*J. Mater. Environ. Sci. 5 (3) (2014) 811-814*
*ISSN : 2528-2028*
*CODEN: JMESCN*



# Identification of the pyrrolizidine alkaloid 1-hydroxymethylpyrrolizidine from *Thesium humile* Vahl

**G. Belakhdar[1], A. Benjouad[2], M. Kessabi[3], E.H. Abdennebi[3]***
[1] *Biochemical and Immunological Laboratory, Faculty of Sciences, University of Mohammed V, Rabat*
[2] *Faculty of Sciences, University of Mohammed V and International University of Rabat*
[3]*Department of Biological and Pharmaceutical Sciences, IAV Hassan II, Rabat*

*Received 9 Dec 2013, Revised 15 Dec 2013, Accepted 15 Dec 2013*
* *Corresponding author. E mail: pr.abdennebi@yahoo.fr*

## Abstract
This work describes the first chemical results obtained from *Thesium humile*, one of the Moroccan poisonous plants to livestock. Dosing mice with different extracts of the plant showed that the aqueous extract was highly toxic while ethyl acetate or methanol extract did not cause any toxicosis, suggesting that the toxic principle is a water soluble compound. After successive separations of the aqueous extract by column chromatography, the relatively clean and more toxic sub-fraction was analyzed by capillary gas chromatography-mass spectrometry and revealed the presence of a major compound identified as 1-hydroxymethylpyrrolizidine, a saturated pyrrolizidine alkaloid (PA).

*Keywords*: *Thesium humile*, pyrrolizidine alkaloids, 1-hydroxymethylpyrrolizidine, poisonous plants.

## 1. Introduction
*Thesium humile* Vahl, known in Morocco as *Ktitila*, belongs to the Santalaceae family. It is an annual small hemiparasite herb that grows mainly in North Africa and other parts of the Mediterranean region [1-3]. In addition of causing damages to cereal crops [4], this plant is also known to be highly toxic to animals, especially to ruminants [5]. *Thesium humile* was reported to cause very acute poisonings, since death of intoxicated animals occurs shortly after feeding. The described clinical signs are mainly digestive and nervous disorders, characterized by abdominal pains, convulsions and severe meteorism [6]. In Morocco, *Thesium humile* has caused cattle poisoning in 2010, in the area of Khemissat where this plant is naturally abundant [7-8]. Elsewhere, such as in South Africa, certain species of the genus *Thesium* have been suspected of causing death to livestock. Experimental poisoning with *Thesium namaquense* has confirmed its toxicity in sheep and laboratory animals [9]. A number of *Thesium* species are employed in African folk medicine. For example, *Thesium hystix roots* are used to cure kidney, bladder and lung infections, *Thesium utile* is used against gastric disorders [10], *Thesium viride* is prescribed to cure ulcers and jaundice [11], and the roots of *Thesium lacinulatum* are used as a remedy for uterine infections [12].
Following cattle poisoning of 2010 by *Thesium humile*, toxicological and phytochemical studies were conducted on this plant at the Institute of Agronomy and Veterinary Medicine of Morocco. The overall objective of these investigations is to identify the active principle(s) of this poisonous plant in order to have a better understanding of its mechanism of toxicity, and, consequently, to be able to suggest any appropriate antidote or therapeutic measures for intoxicated animals by this plant.

## 2. Material and methods
### *2.1. Plant materials*
*Thesium humile* was collected in May 2011 from the region of Khemissat where cases of poisoning by this plant were reported among cattle by animal owners and veterinary practitioners. The identification of the plant was done by comparison with the established National Herbarium specimen with Voucher Number RAB 78257 in the Scientific Research Institute of Morocco, following authentication by the Department of Ecology of the Institute of Agronomic and Veterinary Medicine, Rabat.
Plant material was first dried then powdered. A part of the powder was successively extracted by maceration during 24 hours, first with the ethyl acetate, then methanol and finally with distillated water. The different extracts were evaporated to dryness and kept in the freezer until toxicity testing and chromatographic fractioning and analysis.

