Chiang Mai J. Sci. 2007; 34(1) : 127-133
www.science.cmu.ac.th/journal-science/josci.html
Contributed Paper

Application of Electrocoagulation to the Isolation of Alkaloids

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Received: 18 August 2006
Accepted: 28 October 2006.

ABSTRACT

Electrocoagulation technique was applied to the isolation of seven known alkaloids. The result showed that they could be extracted successfully with a yield comparable to that obtained by conventional methods. However, with respect to the number and amount of organic solvents used in the isolation process, all isolations incorporating electrocoagulation as a fractionating step utilized a minimum number and amount of organic solvents, especially those that were notorious for harming the environment.

Keywords : Electrocoagulation, alkaloids, caffeine, capsaicin, dihydrocapsaicin, reserpine, ajmaline, arecoline, solasodine.

1. INTRODUCTION

Alkaloids comprise the largest single class of secondary plant substances with a wide spectrum of physiological activities, hence their wide use in medicine. Being chemically basic in nature, their isolation normally requires the use of acid-base partitioning, as well as a number of organic solvents, first to extract it out of the raw material, and then to fractionate it by chromatographic methods, before the required alkaloid in a reasonably pure state can be obtained. Those organic solvents are usually not only costly and toxic, but also eventually a burden to the environment even if treated in a proper way.

With the advent of electrocoagulation (EC), it has been known for some time that this process is capable of fractionating in a rather efficient manner a number of both organic and inorganic substances by electrochemically coagulating some of them, while leaving the other components free in the solution. The process comprises an electrolysis setup (Figure 1), normally with aluminium or iron plates being used as both electrodes to generate *in situ* such species as Al^{3+}, Fe^{2+}, Fe^{3+}, OH^-, $Al(OH)_3$, $Fe(OH)_3$, etc., which are capable of selectively coagulating some particles soluble or suspended in the electrolysed solution [1]. The advantage of fractionating by EC is that it is a process in which the use of organic solvents, especially those that are toxic, is dispensed with or kept to a minimum. In our experience in applying this technique to the isolation of natural organic substances, we were successful to some extent in isolating a number of such products as a few glycosides [2-5], a cyclic polyol [6], some triterpenoids [7,8], tannins [9,10], and some phenolic compounds including quinones [10-12]. However, to our

knowledge, no study has been carried out which applies EC to the isolation of alkaloids, another important class of natural products. We thus report here our work on this novel method of isolation of some alkaloids by EC.

2. MATERIALS AND METHODS

2.1 General

The authentic compounds were used as such. Nicotine and reserpine were of laboratory grade and were purchased from BDH (England). Piperine (purum grade) and capsaicin (HPLC grade, > 97.0%) were purchased from Fluka Chemica AG (Buchs, Switzerland). Ajmaline and arecoline were of laboratory grade and were purchased from Merck (Germany) and Sigma (USA), respectively.

All other chemicals and solvents were of commercial grade, laboratory grade, or analytical grade, and were used without further purification. Aluminium plate used for electrodes was purchased from a local store.

Direct current for electrolysis was sustained by a GPR-1810HD or GPS-3030D Model DC power supply from Good Will Instrument Co. Ltd. Absorbance was measured on a Genesys 10 spectrometer.

Melting points were measured on a Mel-Temp II melting point apparatus and were uncorrected. The infrared spectra were recorded as KBr discs or thin films on a Bruker FTIR Tenser27 instrument or a Nicolet FTIR 510 instrument. The UV-VIS spectra were measured on a Genesys 10 spectrometer. The ^{1}H NMR spectra were obtained in deuterochloroform on a Bruker DRX400 (400 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard. Thin layer chromatography (TLC) analyses were performed on silica gel plates (Merck 60 F_{254}, 0.2 mm thickness), and the components were detected by UV light (254, 365 nm). High performance liquid chromatography (HPLC) analyses were carried out with an Agilent Technologies HP1100 Quaternary instrument with a UV detector, an Eclipse XDB-C8 column (4.6x150 mm), and 0.1% trifluoroacetic acid in acetonitrile as eluent (gradient elution). Gas chromatographs were run on an Agilent Technologies 6890 instrument. Mass spectra were obtained on a Hewlett Packard 5973 spectrometer with an ionization potential of 70 eV.

