Mass Spectral, Infrared and Chromatographic Studies on Homologs and Regioisomers of 3,4-Methylenedioxypyrovalerone (MDPV)

by

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Abstract

A series of 10 homologous and regioisomeric aminoketones related to the designer synthetic cathinone derivative MDPV were synthesized. These compounds were all prepared from a common precursor chemical, piperonal (3,4 methylenedioxybenzaldehyde). The analytical properties of these compounds were compared by GC-MS and GC-IR techniques. These aminoketones show major fragments in their mass spectra corresponding to the regioisomeric and homologous immonium cation fragments. All these compounds in this study show equivalent EI MS fragments for the 3,4-methylenedioxybenzoyl fragments (*m/z* 149) and the methylenedioxybenzene fragment (*m/z* 121). The vapor phase infrared spectra allow for the differentiation of the regioisomers of mass spectral equivalence.

The regioisomeric aminoketones yield equivalent mass spectra including mass equivalent regioisomeric immonium cation base peak which is identical to that of the designer drug MDPV. An evaluation of the effects of homologation on gas chromatographic retention showed that addition of a methylene (CH_2) in the nitrogen-containing ring increases retention more than the equivalent group added to the alkyl side-chain.

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List of Abbreviations

µl	Micro liter
µm	Micrometer
°C	Degree centigrade
PPP	α-pyrrolidinopropiophenone
4-MEC	4-Methylethycathinone
4-FMC	4-Flourometh-cathinone
MBDB	2-Methylamino-1-(3,4-methykenedioxyphenyl)butane
MDAI	5,6-Methylenedioxy-2-aminoindane
MDMA	3,4-Methylenedioxymethamphetamine
MDMC	3,4-Methylenedioxy-N-methylcathinone
MDPBP	3,4-Methylenedioxypyrrolidinobutyrophenone
MDPV	3,4-Methylenedioxypyrovalerone
MOPPP	4-Methoxy-α-pyrrolidinopropiophenone
MPPP	4-Methyl-α-pyrrolidinopropiophenone
ATR	Attenuated total reflectance
bk	Benzylketo
CYP-450	Cytochrome- P450
Da	Dalton
EI	Electron impact

ev	Electron volt
f.d.	Film depth
FTIR	Fourier Transform Infrared Spectroscopy
FRNMR	Fourier Transform Nuclear Magnetic Resonance
GC	Gas chromatograpy
GC-IRD	Gas chromatography coupled to infrared detection
GC-MS	Gas chromatography- mass spectrometry
GC-TOF-MS	Gas chromatography with time of flight mass spectrometry
HPLC	High performance liquid chromatography
i.d.	Internal diameter
IR	Infrared
IV	Intravenous
LC	Liquid Chromatography
LC – HR-MS	Liquid chromatography–High resolution-Mass spectrometry
LC/QTOF	Liquid chromatography/quadruple time-of-flight
m	Meter
m-	Meta
min	Minute
ml	Milliliter
mm	Millimeter
MS	Mass spectrometry
MW	Molecular weight

nm	Nanometer
NMR	Nuclear Magnetic Resonance
NRG-1	Naphthylpyrovalerone (Naphyrone)
o-	Ortho
p-	Para
PCC	Pyridinium chlorochromate
ppm	Part per million
TMS	Trimethylsil

1. Literature review

1.1. Introduction

A number of synthetic cathinones (aminoketone type of compounds, see Figure 1) have appeared on the illicit drug market in recent years and these compounds represent a new emphasis in the development of designer drugs. There has been a worldwide rise in the popularity and abuse of these synthetic cathinones. In 2009 and 2010, a significant rise in the abuse of a new group of synthetic cathinones was reported in Western Europe, referred to as "bath salts" or "legal high," which occurred later in the USA [Spiller *et al*, 2011]. "Ivory Wave," "Purple Wave," "Vanilla Sky," and "Bliss" all are among the many street names of a so-called bath salts designer drug which has sparked thousands of calls to poison centers across the U.S. over the past two years [WebMD, 2011].

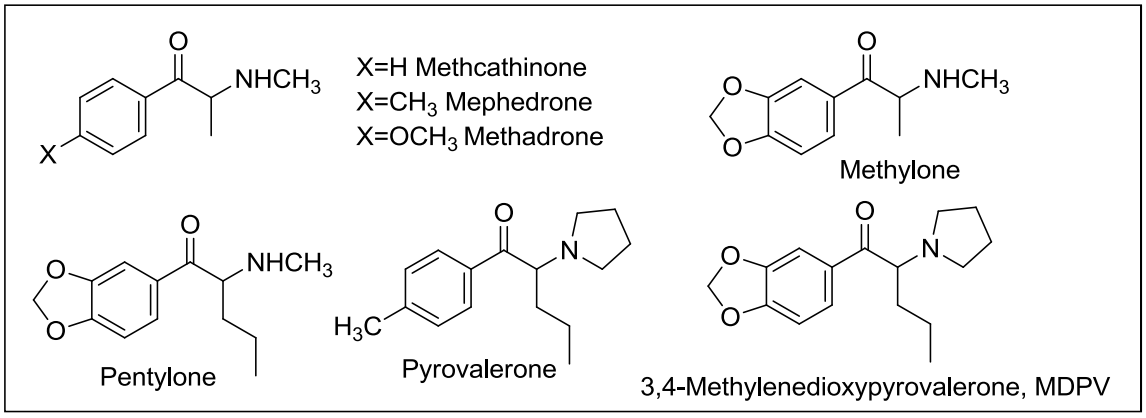


Figure 1: Structures of common cathinone/bath salts reported in recent forensic samples.

Based on the structure of the unsubstituted cathinone molecule, designer modifications are possible in three distinct regions (Figure 2) of the molecule: A) the aromaric ring, B) the alkyl side chain and C) the amino group. Based on the structures in Figure 1, all three of these areas of possible designer modification are currently being explored by designer-type modifications. Legal control of a specific molecule often provides the driving force for clandestine development of additional substituted cathinone designer molecules whose structures place them just outside the chemical boundaries described by the language of the legal control documentation.

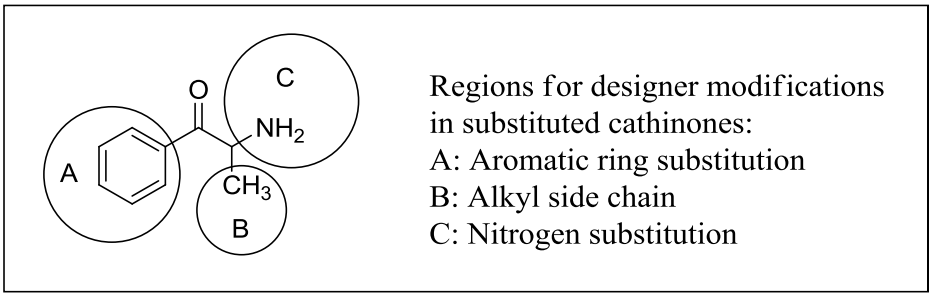


Figure 2: Molecular regions for designer modification in cathinone bath salts.

The goal of clandestine manufacturers is often to prepare substances with pharmacological profiles that are sought after by the user population. Clandestine manufacturers are also driven by the desire to create substances that circumvent existing laws. In Europe, as a result of the substance-by-substance scheduling approach, the appearance of new substances cannot be immediately categorized as illicit drugs. This offers room for clandestine experimentation into individual substances within a class of

drugs with similar pharmacological profiles, perhaps yielding substances of increased potency. In the USA, continued designer exploration has resulted in legislation (Controlled Substances Analogue Act) to upgrade the penalties associated with clandestine use of all compounds of a series.

1.2. History of synthetic cathinones

In 2010 the laboratory of the NIP (The National Institute of Pharmacy) was requested to give expert reports on several unknown bulk powder samples seized by the Hungarian Customs and Finance Guard. Package information and invoices claimed that the samples were pharmaceutical excipients or similar harmless substances. However, analysis of the samples demonstrated that each powder contained the hydrochloride salt of one of the following compounds: mephedrone (4-methylmethcathinone) [Europol-EMCDD, 2010], butylone (beta-keto-MBDB) [Morris, 2010], 3,4-methylenedioxypyrovalerone (MDPV) [Dargan *et al*, 2010], flephedrone (4-fluoromethcathinone) [Torrance and Cooper, 2010] and a new potential designer drug, 4-methylethcathinone (4-MEC) [Windstock *et al*, 2011]. All substances are chemical derivatives of the psychoactive stimulant methcathinone.

Some of these compounds were mentioned in a 2010 joint report of Europol and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) on mephedrone [Europol-EMCDD, 2010]. The report concluded that in many countries mephedrone was marketed as a legal alternative to ecstasy or cocaine. Participants reported seizures of

mephedrone in the form of bulk powder or tablets and provided evidence of toxicity associated with mephedrone use. The conclusion of the study was confirmed by other works reporting undesired side-effects, symptoms of intoxication as well as fatalities associated with mephedrone abuse [Morris, 2010], [Windstock *et al*, 2011]. As a result, mephedrone has been recently controlled in several countries [Morris, 2010].

In the UK, a group of psychoactive drugs called "legal high" derivatives including mephedrone (first generation) have recently been classified as Class B, Schedule I under the Misuse of Drugs Act 1971 [Brandt *et al*, 2010]. Since then, other related products have appeared on different websites, some of these advertised products were collected and analyzed by gas chromatography ion trap mass spectrometry using electron- and chemical ionization modes, nuclear magnetic resonance spectroscopy, and compared with reference standards. These purchased products showed single cathinones or cathinone mixtures including mephedrone, butylone, 4-methyl-N-ethylcathinone (4-MEC), flephedrone (4-fluoromethcathinone or 4-FMC) and MDPV (3,4-methylenedioxypyrovalerone) [Brandt *et al*, 2010]. Earlier studies showing that drug abusers tend to buy such substitutes because of their higher purity than street drugs, with a lower risk of physical harm, and not prosecuted under the Misuse of Drugs Act 1971 [Measham *et al*, 2010]. One of the most common second-generation products, at least in the UK media, is NRG-1 (naphthylpyrovalerone), also called naphyrone, indicating the presence of a naphthalene moiety instead of a substituted benzene ring which is common for cathinone drugs [Brandt *et al*, 2010]. Figure 3 shows some common psychoactive drugs classified according to their appearance into first and second generation.

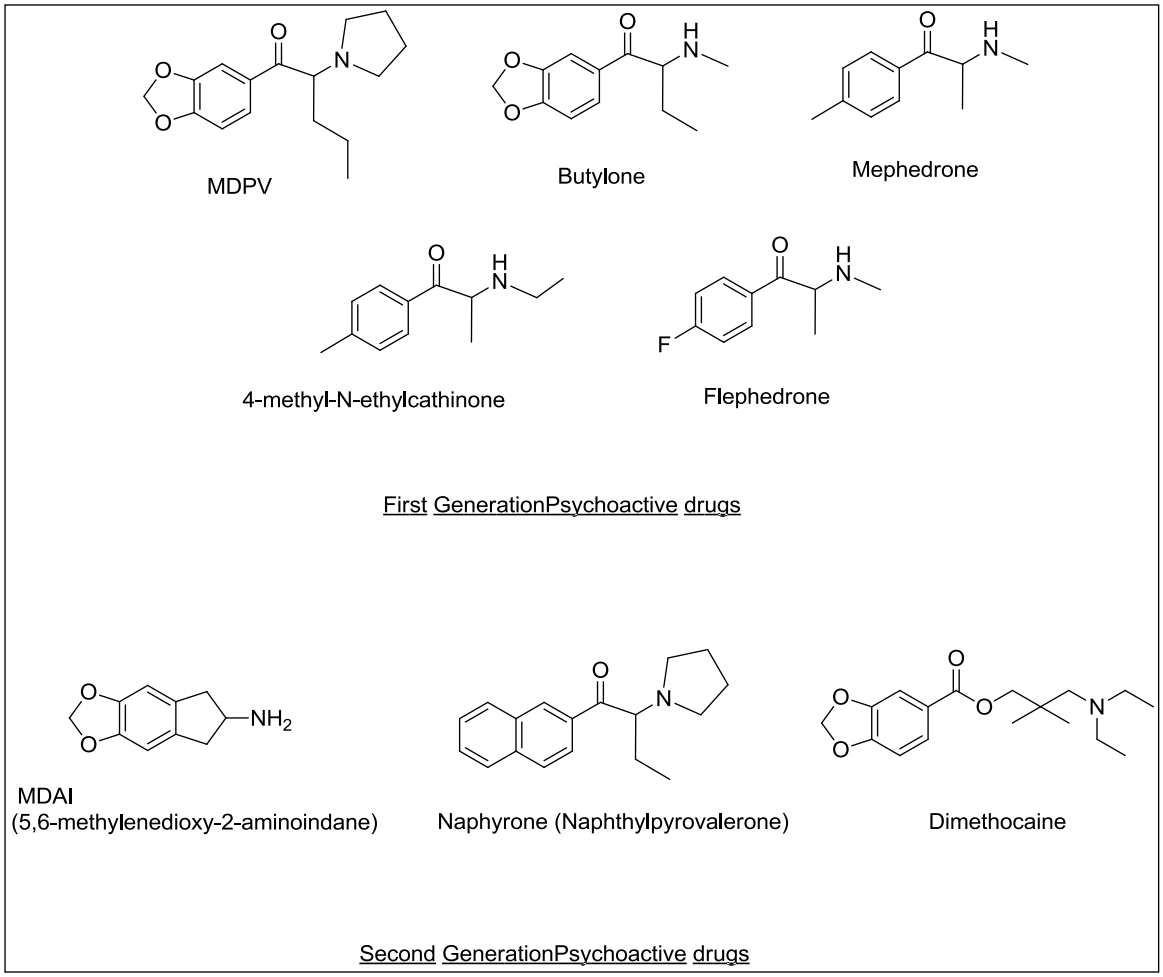


Figure 3: Some common examples of first and second generation psychoactive drugs.

Over the past several years there has been a significant increase in the production, distribution and use of “bath salt” drugs or “synthetic cathinones” across the US and abroad [NDIC Report, 2011]. These drugs are chemical derivatives of cathinone, a Schedule I controlled substance, this substance is extracted from the shrub plant khat (*Catha edulis*) that is native to East Africa and southern Arabia. The khat plant itself is not scheduled under the Controlled Substances Act; however, because one of its chemical

constituents, cathinone, is a Schedule I drug, the Federal Government considers its use illegal [DEA, Fact Sheet, 2011].

The principal constituents responsible for the stimulant effects of khat are widely thought to be the alkaloids (the main psychoactive ingredients) cathine and cathinone, chemicals that are structurally similar to, but less potent than, amphetamine; yet result in similar psychomotor stimulant and subjective effects. Chewing khat leaves can induce a state of euphoria and elation as well as feelings of increased alertness and arousal. The user can also experience an increase in blood pressure and heart rate. The effects begin to subside after about 90 minutes to 3 hours, but can last 24 hours. At the end of a khat session, the user may experience a depressive mood, irritability, loss of appetite, and difficulty sleeping [DEA, Fact Sheet, 2011].

There are a number of adverse physical effects that have been associated with heavy or long-term use of khat, including tooth decay and periodontal disease, gastrointestinal disorders such as constipation, ulcers, inflammation of the stomach, and increased risk of upper gastrointestinal tumors, cardiovascular disorders such as an irregular heartbeat, decreased blood flow, and myocardial infarction. There is also consistent epidemiologic evidence for a weak association between chronic khat use and mental disorders. Although there is no evidence that khat use causes mental illness, chewing khat leaves may worsen symptoms in patients who have pre-existing psychiatric conditions. It is unclear whether khat causes tolerance, physical dependency, addiction, or withdrawal, but nightmares and slight trembling have been reported several days after ceasing to chew [DEA, Fact Sheet,

2011]. It is estimated that as many as 10 million people worldwide chew khat [World Health Organization, 2011]. It is commonly found in the southwestern part of the Arabian Peninsula and in East Africa, where it has been used for centuries as part of an established cultural tradition. In one large study in Yemen, 82 percent of men and 43 percent of women reported at least one lifetime episode of khat use [World Health Organization, 2011].

