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Simple and highly efficient preparation and characterization of (−)-lupanine and (+)-sparteine

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ABSTRACT

In a simple and convenient way, we have improved the non-chromatographic isolation of optically pure (−)-2-oxosparteine ((−)-lupanine) and (+)-sparteine. The fast and efficient method for the determination of the ee of bisquinolizidine alkaloids has been proposed. A relatively simple simple ^{1}H NMR method has been applied for evaluation of the % ee of enantiomers of the lupanines and sparteines with the chiral dibenzoyltartaric acids as the shift reagents. The ^{1}H NMR spectra of the bases and the new salts in polar solvents have been measured.
The results are confirmed by chiral HPLC method. Additionally, for the first time X-ray analysis of the salt of (−)-lupanine has been performed. The improved method of purification of bisquinolizidine alkaloids will considerably facilitate the employment of these alkaloids as chiral ligands in asymmetric reactions and as pharmacological tools.

1. Introduction

Quinolizidine alkaloids with the sparteine, cytisine or lupinine skeleton represent one of the largest groups of compounds distributed within the Leguminosae, the third largest family of flowering plants.[1] The extracts of the lupin alkaloids obtained from the seeds of *Lupinus albus* have been used in traditional medicine for the treatment of diabetes, eczema and as anti-inflammatory agents.[2] The pharmaceutical activity of these extracts is due to the presence of derivatives of sparteine. The main structural types of quinolizidine alkaloids are those with the sparteine skeleton and these alkaloids are very interesting from a stereochemical point of view.[3] They also play an important role in many chemical reactions and (−)-sparteine is still a popular chiral ligand in many reactions.[4]

The second most abundant lupin alkaloid after sparteine is 2-oxosparteine (lupanine).[5] However, the most widespread in nature is the (±)-lupanine enriched with (+)-lupanine isomer[6] optically pure (−)-lupanine $[\alpha]_D^{20}$ −57.5 (*c* 0.15, EtOH) was isolated from another Leguminosae plant—*Lygos raetam* var. sarcocarpa.[7] As well as the above mentioned (−)-lupanine, this extracted mixture was also found to contain (−)-anagyrine, (−)-cytisine and (−)-*N*-methylcytisine (Fig. 1), having the same absolute configuration of the methylene bridge (7*R*,9*R*).

(−)-Lupanine has also been obtained by catalytic hydrogenation of (+)-5,6-dehydrolupanine on PtO_2.[8] It was established that the absolute configuration of (+)-5,6-dehydrolupanine (Fig. 1) was the same as those of (−)-lupanine, (−)-anagyrine, (−)-*N*-methylcytisine and (−)-cytisine (Fig. 1). It is presumed that the compounds have the same intermediates and similar pathways of biosynthesis.[8]

Since (−)-sparteine has found numerous applications in asymmetric reactions, the interest in synthesizing (+)-sparteine has also increased. The synthesis of (+)-sparteine is time consuming, so surrogates of (+)-sparteine were taken under consideration.[9,10] One of them is *N*-methyl-2-deoxotetradehydrocytisine (Fig. 1) that was obtained by reduction of naturally occurring compound (−)-cytisine.[10] The (+)-enantiomers of sparteine derivatives can be obtained by synthetic modification of (+)-2-oxosparteine by introducing selected substituents at the C-2 position. These compounds readily form complexes with metals and can be potential chiral bases for stereochemical reactions.[11] (+)-Sparteine was obtained by resolution of (±)-lupanine with (−)-(1*R*)-10-camphorsulfonate dihydrate and then reduction.[12]

Due to the increasing interest in sparteine enantiomers, we decided to perform the separation of a racemic mixture of 2-oxosparteine enantiomers by crystallization with chiral acids. The next step was reduction of the two enantiomers of lupanine from which the corresponding enantiomers of sparteine were obtained. Up to now, (−)-lupanine has been found in the seeds of *L. albus* cv. Satmarean, in which (±)-lupanine is enriched with (−)-lupanine. The sample was isolated by HPLC using a chiral stationery phase and

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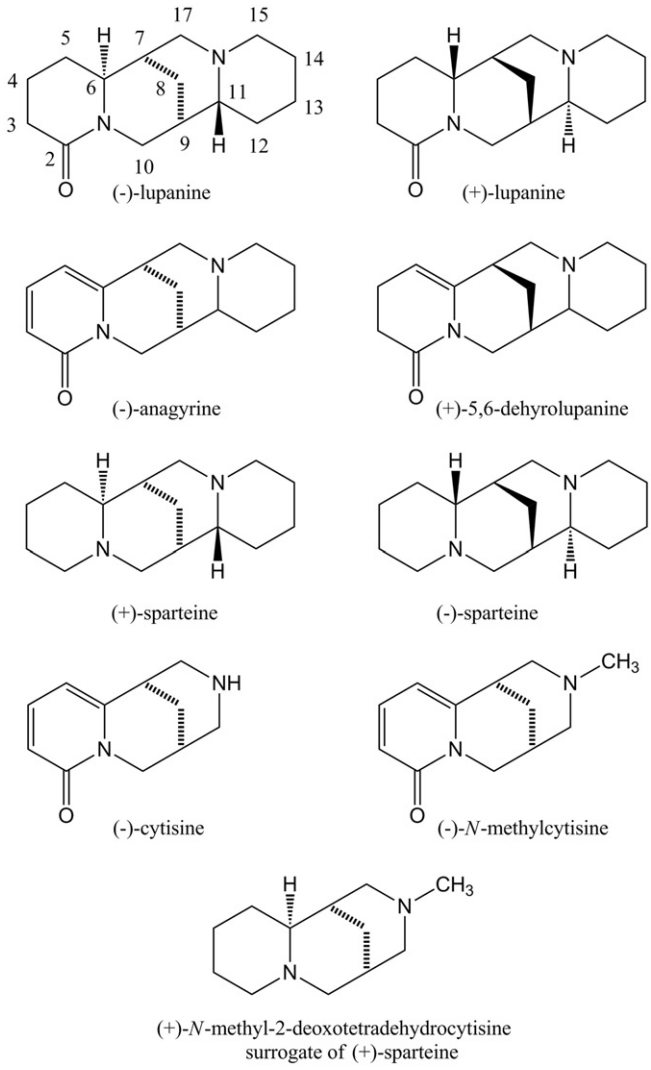

