ELSEVIER SCIENTIFIC PUBLISHERS IRELAND

Forensic Science International 61 (1993) 53–64

Forensic Science International

Gas chromatographic-mass spectrometric identification of chiral derivatives of the alkaloids of KHAT

M.J. LeBelle*, G. Lauriault, A. Lavoie

Bureau of Drug Research, Health and Welfare Canada, Ottawa, Ontario, Canada

(Received 25 January 1993, accepted 9 March 1993)

Abstract

The major alkaloids of the plant khat, *Catha edulis*, were conclusively identified by derivatization with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid. The acid was first reacted with dicyclohexyldicarbodiimide to give in situ the anhydride. The resulting amides formed from reaction with the anhydride were separated by gas chromatography. Separation characteristics of the derivatives of the alkaloids with other chiral acids and an anhydride are also given. Analysis of samples seized in Canada indicated large amounts of the enantiomer of cathinone, the alkaloid chiefly responsible for the activity of the plant. In some samples almost racemic mixtures of the two isomers were detected. These results indicate that racemization of the naturally occurring cathinone in the plant is an important process.

Key words: Khat; Cathinone; Cathine; Derivatization; Identification; Resolution

1. Introduction

Khat, *Catha edulis* Forsk, is a plant which grows primarily in East Africa. The fresh leaves of the plant are consumed by the inhabitants of several Asian and African countries in the region. Recently the use of the drug in other parts of the world has been recognized [1,2] and even in Canada, the drug has been the subject of numerous seizures.

* Corresponding author: Pharmaceutical Chemistry Division, Bureau of Drug Research, Banting Building, Tunney's Pasture, Ottawa, Ontario, Canada K1A OL2.

Elsevier Scientific Publishers Ireland Ltd.
SSDI 0379-0738/93/01314-H

It is now known that the major active principle of the plant is (−)-cathinone, and that this undergoes enzymatic reduction in the plant to (+)-norpseudoephedrine (cathine) and (−)-norephedrine [3]. The cinnamyl analogues [4] of cathinone and cathine were subsequently identified in plants grown in Kenya. The plant can therefore be expected to contain a mixture of these three optically active alkaloids. The amphetamine-like effects of these alkaloids [2,5–9], of the plant have resulted in the international recognition of the need to control them. The Convention on Psychotropic Substances, 1971, lists cathinone on Schedule I and cathine on Schedule III.

A variety of analytical approaches have been applied to the identification and quantification of khat and its alkaloids. Nordal and Laane [10] reported the identification of the plant based on a combination of the morphology, histochemical reactions, thin-layer chromatography (TLC) and gas-liquid chromatography. A TLC method has also been reported for the identification of the plant by Lehmann et al. [11]. Recently gas chromatograpy-mass spectrometry (GC-MS) (O. Morselli et al., pers. commun.) has been used for the identification of plant extracts. This identification was based on the presence of cathinone and cathine in plant extracts. In their study of the khatamine contents of various samples of khat, Geisshüsler and Brenneisen [12] reported the achiral forward phase high performance liquid chromatographic determination of the known alkaloids. However, none of these methods are able to discriminate between the enantiomeric forms of cathinone and its reduction products. Noggle and Clark (F.T. Noggle and C.R. Clark, pers. commun.) reported the HPLC separation of the 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylisothiocyanate (GITC) derivatives of only cathine and its related isomers. The method does not describe the derivatization of cathinone.

The conclusive identification of the plant may present difficulties depending on the jurisdiction. Although all samples seized in Canada have been representative of the traditional method of preparing and packaging the plant [13], the identification of the plant solely on this basis is unacceptable. Khat is the only plant known to produce (−)-cathinone [3] and therefore the identification of this alkaloid in extracts is conclusive proof of the identity of the source plant. However, in older samples of the plant, identification of only the (+)-norpseudoephedrine and (−)-norephedrine alkaloids may be possible and their presence may also be considered conclusive proof of the source plant.

The nomenclature and designation of the substances in this text bears a comment. (−)-Cathinone, (−)-2-aminopropiophenone, has the absolute configuration *S* at the C_2 carbon. It will be referred to as compound **1**S. Its reduction products therefore have the 1*R*,2*S*, (−)-norephedrine, and 1*S*,2*S*, (+)-norpseudoephedrine, configuration. They will be referred to as compounds **2**RS and **3**SS, respectively. The enantiomers of the three alkaloids will be designated **1**R, **2**SR and **3**RR, respectively.