The synthetic cathinones that have appeared in clandestine samples to date include a number of aromatic aminoketones such as MDPV (3,4-methylenedioxypyrovalerone), mephedrone, N-methylcathinone (also known as methcathinone or cat), 4-fluoromethcathinone (also known as flephedrone or 4-FMC), and 3,4-methylenedioxy-N-methylcathinone (also known as methylone, MDMC, bk-MDMA, or M1) [FDA, Jan 2011]. These drugs are also structurally related to several Schedule IV and V prescription drugs including bupropion (Zyban®, Wellbutrin®), diethylpropion (Tenuate®), and pyrovalerone (Centroton®) [FDA, Jan 2011]. Figure 4 shows structures of some common synthetic cathinones and their similarities to schedule IV and V prescription.

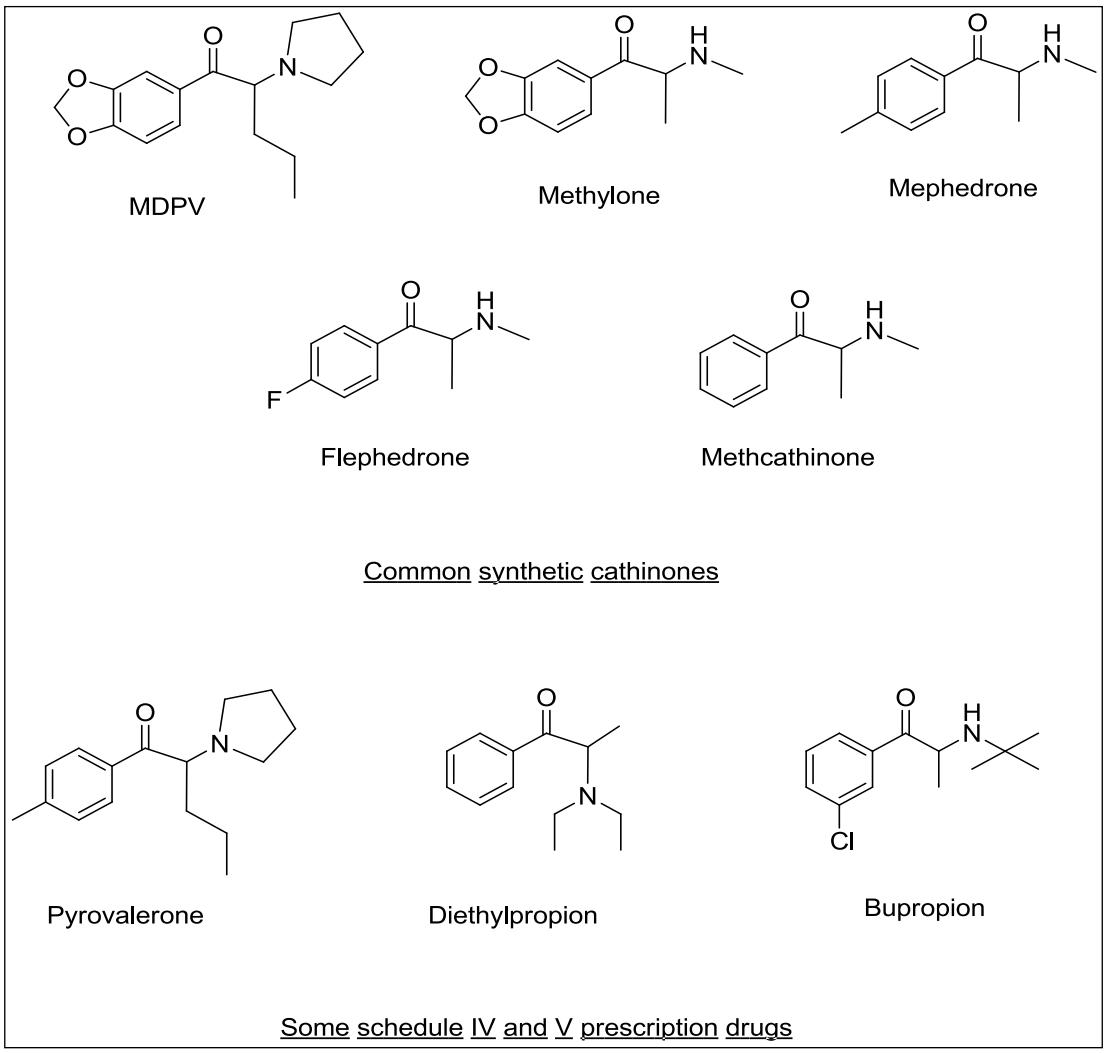


Figure 4: Some common examples of synthetic cathinones and their structure similarities to schedule IV and V prescription drugs.

Like the phenethylamines, cathinone derivatives can exist in two stereoisomeric forms, which may differ in their potencies. The cathinone that occurs naturally in khat is the S-enantiomers. However, it is likely that most ring-substituted derivatives are racemic mixtures. It is also believed that racemization of all cathinone derivatives can occur through keto-enol tautomerism [EMCDDA, 2012].

Synthetic cathinones are typically marketed as “bath salts” as well as plant food/fertilizer, insect repellant, pond cleaner, and vacuum fresheners and “plant food” and are sold under various names (Ivory Wave, Blizzard, etc.) in most areas of the United States [DEA, Synthetic Cathinones, 2011]. The products are generally sold in retail establishments such as adult stores, independently owned convenience stores, gas stations, head shops, and skateboard shops. The synthetic cathinone products, as well as their synthetic precursors, are also sold on many Internet sites, including popular Internet auction sites. Additionally, forensic laboratory analysis of seized clandestine drug products have identified synthetic cathinones being sold as MDMA (“ecstasy”, 3,4-methylenedioxymethamphetamine) as well as in combination with MDMA or other illicit controlled substances [FDA, Jan 2011]. These clandestine products are commonly distributed in powder, crystal, and liquid forms, but they are also available and abused in tablet and capsule forms [DEA, Synthetic Cathinones, 2011]. Abusers typically ingest, inhale, inject, smoke, or snort (insufflate) synthetic cathinone products to experience effects similar to those of amphetamine abuse. Some abusers dissolve the drugs in water or other solvents and proceed to atomize and inhale them, while others apply the solutions to their mucus membranes by placing drops in their eyes or spraying the solutions in their noses [DEA, Synthetic Cathinones, 2011]. The amphetamine like effects of synthetic cathinones is a result of the release of norepinephrine, serotonin, and dopamine and inhibition of their reuptake [Cozzi *et al*, 1999; Kehr *et al*, 2011].

Synthetic cathinone abuse has caused users throughout the country to experience severe adverse effects, and the number of "bath salt" calls to U.S. poison control centers has trended upward over the past several years. On December 21, 2010, the American Association of Poison Control Centers (AAPCC) issued its first warning regarding the dangers of synthetic cathinone abuse, particularly for products marketed as "bath salts" [AAPCC, 2010]. The warning informed the public that as of that date, at least 156 "bath salt"-related calls had occurred in 2010, 85 from Louisiana. Effects reported to the centers included increased blood pressure, increased heart rate, agitation, hallucinations, extreme paranoia, and delusions; no deaths were reported [Louisiana DHH, 2010]. The Louisiana Department of Health and Hospitals also issued a warning regarding synthetic cathinones "bath salts", mentioning several symptoms experienced by hospitalized patients in addition to those mentioned above, including chest pain, headache, and suicidal thoughts. Additionally, some clinical features of synthetic cathinones like psychomotor agitation, delusions, hallucinations, psychosis, hypertension, palpitation, chest pain, seizures, and headaches have been reported [Wood *et al*, 2010]. From January 1 through May 12, 2011, the AAPCC received 2,237 "bath salt"-related calls from poison control centers in 47 states and the District of Columbia, a significant increase from the 303 calls recorded for all of 2010 [AAPCC, 2011].

Available law enforcement reporting data suggest increasing levels of synthetic cathinone availability and abuse, but such information is limited and precise levels are unknown. U.S. Customs and Border Protection (CBP) currently tracks seizures of synthetic cathinones at U.S. ports of entry (POEs), but many synthetic cathinone products

are disguised or mislabeled to impede detection [NDIC Report, 2011]. Also, because common field test kits, drug-detecting canines, and routine urine drug screens do not detect synthetic cathinones, law enforcement officials are challenged in interdicting such drugs and prosecuting their manufacturers and distributors.

At present distributors of synthetic cathinone products evade U.S. Drug Enforcement Administration (DEA) regulation and enforcement because synthetic cathinones are not scheduled under the Federal Controlled Substances Act (CSA). However, possession and distribution of the synthetic cathinones may be prosecuted, albeit with greater difficulty, under the Federal Controlled Substance Analogue Enforcement Act of 1986 (as amended) of the CSA [NDIC Report, 2011]. The availability and suitability of a prosecution under the analogue statute depends on the particular compound being trafficked and the facts of the case. Further, distributors deceptively market synthetic cathinone products as "not for human consumption" to evade U.S. Food and Drug Administration (FDA) scrutiny. Synthetic cathinone products that are introduced into interstate commerce and promoted as alternatives to illicit street drugs may be prosecutable under the Federal Food, Drug, and Cosmetic Act as unapproved new drugs and misbranded drugs [FDA, March 2011]. Additionally, recently Congress has introduced legislation to nationally ban the sale of certain synthetic cathinones, and, as of April 2011, all 50 states and the District of Columbia have introduced or announced plans to introduce legislation banning or restricting the distribution and possession of certain synthetic cathinones and cathinone derivatives [NDIC Report 2011]. However, as synthetic cathinones become more regulated, abusers will likely use the Internet with

greater frequency to purchase cathinone products, the raw chemicals used in their production, and products that contain cathinones not specifically prohibited by enacted legislation.

MDPV is a so-called “designer drug” with stimulant effects similar to cocaine and amphetamine [Kriikku *et al*, 2011]. It is an analogue of pyrovalerone, a psychostimulant that is considered the first commercially available drug from the alpha-pyrrolidinophenone drug class which was synthesized and introduced to the market in the 1960s [Meyer *et al*, 2010] , this stimulant was used to treat lethargy and chronic fatigue in the 1970s, but was later withdrawn from the market because of problems with abuse and dependency [Gardos *et al*, 1971]. MDPV structurally resembles cathinone, found in Khat, and has thus been referred to as a synthetic cathinone derivative [Gibbons *et al*, 2010]. MDPV has no medical use and is said to have exceptionally high dependency potential and high risk of psychosis. At higher doses some users report extremely unpleasant “come-down” effects [Maurer *et al*, 2004]. Police reports indicate that people under the influence of MDPV very often act violently and unpredictably. MDPV is most often sold as a powder, but capsules have also been reported. A wide range of dosage forms and routes of administration are used: oral (capsules or powder dissolved in water), IV, rectal [Maurer *et al*, 2004]. Recently, Ojanperä *et al*. developed a GC-MS method for the quantitative determination of MDPV in the urine of opioid-dependent patients [Ojanperä *et al*, 2011]. Westphal *et al*. confirmed the structure of MDPV in a seized sample in Germany in 2007 with product ion mass spectrometry as well as with 1H and 13C NMR [Westphal *et al*, 2009]. The phase I and II metabolites of MDPV and the

human cytochrome- P450 (CYP) isoenzymes responsible for its main metabolic step(s) were identified using GC – MS and LC – high resolution-MS (LC – HR-MS) [Meyer *et al*, 2010]. Also, a method for the toxicological screening and in vitro metabolism of MDPV was developed [Sabina *et al*, 2010]. The resulting metabolites were analyzed using gas chromatography/mass spectrometry (GC/MS) as trimethylsilyl (TMS) derivatives. The structures of the metabolites were further confirmed by accurate mass measurement using a liquid chromatography/quadruple time-of-flight (LC/QTOF) mass spectrometer. The analysis and characterization of 3,4-methylenedioxypyrovalerone (MDPV) were done using GC-MS, Fourier Transform NMR (FTNMR) spectroscopy, solid phase Fourier Transform IR (FTIR) spectroscopy, and UV spectrophotometry [Yohannan *et al*, 2010]. Analytical and metabolic data of a series of pyrovalerone analogues were already published. Toxicological detection of α-pyrrolidinopropiophenone (PPP) and 4-methyl-α-pyrrolidinopropiophenone (MPPP) was done in urine using GC-MS [Springer *et al*, 2003]. Also, the metabolism and toxicological detection of 4-methoxy-α-pyrrolidinopropiophenone (MOPPP) were studied [Springer *et al*, 2002].

Westphal and coworkers [Westphal *et al*, 2011] recently reported the infrared spectroscopic, the nuclear magnetic resonance spectroscopic and mass spectrometric data of the designer drug 3,4 methylenedioxypyrrolidinobutyrophenone (MDPBP), a homolog of 3,4-methylenedioxypyrovalerone (MDPV). MDPBP was first seized in Germany in the year 2009. The structure elucidation of the aliphatic portion of MDPBP was carried out by product ion spectrometry of the immonium ion with $m/z = 112$ formed after electron

ionization and by one- and two-dimensional 1H- and 13C NMR spectroscopy.

Reports of the designer exploration of the substituted cathinones are continuing in the recent drug chemistry literature. Russell and Bogan [Russell *et al*, 2011] have reported a series of N-substituted beta-keto-analogues of MDMA while two new alkyl side chain homologs of methcathinone were reported by Maheux and Copeland [Maheux *et al*, 2011]. Brandt and coworkers [Brandt *et al*, 2012] have recently described the three regioisomers of trifluoromethyl ring substituted methcathinone. Thus, these recent reports confirm that the exploration of the aromatic substituents, alkyl side chain and nitrogen substitution is continuing in the substituted cathinones.

1.3. Chemistry

The MDPV is a pyrrolidine derivative of the synthetic cathinone pyrovalerone differing for the presence of a 3,4-methylenedioxy group linked to the aromatic ring in substitution of a 4-methyle group [Yohannan *et al*, 2010]. This compound can be named chemically as 1-(1,3-benzodioxol-5-yl)-2-pyrrolidin-1-ylpentan-1-one, the HCl salt has a white color while the free base has a yellow, green or gray color with molecular formula of $C_{16}H_{21}NO_3$ and exact molecular weight of 275.34248 g/mol [Pubchem, 2011]. MDPV is characterized by the presence of a tertiary amino group which makes the compound highly soluble in organic solvent [Caymanchem, 2011]. MDPV as a free base has a high melting point (estimated at 200°C) and is a solid at room temperature [DEA, MDPV, 2011].

