عزل لقلويدات نبات الاناباسيا (*Anabasis aphylla*)

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الخلاصة

درست محتويات الجزء الهوائى لنبات الاناباسيا العراقي من القلويدات . فصل قلويد الاناباسين وقلويد أقلدين من المستخلص الكحولي للجزء الهوائي من نبات الاناباسيا العراقي. تم الفصل باستخدام طريقة الكروموتوغرافيا العمودية (Column Chromatography)، ثم كروماتوغرافيا الطبقة الرقيقة (Thin layer Chromatography) و شخصت القلويدات المعزولة باستخدام طرائق التحليل المختلفة، مثل طيف الاشعة فوق البنفسجية، والاشعة تحت الحمراء، وقياس معامل الانكسار ومقارنة القلويد بالقلويد القياسي باستعمال كروماتوغرافيا الطبقة الرقيقة وتحضير ملح.

Investigation of alkaloids of *Anabasis aphylla* (Chenopodiaceae)

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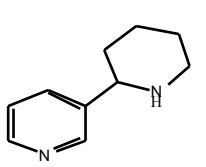
Abstract

The aerial part of Iraqi *Anabasis aphylla* (Chenopodiaceae) had been investigated for its alkaloidal contents.The alkaloid anabasine [2-(3-pyridyl)-piperidine] [1] & aphyllidine were isolated from an ethanolic extract of the plant. Isolation of the alkaloid was done by column chromatography followed by preperative thin layer chromatography. Identification of the isolated alkaloid was done by different spectroscopic methods (UV,IR), refractive index & TLC using authentic sample & preparation of a salt.

Introduction

The Chinopodiaceae contains 102 genera & 1400 species most grow naturally in soils containing much salts (halophytes). Genera include Beta (6spp), *Chenopodium* (100-150), *Salicornia, Atriplex &Anabasis*.(2) Investigation of certain Anabasis species revealed that they contain triterpenoid sapogenins &/or alkaloids. In addition to anabasine (neonicotine)[l],



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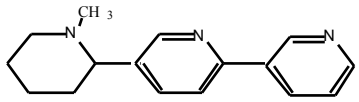

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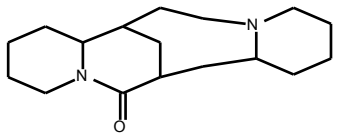

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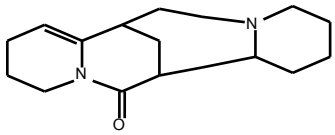

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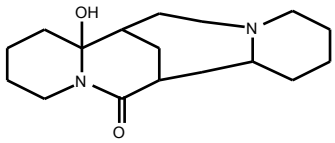


(VI)

anabasamine[ll], *A. aphylla* contains several quinolizidine alkaloids which were identified as aphylline[lll], aphylline N-oxide, aphyllidine[IV] & oxaphylline[V]. Lupinine as well as other alkaloids were also detected in *A. aphylla*.[3, 4, 5]

The presence of the alkaloids above was confirmed by paper chromatography[4]

Although nicotine is the best known alkaloid of tobacco, anabasine is the major alkaloide, as it is in *Nicotiana glauca* & *Anabasis aphylla* & it's large scale isolation from Nioctiana & other genera was extensively studied since anabasine was at one time widely used as insecticide.[6] Anabasine, like lobeline, has antismoking & respiratory muscle stimulatory action, & like nicotine it exhibits insecticidal properties . Anabasine also was used as a mental anticorrosive [7, 8]. Studies revealed that anabasine is teratogenic ,where by it can induce arthogrypotic congenital defect in pigs.[9] Anabasine as well as other minor tobacco alkaloids , nor nicotine & anatabine , are known to posses nicotinic receptor agonist activity, although they are relatively less potent than S-(-)-nicotine , the principal tobacco alkaloid.[10]

Biosyntheticaly the pyridine ring of (-)- anabasine is derived from nicotinic acid, but the piperidine ring is not. This was demonstrated by oxidation of the anabasine to nicotinic acid & decarboxylation of the latter to pyridine .[11]

Anabasine is recommended in the form of its hydrochloride salt for extensive medical use for the treatment of chronic nicotination & technology for its preparation has been developed.

No phytochemical studies had been done in Iraq on this species before, therefore we are reporting here the first phytochemical study in Iraq.

Experimental

Plant material:

The plant material was collected from Al Therthar district, west of Iraq in April & was identified by the Iraqi National Herbarium.

