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Gram-scale purification of aconitine and identification of lappaconitine in *Aconitum karacolicum*

M. Tarbe[a,b,*], H. de Pomyers[a], L. Mugnier[a,b], D. Bertin[b], T. Ibragimov[c], D. Gigmes[b], K. Mabrouk[b,**]

[a] Latoxan, 845 avenue Pierre Brossolette, 26800 Portes lès Valence, France
[b] Aix Marseille Université, CNRS, ICR UMR 7273, 13397 Marseille, Cedex 20, France
[c] Institute of the Chemistry of Plant Substances, Academy of Sciences of Uzbekistan, Tashkent, Uzbekistan

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ABSTRACT

Aconitum karacolicum from northern Kyrgyzstan (Alatau area) contains about 0.8–1% aconitine as well as other aconite derivatives that have already been identified. In this paper, we compare several methods for the further purification of an *Aconitum karacolicum* extract initially containing 80% of aconitine. Reverse-phase flash chromatography, reverse-phase semi-preparative HPLC, centrifugal partition chromatography (CPC) and recrystallization techniques were evaluated regarding first their efficiency to get the highest purity of aconitine (over 96%) and secondly their applicability in a semi-industrial scale purification process (in our case, 150 g of plant extract). Even if the CPC technique shows the highest purification yield (63%), the recrystallization remains the method of choice to purify a large amount of aconitine as i) it can be easily carried out in safe conditions; ii) an aprotic solvent is used, avoiding aconitine degradation. Moreover, this study led us to the identification of lappaconitine in *Aconitum karacolicum*, a well-known alkaloid never found in this *Aconitum* species.

Chemical compounds

Aconitine (PubChem CID: 245,005)
Lappaconitine (PubChem CID: 441,743) (See Schema 1.)

1. Introduction

For centuries, *Aconitum* plants (Ranunculaceae) have been known for their biological properties [1]. On the one hand, *Aconitum* extracts were used as a poison [2]; this toxicity is mainly due to C_{19}-norditerpenoid alkaloids such as aconitine, a diterpenoid alkaloid whose DL_{50} values in mice are 1.8 mg/kg (orally), 0.31 mg/kg (intraperitoneally) and 0.12 mg/kg (intravenously) [3]. On the other hand, *Aconitum* roots have been used in traditional Chinese medicine for their anti-inflammatory and analgesic effects that are also partly attributed to aconitine [1]. This two paradoxical activities lead today scientific research to two main issues about aconites: first, the development of new therapeutic aconite-based treatments whose effects are rigorously monitored by chromatographic technics to avoid the poisoning of patients [4]; secondly, the identification of new aconites derivatives showing the same pharmacological activity as aconitine without the toxicity, such as lipo-alkaloids [5] or C_{20}-diterpenoids [6]. These molecules have been either found in *Aconitum* plants [6–7] but frequently in small quantities or synthesized from natural aconites [6,8]. Indeed, due to the complex structure of the aconite skeleton, the total synthesis of such alkaloids is a great challenge. Thus the access to large amounts of aconitine or derivatives necessarily involves purification of plant extracts.

Since the XIXth century, obtaining pure aconitine has met much controversy; either the pharmacological aspects or therapeutic effects of products named aconitine by their producers were absolutely disparate. Both the different origins and growth ways of *Aconitum* plants and the various extraction processes provided totally different products and so divergent observations. In 1872, Duquesnel first pointed these problems and proposed a limited-steps extraction of aconitine providing crystals from ether [9–10]. Even though other liquid-liquid extraction systems had been developed until the middle of XXth century, the final step purification was a recrystallization from ether or absolute methanol [11–13]. Then, solid-liquid and liquid-liquid chromatography techniques were developed and became the main ways to isolate

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* Corresponding author at: Latoxan, 845 avenue Pierre Brossolette, 26800 Portes lès Valence, France.
** Corresponding author.
E-mail addresses: mariontarbe@hotmail.fr (M. Tarbe), kamel.mabrouk@univ-amu.fr (K. Mabrouk).

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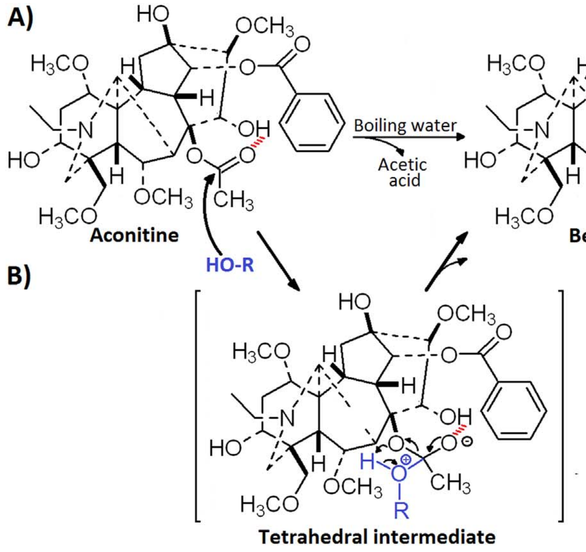

