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**Phytochemical Studies on Khat (*Catha edulis*)**



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**Phytochemical Studies on Khat (*Catha edulis*)**

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## Abstract

Two compounds were isolated from the leaves of *Catha edulis* namely, cathinone (**1**) and flavonoid compound Cae-5 (**49**). The structures of these compounds were determined on the basis of spectroscopic data.

## 1. Introduction

### 1.1 History and Origin of Khat Plant

Khat is a shrub or small to medium-sized evergreen tree, that belongs to Celastraceae family. It was first described by Peter Forskal (1736-1763), a Swedish botanist, on his journey with his friend Karsten Niebuhr to Egypt and Yemen. Karsten Niebuhr called khat as *Catha edulis* Forskal in memory of his friend Peter Forskal [5, 6].

Most researchers believe that khat originates from Ethiopia. Although it has grown for centuries in Yemen, there is no certainty of the date for its introduction [5]. One opinion is that khat was introduced to Yemen during the Ethiopian occupation of Yemen in 525 AD, when it was limited to the invaders. However, this date is believed by others to be too early as Abu Al-Hasan Al-Hamdani in the 10th century (945 AD) does not mention khat in his botanical treatise on the many plants he described [6].

Khat is also mentioned in an Arabic medical book. The author, Najeeb Al-Deen Al-Samargandi, described khat as a treatment for depression, because it led to happiness and excitement. The historian Ibn Fadl Allah Al-Amri (1301-1348 AD) mentioned use of khat in the events of the war between Sabr Al- Deen (the King of Ifat) and the King of Ethiopia (Amdetsion). In the excitement of his victory, King Sabr Al-Deen swore to demolish the residence of the Ethiopian king (Marade) and to plant khat in its place [6].

In view of the above considerations, the consensus of opinion is that khat was probably introduced into Yemen as a social habit in the 13th century. However it was supported by a letter written by a religious scholar, Ahmed Ben Alwan, asking the ruler at that time to legally ban the habit of khat chewing because it prevented Yemenis from performing their prayer, especially the afternoon (Asr) and sunset (Maghreb) prayer periods [6].

## **1.2 Cultivation of Khat Tree**

Khat usually grows up to 7 m but occasionally reaches as high as 15 to 25 m. Leaves are simple, elliptic, and oblong which are glossy green above but lighter below, leathery and stiff tapering to both ends. Flowers are small and white. The fruit is smooth and narrow that splits to release narrowly winged reddish seeds when matured. The stem is straight and slender; the bark has different colors depending on the variety and its age. The young branches are smooth and have green to pink colors but sometimes rougher, grey and darker on older branches and stems [6].

Khat is propagated from branches arising at ground level in the adult tree. The crop can be planted in rows with 2 to 3 m between them in both home gardens and in fields. The first harvesting of chewable leaves is usually after the third or fourth year of growth of a new tree, although it requires a further 6 to 7 years for the tree to attain maturity. A healthy tree will continue to give good harvests for another 50 years [5, 6, 17].

Khat requires well drained field with pH range of 6.0 to 8.2. The optimal altitude and annual rainfall for its growth ranges from 1500 to 2100 m and 1,000 to 1,500 mm, respectively. Wide range of insects, pests, and other animals damage its leaves, stems and roots. Other potential threats that might limit production and marketing of khat include frost, hailstorm and drought [6, 17].

## **1.3 Distribution of Khat Tree**

Khat is widely distributed in Eastern parts of Africa and Yemen. Although the Harar region of Ethiopia is believed to be the centre of khat production, its distribution and cultivation varies in different parts of Ethiopia as shown in the map below [5, 17].

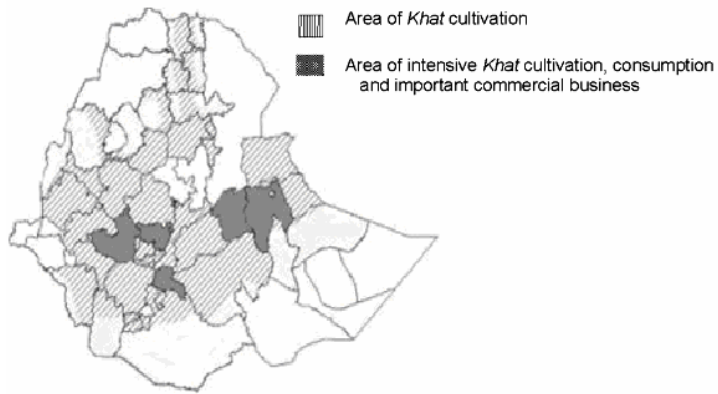


Fig. 1. Map showing rough distribution of khat in Ethiopia [17].

#### 1.4 Consumption and Harvesting of Khat

Khat is harvested by breaking off the young branches from the main branches in early morning or late afternoon. The harvested khat leaves are kept fresh by wrapping in plastic foil, wet clothes, and banana leaves or by immersing the cut end of the stem in water. At best, the leaves can remain in an acceptable condition for up to 5 days maintaining these conditions [6].



Fig. 2. Khat being wrapped with banana leaf [17].

Around 10 million people commonly use khat in East Africa. It has been chewed for recreational purposes (in khat parties, marriage ceremony. . . etc) and has some cultural

and social functions [16]. It is reported that 80–90% of male adult and 10–60% of female adult populations in East Africa consume khat on a daily basis. New patterns of khat consumption, including morning chewing sessions and khat parties, have emerged into East African countries in year 2005 [32].

In Ethiopia, khat is used for direct consumption, local sale and has been exported as value of major commodity to various parts of the world including: Djibouti, UK, Somalia, and a number of Arab countries. Its use of chewing fresh leaves of khat depends on the geographical source where it has been cultivated. For instance Wondo Chat from Wondo, Gelemso and Aweday from Harar, Bahir Dar Chat from Bahir Dar ... etc [6, 17].

Traditionally people in Hararghe also classify Khat into three based on the color of its young shoots such as dimaa for red, dalacha for white and hamarcot for the one between dimaa and dalota in colour [17].



Fig. 3. Typical bundle of khat leaves sold in market (photo by Eshetu Kebede).

### 1.5 Literature Review on Phytochemical Constituents of Khat

Early studies of khat goes back to 1887 when Flückiger and Gerock [34] searching for caffeine as its possible stimulating constituent. However, they found no traces of caffeine but instead discovered an alkaloid that they named katin (**2**). In 1891, Mosso extracted a basic fraction with stimulant-like properties from the plant and called it celastrine. The first comprehensive study on khat was carried out by Beitter who obtained crystalline salts of a

substance that was identical to both Flückiger's katin and Mosso's celastrine [33]. The chemical composition of khat was next studied by Stockman who described three distinct alkaloids without characterizing them structurally. Whereas Alles *et al.* [36] who concluded that only one extractable base found in substantial amount in the plant [32].

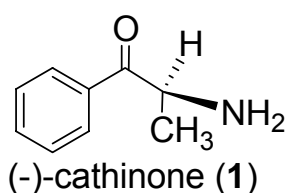
An important step forward was the contribution of Wolfes, who detected the presence of (+)-norpseudoephedrine(NPE) (**2**) in khat, and concluded that this substance corresponded to katin. This result represented the only solid ground for a period of more than three decays in which different groups had taken up the subject with out much substantial progress. Some of them concluded that NPE was the only alkaloid present in khat in appreciable amounts, whereas other found further basic compounds but there was not any proper characterization [4].

Yon Brücke was the first, who suggested the chemistry of khat to be more complex on the basis of simple pharmacological experiments; he felt that the stimulating effect of cathine (**2**) was too small to be exclusively responsible for the effect of the fresh leaves of the plant. Furthermore he addressed the possible presence of a substance with a more powerful stimulating effect. His suggestion was partially supported by Alles [35], and by the fact that consumers show preference and pay a higher price for fresh khat leaves [4, 32].

The first serious attempt was undertaken by Brilla *et al.* [6] who searched for a specific substance in the fresh leaves of the plant that might have a greater activity than cathine. They compared the effect on locomotor activity of synthetic (+)-norpseudoephedrine (**2**) oxalate with that of the oxalates prepared from freeze-dried and air- and sun-dried khat samples. The three preparations had qualitatively similar effects, but the oxalate from the freeze-dried plant sample showed a stronger effect on stimulating activity. Differences were also found in the physical and chemical characteristics of the samples, and they concluded that the substance isolated from the freeze-dried plant was a cathine-like compound, possibly a labile precursor of cathine, for which no correct structure could be proposed on the basis of the data available at that time [4, 6, 33].

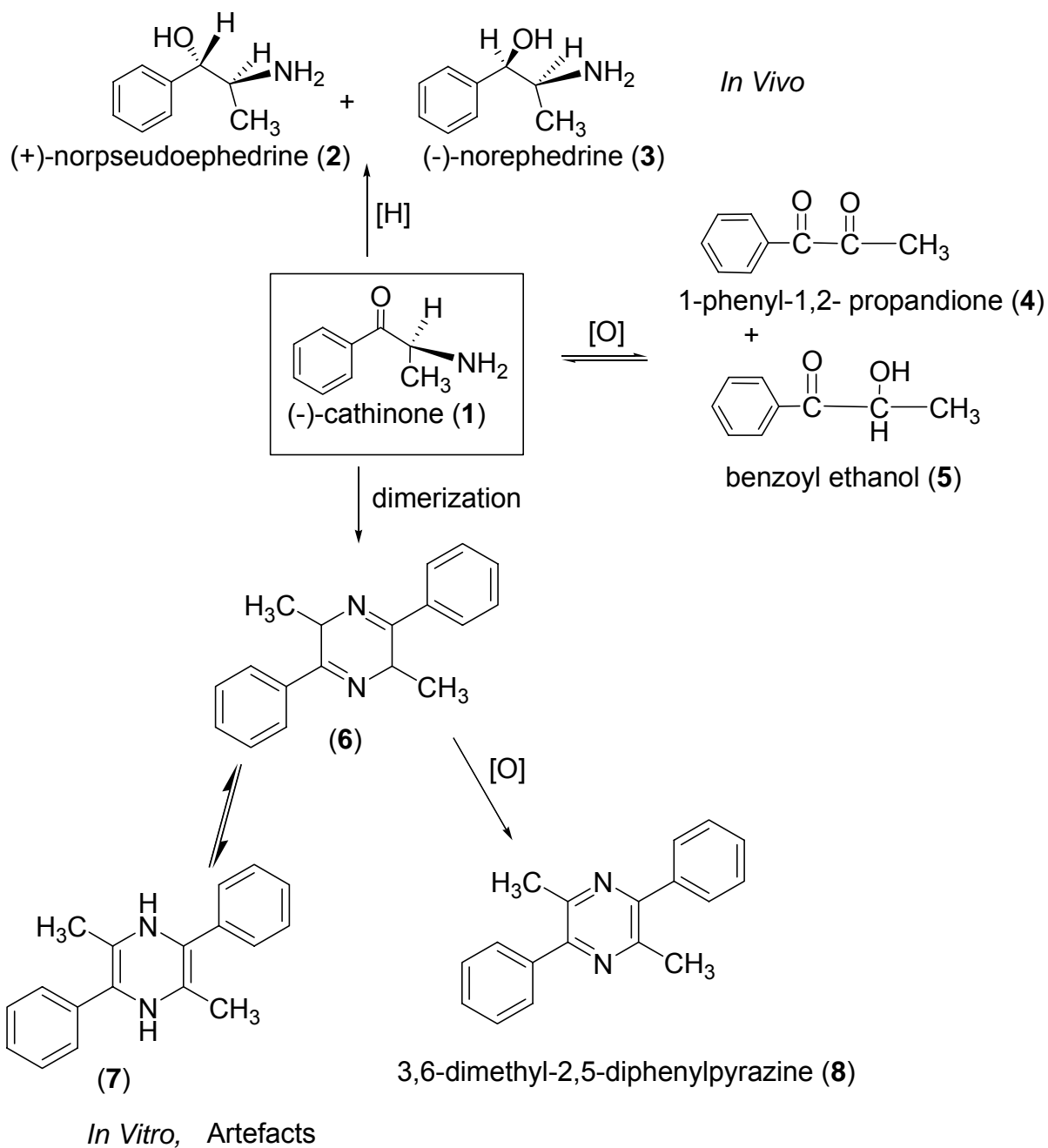
## The Discovery of Cathinone and Structure Elucidation

