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Conformational Studies and Stereochemical Assignments of the Lactarane Sesquiterpenes Furoscrobiculin D and Blennin D[1]

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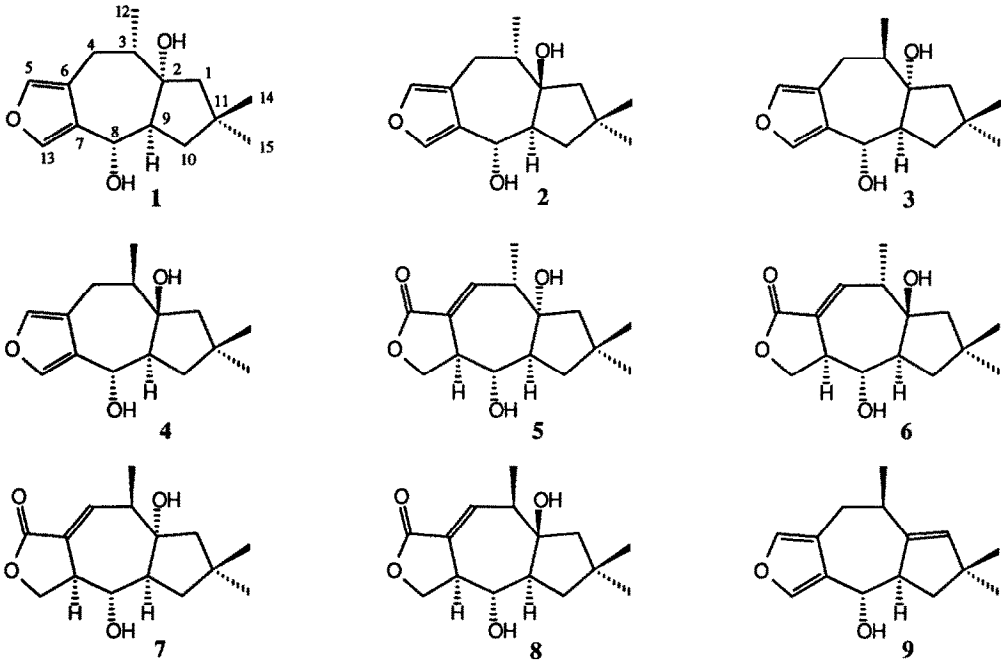
Abstract: Two lactarane sesquiterpenes, furoscrobiculin D and blennin D, previously isolated from the mushrooms *Lactarius scrobiculatus* and *Lactarius blennius*, respectively, have been submitted to conformational analysis by molecular mechanics. The results of the molecular modeling were related to the observed ^{1}H NMR data and allowed to assign stereostructure **4** to furoscrobiculin D, correcting the previous assignment **1**, and stereostructure **5** to blennin D, confirming the previous assignment.

Structure determination of new natural products relies mainly on spectroscopic grounds. Assignment of the relative configuration of the stereogenic centres in a molecule can, in particular, be performed by NMR techniques which often allow a careful description of the molecular shape in solution. However, when conformationally labile compounds are under investigation, spectroscopic data alone do not suffice and sure conclusions can be drawn only with the help of theoretical methods. Some years ago the isolation and characterization of a few lactarane sesquiterpenes obtained from the mushrooms *Lactarius scrobiculatus* and *Lactarius blennius* (Russulaceae) were described.[2,3] The structures **1** and **5** have been tentatively assigned to two of these sesquiterpenes, furoscrobiculin D[2] and blennin D,[3] respectively, on the basis of spectroscopic data. They are the only examples of 2-hydroxy lactarane sesquiterpenes isolated so far from Russulaceae species. Some time ago the configuration at C-3 of furosardonin A (**9**), which has H-3 cis to H-9, was revised.[4] As furosardonin A appears to be the dehydration product of furoscrobiculin D, this makes the configuration at C-3 of formula **1** rather suspicious. The stereochemical assignments of furoscrobiculin D and blennin D need, therefore, a reinvestigation. The seven-membered ring, present in these compounds with the lactarane skeleton, confers them a certain degree of conformational flexibility so that the NMR data need the support of theoretical conformational studies[5-7] in order to confirm, or to correct, such stereochemical assignments. To this aim, the conformational space of furoscrobiculin D and blennin D has been explored through a molecular mechanics approach and the results are reported here.

