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ASTERISCUNOLIDES A, B, C AND D, THE FIRST HUMULANOLIDES; TWO PAIRS OF CONFORMATIONALLY STABLE STEREOISOMERS

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Abstract: Following a preliminary comunication on Asteriscunolide A (the first reported natural humulanolide), three more configurationally and/or conformationally isomeric Asteriscunolides (B, C and D) from the same plant (Asteriscus aquaticus L.) are described. The absolute configurations and the stable conformations of all four Asteriscunolides are established by an array of spectroscopic methods (CD, ^{1}H and ^{13}C NMR) without resorting to X ray analysis.

Introduction

Asteriscus aquaticus L. Less (Familly Compositae, Tribe Inuleae) is an odoriferous, herbaceous and annual plant that grows on calcareous soils from Portugal to the South of Bulgaria. (Synonyms: Buphtalmun aquaticum L. Asteriscus citriodorus Held & Halacsy and Bubonium aquaticum L. Hill).

Previously [1] we have reported in a preliminary communication the isolation and structural determination of Asteriscunolide A 1 the first described sesquiterpene lactone with humulane skeleton. In this paper we wish to report the results obtained from the study of structures and stereochemistries of Asteriscunolides B 2, C 3 and D 4, which have been isolated, through chromatographic separations and crystallizations, from the same extract of the aerial part of Asteriscus aquaticus L.

Methods and Results

The three new compounds shows physical and spectroscopic properties closely related to those of Asteriscunolide A; namelly, all of them are crystalline compounds, display negative optical rotation, show only small differences in their ^{1}H and ^{13}C NMR spectra (tables I and II). These concordances readily suggest that the new substances have the same constitution as Asteriscunolide A and the possible differences between all of them should occur at the stereochemical level.

Of the three olefinic bonds in each molecule, the Δ^{2} double bond must obviously have the Z configuration due to the lactone ring and Δ^{9} double bond must have the E configuration as deduced from the value of 14 to 16 Hz for the coupling constants $J_{9,10}$ observed in the four cases (table I). As the specific rotations are all greatly negative the configuration at the only chiral center C_1 must be equal in all four isomers, and the remaining possibilities for explaining the existence of four compounds with the same constitution are changes in the Δ^{6} double bond configuration and in the conformation of the eleven-membered ring with humulatriene skeleton.

Since Asteriscunolides A and C show fair positive $H_6[H_{13}]$ NOEs they must have the *Z*-6 configuration, while Asteriscunolides B and D lack this effect, and their ^{13}C NMR off resonance spectra show shielded quartet for C_{13} (10.9 and 12.6 ppm respectively, table II) which agree with their *E*-6 configuration.

Each one of the Asteriscunolides is conformationally quite stable even in Et_2O, chloroform and other common solvents at room temperature and, as far as we know, these are the first reports on the isolation of crystalline natural conformers which are no interconvertible in solution. This could be explained by the high rotational barriers [2] of the polyunsaturated eleven-membered humulane skeleton[#], which are higher than those of the conformationally equilibrating ten-membered germacratriene skeleton [3-6].

The conformational isomerism between **1** and **3** was confirmed by interconversion. Thus, Asteriscunolide A in DMSO solution, in an NMR variable temperature experiment, changes to Asteriscunolide C and approximately 30% of the later was present in the sample after heating at 160° for five minutes. Furthermore, when the Asteriscunolide A was heated in its melted state at 200°C for five minutes and allowed to cool to room temperature it was quantitatively changed (as observed in the ^{1}H NMR spectrum of the sample no further purified) to Asteriscunolide C. Asteriscunolide C heated both, in a NMR variable temperature experiment (150°C, in DMSO) and in the melted state (200°C) does no appreciably change to Asteriscunolide A.