1.4. Pharmacology

The synthetic cathinones produce similar effects as amphetamine, ecstasy, and cocaine. They are characterized as central nervous system (CNS) stimulants and dopamine reuptake inhibitors, therefore considered stimulants drugs with psychedelic psychoactive properties [Norchem, Information Sheet, 2011]. As with phenethylamines, in the absence of ring-substitution, cathinones have a lower potency than the corresponding phenethylamine analogues. The lower potency is caused by the β-keto group creating a more polar molecule less able to cross the blood–brain barrier [EMCDDA, 2012]. According to the Norchem, a Drug Testing Lab and Case Management System, the synthetic cathinones produce physiological and psychological effects:

Physiological effects [Norchem, Information Sheet, 2011]:

- Over stimulation of the cardiovascular system with risk of heart and circulatory problems rapid mood elevation
- Overstimulation of the nervous system with risk of agitation and enhanced appreciation of music
- Nose bleeds and burns skin tingling
- Dangerously raised body temperature
- Rashes
- Dilation of pupils
- Altered blood pressure
- Breathing difficulties
- Bruxism
- Loss of appetite

- Discoloration of the extremities (cold and/or blue fingers)
- Rapid heartbeat
- Profuse sweating
- Loss of bowel control
- Muscle damage
- Renal failure
- Myocardial infarction
- Headaches, nausea and seizures

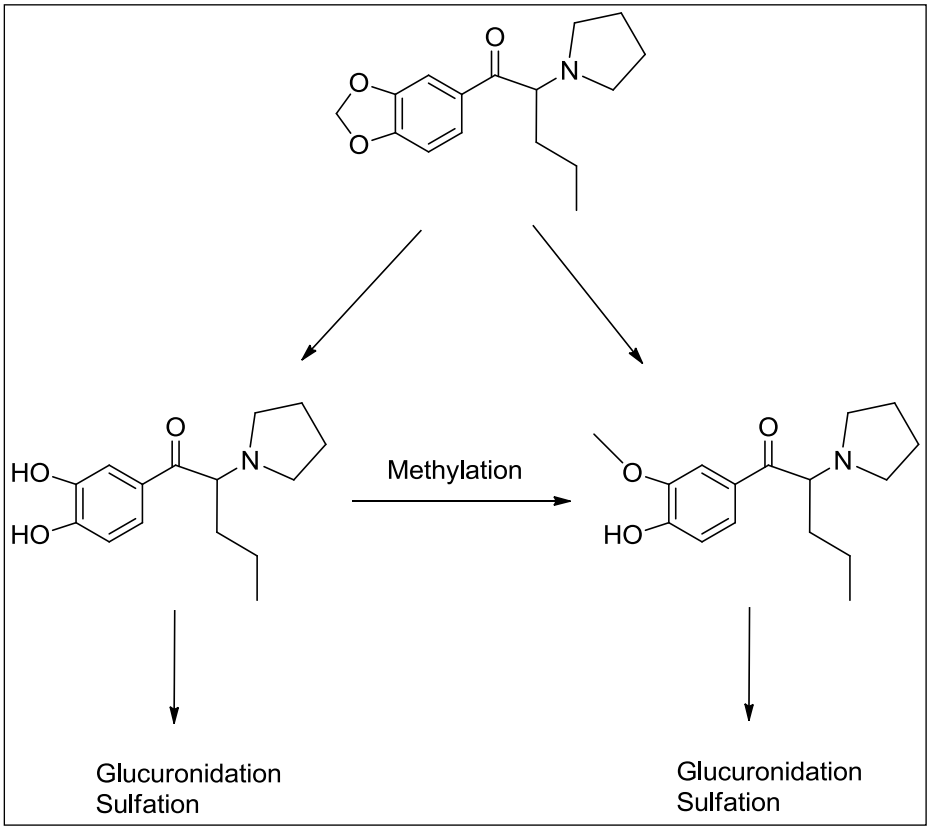
Psychological effects [Norchem, Information Sheet, 2011]:

- Euphoria
- Talkativeness
- Alertness
- Elevated mood
- Mild sexual stimulation
- Increased motivation
- Sever agitation/aggression
- Depression
- Sever paranoia
- Hallucinations (auditory and visual)
- Delusion
- Anxiety
- Tinnitus
- Prolonged panic attack
- Potential for developing personality disorders

New Jersey Office of the Attorney General also reported that the use of designer drugs labeled as “bath salts” has been associated with extreme anxiety, extreme paranoia, delusional thinking, and visual and auditory hallucinations, leading to violent outbursts, self-mutilation, and suicidal thoughts; increased blood pressure and heart rate; chest pains so severe the persons fears they are dying; and jerky muscle movements. Some authorities believe the patients they have seen will suffer long-term if not permanent effects due to their use of designer drugs labeled as “bath salts.” [New Jersey Office of the Attorney General, 2011].

There are very little data about the human pharmacokinetics and pharmacodynamics of cathinone derivatives. As mentioned earlier, synthetic cathinones exert their stimulant effects via increasing synaptic concentration of catecholamines such as dopamine, serotonin and norepinephrine (amphetamine like effecs). These molecules are able to inhibit monoamine uptake transporters producing a decreased clearance of the neurotransmitters from the synapse. Furthermore, they may cause release of biogenic amines from intracellular stores [Cozzi *et al*, 1999]. In rat study has shown that the activities of two neurotransmitter biosynthetic enzymes, tyrosine hydroxylase and tryptophan hydroxylase, are decreased following methcathinone administration, leading to reductions in the concentrations of dopamine and serotonin and their respectively metabolites in frontal cortex, hippocampus and neostriatum [Gygi *et al*, 1996].

MDPV is a monoamine uptake inhibitor with greater potency than other cathinone compounds [Meltzer *et al*, 2006]. The higher lipophilicity of MDPV is mainly because of the pyrrolidine ring and tertiary amine group which allows MDPV to easily penetrate blood brain barrier [EMCDDA, 2012]. The studies on the metabolism of synthetic cathinones have shown that they are N-demethylated, the keto group is reduced to hydroxyl and ring alkyl groups are oxidized [Meyer and Maurer, 2010]. The primary metabolism of methylone, ethylone and butylone starts with demethylation of the methylenedioxy ring, followed by O-methylation into 4-hydroxy-3-methoxy or 3-hydroxy-4-methoxymethcathinone mediated by catechol-O-methyltransferase. These metabolites are partially conjugated with glucuronides and sulfates and excreted in the urine together with unmetabolized molecules. N-dealkylation appears to be a minor pathway for the metabolism of these molecules [Zaitus *et al*, 2009]. Mephedrone is N-demethylated to a primary amine, subsequently, ketone moieties are reduced to alcohols, and finally, totyl group is oxidized to the corresponding alcohol. Some of the alcohols are conjugated with glucuronides and sulfates and excreted in the urine [Meyer *et al*, 2010]. The metabolism of MDPV was evaluated in vitro using human liver microsomes and S9 cellular fractions for CY450 phase I and uridine 5-diphosphoglucuronosyltransferase and sulfotransferase for the phase II metabolism. This study has demonstrated that the main metabolites of MDPV are catechol and methyl-catechol pyrovalerone which are in turn sulfated and glucuronated, this was done by GC-MS and LC-GTOF. The structures of these metabolites are shown in Scheme 1 [Strano-rossi *et al*, 2010].



Scheme 1: Structures of MDPV metabolites.

1.5. Toxicology

Synthetic cathinones have received large popularity, particularly among young people, for their cocaine and amphetamines like effects. In particular, the stimulant effects of these drugs have been compared to methylphenidate, at low doses, and cocaine or amphetamines, at high doses [Scribd, 2012]. The information currently available about the short and long-term human toxicological effects of these designer drugs of abuse is very limited. Desired effects reported by users include: increased sociability, energy, libido sexual performance and capacity of work, limited euphoria, empathy [Winstock *et*

al, 2011]. Users also reported untoward effects such as: prolonged panic attack, tremor, agitation, insomnia, nausea, headache, tinnitus, vertigo, muscle twitching, dizziness, increased heart rate, altered vision, confusion, short-term memory difficulty, anhedonia, depression, suicidal thoughts, psychosis, tolerance and dependence [Winstock *et al*, 2010]. In literature have been reported several cases of severe acute toxicity and deaths related to the consumption of synthetic cathinones [Wood *et al*, 2010; Gustavsson and Escher, 2009]. Acute toxicity mainly includes neurological, cardiovascular and psychopathological symptoms such as: psychomotor agitation, motor automatisms, parkinsonism, tremors, tachycardia, chest pain, S–T segment changes, hypertension, hyperthermia, mydriasis, dizziness, delusions, paranoid psychosis, depression, panic attacks, long term changes in cognition and emotional stability, rhabdomyolysis, abdominal pain, vomiting, kidney damage, hyponatremia, headache, cerebral edema and seizures [Borek and Holstege, 2012; Durham, 2011; CDC, 2011; Panders and Gestring, 2011; Regan *et al*, 2011]. Patients with acute intoxication related to mephedrone assumption have also shown serious vasoconstriction in extremities, skin rashes, decoloration of the skin and bruxism [Durham, 2011; CDC, 2011; Panders and Gestring, 2011; Regan *et al*, 2011]. The therapeutic treatment generally includes low or moderate doses of benzodiazepines to control agitation or seizures and antipsychotics or propofol to control severe agitation and psychotic symptoms. Hyperthermia should be treated with aggressive cooling and hyponatremia should be treated with hypertonic saline and water restriction [Spiller *et al*, 2011]. Recently, a case of MDPV induced serotonin syndrome has been reported. The patient was treated with benzodiazepines and cyproheptadine for 8 days with slow resolution of the symptomatology [Mugele *et al*, 2012].

Although there is limited information regarding MDPV toxicology, it has been reported that MDPV has a stimulant effect which is similar to methphenidate at low doses and cocaine and methamphetamine at high doses [Scribd, 2011] and more potent than cocaine and amphetamine [Drugs-Forum, 2011; Erowid, 2011] because of the lipophilic properties of the pyrrolidine ring and the tertiary amino group in MDPV [EMCDDA, 2012]. There was only one death case reported due to isolated confirmed MDPV intoxication, the case was a 40-year old male, he developed hyperthermia, coagulopathy, acidosis, brain injury and then died [Murray *et al*, 2012].

1.6. Fatalities

Fatal intoxication have been associated with various molecules such as mephedrone, MDPV, methedrone, butylone and methcathinone, but in many cases laboratory analysis revealed the presence of multiple drugs of abuse [Prosser and Nelson, 2012]. In confirmed mephedrone related deaths, the postmortem blood concentration detected was between 0.13 and 22 mg/L, while in confirmed butylone related deaths the postmortem blood concentration detected was between 0.435 and 1.2 mg/L [Maskell *et al*, 2001; Torrance and Cooper, 2010; Carter *et al*, 2000]. In a recent case of confirmed MDPV related death, the serum and urine concentration was 82 ng/mL and 670 ng/mL, respectively [Murray *et al*, 2012].

1.7. Tolerance, dependence and withdrawal syndrome

Data currently available have shown that the frequent consumption of high doses of synthetic cathinones induces tolerance, dependence, craving and withdrawal syndrome after sudden suspension [CDC, 2011]. Although the typical doses range of MDPV appear to be between 5 and 30 mg in a single ingestion, some users reported tolerance with consumption of doses higher 200 mg in a single session [Coppola and Mondola, 2012]. Several users have reported a withdrawal syndrome after abrupt cessation of long-term use of methcathinone, mephedrone and MDPV. This syndrome includes: depression, anergia, anhedonia, anxiety, sleep disorders fatigue and craving. Craving, anhedonia and anergia can last for several weeks [CDC, 2011; Winstock *et al*, 2011].

1.8. Prevalence

Although the recreational use of synthetic cathinones is not new (e.g. methcathinone in the ex-Soviet Union in 1970s and 1980s and in the United States in 1990s), information about the currently prevalence of synthetic cathinones misuse in the population are very limited [NAMSDL, 2012]. The emergence of six synthetic cathinones, all closely related to pyrovalerone, was reported in Germany between 1997 and 2004 [NAMSDL, 2012], but the use of Google Insights, an internet application used to track search terms, shows almost no searches for synthetic cathinones before 2008 [Google/insights, 2012]. A significant increase of searches there was between 2009 and 2010 when the United Kingdom Poison Information Service received a number of inquiries regarding synthetic cathinones comparable to those for cocaine and MDMA

[James *et al*, 2011; Google/insights, 2012; NAMSDL, 2012]. A Finnish study which analyzed blood from suspected by police to drive under the effect of drugs found that 286 of 3000 specimens submitted for analysis contained 3,4-methylenedioxypyrovalerone (MDPV) (8.6%) [Kriikku *et al*, 2011]. An Irish study which analyzed urine samples collected from patients receiving methadone maintenance found that 14% were positive to mephedrone and 3% were positive to methylone [McNamara *et al*, 2010]. A self-report study on students of UK high school and college revealed that 20% had used mephedrone on at least one occasion, 4% reported daily use and all daily users were less than 21 years of age [Dargan *et al*, 2010].

An online survey of club-goers in the UK found that 41% had used methedrone and 10% had used methylone. A third had used methedrone in the last month and 14% reported weekly assumption [Winstock *et al*, 2011]. In an online survey conducted in late 2010 in collaboration with the UKs dance music magazine Maxmag, mephedrone was the fourth most-commonly used drug in the past year after cannabis, ecstasy and cocaine and it had been tried by 61% of respondents [EMCDDA, 2012]. The American Association of Poison Control Centers reported 303 calls related to synthetic cathinones in 2010 and 6072 in 2011 [AAPCC, 2011]. Although limited, prevalence data currently available show a progressive increase in the spread of these substances justifying the concerns in the fields of drug policy, forensic toxicology and public health.

1.9. Illicit distribution and statistics

The National Forensic Laboratory Information System (NFLIS) indicates that, local, federal, and state law enforcement officials reported around 34 states with illegal use of MDPV. The statistics show an increased number of MDPV exhibits from 2 in 2009, to 338 in 2010, and within 9 months in 2011 from January through September about 911 MDPV exhibits have been reported in the NFLIS database [DEA, MDPV, 2011].

1.10. Legal status

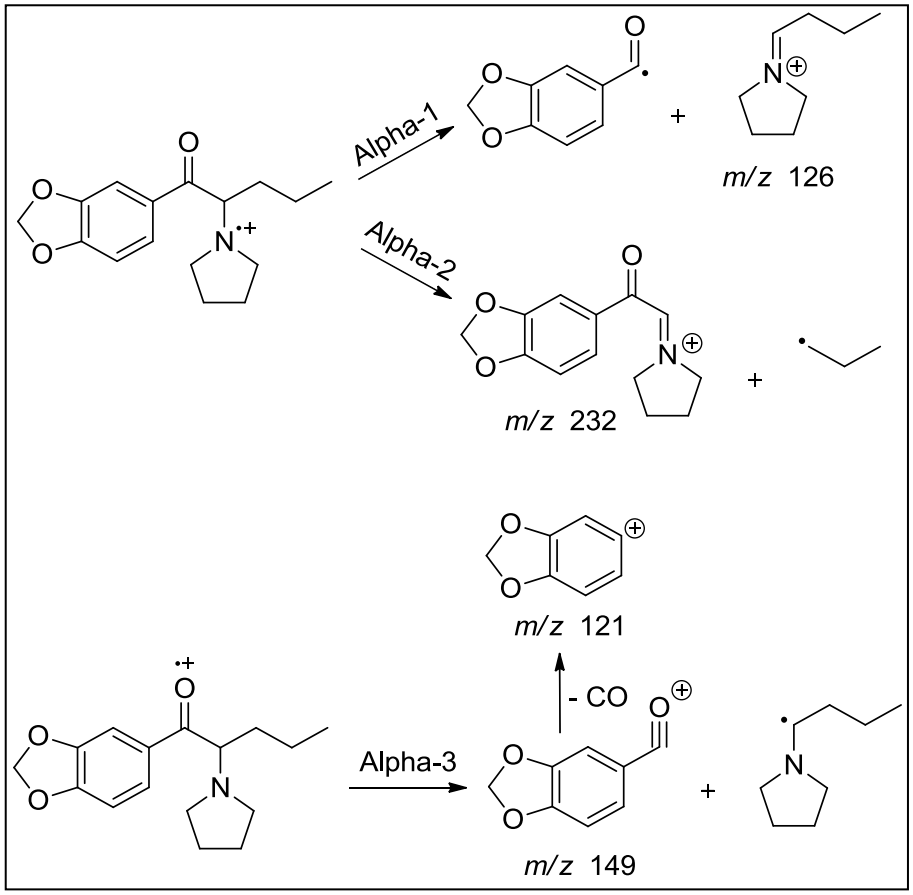
It has been reported that MDPV is a controlled substance in Sweden (2010), Denmark (2009), UK (2010), Germany (2010), Australia (2010), and Finland (2010). Some states in the USA such as Alabama, Florida, Michigan, Louisiana, North Dakota, Utah, Mississippi, New Jersey, Idaho, and North Carolina (2011) [Drugs-Forum, 2011]. Additionally, MDPV, salts, its isomers, and salts of isomers have been temporarily controlled as schedule-I substances in addition to mephedrone and methylone in the USA [DEA, MDPV, 2011].