RESULTS AND DISCUSSION

The structure **1** has been assigned[2] to furoscrobiculin D on the basis of its spectroscopic (IR, MS and NMR) data. In particular, the ^{1}H NMR spectral data have suggested for the stereogenic centres C-2, C-3, C-8 and C-9 the configuration indicated in the formula **1**. However, while the configurations at C-8 and C-9 are sure enough, the configurations at C-2 and C-3 have been assigned only tentatively on the basis of induced solvent shifts and in analogy with similar compounds. Therefore, we performed molecular mechanics



calculations using the MM2 program[8] for the search of the conformational space of compound **1** and of its diastereoisomers **2**, **3**, and **4**. The conformational search strategy was mainly focused on the seven-membered ring whose puckering was investigated through the use of the single and the double driver option of the MM2 program extensively applied to the torsional angle of this ring. After location of all the conformers deriving from its puckering, single driving of a proper torsional angle inside the cyclopentane ring explored the possibility of the existence of further minima deriving from the different geometries of this ring. Moreover, full OH rotamer analysis was made for all conformers giving rise to a cluster of nine combinations of possible orientations of the two hydroxyl groups; only the data of the lowest energy conformation in each cluster are reported in the following discussion.

Table 1 reports the relative energies, the equilibrium percentages and selected torsional angles of the conformers of **1-4** found in a range of 5 kcal/mol above the global minimum of each stereoisomer. In three out of the four stereoisomers a conformer (**2A**, **3A**, and **4A**) accounts for more than 90% of the overall population while the stereoisomer **1** has two significantly populated conformations (**1A** and **1B**). More interestingly, differences in the geometries at both the seven and the five membered ring can be observed for the four diastereoisomers as can be seen from the selected torsional angles in table 1 and in the three-dimensional plots in figure 1. For each diastereoisomer the ^1H NMR vicinal coupling constants for the hydrogen atoms at C-3, C-4, C-8, C-9, and C-10 were then calculated[9] as weighted averages of the values of each conformer and are reported in table 2. It can be seen that compounds **1-4** have different patterns of coupling constants; a sure stereochemical assignment of furoscrobiculin D should be possible if all the five coupling constants of table 2 could be read in its ^1H NMR spectrum. A ^1H NMR spectrum of furoscrobiculin D, obtained at 100 MHz, is reported in reference 2. Several signals are described as multiplets and several coupling constants are not measurable on the spectrum. Therefore, a new spectrum of furoscrobiculin D was recorded in $CDCl_3$ on a high field instrument (500 MHz); however, a second order multiplet at 1.8-2.0 ppm due to H-9 and H_2-10 was still present. Nevertheless, the coupling constants and the chemical shifts were obtained by a Laocoon III simulation of the five spin system H-8, H-9, H_2-10, H-13 which gave all the spectral data (table 3) needed for the comparison with the values calculated for **1-4**. The close agreement of the experimental data with the values

Table 1. Relative Energies (kcal/mol), Equilibrium Percentages and Selected Torsional Angles (degrees) for the Conformers of Compounds **1-8**.

Conf.	E_{rel}	%	C-2—C-9—C-8—C-7	C-2—C-3—C-4—C-6	C-2—C-1—C-11—C-10	C-1—C-2—C-9—C-10
1A	0.00	63.6	79	79	-7	-38
1B	0.37	34.1	-67	73	9	45
1C	2.13	1.7	-63	-76	-21	23
1D	2.79	0.6	65	-75	-15	-43
2A	0.00	90.4	70	-72	8	41
2B	1.35	9.3	75	76	12	46
2C	3.35	0.3	42	44	-24	20
3A	0.00	99.1	-70	-73	-25	21
3B	3.31	0.4	68	-74	-14	-42
3C	3.40	0.3	73	80	-13	-41
3D	3.80	0.2	-53	77	-2	38
4A	0.00	95.5	74	-71	11	42
4B	1.85	4.2	44	43	-26	20
4C	3.45	0.3	72	74	12	45
5A	0.00	68.3	60	36	-33	14
5B	0.49	29.9	45	34	18	40
5C	2.15	1.8	56	-58	-16	-44
6A	0.00	100.0	65	-53	3	39
7A	0.00	90.4	48	32	-15	28
7B	1.57	6.4	-67	-68	-30	15
7C	1.97	3.2	54	-65	-17	-43
8A	0.00	100.0	67	-56	12	42

calculated for **4** ensures that furoscrobiculin D has the relative configuration indicated by this structure. So, the previous assignment of structure **1** to natural furoscrobiculin D should be revised into **4**.