Careful observation of models of Asteriscunolides leads to a conclusion, already predicted by theoretical calculations on the closely related ring system of α-humulene [2], namely that the rotational barriers for the interconversion between conformers having the *E*-6 configuration are much higher than those of conformers having the *Z*-6 configuration. Moreover, under experimental conditions similar to those described for the transformation of Asteriscunolide A into Asteriscunolide C, no appreciable change in any direction was observed between Asteriscunolides B and D, though some structural degradation occurs in the case of Asteriscunolide B, which also degrades upon standing for a week in $CDCl_3$ solution.

The conformation of Asteriscunolide A **1** was stablished [1] according to the observed NOE between H_{13} and H_{10} and the values of the coupling constants $J_{5\alpha,6}$ and $J_{5\beta,6}$ (table I). Similarly, the absorption pattern of H_6 in the ^{1}H NMR spectrum of Asteriscunolide C as a broad doublet ($J_{5\beta,6}$= 12.7Hz) implies a value from 70 to 110° for the dihedral angle between $H_{5\alpha}$ and H_6. Furthermore, the irradiation on the H_{13} and $H_{5\beta}$ resonances at 1.86 δ produces a NOE at the resonances of H_6 (15%), H_{10} (4.2%), H_2 (3.5% and H_9 (2.4%) and upon irradiation at the resonances of $H_{4\alpha}$, $H_{4\beta}$ and $H_{5\alpha}$ (ca. 2.4 ppm) almost no change in the spliting of the H_6 resonance was observed. These data support conformation **2** for Asteriscunolide C. Nevertheless, the broadened shape of the H_6 resonance could be interpreted as produced by a certain degree of conformational mobility in the C_4-C_5 fragment of the molecule. Thus, when the temperature of the sample was raised to 100°C some changes in the ^{1}H NMR spectrum were observed, namely the H_6 resonance that changed to four quartets ($J_{5\alpha,6}$= 4.4, $J_{5\beta,6}$= 5.9 and $J_{6,13}$= 1.6 Hz) together with the modification of the H_5 and H_4 absorption patterns.

The shape and height of the signals of H_{10} and H_9, influenced by couplings with hydrogen atoms at C_{14} and C_{15} also change with the variation of the temperature. Thus, at 33°C H_{10} shows signals sharper than those of H_9, at 60°C both doublets show the same height and at 100°C the signals of H_9 are sharper than those of H_{10}. From these data the existence of a small degree of mobility at C_{11}-C_9 fragment is also deduced. Furthermore, irradiation at 1.28 ppm corresponding to H_{15}, the β-quaternary methyl group at C_{11} (as was shown in a $[H_2]$ NOE diff. experiment), showed that a long-range coupling between H_{10} and H_{15} exists and this fact established the antiperiplanar conformation of H_9 and C_{14} at 33°C (fig. 1).

The conformational mobility of the *E*-6 isomers, Asteriscunolides B and D, is much lesse than that of Asteriscunolides A and C. In fact

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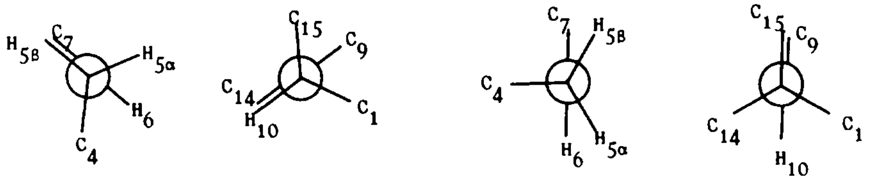
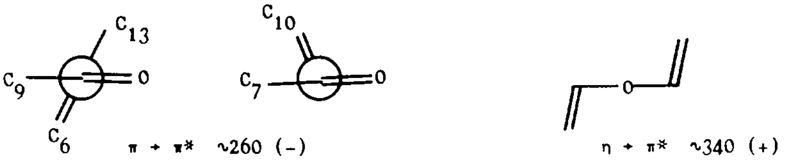
[#] Experimental $\Delta G^{\#}$ values: 10.6 kcal mole^{-1} for humulene and 15.8 kcal mole^{-1} for zerumbone (S. Dev, J.E. Anderson, V. Comier, N.P. Damodaran and J.D. Roberts, 1968 *J. Amer. Chem. Soc.* 90, 1246). Calculated $\Delta H^{\#}$ value: 14.2 kcal mole^{-1} for humulene [2]. These values should increase considerably when taking into account the extra rigidity associated with the butenolide ring featured by all Astericunolides.