### *2.2. Fractioning and bioassay testing*
The crude aqueous extract, which was toxic to mice, was subjected to successive chromatographies on silica gel to give different fractions. Elution was done by a solvent mixture of butanol, acetic acid and water (6/3/1) and collection was accomplished by an automatic fraction collector (LKB2211). The obtained Fractions (F1 to F5) were controlled by thin-layer chromatography (TLC) (Kieselgel 60 $F_{254}$, 0.20 mm, Merck) using butanol/acetic acid/ water (6/3/2) as the migration solvent

and Ninhydrin and Dragendorff as the spraying reagents. Each fraction was then tested for its toxicity in Swiss albino mice kept under standardized environmental conditions and had free access to food and water according to procedures of animal research guidelines. After solubilization, each fraction was given by subcutaneous injection of an equivalent of 2g/kg, taking into account the extraction ratios. Immediately after treatment, the animals were closely monitored for any behavioral changes or clinical signs of toxicity during a period of 24 hours. Fraction F3, the most toxic, was again chromatographed in the same condition as above to give subfractions F3a to F3e. The latter subfraction (F3e) was separated into four parts (F3e1 to F3e4) by Sephadex LH20 column chromatography.

*2.3. Gas chromatography- mass spectrometry analysis*

The sub-fraction F3e3 was analyzed by gas chromatography associated with a mass spectrometry (GC-MS) using an Agilent apparatus (6890N GC and 5973 inert MSD) equipped with a split less injector and a capillary DB-5 MS column [30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)]. The injection port temperature was 285℃ and the column oven temperature program was from 70℃ to 220℃ (15℃/min), then to 305℃ (8℃/min). The carrier gas was Helium (1ml/min) and the mass spectra were recorded at 70 eV. The alkaloid was identified by its retention index (RI) and mass fragmentation patterns in comparison to those of the reference data. Retention index (RI) was calculated using co-chromatographed hydrocarbon standards (C8-C28) according to Kovats [13].

## 3. Results and discussion

The results of toxicity tests of the extracts of *Thesium humile* revealed that only the aqueous extract was toxic to mice. After subcutaneous injection, the animals presented pronounced signs of asthenia, weakness, trembling then general paresis. Depending on the injected fraction, death occurred 10 minutes to 24 hours after treatment as a consequence of convulsive seizures and respiratory arrest.

From the first column chromatography, F3 was the highly toxic to mice, since the survival time was less than 20 minutes. TLC of this fraction showed brown-orange spots with Dragendorff's reagent suggesting the presence of alkaloids type of compounds. TLC of F3e3 subfraction revealed the presence of a single spot reacting with the Dragendorff's reagent with an Rf of 0.37. GC-MS chromatogram of this subfraction showed a major peak with a retention time of about 6.7 minutes and a retention index of 1294 (figure 1).

The mass fragmentations of this compound are m/z (%): 141(23, $M^+$), 124 (16), 110 (11), 97 (3), 83 (100), 70 (6), 55 (40), corresponding to the molecular formula of $C_8H_{15}NO$ and a molecular weight of 141.12 (figure 2). The comparison of these data with the defined reference data from our database (PMW-Tox2. I and Wiley 275.I) and with those obtained from the literature suggest that this compound is most likely 1-hydroxymethylpyrrolizidine, a saturated pyrrolizidine alkaloid (PA), with a quality level of 98%. The chemical structure of this compound is given in figure 3.

