds apart) H<sub>6</sub>, H<sub>9</sub> and H<sub>13</sub>, and the carboxyl group near H<sub>1</sub>, H<sub>9</sub> and H<sub>4</sub>. Furthermore, methyl protons H<sub>14</sub> and H<sub>15</sub> simultaneously correlate with carbons C<sub>1</sub>, C<sub>2</sub> and C<sub>11</sub>, thus demonstrating the existence of the *gem*-dimethyl group at C<sub>11</sub>.

The assignment of the relative stereochemistry for 1 was simple due to the restrictions involved by the fusion of several rings. The only position to be defined was C<sub>9</sub>, whose configuration was easily deduced to have a H<sub>9</sub>/H<sub>10</sub>-*trans* relationship due to the absence of coupling between both protons, corresponding to the observed 90° dihedral angle. Some NOE-difference experiments, whose results are summarized in fig. 2, served to confirm the structure and to unequivocally assign the signals of methyls 14 and 15.

The conformation depicted in fig.2 for the seven-membered ring was deduced from the NOE detected on both H<sub>5a</sub> and H<sub>5b</sub> upon irradiation of {H<sub>6</sub>} and from the excision pattern of these three protons. After assuming this conformation, application of empirical rules<sup>3</sup> to the analysis of the CD spectrum of aquatolide, showing Cotton effects at 245 (  $\Delta\epsilon = -16.4$ ) and 318 nm (  $\Delta\epsilon = +5.6$ ), permitted us to establish the absolute stereochemistry shown in Fig.2 for this compound, which also agrees with those of Asteriscunolides A-D and Asteriscanolide, previously isolated from the same plant<sup>4,5</sup>.

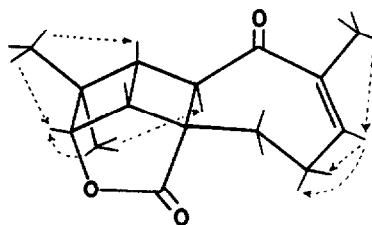


Fig.2 NOEs observed for aquatolide

Table I. <sup>13</sup>C and <sup>1</sup>H NMR data for Aquatolide. CDCl<sub>3</sub>. TMS as int. standard. J in Hz.

N <sup>o</sup>	<sup>13</sup> C $\delta$	<sup>1</sup> H $\delta$	multipl.(J)	N <sup>o</sup>	<sup>13</sup> C $\delta$	<sup>1</sup> H $\delta$	multipl.(J)
1	84.12	4.48	dd ( 2.5, 1.9)	7	135.07		
2	54.48	3.25	dd ( 7.2, 2.5)	8	211.58		
3	62.77			9	54.37	2.92	s
4	22.07	4a; 2.50	dd(13.9, 6.5)	10	62.57	2.64	dd (7.2, 1.9)
		4b; 2.02	m	11	41.73		
5	28.53	5a; 2.38	m	12	177.27		
		5b; 2.07	m	13	22.00	1.86	m
6	130.94	5.84	m	14	22.73	1.18	s
				15	22.46	1.04	s

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## REFERENCES AND NOTES

1. Mp. = 142-143° (ether);  $[\alpha]_D = + 66.3^\circ$  (c, 0.45, CHCl<sub>3</sub>).
2. EIMS, *m/z* (rel. abund. %): 246(2), 203(3), 185(4), 161(14), 150(27), 135(42), 107(42), 105(39), 91(100).
3. Yoshioka H., Mabry T.J. and Higo A.; *J. Am. Chem. Soc.* **92**, 923 (1970).
4. San Feliciano A., Barrero A.F., Medarde M., Miguel del Corral J.M., Aramburu A., Perales A, Fayos J. and Sanchez-Ferrando F.; *Tetrahedron* **41**, 5711 (1985).
5. San Feliciano A., Barrero A.F., Medarde M., Miguel del Corral J.M., Aramburu A., Perales A and Fayos J.; *Tetrahedron Letters* **26**, 2639 (1985).
6. *Homonuclear <sup>1</sup>H correlations.*- Pulse sequence COSY.AU, from Bruker DISNMR software, was used. 256 FIDs of 16 scans each, with 1 sec recycle delay and incrementing *t*<sub>1</sub> from 5 μsec to 180 msec, were acquired on a 0.3M solution of **1**, at 24° C. Fourier Transforming was performed after sinebell filtration in both time domains, leading to a 256K matrix which was symmetrized. Digital resolution was DR=2.9 Hz/point. For long-range correlation the sequence COSYLR.AU was used. Most experimental conditions were unchanged, except that the spectral width was enlarged and the carrier placed at the end of the spectrum to avoid ghost peaks. A fixed delay of 0.6 sec was introduced to emphasize long-range couplings. DR was 5.8 Hz/pt.
7. *Heteronuclear long-range <sup>1</sup>H/<sup>13</sup>C correlation.*- Pulse sequence HCCORR.AU was used. 128 FIDs of 560 scans each, with 1 sec recycle delay and incrementing *t*<sub>1</sub> from 3 μsec to 92 msec, were acquired on the same sample. The F<sub>1</sub> carrier was placed at the end of the <sup>1</sup>H spectrum to avoid folding and fixed delays were tuned for  $^nJ_{C,H} = 8.3$  Hz. Improvement of resolution in F<sub>2</sub> was achieved by introduction of one degree of zero-filling in the *t*<sub>2</sub> domain. DR were 5.1 and 14.1 Hz/pt in F<sub>2</sub> and F<sub>1</sub> respectively.
8. Djerassi C., Records R., Bunnenberg K., Mislow K. and Moscovitz A.; *J. Am. Chem. Soc.*; **84**, 870 (1962).

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