

One- and Two-Dimensional NMR in the Structure Determination of 3 β -Acetoxy-17 β ,21 β -epoxyhopane from *Centaurea chilensis*

Rudolf Hartmann and Eberhard Breitmaier

Bonn, Institut für Organische Chemie und Biochemie der Universität

Rodrigo C. Camargo, Rosa Negrete, Nadine Backhouse and Carla Delporte

Santiago (Chile), Laboratorio de Farmacognosia, Facultad de Ciencias Químicas y Farmaceuticas, Universidad de Chile

Bruce K. Cassels

Santiago I (Chile), Departamento de Química, Facultad de Ciencias, Universidad de Chile

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Introduction

The genus *Centaurea* (*Asteracea-Cyanareae*) is represented in Chile by the native *C. chilensis* and *C. floccosa* and the introduced weeds *C. melitensis* and *C. solstitialis* as well as the cultivated *C. cyanus*. Earlier chemical studies on *C. chilensis* Hook et Arn. have afforded β -sitosterol and stigmasterol, the guaianolides dehydrocostus lactone and 8 α -hydroxydehydrocostuslactone [1], 8 α -acetoxydehydrocostuslactone [2], and 11 β H-11,13-dihydrodesacylcynaropicrin-8 β -D-glucoside, as well as the flavonoids apigenin-7-O-glucoside, chrysoeriol-7-O-glucoside, chrysoeriol, luteolin, kaempferol, quercetin, and hispidulin [3]. Further study of the chloroform extract yielded a compound which strained bright pink with Liebermann-Burchard reagent and was thus presumed to be a triterpene. The mass spectrum and an extensive ^1H and ^{13}C one- and two-dimensional NMR study [4, 5] led to the 3 β -acetoxy-17 β ,21 β -epoxyhopane structure (1–4).

Experimental

General experimental procedures

Melting points were determined on a Kofler-Leitz hot stage apparatus. IR spectra (KBr disc) were recorded utilizing a Leitz III G infrared spectrometer. NMR spectra in CDCl_3 with TMS as internal standard were recorded at 400 MHz (^1H) and 100.5 MHz (^{13}C) on a Bruker AM 400 spectrometer, using commercially available programs for multipulse experiments (DEPT), shift correlations (H,H-COSY, C,H-COSY, C,H-COLOC) and H,H-NOE-difference spectroscopy (NOEDS) [4, 5]. The high-resolution electron impact mass spectrum (HREIMS) was recorded on a Kratos instrument.

Plant material

Aerial parts of *Centaurea chilensis* were collected in December (early summer) in the foothills of the Andes near Rio Blanco (32° 50' S latitude), Chile. A voucher specimen (Herbarium No. 16642) is on deposit in the Botany Laboratory of the Faculty of Chemical and Pharmaceutical Sciences, University of Chile.

Extraction and Isolation of 3 β -Acetoxy-17 β ,21 β -epoxyhopane (1)

The air-dried, ground plant material (4.3 kg), was extracted successively with light petrol and CHCl_3 yielding respectively 150 and 300 g of residue after removing the solvents. The chloroform extract was chromatographed on a silica gel G column, eluting with light petrol / CHCl_3 mixtures in which the polarity was increased by 5 % (v/v) increments of the latter solvent at each stage. The triterpene discussed here eluted in the 60 % to 70 % CHCl_3 fractions. This product was purified by crystallization in MeOH; white microcrystals, m. p. 290 °C, readily soluble in CHCl_3 , slightly so in MeOH, practically insoluble in light petrol, giving a bright pink Liebermann-Burchard reaction. HREIMS m/z (rel. int.) 484.3930 [M^+ , calcd. $\text{C}_{32}\text{H}_{52}\text{O}_3$ 484.3916] (100). NMR data: formulae 1–4.

Results and Discussion

The HREIMS of this product showed a molecular ion at m/z 484.3930 (100 %), corresponding to the molecular formula $\text{C}_{32}\text{H}_{52}\text{O}_3$, with seven double bond equivalents. The proton broadband decoupled ^{13}C NMR spectrum showed 32 signals, all of which but one at 171.16 ppm (assignable to an ester or carboxylic acid carbonyl) lie in the 16–81 ppm range. As no olefinic carbon resonances appear in the

Table 1 ^{13}C and ^1H n.m.r. chemical shifts from the 100 MHz ^{13}C and 400 MHz ^1H n.m.r. spectra. Assignments are based on the 100/400 MHz proton-decoupled C,H-COSY.