Fig. 1. Quinolizidine alkaloids: (−)- and (+)-lupanine, (−)-anagyrine, (+)-5,6-dehydrolupanine, (+)- and (−)-sparteine, (−)-cytisine, (−)-*N*-methylcytisine and (+)-*N*-methyl-2-deoxotetradehydrocytisine.

the optical purity 99% of (−)-lupanine $[\alpha]_D^{20}$ −80.8 (*c* 0.51, EtOH) was determined.[6]

This work presents an improved methodology for extraction of lupanine from *L. albus*, followed by isolation of the optically pure enantiomers of (+)-lupanine and (−)-lupanine on a preparative scale by resolution using dibenzoyltartaric acids. The reduction of (+)-lupanine and (−)-lupanine then led to (−)-sparteine and (+)-sparteine, respectively. Detailed NMR spectroscopic and X-ray crystallographic analyses of these alkaloids are performed.

2. Results and discussion

Our aim was to develop a relatively fast and effective method for separation of the pure enantiomers of lupanine, as the literature procedure was time-consuming and ineffective on a large scale. The extraction of a racemic mixture of naturally occurring lupanine from *L. albus* as well as the optical resolution of the enantiomers is described in the Experimental section. The NMR spectroscopic data of lupanine and sparteine are given in Tables 1–3.

By crystallisation of the racemic mixture of lupanine with (+)-dibenzoyltartaric acid we obtained (−)-lupanine with $[\alpha]_D^{20}$ −81.2 (*c* 1, EtOH) in 37% yield. An ee of 99.9% ee was found using HPLC analysis with a chiral column (rt 26.139 min). We also obtained (+)-lupanine $[\alpha]_D^{20}$ +81.2 (*c* 1, EtOH) in 32% yield by crystallising the (+)-lupanine from the mother liquid with (−)-dibenzoyltartaric acid. (+)-Lupanine was also formed in 99.9% by chiral HPLC (rt 28.120 min).

The same size optical rotations of the (−) and (+) enantiomers in the opposite directions suggested a complete resolution. However, we required an independent confirmation of enantiopurity of the isomers. Our methodology of evaluation of enantiopurity as described below showed that lupanine enantiomers (−) and (+) were more than 99% pure.

We have also developed a simple NMR method for determining the % ee of the lupanines. The method relies on salt formations. We used the same optically pure chiral acids that were applied for the resolution of the (−) and (+)-lupanine. The quantitative and rapid formation of salts offers an alternative to other more expensive reagents, such as chiral lanthanide shift reagents.

Well-separated peaks of H10(eq) between 4.47 and 4.27 ppm were observed in the ^{1}H NMR spectrum (500 MHz) of (±)-lupanine with (+)-2,3-dibenzoyl-D-tartaric acid (Fig. 2b). The important signal of H10(eq) as a doublet of triplets is shown in Fig. 2. The salt of each enantiomer displayed a signal that overlapped with some of those observed in the diastereomeric salts of the racemic mixture (Fig. 2). Such overlaps occurred at 4.46 and 4.44 ppm for the (−)-isomer (99% ee) and at 4.44 and 4.42 ppm for the (+)-isomer in the selected area (99% ee).

For the first time, the NMR spectra of lupanine were measured in polar solvents, such as MeOD and DMSO. On the basis of these data, the solvent effects were measured and the results are shown in Table 1.

Upon reduction of (−)-lupanine and (+)-lupanine, the isomers (+)-sparteine and (−)-sparteine were obtained in 84% and 85% yield, respectively (Fig. 3). (+)-Sparteine is the target model compound used in stereoselective reactions.[5]

The changes in the ^{1}H NMR spectra of (±)-sparteine (synthesised from (±)-lupanine) with a chiral acid were similar to those observed for the lupanine salts. However, the best resolution of the key signals of H-17(eq) (2.38 ppm) and H10(eq) (2.56 ppm) was obtained with (−)-2,3-dibenzoyl-D-tartaric acid (Fig. 4, Table 3). The salts of each enantiomer of sparteine with (−)-2,3-dibenzoyl-D-tartaric acid displayed a signal that overlapped with some of those observed in the diastereomeric salts of the racemic mixture (Fig. 4).

The signal of H17(eq) occurred at 2.70 ppm as a doublet of doublets for the salt of the (−)-isomer and at 2.65 ppm for the salt of (+)-isomer in the selected area (Fig. 4). Another signal that can differentiate between the enantiomers is the chemical shift of H-10. In the spectrum of the (−)-sparteine salt it is found at 2.60 as a broad multiplet, while in the spectrum of (+)-sparteine it is at 2.53 as a doublet (Fig. 4).