1.11. Analytical methods used to separate and identify aminoketones

1.11.1. Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is the main tool used for the detection and identification of unknown drugs in forensic and other drug screening laboratories. [Westphal *et al*, 2009] reported the electron impact (EI) mass spectrometric fragmentation pathway for 3,4-methylenedioxypyrovalerone (MDPV). The ions of significant relative abundance are likely from fragmentation of the pyrrolidine ring and are shown in Scheme 2. The mass spectrum of MDPV shows the fragment ions at *m/z* 126, 149, and 121 as well as other ions of low relative abundance. The structures of these fragment ions as proposed by Westphal et al are shown in Scheme 2 [Westphal *et al*, 2009].

The radical electron of the nitrogen atom induces a fast alpha-cleavage reaction (alpha-1) of the benzyl bond and produces a base peak immonium cation at *m/z* 126. The alternative alpha-cleavage reaction (alpha-2) produces an immonium cation at *m/z* 232 with low intensity by the loss of a propyl radical. M-15 and M-29 alpha-cleavage fragments are found with low intensities at *m/z* 260 and *m/z* 246, respectively. Ionization at the carbonyl oxygen atom and alpha-cleavage reaction (alpha-3) yields a methylenedioxybenzoyl cation at *m/z* 149, and a subsequent (CO) loss is responsible for the ion at *m/z* 121 [Westphal *et al*, 2009].



Scheme 2: EI-MS fragmentation pathway for MDPV as proposed by Westphal *et al* [Westphal *et al*, 2009].

1.11.2. Gas chromatography coupled with infrared detection (GC-IRD)

The absorption of IR radiation is also considered one of the non-destructive techniques that can be used for the identification of organic molecules. The region from 1250 to 600 cm^{-1} is generally classified as the "fingerprint region" and is usually a result of bending and rotational energy changes of the molecule as a whole. However since the clandestine samples are usually impure, overlapping absorptions of different molecules present in the sample becomes a possibility. Hence, this region is not useful

for identifying functional groups, but can be useful for determining whether or not samples are chemically identical. Infrared spectrometry is often used as a confirmatory method for drug identification in forensic drug analysis. Infrared detection should provide compound specificity without the need for chemical modification of the drug molecule.

1.11.3. Nuclear magnetic resonance (NMR)

NMR is a nondestructive flexible technique that can be used for the identification of pure compounds and even mixtures of compounds in one sample. Its advantages, compared to GC-MS techniques, include stereochemical differentiation and the capability to analyze nonvolatile compounds. However, the lack of use in forensic laboratories can be attributed to the high cost of instrumentation and the poor sensitivity of NMR. Solid state NMR also can be used for analytical purposes in much the same way as solution NMR. The observed chemical shifts however differ in the solution and solid states because of conformational freezing and packing effects. Westphal et al. confirmed the structure of MDPV in a seized sample in Germany in 2007 with 1H and 13C NMR [Westphal *et al*, 2009].

1.12. Project rational

A number of aminoketones or benzylketo compounds (bk-amines) have appeared on the illicit drug market in recent years including methcathinone, mephedrone, methylone and MDPV (3,4-methylenedioxypyrovalerone). These substances represent a variety of aromatic ring substituent, hydrocarbon side chain and amino group modifications of the basic cathinone/methcathinone molecular skeleton.

There has been a worldwide rise in the popularity and abuse of these synthetic cathinones. In 2009 and 2010, a significant rise in the abuse of a new group of synthetic cathinones was reported in Western Europe, referred to as “bath salts” or ”legal high,” which occurred later in the USA [Spiller *et al*, 2011]. Additionally, over the past several years there has been a significant increase in the production, distribution and use of “bath salt” drugs or “synthetic cathinones” across the US and abroad [NDIC Report, 2011]. The synthetic cathinones that have appeared in clandestine samples to date include a number of aromatic aminoketones such as MDPV (3,4-methylenedioxypyrovalerone), mephedrone, N-methylcathinone (also known as methcathinone or cat), 4-fluoromethcathinone (also known as flephedrone or 4-FMC), and 3,4-methylenedioxy-N-methylcathinone (also known as methylone, MDMC, bk-MDMA, or M1) [FDA, Jan 2011].

Synthetic cathinone abuse has caused users throughout the country to experience severe adverse effects, and the number of “bath salt” calls to U.S. poison control centers has trended upward over the past several years. On December 21, 2010, the American

Association of Poison Control Centers (AAPCC) issued its first warning regarding the dangers of synthetic cathinone abuse, particularly for products marketed as “bath salts” [AAPCC, 2010].

Exploration and designer development in the aminoketone drugs using models based on substituted amphetamines and related phenethylamines is likely to continue for many years. Current clandestine designer drug development concepts used for amphetamine-type molecules can be applied directly for aminoketone development. Clandestine production of these drugs can be based on common precursor chemicals. Therefore, legal control of the key precursor substance is not likely and would not prevent the further clandestine/designer exploration of this group of compounds. It could be argued that isomer differentiation is not necessary in forensic drug science because of the Controlled Substance Analogue Act. However, the forensic chemist must identify the compound in order to know if it is an analogue of a controlled substance. These circumstances all point to the strong need for a thorough and systematic investigation of the forensic chemistry of these substituted aminoketones.

1.13. Statement of research objectives

This project addresses issues of resolution and discriminatory capabilities in controlled substance analysis providing additional reliability and selectivity for forensic evidence and analytical data on new analytes of the so-called bath salt-type drugs of abuse. The purpose of this project is to develop regioisomer and homolog specific methods for the identification of ring substituted aminoketone compounds (cathinone derivatives). This will be accomplished by

1) The chemical synthesis of all regioisomeric and homologous forms of selected aromatic ring substituted aminoketones,

2) Generation of a complete analytical profile for each compound.

3) Chromatographic studies to separate/resolve all regioisomeric and homologous aminoketones having overlapping analytical profiles.

4) Design and validation of confirmation level methods to identify each compound to the exclusion of other regioisomeric and homologous forms.

The broad objective of this research is to improve the specificity, selectivity and reliability of the analytical methods used to identify ring substituted aminoketones and related compounds. This improvement will come from methods which allow the forensic analyst to identify specific regioisomeric forms of substituted aminoketones among many isomers of mass spectral equivalence. Mass spectrometry is the most common method of confirmation in forensic analysis. This project will provide methodology and analytical data to discriminate between those regioisomeric and isobaric molecules having the same molecular weight and major fragments of equivalent mass. Furthermore, this work will

anticipate the future appearance of some designer aminoketones and develop analytical reference data and analytical reference standards for these compounds.

The initial phase of this work is the organic synthesis of aminoketones of varying hydrocarbon side chains and amino groups. In this phase of the work about 13 substituted aminoketones of potential forensic interest will be evaluated. The analytical phases will consist of chemical characterization, using tools common to forensic science labs such as MS and IR and these studies will be carried out on each of the compounds. The chromatographic retention properties for each series of regioisomers and homologs will be evaluated by gas chromatographic techniques on a variety of stationary phases. These studies will establish a structure-retention relationship for the regioisomeric and homologous aminoketones.

The results of this project will significantly increase the forensic drug chemistry knowledge base for aminoketone-type designer drugs. When compounds exist which produce the same mass spectrum (same MW and fragments of equivalent mass) as the drug of interest, the identification by GC-MS must be based entirely upon the ability of the chromatographic system to resolve these substances. This project involves the synthesis and generation of complete analytical profiles as well as methods of differentiation for regioisomeric and homologous substances related to the substituted aminoketones. This project will provide proactive data for the forensic drug analyst and describe a unified approach for specific drug identification based on structure correlated analytical results.

Based on Figure 2 (section 1.1, page 2), the (A) parameter will be the same for all compounds, which is the 3,4-methylenedioxy moiety. The other two parameters (B and C) will be varied and the entire group will be classified as regioisomeric and homologous aminoketones based on the MDPV structure. The general structures for these aminoketones in this study are shown in Figure 5.

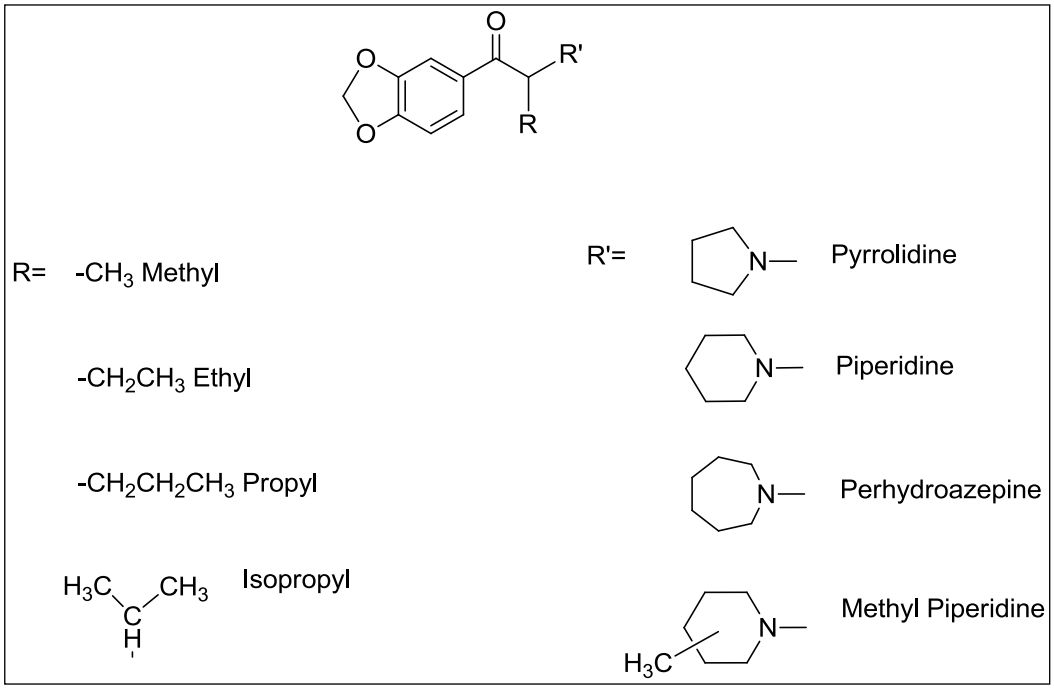


Figure 5: General structure for the aminoketones in this study.

2. Results and discussion

2.1. Introduction

Regioisomeric and homologous substances are considered a significant challenge for the analytical techniques used to identify specific molecules. This is considered extremely important when some of these molecules are legally controlled drugs and others may be uncontrolled, non-drug species or imposter molecules. MDPV can have regioisomeric equivalents of equal molecular weight and fragmentation products of identical mass. The regioisomers are those substances containing the methylenedioxybenzoyl ring system which yields an immonuium ion fragment as the base peak ($C_8H_{16}N+$, *m/z* 126) in their mass spectra, The homologous series can be defined as a series of compounds with similar general formula, possessing similar chemical properties due to the presence of the same functional group, and showing a gradation in physical properties as a result of increase in molecular size and mass, each compound in a homologous series vary by an extra methylene moiety from the previous compound.

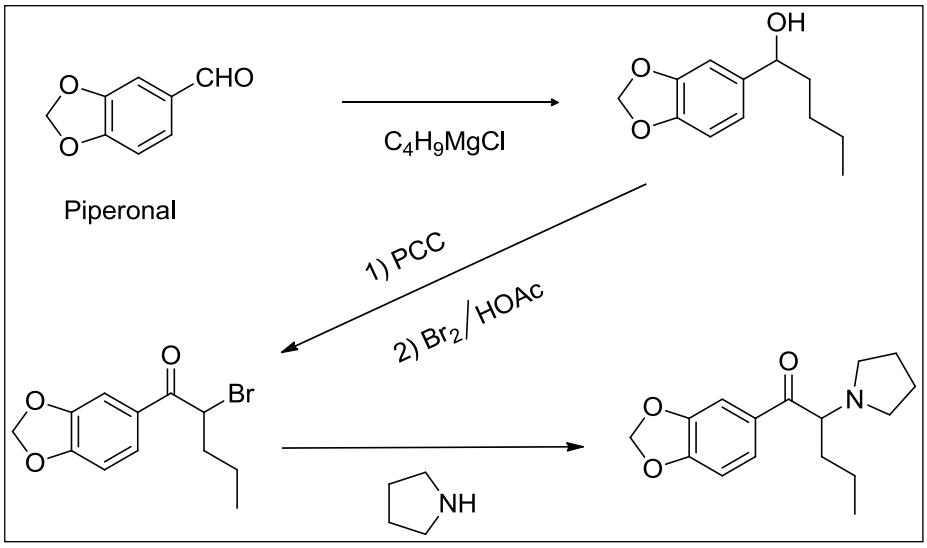
In this chapter the synthesis of these regioisomeric and homologous substances related to MDPV are described while their analytical properties including chromatographic separations are discussed in section 2.3.

2.2. Synthesis of the regioisomeric and homologous aminoketones

The key to the successful completion of this study is the chemical synthesis of each of the desired compounds. The synthetic methods needed to complete the preparation of the various isomeric and homologous aminoketones are well established in the chemical literature and in our laboratory. Many of the desired compounds can be prepared from the substituted benzaldehydes via a 4-step synthetic procedure (Scheme 3). The condensation of alkylmagnesium halides (Grignard reagents) with substituted benzaldehydes to yield ring substituted benzyl alcohols. Oxidation of the benzyl alcohol with PCC yields the ring substituted ketones (alkylphenones). Alpha-bromination of the ketones yields the alpha-bromoketones and eventually subsequent displacement with the appropriate cyclic secondary amine yields the desired aminoketone final products. The products are isolated by solvent extraction and recrystallization of the HCl salts.

For the compounds shown in (Figure 6), compounds 1, 2, 3, 10, 11, and 12, the starting compound for their synthesis was the commercially available 3,4-methylenedioxypropiophenone. In the last two synthetic steps, alpha bromination and subsequent displacement with the cyclic secondary amine were carried out to obtain the desired final products. Regarding compounds 4, 5, and 6, the commercially available 3,4-methylenedioxybutyrophenone was the substrate molecule and the remaining modification in the steps outlined in (Scheme 3) was employed. In the case of compounds 7, 8, and 9, the full sequence shown in (Scheme 3) using piperonal and butylmagnesium chloride as starting materials was employed. Finally, compound 13 was prepared following the steps outlined in (Scheme 3) except that isobutylmagnesium bromide was

used as a starting material. The general synthetic method for these aminoketones is shown in the (Scheme 3) with MDPV as an example.



Scheme 3: General synthesis for the bath salt aminoketones using the synthesis of MDPV as an example.

Looking at the structures in Figure 6, in each horizontal row (compare compounds 1, 2 and 3) the compounds are homologs in the nitrogen containing ring. These compounds were made from the commercially available amines, pyrrolidine, piperadine and perhydroazepine. In each vertical column (compare compounds 1, 4 and 7, for example), the compounds are homologs in the alkyl side chain and prepared from commercially available alkyl magnesium bromide or chloride reagents. Thus, these compounds are potential future designer drug substances based on the commercial availability of precursor materials and common methodology. Looking at the structures in a diagonal manner (specifically compare compounds 3, 5 and 7) these are regioisomeric substances

of equal molecular weight and identical elemental composition $C_{16}H_{21}NO_3$ as well as yielding regioisomeric base peaks of equal mass in their EI mass spectra *m/z* 126. Thus, based on mass spectra alone and without reference materials to validate a chromatographic separation any of these compounds could be identified as MDPV. Figure 6 shows the chemical structure of regioisomers and homologs prepared in this study arranged in terms of homologous and regioisomeric properties.