Similarly to furoscrobiculin D, blennin D has been assigned[3] the stereostructure **5** on spectroscopic grounds. Also in this case the configurations at C-2 and C-3 need to be confirmed and a conformational study was performed with the same strategy previously applied to furoscrobiculin D.

Tables 1 and 2 and figure 1 report the results of these calculations. In addition, a new ^{1}H NMR spectrum of blennin D was recorded at 500 MHz. A simple first order approach allowed us to determine all the spectral parameters reported in table 3. A comparison of the calculated[9,10] and experimental coupling constants indicates that the previously assigned structure **5** is correct; in particular the experimental $J_{3,4}$ is close to the coupling constant calculated for **5**. This structure was further supported by the n.O.e. enhancement of H-3 (2.2 %), H-13β (1.8 %) and H-10β (1.7 %) on irradiation of H-8. In fact, the distance H-3—H-8 is calculated to be 2.44 and 2.53 Å for the two populated conformers **5A** and **5B**, whereas it is 4.56, 4.15, and 4.13 Å for **6A**, **7A**, and **8A**, respectively; therefore, the last three structures can confidently be excluded. Moreover, also the observed high (4.5 Hz) homoallylic coupling constant $^4J_{3,7}$ allows to exclude conformers **6A**, **7A**, and **8A** as the calculated torsional angles H-3—C-3—C-4—C-6 and C-4—C-6—C-7—H-7 are -168 and -71°, 146 and -90°, 63 and -68°, respectively, while they are both very close to 90° in **5A** and **5B** (-84 and -93°, -86 and -90°, respectively). Finally, the $^4J_{1\alpha,10\alpha}$ = 2 Hz cannot be explained by the torsional angles H-1α—C-1—C-11—C-10 and C-1—C-11—C-10—H-10α of conformers **6A**, **7A**, and **8A**, as at least one of them is far from 180° (-116 and 145°, -138 and 156°, -105 and 138°, respectively) compared to -159 and 164° in conformer **5A**.