The sample for the isolation of cathinone and other phenylpropylamines were fresh and dried plants from *Catha edulis* cultivation in the area of Meru, Kenya, in particularly young shoots and leaves of varying age and states of development as used for consumption. The phenylpropylamines have been isolated predominantly as oxalates or as N-acetates using various extraction procedures. The structure of cathinone was determined based on its spectroscopic data (IR, UV,  $^1\text{H}$  NMR (Table 2), MS and CD).



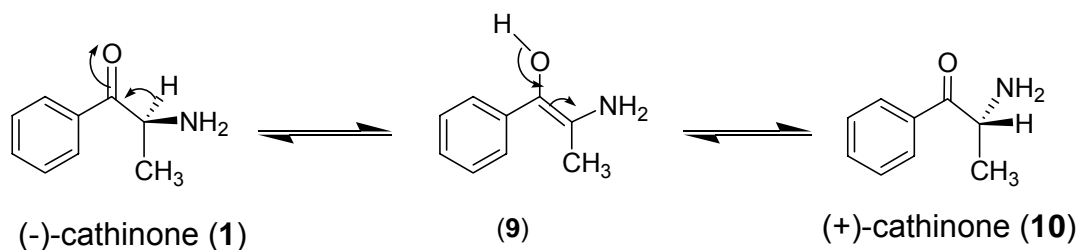
It was separated, rapidly identified as (-)- $\alpha$ -propiophenone(**1**) and named as cathinone and suggested that, it is a remarkable natural phenylalkylamine derivative, unstable, and prone to enzymatic reduction that transforms into the less active cathine, (+)- norp-seudoephedrine (**2**) and (-)-norephedrine (**3**) [4]. There are major metabolites of (-)-S-cathinone which are slowly absorbed and then excreted mainly in the unchanged form within about 24 h. As a result fresh khat was suggested to contain hundred times more cathinone than dried material. In other word cathine content increases when khat leaves get dry [22].

The oxidation product 1-phenyl-1,2-propanedione (**4**) can be detected even in the benzoyl ethanol (**5**) from fresh plants. There is an opportunity for this diketone to be formed via enzymatic deamination or by photolysis. The cathinone dimers 3,6-dimethyl-2,5-diphenylpyrazine (**8**) and its dihydroderivatives (**6** and **7**) are artifacts. This instability of the khat principle cathinone declares why about 100 years of chemical research were needed to identify this structurally simple component and why khat users prefer the fresh one. The possible *in vivo* and *in vitro* biotransformation product of cathinone is summarized in Scheme 1 [4, 12, 33].



Scheme 1. *In vivo* and *in vitro* transformation products of cathinone.

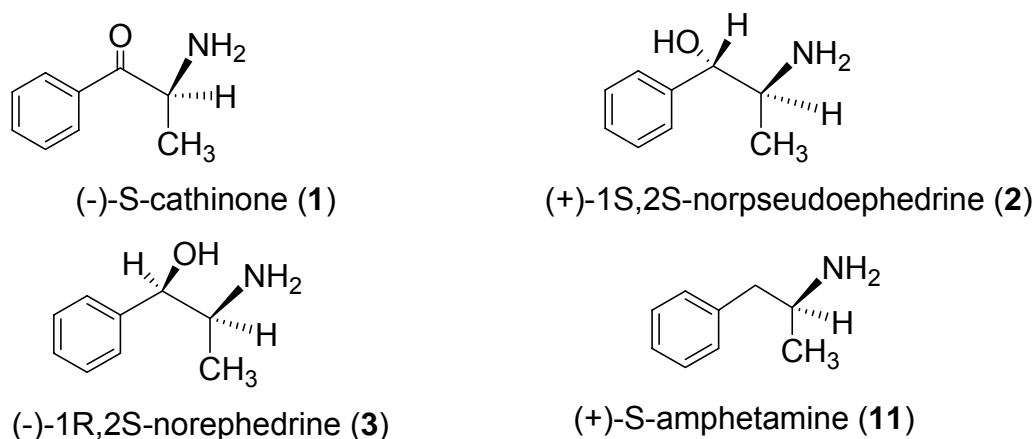
Because of the easy enolization of the ketone group, (-)-cathinone (1) racemizes quickly. In contrast its oxalate salts are stable in solid form [4, 13].



Scheme 2. Racemization of cathinone (**1**).

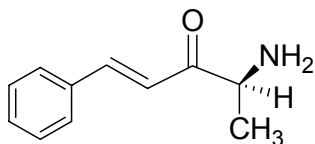
One study conducted in Yemen indicated that the concentration of cathinone and tannic acid varies in the khat depending on the geographical location. Thirty nine samples (fresh khat leaves) from different region of Yemen were analyzed for cathinone content. Among these 10 samples contained 200-300 mg/100 g cathinone and 6-9 g/100 g tannic acid whereas the rest had less than 200 mg/100 g cathinone and 3-5 g/ 100 g of tannic acid. The tannic acid concentration is considerably high and determines the taste of khat [5].

The three major alkaloids present in khat leaves are: (-)-S-cathinone (S- $\alpha$ -aminopropiophenone) (**1**), norpseudoephedrine (cathine) (**2**) and norephedrine (**3**) which are phenylpropylamines and structurally related to amphetamine (**11**). The phenylpropylamine composition varies depending on the region and country of origin of the plant [17, 18, 19, 20].

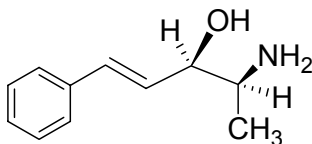


Further studies of the CNS-active khatamines from *Catha edulis* indicated the presence of phenylpentenylamines: merucathinone (**12**), pseudomerucathine (**13**) and merucathine

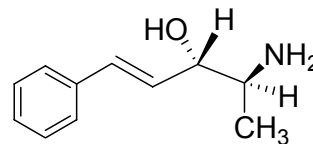
(14), were isolated from the fresh plant material cultivated in the Meru region of Northern Kenya [20].



(+)-S-merucathinone (12)



(-)-3R,4S-pseudomerucathine (13)

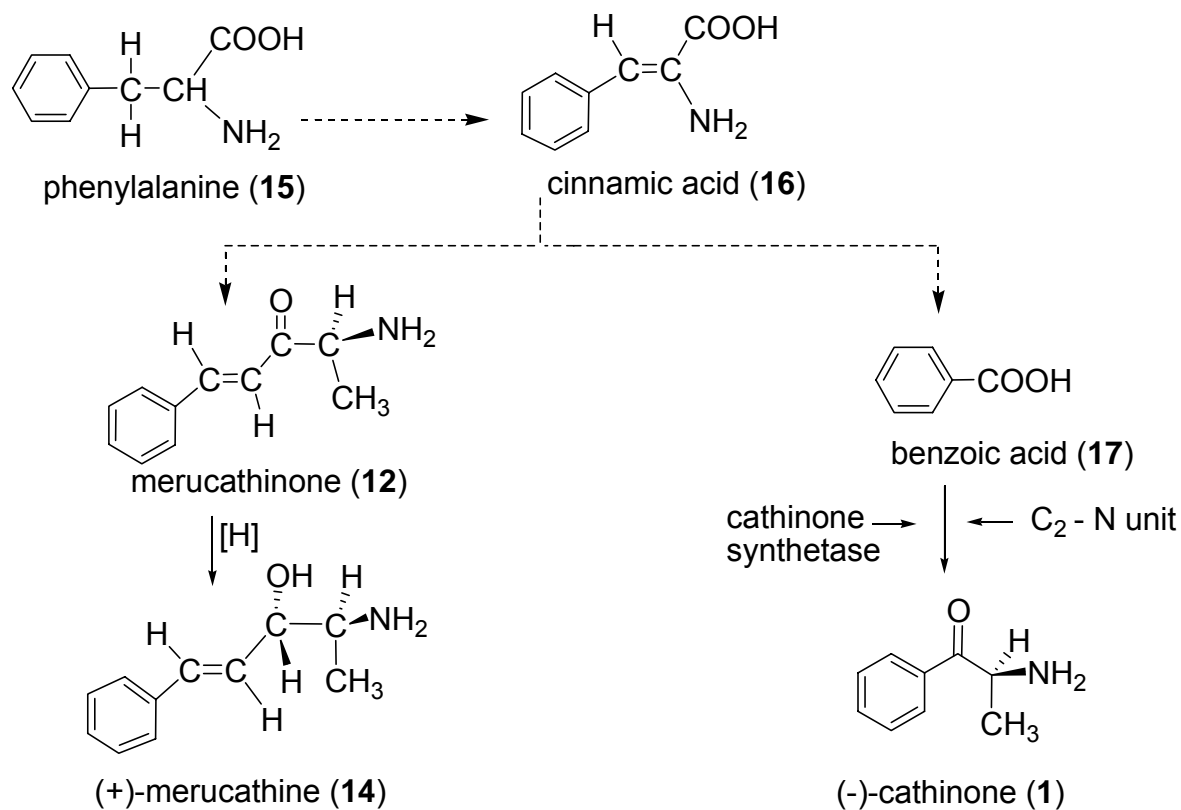


(+)-3R,4S-merucathine (14)

### Biosynthesis of Cathinone

A biosynthetic pathway for cathinone was postulated by Shiata and co-workers in the early seventies. It was considered as a hypothetical intermediate for other alkaloids which are fully identical with the cathinone isolated from *Catha edulis*. At the time this study was conducted cathinone was not isolated from plants. According to the investigations, nitrogen is not incorporated via phenylalanine directly rather via still undefined C<sub>2</sub> - N unit. Cinnamic acid is the most probable precursor for the phenylpentylamine, merucathinone and merucathine [13].