The GC-MS identification of optically active amines of interest has been performed with *N*-trifluoroacetyl-(*S*)-(−)-prolyl chloride (TPC). Liu and Ku [14] described the GC-MS determination of TPC derivatives of amphetamine with chiral and achiral columns. However, this reagent is stereochemically impure; it usually contains about 6% of the other optical isomer. More recently McKibben [15]

reported the use of TPC for the separation of some enantiomeric amines related to clandestine laboratory synthesis. Successful derivatization and separation was reported for enantiomers of amphetamine, methamphetamine and ephedrine as well as other amphetamine analogues. We therefore investigated the application of this previously successful reagent to the derivatization of the khat alkaloids. At the same time we realized that the inherent impurity of the TPC reagent could be a problem in regard to the number of detectable alkaloid derivatives. It seemed that if other pure optically active acids were commercially available, they would be an alternative to the use of TPC. We considered that the condensation of an optically active acid with an amine could be carried out with dicyclohexyldicarbodiimide (DCC). We report here the results of the derivatization of khat extracts, and pure alkaloids using TPC and a number of these acids as well as one commercially available optically active anhydride.

2. Experimental

2.1. Materials and supplies

The following reagents were obtained from Aldrich Chemical Co.: (*S*)-(−)-*N*-(trifluoroacetyl)prolyl chloride 0.1 M solution in dichloromethane, (*R*)-(+)-2-chloropropionic acid, (*S*)-(+)-2-methylbutyric acid, (*R*)-(−)-2-phenylpropionic acid, (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid, (*R*)-(−)-2-phenylbutyric acid and (*R*)-(−)-*O*-acetylmandelic acid, (+)-diacetyl-L-tartaric anhydride, (+,−)-cathinone hydrochloride, which is sold as 2-aminopropiophenone hydrochloride, (+,−)-norephedrine hydrochloride, (1*R*,2*S*)-(−)-norephedrine, **2**RS, (1*S*,2*R*)-(+)-norephedrine hydrochloride, **2**SR, (1*S*,2*R*)-(+)-norephedrine, **2**SR, and (1*R*,2*R*)-(−)-norpseudoephedrine, **3**RR, hydrochloride. Cathine hydrochloride, **3**SS, and cathinone hydrochloride, **1**S, were obtained from the United Nations Drug Control Program Laboratory, Vienna. (+)-Amphetamine and (−)-amphetamine were available from the Branch's collection of drug standards.

2.2. Instrumentation

Polarimeter. The polarimeter was a Perkin Elmer model 241MC. Temperature was maintained by a Haake GH circulating bath. Specific rotations were determined in a temperature-controlled cell with a path length of 10 cm at 589.0 nm and 20°C. The instrumental operation was verified using aqueous solutions of related substances with known specific rotations.

Gas chromatograph-mass spectrometer. The GC-MS was a Carlo Erba Vega Series 6000 equipped with a Grob-type split/splitless injector and a capillary column. The column, DB-5, 15 m × 0.25 mm, 0.25 μm film thickness, was obtained from J & W Scientific, Rancho Cordova, CA. The column was operated at a head pressure of 40 kPa of helium (linear velocity = 60 cm/s). The injector was maintained at 275°C. Two sets of column conditions were used depending on the volatility of the derivatives. Condition A was: start, 160°C, immediately increase temperature at 5°/min to 220°C, then immediately increase temperature at 20°/min to 260°C. Condition B was: start, 190°C, hold for 1 min, increase temperature at 2°/min to 220°C, hold for 1 min, then increase temperature at 20°/min to 275°. Injections (1 μl) were

made at a split ratio of approximately 25:1. The mass spectrometer was a Finnigan Mat 800 Ion Trap Detector. The gas chromatograph's column effluent was introduced to the spectrometer by direct attachment of the column to the Ion Trap detector; no splitting occurred between the gas chromatograph and the detector. The Ion Trap was set to acquire data at 1 scan/s over a mass range of 40–500 amu. Chemical ionization spectra were acquired with methane using the standard operating procedures specified by the manufacturer.

Gas chromatography. The gas chromatograph was a model HP 5890 equipped with a flame ionization detector, a split/splitless injector and a HP 5895A ChemStation data system/system controller. The column was identical to that used in the GC-MS. The instrument was operated at a head pressure of 12 p.s.i. with a flow of 1.5 ml/min of helium. The detector was operated with a mixture of air at 400 ml/min and hydrogen 30 ml/min. Nitrogen was used as make-up gas at a flow of 30–40 ml/min. One-milliliter injections were made in split mode. The column temperature program was as specified in Condition B for the GC-MS.