C-Atoms.	$\delta^{13}\text{C}$ [ppm]	CH_n	$\delta^1\text{H}$ [ppm]		$^3J_{\text{HH}}$ -coupling [Hz]			
31	171.16	C						
3	81.04	CH	4.48		10.0	d;	6.5	d
17	76.49	C						
21	75.88	C						
5	55.31	CH	0.79					
9	50.46	CH	1.25					
18	43.44	C						
13	43.32	CH	1.80		12.5	d;	3.5	d
8	42.17	C						
14	41.88	C						
1	38.50	CH_2	1.69	1.00				
4	37.86	C						
10	37.12	C						
19	34.62	CH_2	1.15	0.99	11.8	d;	7.5	d
7	33.29	CH_2	1.47	1.30				
15	29.25	CH_2	1.75	1.25	13.0	d;	8.5	d
22	28.59	CH	1.69		7.0			sep
23	28.05	CH_3	0.85					
2	23.77	CH_2	1.62	1.62				
20	23.33	CH_2	1.72		14.0	d;	8.0	d
					14.0	d;	10.0	d
					7.5			d
12	23.27	CH_2	1.43	1.33				
32	21.42	CH_3	2.04					
11	21.06	CH_2	1.51	1.34				
16	20.25	CH_2	2.04	1.28	14.0	t;	4.8	d
29	19.22	CH_3	0.94		7.0			d
30	18.50	CH_3	1.05		7.0			d
6	18.32	CH_2	1.52	1.39				
28	17.99	CH_3	0.82		1.0			d
26	16.65	CH_3	1.02					
24	16.56	CH_3	0.83					
25	16.05	CH_3	0.84					
27	15.93	CH_3	1.03		0.7 d			

spectrum, the compound must have a hexacyclic structure. The DEPT subspectra, recorded with 90° and 135° polarization transfer pulses [4], showed the presence of five CH, ten CH_2 and nine CH_3 groups accounting for all the hydrogen atoms which are therefore directly bonded to carbon. Comparison with the broadband decoupled spectrum pinpointed eight quaternary carbon resonances including the signal at 171.2 ppm which could now be assigned unambiguously to an ester function. The molecular formula, the number of rings and the fairly large number of methyl groups suggested at this point that the compound is a saturated pentacyclic triterpene derivative with an extra ring. Three downfield signals at 81.04 (CH), 76.49 (C) and 75.88 ppm (C) are indicative of the expected alcohol moiety of the ester and an oxiran ring.

All the signals in the ^1H NMR spectrum, with the exception of the one at 4.48 ppm, corresponding to a proton which must be deshielded by an oxygen atom and therefore should be bonded to the 81.0 ppm carbon nucleus, lie within the 8.0–2.1 ppm range (Table 1). A strong singlet at

2.04 ppm can be assigned immediately to the acetyl group. The other eight methyl groups (according to the DEPT subspectra) give ten signals, and therefore two of these groups must be bonded to one or more CH groups, appearing as doublets. In fact, two strong doublets ($J=7.0$ Hz) can be identified at 0.94 and 1.05 ppm. Owing to the dense sequence of signals and the expected high multiplicity of most of them, a C,H-COSY with proton-proton decoupling [6], which only shows geminal splittings in the proton dimension, was helpful to assign all the proton chemical shifts (Table 1).

The H,H-COSY indicates the presence of an isopropyl group with methyl protons resonating at 0.94 and 1.05 ppm, correlated with the methine proton at 1.69 ppm. Some other partial structures (two $\text{CH}_2\text{-CH}_2$ moieties with $\delta=1.75-1.25/2.04-1.28$ and $1.15-0.99/1.72-1.62$ and a $\text{CH}_2\text{-CH}$ fragment with $\delta=1.43-1.33/1.80$) can also be deduced from the spectrum. Further interpretation is not possible due to the high signal density even in the 400 MHz H,H-COSY. In all, twenty CH and CH_2 proton signals overlap in

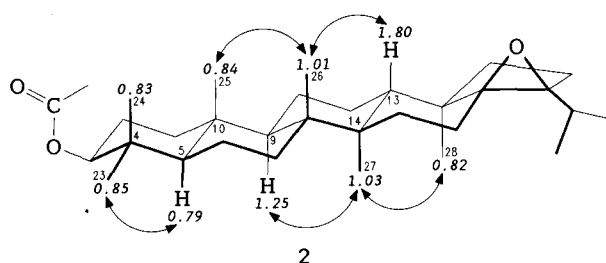
Table 2 ^{13}C n.m.r. chemical shifts of carbon 1–10 and 23–25 of 3 β -acetylupane [7, 8] and compound **1** isolated from *Centaurea chilensis*