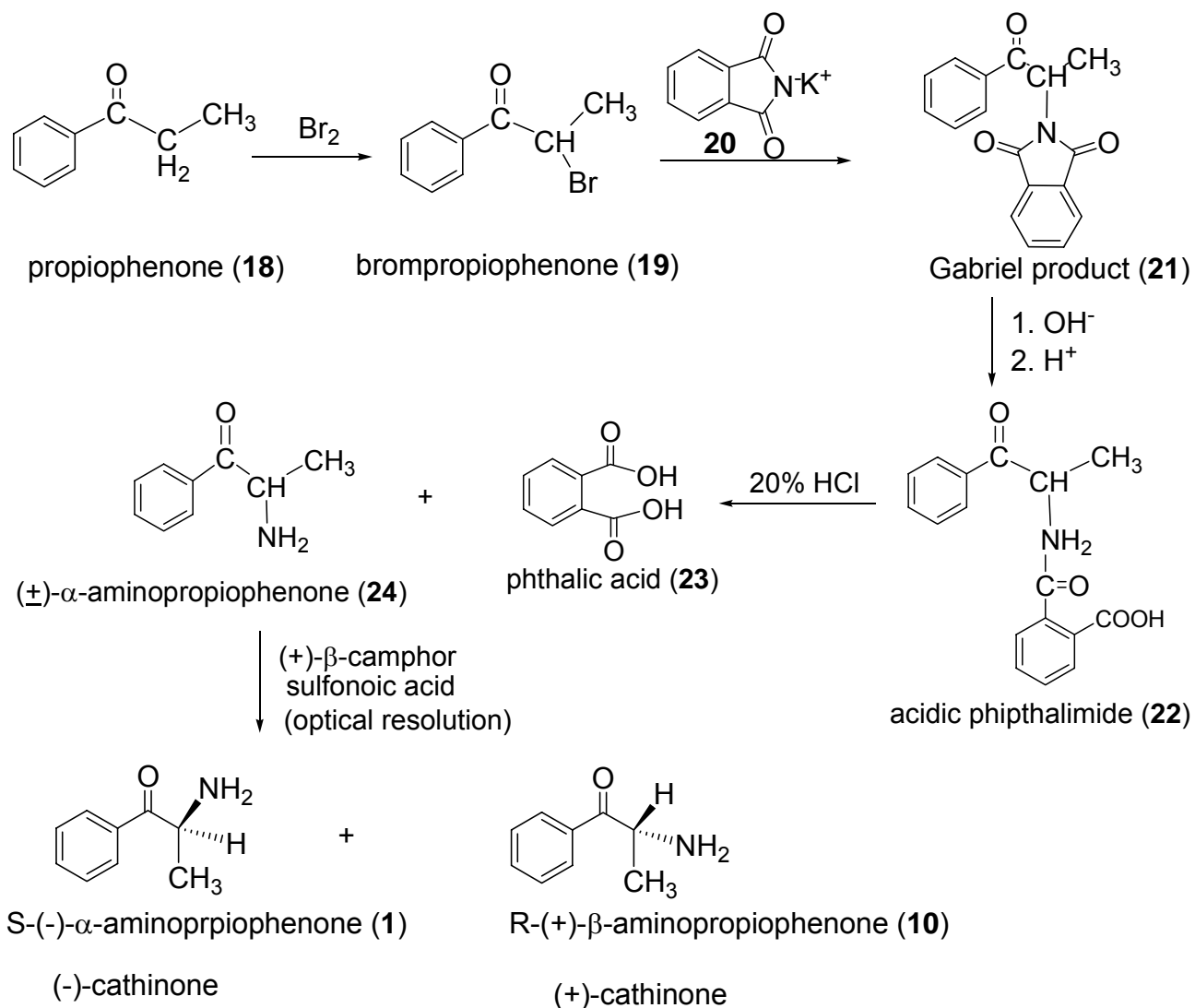
The absolute configuration of the asymmetric centre of (-)-cathinone is S, the same as that of the corresponding centre of (+)-norpseudoephedrine. This suggests that the two substances may be biogenetically closely related. Based on its structural relationship to cathine, and high instability, cathinone may be considered as the suspected and long-sought labile precursor in khat. However, it is not clear yet under what conditions it could be converted to cathine in the plant [4, 33].



Scheme 3. Suggested biosynthetic pathway of cathinone.

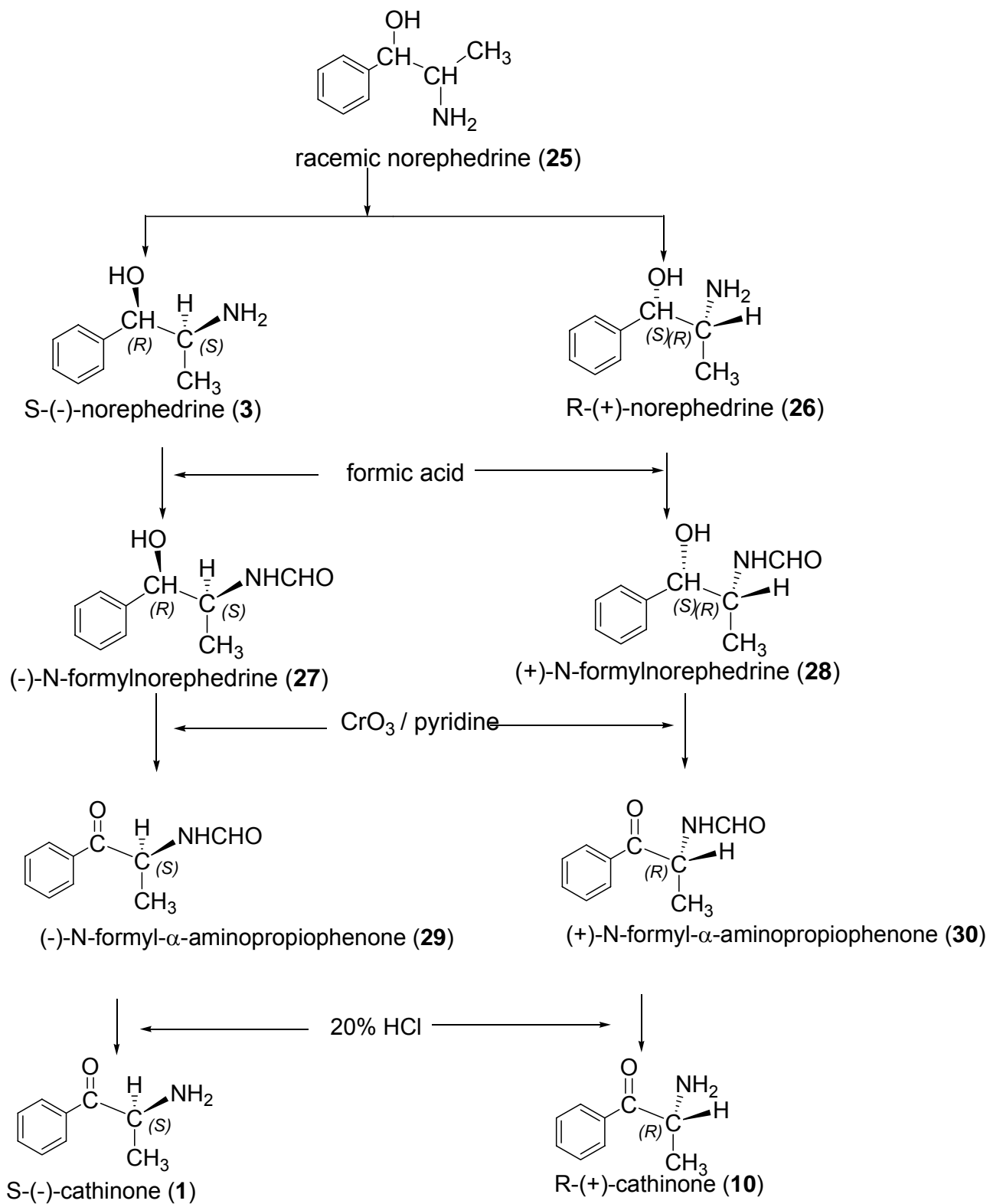
### Synthesis of Cathinone

The observed CNS activity resulted in increased interest of cathinone synthesis. Consequently two stereospecific and racemate syntheses of cathinone were known for almost 100 years [4, 17]. Racemates of cathinone were synthesized by Schorno [4] via modified the Gabriel synthesis using propiophenone (**18**) as starting material. The yield was about 85% relative to Gabriel product. The subsequent separation of the racemate of cathinone was achieved using (+)- $\beta$ -camphorsulfonic acid.



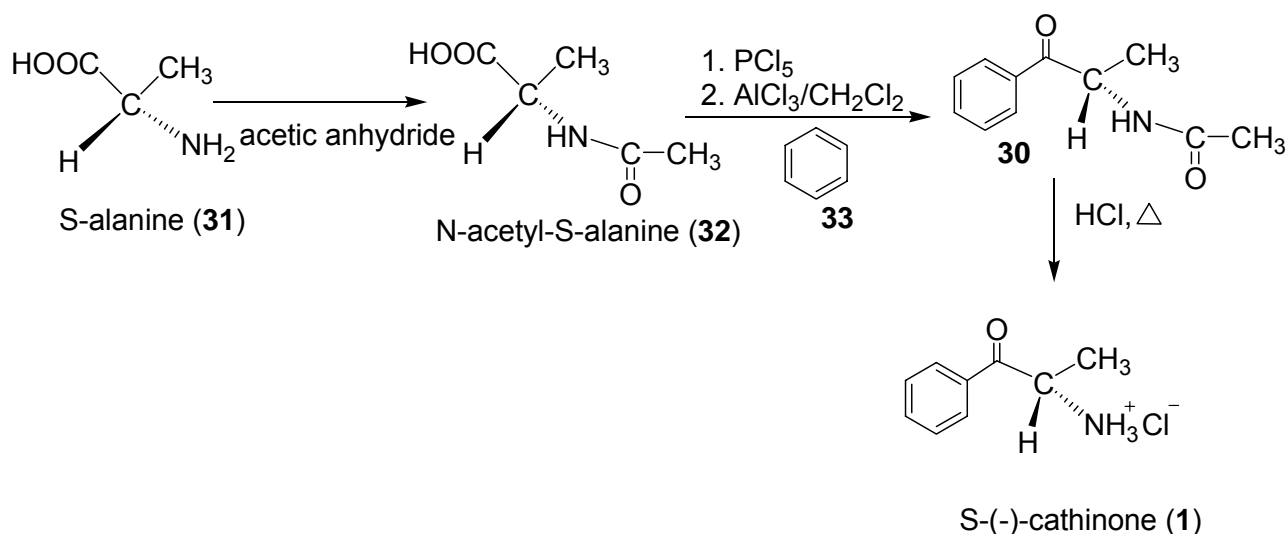
Scheme 4. Cathinone racemate synthesis.