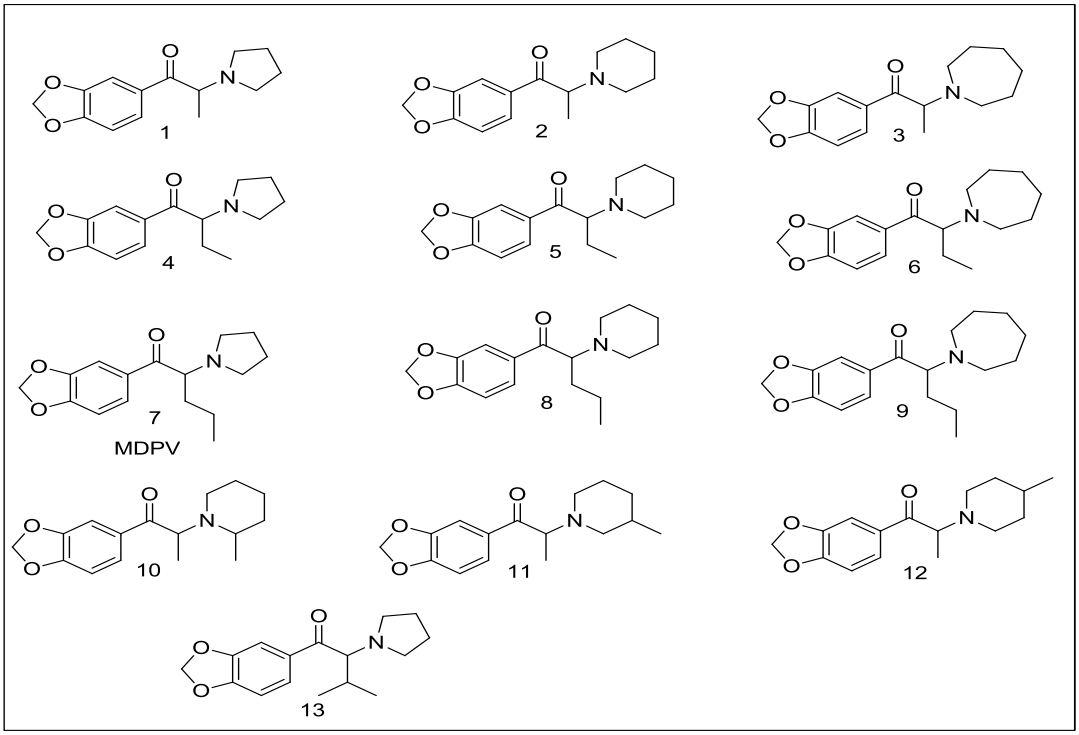


Figure 6: Chemical structures of regioisomers and homologs prepared in this study.

2.3. Analytical studies of the regioisomeric and homologous aminoketones

The ability of the analytical method to distinguish between drugs of abuse and regioisomers directly enhances the specificity of the analysis for the target drugs. The mass spectrum is often the confirmatory piece of evidence for the identification of drugs of abuse in the forensic laboratory. While the mass spectrum is often considered a specific "fingerprint" for an individual compound, there may be other substances, not necessarily having any known pharmacological activity, capable of producing very similar or almost identical mass spectra. These imposter substances provide the possibility for misidentification as the drug of abuse itself. In the case of the aminoketones, there may be many positional regioisomers which yield similar mass spectra. A compound co-eluting with the controlled drug and having the same mass spectrum as the drug of abuse would represent a significant analytical challenge. The ultimate concern then is "if the forensic scientist has never analyzed all the non-drug substances, how can she/he be sure that any of these compounds would not co-elute with the drug of abuse?" The significance of this question is related to many factors, chief among these is the chromatographic system separation efficiency and the number of possible counterfeit substances (imposter molecules). Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of abuse.

NMR is a nondestructive flexible technique that can be used for the simultaneous identification of pure compounds and even mixtures of compounds in one sample. Its advantages, compared to GC-MS techniques, include stereochemical differentiation and the capability to analyze non volatile compounds. However, the lack of use in forensic laboratories can be attributed to the high cost of instrumentation and the poor sensitivity of NMR. In addition, NMR requires pure samples which are hard to satisfy in the case of biological samples.

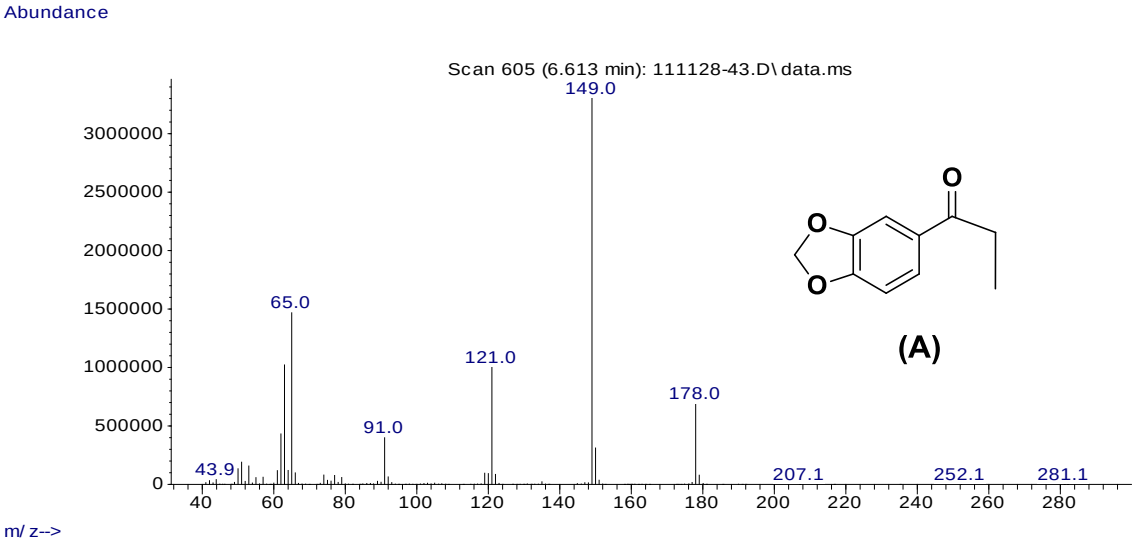
Infrared spectroscopy is considered a confirmation method for the identification of organic compounds due to the uniqueness of infrared spectra for very similar organic molecules. Gas chromatography with infrared detection (GC-IRD) is characterized by scanning quickly enough to obtain vapor phase IR spectra of compounds eluting from the capillary GC column. All the regioisomers, direct and indirect have a strong possibility to be misidentified as the controlled drug, by some analytical methods especially mass spectrometry.

2.3.1. Differentiation of the precursor ketones by GC-MS and GC-IRD

2.3.1.1. Mass spectral studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Although legal control of the key precursor substance is not likely and would not prevent the further clandestine/designer exploration of this group of compounds (aminoketones), finding these precursor substances (ketones) as contaminants or impurities in suspected samples could be an indication for the presence of aminoketones and also to understand fragmentation mechanisms, because clandestine production of these drugs is based on these common precursor ketones.

The first phase of this project was to make an analytical comparison between the four precursor ketones by looking at their mass spectral pattern and explaining the major EI fragments that came out of these ketones, then chromatographic separation of these ketones. Figure 7 shows the EI mass spectra of the four precursor ketones. The mass spectra of these compounds (A, B, C and D) show the fragment ions at *m/z* 149, 121 in common as well as their individual molecular ion peaks at 178, 192, and 206 Da. The homologous side chain series of ethyl, n-propyl and n-butyl in ketones A, B, C and D respectively account for the increase in MW of this series. The n-butyl and isobutyl side chain in ketones C and D are regioisomeric and present very similar mass spectra (Figure 7, C and D) as well as equivalent MW and elemental composition.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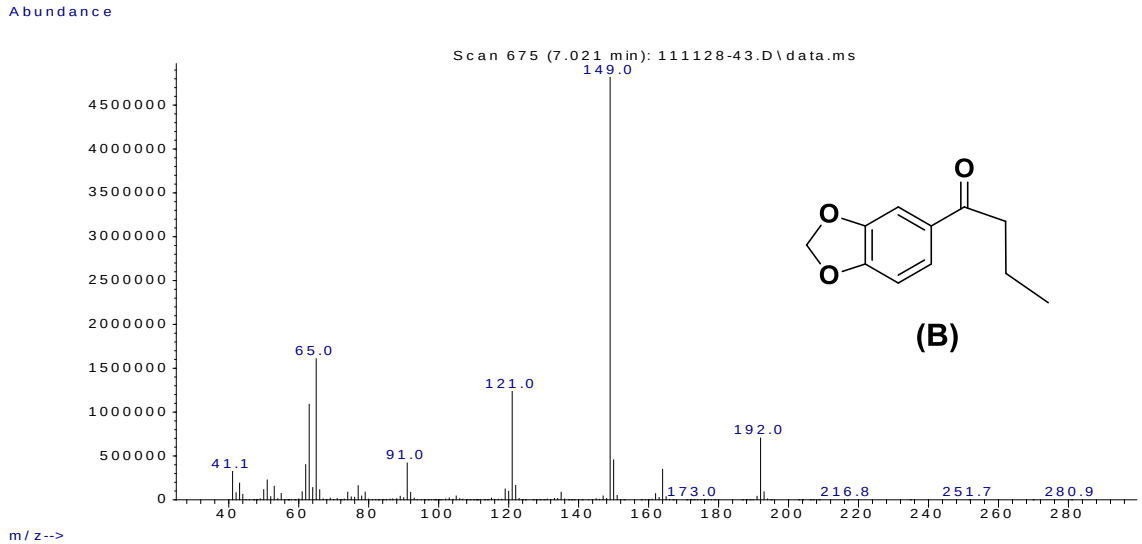
(A)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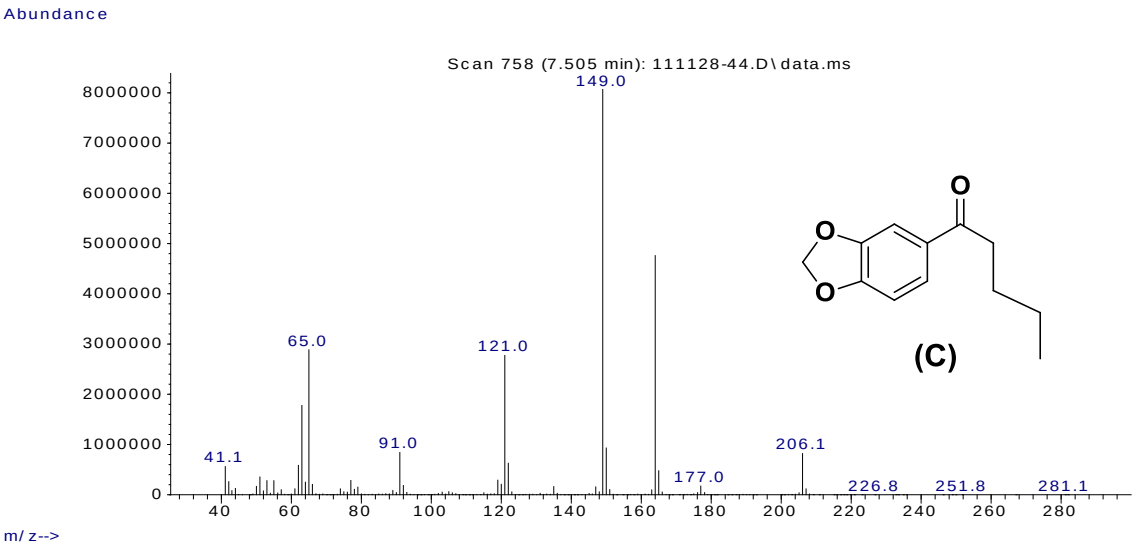
(B)



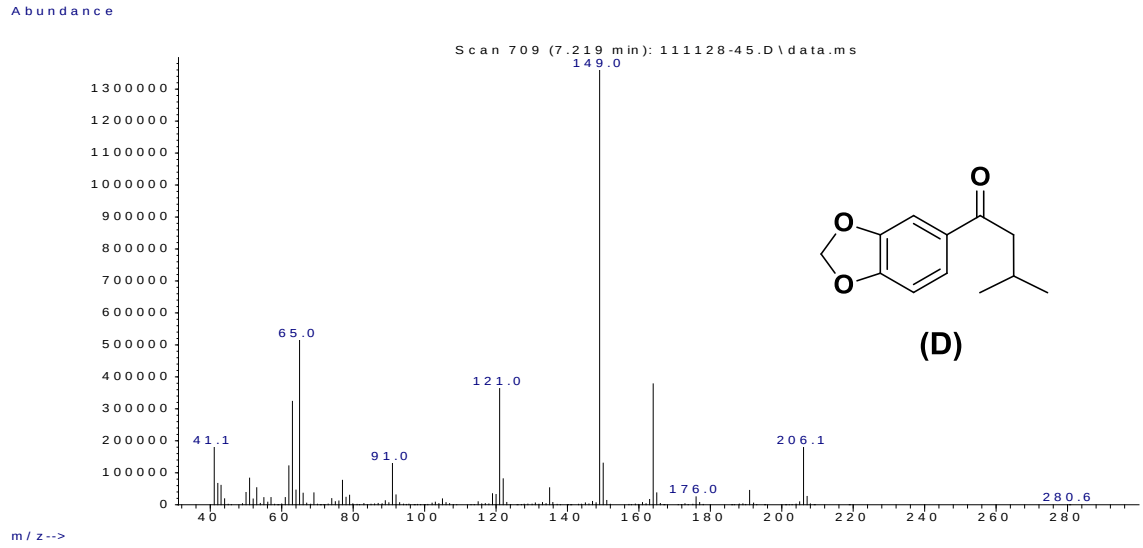
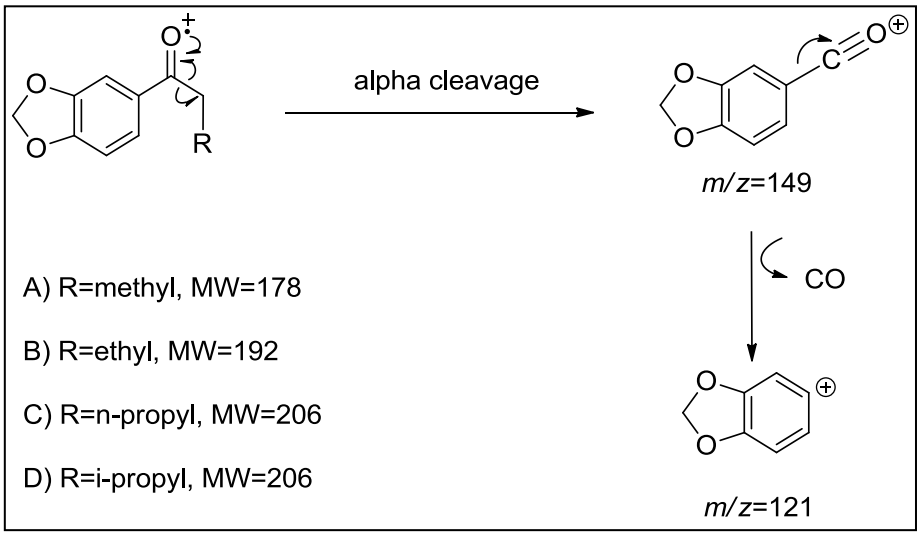


Figure 7: EI Mass spectra of the four precursor ketones.

All four ketones fragment via an initial ionization at oxygen followed by loss of the alkyl side carbonyl carbon. This fragmentation yields the common major fragments observed in all four of the mass spectra in Figure 7. The base peak (*m/z* 149) in all these four spectra is the 3,4-methylenedioxybenzoyl cation resulting from the loss of the alkyl side chain via the oxygen initiated alpha-cleavage fragmentation.

The *m/z* 149, 3,4-methylenedioxybenzoyl cation fragments further via loss of carbon monoxide (CO) to yield the methylenedioxybenzene cation at *m/z* 121. The mechanistic detail of this common fragmentation pathway for the ketones is shown in Scheme 4.

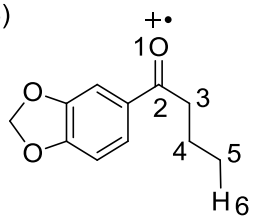


Scheme 4: Mass spectral fragmentation pattern of the precursor ketones under EI (70eV) conditions.

