CYCLOBAKUCHIOLS A AND B FROM *PSORALEA GLANDULOSA*

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Abstract — Bioactivity guided fractionation of the dichloromethane extract from *Psoralea glandulosa* yielded the isolation of a diasteromeric mixture of cyclobakuchiols A and B as possible anti-inflammatory active compounds.

INTRODUCTION

As a part of our continuing research on the composition and bioactivity of Chilean medicinal plants and within a number of species preliminarily tested, we selected the so called 'culén', *Psoralea glandulosa* L. for a systematic study of its anti-inflammatory properties and chemical composition. Three species of this genus grow in Chile and are presumably antipyretic and/or anti-inflammatory. An infusion of *P. glandulosa* is used for the treatment of wounds, stomach ailments and fever [1].

Previous research on the secondary metabolites of this plant led to the isolation of two furocoumarins: angelicin and psoralene, together with the methyl ester of drupamine and bakuchiol [2] previously isolated from *P. corylifolia* [3]. Recently we have reported the antipyretic and anti-inflammatory effects of the infusion and the methanol extract prepared from the dried aerial part [4].

RESULTS AND DISCUSSION

Anti-inflammatory tests performed on successive petrol, methylene chloride and methanol extracts from the aerial part of the plant led us to select the most potent methylene chloride extract for phytochemical studies. Bioactivity-guided chromatography of this extract resulted in the isolation of a viscous homogeneous product, giving one spot under several TLC conditions, but, actually being a mixture of two isomeric compounds in an approximate 3:2 proportion. The compounds were a diastereomeric pair and their structures were established through complete IR, UV, and 1D and 2D-NMR and mass spectral analyses. We called these compounds cyclobakuchiols A (major) and B.

The molecular formula of cyclobakuchiols was deduced from the $[M]^+$ at m/z 256.1822 in the HREI-mass spectrum and corresponded to $C_{18}H_{24}O$, in agreement with calculations based on ^{13}C BB and DEPT spectra. The IR spectrum of the mixture showed bands for a phenol fragment (OH: 3600, 3400, arom: 3030, 1600, 1580, 1500 cm^{-1}) as well as for vinyl and vinylidene unsaturations (3080, 1640, 990, 910, 890 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table 1) of cyclobakuchiols showed proportionally duplicated signals for two methyls, one on a quaternary carbon and the other on an olefinic non-protonated carbon, for the mentioned vinyl and vinylidene fragments and for a *p*-disubstituted benzene ring. These data prompted us to relate the structure of cyclobakuchiols to that of bakuchiol (1), a meroterpenoid, and to consider the new natural compounds as cyclized derivatives of 1. The proposal of structures 2 and 3 for cyclobakuchiols A and B, respectively, was based on the complete analysis of homonuclear (COSY) and heteronuclear (HMQC, HMBC) 2D-NMR correlations, whose results were also the basis for the assignments summarized in Table 1.

The determination of the relative stereochemistries for cyclobakuchiols was based on several NOE difference experiments, which showed the spatial proximity between the H-10 methyl protons and those H-2, H-6, H-11 and H-12a in the case of 2, whereas in the case of 3 irradiation of H-10 methyl protons provoked NOE enhancement on the signals of H-3, H-5, H-11 and H-12b.

Finally, the proposal for the absolute stereochemistry of cyclobakuchiols, was based on the presence of (*S*)-(+)-bakuchiol in the same plant, whose absolute configuration was previously determined [5], and its clear nature of being the biogenetic precursor of the cyclic compounds.

Several studies relating the cyclization of synthetic racemic bakuchiol [6] and on its methyl ether derivative under strong acidic conditions (TsOH) and heating (80°) have been reported [7]. Nevertheless, the possible arte-

