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ACHILLEOL B: A NEW TRICYCLIC TRITERPENE SKELETON FROM *ACHILLEA ODORATA* L.

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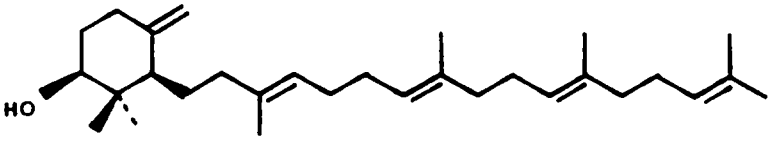
Abstract: Achilleol B (**2**), a new tricyclic triterpene, has been isolated from *Achillea odorata* L. and its structure established on the basis of its NMR data obtained through n.O.e., short and long-range homo and heteronuclear correlation experiments and other spectroscopic evidence.

In a previous paper[1] we reported on the isolation from *Achillea odorata* L. of a minor component, with a monocyclic triterpene skeleton, named Achilleol A (**1**), which could be biosynthesized in the first step of cyclization of squalene oxyde[2-4].

Now we describe the isolation from the same specie of a new minor metabolite, Achilleol B (**2**), structurally related to **1**.

The molecular formula ($C_{30}H_{50}O$) was determined from its HREIMS, that contained a molecular ion at m/z 426.3870, suggesting it to be an isomer of **1**. The IR spectrum showed absorption bands for hydroxyl (3394 cm^{-1}), exocyclic terminal methylene (1644 and 892 cm^{-1}), trisubstituted carbon-carbon double bond (1662 cm^{-1}) and gem-dimethyl (1385, 1365 and 1195 cm^{-1}) groups. The NMR ^1H spectrum (Table 1) contained signal for protons related with those present in **1**. Thus, a signal at δ 3.40 (dd, 1H, J=10.0 and 4.0 Hz) was related to a geminal proton to an equatorial hydroxyl group in a cyclohexane ring, whereas the broad singlets at δ 4.62 and 4.88 were assigned to an exocyclic methylene group. The presence of a gem-dimethyl group (two singlets at δ 0.72 and 1.02 ppm) was established on the basis of long range proton-proton and also reverse carbon-proton correlation. Finally, the singlet and broad triplet at δ 1.60 and 5.12 were correlated with a methyl group and a vinyl proton of a trisubstituted carbon-carbon double bond, respectively. These structural features confirmed the presence of the partial structure ⓐ in compound **2**, as could be also shown by the comparison of its ^{13}C NMR spectrum (Table 1) with that of **1**.



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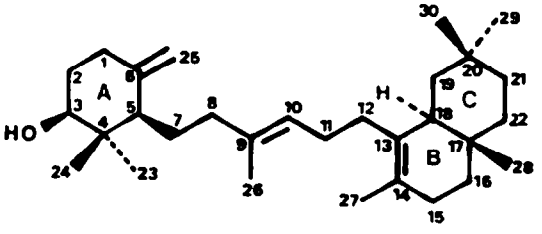


2

A first indication of the structure of the R moiety in ⓐ was obtained from the study of the remaining signals in the ^1H and ^{13}C NMR spectra. Thus, four methyl singlets, three of them on a sp^3 (δ 0.81, 0.86 and 0.88) and one on a sp^2 carbon (δ 1.56), together with the absence of olefinic protons, were observed. Furthermore, the ^{13}C NMR spectrum, using DEPT and J-Modulation experiments, showed the existence of 4 CH_3, 6 CH_2 (sp^3), 1 CH (sp^3), 2 C (sp^3) and 2 C (sp^2). This data together with the molecular formula suggested the presence in R of a bicyclic system containing a tetrasubstituted carbon-carbon double bond.

The structural elucidation of the bicyclic moiety in **2** was established using bidimensional experiments, including homo- (LRCOSY[5], DQFCOSY[6], HOHAHA[7]) and heteronuclear (normal, long range and reverse long range[8] C-H correlations). The directe C-H correlation experiment allowed the unambiguous assignement of the most of the signals of ^1H and ^{13}C NMR spectra, corresponding to the bicyclic moiety in **2**. Furthermore, the results of the various 2D correlations, mainly DQFCOSY (Fig. 1), not only confirmed the above assignements, but also stated the following fragments for that moiety:

I: q_2-CH(18)-CH_2(19α,19β)-q II: q-CH_2(21α,21β)-CH_2(22α,22β)-q

III: q-CH_2(15α,15β)-CH_2(16α,16β)-q IV: CH_3(29)-q-CH_3(30)

V: CH_3(28)-q VI: CH_3(27)-q=q

The double quantum filtered (DQF)COSY sequence was selected in order to avoid the methyl-group crowd on the diagonal. The neatness of the 0.7-2.0 region, once the methyl groups removed, shows the great usefulness of this technique.