Dry tea leaves, dry tobacco leaves, dry black pepper fruits, areca nuts, and the fruits of *Capsicum frutescens* were purchased from a local market. The roots of *Rauwolfia serpentina* were purchased from a local herbal drug store.

2.2 Electrocoagulation Experiments

Solutions of each of the five alkaloids (0.005-0.025% w/v), viz. caffeine, capsaicin, reserpine, ajmaline, and arecoline, were prepared in water or aqueous ethanol. A pair of 15 x 4 cm aluminium plates spaced 3 cm apart was immersed 6.5 cm into each alkaloid solution (200 ml) contained in a 250-ml beaker. The solution was agitated throughout the experiment with a magnetic stirrer. Sodium chloride (0.4 g or 0.2% w/v) was added as supporting electrolyte. Direct current (0.2-2.6 A, 19-31 V) was passed through the solution *via* the two electrodes. At 15-minute intervals over a 2-hour period of electrolysis, a 5-ml aliquot of the solution was withdrawn, centrifuged, and the absorbance of the supernatant solution was measured at an appropriate wavelength corresponding to the absorption maximum for each alkaloid solution as follows: caffeine 275 nm, capsaicin 230 nm, reserpine 270 nm, ajmaline 245-250 nm, and arecoline 205 nm.

2.3 Purification by Electrocoagulation (EC)

Previous procedures [3-6,11] for EC were generally followed. An aqueous, aqueous ethanolic, or aqueous methanolic extract of each studied plant part, usually obtained by a Soxhlet extraction or maceration, was rid of undesirable impurities, e.g. tannins, pigments,

by electrolysis. For this process, a pair of aluminium or iron plates of suitable dimension placed 1.5-3 cm apart was used as electrodes and sodium chloride (0.2%) was used as supporting electrolyte. Direct current at low voltage (up to 8 A and 31 V) from a power supplier was passed through the magnetically-stirred extract for 0.5-2.5 hours (Figure 1). The resulting mixture was filtered and the filtrate normally was rid of solvent by a rotary evaporator. The resulting residue which contained the added salt was extracted with a small amount of ethanol or acetone and the ethanolic or acetone solution obtained was evaporated to dryness to give a crude alkaloid, which was subsequently purified by crystallisation, or directly analysed by a suitable method.

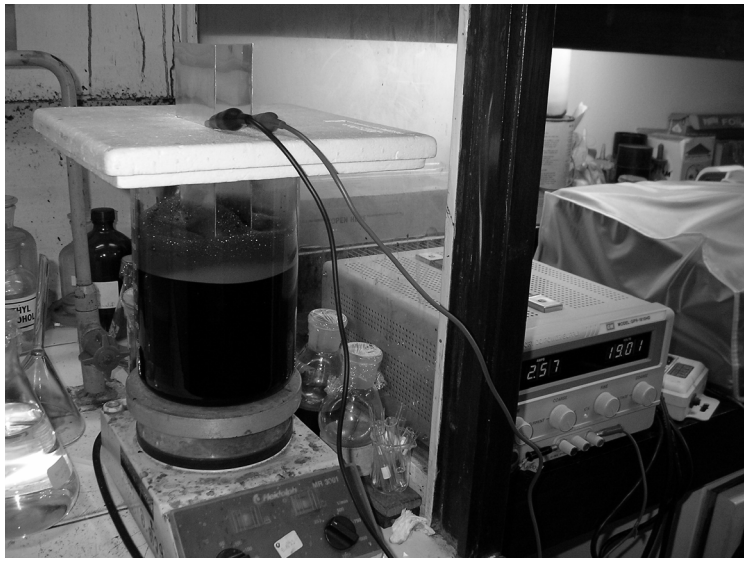

Figure 1. A typical electrocoagulation setup.