Table 1. 1D and 2D NMR spectral data (500/125 MHz) for compounds 2 and 3‡

C	CH_n	δ_C	Attached protons	long-range connected protons	δ_C	Attached protons	Long-range connected protons
				2			**3**
1	C	37.8		10,12a,b	37.0		10, 12a, b
2	CH_2	47.5	a:1.40 m	3, 4, 10, 11	46.5	a:1.47 m	10
			b: 1.82 m			b: 1.58 m	
3	CH	42.8	2.68td(12, 3.6)	4, 2', 6'	42.3	2.74td(12,3.6)	4,2',6'
4	CH	51.6	2.23 td(12,3.6)	8	51.5	2.21 td (12,3.6)	
5	CH_2	29.0	a:1.59 m		28.4	a:1.59 m	
			b: 1.64 m			b: 1.74 m	
6	CH_2	37.6	a:1.42 m	10	36.7	a, b:1.53 m	10
			b: 1.82 m				
7	C	148.6		4,9	148.4		4,9
8	CH_2	111.2	a:4.52 bs	3,9	111.3	a:4.56 bs	3,9
			b:4.55 bs			b: 4.58 bs	
9	CH_3	19.7	1.50 s	8	19.8	1.55 s	8
10	CH_3	31.6	0.99 s		22.2	1.16 s	
11	CH	146.3	5.87 dd(17,10)	10,12a,b	150.7	5.83 dd(17,10)	10,12a,b
12	CH_2	112.6	a:5.08 dd(17,1.3)		109.2	4.88 dd (17,1.3)	
			b:5.16dd(10,1.3)			4.91 dd (10,1.3)	
1'	C	138.2		3',5'	138.0		3', 5'
2',6'	CH	128.6	7.00 d (8.5)	3	128.6	7.01 d (8.5)	3
3',5'	CH	115.1	6.73 d (8.5)	2', 6'	115.1	6.73 d(8.5)	2', 6'
4'	C	153.5		2', 6', 3', 5'	153.6		2', 6', 3', 5'
4'-OH			4.73			4.73	

‡ Data from ^{1}H, COSY, ^{13}C, BB, DEPT, HMQC spectra (CDCl$_3$. TMS as internal standard).
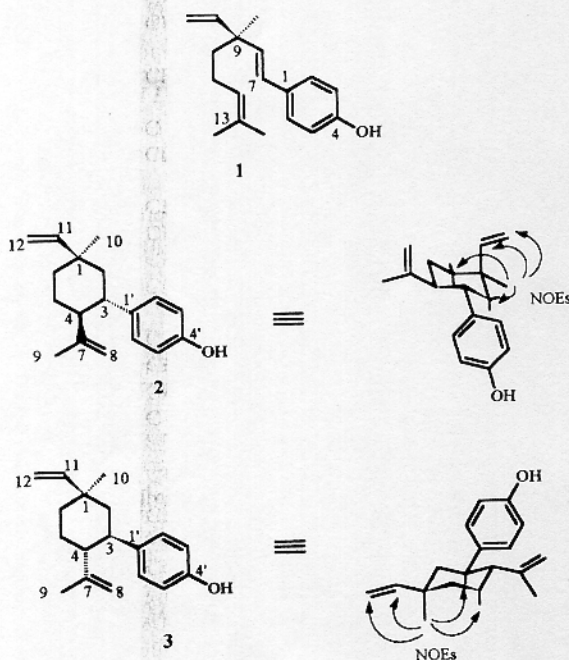
EXPERIMENTAL

IR: CCl$_4$. ^{1}H NMR (500 MHz) : CDCl$_3$ and C$_6$D$_6$, ^{13}C NMR (125 MHz). HREI-MS: 70 eV. TLC spots were detected under UV (254 and 365 nm) and heating the plates to 110° after spraying with Liebermann–Burchard reagent.

Plant material. The aerial part of *P. glandulosa* was collected in the upper Maipo valley (Cajón del Maipo), SE of Santiago, 34° S lat., Chile, in early summer (December), and identified by Professor Carla Delporte. A voucher specimen is kept in the herbarium of the School of Chemistry and Pharmacy (SQF: 17273).

Extraction and isolation. Air-dried and ground plant material (4 kg) was extracted successively at room temp with petrol, CH$_2$Cl$_2$ and MeOH yielding after removal of the solvents *in vacuo* 80, 98 and 300 g of crude extracts, respectively. All 3 extracts were submitted to anti-inflammatory and antipyretic assays in guinea-pigs and in rabbits, respectively, to monitor potential activities (Backhouse, N., and Delporte, C., unpublished results). The CH$_2$Cl$_2$ extract proved to be the most active and, therefore, it was submitted to CC on silica gel, eluting with petrol and petrol–CH$_2$Cl$_2$ mixtures of increasing polarity, being bioactivity guided. Two anti-inflammatory active fractions were obtained: B2 (20.0 g) eluted with petrol and B7 (3.0 g) with petrol–CH$_2$Cl$_2$ (19:1). The first fraction B2 was purified by further dry CC on silica gel, yielding PSN2 (4.5 g) eluted with petrol–CH$_2$Cl$_2$ (4:1)

factual nature of cyclobakuchiols should be discarded as the extracts and fractions used in this study were neither subjected to acidic conditions nor heating above 45° during their manipulation.

which submitted to a new CC yielded a small representative sample (40 mg) of the mixture of cyclobakuchiols A (**1**) and B (**2**) eluted with petrol–CH_2Cl_2 (43 : 7 to 41 : 9).

This component seemed to be homogeneous in TLC (silica gel, petrol–EtOAC, 7 : 3); R_f 0.6 and was viscous white-yellowish and highly soluble in $CHCl_3$ and CH_2Cl_2, slightly soluble in petrol, giving a green Liebermann–Burchard reaction.

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