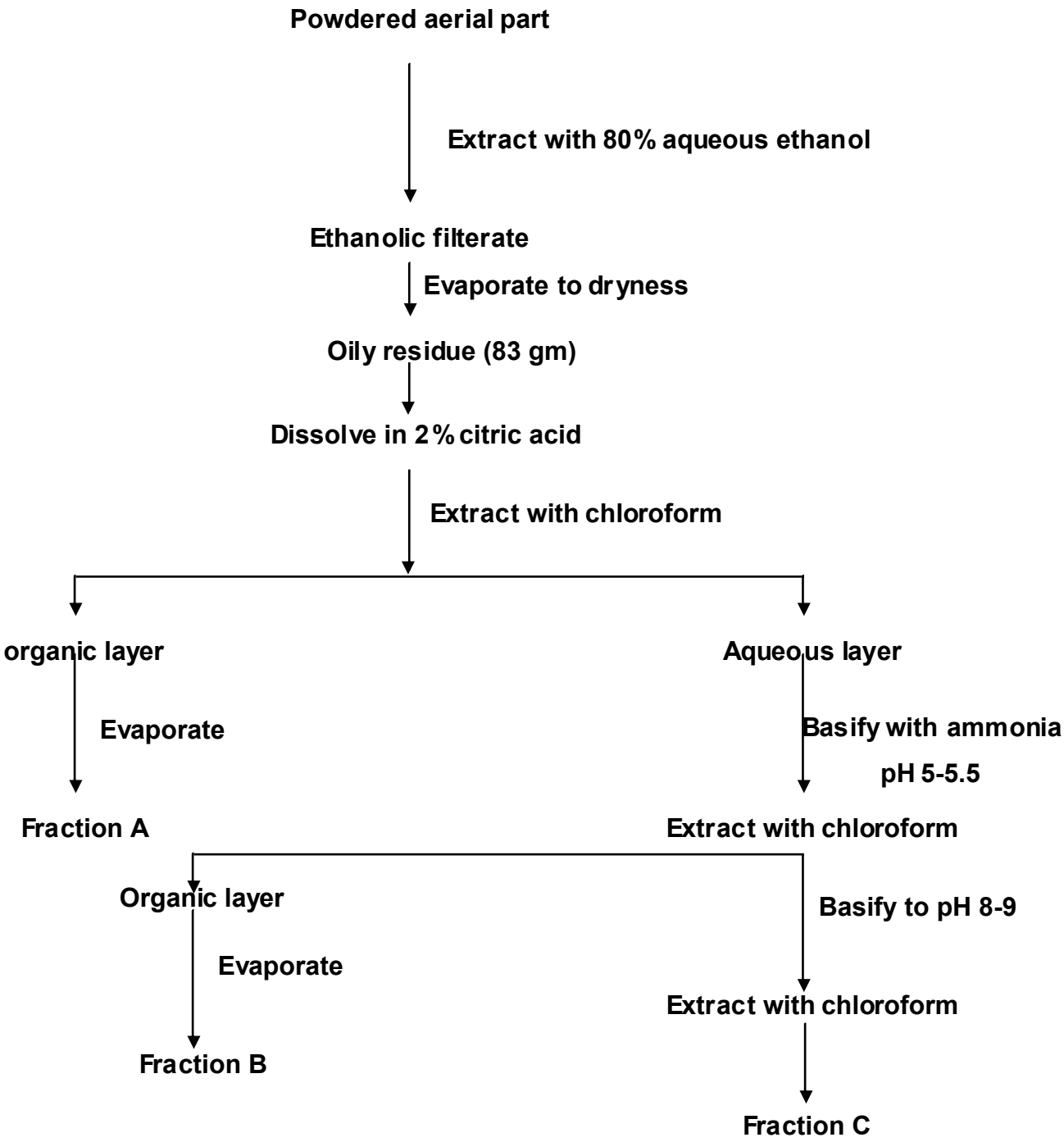
Apparatus

UV spectra were recorded using Shimadzu UV-300 spectrophotometer. lR were measured by using Beckman Acculab -8 spectrophotometer . n_D was measured by using Abb'8 refractometer, TLC was performed on a pre coated silica gel plates & PLC was carried out on silica gel GF_{254} plates 20X20 cm, 0.5 mm thickness.

Extraction & isolation

The aerial parts of the plant were air dried & ground into a fine powder. About (700 gm) of the powder was extracted exhaustively with 80% aqueous ethanol in a mixer. The extract was filtered & evaporated to dryness, to yield 83 gm oily residue. The residue was dissolved using 2% citric acid (pH 3-4), filtered & extracted with chloroform (3x250ml) , the chloroform layers were combined , filtered, dried over un hydrous sodium sulfate & evaporated to dryness (Fraction A).