As the bicyclic system of the molecule only has two sp^3 quaternary carbon, it follows that the fragment I must be linked to VI, and since the H-19α and H-19β have chemical shifts different to those expected for allylic

Table 1. Proton and Carbon-13 NMR Data for Achilleol B

C	^{1}H	^{13}C	COSY*	long-range coupling H/H	long-range coupling C/H
1 α	2.32 ddd (13.2,4.4,4.4)	33.0		2β,3,7,25	
β	1.95 m				
2 α	1.47 m	32.2		1α,3	
β	1.85 m			1α,3,7	
3	3.40 dd (10.0,4.5)	77.3		1α,2β,7,24	
4		40.5			
5	1.62 m	51.0		24,25,25'	23,24
6		147.3			
7	1.47 m	23.7		10	5,8,8'
	1.60 m				
8	1.75 ddd (13.2,10.3,6.5)	38.6		7,8',10	
	2.07 ddd (13.2,10.3,3.8)			7,8	
9		135.2			
10	5.12 m	124.6		7,8,11'	
11	1.87 m	29.5		8	15α,15β,27
	1.96 m				
12	1.70 m	31.6			11'
	2.20 ddd (13.1,10.0,6.6)				
13		133.7			
14		123.9			
15 α	0.80 m	26.5		16α,16β,18	11,11',28
β	1.90 m			27	
16 α	1.85 m	27.1		15α,28	28
β	1.95 m			15α,28	
17		31.4			21α,28
18	1.62 m	42.3	19α,19β	15α,16β,19β,24	19α,22β,28
19 α	1.37 ddd (13.0,3.7,2.6)	43.0	18,19β	21α,29,30	21α,30
β	0.95 dd (13.0,13.0)		18,19α	18,30	
20		31.0			15α,15β,19β,22β,29,30
21 α	1.10 m	34.6	21β,22α,22β	19α,21β,22α,22β	21β,22α,22β,30
β	1.33 dd (13.8,3.9)		21α,22α,22β	21α,22α,22β,29,30	
22 α	1.48 dd (13.3,4.2)	36.5	21α,21β,22β	21β,22β	19α,22β,28
β	1.21 ddd (13.3,3.5,3.5)		21α,21β,22α	21α,21β,22α,28	
23	0.70 s	25.8		5,24	
24	1.02 s	15.4		23	
25	4.62 m	108.3		1,5,25'	
	4.88 m			5,25	
26	1.60 bs	16.0		8	8,8'
27	1.56 bs	18.6		15β	16β
28	0.81 s	26.9		16β,22β	22α
29	0.88 s	24.2		19α,21β	19β,30
30	0.86 s	33.2		19α,19β,21β	29

* Only correlations for ring C are indicated.