A significant and diagnostic high mass fragment at *m/z* 164 occurs in the spectra for the longer alkyl chain ketones and is a major fragment for ketone C, 1-(3,4 methylenedioxyphenyl)pentan-1-one. This ion is absent from the spectrum of ketone A and only a low intensity ion for ketone B. The spectra for ketones C and D show higher relative amounts of the *m/z* 164 ion and it is the second most abundant peak in ketone C.

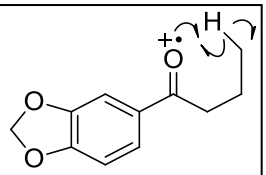
The peak at *m/z* 164 is an odd electron ion resulting from a hydrogen rearrangement reaction of the molecular ion. This ion does not form in the first ketone (3,4-methylenedioxypropiophenone, ketone A) because it lacks the necessary 1,6 unsaturated ring transition state which is essential for the hydrogen rearrangement. However, in the other three ketones we can see this peak with some differences in the relative abundance for each compound from the other because they all can form the unsaturated transition state of six membered ring. 1(3,4-methylenedioxyphenyl)pentane-1-one, ketone C has the highest relative abundance for the ion at *m/z* 164 because of the secondary (CH) radical stability compound to the less stable primary CH_2 radical from ketones B and D. Scheme 5 shows the mechanism of formation of the ion at *m/z* 164 in the mass spectra of the three ketones B, C, and D as assigned on the top of each mechanism.

(Ketone-B)



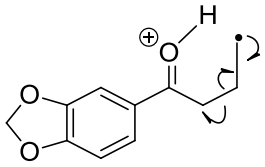
Unsaturated ring transition state

(Pseudo 6 membered ring)

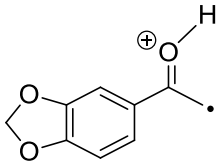


3,4-Methylenedioxybutyrophenone
MW=192
Even Mass

1,6 H-rearrangement



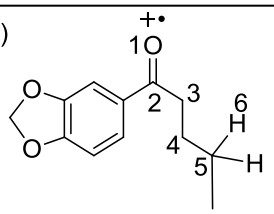
Alpha-Cleavage



(CH$_2$ -Active radical)

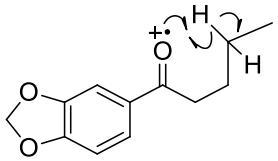
The Mass=164
Even Mass
Odd electron ion(OE)

(Ketone-C)



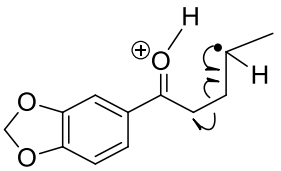
Unsaturated ring transition state

(Pseudo 6 membered ring)

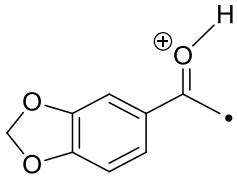


1(3,4-Methylenedioxyphenyl)pentan-1-one
MW=206
Even Mass

1,6 H-rearrangement



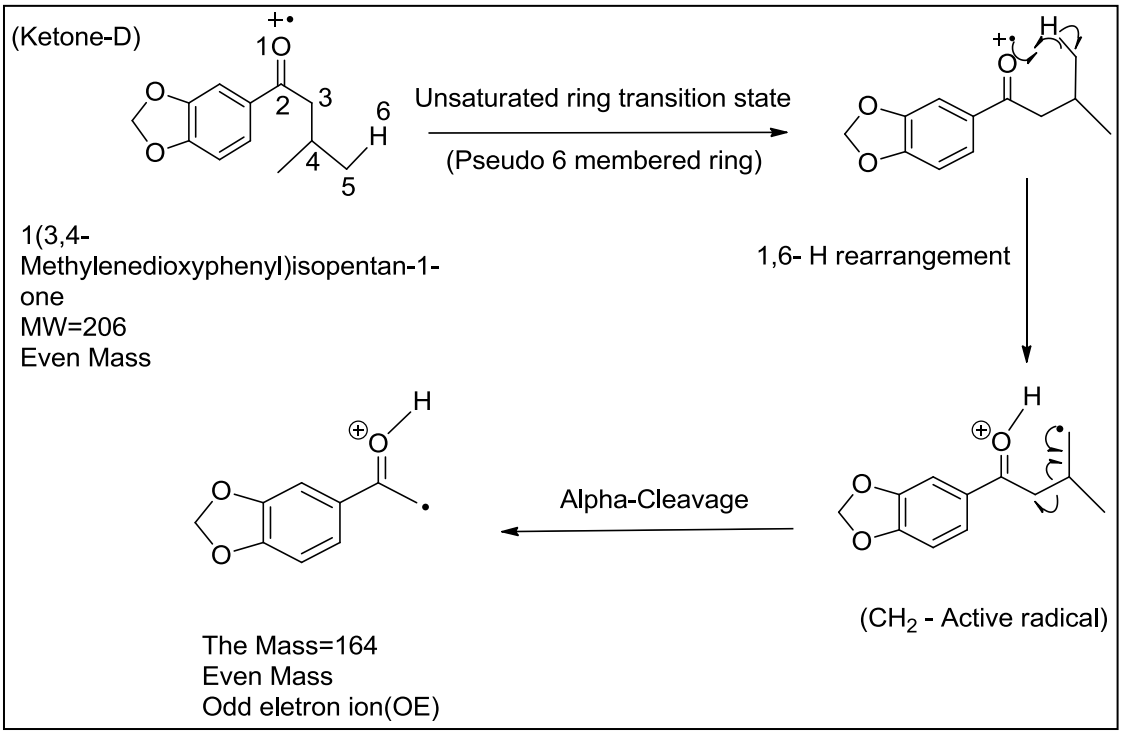
Alpha-Cleavage



More substituted radical (CH)

(More Active)

The Mass=164
Even Mass
Odd electron ion(OE)



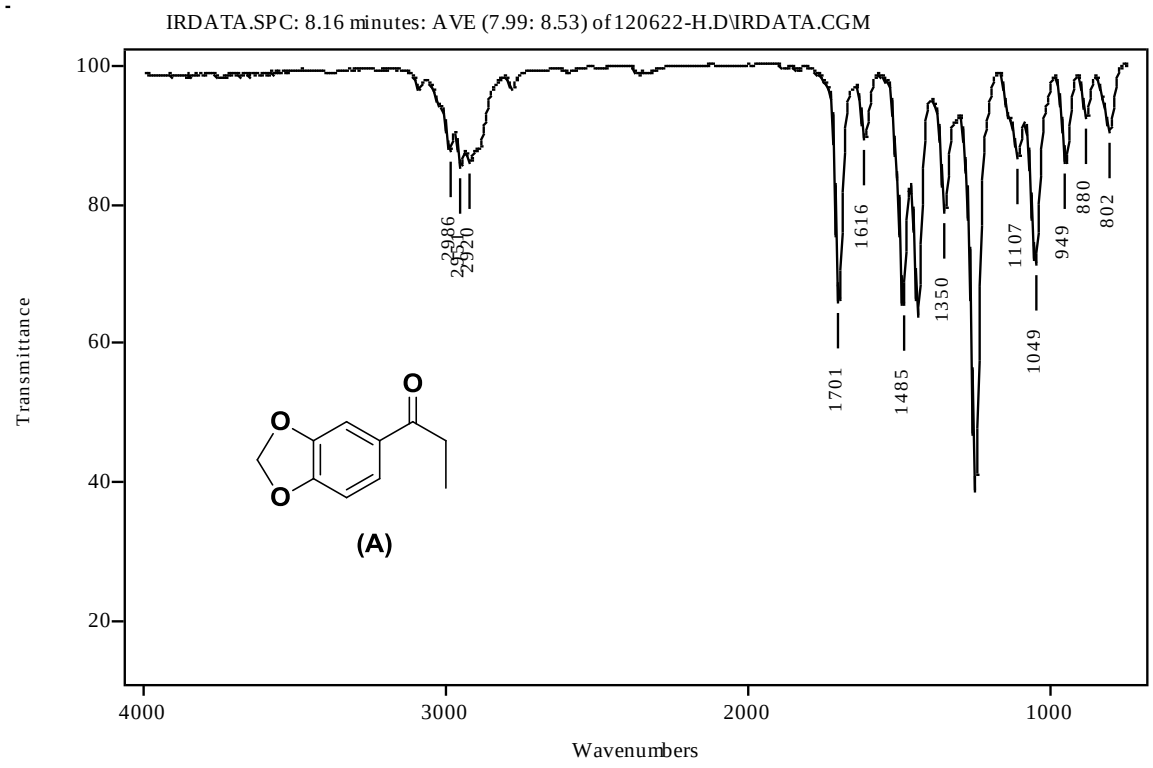
Scheme 5: Mechanism of formation of the ion at *m/z* 164 in the mass spectra of the ketones B, C and D.

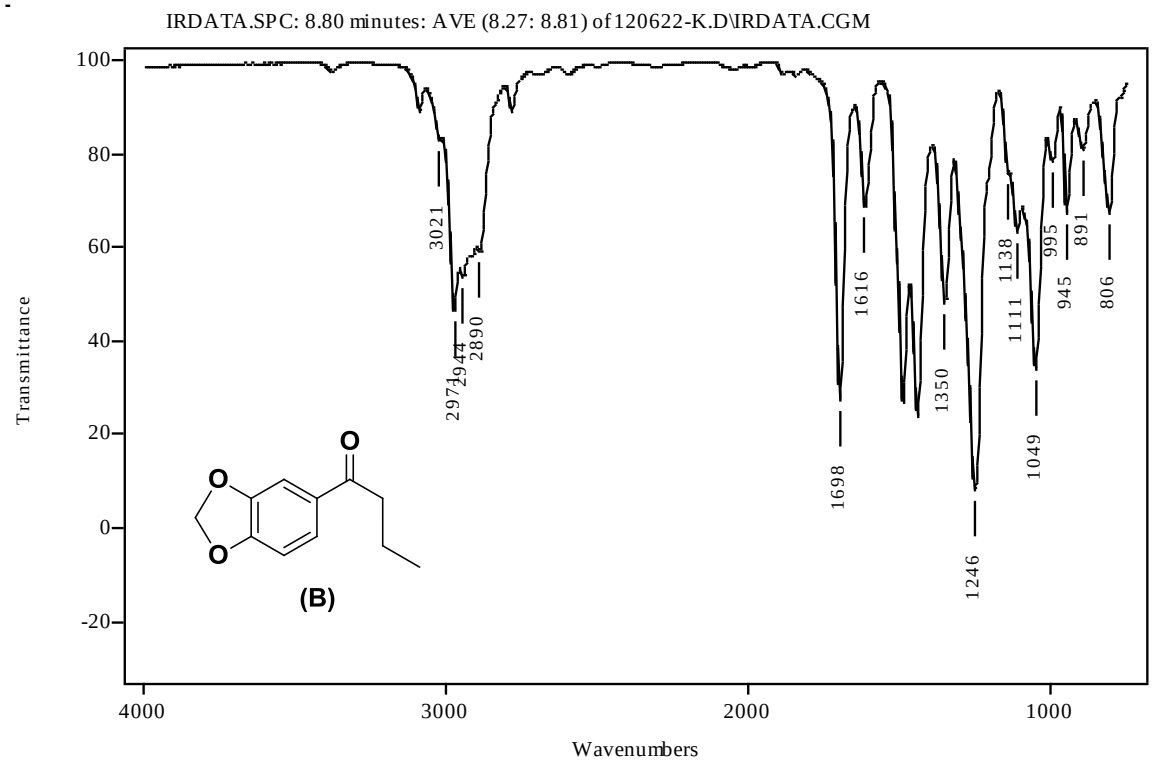
2.3.1.2. Vapor phase infrared spectroscopy

The absorption of IR radiation is also considered one of the non-destructive techniques that can be used for the identification of organic molecules. The region from 1250 to 600 cm^{-1} is generally classified as the "fingerprint region" and is usually a result of bending and rotational energy changes of the molecule as a whole. However since the clandestine samples are usually impure, overlapping absorptions of different molecules present in the sample becomes a possibility. Hence, this region is not useful

for identifying functional groups, but can be useful for determining whether or not samples are chemically identical.

Infrared spectroscopy is often used as a confirmatory method for compound identification in forensic drug analysis. Gas chromatography coupled with infrared detection (GC-IRD) was evaluated for differentiation among the four ketones. Infrared analysis should provide compound specificity without the need for chemical modification of the parent molecule. The vapor phase infrared spectra for the four ketones are shown in Figure 8. The spectra were generated in the vapor phase following sample injection into the gas chromatograph. Each compound shows a vapor phase IR spectrum with bands in the regions 650 –1700 cm^{-1} and 2700 – 3100 cm-1 which represent the carbonyl and the amine groups respectively. The vapor phase infrared spectra for the precursor ketones are shown in Figure 8.


IRDATA.SPC: 8.16 minutes: AVE (7.99: 8.53) of 120622-H.D\IRDATA.CGM
Transmittance
Wavenumbers
100
80
60
40
20
4000
3000
2000
1000
2986
2920
1701
1616
1485
1350
1107
1049
949
880
802
O
O
O
(A)


IRDATA.SPC: 8.80 minutes: AVE (8.27: 8.81) of 120622-K.D\IRDATA.CGM
Transmittance
Wavenumbers
100
80
60
40
20
0
-20
4000
3000
2000
1000
3021
2974
2944
2890
1698
1616
1350
1246
1138
1111
1049
995
945
891
806
O
O
O
(B)

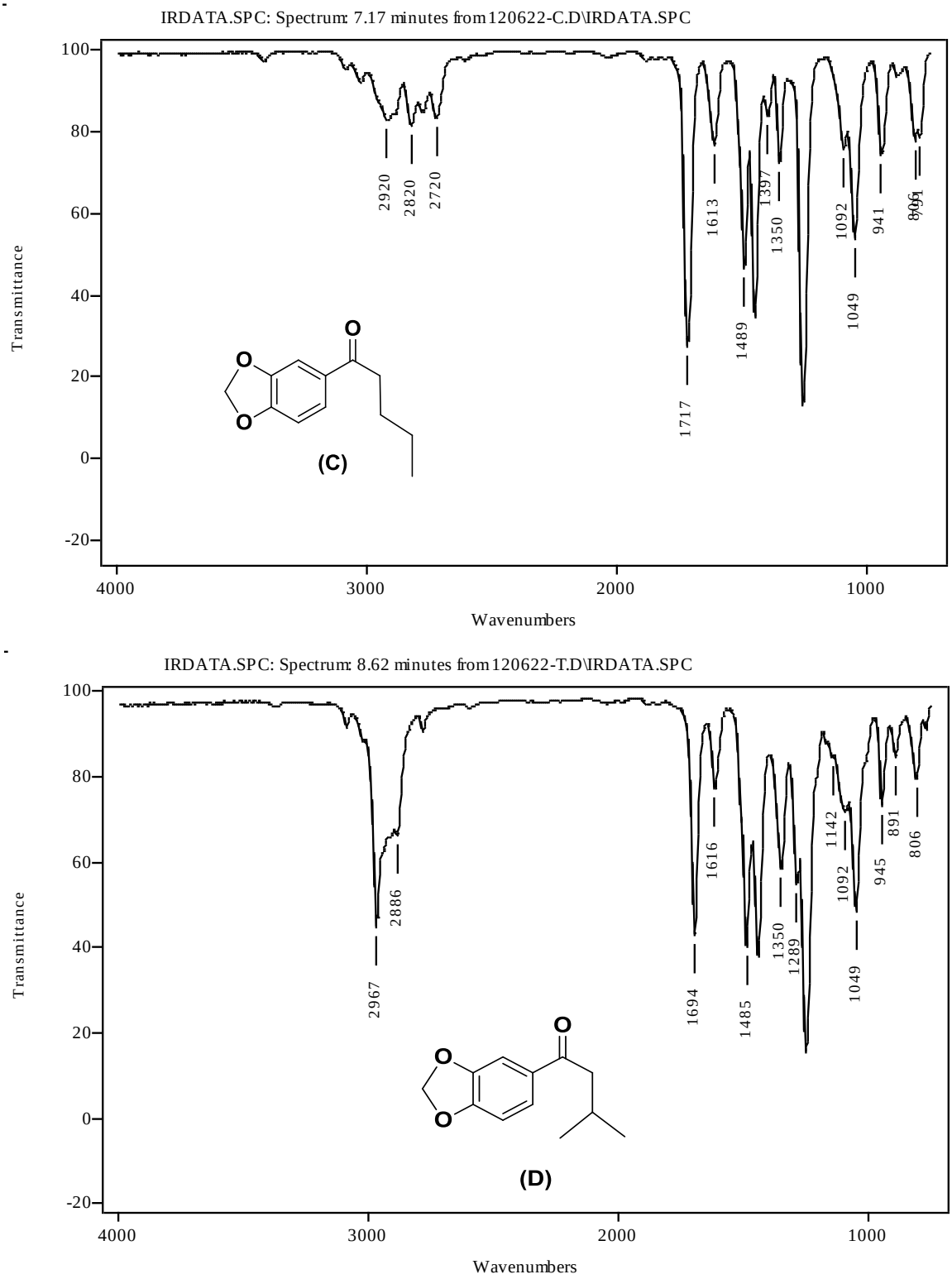


Figure 8: Vapor phase IR spectra of the four precursor ketones.