Optically pure cathinone was synthesized using commercially available racemate of norephedrine as starting material. Beginning with racemate separation followed by protection of the amino group and then oxidation of the hydroxyl group resulted in optically pure cathinone as shown in Scheme 6 [23].



Scheme 5. Synthesis of optically pure cathinone.

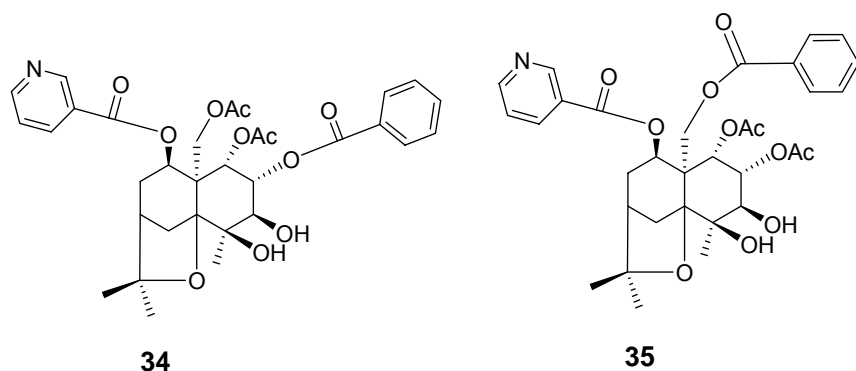
Another stereospecific synthesis of cathinone was used Friedel Craft's alkylation of the N-protected S-alanine to get optically pure HCl derivative of (-)-cathinone [10].



Scheme 6. Stereospecific synthesis of cathinone.

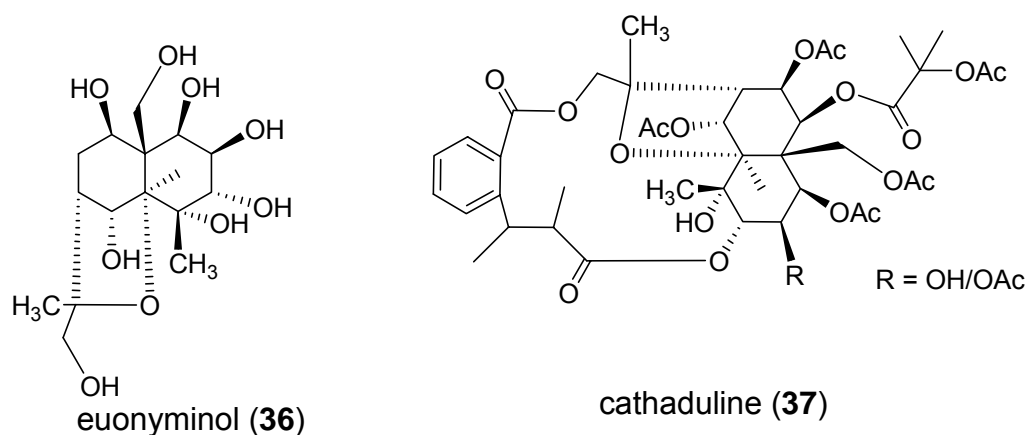
### Sesquiterpene and Miscellaneous Alkaloids of Khat

The presence of water-insoluble alkaloids in khat were detected and carried out with extensive extraction and separation experiments on a weakly basic khat alkaloid fraction. Among a mixture of closely related alkaloids, cathidine (34) was isolated in crystalline form and its partial structure was proposed. It might be related to other alkaloids originating from the same plant family [32].



Luftmann and Spiteller established the structure of the sesquiterpene core of euonyminol (36) using an old "cathidine" sample as starting material, that is, an alkaloid mixture which

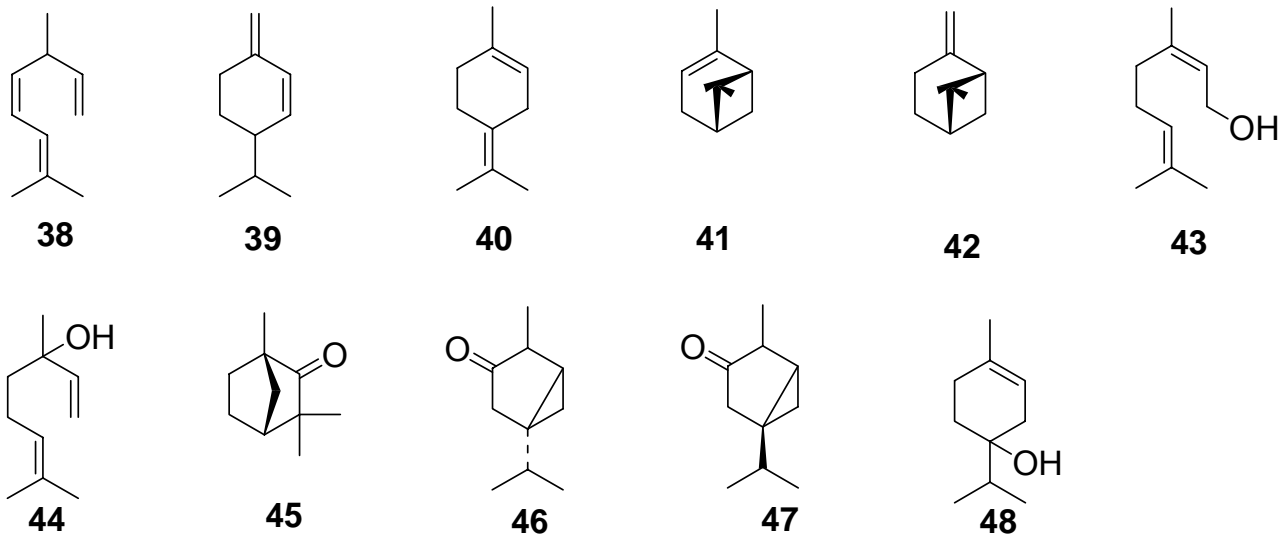
in the best case might correspond to the original mixture in the plant. It was thought to be a mixture of polyesters of a sesquiterpene polyol, euonyminol (**36**), and various acids such as acetic acid, benzoic acid, gallic acid trimethyl ether, and nicotinic acid. However, none of these alkaloids were isolated from the mixture [28].



In addition to the above chemical composition, it has been reported that fresh khat leaves contain: ascorbic acid, thiamin, niacin, riboflavin, beta-carotene, calcium, iron, ash, protein, and fiber [33].

### Volatile Components of Khat

Khat has moderate characteristic odour and weak aromatic taste. Its volatile oil content is fairly low. Yellow ethereal oil was obtained in yields varying from 0.03% to 0.08% depending up on the nature of the plant. It was analyzed to show the presence of components mostly terpenoids and aromatics. Eleven of these compounds were identified as ocimene (**38**),  $\beta$ -phellandrene (**39**), terpinolene (**40**),  $\alpha$ - and  $\beta$ -pinene (**41**) and (**42**), nerol (**43**), linalool (**44**)  $\alpha$ -terpineol (**45**),  $\alpha$ - and  $\beta$ -thujone (**46**) and (**47**), and fenchone (**48**) [5, 6, 32].



Structures of some of volatile components isolated from khat.

### 1.6 Adverse Effects of Khat Use

When chronic khat chewers stop chewing the leaf, they may feel hot, especially in their lower extremities, lethargic and gripped with a desire to chew in the first two days. During sleep usually a person may suffer from a nightmare. This takes the form of seeing himself and facing a dangerous situation unable to shout or move. The nightmare lasts only for 1 to 2 nights, after which sleep returns to normal. Then a person becomes at the state of longer and deeper sleep, better appetite, less constipation and less need of smoke [2, 6, 20].

Khat chewing has resulted in so complicated and long-lasting adverse effects. This is because chewers get relief of fatigue, increased alertness, reduced sleepiness, mild euphoria, excitement and improved ability to communicate while they are using it.

Different reports confirm that the prevalence of the effects and their severity increased with frequency and duration of khat use. The effects on the nervous system resemble those of amphetamine with differences being quantitative rather than qualitative [5, 7]. Suggested adverse effects of khat in humans are summarized in Table 1 [3, 8, 9, 10, 16].

Table 1. Adverse effects of khat chewing in humans

Central nervous system	dizziness, fine tremor, insomnia, headache
Obstetric effects	low birth weight, stillbirths, impaired lactation
Metabolic and endocrine effects	hyperthermia, perspiration, hyperglycaemia
Ocular effects	blurred vision, mydriasis
Genito-urinary system	urinary retention, impotence, libido change
Psychiatric effects	lethargy, irritability, psychotic reaction, depression
Cardiovascular system	hypertension, pulmonary edema
Respiratory system	tachypnoea, bronchitis
Gastro-intestinal system	dry mouth, dental caries, periodontal disease, chronic gastritis, constipation, hemorrhoid

### 1.7 Objectives of the Study

Review of the literature indicates the lack of phytochemical studies on khat leaves since the discovery of cathinone in 1979. We have therefore set out to isolate cathinone and other secondary metabolites of khat including non-alkaloidal compounds, which up to now have not been given sufficient attention.

## 2. Results and Discussion

Different methods of extractions were used to isolate compounds from *Catha edulis*. However, all methods used can be classified into three. The first way was extracting the fresh leaves of khat with acid followed by solvent-solvent extraction. The second way was extracting the plant material with methanol and applying the extract to column chromatography in order to isolate components. The last way was a combination of the two, which is extracting the fresh leaves of khat starting with methanol followed by acid-base extraction.

### 2.1 Characterization of Alkaloid Oxalate

The powdered fresh leaves of khat was suspended on 0.1 N HCl, sonicated for 30 min, shaken on the shaker for 10 min and then filtered by using suction filtration. The filtrate was extracted with Et<sub>2</sub>O (2x) and separated by separatory funnel. The acidic aqueous portion was again extracted with C<sub>6</sub>H<sub>6</sub> (2x) and separated in similar way as Et<sub>2</sub>O portion above.

10% NaOH was added to the acidic aqueous portion (drop wise) to attain pH 10 and extracted with Et<sub>2</sub>O (2x). Oxalic acid (1% in Et<sub>2</sub>O) was added (drop wise) to the Et<sub>2</sub>O portion and then white precipitate formation was observed. The mixture was left to stand for 20 h in the refrigerator (6 °C), filtered and dried at RT.