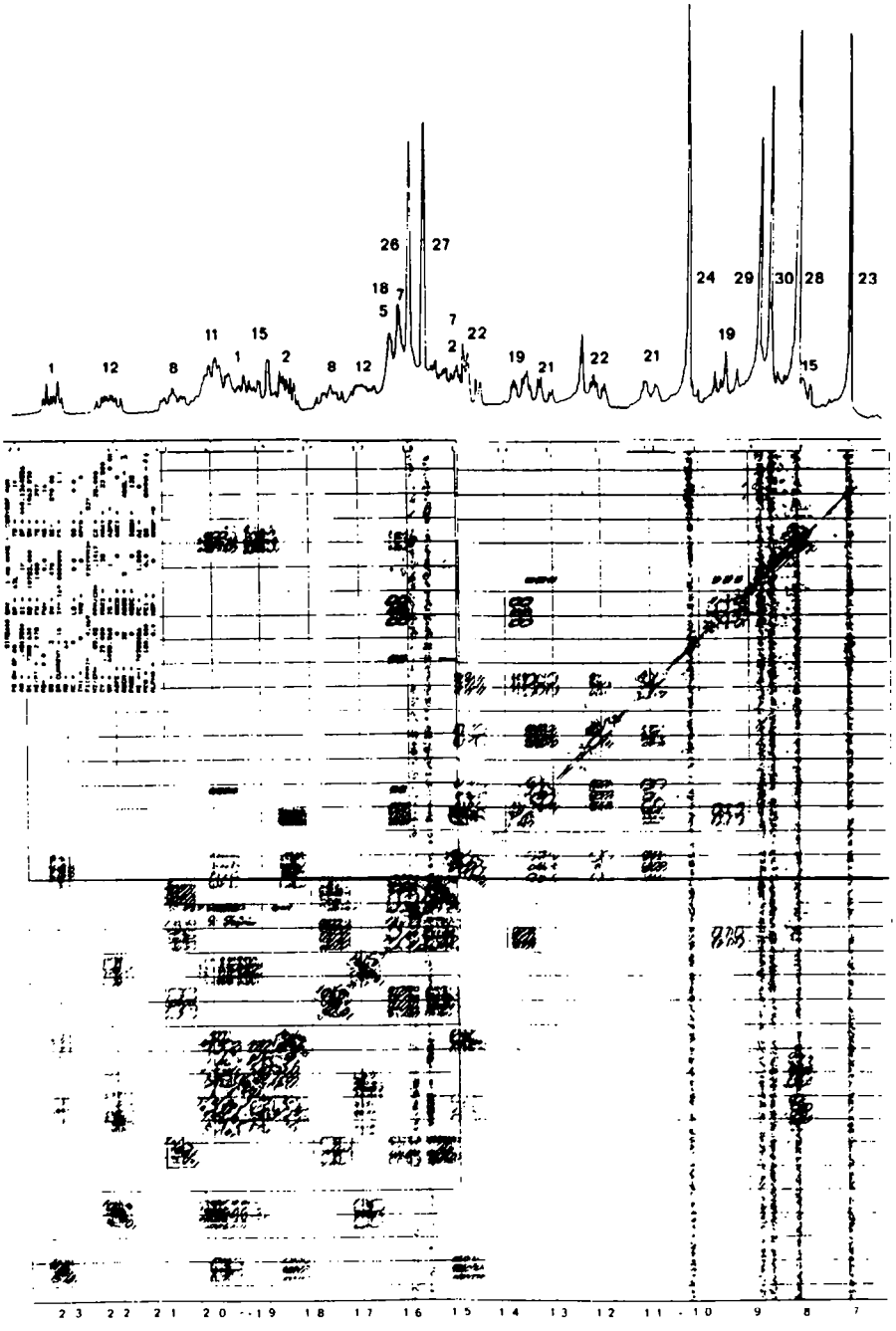
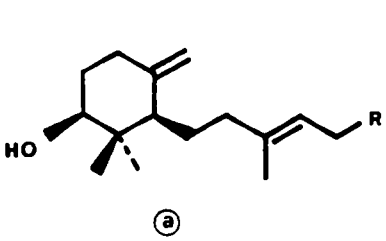


Figure 1. Double quantum filtered (DQF) COSY of Achilleol B



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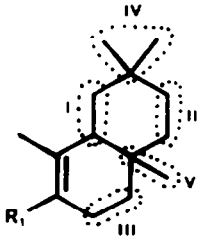

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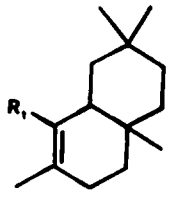


ⓒ

protons, they must be linked through C-18. The connections of the remaining fragments were established on the basis of the data found in the H-H long range correlation NMR experiments. Correlation between H-19α with H-21α and Me-29, and the later with H-21β, showed that the fragment I was connected to IV, and this with II, giving a moiety of the type $CH-CH_2-C(CH_3)_2-CH_2-CH_2-$. On the other hand, interaction of H-22β with Me-28 connect II with V, whereas that of H-18 with H-15α (homoallylic coupling) revealed the connection between III and the above fragments. According to these findings, partial structures ⓑ or ⓒ could be assigned to Achilleol B. The reverse long range C-H correlation spectrum not only confirmed the proposed structures, but also allowed to complete the assignement of the signals in the ^{13}C NMR spectrum.

A $^1H-^1H$ homonuclear correlation using the HOHAHA experiment (Table 2) showed to be very useful for structural elucidation of the acyclic moiety of the molecule, allowing the unambigous assignement of H-8 through H-12 signals.

Table 2. H-H correlations in the Homonuclear Hartman Harn (HOHAHA) spectrum of Achilleol B*

H	H			
1α	1β	2α	2β	3
1β	1α	2α	2β	3
2α	1α	1β	2β	3
2β	1α	1β	2α	3
3	1α	1β	2α	2β
7	8	10	11	12
8	7	10	11	12
10	7	8	11	12
11	7	8	10	12
12	7	8	10	11

*Only easily recognizable correlations are indicated.