2.3. Sample extraction

The extraction procedure was similar to that described by Geisshüsler and Brenneisen [12]. Approximately 200 mg of dried leaves were sonicated with 5 ml of 0.1 M hydrochloric acid at room temperature for 15 min. The insoluble material was allowed to settle and the aqueous solution was drawn off. It was extracted once with a 3-ml portion of dichloromethane and the organic phase was discarded. When sequential extractions were performed all of the aqueous extracts were combined and the combined extracts extracted with one 3-ml portion of dichloromethane. Sufficient saturated sodium bicarbonate was added to render the aqueous solution barely basic (pH 7–8) and the aqueous solution extracted as quickly as possible with dichloromethane. The dichloromethane solution was then transferred to a tube and evaporated to dryness. A volume of 100 μl of dichloromethane was immediately added to the tube to re-dissolve the alkaloids. Berrang et al. [16] previously confirmed the enantiomeric stability of (−)-cathinone in dichloromethane for 24 h.

2.4. Derivatization

Anhydrides. The anhydrides of the acids were prepared at a concentration of 0.025 M in dichloromethane by mixing equal volumes of a 0.05-M solution of the acid and a 0.05-M solution of DCC. The completeness of the conversion of the acid to the anhydride was confirmed by injecting this solution into the GC-MS. Peaks attributable to the acid, anhydride, DCC and dicyclohexylurea could be detected for all acids. The reactions were complete in 5 min or less.

This resulting solution was used for derivatization of the plant alkaloids by addition of a 200-μl aliquot to the plant extract.

TPC. About 1 mg of each standard was placed in a culture tube (about 15 cm × 0.8 cm i.d.) and 2 ml of chloroform was added. One ml of the TPC solution was added followed immediately by 1 ml of a saturated solution of sodium bicarbonate. The reaction mixture was stirred vigorously at room temperature with a small magnetic bar for 5 min. The aqueous phase was removed with a pipette and 1 ml of water was added and the mixture stirred for another minute. The aqueous

Table 1
Drying and storage conditions of the samples

Sample No.	Date received	Sample history[a]	Drying conditions
1a	07/91	1d	Freeze dried
1b	07/91	4d	Freeze dried
1c	07/91	7d	Freeze dried
1d	07/91	5d	Air dried
2	11/91	3d	Freeze dried
3	01/92	2d	Freeze dried
4a	09/92	1d	Air dried
4b	09/92	1d	Freeze dried

[a–d]refers to the number of days the plant material was stored at approximately 10°C before drying.

layer was again removed and the organic phase dried with a small quantity of anhydrous sodium sulphate. This method differs significantly from that of Liu and Ku [14] who converted the amphetamine salts to the bases with 0.1 N NaOH. The in situ generation of the bases after the addition of the TPC reagent solution was intended to minimize the time the alkaloidal base was in solution. Cathinone is known to rapidly racemize [16] in the presence of protic solvents.

2.5. The samples

The samples which were suspected of being khat had been trans-shipped from different European cities and were seized at Ottawa International Airport. Their appearance was consistent with the traditional presentation [13]. Visual inspection of the samples upon receipt in our laboratory indicated they were not excessively decomposed. They were either lyophilized or air dried in a fume hood on receipt. Table 1 gives the sample information. They were then stored for up to 1 year in screw-capped jars at room temperature or in a freezer (−20°C).

3. Results and discussion

The optical purity of the cathinone hydrochloride standard was estimated by determining its optical rotation. The specific rotation of the cathinone standard

Table 2
Retention times[a] of derivatives of TPC

Derivative (retention time (s))					
2-Aminopropiophenone **1**		Norephedrine **2**/norpseudoephedrine **3**			
1S	**1R**	**2SR**	**3RR**	**3SS**	**2RS**
415	358	377	384	410	413

[a]Chromatographic condition B.

Table 3
Retention times[a] of derivatives of 2-methylbutyric and 2-chloropropionic acids

Acid	Derivative (retention time (s))					
	2-Aminopropiophenone **1**		Norephedrine **2**/norpseudoephedrine **3**			
	1S	**1R**	**2SR**	**3RR**	**3SS**	**2RS**
(*S*)-(+)-2-Methylbutyric acid	252	257	299	299	298	299
(*R*)-(+)-2-Chloropropionic acid	239	248	266	266	266	263

[a]Chromatographic condition A. See text for details.