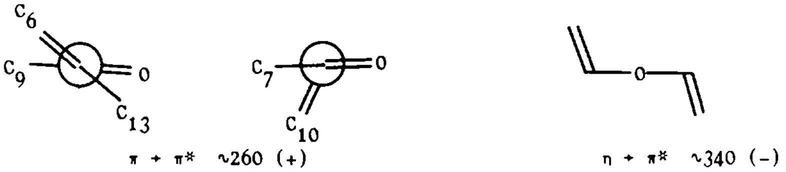


at 33°C at 100°C

Fig.1 Partial C_7-C_{11} conformations of Asteriscunolide C



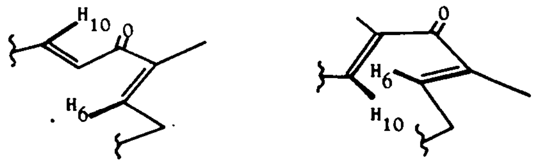
Asteriscunolides A and B



Asteriscunolides C and D

Fig.2 Partial conformations of Asteriscunolides A, B, C and D

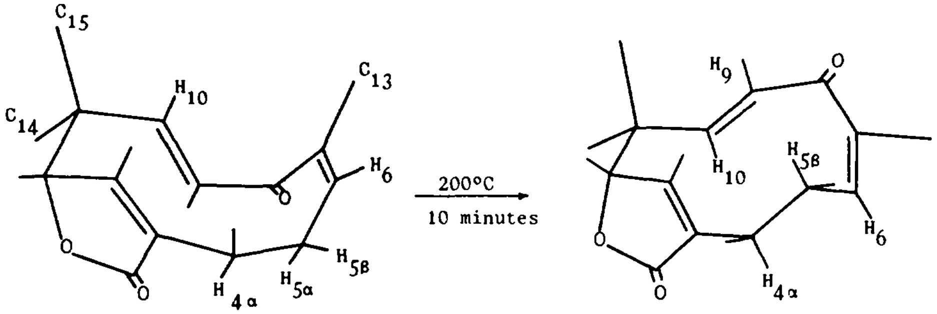
examination of their Dreiding models, and the empirical force field calculations for α-humulene [2] show that the posible conformers could be reduced to two: one with the H_6 over the H_{10} and another with H_{10} over H_6, and the interconversion should be greatly restricted because both nuclei would lie very close (less than 0.5 Å) when the hypothetical C_6-C_{10} planar or almost planar transition states were reached.



The conformation of Asteriscunolides B and D have been directly deduced from their CD curves. Asteriscunolide B, as well as Asteriscunolide A, shows a negative Cotton effect near 260 nm ($\pi \rightarrow \pi^*$ transition of the enone) and a positive Cotton effect near 340 nm ($n \rightarrow \pi^*$ transition of the enone), while Asteriscunolide D, as well as Asteriscunolide C, shows positive Cotton effect near 260 nm and a negative Cotton effect near 240 nm, and all of the Asteriscunolides have a negative Cotton effect associated to the $\pi \rightarrow \pi^*$ transition of the conjugated lactone. From these data we concluded that the spacial dispositions of the cross conjugated ketone groups are those represented in Fig.2