The choice of the proper structure rests on the δ value (18.6 ppm) of the methyl group at the carbon-carbon double bond. This value is consistent with structure ⓒ since such methyl group should be affected by a δ-gauche interaction with C-19 in ⓑ, and show a corresponding up-field shift. In addition, the structure ⓒ would be in accordance with the biogenetic mechanism proposed for the formation of ring E in some pentacyclic triterpenes[9].

The stereochemistry of the bicyclic system could be established from the value of the coupling constants in the ^{1}H NMR spectrum. Thus, $J_{18,19\beta}$=13 Hz indicated antiperiplanar disposition for such protons and so a *trans* ring junction, whereas the chair conformation of ring C was confirmed by the $J_{21\beta,22\alpha}$=13.7 Hz, the long-range coupling between H-19α and H-21α ("W" coupling) and the homoallylic coupling of H-18 with H-15α.

Ring B can adopt either a half-chair or a sofa (1,2-diplanar) conformation. Examination of the Dreiding model (combined with an MM2 calculation[10]) revealed that the axial methyl groups should show a strong n.O.e. effect with the suitably situated ring protons. Indeed, the 1D NOEDIFF experiments revealed the spatial proximity depicted on Fig. 2 as expected. There was no n.O.e. between H-18 and C-17 methyl group which implies a *trans* ring junction in agreement with the coupling information.

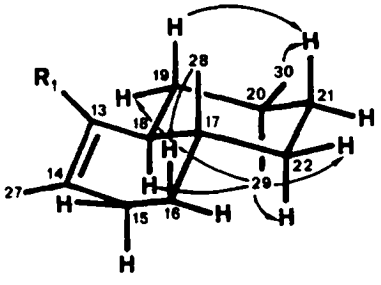
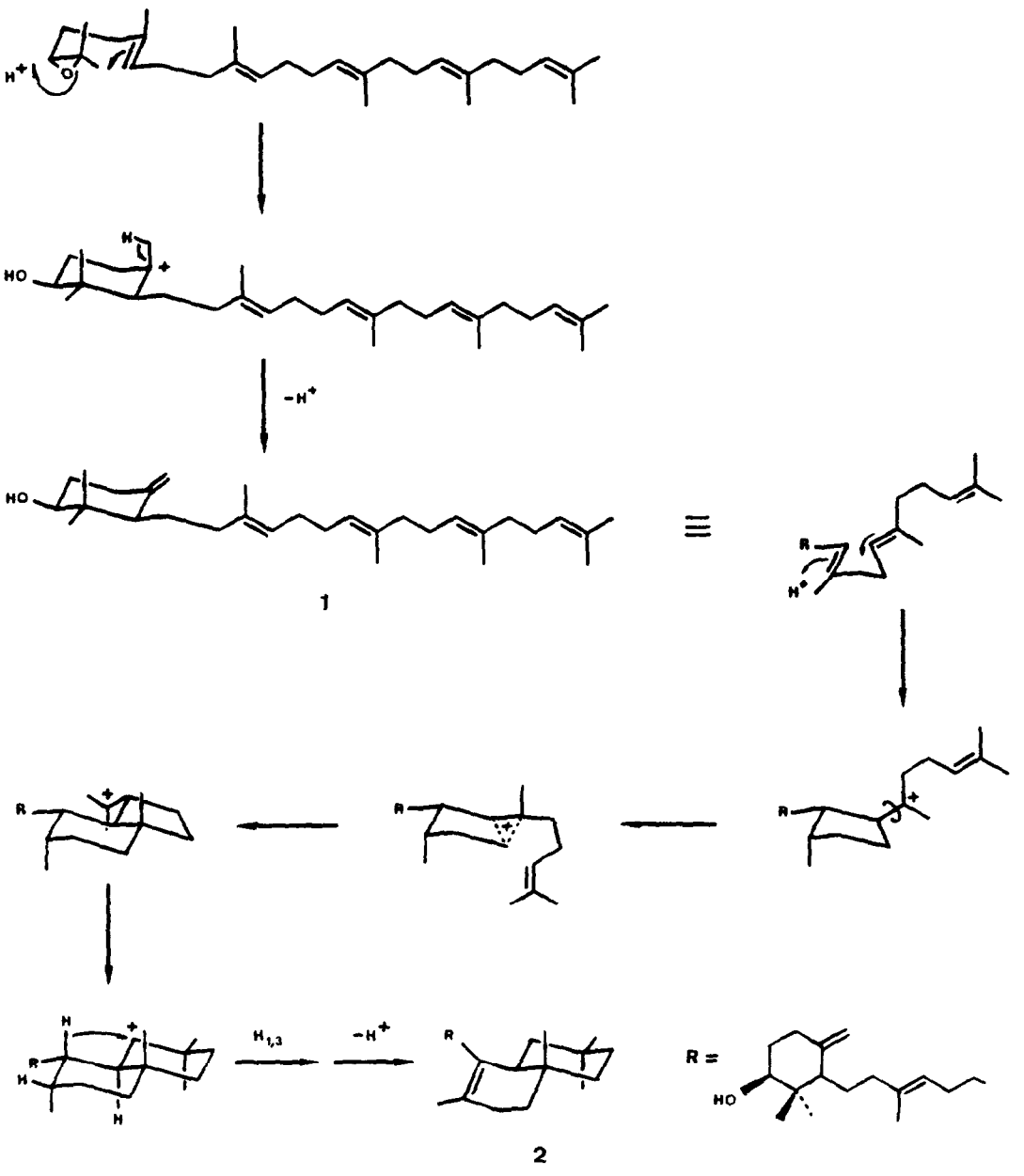