C-Atoms.	3 β -Acetylupane	compound 1	CH _n
1	38.3	38.50	CH ₂
2	23.6	23.77	CH ₂
3	80.7	81.04	CH
4	37.7	37.86	C
5	55.3	55.31	CH
6	18.2	18.32	CH ₂
7	34.1	33.29	CH ₂
8	40.7	41.88	C
9	50.2	50.46	CH
10	37.0	37.12	C
23	27.9	28.05	CH ₃
24	16.5	16.56	CH ₃
25	16.1	16.05	CH ₃

the 1.2–1.8 ppm region. – Comparing the measured ^{13}C chemical shifts (Table 2) with literature values [7, 8] for pentacyclic triterpenes such as lupane of hopane derivatives, which also carry an isopropyl group, good agreement is found with the C-1 to C-10 resonances of compound bearing a hydroxy or an acetoxy group at C-3.

A C,H-correlation via two- and three-bond CH coupling (C,H-COLOC, Table 3), optimized for an average CH coupling constant of 6 Hz, was recorded in order to complete the structure assignment. The C,H-COLOC led to the unambiguous identification of several partial structures and to the assignments of C-1 to C-10 in rings A and B (formula **1**). At this point, NOE-difference spectra (NOEDS [5] were also useful. Irradiating the methyl protons (except the acetyl and isopropyl ones) at low power, significant NOE enhancements can be observed for the neighboring protons (formula **2**, arrows). These spectra indicate that methyl groups 27 and 28 are cis-coaxial to each other, and are trans with regard to the also coaxial methyl groups 25 and 26. The protons resonating at 1.80 and 1.75 ppm (15-H_a) are axial and cis with regard to methyl 26. Those resonating at 2.04 (16-H_a) and 1.25 (9-H) ppm are axial and cis with regard to methyl 28.

Comparison of the ^{13}C chemical shifts of C-12 and C-13 [8] in lupane and hopane derivatives shows that in the lupane series C-13 is clearly shielded and C-12 is deshielded by the axial methyl group 28 at C-17. In the hopane series C-12 is shielded and C-13 is deshielded, as here the axial

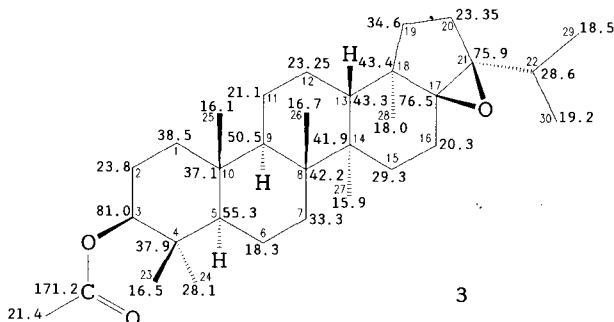
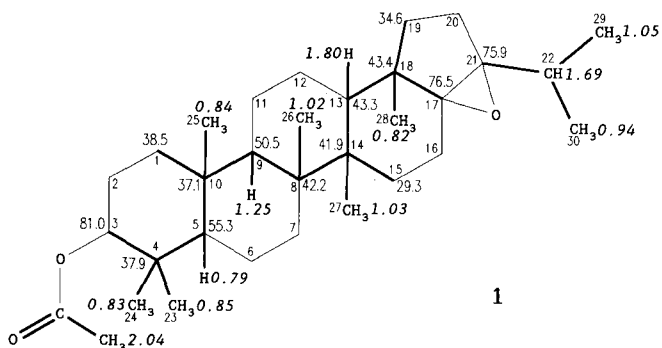


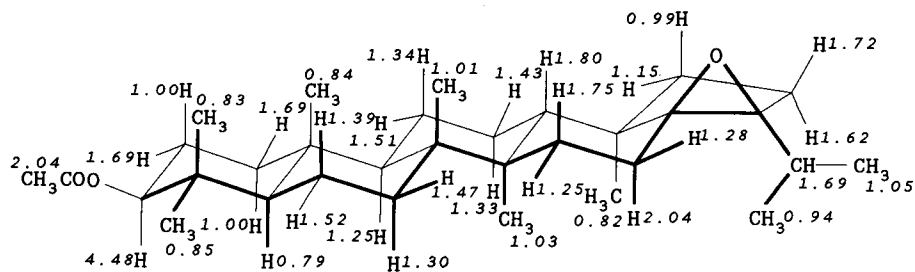
methyl group 28 is bonded to C-18. According to the NOEDS, the substance from *C. chilensis* is a hopane-type compound, with similar C-12 and C-13 chemical shifts. Combining all the aforementioned structure elements in a hopane skeleton, formula **1** can be deduced, in which the C-9/C-11/C-12, C-14/C-15, C-16/C-17/C-21/C-20 and C-18/C-19 bonds have not yet been determined.

