Epimerization of Lupinine to Epilupinine and *vice versa*. Reexamination of the Structures of Lupinal and Epilupinal

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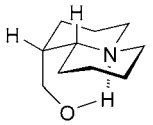
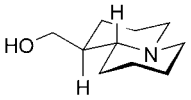
Although the epimerization of lupinine (**1**) has been largely investigated, a previously not observed compound of formula $C_{10}H_{17}NO$ was now isolated from the mixture of alkaloids that remains after the separation of epilupinine (**2**). It is insoluble in dry Et_2O but soluble in EtOH, from which it is recovered as an Et_2O-soluble oil that slowly returns to the Et_2O-insoluble solid form. For these characteristics and based on GC/MS, ^{1}H-NMR, and IR data, it is considered as the inner salt **6** of the common enolic form **5** of lupinal (**3**) and epilupinal (**4**), with which it is in equilibrium when standing in solution (see *Scheme 1*). The oily form, but not the solid one, is able to improve the conversion of **1** to **2**, establishing the role of the aldehydes in the epimerization process. It was observed that also **2** can be converted to **1**. Finally, the solid lupinal described by *Zaboev* should be considered as being identical to the now isolated inner salt **6**, while the oily epilupinal of *Wicky* and *Schumann* is, indeed, a mixture of epilupinal (**4**) with a minor amount of lupinal (**3**), which, on standing, is converted to the inner salt **6** of the common enolic form **5**.

Introduction. – For a long time, we have been using (−)-lupinine (**1**) and (+)-epilupinine (**2**) as the starting material for the preparation of octahydro-*2 H*-quinolizine (quinolizidine) derivatives of pharmacological interest.

While lupinine (**1**) has been extracted from seeds of either cultivated bitter *Lupinus luteus* L. or wild Sardinian *Lupinus hispanicus* BOISS et REUT. [1], epilupinine (**2**) has been obtained through epimerization of **1**.



1 (–)-lupinine



2 (+)-epilupinine

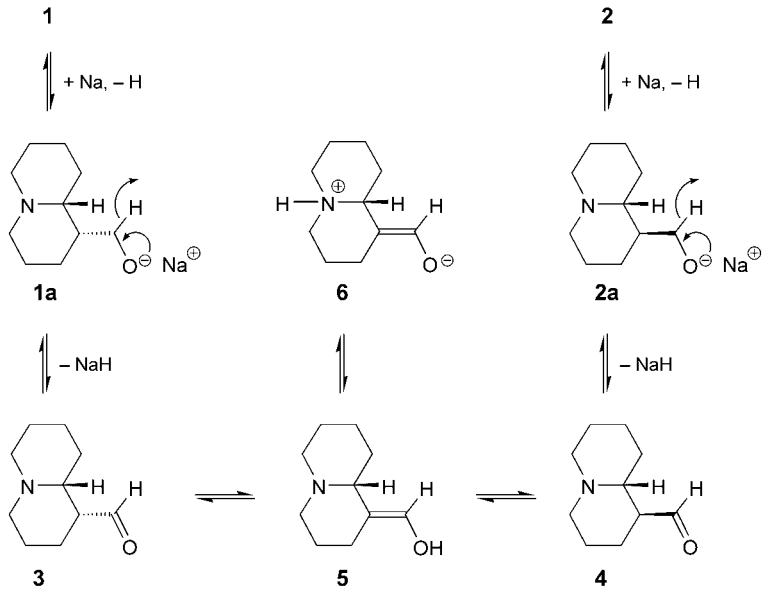
The epimerization of **1** to **2** has been accomplished by several authors by heating its benzene solution for 3 d in the presence of Na [2–5]. Having repeated several times this experiment, *Sparatore* and *Boido* [6] observed that the yield of **2** was largely variable in an unaccountable way. This observation conformed to that of *Clemo* and *Rudinger* [3], but was in contrast to that of *Galinovski* and *Nesvadba* [4].

Better results in the conversion of lupinine (**1**) to epilupinine (**2**) were obtained by *Boido* and *Sparatore* [7] by refluxing the xylene solution of **1** for 5 h in the presence of either NaH, $NaNH_2$ or NaOEt, followed by the isolation of the main portion of **2** and

repeating twice the same treatment with the residual mixture **1**/**2**; a conversion higher than 90% was finally obtained.