The percentage of enantiomeric contamination was measured according to a literature method[13] to be 99%. In order to measure % ee, to each sample, we added an opposite isomer together with the chiral reagent in steps of 0.5% (wt %). This amount did not cause detectable changes in the signals. Although, after addition of 1% a weak signal of the minor isomer, which was distinguishable from the background noise could be observed. These indicate that the detection limit of % ee was between 1 and 0.5%. Thus, our resolved compounds were at least 99% optically pure. That finding was confirmed by chiral HPLC method.

The structure of the newly obtained salt of (−)-lupanine was confirmed by X-ray crystallographic analysis (Fig. 5). The lupaninium cation is protonated at the N16 nitrogen atom, and one of the carboxylic groups of the tartrate anion is deprotonated. This was proved by the successful location of these hydrogen atoms as well as by the bond length (carboxylate) and angle (lupaninium) patterns. The crystal structure also contains two water molecules per cation–anion pair. All these structural components are connected

Table 1
^{1}H NMR and ^{13}C NMR–chemical shifts of lupanine in MeOD and DMSO

Atom C	δ [ppm]			Atom H	δ [ppm]		δ [ppm]	
	MeOD	DMSO	$\Delta=\delta_{MeOD}-\delta_{DMSO}$		MeOD	*J* [Hz]	DMSO	*J* [Hz]
2	174.00	169.7	4.28	—	—	—	—	—
3	33.7	32.6	1.09	3α ax 3β eq	~2.35 m	m; 2H	~2.21	m; 2H; 8.7; 9.3; 4.0; 4.6; 0.6
4	20.3	19.2	1.14	4α eq	1.64–1.58	m; 3H	1.52	m
				4β ax	1.83	dddd 11.4; 7.6; 4.8; 2.7; 2.1	1.70	ddd 8.3; 3.4; 1.6
5	28.1	26.7	1.45	5α ax	1.64–1.58	m; 3H	1.45	m
				5β eq	1.81	dddd 9.5; 5.8; 3.6; 2.3; 1.1	1.71	ddd 10.0; 4.0; 2.4
6	62.3	59.8	2.47	6 ax	3.42	dddd 11.2; 7.0; 5.0; 2.0	3.28	dddd 11.1; 7.0; 5.0; 1.9
7	33.6	31.6	1.93	7 equiv	~2.12	m	1.98	m
8	27.3	26.2	1.1	8α eq	2.14	dddd 12.4; 4.2; 4.1; 2.2	2.00	dddd 11.7; 4.1; 4.0; 2.1
				8β ax	1.36	ddd 12.4; 4.6; 2.3	1.19	ddd 11.7; 4.6; 2.4
9	36.3	34.5	1.78	9 eq	1.65	m; br s	1.51	m
10	48.0	45.9	2.12	10α eq	4.42	ddd 13.2; 4.9; 2.5	4.29	ddd 12.9; 4,8; 2,4
				10β ax	2.58	dd 13.2; 2.8	2.43	dd 12.9; 2.07
11	65.7	63.4	2.27	11 ax	1.63	m	1.46	m
12	34.2	32.8	1.37	12α eq	1.64–1.58	m; 3H	1.47	m
				12β ax	1.40	dddd 14.7; 12.8; 11.2; 3.7	1.26	dddd 14.6; 13.2; 11.6; 3.8
13	25.7	24.4	1.35	13α ax	1.30	dddd 12.8; 9.0; 7.9; 4.2; 3.9	1.15	ddddd 12.7; 9.1; 8.0; 4.3; 3.7
				13β eq	1.73	dddddd 12.8; 4.7; 4.1; 3.6; 3.3; 1.4	1.64	m 12.7; 6.2; 4.8; 2.8; 2.6; 1.4
14	25.9	24.8	1.08	14α eq 14β ax	1.58–1.55	m; 2H	1.43–1.37	m; 2H
15	56.8	54.8	1.99	15α ax	1.93	ddd 12.0; 11.5; 4.5	1.79	ddd 11.5; 11.4; 3.2
				15β eq	2.79 d	ddd 11.5; 5.6; 4.5; 3.0	2.68	d 11.5
17	53.9	52.1	1.75	17α ax	1.95	dd 12.2; 3.9	1.82	dd 11.4; 3.7
				17β eq	2.87	dd 12.2; 10.1; 2.1	2.72	dd 11.4; 10.1; 1.3

Table 2
Comparison of the chemical shifts (^{13}C NMR, TMS) of sparteine measured in different solvents

At C	C_6D_6[14]	$CDCl_3$[15]	MeOD	D_2O pH 10[16]	$\Delta=\delta_{CDCl3}-\delta_{C6C6}$	$\Delta=\delta_{MeOD}-\delta_{C6C6}$	$\Delta=\delta_{MeOD}-\delta_{CDCl3}$	$\Delta=\delta_{D2O}-\delta_{CDCl3}$	$\Delta=\delta_{D2O}-\delta_{MeOD}$
2	56.48	56.0	57.41	58.45	0.48	0.93	1.41	2.45	1.04
3	26.23	25.7	26.19	27.50	0.53	0.04	0.49	1.80	1.31
4	25.10	24.5	25.70	26.00	0.60	0.60	1.20	1.50	0.30
5	29.56	29.1	30.46	31.88	0.46	0.90	1.36	2.78	1.42
6	66.51	66.2	68.02	68.70	0.31	1.51	1.82	2.50	0.68
7	33.75	33.0	34.30	35.35	0.75	0.55	1.30	2.35	1.05
8	28.02	27.4	28.34	28.90	0.62	0.32	0.94	1.50	0.56
9	36.97	36.0	37.25	35.51	0.97	0.28	1.25	−0.49	−1.74
10	62.29	61.8	62.95	63.88	0.49	0.66	1.15	2.08	0.93
11	64.40	64.1	65.63	65.85	0.30	1.23	1.53	1.75	0.22
12	35.18	34.5	34.96	25.46	0.68	0.22	0.46	−9.04	−9.5
13	25.51	24.7	25.89	25.75	0.81	0.38	1.19	1.05	−0.14
14	26.58	25.8	26.72	20.69	0.78	0.14	0.92	−5.11	−6.03
15	55.78	55.2	56.55	55.49	0.58	0.77	1.35	0.29	−1.06
17	54.00	53.4	54.19	49.87	0.60	0.19	0.79	−3.53	−4.32