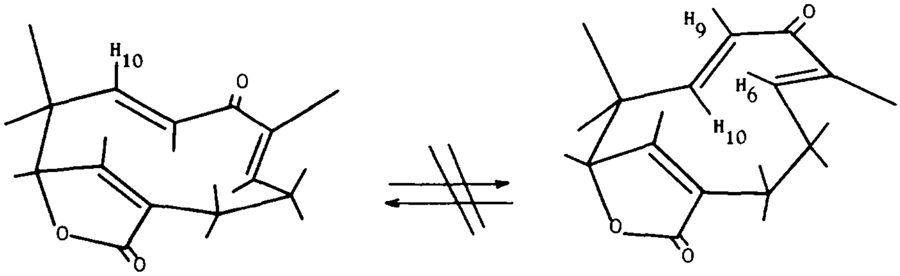
Furthermore, the conformation of Asteriscunolide D has been confirmed by means of an [H_2] NOE difference experiment , in which enhancements were measured on H_6(6.2%), H_1(5.9%) and H_{15}(6.0%). Complementary NOE difference spectra also helped in the assignment of the ^1H NMR signals of Asteriscunolide D. Thus, irradiation at H_{15} resulted in enhancement at H_2(7.0%), H_9(8.4%), H_6(2.5%) and H_1(7.8%), while upon irradiation of H_{14} only H_{10}(8.6%) and H_1 (8.2%) displayed enhanced intensity. These results confirm the unexpected downfield shift for H_9 (and upfield for H_{10}) reported in Table I.

Other experimental observations, as the



1
Asteriscunolide A

3
Asteriscunolide C



3
Asteriscunolide B

4
Asteriscunolide D

solvent and lanthanide induced shifts in the ^1H NMR spectra and the shielding of H_6 in each case, agree with the above proposed stereochemistries. A few experimental observations remain unexplained. Thus, the shifts δ H_9 and δ H_{10} in lactones 1 - 4 cannot be predicted using simple additive parameters from tables. Furthermore, δ H_6 seems to depend on the relative conformations of double bonds Δ^2 and Δ^9 across the eleven membered ring (parallel for A and B; crossed for C and D), rather than the Z or E configuration of the Δ^6 double bond. In the ^{13}C NMR spectra, a similar dependence is apparent for the chemical shifts of the pairs of carbons C_9-C_{10} and C_{14}-C_{15}, although in this case the proximity of the interacting double bonds to the carbons suffering the effect is far greater.

The assignments of the signals in the ^{13}C NMR spectra have been performed by their chemical shifts, by the values of their residual coupling constants in their off resonance spectra and, when needed, by ^1H selective irradiation. Particularly C_3 and C_7 have been assigned through selective H_2, H_6 and H_{13} irradiation.

EXPERIMENTAL

Mps are uncorrected. IR spectra were recorded in $CHCl_3$ solution on a Beckmann IR-33 instrument. ^1H and ^{13}C NMR spectra were run with internal TMS on a Bruker WP200SY spectrometer. EIMS were obtained at 70 eV with a Hewlett-Packard 5930A. UV spectra were recorded in EtOH on a Hitachi 100-60 spectrophotometer. CD measurements were made on a Jobin-Yvon Dichrograph III in dioxan solution. Analytical TLC was performed on Si gel G (Merck 7331) and CC on Si gel (Merck 7734).

Flowering plant material was collected in July at Almenara de Tormes (Salamanca. Spain). Air-dried material (11Kg) was extracted in a Soxhlet with n-hexane. After successive separation of the cool n-hexane insoluble fraction, MeOH-insoluble fraction and the acidic compounds (42.5g), by 10% aq. NaOH extraction, 114.5 g of neutral part were obtained.

Compounds 1-4 were isolated by CC (Si gel) of 8.9 g of the neutral fraction and purified by CC (Si gel-20%$AgNO_3$) and/or crystallization from EtO_2.