The white solid was characterized as cathinone based on the evidence below. Its melting point 172 - 174 °C and  $[a]_D^{25}$  -39° whereas its literature value of mp 172 - 175 °C and  $[a]_D^{25}$  -40.5°, R<sub>f</sub> 0.45 with solvent system EtOAc : MeOH : NH<sub>4</sub>OH (8.5 : 1 : 0.5). The <sup>1</sup>H NMR spectrum (Appendix 1) showed signals at δ 7.89 (dd, 2H), 7.64 (m, 2H), and 7.49 ppm (*m*, 1H) which are *ortho*, *para*, and *meta* aromatic protons respectively. It also showed a signal at δ 5.07 (*q*, 1H) and 1.47 ppm (*d*, 3H). Moreover the intense singlet peak at δ 4.70 ppm stands for water as the NMR data was generated in D<sub>2</sub>O media and the signal at δ 3.22 ppm is due to NH<sub>2</sub>.

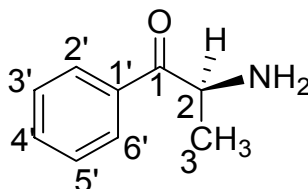
The <sup>13</sup>C NMR displayed signals at δ 198.1 ppm for carbonyl, at δ 165.5, 129.3, 128.8 and

135.2 ppm for monosubstituted aromatic functionality, at  $\delta$  51.6 ppm for CH and at 16.4 ppm for CH<sub>3</sub> (which are close to electron withdrawing group), There are only two quaternary carbons which is further confirmed by DEPT spectrum with the absence of the corresponding signals. There is no CH<sub>2</sub> group (as indicated by DEPT). Thus, all the NMR data constructively confirms that the isolated compound was cathinone in its oxalate salt form.

Table 2. <sup>1</sup>H NMR (observed and reported data) <sup>13</sup>C NMR and DEPT of cathinone.

C	$\delta_H$		$\delta_C$		Remark
	Observed (D <sub>2</sub> O)	Reported [4] (400 MHz, DMSO-d <sub>6</sub> )	<sup>13</sup> C NMR data, (D <sub>2</sub> O)	DEPT 135 data (D <sub>2</sub> O)	
1			198.1		C (carbonyl)
2	5.07 <i>q</i>	5.13 <i>q</i>	51.6	51.6	C-H
3	1.47 <i>d</i>	1.48 <i>d</i>	16.4	16.4	CH <sub>3</sub>
1'			165.5		C
2', 6'	7.89 <i>dd</i>	8.01 <i>dd</i>	129.3	129.3	C-H ( <i>ortho</i> )
3', 5'	7.49 <i>m</i>	7.57 <i>m</i>	128.8	128.8	C-H ( <i>meta</i> )
4'	7.64 <i>m</i>	7.75 <i>m</i>	135.2	135.2	C-H ( <i>para</i> )

From the above spectroscopic data, the structure of alkaloid was established as cathinone (1).



Structure of (S) - Cathinone (1)

## 2.2 Characterization of Non-Alkaloidal Constituents of Khat

The methanol extract (8 g) of khat leaves was applied on a column of silica gel. Elution was done with DCM : methanol by increasing polarity (95 : 5, 90 : 10, 80 : 20, 70 : 30, 60 : 40) as indicated in Table 5. The extract on TLC showed different spots and the chromatogram was seen using UV lamp at 254 nm.

Among five fractions from FCC, fr-4 (3 g) contained all components of fr-3 and fr-5. Hence all of fr-4 was again applied to FCC. Elution was done with DCM : methanol by increasing polarity (100:0, 95:5, 90:10,...) as shown in Table 6 and seven combined fractions were obtained. Fraction E (Fig. 6) containing the major component was further subjected to column chromatography and resulted in a pure compound Cae-5.

Cae-5 was yellow solid, soluble in methanol, melting point 148 - 150 °C,  $[\alpha]_D^{25} +17^{\circ}$ . The  $^1\text{H}$  NMR spectrum shows signals at  $\delta$  2.16 (sharp, s, 3H),  $\delta$  4.48 (d, 1H),  $\delta$  4.86 (d, 1H), 5.89 (s, 1H, aromatic), 5.92 (s, 1H, aromatic), and 6.56 ppm (sharp, s, 2H, aromatic). The  $^{13}\text{C}$  NMR spectrum shows fourteen signals. From which three signals at  $\delta$  72.3, 106.7, and 143.4 ppm are more intense than the others. The three signals in the chemical shift range from 120 – 150 ppm, which belong to aromatic carbons and one signal at  $\delta$  196.9 ppm due to carbonyl group.

DEPT spectrum has only six signals indicating the presence of CH and CH<sub>3</sub> group, one carbonyl and seven quaternary carbons in the compound.

Table 3.  $^1\text{H}$  NMR  $^{13}\text{C}$  NMR and DEPT data of compound Cae-5

C	$\delta_{\text{H}}$	$\delta_{\text{C}}$		Remark
	Observed (Methanol- $\text{d}_4$ )	$^{13}\text{C}$ NMR data, (Methanol- $\text{d}_4$ )	DEPT 135 data (Methanol- $\text{d}_4$ )	
2	4.86 <i>d</i>	83.8	83.8	C-H
3	4.48 <i>d</i>	72.3	72.3	C-H
4		196.1		
4a		100.4		
5		163.9		
6	5.89 <i>s</i>	94.9	94.9	C-H
7		167.3		
8	5.92 <i>s</i>	95.9	95.9	C-H
8a		163.0		
1'		133.5		
2', 6'	6.56 <i>s</i>	106.5	106.5	C-H
3', 5'		145.5		
4'		127.7		
1''	2.16 <i>s</i>	29.4	29.4	

## 2D NMR Spectrum Analysis for Compound Cae-5

As stated above there are six chemical environments for the protons, however the  $^1\text{H} - ^1\text{H}$  correlation spectroscopy indicated that only protons at  $\delta$  4.86 and 4.48 ppm are coupled with each other.

The HSQC was used to correlate protons with carbon atoms to which they are directly attached. In addition to these the HMBC together with proton multiplicity gave so valuable information to identify and define the connectivity of different groups in the compound as shown in Table 4.

Table 4. HSQC and <sup>1</sup>HMBC data of compound Cae-5

Proton position	HSQC	HMBC
H-2	H-2↔C-2	H-2 ↔ C-3, C-4', C-4, C-2', 6'
H-3	H-3↔ C-3	H-3 ↔C-2, C-2',6',C-4, C-4'
H-6	H-6↔ C-6	H-6↔C-6,C-4a,C-8a, C-7
H-8	H-8↔ C-8	H-8↔ C-8, C-4a,C-8a,C-7
H-2', 6'	H-2',6'↔ C-2',6'	H-2', 6'↔ C-2',6',C-2,C-4',C-1',C3',5'
H-1''	H-1''↔ C1''	H-1'' ↔ C-1''

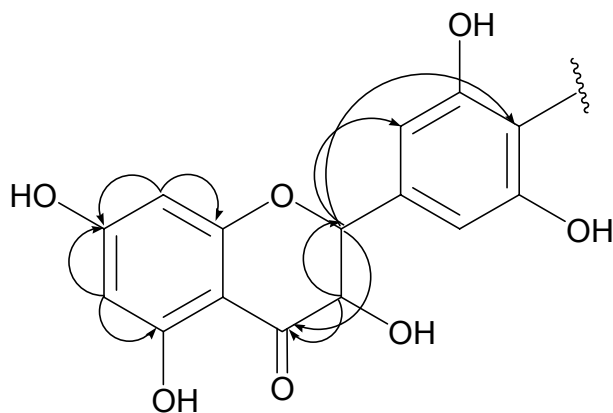
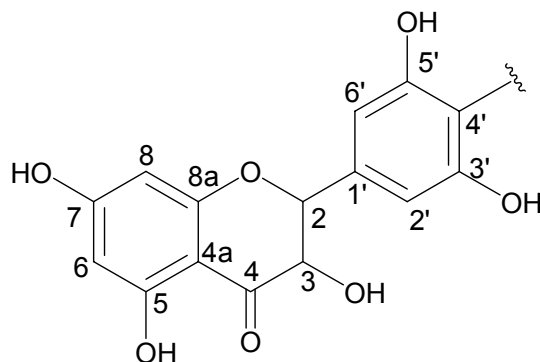


Fig. 4. Selected HMBC correlation of compound Cae-5

Depending on all the NMR data above structure is suggested for Cae-5 which belongs to a group of natural product called flavonoids.



Partial structure of compound Cae-5 (49)

### **3. Conclusion**

From the two compounds, which were isolated from *Catha edulis*, Cathinone was identified in its oxalate salt form. The acid extraction followed by solvent-solvent extraction is fast and easy to handle with considerably good yield (31 mg from 100 g of fresh leaves of the plant within two working days) even though its isolation using different methods has been reported. Cae-5 was isolated and characterized as it was a flavonoid type although it was not possible to propose a complete structure.

### **4. Experimental**

#### **4.1 Materials and Methods**

Mortar and pestle, sonic bath, rotary evaporator, TLC plate (pre-coated aluminum sheet silica gel 60 F<sub>254</sub>), UV lamp, shaker, column, silica gel (70 – 230 mesh), separatory funnel, NMR machine ( Bruker Avance 400 NMR spectrometer at 400 MHz), Autopolo IV polarometer, thiel tube, visualizing agents: 1% ninhydrin solution in ethanol and EtOH : anisaldehyde : conc.H<sub>2</sub>SO<sub>4</sub> : AcOH (9 :0.5 : 0.5 : 0.1) were used.

#### **4.2 Plant Material**

Fresh khat sample, which has different geographical source, was bought from private shops at Arat-kilo nearby Tourist Hotel.

#### **4.3 Acid – Base Extraction Followed by Solvent-Solvent Extraction**

##### **4.3.1 Sample Preparation**

Fresh khat leaves were frozen by pouring liquid nitrogen and powdered by using mortar and pestle.

### 4.3.2 Extraction and Isolation

The powdered sample (100 g) was suspended on 150 mL of 0.1 N HCl in 400 mL erlenmeyer flask. The mixture was sonicated for 30 min, shaken on the shaker for 10 min ( $120 \text{ min}^{-1}$ ) and then filtered by using suction filtration. The remaining plant residue was washed by 70 mL of 0.1 N HCl.

The filtrate was extracted 2x with  $\text{Et}_2\text{O}$  (2x50 mL) and the  $\text{Et}_2\text{O}$  portion was separated by using 500 mL separatory funnel. The acidic aqueous portion was again extracted 2x with  $\text{C}_6\text{H}_6$  (2x50 mL) and the  $\text{C}_6\text{H}_6$  portion was separated in similar way as  $\text{Et}_2\text{O}$  portion above.