A reasonable mechanism for the epimerization was reported in 1972 by *Mnatsakanyan et al.* [8], who postulated the initial conversion of lupinine (**1**) to the corresponding aldehyde, lupinal (**3**); the latter would then be tranformed, *via* the enol form **5**, to epilupinal (**4**), which would finally be reduced to epilupinine (**2**) (*cf. Scheme 1*). All steps are considered unidirectional.

Scheme 1



In the present communication, we illustrate experimental results that substantiate the proposed mechanism, establishing, however, that it is bidirectional since also epilupinine (**2**) can be converted to lupinine (**1**). The structures of compounds previously described as lupinal and epilupinal are also reexamined.

Isolation of (Quinolizidin-1-ylidene)methanol Inner Salt (**6**). – In an attempt to recover some more epilupinine (**2**) from the residues of the many epimerization experiments with lupinine (**1**) performed so far, we isolated a new compound **6**, which throws some light on the epimerization process **1** → **2**. Compound **6** (m.p. 100.5–103°) is very poorly soluble in dry Et_2O and its elemental analysis revealed a formula $C_{10}H_{18}NO_{1.5}$ corresponding to the hemihydrate of a compound with two H-atoms fewer than **1** or **2** ($C_{10}H_{17}NO \cdot 0.5\ H_2O$). Therefore, **6** could be considered a didehydro derivative of either **1** or **2** but is different from lupinal (**3**; m.p. 93–96°) [9] and epilupinal (**4**; oil) [10]. The molecular formula of **6** was confirmed by MS, which exhibited a molecular-ion peak at *m/z* 167, *i.e.*, at two mass units lower than that of **1** and **2** (*m/z* 169). The ^{1}H-NMR spectrum ($CDCl_3$) of **6** suggested the presence of an enol besides traces of an aldehyde.

The MS of **6** was largely coincident with the spectra of (**1**) and epilupinine (**2**) [11], which, in turn, differ from each other only slightly in the relative abundance of fragment-ion peaks. Nevertheless, two main differences should be pointed out. The relative intensity of the M^+ of **6** was very low (3%), while that of **1** and **2** is very high (60 and 90%, resp.). Moreover, the fragment at *m/z* 152 is the base peak (100%) of **1** and **2** arising from the loss of the OH group, whereas, very significantly, this fragment ion was lacking in the MS of **6**. Instead, a peak at *m/z* 150, corresponding to the loss of an OH group, was present with a very low abundance (4%), suggesting a quite different molecular entourage in **6** from that of the enol form of the aldehyde.

The ^{1}H-NMR spectrum of **6** exhibited a *d* at δ 9.59 ($J = 3.5$ Hz), that might be related to the proton of an aldehyde group coupling with H–C(1) of the quinolizidine ring. However, the integration of the *d* accounted for only 0.14–0.31 H, depending on the concentration of the $CDCl_3$ solution. The low intensity of this peak could be due either to an equilibrium between the carbonyl and the enol forms or by a reversible interaction of the solvent with the carbonyl group. The presence of exchangeable protons around δ 1.9 (superimposed to the quinolizidine protons) might support the presence of an enol OH group, though no quantitative evaluation could be performed due to the presence of H_2O, which could not be removed completely from the hemihydrate.

The IR spectrum (KBr) of **6** exhibited a low-intensity carbonyl band at 1722 cm^{-1} and a strong broad band around 3260 cm^{-1}, supporting further the existence of an equilibrium between an aldehyde and its enol form, either as such or as a salt with the strongly basic quinolizidine N-atom. The formation of a salt would account for the very poor solubility in dry Et_2O.

Compound **6** was very soluble in EtOH, but the oily residue obtained upon evaporation of the EtOH solution was now also soluble in dry Et_2O. After evaporation of the Et_2O solution, the oily residue exhibited the same ^{1}H-NMR and IR spectra as those already discussed for the Et_2O-insoluble compound, but the intensities of the aldehyde signals (δ 9.6; 1722 cm^{-1}) were enhanced, while those of the enol signals (δ 1.9; 3260 cm^{-1}) were reduced. On standing, the oily compound returned slowly to the Et_2O-insoluble solid form.