(c = 1, H_2O) was determined to be −35.1°. The specific rotation of the pure hydrochloride salt has been reported to be −45°, c = 0.35, H_2O [17] and −46.9°, c = 1, H_2O [16]. Correcting the observed value for the presence of about 5% of an impurity (see below) the optical purity of the standard can be estimated to be about 89% **1S**. (Dr. Watanabe of the U.N. laboratory had provided the sample on the understanding that it was quite old and of unknown purity.)

Table 2 gives the retention times of the TPC derivatives of the two cathinone enantiomers and the four isomers of the norephedrine/norpseudoephedrine series. Satisfactory resolution was obtained for the two aminopropiophenone isomers. However, inadequate separation was obtained for the two norephedrine/norpseudoephedrine diastereoisomers of interest. The derivatives of **3SS** and **2RS** isomers were nearly coincident, 410 and 413 s, respectively, and were also only marginally resolved from the aminopropiophenone isomer of interest at 415 s. All of the chromatograms of the pure standards of the norephedrine/norpseudoephedrine series contained small peaks attributable to the diastereomeric derivative

Table 4
Retention times[a] of derivatives of other acids and anhydrides

Acid	Derivative (retention time (s))					
	2-Aminopropiophenone **1**		Norephedrine **2**/norpseudoephedrine **3**			
	1S	**1R**	**2SR**	**3RR**	**3SS**	**2RS**
(*R*)-2-Phenylpropionic	471	437	494	501	515	516
(*R*)-2-Phenylbutyric	551	515	573	582	604	604
(*R*)-α-Methoxy-α-trifluoromethylphenylacetic	458	482	544	554	534	516
(*R*)-*O*-Acetylmandelic	752	764	851	853	834	834
(+)-Diacetyl-L-tartaric anhydride	459	479	467	536	523	467

[a]Chromatographic condition B. See text for details.

resulting from the presence of a small amount of the TPC enantiomer in the reagent. The average of the ratios of the two derivatives for each of the four isomers was 6%. This is similar to the amount (%) of the (*R*)-TPC isomer found by Liu and Ku [14]. The (−)-cathinone standard on the other hand indicated about 16% of a peak which could be ascribed to the combination of enantiomeric *SR* and *RS* derivatives. This implied that 6% of this 16% was due to the presence of the (*R*)-TPC in the reagent and the remaining 10% of the derivative would be due to the presence the (*R*)-isomer of cathinone in the standard. This agrees well with the presence of about 11% of **1R** in the standard calculated from the specific rotation.

Tables 3 and 4 give the retention times of the derivatives of the khat-related substances using the anhydride derivatization method with a number of different optically active acids. Table 3 shows the retention times of the derivatives of 2-methylbutyric acid and 2-chloropropionic acid. As the derivatives of these acids were more volatile than others, chromatographic conditions involved starting the column at a lower temperature. Both acids formed derivatives of the (*R*)- and (*S*)-isomers of cathinone that were separable. However, almost no separation was obtained with derivatives of norephedrine and norpseudoephedrine. Table 4 is a summary of the retention times of the derivatives of the remaining acids. Once more all of these acids yielded derivatives of cathinone that were easily separable. However, only (*R*)-α-methoxy-α-trifluoromethylphenylacetic (MTPA) gave derivatives of the **2RS** and **3SS** isomers that were readily separable from each other and the other possible isomers. Even though the four isomers of the norephedrine/norpseudoephedrine series are not completely resolved — only marginal separation was obtained between the **2SR** and **3RR** isomers — analysis using the derivatives can conclusively identify the alkaloids found in khat.

For substances with multiple centres of optical activity, inversion of all of the centres yields enantiomers. It is therefore possible to predict the retention characteristics of the derivatives of the alternate enantiomers of all of the acids used. The cathinone series is of course trivial as only two sets of diastereomers exist. Therefore the derivative of **1S** with an *R* acid will be the enantiomer of **1R** with the *S* acid. However, in the norephedrine/norpseudoephedrine series, where four pairs of enantiomers are possible, the correspondence between derivatives is not as simple. For example, **3SS** with an *S* acid will give a derivative with the same retention time as the **3RR** with an *R* acid. In the case of the MTPA, the *S*-enantiomer of this acid would give derivatives of **3SS** and **2RS**, the alkaloids of interest, with retention times of 554 and 544 s, respectively. By inspection of the retention times in the tables of the derivatives for this and the other derivatizing agents, it can be seen that none of the other enantiomers of the acids investigated would have yielded derivatives with superior resolution.