10% NaOH was added to the acidic aqueous portion (drop wise) to attain pH 10 which was monitored by using "universal indicator paper pH 1-14". The basic mixture was extracted two times with  $\text{Et}_2\text{O}$  (2x50 mL). The  $\text{Et}_2\text{O}$  extract was separated by using 500 mL separatory funnel. Oxalic acid (1% in  $\text{Et}_2\text{O}$ ) was added (drop wise) to  $\text{Et}_2\text{O}$  portion and white precipitate formation was observed.

The mixture with precipitate was left to stand for 20 h in the refrigerator ( $6^\circ\text{C}$ ) and then filtered. The precipitate was dried at RT and obtained 31 mg within two working days. It was white solid, mp  $172-175^\circ\text{C}$ ,  $[\alpha]_D^{25} -39^\circ$ .  $^1\text{H NMR}$  ( $\text{D}_2\text{O}$ ):  $\delta$  7.89 (m, 2H), 7.64 (m, 2H), 7.49 (m, 1H) which are *ortho*, *para*, and *meta* aromatic protons respectively, 5.07 (q, 1H), 1.47 (d, 3H), and 3.22 ppm is due to  $\text{NH}_2$ .  $^{13}\text{C NMR}$  ( $\text{D}_2\text{O}$ ):  $\delta$  198.1 (C-1), 51.6 (C-2), 16.4 (C-3), 165.5 (C-1'), 129.3, (C-2',6'), 128.8 (C-3',5'), 135.2 (C-4').

## 4.4 Methanol Extraction Followed by Column Chromatography

### 4.4.1 Extraction and Isolation

The powdered sample (100 g) was mixed with MgO (100 mg). The mixture was suspended on 200 mL MeOH which was sonicated for 40 minutes, shaken on the shaker for 20 minutes ( $120 \text{ min}^{-1}$ ) and then filtered using suction filtration. The remaining plant residue was washed with 80 mL (2x40 mL) methanol.

The filtrate was concentrated to dryness on the rotary evaporator at 40 °C and obtained 8 g crude MeOH extract. All of the crude extract was dissolved with MeOH and adsorbed on 3 g silica gel (70 - 230 mesh). The dried and adsorbed sample was applied on the FCC that packed with 130 g silica gel (70 - 230 mesh). Elution was done with DCM : methanol by increasing polarity to obtain the following five fractions indicated Table 5.

Table 5. Different fraction of methanol extract

Solvent (CHCl <sub>3</sub> : MeOH)	95:5	90:10	80:20	70:30	60:40
Fractions	1	2	3	4	5
Mass of fractions	187 mg	102 mg	57 mg	3 g	3 g

#### 4.4.2 TLC Analysis of Fractions of Methanol Extract (fr 1 – 5)

Pre-coated TLC plate (Aluminum sheet silica gel 60 F<sub>254</sub>, 5 x 6 cm, 0.25 mm layer thickness, Merk) was used for the analysis. The developing solvent system was CHCl<sub>3</sub> : MeOH (8.5 : 1.5). The TLC developing chamber was saturated with the solvent for 10 – 15 minutes at room temperature. The chromatogram on the TLC plate was seen using UV lamp at 254 nm.

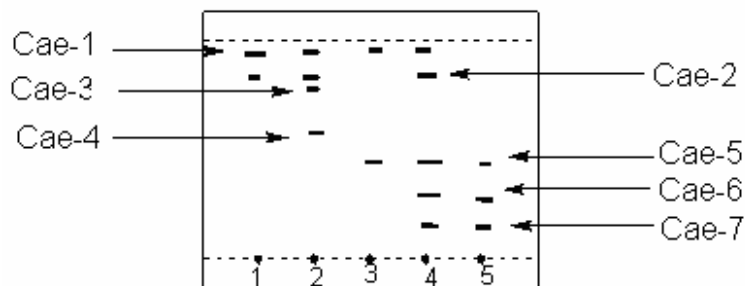


Fig. 5. TLC Analysis of fractions of methanol extract

Both TLC analysis and NMR data indicated as fr-4 contained all components of fr-3 and fr-5. Because of this all of fr-4 (3 g) was dissolved with methanol and adsorbed on 2 g Silica gel (70 - 230 mesh) which was dried on rotary evaporator at 40 °C. The dried and adsorbed sample was applied on the column which was packed with 80 g Silica gel (70 - 230 mesh). DCM : methanol solvent system was used as eluent and eighteen fractions were obtained. Fractions which had the same R<sub>f</sub> on the TLC plate was merged in to one and that resulted in seven combined fractions as indicated Table 6.

Table 6. Different fractions from fr-4 above

Solvent CH <sub>2</sub> Cl <sub>2</sub> /MeOH	100:0	95:5	90:10	85:15	80:20	75:35	70:30
Combined frs	A	B	C	D	E	F	G
Volume of combined frs (mL)	60	70	100	50	40	60	50
Mass of combined frs (mg)			30	16	1200	70	856

#### 4.4.3 TLC Analysis of frs A – G

For this TLC analysis pre-coated TLC plate (Aluminum sheet silica gel 60 F<sub>254</sub>, 9 x 6 cm, 0.25 mm layer thickness, Merk) was used. The developing solvent system was CH<sub>2</sub>Cl<sub>2</sub> : MeOH (8 : 2) and the developing chamber was saturated for 10-15 minutes at room temperature. The chromatogram of the combined fractions was seen using UV lamp at 254 nm wave length.

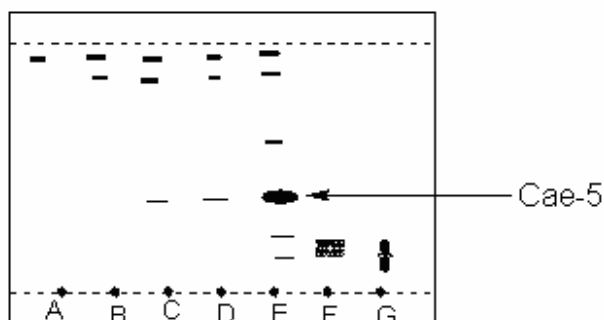


Fig. 6. TLC analysis of fractions from fr-4

As indicated in the above chromatogram, the combined fraction **E** contains one major compound together with other components. This fraction was subjected to column chromatography in the same procedure as fr-4 and three fractions were obtained.

#### 4.4.4 TLC Analysis of the Three Fractions from E

This TLC analysis was also done by keeping all the conditions and parameters the same, including the stationary as well as the mobile phases, with the above one.

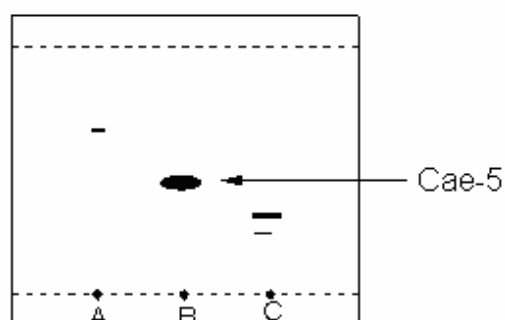


Fig. 7. TLC analysis of compound Cae-5

Finally the major component Cae-5 (55 mg) was isolated. It was yellow solid, soluble in methanol, mp 148 - 150 °C,  $[a]_D^{25} +17^{\circ}$ .  $^1\text{H}$  NMR (methanol- $d_4$ ):  $\delta$  2.16 (sharp, s, 3H), 4.48 (d, 1H), 4.86 (d, 1H), 5.89 (s, 1H), 5.92 (s, 1H), and 6.56 ppm (sharp, s, 2H).  $^{13}\text{C}$  NMR (methanol- $d_4$ ):  $\delta$  83.8 (C-2), 72.3 (C-3), 196.7 (C-4), 100.4 (C-4a), 163.9 (C-5), 95.9 (C-6), 167.3 (C-7), 94.9 (C-8), 163.0 (C-8a), 133.5 (C-1'), 106.7 (C-2', 6'), 145.5 (C-3', 5'), 127.7 (C-4'), 29.4 (C-1'').

## 4.5 Methanol Extraction Followed by Acid-Base Extraction

### 4.5.1 Extraction and Isolation

The powdered fresh khat leaves (100 g) was extracted with methanol, filtered and obtained 140 mL filtrate. It was concentrated into 10 mL using rotary evaporator at 40 °C and acidified with 0.1 N HCl to attain pH 3 (the pH change was monitored by "1 reel universal indicator paper pH 1-14"). This acidified methanol extract was diluted with distilled water into 40 mL which was extracted with n-hexane (3x40 mL). The n-hexane portion was separated by using 125 mL separatory funnel.

The HCl aqueous MeOH portion was basified with 10% NaOH to pH 10 and extracted with Et<sub>2</sub>O (3x40 mL). The Et<sub>2</sub>O portion was concentrated under reduced pressure at 30 °C and 99 mg was obtained. This was sent to NMR and found to be mixture of compounds.

The remaining basic aqueous - MeOH portion was again extracted with CHCl<sub>3</sub> (3x40 mL). The CHCl<sub>3</sub> portion was concentrated under the same condition as Et<sub>2</sub>O portion above and 33 mg was obtained. This was also submitted to NMR and assured to be mixture of compounds.