Schema 1. A) Degradation of aconitine in boiling water; B) Mechanism proposed for the degradation of aconitine in water and alcoholic solvent at room temperature.

natural substances. The former, alkaloids from *Aconitum* extracts including *Aconitum karacolicum* were purified by several consecutive chromatography columns differing from the stationary phase (Silica, Sephadex,…) [7,14]. The latter, counter-current chromatography, a liquid-liquid chromatography technique based on partition coefficient of a molecule in different solvents, has been used to isolate aconitine from *Aconitum forrestii* [15] and *Aconitum duclouxii* [16]. Finally, a green chemistry technology, macroporous resins have been used to isolate aconitine and other alkaloids from *Aconiti kusnezoffi* radix [17]. Each of these publications is individually interesting but, because of both the broad range of *Aconitum* Genus and the nature of the initial crude used to isolate aconitine and other alkaloids, it is impossible to really compare the efficiency of one technique from another. Indeed, the *Aconitum* genus includes over 100 species. Among them, the *Aconitum karacolicum* which comes from the North of Kirghizstan (Alatau area) contains about 0.8–1% of aconitine [7] and several other aconites including karakoline, karakolidine, zongorine and zongoramine as major component and nappeline, aconifine, acetylnapelline, karaconitine, 3-deoxiaconitine, indacontine and chasmaconitine as minor one [18–20]. In the present study we compare different methods of purification of aconitine from an *Aconitum karacolicum* extract particularly rich (14.4%) in an alkaloid presenting physico-chemical properties similar to aconitine. Furthermore, the impurity has been identified as lappaconitine, a well-known diterpenoid from *Aconitum* genus, but never isolated from *Aconitum karacolicum* specie.

2. Material and methods

2.1. Safety cautions

Dry aconite extracts were manipulated on no draught area and the experimenter was equipped with safety glasses, powder mask and gloves. As far as possible, solutions of aconites were manipulated under hood. Aconite extracts were stored in safety place in order to be accessible only by authorised staff and all areas and materials in contact with these substances have been carefully cleaned after each procedure.

2.2. Plant material

Aconitum karacolicum roots were cleaned by water from dust impurities, dried on open air and grounded to obtain fragments under 10 mm length. Ground roots (10 kg) were damped with 5% aqueous ammonia for 2 h and then extracted with dichloromethane 8 times (in 12 h). After then, obtained extracts were concentrated and treated with a 5% aqueous solution of sulphuric acid. The acidic solution was cooled down to 0 °C and treated with a 25% aqueous ammonia solution. This aqueous mixture was extracted with ether 4 times in presence of K_2CO_3. Obtained solution were dried on $MgSO_4$, filtered and distilled. Crude sample was dissolved in chloroform and loaded on an aluminium oxide column chromatography (chloroform – methanol, 4:1). Fractions containing aconitine were collected, evaporated and the dry residue was submitted to recrystallization process in methanol. Crystals were filtered and dried at 25 °C (126 g, 1.26%). These extract mainly containing aconitine has been utilised to compare separative methods of aconitine from an unknown impurity (Fig. 1).

2.3. High pressure liquid chromatography

RP-HPLC analyses were carried out on an Alliance 2695 system (Waters) using a Zorbax SDB-C8 column (4.6 × 250 mm, 5 μm). The mobile phase was composed of 10 mM of ammonium acetate, pH = 8.9 (Solvent A) and methanol (Solvent B). A gradient elution (0–35 min: 40 to 100% of B) was applied at a flow rate of 1.0 mL/min. Column effluent was monitored by UV detection at 233 nm using a 2998 Photodiode array detector (Waters).

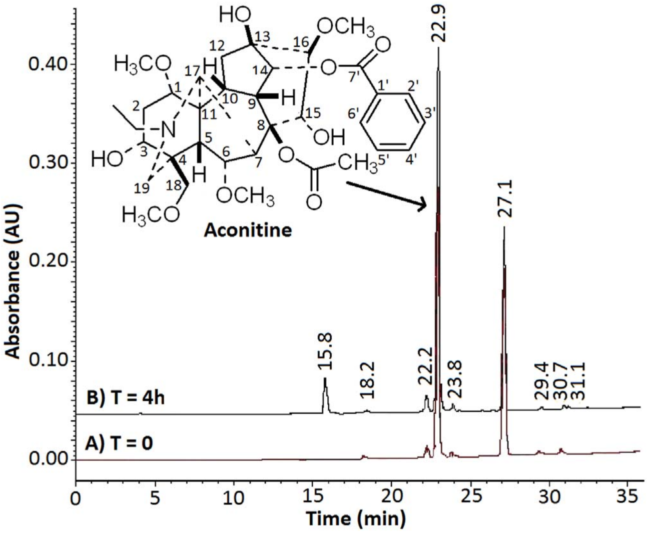

Fig. 1. RP-HPLC of impure batch A) Freshly prepared, B) After 4 h at room temperature in the dark.