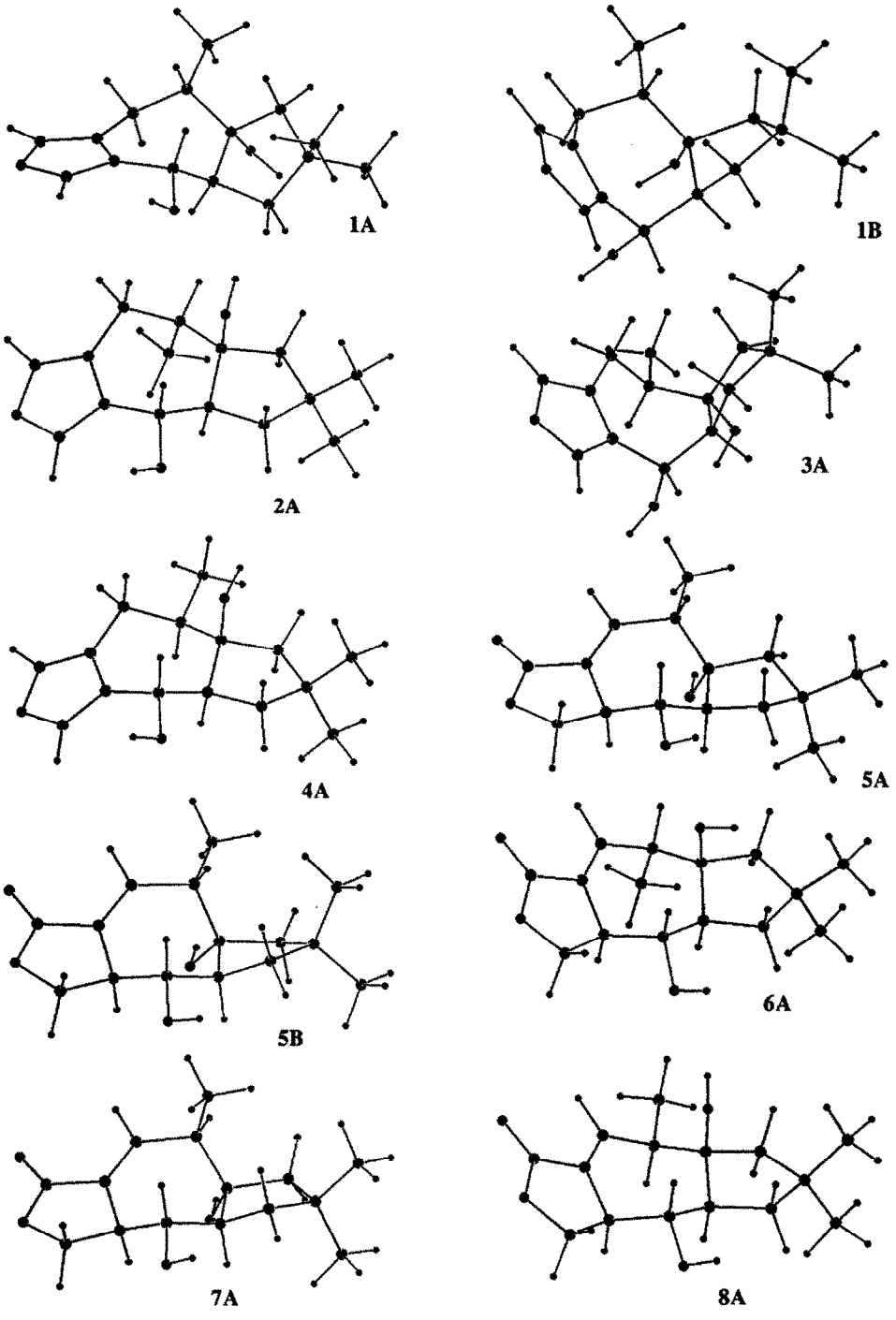


Fig. 1. Three-dimensional plots of the most populated conformations of compounds **1-8**.

Table 2. Calculated ^{1}H NMR Vicinal Coupling Constants (Hz) of Compounds **1-8**.

Compound	$J_{3,4\alpha}$	$J_{3,4\beta}$	$J_{8,9}$	$J_{9,10\alpha}$	$J_{9,10\beta}$		
1	11.5	1.4	8.9	6.2	5.0		
2	4.9	2.3	10.9	5.5	11.6		
3	2.6	12.3	5.7	5.5	11.7		
4	2.1	11.7	10.9	5.6	11.6		
Compound	$J_{3,4}$	$J_{7,13\alpha}$	$J_{7,13\beta}$	$J_{7,8}$	$J_{8,9}$	$J_{9,10\alpha}$	$J_{9,10\beta}$
5	2.7	10.7	8.0	10.9	9.5	6.0	11.3
6	6.4	8.5	9.4	10.4	10.5	5.5	11.6
7	5.3	10.5	8.1	10.2	8.6	5.2	11.6
8	4.2	8.1	9.5	10.6	10.7	5.6	11.6

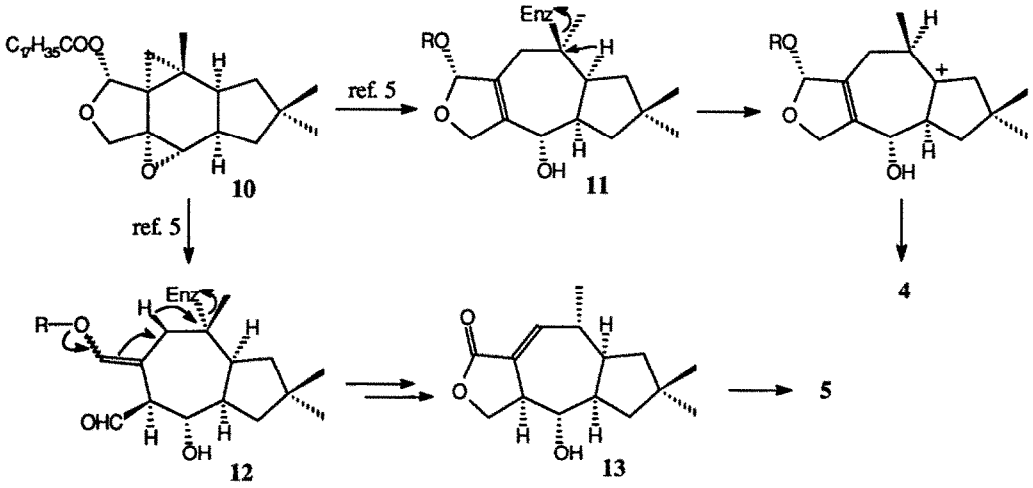
Table 3. ^{1}H NMR Spectral Data ($CDCl_3$, 500 MHz) for Compounds **4** and **5** (δ_H values in ppm, J in Hz).