into a three-dimensional network by means of the relatively strong hydrogen bond network (Table 4). The NH group acts as a donor for a hydrogen bond with the deprotonated carboxylate group and the carboxylic group acts as a donor for a hydrogen bond with the oxygen atom from one of the water molecules. The water molecules are involved in hydrogen bonds with the remaining oxygen atoms.

The lupaninium cation (Fig. 6) has the typical conformation of the rings: half-chair, chair, boat, chair—ring C is inverted from the boat conformation typical for the free base (e.g., lupanine itself[17]).

Additionally, to analyze the influence of solvent on the chemical shifts of sparteine, the NMR data obtained in MeOD were compared with those available in the literature (only in non-polar solvents, such as $CDCl_3$ and C_6D_6). On the basis of this comparison, the differences in the chemical shifts were calculated. The solvent effect is illustrated in Tables 2 and 3. This bisquinolizidine compound was chosen as a model compound with ring C as a pure boat conformer. According to Brukwicki the chemical shifts of C-12 and C-14 as well as the $J_{H7}-H_{17\beta}$ coupling constant of sparteine permit the calculation of the conformational equlibria in bisquinolizidine system.[3] The new values of the chemical shifts C-12, C-14 and $J_{H7}-H_{17\beta}$ for the model with 100% boat conformer are: 34.9 ppm, 26.0 ppm and 10.8 Hz, respectively. While for the model with 100% chair conformer, the corresponding values are: 21.0 ppm (C-12), 18.0 ppm (C-14) and 1.9 Hz ($J_{H7}-H_{17\beta}$) (Tables 2 and 3). The calculations based on the Haasnoot equation have been published.[3] However, it seems that these data are correct for the NMR spectra measured only in $CDCl_3$, because the values of chemical shifts and coupling constant in MeOD are higher than those mentioned above needed for the calculation: 35.0 ppm, 26.7 ppm and 11.4 Hz, respectively. For this reason it is impossible to use the above-mentioned equation for the calculation of conformational equilibrium of sparteine in polar solvents, such as MeOD or DMSO. This result emphasises that for the identification of conformational equilibrium it is necessary to take under consideration the influence of the solvent and compare the values of chemical shifts and coupling constants obtained in the same solvent.

3. Conclusions

The most abundant lupin alkaloid besides sparteine is 2-oxosparteine (lupanine). In order to provide ready access to gram

Table 3
Comparison of the chemical shifts of sparteine (^{1}H NMR, TMS) measured in different solvents

At H	C_6D_6[14]	$CDCl_3$[15]	MeOD	D_2O pH 10[16]	$\Delta=\delta_{CDCl3}-\delta_{C6C6}$	$\Delta=\delta_{MeOD}-\delta_{CDCl3}$	$\Delta=\delta_{D2O}-\delta_{MeOD}$
2α eq	2.62	2.64	2.70 dd 11.04; 5.7	2.92	0.02	0.06	0.22
2β ax	1.88	1.89	1.98 ddd 15.3; 11.3; 4.1	2.10	0.01	0.09	0.12
3α ax	1.56	2.01	1.59 m	1.50	0.45	−0.42	−0.09
3β eq	1.44	2.01	1.59 m	1.62	0.57	−0.42	0.03
4β ax	1.12	1.30	1.30–1.25 m (2H)	1.40	0.18	—	—
4α eq	1.62	1.67	1.74–1.71 m (2H)	1.77	0.05	—	—
5α ax	1.40	1.35	1.40 ddd 16.7; 12.7; 3.8	1.55	−0.05	0.05	0.15
5β eq	1.09	1.20	1.30–1.25 m (2H)	1.38	0.11	—	—
6	1.62	1.67	1.79 bd 11.3	2.55	0.05	0.12	0.76
7	1.74	1.84	1.85 m	1.87	0.1	0.01	0.02
8β ax	1.02 ax	1.00	1.13 dt 12.3; 4.7	1.60	−0.02	0.13	0.47
8α eq	2.34 equiv	2.01	2.07 m	2.07	−0.33	0.06	0
9	1.41	1.42	1.50 m	1.89	0.01	0.08	0.39
10β ax	1.96	1.96	2.04 dd 11.1; 2.5	2.64	0	0.08	0.60
10α eq	2.47	2.48	2.56 ddd 11.1; 4.4; 2.2	3.04	0.01	0.08	0.48
11	2.11	1.90	2.10 m	3.44	−0.21	0.20	1.34
12β ax	1.45	1.45	1.53 m	2.03	0	0.08	0.50
12α eq	1.37	1.34	1.38–1.33 m (2H)	1.48	−0.03	—	—
13α ax	1.25	1.67	1.38–1.33 m (2H)	1.52	0.42	—	—
13β eq	1.66	1.67	1.74–1.71 m (2H)	1.87	0.01	—	—
14β ax	1.65	1.51	1.55 m (2H)	1.77	−0.14	0.04	0.22
14α eq	1.51	1.51	1.55 m (2H)	1.52	0	0.04	−0.03
15α ax	2.04	1.89	2.05 m	3.1	−0.15	0.16	1.05
15β eq	2.76	2.64	2.78 bd 11.4	3.1	−0.12	0.14	0.32
17α ax	2.47	2.30	2.38 dd 11.4; 3.4	3.58	−0.17	0.08	1.20
17β eq	2.67	2.65	2.72 dd 11.4; 11.4	3.11	−0.02	0.07	0.39