3. RESULTS AND DISCUSSION

When a pure alkaloid sample chosen for this study was subjected to EC under a normal condition, the result shows that the alkaloid is little affected by the process and remains in the electrolysed solution (Figure 2). This result is more or less the same as that obtained when EC is applied to glycosides [2-4,11]. Thus, it appears that, like glycoside, the amine function in the alkaloid seems to be unreactive to coagulation under the electrochemical condition used in our operation. EC in this case will then serve to clear up the undesirable impurities, e.g. tannins and pigments, by coagulating them out. What remains in the solution is therefore a purer alkaloid fraction, which can be easily separated. In most cases, this is done simply by evaporating out the solvent from the filtrate obtained after filtering out the coagulated impurities. The residual crude alkaloid, still containing the salt added as supporting electrolyte, is dissolved in a little nonaqueous solvent, usually ethanol or acetone, to rid it of salt in the final step.

By using this general method, we could extract crude caffeine from tea in a purer form (white powder, higher m.p.), although in a somewhat lower yield (0.4 vs. 0.7%), than that obtained by a classical laboratory procedure [13]. However, the latter method requires a large amount of chlorinated solvent

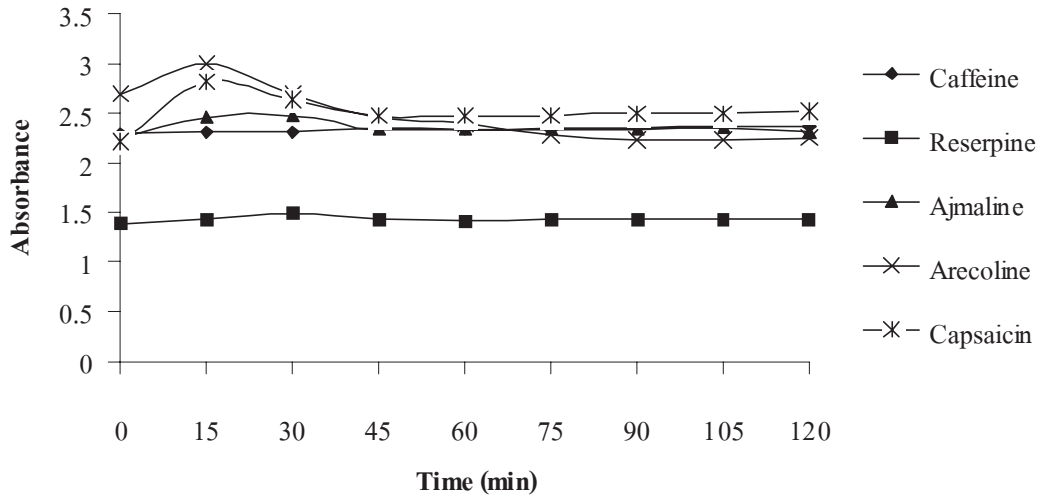

Figure 2. Plot of absorbance against electrocoagulation time for each alkaloid solution (Due to an increase in pH of the electrolysed solution, this might initially affect the absorbance of a strong base like arecoline slightly. Similar phenomenon may occur also to capsaicin, the only alkaloid which has a phenolic function).

(usually dichloromethane) to partition the caffeine out of the aqueous tea solution before a crude slightly green-coloured caffeine is gotten. Similarly, capsaicin and dihydrocapsaicin, the major pungent alkaloids from red chilli, were isolated electrolytically using only 75% ethanol as solvent. With a typical conventional method [14], four organic solvents, viz. acetone, toluene, benzene, and 50% ethanol, were utilized before the same alkaloids were obtained in comparable quantity (0.3%) and purity (as characterised by NMR and determined by GC, Figure 3).