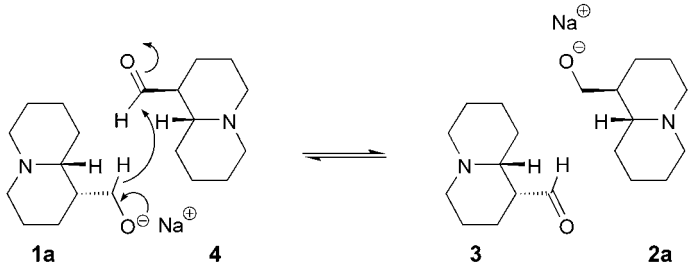
Finally, reduction of **6** with $NaBH_4$ gave rise to a mixture of lupinine (**1**) and epilupinine (**2**); **1** representing a quite minor component of the mixture, as indicated by the integration of its peculiar ^{1}H-NMR signal at δ 4.17 (*ddd*, $J = 10.4$ and 4.6 Hz) [12], due to the long-range coupling of one of the protons of the exocyclic methylene group with the OH proton bound to the tertiary N-atom, as depicted in structure **1**.

All these observations allowed us to attribute to the novel compound **6** the structure of the inner salt of (quinolizidine 1-ylidene)methanol, which is the common enol form of both lupinal (**1**) and epilupinal (**2**) and, with which **6** is in a tautomer equilibrium when it is dissolved in EtOH or $CDCl_3$.

Mechanism of Epimerization of Lupinine (1) and Epilupinine (2). – The always incomplete conversion of lupinine (**1**) to epilupinine (**2**) suggests the presence of a reversible process tending to an equilibrium. To substantiate this assumption, we heated separately for 6 h at 165° in closed tubes very pure **1** and **2** in xylene solution in the presence of Na. In both cases, we obtained a mixture **1**/**2**, thus establishing the reversibility of the epimerization process. The conversion **1** → **2** was *ca.* 30%, while **2** → **1** was 11–14% (result of several experiments). The addition of a small amount of (quinolizidin-1-ylidene)methanol inner salt (**6**) did not improve the conversion **1** → **2**; however the addition of the oily form of **6** (see above) raised the yield of **2** to 67%. Extending the heating time to 9 h, the yield increased to 85%, but further heating did not improve it anymore .The conversion **2** → **1** was not affected by the addition of the oily form of **6**.

Therefore, the reciprocal epimerization of **1** and **2** may be represented by *Scheme 1.* Lupinal (**3**), required to initiate the epimerization of **1**, could be formed either by air oxidation or, better, by thermal elimination of NaH from sodium lupinine (**1a**); then epilupinal (**4**) by reacting with sodium lupinine (**1a**) forms sodium epilupinine (**2a**) and a new amount of lupinal (**3**) that will propagate the reaction (*Scheme 2*). Starting from epilupinine (**2**) the sequence will proceed in the reverse direction. The formation of the insoluble inner salt **6**, by subtracting the aldehydes from the system, is detrimental on the epimerization process which can proceed only if new lupinal is produced.