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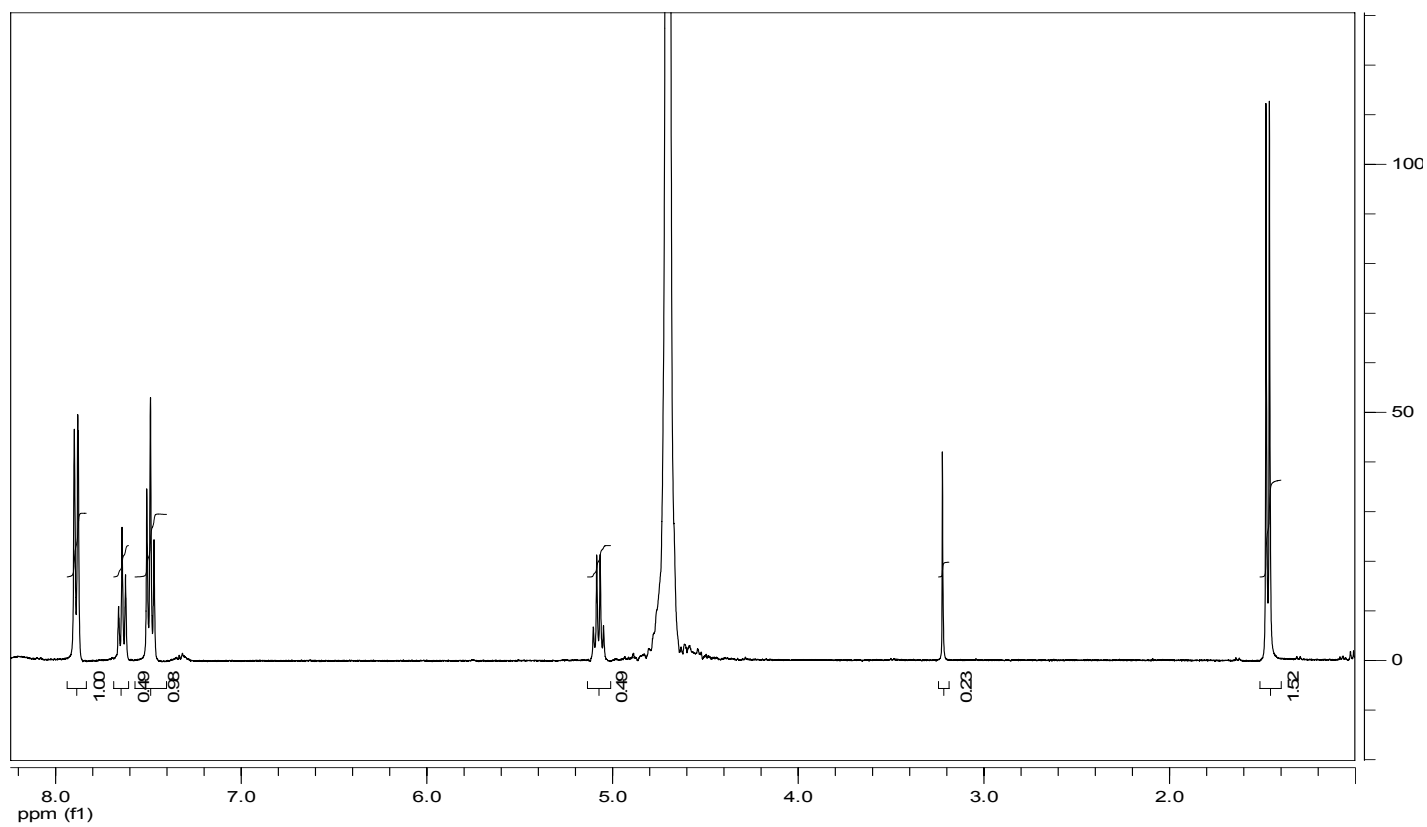
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#### **Availability of the above cited references**

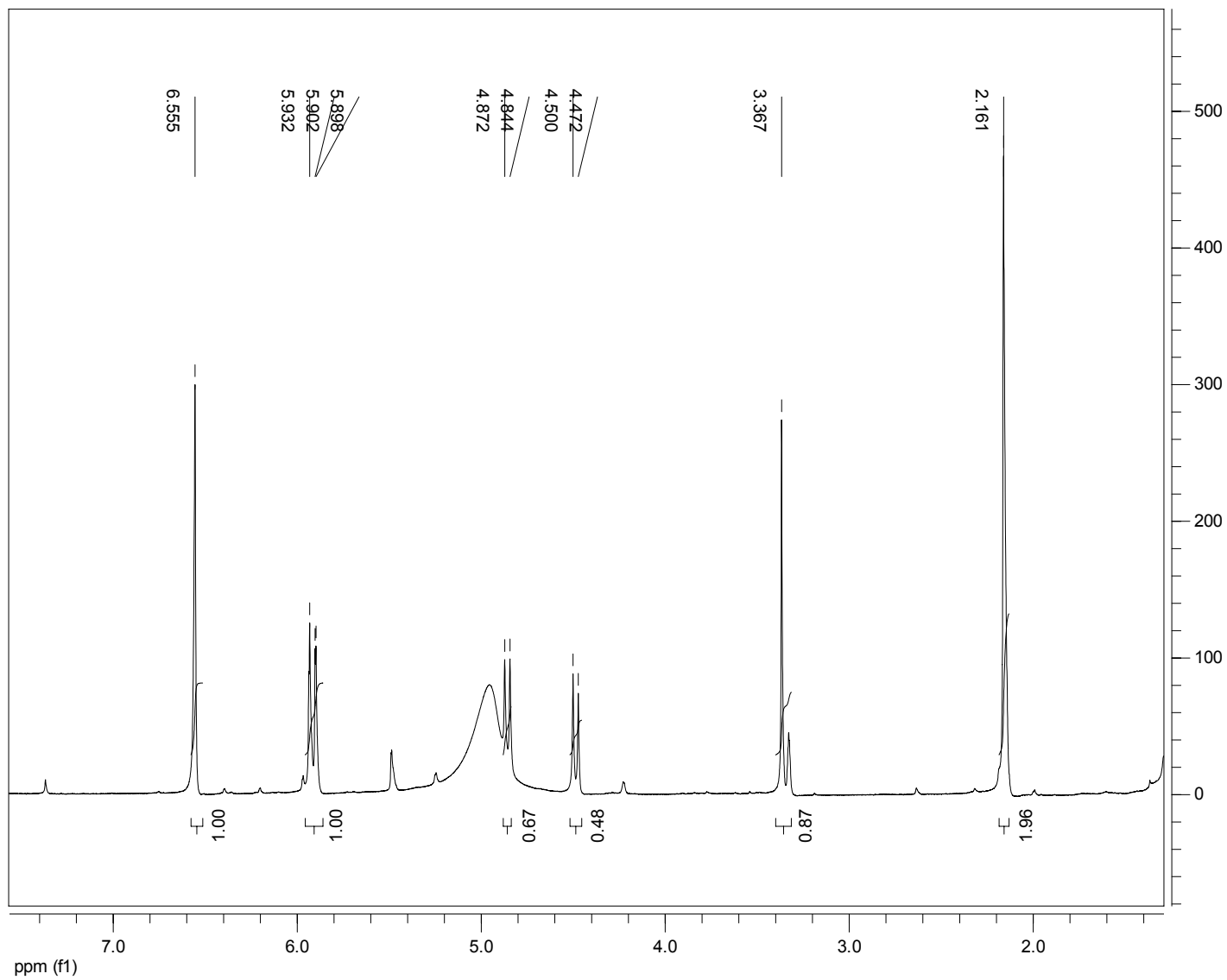
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- b. available in chemical information center
- c. available in ALNAP library Urael, A A
- d. available in science library

## 6. Appendices

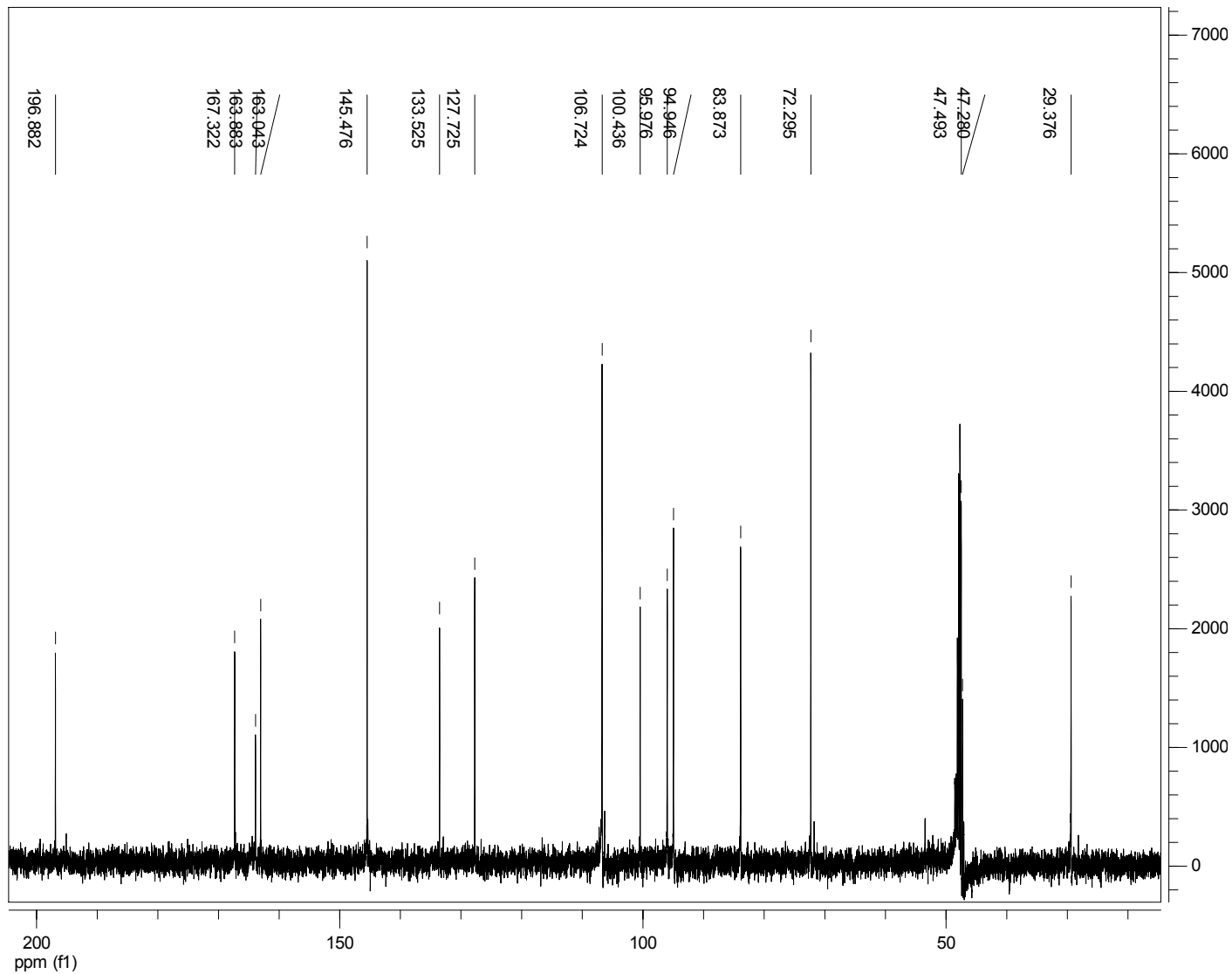
### Appendix-1 $^1\text{H}$ NMR spectrum of cathinone in $\text{D}_2\text{O}$