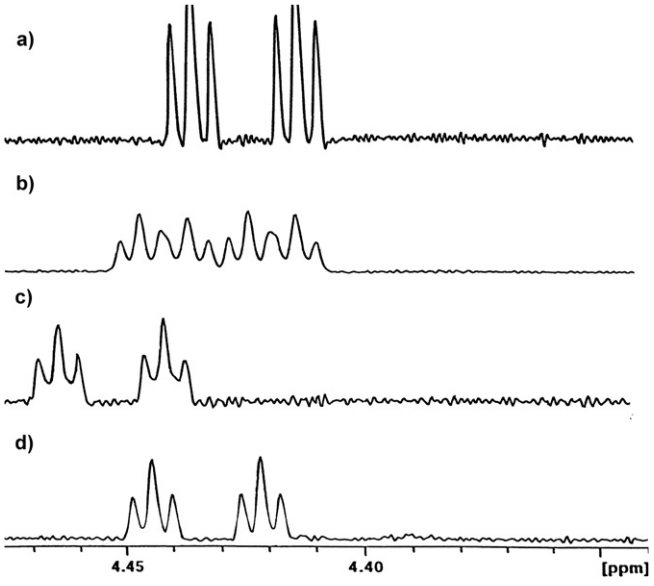

Fig. 2. The chemical shift of H10α(eq) in the spectra of: (a) (±)-lupanine; (b) the salt of (±)-lupanine and (+)-2,3-dibenzoyl-D-tartaric acid; (c) the salt of (−)-lupanine and (+)-2,3-dibenzoyl-D-tartaric acid; (d) (+)-lupanine and (+)-2,3-dibenzoyl-D-tartaric acid (^{1}H NMR, MeOD, TMS, 400 MHz).

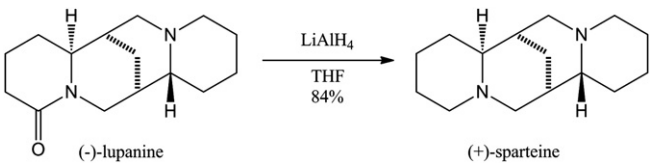

Fig. 3. Reduction (−)-lupanine to (+)-sparteine.

quantities of the optically pure 2-oxosparteine, we improved the resolution of (±)-lupanine and determined the optical purity of its enantiomers by optical rotation and using relatively simple ^{1}H NMR method with optically pure acids: (−)-2,3-dibenzoyl-D-tartaric acid and (+)-2,3-dibenzoyl-D-tartaric acid was developed. This quantitative and rapid formation of salts offers an alternative to other much more expensive used reagents, such as chiral lanthanide shift reagents.

In this work, a new method of obtaining (+)-sparteine by optical resolution of racemic mixture of lupanine followed by reduction is proposed as well as a new application of the well known chiral dibenzoyltartaric acids has been introduced.

Additionally, the influence of solvents on the NMR spectra of lupanine and sparteine has been analyzed (Tables 1–3).

The improved method of resolution of bisquinolizidine alkaloids will considerably facilitate the employment of these alkaloids as chiral ligands in asymmetric reactions and as pharmacological tools.

4. Experimental

4.1. General techniques

Plant material: The lupin seeds of *L. albus* cv. BAC (Leguminosae plant), Gene Bank, Experimental Station of Plant, Wiatrowo, 62–100 Wągrowiec, Poland.

Thin layer chromatography (TLC) was performed using aluminium sheets precoated with silica gel 60 F_{254} (Merck). Flash chromatography was carried out on silica gel 60 G F_{254} (Merck). Melting points were determined on a Boetius apparatus (PHMK 05 VEB Wagetechnik Rapido, Radebeul). Low- and high-resolution electron ionization (EIMS) mass spectra were recorded using a model 402 two-sector mass spectrometer (AMD Intectra GmbH, Harpsted, Germany; ionising voltage 70 eV, accelerating voltage 8 kV, resolution 1000 for low-resolution and 10,000 for high-resolution mass spectra). IR spectra were obtained on an FTIR Bruker IFS 113 v spectrometer (KBr pellets technique).

NMR spectra: 1D and 2D correlation spectra: ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC were recorded on a Bruker AVANCE II 400 (400.13 MHz for ^{1}H and 100.63 MHz for ^{13}C) spectrometer, with a 5 mm broadband probe head with actively shielded *z* gradient coil (90° ^{1}H pulse width 10.8 μs, ^{13}C pulse width