Figure 2. N.O.e.'s deduced from 1D NOEDIFF

From a biosynthetic point of view it seems to be reasonable that this tricyclic triterpene might be formed from Achilleol A. The bicyclic system in Achilleol B could arise in analogous way to that of the D and E ring in some pentacyclic triterpene. The anomalous *trans* ring junction in the triterpene reported herein must imply a deviation in the last steps of the biosynthetic pathway. The carbocationic intermediate proposed in the β-amirine type pentacyclic triterpene biosynthesis, where a 1,2 hydrogen migration is postulated, must proceed in a different way. Thus, a 1,3 hydride shift leading to a subsequent proton elimination should afford Achilleol B (Scheme 1). The absolute stereochemistry for rings B and C is based on the precedent biosynthetic pathway.



Scheme 1. Proposed biosynthetic pathway from squalene oxide to Achilleol B

Experimental

General. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. IR spectra were obtained on a Perkin-Elmer 983 G spectrophotometer. High resolution Mass spectral data were recorded on a Varian MAT 311 instrument. ^1H NMR spectra were determined on a Brucker AM 400 or AM 600 spectrometer (400 and 600 MHz respectively), whereas ^{13}C NMR spectra were obtained on a Bruker AM 300 instrument. 2D NMR and 1D NOEDIFF experiments were performed on a Bruker AM 400 spectrometer.

Determinations of n.O.e.'s by the NOEDIFF method were performed with the aid of Aspect 3000 microprograms which allowed direct accumulations of difference FID's. HOHAHA was recorded according to reference 7. The data matrix was 256x1024 pts (NE 256, SI 1K) zero filled to 1024x1024 (two level zero filling) before FT, total mixing time: 45 ms, filters S in both dimensions, SSB 4 in both dimensions, MC2 W, SW1=SW2=2000 Hz. For the reverse LRCH correlation[8] the data matrix was 1024x1024 (zero filled in F1 dimension before FT), SW2=1915 Hz, SW1=25000Hz, NS=128, DS=2, filters: cos^2 in both dimensions. For the DQF the matrix size was 512x1024 pts zero filled to 1024x1024 before FT. The surface is shown in phase sensitive manner with the TPPI method[11]. The double quantum filter was preferred to normal COSY, since it yields in-phase diagonal and correlation peaks, which appear sharper and also cleans-up the diagonal. We used the usual $90°-t_1-90°-90°$ sequence.

Extraction and Isolation. Specimens of *Achillea odorata* L. were collected in May 1987 in Sierra Nevada (1700 m altitude), Granada (Spain). Extraction with hexane in a Soxhlet system of air dried plant (1580 g) gave a residue (67.0 g), which was defatted ($HCCl_3$-MeOH) to yield a crude (36.4 g), that after saponification and subsequent chromatographies on silica gel columm, using hexane-diethyl ether mixtures as eluant, afforded **2** (60 mg).

Achilleol B (**2**) was isolated as a viscous oil, $[\alpha]_D$ -8.1° (c 1.10, $HCCl_3$); HREIMS m/z (%): 426.3870 [M$^+$, calc. for $C_{30}H_{50}O$, 426.3861], 411 (16), 409 (5), 393 (5), 205 (10), 191 (21), 189 (23), 175 (37), 135 (42), 109 (100), 95 (60). IR (neat) ν_{max} cm^{-1}: 3394, 3080, 1662, 1644, 1385, 1365, 1195, 1085, 892. ^1H and ^{13}C NMR (Table 1).

Acknowledgements

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