2.3.1.3. Gas chromatographic separation

Figure 9 shows the gas chromatographic separation of the precursor ketones. It was carried out using the stationary phases which has the following features, a 30 m × 0.25 mm i.d. capillary coated with 0.50 µm film of 100% dimethyl polysiloxane (Rtx-1). The temperature program consisted of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes. The elution order was parallel to the number of carbon atoms in the side chain with the 3,4-methylenedioxypropiophenone (ketone A) eluting before the next higher homolog, 3,4-methylenedioxybutyrophenone (ketoneB). The ketone with the branched C_4 side chain (ketone D) eluted before the regioisomeric straight chain one (ketone C).

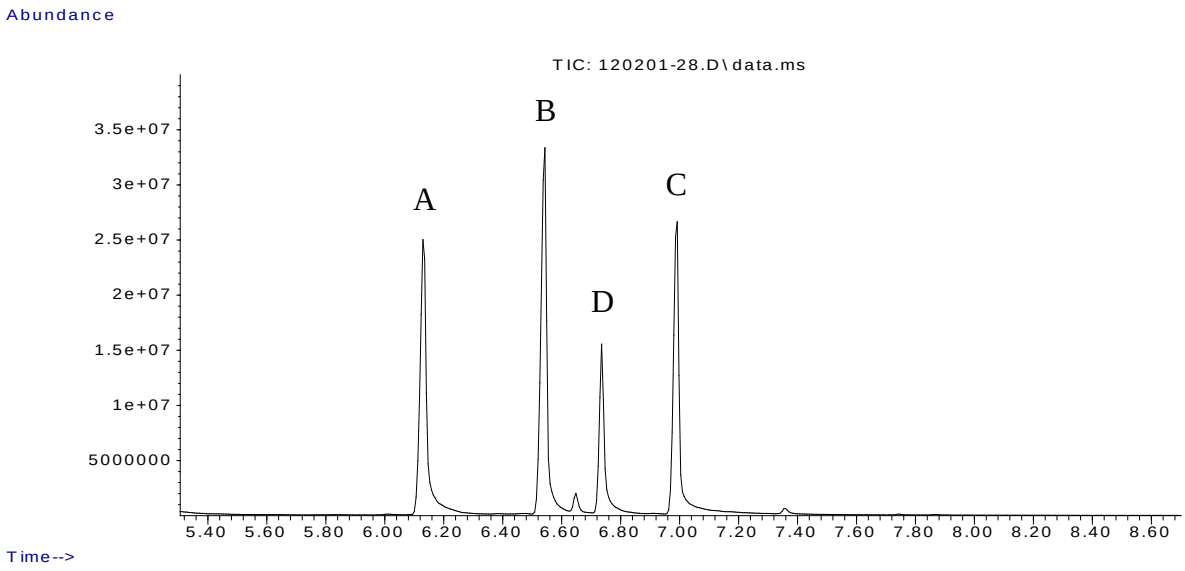


Figure 9: Gas chromatographic separation of the precursor ketones A, B, C and D on Rtx-1 stationary phase.

The four precursor ketones show similar mass spectra pattern with the same fragmentation process producing ions at *m/z* 149 and 121. Each compound shows a vapor phase IR spectrum with the band in the regions 650 –1700 cm-1 which represents the carbonyl group. The ion at *m/z* 164 is an odd electron ion resulting from a hydrogen rearrangement reaction of the molecular ion. This ion occurs in ketones B, C and D and it is most abundant in the mass spectrum of (ketone C) because of the stable primary (CH) radical which is responsible for radical site alpha-cleavage reaction. This set of four ketones is well resolved on the Rtx-1 column according to their homologous order from lower to higher, the ketone with isobutyl side chain (D) eluted before the one with the straight butyl chain (C).

2.3.2. Differentiation of the homologous and regioisomeric aminoketones by GC-MS and GC-IRD

Figure 6 shows the chemical structures of all compounds involved in this study. In each horizontal row (compare compounds 1, 2, and 3 for example) the compounds are homologs in the nitrogen containing ring. These compounds were made from the commercially available amines, pyrrolidine, piperidine, perhydroazepine and the precursor ketone (3,4 methylenedioxypropiophenone, A) described in the previous section (2.3.1., p 39) . In each vertical column (compare compounds 1, 4 and 7 for example), the compounds are homologs in the alkyl side chain and prepared from appropriate ketones (A, B and C) described in the previous section (2.3.1., p 39) and the commercially available pyrrolidine. Thus, these compounds are potential future designer

drug substances based on the commercial availability of precursor materials and common methodology.

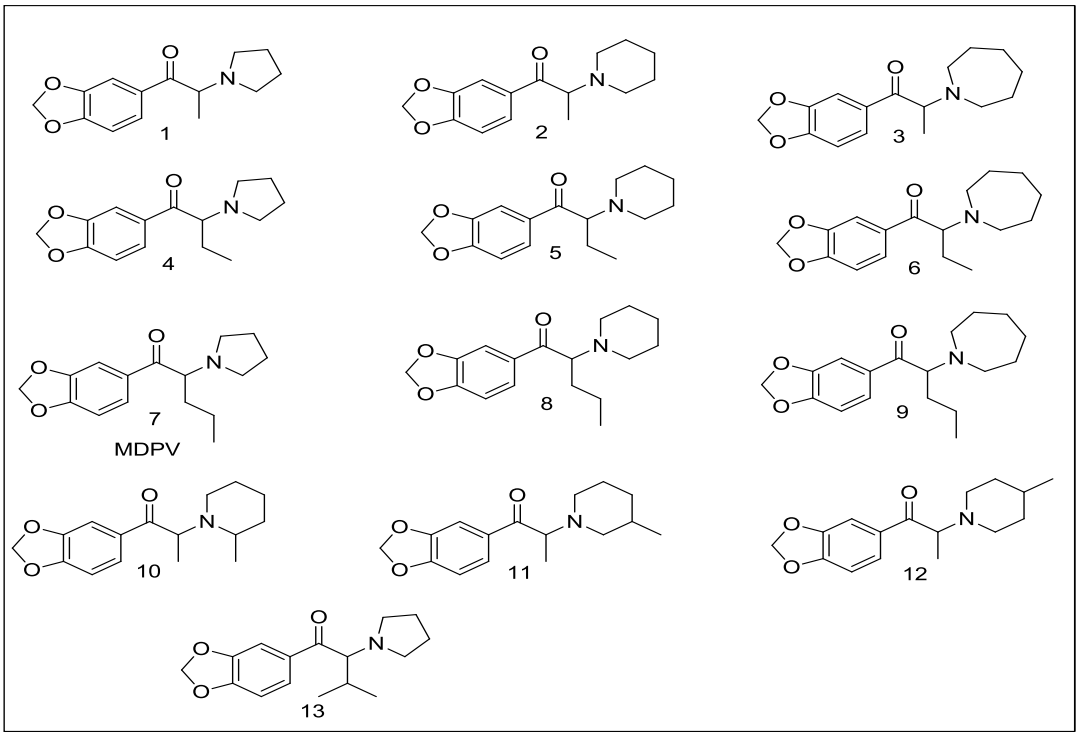
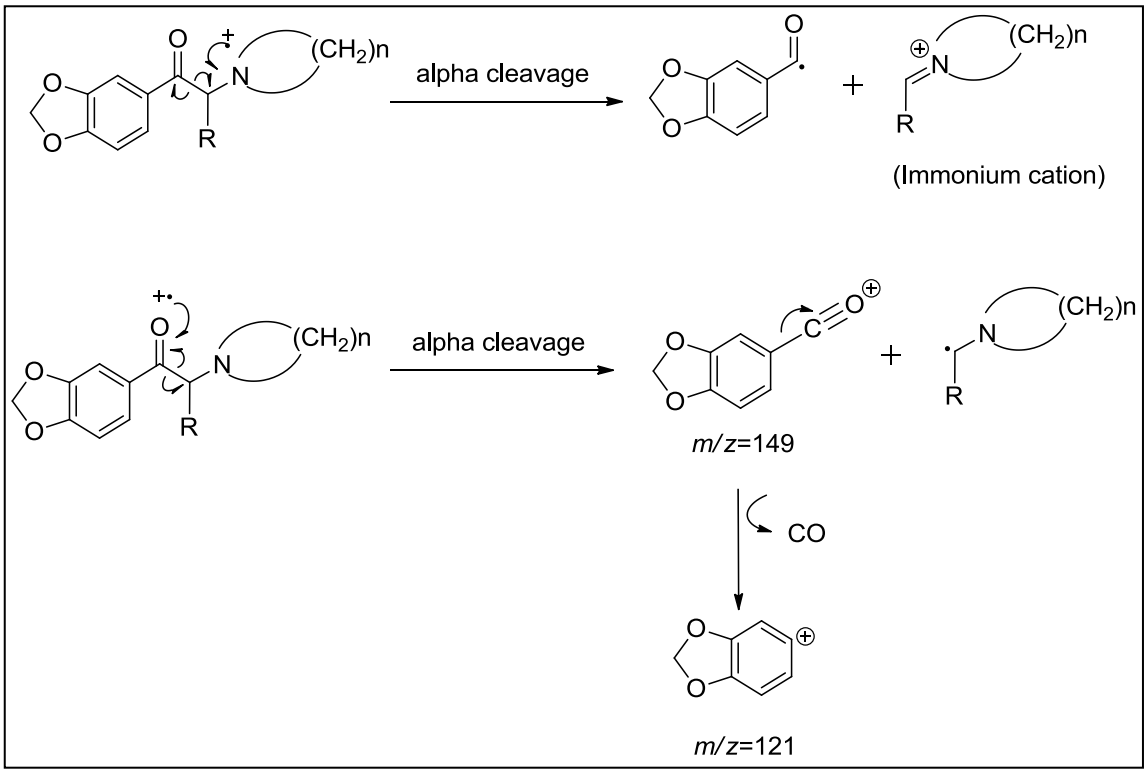


Figure 6: Chemical structures of the regioisomers and homologs prepared in this study.

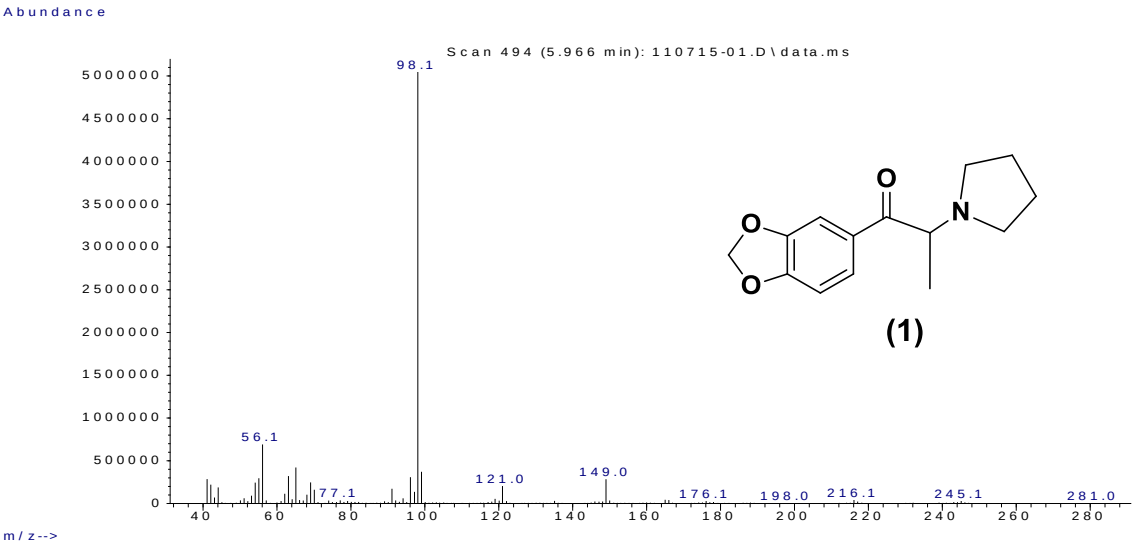
2.3.2.1. Mass spectral studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Scheme 6 shows the general fragmentation process for the formation of the ions of major relative abundance for the aminoketones. Initial ionization at the nitrogen followed by alpha-cleavage initiated by the radical site yields the homologous and/or the regioisomeric substituted immonium cations of varying mass. Initial ionization at the oxygen atom of the carbonyl group followed by a similar radical site initiated alpha-cleavage yields the loss of the alkyl group side of the carbonyl. The cation formed in this process is the 3,4-methylenedioxybenzoyl cation at *m/z* 149. This fragment occurs at the same mass in all the compounds in this study. The loss of carbon monoxide (CO) from the *m/z* 149 ion yields the methylenedioxybenzene cation at *m/z* 121 in all these spectra. The proposed structures of these fragment ions are shown in Scheme 6.



Scheme 6: The general fragmentation pathways for the proposed 13 compounds prepared in this study.

Figure 10 shows the EI mass spectra of all thirteen isomeric and homologous aminoketones (Compounds 1-13). The base peaks in the mass spectra of all compounds are the result of the same alpha-cleavage process producing fragments of identical mass in case of regioisomers and homologous fragments in case of the homologs. The base peak in all these spectra occurs at a range between *m/z* 98 and *m/z* 154 with a fourteen mass units being added in every next homologous compound. The additional high mass ions of significant relative abundance common to the thirteen compounds likely arise from fragmentation initiated by the radical cation at the carbonyl oxygen. These fragment ions are at *m/z* 149 and 121, and other ions of low relative abundance.