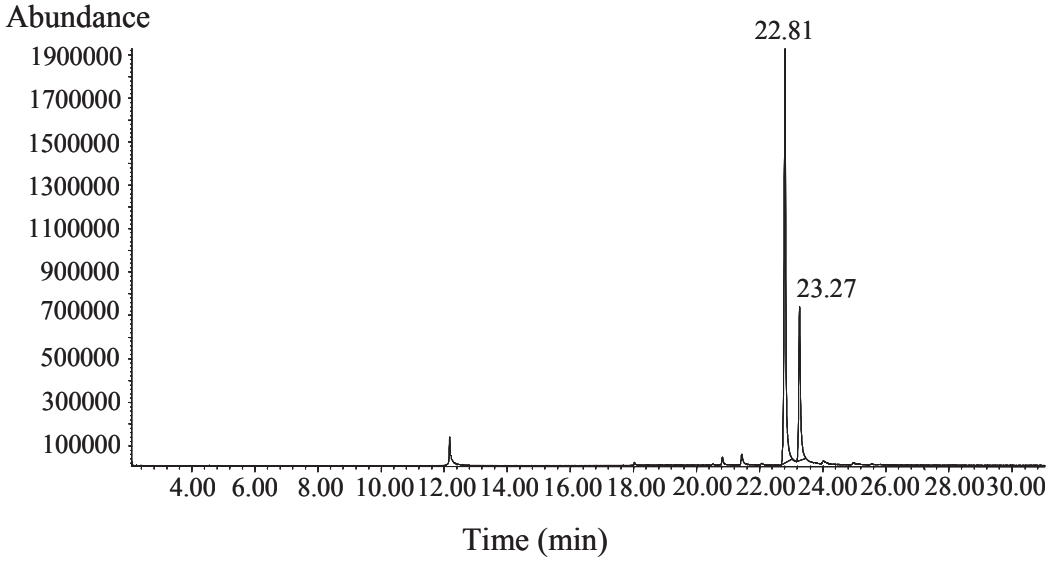

Figure 3. GC of red chilli crude extract obtained by EC method, showing capsaicin (retention time 22.81 min.) and dihydrocapsaicin (retention time 23.27 min.).

In order to isolate the medicinally important alkaloids (reserpine and ajmaline) from the root of *Rauvolfia serpentina*, it usually has to be soaked with dilute ammonia before being extracted with an organic solvent (e.g. ether) [15]. The extracted alkaloids were then partitioned with an acid solution, converted back into the free bases, and taken into another water-insoluble organic solvent (e.g. benzene) to give, after evaporation, the crude alkaloid extract. With our EC method, we directly extracted the root with ethanol, diluted the ethanolic extract with a small amount of water before subjecting it to EC for 2 hours. The resulting electrolysed mixture was filtered and the filtrate evaporated to dryness. Dissolving the resulting residue with a small amount of ethanol directly afforded, after evaporating off the ethanol, the crude extract containing the two alkaloids in about the same amounts (although in a more dilute form) as those in the above extract obtained conventionally (0.1% and 0.8% of the root for reserpine and almaline respectively, as determined by HPLC, Figure 4).