Scheme 2

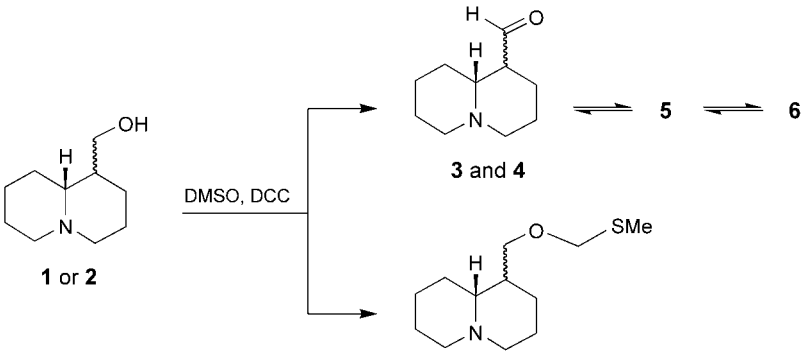


Reexamination of the Structures of Lupinal (3) and Epilupinal (4). – So-called lupinal was obtained by *Zaboev* [9] by oxidation of lupinine (**1**) with CrO_3 in AcOH and was described as an Et_2O-insoluble compound melting at 93–96°, only a few degrees lower than our inner salt **6** of (quinolizidine-1-ylidene)methanol. On the other hand, so-called epilupinal was obtained by *Wicky* and *Schumann* [10] on oxidation of epilupinine (**2**) with DMSO in the presence of dicyclohexylcarbodiimide and anhydrous phosphoric acid according to the method of *Pfitzner* and *Moffatt* [13]. This epilupinal was described as an oil, and, for its characterization, the 2,4-dinitrophenylhydrazone was prepared (probably by the use of a sulfuric acid solution of the hydrazine); however, the elemental analysis of this derivative did not account for the proposed structure. If the given values for H and N are exchanged, the analytical results agree with those required for the hydrogen sulfate of this epilupinal 2,4-dinitrophenylhydrazone. Nevertheless, the MS of this oily aldehyde is in reasonable accordance with that of our ether-insoluble compound **6**.

To clarify the situation, we oxidized lupinine (**1**) and epilupinine (**2**) with DMSO following the procedure of *Wicky* and *Schumann* [10]. In both cases, identical oily mixtures of the two aldehydes **3** and **4** with an M^+ at *m/z* 167 were obtained, as established by GC/MS (*Scheme 3*). The two aldehydes, differing in retention times by only 0.57 min, were present in a ratio ranging from 5 : 1 to 9 : 1 in repeated experiments. The fragmentation patterns of the two aldehydes were identical and were also identical to those of the product isolated from the epimerization mixtures. The initially oily mixture obtained from DMSO oxidation of **1** and **2** exhibited a clear carbonyl band in the IR spectrum, but, on standing, the mixtures solidified slowly with concomitant decrease in the intensity of the carbonyl band; finally they became completely solid and Et_2O-insoluble. Since identical aldehyde mixtures were obtained from **1** and **2**, the

interconversion of the two epimeric aldehydes to reach an equilibrium must be very easy. The $NaBH_4$ reduction of the initially oily aldehyde mixtures gave mixtures of lupinine (**1**) and epilupinine (**2**), with the latter largely prevailing over the former.

Scheme 3



DMSO = Me_2SO, DCC = dicyclohexylcarbodiimide

Therefore, it seems sound to conclude that the solid so-called lupinal obtained by *Zaboev* is, indeed, the inner salt **6** of the enol form **5**, while the oily so-called epilupinal of *Wicky* and *Schumann* is a mixture of the two epimeric aldehydes, epilupinal (**4**) and lupinal (**3**), not yet converted to the inner salt **6** of the common enol form **5**.

It is worth noting that the oxidation of **1** and **2** with DMSO was accompanied by the formation of an S-containing compound of molecular mass 229 that might be the (methylthio)methyl ether of the starting alcohol (see *Scheme 3*). Indeed, traces of such (methylthio)methylethers of the starting alcohol have been already isolated by *Pfitzner* and *Moffatt* [13], who also proposed the mechanism for their formation. In our case, particularly from **1**, the supposed (methylthio)methyl ethers were formed in substantial quantities, accounting for the relatively low yield of the aldehydes.

Experimental Part

General. All commercially available solvents and reagents were used without further purification, unless otherwise stated. TLC: aluminium oxide $60F_{254}$, neutral (*Merck*). M.p.: *Büchi* apparatus; uncorrected. Optical activity; *Perkin-Elmer 241 MC* polarimeter; sodium lamp, λ 589 nm; tube length 10 cm; EtOH solns. IR Spectra: *Perkin-Elmer Paragon-1000-PC* spectrophotometer; KBr pellets for solids, and neat for liquids; $\tilde{\nu}$ in cm^{-1}. ^{1}H-NMR Spectra: *Varian Gemini-200* spectrometer; in $CDCl_3$ with Me_4Si as internal standard; δ in ppm, J in Hz. GC/MS: *Hewlett-Packard 6890/5973* equipment; *m/z* (rel.%). Elemental analyses were performed on a *Carlo Erba EA-1100* CHNS-O instrument in the Microanalysis Laboratory of the Department of Pharmaceutical Sciences of Genoa University.