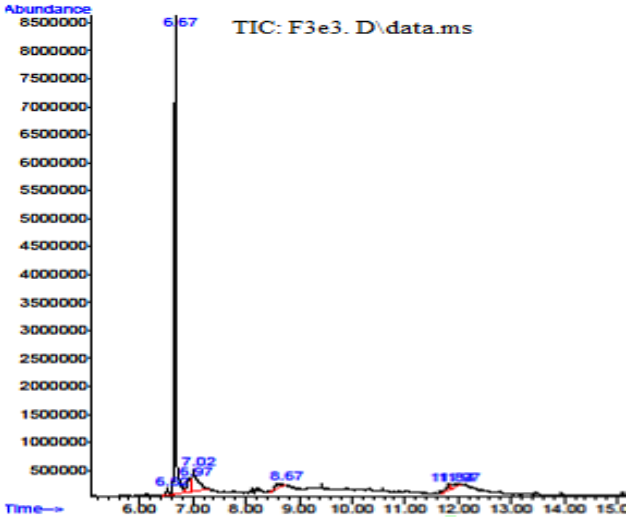


**Figure 1**: GC-MS chromatogram of the fraction F3e3

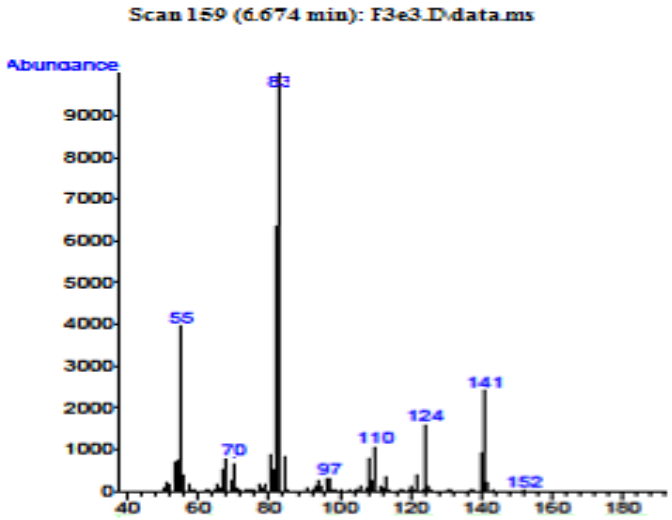


**Figure 2:** Mass spectrum of the major compound of F3e3

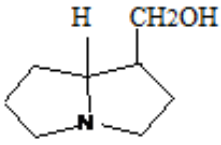


**Figure 3:** Chemical structure of 1-hydroxymethylpyrrolizidine

To our knowledge, only Balaguer and García-Marquina [14] and Garbo et al. [15] have suggested the presence of alkaloids in *Thesium humile* as detected by TLC. The identification of 1-hydroxymethylpyrrolizidine from this herb may be considered as an important step in the determination of its active(s) principle(s) and for the understanding of its mechanism of toxicity. According to Witte et al [16], capillary gas chromatography of underivatized PAs combined with mass spectrometry, as adopted in this study, is the most widely used technique for the analysis of this type of compounds. However, it has to be underlined that, depending on the spatial position of (-H) and ($CH_2OH$) groups, 1-hydroxymethylpyrrolizidine has four stereoisomers: (-)-trachelanthamidine, (-)-isoretronecanol, laburnine [(+)-trachelanthamidine] and lindelofidine [(+)-isoretronecanol]. Consequently, the isolated alkaloid from *Thesium humile* would be one of the Isoretronecanols or of the trachelanthamidines. The exact identity of this alkaloid is yet to be determined by more specific chemical analysis.
Biosynthesis of 1-hydroxymethylpyrrolizidines by plants starts with decarboxylation of the amino acids L-arginine and L-ornithine by the action of arginine and ornithine decarboxylase leading to the formation of putrescine [17]. From two putrescine molecules homospermidine is formed. This step is the most important in the biosynthesis of alkaloids and is catalyzed by the specific enzyme homospermidine synthetase (HS) [18]. Homospermidine is cyclized to the corresponding intermediate iminium ion which is reduced with further cyclization to the 1-hydroxymethylpyrrolizidines. Each of the1-hydroxymethylpyrrolizidine isomer is a specific precursor of other pyrrolizidine alkaloids as shown in figure 4. (-)-trachelanthamidine is the specific precursor of retronecine, by far the most widespread necine base of the toxic pyrrolizidine alkaloids [19]. (-)-isoretronecanol is specifically incorporated into the rare 2-hydroxylated PA such as rosmarinine [20]. Cynaustraline, one of the very rare examples of a PA with 8β-stereochemistry, was found to be formed via lindelofidine [(+)-isoretronecanol] in *Cynoglossum officinale* [21-23].