The chiral purity of MTPA was verified with a number of optically active amines. The ratios of the areas of the peaks of the derivatives of (+)- and (−)-amphetamine, **2RS**, **2SR**, **3SS** and **3RR** were used to calculate the percent content of the other diastereomer due to the presence of the (*S*)-isomer in MTPA. The values ranged from 0.63% (**3SS**) to 2.7% (**3RR**) with an average of 1.6%. Based on the lowest values obtained, it would seem acceptable to declare that the reagent contained less than 1% of the (*S*)-isomer or less than 1% epimerization of the alkaloid or the derivative occurred during the derivatization reaction.

The purity of the cathinone standard was also verified by injection of the dichloromethane solution derived by the extraction procedure with and without derivatization. By injection without derivatization, the standard was shown to contain about 5% (relative to cathinone) of 1-phenyl-1,2-propanedione, cathinone's recognized [13] oxidation product. The identity of this substance was inferred from its mass spectrum (M^+ = 148, 105, 77) and by comparison with the spectrum in the NBS library. By injection after derivatization with MTPA, the standard was shown to contain about 12% of the (*R*)-isomer, **1R**. This agrees favourably with the results from the determination of the optical purity of the standard with TPC and by specific rotation when approximately 1% of the alternate derivative can be ascribed to the presence of the alternate isomer of the acid.

All of the derivatives of MTPA and the alkaloids gave spectra with weak M + 1 ions in electron ionization mode. This is a phenomenon of the Ion Trap which occurs and is unavoidable with some substances, particularly amines [18]. Acquisition in chemical ionization mode (methane) gave, in the case of **1S**, the M + 1, 366, as the base peak in the spectrum. However, in the norephedrine/norpseudoephedrine series loss of water from the M + 1 ion (10%) was favourable with 350 as base peak. Figures 1 and 2 are the electron impact spectra of the derivatives of **1S** and **3SS** with MTPA, respectively. Most of the fragments can be easily ascribed as both ions from the fragmentations are detected. Figure 3 shows the proposed fragmentation points of the **1S** derivative.

Fig. 4 is a chromatogram of a plant extract after derivatization with MTPA. Peaks 1 and 2 are due to the derivatives of **1S** and **1R**, respectively. Peaks 3 and 4 are the derivatives of **2RS** and **3SS**, respectively. The two smaller peaks which elute immediately after peak 4 are the two derivatives of **2SR** and **3RR**, respectively. We pro-

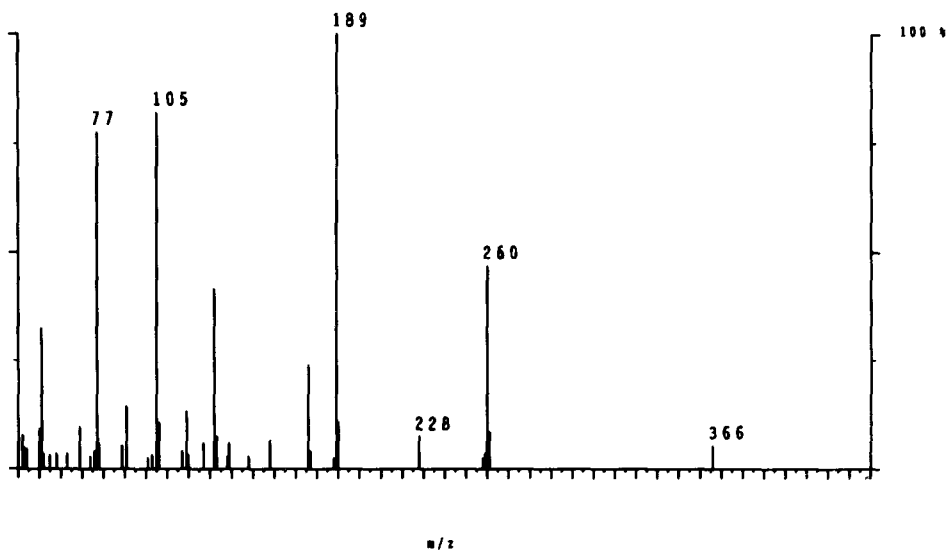

Fig. 1. Electron impact mass spectrum of the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid derivative of cathinone.