*Lupinine Epimerization: (Hexahydro-2*H*-quinolizin-1(6*H*)-ylidene)methanol Inner Salt* (**6**). To a soln. of (−)-lupinine (**1**; 8 g) in dry xylene (40 ml), NaH (3 g of 60% dispersion in mineral oil) was added, and the mixture was heated under reflux for 5 h under stirring (e.m.). On reaching the boiling temp., the mixture became pasty. After further stirring overnight at r.t., the mixture was extracted with 6N HCl (25 ml) and 0.3N HCl (2×25 ml), and then with H_2O. The acid soln. was extracted twice with Et_2O, strongly basified with 30% KOH soln., and extracted with Et_2O. The aq. phase was saturated with K_2CO_3 and further extracted with Et_2O. The Et_2O soln. was dried (Na_2SO_4) and evaporated. The obtained oil (7.6 g) was dissolved in dry Et_2O (5 ml) and left in the cold. Crystals were collected and washed with cold dry Et_2O/light petroleum ether 3:7. On

concentration of the Et_2O soln., a second crop of crystals was collected and washed as above: 4.56 g (57%) of (+)-epilupinine (**2**). M.p. 74.4–75.4°.

After evaporation of the remaining Et_2O soln., 3 g of oily crystals were recovered, which (eventually joined with analogous fractions obtained from other epimerization experiments) were treated again with NaH as above. The whole procedure might be repeated improving the yield of **2**. The further crops of crystals of **2** melted at 72–78° with a few crystals melting around 90°; these portions of **2** were dissolved in dry Et_2O leaving a whitish residue (m.p. 100–103°, see below), while from the evaporated soln., purer **2** was recovered. M.p. 77–78° (petroleum ether) $[\alpha]_D^{20} = +34.53$ (95°/EtOH, $c = 0{,}94$).

The whitish, Et_2O-insoluble (*ca.* 200 mg from each batch (8 g) of **1** used; *i.e.*, 2.5% yield), was boiled several times with dry Et_2O to eliminate any residual **1** and **2**: pure **6**[1]) M.p. 100.5–103°. IR (KBr): 1722 (C=O), 3150, 3250 (OH and/or NH^+). ^{1}H-NMR ($CDCl_3$): 1.1–2.2 (*m* with superimposed *s* at 1.9, *ca.* 13.7 H, 13 H of quin. *ca.* 0.7 H of OH collapsing with D_2O); 2.7–2.9 (*m*, 2 H, α to N); 3.50–3.74 (*m*, 1 H of quin.); 9.59 (*d*, J = 3.5, *ca.* 0.3 H, CH=O). MS: 167 (3, M^+), 166 (12, $M-H]^+$), 150 (4, $[M-OH]^+$), 138 (62, $[M-CH{=}O]^+$), 124 (10), 110 (42), 96 (51), 83 (100), 67 (3), 55 (11). $[\alpha]_D^{20} = +10.08$ (95% EtOH, $c = 0.594$). Anal. calc. for $C_{10}H_{17}NO \cdot 0.5\ H_2O$: C 68.14, H 10.29, N 7.95; found: C 68.26, H 9.94, N 8.16.

Reduction of Compound **6**. To a soln. of **6** (150 mg) in 80% EtOH (5 ml), $NaBH_4$ (50 mg) was added. The soln. was refluxed for 3 h and then evaporated. The residue was taken up in H_2O, treated with a few drops of 6N NaOH and extracted with Et_2O. The org. phase was dried (Na_2SO_4) and evaporated. 135 mg of **1/2** as oily crystals. TLC (alumina, ^{i}PrOH): R_f 0.32 (**1**; minor) and 0.44 (**2**). ^{1}H-NMR ($CDCl_3$): 4.10–4.25 (*m*, *ca.* 0.15 H) indicating a 15% content of **1**; for pure **1** this signal is at 4.17 (*ddd*, J = 10.4 and 4.6); it is absent in the spectrum of **2**.