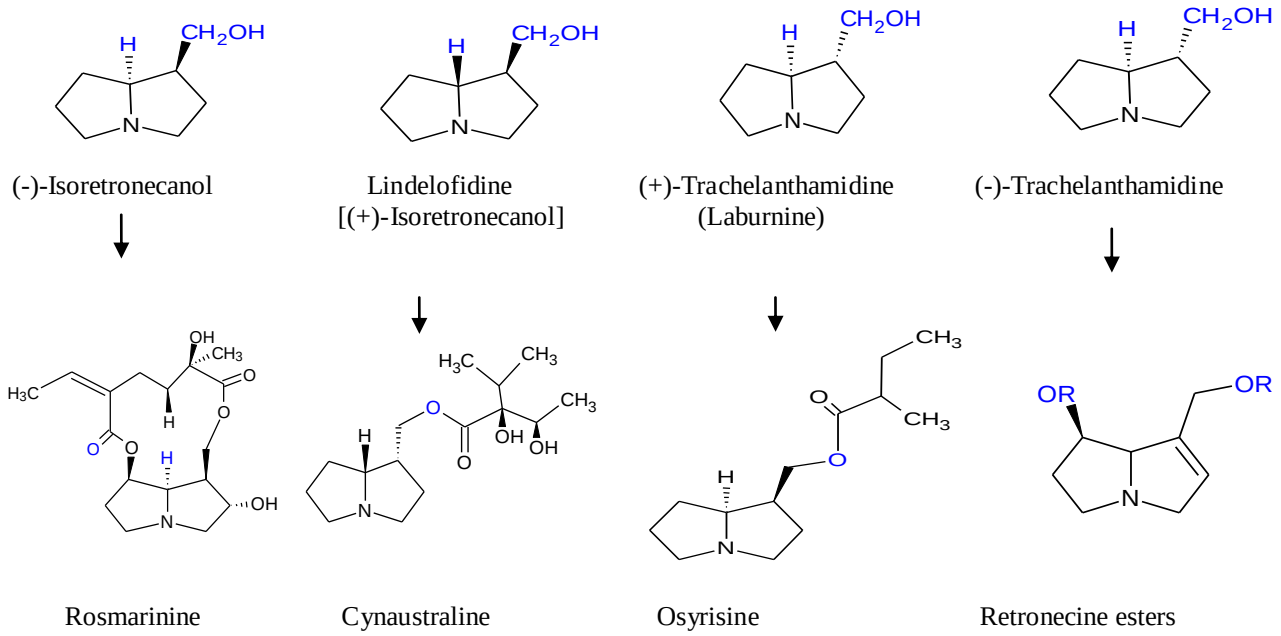


**Figure 4:** Necine esters obtained from 1-hydroxymethylpyrrolizidine isomers [19-21].

Up to now, more than 500 different pyrrolizidine alkaloids are identified from six main plant families: Apocynaceae, Boraginaceae, Asteraceae (Compositae), Leguminosae (Fabaceae), Ranunculaceae, and Scrophulariaceae [24]. PA-containing plants are probably the most common poisonous plants affecting livestock, wildlife and humans [25-27]. A large number of people in India [28] and Afghanistan [29] had contracted severe liver disease and many died after consuming cereals contaminated with seeds of *Crotalaria* sp. and *Heliotropium* sp., respectively. This type of chemicals is well known for their remarkable biological activities, including carcinogenic, mutagenic and/or hepato-toxic properties [30-31]. These adverse effects occur after metabolic activation by microsomal enzymes (P450), due to the presence of a 1,2-double bond in the pyrrolizidine ring system and esterification at C-9 or C-7 [32-35]. This bioactivation converts the protoxic alkaloids into pyrrolic intermediates that easily react with biological nucleophiles, causing severe cell toxicity and liver cancer [34]. Some saturated pyrrolizidine alkaloids are also cholinesterase inhibitors and interact with neuroreceptors [36-37] and, thus, affect the nervous systems by causing respiratory disorders, trembling, unconsciousness and death due to paralysis [38].
Chemical studies on the *Thesium* species are very limited and, thus, their active principles remain a matter of speculation. Only *Thesium lineatum* and *Thesium minkwitzianum* were relatively investigated. In the first, bufadienolide, a cardiac glycoside, has been identified by Anderson *et al.*, [39], while in the second three alkaloids (thesine, thesinine and thesinicine) were isolated from its aerial parts and two alkaloids (thesine and lindelofidine [(+) - isoretronecanol]) from its underground

parts. Thesine, an extremely toxic alkaloid, is the lindelofidine ester of thesininic acid, while thesinine is the lindelofidine ester of hydroxycinnamic acid [40].

## Conclusion

Preliminary investigations on *Thesium humile*, reported in this paper, confirm the toxicity of this plant in laboratory animals (mice) and revealed the presence of alkaloids in its aqueous extract. One of these compounds is identified by capillary gas chromatography-mass spectrometry as the pyrrolizidine alkaloid 1-hydroxymethylpyrrolizidine. The presence of this substance will certainly add this herb into the growing list of the pyrrolizidine alkaloids-containing plants and gives some light on the understanding of its mechanism of toxicity. Studies are underway to isolate and identify the other alkaloids detected by TLC.