Asteriscunolide A (1). By Si gel CC 1.02 g of 1 were eluted with n-hexane-AcOEt

Table I. ^{1}H NMR spectral data of Asteriscunolides A, B, C and D
(200 MHz, $CDCl_3$ solution, Chem. shifts are in δ-values from TMS, J in Hz)

	H-1	H-2	H-4,5	H-6	H-9	H-10	H-13	H-14	H-15
A (1)	4.68 bs	7.00 bs	2.0–2.8 m	6.00 bdd $J_{6,5\alpha}$=10.0 $J_{6,5\beta}$= 7.0	5.29 d $J_{9,10}$=13.8	6.16 d	1.94 bs	1.20 s *	1.41 s*
B (2)	4.70 bs	6.79 bs	2.5–2.9 m	6.17 bdd^{+} $J_{6,5\alpha}$= 6.4 $J_{6,5\beta}$= 8.4	5.49 d $J_{9,10}$ =14.1	6.17 d	1.80 bs	1.38 s *	1.04 s*
C (3)	4.71 bs	6.97 bs	1.7–2.6 m	5.48 bd $J_{6,5\beta}$=12.7	5.91 d $J_{9,10}$=16.5	6.27 d	1.86 bs	1.36 s	1.28 s
#	*4.65 bt* $J_{1,2}$=*1.5*	*6.90 m*	*1.7–2.6 m*	*5.47 ddq* $J_{6,5\alpha}$= *4.4* $J_{6,5\beta}$= *5.9* $J_{6,13}$= *1.6*	*5.91 d*	*6.20 d*	*1.84 m*	*1.36 s*	*1.27 s*
D (4)	4.78 bs	7.08 bs	2.3–2.8 m	5.54 bd $J_{6,5\beta}$= 8.1	5.74 d $J_{9,10}$=16.5	6.10 d	1.77 bs	1.28 s	1.30 s

\# Spectrum of 3 at 373°K $^{+}$ Overlapped with H_{10}
* Assigned by solvent and lanthanide induced shifts

NOEs (%)		H_1	H_2	H_6	H_9	H_{10}	H_{15}
Asteriscunolide A	$\lvert H_{13}\rvert$			14		8.5	
Asteriscunolide B	$\lvert H_{13}\rvert$					3.2	
Asteriscunolide C	$\lvert H_{13}+H_{5\beta}\rvert$		3.5	15	2.4	4.2	
Asteriscunolide D *	$\lvert H_2\rvert$	5.9		6.2			6.0
	$\lvert H_{15}\rvert$	7.8	7.0	2.5	8.4		
	$\lvert H_{14}\rvert$	8.2				8.6	

* values calculated from NOE and NOE difference experiments

Table II. ^{13}C NMR spectral data of Asteriscunolides A, B, C and D
(50.3 MHz, $CDCl_3$ solution, Chem. shifts are in δ-values from TMS)

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15
A (1)	88.1	147.9	138.8	23.0	29.9	138.3	133.7	198.5	136.5	133.0	42.5	173.5	21.3	24.7	30.0
B (2)	88.7	151.6	138.3	21.7	23.9	153.1	131.8	199.7	136.8	133.1	42.7	173.7	10.9	23.9	31.1
C (3)	85.6	149.7	138.6	22.8	33.9	128.4	135.6	202.6	129.5	156.4	40.7	173.7	21.1	24.6	21.0
#	*85.8*	*148.0*	*139.6*	*21.9*	*32.9*	*128.4*	*136.3*	*201.4*	*129.7*	*155.7*	*41.1*	*173.0*	*20.9*	*23.8*	*23.6*
D (4)	87.3	150.3	139.3	22.8	24.5	145.2	133.9	202.9	129.8	155.3	41.0	172.5	12.6	24.5	21.2

\# Spectrum of 3 at 373°K

(9:1). Mp 158° (Et_2O).

$$[\alpha]^{\lambda}_{24°} = \frac{589 \quad 578 \quad 546 \quad 435}{-335.5° \quad -354° \quad -406° \quad -712°} \ (c1.40)$$

IR ν_{max} cm^{-1}: 3030, 1755, 1680, 1640, 1370, 1360, 1100, 1060, 1040, 1015, 980, 900, 860, 840. UV λ^{EtOH}_{max} nm ($\log \epsilon$): 243 (7040), 237 (7500), 223 (9470). EIMS m/z (rel.int.): 246 M^+ (2), 203 (20), 201 (2), 185 (3), 173 (14), 150 (70), 135 (100), 107 (28), 91 (17).