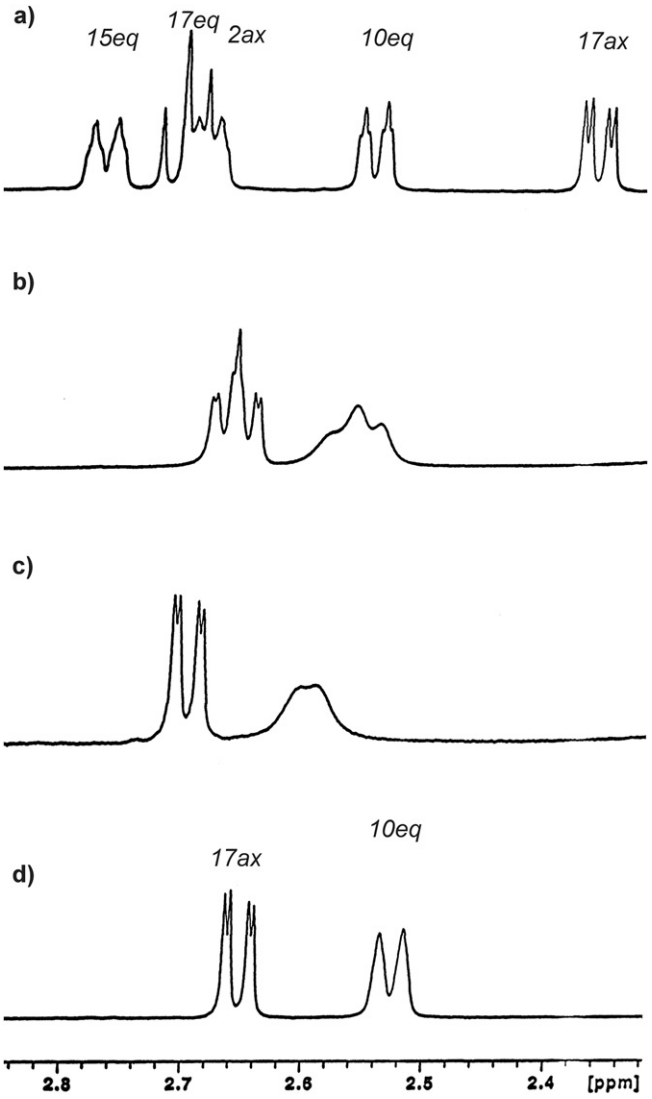

Fig. 4. The chemical shift of H10α(eq) and H17β(eq) in the spectra of: (a) (±)-sparteine; (b) salt of (±)-sparteine and (−)-2,3-dibenzoyl-D-tartaric acid; (c) salt of (−)-sparteine and (−)-2,3-dibenzoyl-D-tartaric acid; (d) (+)-sparteine and (−)-2,3-dibenzoyl-D-tartaric acid (^{1}H NMR spectra, MeOD, TMS).

12 μs). All 2D spectra were acquired and processed using standard Bruker software. Spectral width of 4000 Hz and 16,666.7 Hz in ^{1}H−^{13}C HSQC, and 4000 Hz and 22,321.4 Hz in the case of ^{1}H−^{13}C HMBC were used for ^{1}H and ^{13}C, respectively. Relaxation delays of 1.0 s were used for all 2D experiments. All 2D spectra were collected with 2K points in F2 and 256 increments (F1) with 4 (g-COSY) and 64 (g-HSQC) transients each.

The HPLC analysis was performed with a Waters HPLC system with an analytical column Chiracel OD-H and the solvent system: hexane/2-propanol (19:1); flow rate 0.5 mL/min; detection at UV light, l=220 nm.[6] Optical rotation was measured using a Perkin−Elmer 242B polarimeter at 20 °C in ethanol solution.

4.2. Extraction and isolation of (±)-lupanine

(±)-Lupanine was obtained from the lupin seeds (*L. albus* cv. BAC) according to a literature procedure,[18] but modified to obtain only quite pure lupanine. For this reason 2.5 kg of the grounded seeds were defatted in a soxhlet with hexane (or with cheaper petroleum ether) for 40 h. After drying, the 2.2 kg of the powdered seeds were macerated for 3 h with 25% $KOH_{(aq)}$ (2 L) in order to sufficiently destroy the tissues and release the alkaloids from their ester forms or salts. A longer time of maceration can lead to the on hydrolysis of the lactam group to give lupaninic acid.

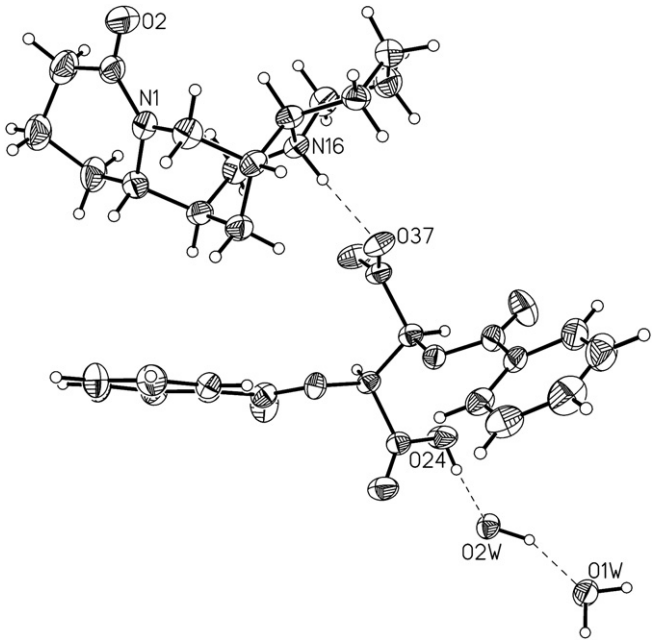

Fig. 5. X-ray of the salt of (−)-lupanine and (+)-2,3-dibenzoyl-D-tartaric acid. Elipsoids are drawn at the 33% probability level, hydrogen bonds are spheres of arbitrary radii. Hydrogen bonds are depicted as dashed lines.