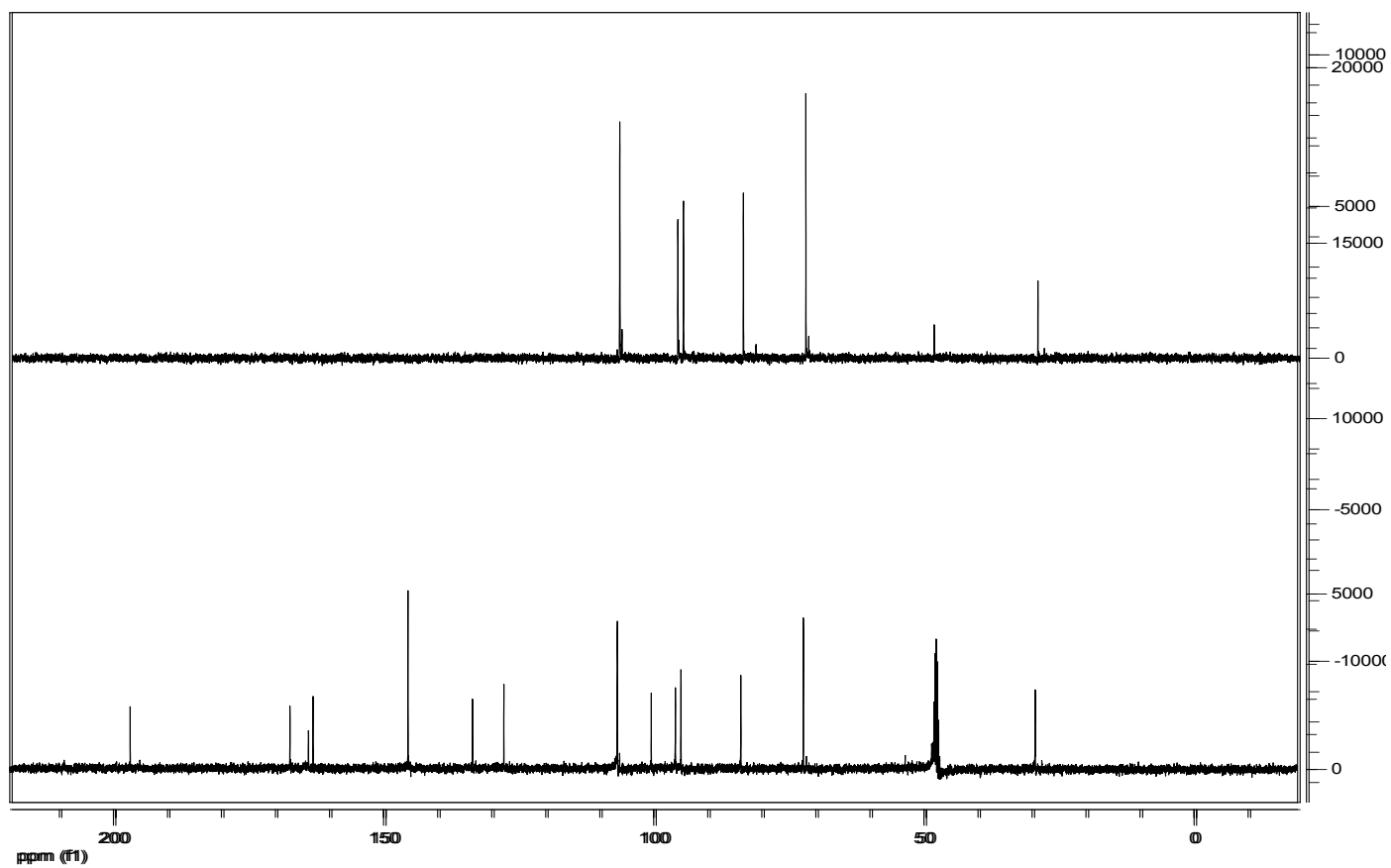
Appendix-3  $^1\text{H}$  NMR spectrum of Compound Cae-5



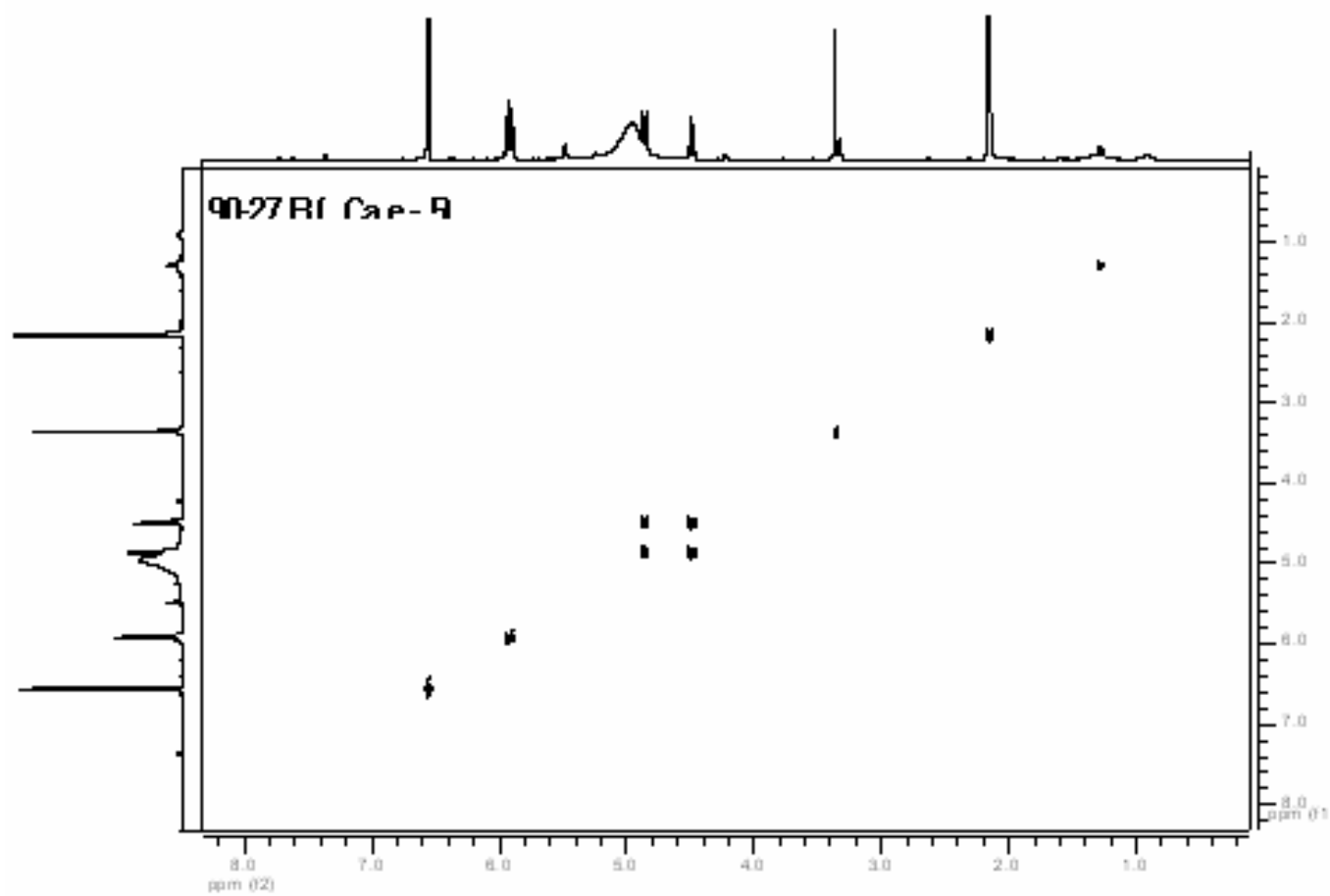
Appendix-4  $^{13}\text{C}$  NMR spectrum of Compound Cae-5



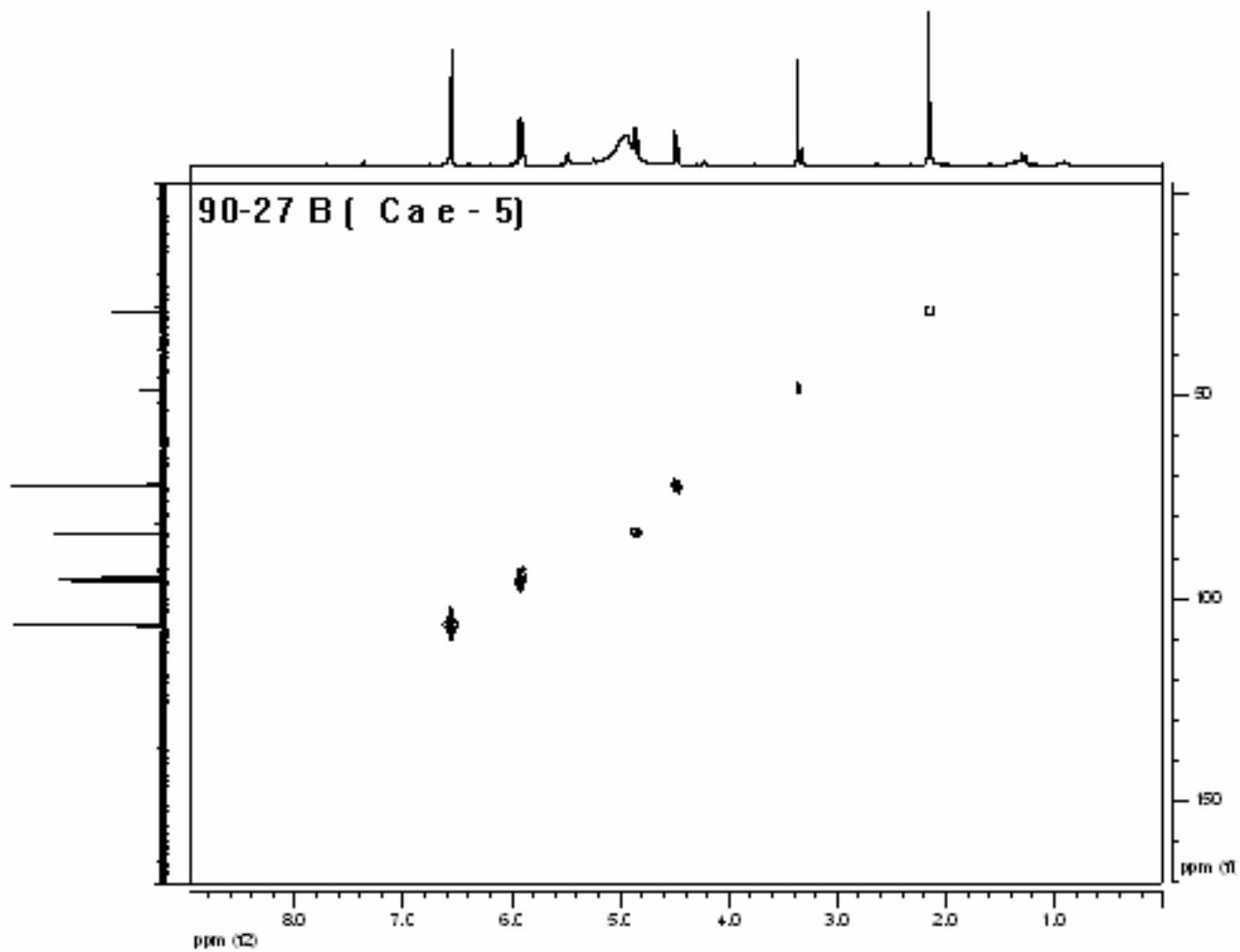
Appendix-5  $^{13}\text{C}$  NMR and DEPT spectrum of Compound Cae-5



Appendix-6  $^1\text{H}$  -  $^1\text{H}$  COSY of Compound Cae-5



Appendix-7  $^1\text{H}$  NMR and DEPT spectrum of compound Cae-5



Appendix-6 HMBC of Compound Cae-5