Reciprocal Conversion of Lupinine (**1**) *and Epilupinine* (**2**). Pure **1** (500 mg, m.p. 69–70°, $[\alpha]_D^{20} = -21.49°$) was dissolved in dry xylene (3 ml) in an *Aldrich* pressure tube. Na (70 mg) was added, and the closed tube was heated in an oil bath at 165° for 6 h. The metal slowly disappeared, while a viscous mass was formed. After cooling, the mixture was diluted with Et_2O and extracted several times with dil. HCl soln. The acid soln. was alkalinized with 6N NaOH, saturated with NaCl, and extracted with Et_2O. The org. phase was dried (Na_2SO_4) and evaporated, leaving 478 mg of an oil of. $[\alpha]_D^{20} = -5.00$ (95% EtOH, $c = 0.956$), corresponding to a mixture of 70.6% of **1** and 29.4% of **2**.

Repeating the same experiment (470 mg of **1**) in the presence of the solid **6** (30 mg), the degree of conversion of **1** to **2** was practically the same.

In a further experiment, with 30 mg of the oily compound obtained from the EtOH soln. of **6** or from oxidation of **1** (see below), 464 mg of an oil of $[\alpha]_D^{20} = +16.04$, were obtained, corresponding to a mixture of 33% of **1** and 67% of **2**. Extending the heating time to 9 h, the yield raised to 85%.

Starting from pure **2** (500 mg; m.p. 77–78°, $[\alpha]_D^{20} = +34.53$) and operating under the same conditions, 456 mg of an oil of $[\alpha]_D^{20} = +26.85$ (95% EtOH, $c = 0.912$) were obtained that soon crystallized, corresponding to a mixture of 13.7% of **1** and 86.3% of **2**. Also in the presence of the oily aldehyde, the yield of **1** remained at *ca.* 15%.

The optical activity of mixtures of **1** and **2** changed linearly with composition.

Oxidation of Lupinine (**1**). Pure **1** (1 g, 5.9 mmol) was dissolved in anh. DMSO (40 ml; distilled *in vacuo* and stored over 4-Å molecular sieves) containing dicyclohexylcarbodiimide (3.2 g, 16 mmol). Anh. H_3PO_4 (1.2 g, 12 mmol) was added, and the mixture was stirred at r.t. for 1 h. Under ice cooling, 2N NaOH (20 ml) was added, and the white precipitate was centrifugated. The precipitate and the aq. DMSO soln. were extracted with petroleum ether. The combined org. phase was filtered and evaporated and the oily residue taken up in dil. HCl soln. and washed with Et_2O. The acid soln. was basified and extracted with Et_2O, the extract evaporated, and the residue (0.76 g) bulb-to-bulb distilled at 0.3 Torr. At 110–115° (air-bath temp.), 0.54 g of colorless oil were collected. GC/MS: two aldehydes with M^+ at m/z 167 (t_R 15.1 and 15.6; ratio 7:1, varying to up to 9:1 in further experiments), **1** with M^+ at m/z 169 (t_R 16.4), and traces of the supposed *O*-[(methylthio)methyl]lupinine with M^+ at m/z 229 (t_R 20.0), the latter being the main component of the distillation tail. On standing, the distilled oil solidified, and after repeated washings with dry Et_2O, a white powder (230 mg, *ca.* 23% yield; m.p. 95–96°) was obtained, which exhibited a unique peak in the GC/MS: M^+ at m/z 167. The MS fragmentation patterns of both oily and solid aldehydes were identical with that of the compound isolated from the epimerization mixture. IR (KBr): 3260*s* (OH and/or NH^+), 1722*w* (C=O).

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[1]) This compound is very soluble in EtOH, from which it is recovered as on oil with practically the same spectral characteristics as described above.

Oxidation of Epilupinine (**2**). Pure **2** was oxidized as described for the oxidation of **1** with practically the same results, apart from a higher yield (40–50%) of solid aldehyde. Also in this case, the GC/MS of the initially oily aldehyde indicated the presence of two fractions with M^+ 167 in a ratio 7–9:1.

Reduction of Prepared Aldehydes. The $NaBH_4$ reduction of either the oily or solid aldehydes obtained from both **1** and **2** gave always similar mixtures of **1** and **2** (TLC), with a large prevalence of the latter, similar to the reduction of **6** isolated from the epimerization mixture.

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Received October 25, 2004