Table 4
Hydrogen bond data (Å, °)

D	H	A	D−H	H···A	D···A	D−H···A
N16	H16	O37[a]	0.91	1.86	2.765 (4)	177
O24	H24	O2W	0.82	1.76	2.551 (4)	161
O1W	H1W1	O36[b]	1.00	1.80	2.784 (4)	165
O1W	H1W2	O2[c]	0.94	1.86	2.769 (4)	162
O2W	H2W1	O37[d]	0.84	1.99	2.803 (4)	165
O2W	H2W2	O1W	0.99	1.78	2.704 (4)	154

Symmetry codes:
[a] $1+x,-1+y,z$.
[b] $1+x,y,z$.
[c] $1+y,1-x,1/4+z$.
[d] $y,1-x,0.25+z$.

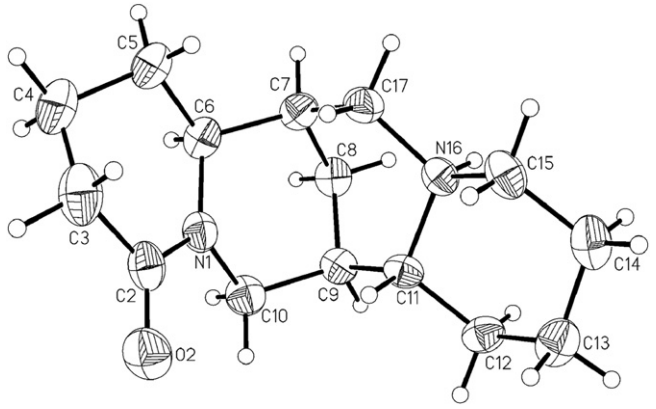

Fig. 6. The perspective view of the (−)-lupaninium cation. Elipsoids are shown at the 33% probability level.

The pulp was mixed with 1 kg of Celite (diatomaceous earth) to increase the surface of the mixture and to absorb the excess of water. Such mass was poured into a linen sack and placed into a soxhlet. The mixture of (±)-lupanine and (+)-lupanine (1:1)[18] were eluted for 40 h with petroleum ether to avoid the extraction of the minor more polar alkaloids. This yielded 23.2 g of a yellow solidifying oil. This extract was crystallized from diethyl ether to obtain 12.46 g of yellow crystals of (±)-lupanine that was recrystallized from hexane to get 10.3 g of white crystals of (±)-lupanine.

4.3. Compounds

4.3.1. (±)-Lupanine. White crystals, mp 98–99 °C, ^{1}H NMR and ^{13}C NMR (MeOH, DMSO) in Table 1, ^{13}C NMR ($CDCl_3$): 171.0, 63.9, 60.9, 55.4, 52.9, 46.7, 34.9, 33.7, 33.1, 32.4, 27.4, 26.8, 25.4, 24.5, 19.7; IR (KBr): ν C=O 1632 cm^{-1}, *trans*-band[11] 2810–2760 cm^{-1} with maxima at 2756 cm^{-1} and weaker at 2820 cm^{-1}; *m*/*z* (rel int.): 248 (28%), 136 (100%), 149 (52%), 150 (36%), 98 (25%). The ^{13}C NMR and EIMS data were consistent with those in the literature.[19,20]

4.3.2. (+)-Lupanine. The petroleum ether mother liquor after removing (±)-lupanine was concentrated to give 10.86 g of solidified oil, which was dissolved in MeOH (50 mL). To the solution 10 mL of 10% $HClO_4$ from MeOH was added (pH=6) to obtain the perchlorate of lupanine (11.6 g) that was recrystallized from MeOH to yield 10.8 g of lupanine perchlorate as a mixture enriched with the dextrorotary enantiomer. Mp 251–252 °C of the $HClO_4$ salt; IR (KBr) ν C=O 1620 cm^{-1}, ν N$^+$–H 3140 cm^{-1}. The free base of lupanine $[\alpha]_D^{20}$ +21.1 (*c* 1.0, EtOH); NMR—Table 1 and Fig. 2.

4.3.3. Optical resolution of (−)-lupanine and (+)-lupanine. Optical resolution of the two enantiomers was performed with (+)-2,3-dibenzoyl-D-tartaric acid. To the solution of (±)-lupanine (2.48 g, 10 mmol) in MeOH (10 mL) (+)-2,3-dibenzoyl-D-tartaric acid (3.58 g, 10 mmol) in MeOH (10 mL) was added. The mixture was heated, then cooled down to rt. After half an hour white crystals started to precipitate.

The white crystals were recrystallized from EtOH (10 mL) to yield 2.37 g of (−)-lupanine salt, mp 144–145 °C. The obtained tartrate salt was transformed into the free base of (−)-lupanine by dissolving the salt in water (20 mL) and basified with KOH to pH=14. The alkaloid was extracted with diethyl ether (5×5 mL, the alkaloids in the eluent were monitored in Dragendorf reagent) to give (−)-lupanine (0.92 g, 37%) $[\alpha]_D^{20}$ −81.8 (*c*=1, EtOH); HPLC rt 26.139 min (Supplementary data). X-ray—Figs. 5 and 6.

After removal of (−)-lupanine the mother liquor was concentrated and dissolved in water (20 mL), basified with KOH to pH=14, and (+)-lupanine was extracted with diethyl ether (5×5 mL, the alkaloids in the eluent were monitored in Dragendorf reagent) to give 1.56 g of the crude oil that was dissolved in MeOH (10 mL) and (−)-2,3-dibenzoyl-L-tartaric acid (1.79 g, 5 mmol) was added. The mixture was heated, then cooled down to rt. After 1 h white crystals started to precipitate. The crystals were recrystallized from EtOH (10 mL) to yield white crystals 2.34 g, mp 143–144 °C. Free base of (+)-lupanine (0.87 g, 35%) $[\alpha]_D^{20}$ +80.9 (*c*=1, EtOH); HPLC rt 28.120 min (Supplementary data); NMR—Table 1 and Fig. 2.