Asteriscunolide B (2). 900 mg were eluted with n-hexane-AcOEt (9:1) from a Si gel-20%NO_3Ag CC. Mp 175° (Et_2O).

$$[\alpha]^{\lambda}_{24°} = \frac{589 \quad 578 \quad 546 \quad 435}{-381° \quad -401° \quad -452° \quad -665°} \ (c0.98)$$

IR ν_{max} cm^{-1}: 3030, 1755, 1660, 1645, 1385, 1370, 1085, 1070, 1015, 980, 905, 845, 840. UV λ^{EtOH}_{max} nm ($\log\epsilon$): 245 (1800), 232 (210), 222 (1940). EIMS m/z (rel.int.): 246 M^+ (2), 230 (2), 203 (1), 185 (1), 182 (2), 150 (100), 135 (91), 107 (20), 91 (11).

Asteriscunolide C (3). 700 mg were eluted with n-hexane-AcOEt (4:1) from the same Si gel-20%NO_3Ag CC that 2. Mp 164° (Et_2O).

$$[\alpha]^{\lambda}_{24°} = \frac{589 \quad 578 \quad 546 \quad 435}{-260° \quad -277° \quad -335° \quad -863°} \ (c0.90)$$

IR ν_{max} cm^{-1}: 3030, 1765, 1660, 1380, 1370, 1080, 1055, 1010, 985, 900, 860, 840. UV λ^{EtOH}_{max} nm ($\log\epsilon$): 217 (8970). EIMS m/z (rel. int.): 246 M^+ (2), 221 (3), 207 (3), 175 (3), 150 (100), 135 (77), 107 (50), 91 (34).

Asteriscunolide D (4). 700 mg were eluted with n-hexane-AcOEt (4:1) from the Si gel CC. Mp 145° (Et_2O)

$$[\alpha]^{\lambda}_{24°} = \frac{589 \quad 578 \quad 546 \quad 435}{-158° \quad -172° \quad -223° \quad 855°} \ (c0.37)$$

IR ν_{max} cm^{-1}: 3030, 1760, 1660, 1375, 1365, 1090, 1060, 1010, 980, 900, 860, 840. UV λ^{EtOH}_{max} nm ($\log\epsilon$): 247 (4674), 221 (8036). EIMS m/z (rel. int.): 246 M^+ (5), 228 (1), 218 (1), 203 (1), 185 (1), 175 (2), 150 (100), 135 (87), 107 (96) 91 (40).

CD curves of all four Asteriscunolides are compared in the following table ($\Delta\epsilon$ in dioxan).

Ast. A	+1.5 (340nm)	- 5.0 (268nm)	-28.5 (228nm)
Ast. B	+4.6 (340nm)	-34.0 (249nm)	---
Ast. C	-3.4 (343nm)	+ 6.9 (263nm)	-59.8 (227nm)
Ast. D	-3.1 (339nm)	+10.4 (257nm)	-20.7 (225nm)

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REFERENCES AND NOTES

1. A. San Feliciano, A.F. Barrero, M. Medarde, J.M. Miguel del Corral, E. Ledesma and F. Sánchez-Ferrando (1982) *Tetrahedron Letters* 3097-3100.
2. H. Shirahama, E. Osawa and T. Matsumoto (1980) *J. Am. Chem. Soc.* 102, 3208-3213.
3. K. Tori, I. Horibe; Y. Tamura, K. Kuriyama, H. Tada and K. Takeda (1976) *Tetrahedron Letters* 387-390.
4. R.K. Bentley, J.G. St. Buchanan, T.G. Halsall and V. Thaller (1970) *Chem. Commun.* 435-436.
5. K. Tori and I. Horibe (1971) *J. Chem. Soc.* B 1084-1088.
6. K. Tori, I. Horibe, K. Kuriyama and K. Takeda (1970) *Chem. Commun.* 957-959.