Furoscrobiculin D (**4**)					Blennin D (**5**)				
Proton			J		Proton			J	
1α	1.644	d	1α,1β	14.3	1α	1.642	dd	1α,1β	14.0
1β	1.676	d	3.4α	2.3	1β	1.843	d	1α,10α	2.0
3	1.561	ddq	3,4β	11.8	3	2.556	ddq	3,4	2.5
4α	2.225	dd	3,12	7.0	4	6.486	dd	3,7	4.5
4β	2.554	ddd	4α,4β	14.8	7	3.426	m	3,12	7.5
5	7.136	t	4β,5	1.8	8	3.611	td	4,7	3.0
8	4.804	m	5,13	1.8	9	2.454	ddd	7,13α	9.2
9	1.931	m	8,13	1.7	10α	1.957	ddd	7,13β	9.2
10α	1.860	m	8,9	10.2	10β	1.285	t	13α,13β	9.2
10β	1.926	m	9,10α	7.0	12	1.240	d	7,8	10.0
12	0.980	d	9,10β	12.7	13α	4.538	t	8,9	9.5
13	7.265	t	10α,10β	12.4	13β	4.079	t	8,OH	4.0
14	1.001[a]	s			14	1.043[b]	s	9,10α	6.5
15	1.173[a]	s			15	1.154[b]	s	9,10β	12.5
								10α,10β	12.5

[a,b] These assignments may be interchanged.

It is worthy of note that the different electronic environment determined by the configurations at C-2 and C-3 of furoscrobiculin D and blennin D heavily influences the chemical shifts of the cyclopentane protons. A comparison of the data in table 3 shows the expected deshielding of H-10β in **4** with respect to **5** (1.926 vs 1.285 ppm) and of H-9 in **5** with respect to **4** (2.454 vs 1.931 ppm).

In conclusion, both furoscrobiculin D (**4**) and blennin D (**5**) have the 2-OH and the 3-Me cis to each other; however, the two substituents are trans to H-9 in the former compound and cis in the latter lactarane lactone. These subtle stereochemical details are extremely important in defining the possible biosynthetic routes to Russulaceae sesquiterpenes. It has been shown in several instances[5,11,12] that velutinal esters (*e.g.* **10**) are the enzymatic precursors of the furane and lactone lactaranes. The conversion of velutinal derivatives to furoscrobiculin D (**4**) in *L. scrobiculatus* and blennin D (**5**) in *L. blennius* involves, among other things, the opening of the cyclopropane ring and the introduction of a H-3 not present in the original marasmane skeleton. Recently, we have proposed a comprehensive biosynthetic scheme for all lactarane sesquiterpenes;[5] it indicates that H-3 in the lactaranes may originate from **10** itself, although through to two different mechanisms. According to this picture, the stereochemistry at C-3 of furoscrobiculin D (**4**), as well as that of other furane



lactaranes as furosardonin A (**9**),[4] may originate from an intermediate like **11** (or a carbocation equivalent at C-3)[13] through a hydride shift from C-2 to C-3. This would leave a formal carbocation at C-2 which can be eventually hydroxylated by an external nucleophile. On the contrary, the stereochemistry at C-3 of blennin D (**5**) may occur by a β-hydride shift from C-4 to C-3 and a double bond migration in an intermediate like **12**. An internal Cannizzaro reaction would eventually install the lactone ring of **5**, while the mechanism of the C-2 oxidation appears intriguing, as it occurs with retention of the original H-2 configuration of sesquiterpene **10**. Indeed, blennin D (**5**) may be considered the 2-OH derivative of blennin A (**13**).[3] Therefore, it is likely that the hydroxylation step leading to blennin D (**5**) takes place with a different mechanism than for furoscrobiculin D (**4**), thus confirming the intricate biochemistry of Russulaceae sesquiterpenes.[5]

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