4.3.4. (+)-Sparteine. (−)-Lupanine (248 mg, 1 mmol) was dissolved in dry THF (10 mL) and cooled to 0 °C. $LiAlH_4$ (309 mg, 8 mmol) was added in one portion. The reaction mixture was stirred and refluxed in 100 °C for 17 h and then was cooled to 0 °C Et_2O (10 mL) was added. Then, saturated $Na_2SO_{4(aq)}$ was added dropwise until gas evolution ceased. The layers were separated in a separating funnel. The aqueous layer was extracted with CH_2Cl_2 (5×20 mL). The organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give light oil of (+)-sparteine (196 mg, 84%); $[\alpha]_D^{20}$ +21.2 (*c* 1.6, EtOH) compared to mmc3 −20.7 (*c* 1.8, EtOH) measured for the laevorotatory material purchased from Aldrich and lit.[10] $[\alpha]_D^{20}$ +21.3 (*c* 1.7, EtOH); mp 128–129 °C of (+)-2,3-dibenzoyl-L-tartaric acid salt. Examination of the substance under polarized light showed that the sample does not contain single crystals. NMR in Tables 2 and 3, and Fig. 4. EIMS *m*/*z* 234 (15%), 137 (100%), 98 (73%), 136 (50%), 193 (35%), 110 (20%).

4.4. X-ray diffraction

X-ray diffraction data were collected on a KUMA KM4CCD diffractometer, using graphite-monochromated Mo Kα radiation (λ=0.71073 Å) at room temp. The unit-cell parameters were determined by the least-squares fit of the positions of the 11,388 most intense reflections chosen from the whole experiment. The Lorentz-polarization and absorption corrections were applied.[21] The structures were solved with SIR92[22] and refined with the full-matrix least-squares procedure on F^2 by SHELXL97.[23] Scattering factors incorporated in SHELXL97 were used. The function $\Sigma w(|F_o|^2-|F_c|^2)^2$ was minimized, with $w^{-1}=[\sigma^2(F_o)^2+(0.0387\ P)^2+0.5248\ P]$, where $P=[\mathrm{Max}\ (F_o^2, 0)+2F_c^2/3]$. Table 5 lists relevant crystal data and refinement details. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms from water molecules were found in difference Fourier maps and refined as a riding model in positions found; all other hydrogen atoms were placed geometrically, in idealized positions, and refined as rigid groups the U_{iso} values for all hydrogen atoms were set at 1.2 times U_{eq} of the appropriate carrier atom. Absolute configuration could not be determined solely on the basis of X-ray data and was assigned on the basis of known configuration of tartaric acid.

Table 5
Crystal data, data collection and structure refinement

Compound	**1**
Formula	$C_{15}H_{25}N_2O^+\cdot C_{18}H_{13}O_8^-\cdot 2H_2O$
Formula weight	642.70
Crystal system	Tetragonal
Space group	$P4_3$
a(Å)	10.906 (2)
c(Å)	27.233 (4)
V(Å^3)	3239.1 (9)
Z	4
D_x(g cm^{-3})	1.32
F(000)	1368
μ(mm^{-1})	0.099
Crystal size (mm)	0.2×0.2×0.1
Θ Range (°)	1.87–25.50
hkl Range	$-13\le h\le 13$
	$-13\le k\le 13$
	$-32\le l\le 32$
Reflections:	
collected	59,234
unique (R_{int})	3086 (0.080)
with $I>2\sigma(I)$	2430
Number of parameters	417
$R(F)$ [$I>2\sigma(I)$]	0.051
$wR(F^2)$ [$I>2\sigma(I)$]	0.092
$R(F)$ [all data]	0.072
$wR(F^2)$ [all data]	0.098
Goodness of fit	1.131
max/min $\Delta\rho$ (e Å^{-3})	0.14/−0.13

Crystallographic data (excluding structure factors) for the structural analyses have been deposited with the Cambridge Crystallographic Data Centre, CCDC Nos. 819,528 Copies of this information may be obtained free of charge from: the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK. Fax: 44 1223 336 033, e-mail: deposit@ccdc.cam.ac.uk, or www.ccdc.cam.ac.uk.

4.4.1. The salt of (−)-lupanine and (+)-2,3-dibenzoyl-D-tartaric acid. $C_{15}H_{25}N_2O^+ \cdot C_{18}H_{13}O_8^- \cdot 2H_2O$, MW=642.70, tetragonal, $P4_3$, a=10.906(2) (Å), c=27.233(4) (Å), V=3239.1(9) (Å^3), Z=4, D_x=1.32 (g cm^{-3}), $F(000)$=1368, μ=0.099 (mm^{-1}), crystal size 0.2×0.2×0.1 (mm), Θ range 1.87–25.50 (°), *hkl* range: $-13\leq h\leq 13$, $-13\leq k\leq 13$, $-32\leq l\leq 32$; reflections: collected 59,234, unique 3086 (R_{int}=0.080), with $I>2\sigma(I)$ 2430. Number of parameters 417, $R(F)$ [$I>2\sigma(I)$]=0.051, $wR(F^2)$ [$I>2\sigma(I)$]=0.090, $R(F)$ [all data]=0.072, $wR(F^2)$ [all data]=0.098, goodness of fit 1.131, max/min $\Delta\rho$ 0.14 and −0.13 e Å^{-3}.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.07.080.

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