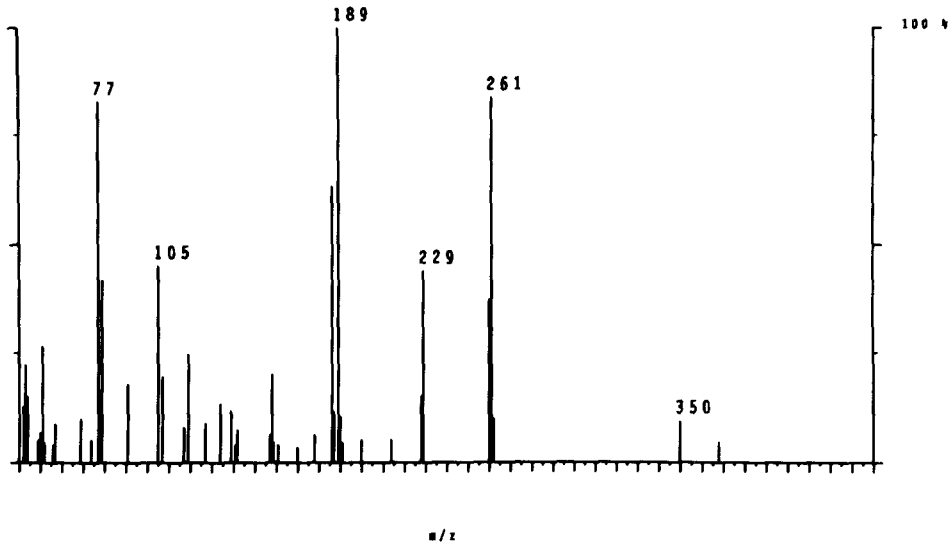

Fig. 2. Electron impact mass spectrum of the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid derivative of cathine.

pose that the small peak at 910 scans is the derivative of merucathine. Although no standard for this substance was available, its mass spectrum was very similar to those of the derivatives of **3SS** and **2RS**. In addition the chemical ionization mass spectrum of the peak indicated an M + 1 of 394 which would be consistent with a cinnamyl analogue. However, no peaks in the chromatogram suggestive of the presence of merucathinone could be identified.

The effect of the extraction procedure on the relative amounts of the derivatives of the respective aminopropiophenone isomers was determined. Complete and incomplete evaporation of the methylene chloride extracts had no effect on the relative amounts of the detected derivatives. In addition, no dimethyldiphenylpyrazine was detected in any of the chromatograms. This substance eluted before the **1S** derivative at 429 s. The stability of the extracts was confirmed by preparing an extract of 1 g

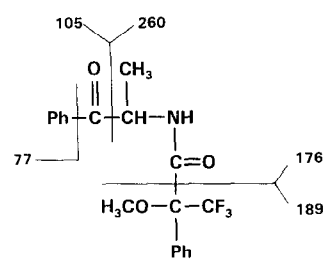

Fig. 3. Suggested fragmentation pathways of the derivative of cathinone with MTPA.