The C,H-COLOC completes the necessary information: The carbon atom which shows a chemical shift of 42.17 ppm correlates over two and three bonds with the methyl protons at 1.02, 1.03 ppm and one proton of CH₂-11 at 1.51 ppm, and can thus be unequivocally assigned to C-8. The 41.88 ppm resonance, in contrast to the literature (Table 2), must be assigned to C-14, as it is coupled to CH₂-16 resonating at 1.28 ppm. C-17 correlates with 15-H. The signal at 43.44 ppm correlates with the proton resonance at 1.72 ppm, bonded to C-20, and a similar correlation can be seen between C-21 and the 19-H signal at 1.15 ppm. An oxiran ring must therefore involve C-17 and C-21, as the molecule, with seven double bond equivalents, must have six rings. The bond between C-11 and C-12 is not evidenced in this experiment.

The proton with chemical shift 4.48 ppm resonates as a doublet of doublets with coupling constants of 10 and 6.5 Hz, corresponding to an anti coupling with 2_a-H and a syn coupling with 2_e-H. 3-H must therefore be axial, and the acetoxy group equatorial. The orientation of the methyl groups is clear from the NOEDS, so that finally the configurations of the isopropyl group and the oxiran ring remain to be determined. Molecular models only allow a configuration in which the C-28 methyl group at C-18 is axial. The oxiran ring can only lie trans with regard to methyl-18, as a cis orientation would involve excessive steric compression between the isopropyl group and the axial 13-H and 15-H, and the oxiran ring tension would be too large.

The relative configuration of only some of the protons (with resolvable couplings) can be determined on the basis of the NOEDS. The signal at 1.15 ppm (19-H) exhibits a geminal coupling (11.8 Hz) and a vicinal coupling (7.5 Hz) with the signal at 1.62 ppm; an NOE shows that this proton





4

Table 3 Correlation signals from 100/400 MHz C,H-COLOC of compound 1. The number in brackets shows the number of bonds separating the coupled C and H nuclei.

^{13}C - $^1\text{J}_{\text{CH}}$	^1H -Correlation-signals $^{2,3}\text{J}_{\text{CH}}$
171,16	2.04 ⁽²⁾
81.04	4.48
76.49	0.85 ⁽³⁾
75.88	0.82 ⁽³⁾ ; 1.75 ⁽³⁾
	0.94 ⁽³⁾ ; 1.05 ⁽²⁾
	1.15 ⁽³⁾ ; 1.69 ⁽²⁾
55.31	0.79
50.46	1.26
43.44	0.84 ⁽³⁾ ; 1.02 ⁽³⁾
43.32	1.80
42.17	0.82 ⁽²⁾ ; 1.72 ⁽³⁾
41.88	0.82 ⁽³⁾ ; 1.03 ⁽³⁾
38.50	1.69/1.00
37.86	0.84 ⁽³⁾
37.12	1.02 ⁽²⁾ ; 1.03 ⁽³⁾ ; 1.51 ⁽³⁾
34.62	1.15/0.99
33.29	1.47/1.28
29.25	1.75/1.25
28.59	1.02 ⁽³⁾ ; 1.03 ⁽²⁾ ; 1.28 ⁽³⁾
19.22	1.69
18.50	0.84 ⁽³⁾
	0.79 ⁽²⁾ ; 0.85 ⁽²⁾ ; 0.83 ⁽²⁾
	0.79 ⁽²⁾ ; 0.84 ⁽²⁾
	0.82 ⁽³⁾
	1.02 ⁽³⁾
	1.03 ⁽³⁾
	0.94 ⁽²⁾ ; 1.05 ⁽²⁾
	1.05 ⁽³⁾
	0.94 ⁽³⁾

proton at 1.62 ppm (20-H), similarly, is cis with regard to C-28. The proton at 1.72 ppm (also 20-H) must make an angle of about 90° with 19-H (1.15 ppm). The axial 15-H (1.75 ppm) and 16-H (2.04 ppm) can also be assigned clearly. The isolated compound is therefore 3 β -acetoxy-17 β ,21 β -epoxyhopane (3 β -acetoxy-17 β ,21 β -epoxy-A'-neogammacerane, 3-4), a previously undescribed substance.

On the other hand, both 3-acetoxyhopane and 17,21-epoxyhopane are known [8]. Assignments of ^{13}C and proton shifts are summarized in formulae 3 and 4.

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Korrespondenzanschrift:

Prof. Dr. E. Breitmaier
 Institut für Organische Chemie und Biochemie
 der Universität
 Gerhard-Domagk-Str. 1
 W-5300 Bonn 1, Bundesrepublik Deutschland