The acidic fraction was basified to pH 5-5.5 with 10% ammonia solution (pH meter) , then extracted with chloroform , the chloroform layers were combined , filtered, dried over unhydrous sodium sulfate & evaporated to dryness (Fraction B). The aqueous layer was further basified to PH 8-9 with 10% ammonia solution & extracted with $CHCl_3$, the chloroform layers were combined , filtered, dried over unhydrous sodium sulfate & evaporated to dryness (Fraction C). The overall method of extraction is shown in scheme 2.



Scheme (2): Method of extraction and fractionation of Anabasis aphylla aerial part

Isolation of aphyllidine

Fraction A gave a negative test for alkaloids (Mayer 's reagent), fraction B gave a positive Dragendorff's & Mayer's reagent, it revealed the presence of two minor spots showed positive reaction. About 0.4gm of this fraction was further fractionated by column chromatography using a column of alumina (Grade II ,50gm), eluted with benzene, then with benzene-MeOH 1/2,3&5% [12]. The benzene fractions were further purified by PLC on silica gel GF_{254} plates using (acetone –water 100-8) as a mobile phase ,to reveal 19 mg crystals (m.p.110-113 °C)which is identical with that reported for aphyllidine. [12] Aphyllidine was further identified by UV λ_{max} 238nm; IR V_{max} at 1630 cm^{-1} (C=0), 2920 and 2845 cm^{-1} (methylene CH) and by TLC using Acetone-water 100:8 on silica gel; ether- $CHCl_3$ 100:70 on alumina to give identical R_f values with the reported one. [13, 14]

Isolation of anabasine:

Fraction C showed positive tests with both Mayer & Dragendorff's reagent . About 1 gm of this fraction was fractionated by passing it through a column of silica gel (60-120 mesh) using about 70 gm of silica gel mixed with hexane & packed in a column (2 cm diameter x 80 cm hight) . The column was eluted with hexane, then with $CHCL_3$ then with $CHCL_3$-MeOH 1,2,5,10%. About 7-10 ml fractions were collected. Similar fractions (TLC) were combined . Fractions containing anabasine (authentic) were further fractionated by PLC on silica gel using ($CHC1_3$-MeOH-NH_4OH 60:10:1) as a mobile phase. [15, 16] (Mode of separation by column chromatography is shown in figure 1).
Anabasine ($C_{10}H_{14}N_2$) was isolated as an oil (73 mg), B. P. 105-107 ^0C
n20/D 1.500, UV λ_{max} 210, 260 nm, IR V_{max} 3450, 3100-2900, 1580, 1490-1410, 1300-1100, 800, 715 cm^{-1}. Anabasine HC1 m.p. 213-216^oC.

Results and Discussion

The differences in basicity of alkaloids of *Anabasis aphylla* gave opportunity for fractionation of the mixture of alkaloids by step wise basification.
Column and TLC of fraction B revealed the presence of two alkaloids, the one eluted from the benzene fraction was confirmed to be aphyllidine which showed UV absorption at 238 nm which is characteristic for the chromophore C=C-N-C=O, in addition the IR spectrum showed absorption band at 1650 cm^{-1} due to the lactam carbonyl.
Column & thin layer chromatography of fraction C revealed the presence of not less than three alkaloids the major band of them was isolated & identified.
The identification started by comparing the isolated alkaloid with standard anabasine by TLC using five different solvent systems / using silica gel GF_{254} as a stationary phase , the solvent systems are: [16, 17]

1- CCl_4 : Me_2CO : MeOH 3: 7 :0.5

II- $CHCI_3$: MeOH: NH_4OH 60:10:1

III -$CHCL_3$: MeOH: Acetic acid 60:10:1

IV-Toluene : Methanol: chloroform 90: 30 : 10 (on basic SG 0. IN KOH)

V-CHCl3-EtOH 9:l

The isolated alkaloid gave identical R_f values with the standard alkaloid, using single & mixed

spots (HR_f values are shown in table I).

The UV spectrum showed absorption maxima at 210 & 260 nm (figure 1) which is identical for compounds containing the pyridine-piperidine moieties. IR showed bands at 3450cm^{-1} (N-H stretching vibration), 1300-1100 cm^{-1} (C-N stretching), (figure 2), [18, 19, 20, 21].

Further identification of anabasine was confirmed by preparation of a salt which is anabasine HC1 which showed m.p at 213 -216 °C which is identical with the reported m.p .