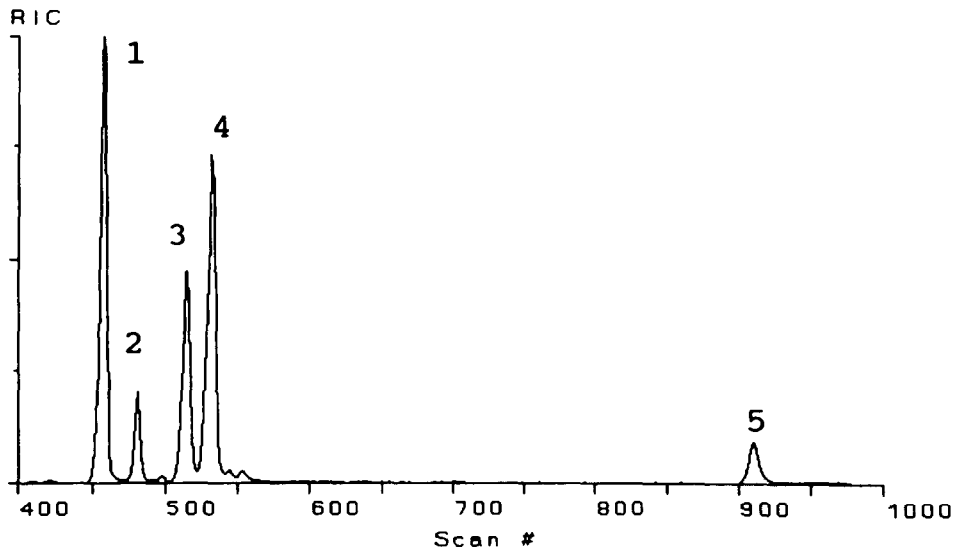

Fig. 4. Total ion chromatogram of an extract of the plant, *Catha edulis* after derivatization with MTPA. Peak 1, (*S*)-cathinone derivative; Peak 2, (*R*)-cathinone derivative; Peak 3, 2*R*,3*S*-norephedrine derivative; Peak 4, 2*S*,3*S*-norpseudoephedrine derivative; Peak 5, merucathine derivative (unconfirmed).

of plant material. The extract was analyzed on the day of preparation and after 2 days at 5°C. No difference in the ratios of the relative amounts of the respective isomers could be detected. Although known to racemize in the presence of protic solvents [16], cathinone maintains its enantiomeric integrity in dichloromethane for at least 2 days. These findings agree with those of Berrang [16].

The completeness of extraction was determined by the sequential extraction of a sample of plant material and gas chromatographic analysis of each of the extracts. The degree of extraction of the alkaloids into the aqueous phase was determined by extracting the same portion of the plant four times with fresh aliquots (5 ml) of hydrochloric acid. After each extraction as much as possible of the aqueous solution was removed. The dichloromethane wash of the acidic aqueous solution contained none of the alkaloids of interest. Injection of this washing indicated the presence of substances with mass spectra indicative of hydrocarbons as well as other unidentified substances. Each of the aliquots was made basic with bicarbonate and extracted two times with 3 ml of dichloromethane. These extracts were injected directly into the gas chromatograph and the areas of the peaks for cathinone and the norephedrine/norpseudoephedrine peaks recorded. The percent of the areas of each of the peaks in each of the extracts versus the total areas of each of the peaks was determined. It could be estimated that the fourth extract contained only 4% of the total cathinone and 2% of the total norephedrine/norpseudoephedrine alkaloids indicating near complete extraction. The degree of extraction of the alkaloids from the aqueous phase was determined in the same way. Four extractions of 4 ml each with dichloromethane indicated the fourth extract contained less than 1% of the total amount of cathinone. However, in the case of the norephedrine/norpseudoephedrines the

Table 5
Cathinone enantiomer ratio in samples

Sample No.	% **1R**
1a	13
1b	42
1c	38
1d	47
2	36
3	36
4a	23
4b	18

fourth extract still contained about 20% of these alkaloids indicating somewhat incomplete extraction.

The ratios of the cathinone isomers present in the samples shown in Table 1 were determined. A portion (200 mg) of each of the samples was extracted sequentially with 4–5-ml aliquots of 0.1 N HCl for 15 min in an ultrasonic bath. The aqueous solutions were combined and washed with 3 ml of dichloromethane. Each of the combined aqueous phases was then made basic and extracted with four 3-ml portions of dichloromethane. The dichloromethane was evaporated under a stream of nitrogen and the derivatization carried out as described above. The percent of the **1R** isomer in relation to the total of the **1R** and **1S** contents is given in Table 5. The relative amounts of the two enantiomers varied widely. The two samples, 1a and 4b, which were freeze-dried immediately after receipt showed the lowest amounts, 13 and 18%, of the cathinone enantiomer. The other samples showed considerable amounts of the cathinone enantiomer. In the case of sample 1d which had been freeze-dried 7 days after receipt, an almost racemic mixture of the two enantiomers (47% **1R**) was present. It would appear that racemization in the plant is a predominant phenomenon. The results from the sample-1 series also would seem to indicate that after drying the rate of racemization of the cathinone is retarded. Sample 1a which had been stored for more than 1 year after drying contained the lowest amount of the *R*-enantiomer of cathinone. The popularity of freshly harvested khat has been ascribed to the speed of reduction of cathinone to yield the less active alcohols but it would appear that the loss of potency of khat after harvest may also be attributed to the racemization of the active principle.