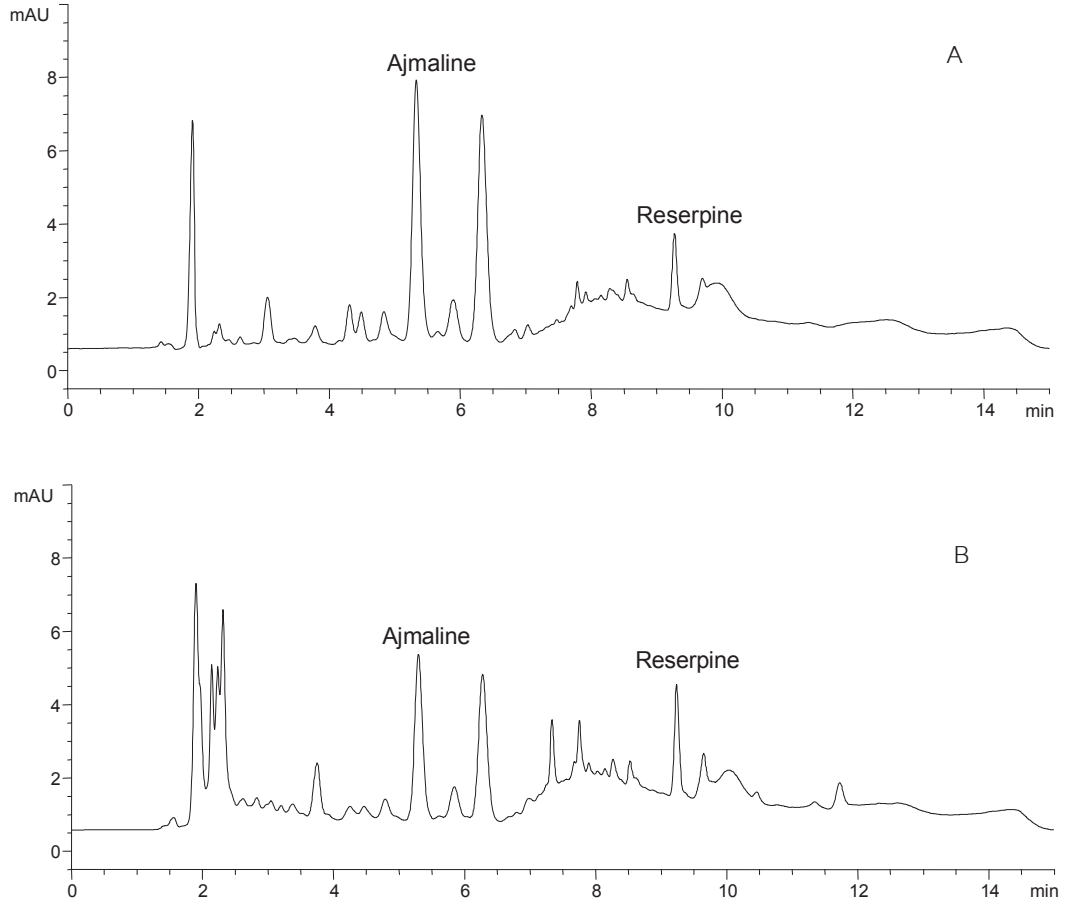

Figure 4. HPLC of crude extracts of *Rauvolfia serpentina* roots obtained by conventional method (A) and EC method (B) [Extra peaks in B might stem from alcohol, a solvent with a wider extracting range, being used as extracting solvent].

Similarly, arecoline was electrochemically extracted from arecanut in approximately the same percentage yield (0.1%) as by a normal method in the literature [16], but with less use of organic solvent (ether). Moreover, a red dye, which readily coagulated out, was obtained as a by-product. Finally, when solasodine, a steroidal alkaloid from the leaf of *Solanum laciniatum*, was extracted using EC as the dechlorophyllation step [5] in place of the conventional dechlorophyllation by organic solvent (e.g. chloroform) [17,18], we obtained the pure alkaloid (matching m.p., IR, NMR with authentic sample) in a much better yield (1% vs. 0.5%). This steroidal alkaloid is an important starting material for the synthesis of many steroids.

4. CONCLUSION

It has been demonstrated that EC can be successfully applied to the isolation of some alkaloids, with an important advantage over conventional isolation methods. This is the reduction in both the number and amount of toxic organic solvents involved in the isolation process. This method of isolation of alkaloids therefore seems to be a novel alternative method, which is also a more environmentally friendly one.

ACKNOWLEDGEMENTS

This research study is supported financially by TRF (Thailand Research Fund) in the form of Medicinal Chemistry Grant (DBG4780011) and Royal Golden Jubilee PhD Grant for K. J., and by the Commission on Higher Education PhD Grant for N. C. We also thank the Graduate School, Chiang Mai University, for partly supporting N. P., K. J., and N. C. financially.

We are grateful to Dr. Jiradej Manosroi of the Faculty of Pharmacy, Chiang Mai University, for supplying the leaves of *Solanum laciniatum*.

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