As a conclusion ethanolic extract of the aerial part of *Anabasis aphylla* revealed the presence of about five compounds showed a positive reactions for alkaloids, the major one was isolated from fraction C & was proved to be anabasine . A minor one was isolated from fraction B which was confirmed to be aphyllidine. These alkaloids are reported here in the Iraqi species for the first time.

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Table (1): HR_f value of standard and isolated anabasine

Solvent system	HR_f standard	HR_f sample
I	35	33
II	50	52
III	06	04
IV	83	80
V	34	37

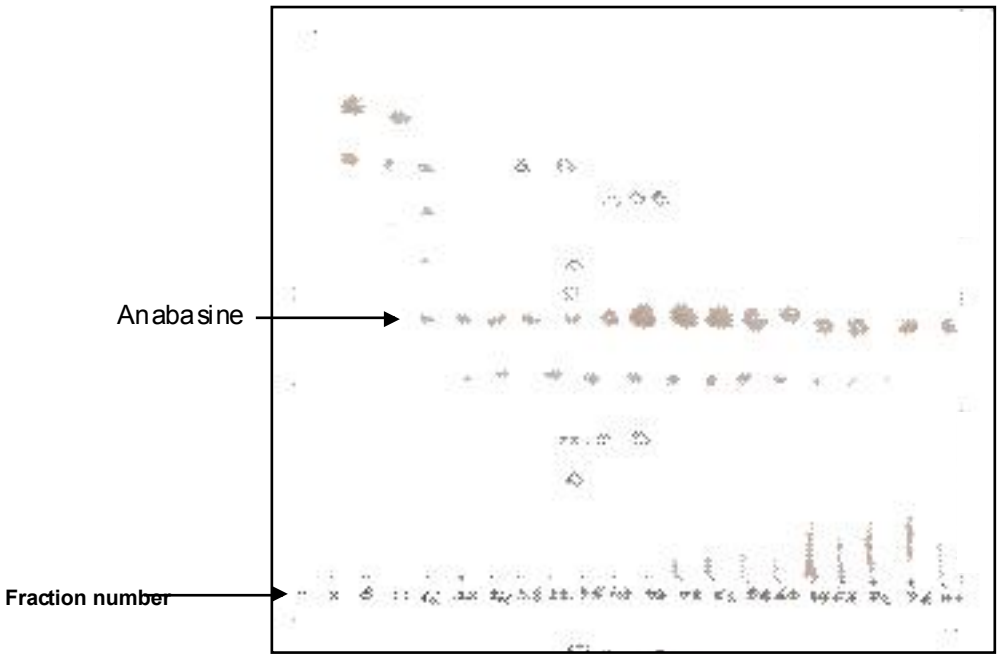


Fig.(1): Mode of separation of fraction C by column chromatography
Adsorbent : Silica gel GF_{254}

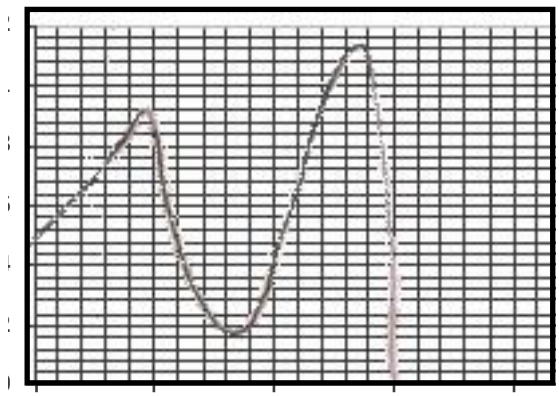
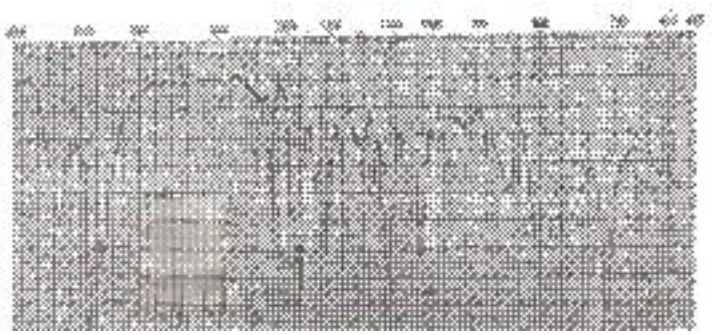


Figure 1: UV spectrum of anabasine



Fig(3): IR spectrum of anabasine

Fig(2):