The results further indicate that the rate of reduction of the (*R*)- and (*S*)-isomers of cathinone in the plant are significantly different. The relative amounts of the **2RS** and **3RR** isomers were not consistent with the relative amounts of the **1S** and **1R** isomers. That is, the total relative amount of the **2RS** and **3RR** isomers was 5% or less of the total amount of all the reduced alkaloids. For example, sample 1d contained 47% of **1R** relative to the total of **1R** and **1S**. However, the total of **2RS** and **3RR** relative to the total of all isomers of **2** and **3** represented only 3%. This may be due to incomplete extraction but it is unlikely that the four isomeric alcohols would exhibit such great differences in extractability.

In conclusion, the method described is effective for the conclusive identification of the alkaloids found in the khat plant. At the same time the ratios of the relative amounts of the epimers of cathine can be determined.

4. References

1 P. Kalix, Une plante psychotrope, le khat. *Psychotropes,* III (1986) 13–22.
2 P. Kalix, S. Geisshüsler, R. Brenneisen, U. Koelbing and H.-U. Fisch, The khat alkaloid cathinone has amphetamine-like effects in humans. *Eur. J. Pharmacol.,* 183 (1990) 457.
3 K. Szendrei, The chemistry of khat. *Bull. Narcotics,* XXII (1980) 5–35.
4 R. Brenneisen, S. Geisshüsler and X. Schorno, Merucathine, a new phenylalkylamine from *Catha edulis. Planta Med.,* 50 (1984) 531.
5 J.L. Zelger, Hj.X. Schorno and E.A. Carlini, Behavioral effects of cathinone, an amine obtained from *Catha edulis* Forsk: comparisons with amphetamine, norpseudoephedrine, apomorphine and nomifensine. *Bull. Narcotics,* XXXII, (1980) 67–81.
6 P. Kalix and R.A. Glennon, Further evidence for an amphetamine-like mechanism of action of the alkaloid cathinone. *Biochem. Pharmacol.,* 35 (1986) 3015–3019.
7 R.A. Glennon, M. Yousif, N. Naiman and P. Kalix, Merucathinone: A new and potent amphetamine-like agent. *Pharmacol. Biochem. Behav.,* 26 (1987) 547–551.
8 R. Brenneisen, H.-U. Fisch, U. Koelbing, S. Geisshüsler and P. Kalix, Amphetamine-like effects in humans of the khat alkaloid. *Br. J. Clin. Pharmacol.,* 30 (1990) 825–828.
9 P. Kalix, Cathinone, a natural amphetamine. *Pharmacol. Toxicol.,* 70 (1992) 77–86.
10 A. Nordal and M.M. Laane, Identification of Khat. *Medd. Nor. Farm. Selsk.,* 40 (1978) 1–18.
11 T. Lehmann, S. Geisshüsler and R. Brenneisen, Rapid TLC identification test for khat (*Catha edulis*). *Forensic Sci. Int.,* 45 (1990) 47–51.
12 S. Geisshüsler and R. Brenneisen, The content of psychoactive phenylpropyl and phenylpentenyl khatamines in *Catha edulis* Forsk of different origin. *J. Ethnopharmacol.,* 19 (1987) 269–277.
13 R. Brenneisen and S. Geisshüsler, Psychotropic drugs, III. Analytical and chemical aspects of *Catha edulis* Forsk. *Pharm. Acta. Helv.,* 60 (1985) 290–301.
14 J.H. Liu and W.W. Ku, Determination of enantiomeric *N*-trifluoroacetyl-L-prolyl chloride amphetamine derivatives by capillary gas chromatography/mass spectrometry with chiral and achiral stationary phases. *Anal. Chem.,* 53 (1981) 2180–2184.
15 T. McKibben, Separation and identification of drug enantiomers via *N*-TFA-(*S*)-prolyl chloride derivatization. *J. Clan. Lab. Investig. Chemists Assoc.,* 2 (1992) 13–20.
16 B.D. Berrang, A.H. Lewin and F.I. Carroll, Enantiomeric α-aminopropiophenones (cathinone): preparation and identification. *J. Org. Chem.,* 47, (1982) 2643–2647.
17 J.-P. Wolf and H. Pfander, Synthese und strukturaufklärung von Merucathinon and synthese von Cathinon, inhaltsstoffe von *Catha edulis* Forsk. *Helv. Chim. Acta.,* 69 (1986) 1498–1504.
18 A.W. By, M.J. LeBelle, B.A. Lodge and W.L. Wilson, Analysis and identification of Canadian designer drugs, presented at the 103rd Assoc. Off. Anal. Chem. Meeting, September, 1989 St. Louis.