## References

1. Negre R., *Centre National de la Recherche Scientifique* (CNRS.), Paris (1961) 160.
2. Simier P., Renaudin S., Fer, A. J., *Plant Physiol*. 143 (1994) 33-38.
3. El Hamri D., *Mémoire d'Ingénieur d'Application*, IAV Hassan II, Rabat, Maroc (1988).
4. Fer A., Simier, P., Arnaud M.C., Rey L., Renaudin S., *Plant Physiol*. 20 (1993) 15-24.
5. Curasson G., *Rev. Elevage Méd. Vét. Pays Trop*. 10 (1957) 53-62.
6. Fennane M., Ibn Tattou Ouyahya A., EL Oualidi J., *Flore pratique du Maroc*, 38 (2007) 636 pp.
7. Nahi H., *Thèse Doct. Vét.* IAV Hassan II, Rabat Maroc (1997).
8. Taleb A., Bouache M., Rzozi S., *Actes Inst. Agron. Vét. (Maroc)*, 18 (1998) 121-130.
9. Steyn D.G., *Onderstepoort J. Vet. Sci. Anim. Indus*. (1937) 9 111-124.
10. Watt Breyer-Brandwijk, E, Livingstone Ltd., Edinburgh-London, 2nd Edition (1962).
11. Polhill R.M., *Flora of Tropical East Africa*, Royal Botanic Gardens, Kew, Richmond, United Kingdom (2005) 27 pp.
12. Von Koenen E., *Klaus Hess Verlag*, Göttingen, Germany (2001) 336 pp.
13. kovats E., *Helv.Chim. Acta*, 41 (1958) 1915-1932.
14. Balaguer R., García-Marquina J.M., *Farmacognosia* 24 (1964) 75-84.
15. Gharbo S.A, Saleh M.R, Shukry E.M., *Planta Med.* 17 (1969) 236-44.
16. Witte L., Rubiolo, P., Bicchi, C., Hartmann, T., *Phytochem.* 32 (1993) 187-196.
17. Robins D.J. *Experientia*, 47 (1991) 1118-1122.
18. Böttcher F.,Adolph, R. D.,Hartmann, T., *Phytochem.* 32 (1993) 679-689
19. Leete E., Jatinder R., *J. Nat. Prod*. 49 (5) (1986) 838-844.
20. Kunec E.K, Robins D.J., *J. Chem. Soc.* 1 (1989) 1437-1441.
21. Van Dam N.M., Verpoorte R., van der Meijden, E., *Phytochem.* 37 (1994) 1013-1016.
22. Finian J., Leeper, Vederas J.C., *Aromatic Polyketides, Isoprenoids and Alkaloids*, published by Springer Berlin Heidelberg, 29 (2000).
23. Becerra Jimenez, J., *Doctorate Dissertation*, Rheinische Friedrich-Wilhelms-Universitat Bonn (2013).
24. Weiedenfeld, H., *Natural products*, edited by Ramawat & Mérillon , Springer-Verglag Berlin Heidelber (2013).
25. Roeder E., *Curr. Org. Chem.* 3 (1999) 557-576.
26. Roeder E., *Pharmazie*, 64 (2009) 699-716.
27. Cheeke P.R., *In Toxicants of Plant Origin*, edited by Cheeke P.R., CRC Press Florida (1989).
28. Tandon B.N., Tandon H.D., Tandon R.K., Narndranathan M., Joshi Y.K., *Lancet*, 2 (1976) 271-272.
29. Mohabbat O., Younos M.S., Merzad A.A., Srivastava R.N., Sediq G.G., Aram, G.N., *Lancet*, 7 (1976) 269-71.
30. Peterson J. E., Culvenor C.C.J., *Handbook of Natural Toxins*, edited by Keeler & Marcel Dekker, New York,1 (1983) 637.
31. Hartman T., Witte, L., *Chemical and Biological Perspectives*, edited by Pelletier, Pergamon, Oxford, 9 (1995) 115.
32. Stegelmeiern B.L., Edgar J.A., Colegate S.M., Gardner D.R., Schoch T.K., Coulombe R.A., Molyneux R.J., *Nat. Toxins* 8 (1999) 95.
33. Fu P.P., Xia Q., Lin G., Chou M.W., *Int. J. Mol. Sci.* 3 (2002) 948-964.
34. Fu P.P., Xia Q., Lin G., Chou M.W., *Drug Metab Rev* 36 (2004) 1-55.
35. Xia Q., Chou M.W., Edgar, J.A., Doerge, D.R., Fu, P.P., *Cancer Lett.* 231(2006) 138-145
36. Atal C.K., *Lloydia* 41 (1978) 315–326.
37. Schmeller T., El-Shazly, A., Wink, M., *J. Chem. Ecol*. 23 (1997) 399-416.
38. Roberts M.F., Winks, M., *Alkaloids: Biochemistry, ecology and medical applications*, edited by Roberts & Wink, Plenum Press, New York (1998) 397-424.
39. Anderson L.A., Joubert J.P., Schultz R.A., Kellerman T.S., Pienaar B.J., *Onderstepoort J. Vet. Res.* 54 (1987) 645.
40. Arendaruk Proskurinna, Konovaliva, *Zhur. Obs. Khim.* 30 (1960) 670.