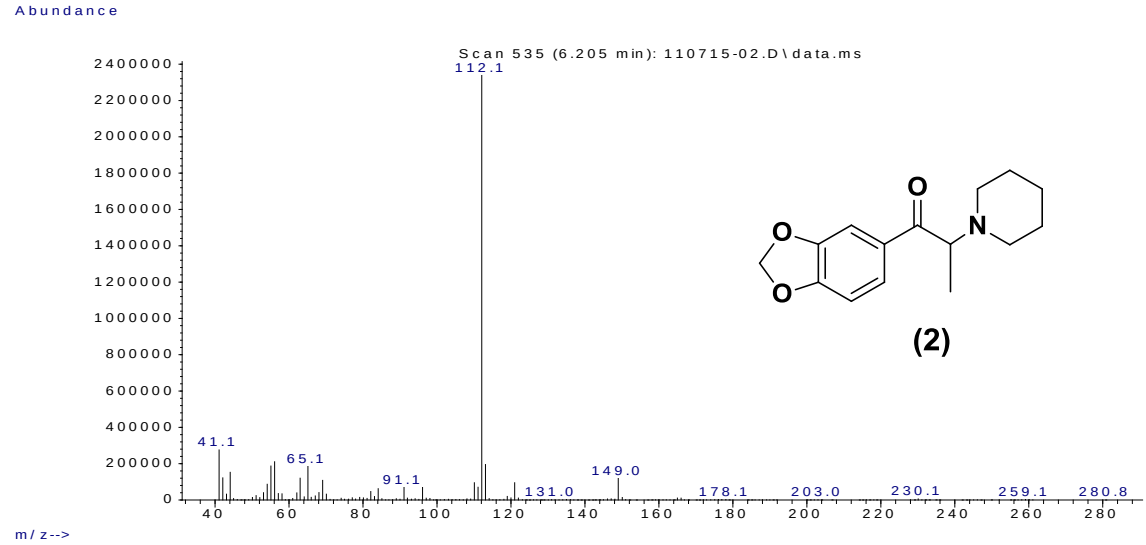
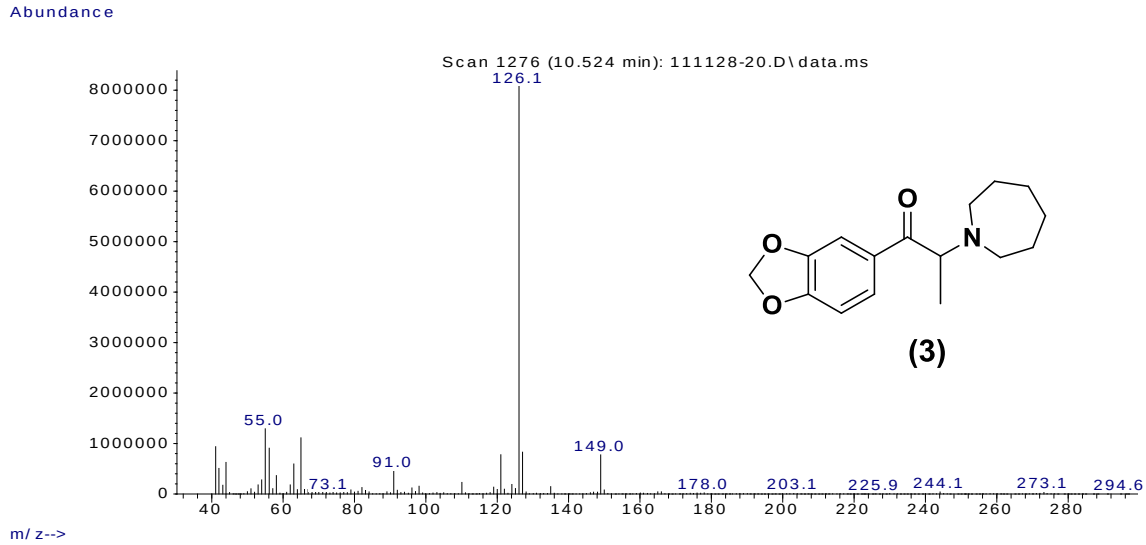
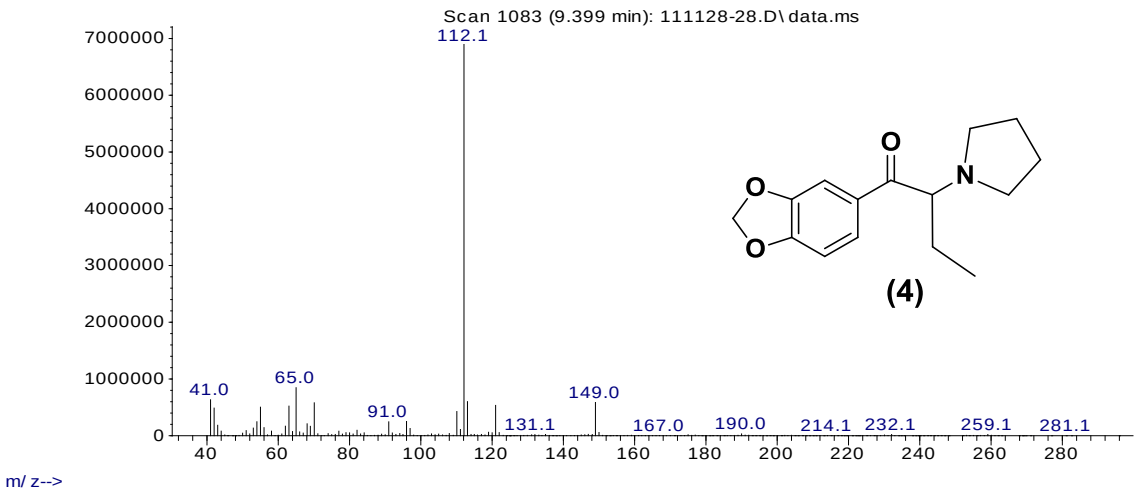


Figure 10: Mass spectra of 13 regioisomeric and homologous aminoketones prepared in this study.



(3)



(4)

Figure 10: Mass spectra of 13 regioisomeric and homologous aminoketones prepared in this study.

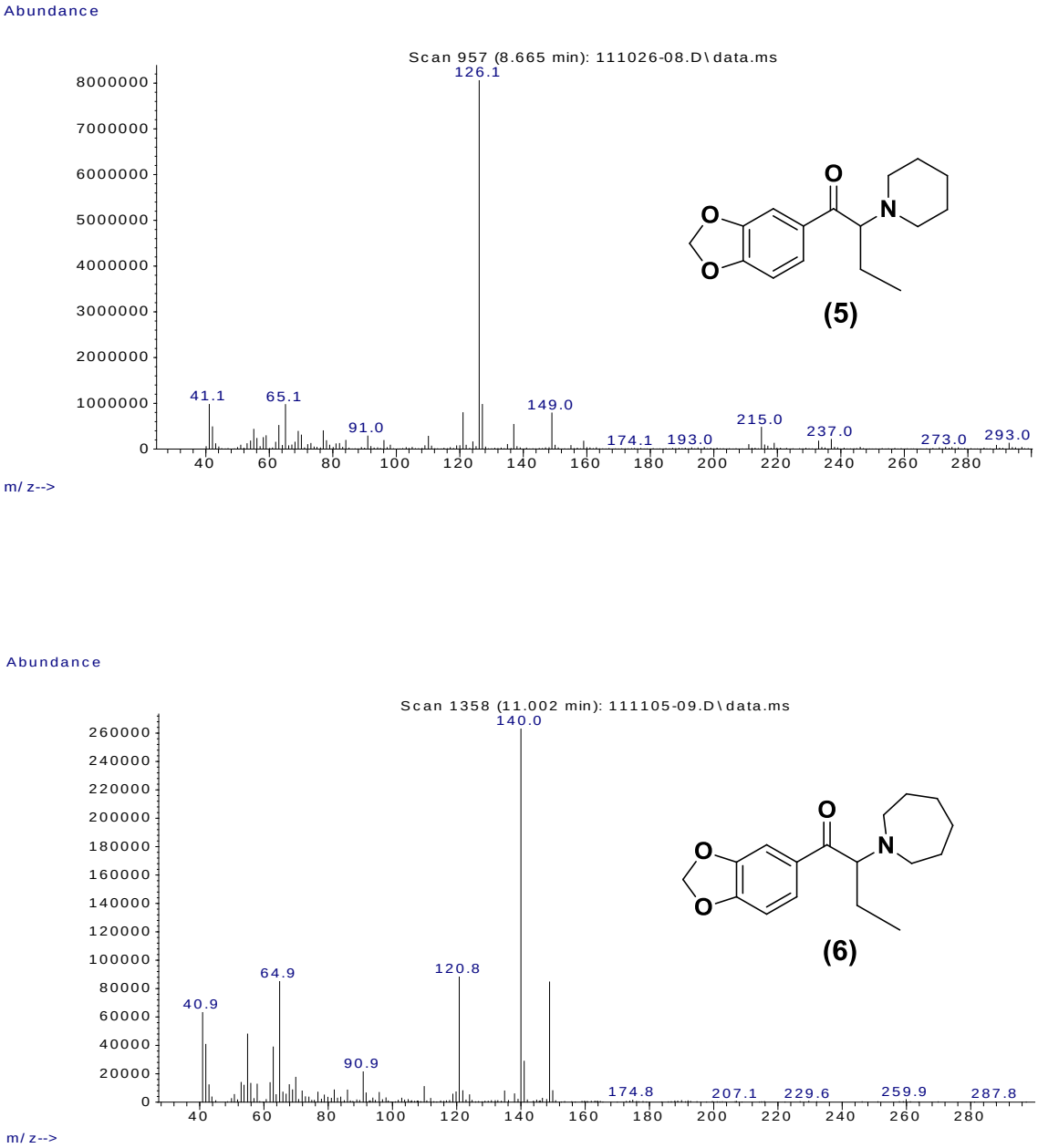


Figure 10: Mass spectra of 13 regioisomeric and homologous aminoketones prepared in this study.

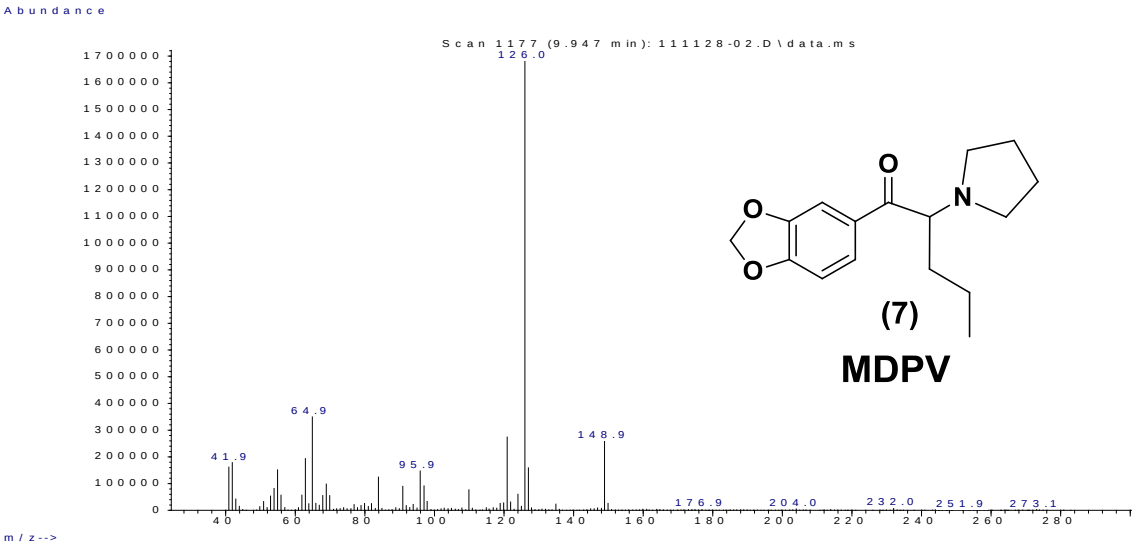
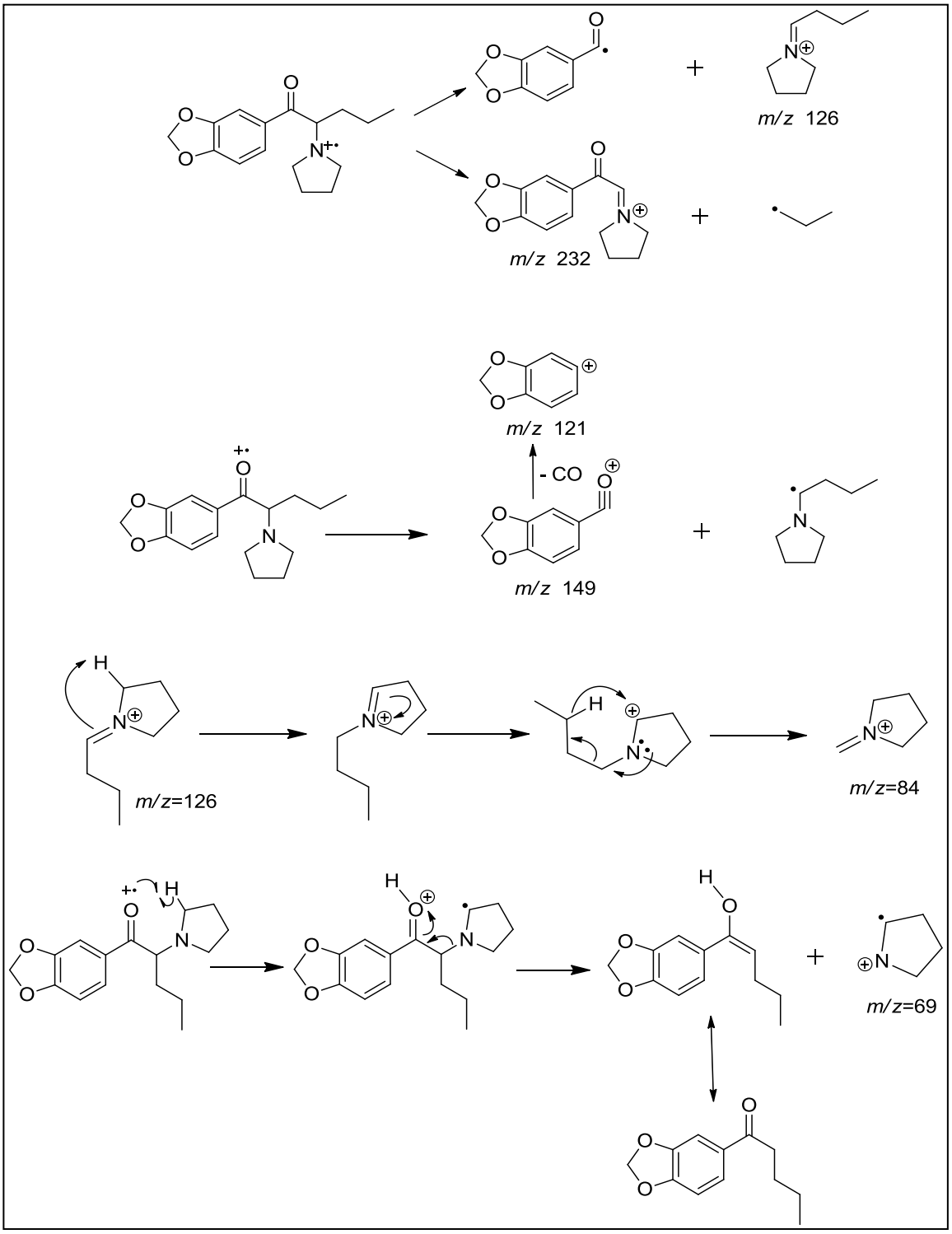


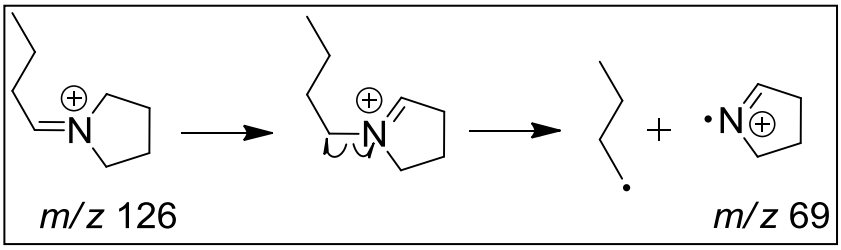
Figure 10: Mass spectra of 13 regioisomeric and homologous aminoketones prepared in this study.

The fragmentation pathway that was described in Scheme 2 (section 1.11.1., p 26) and characteristic for aminoketones is also described with more details in Scheme 7. This detailed pathway for MDPV has fragment ions at *m/z* 232, 126, 149, and 121, as well as 80 and 69. The first four ions are resulting from an initial ionization followed by radical site alpha-cleavage of the nitrogen and the carbonyl oxygen, respectively.

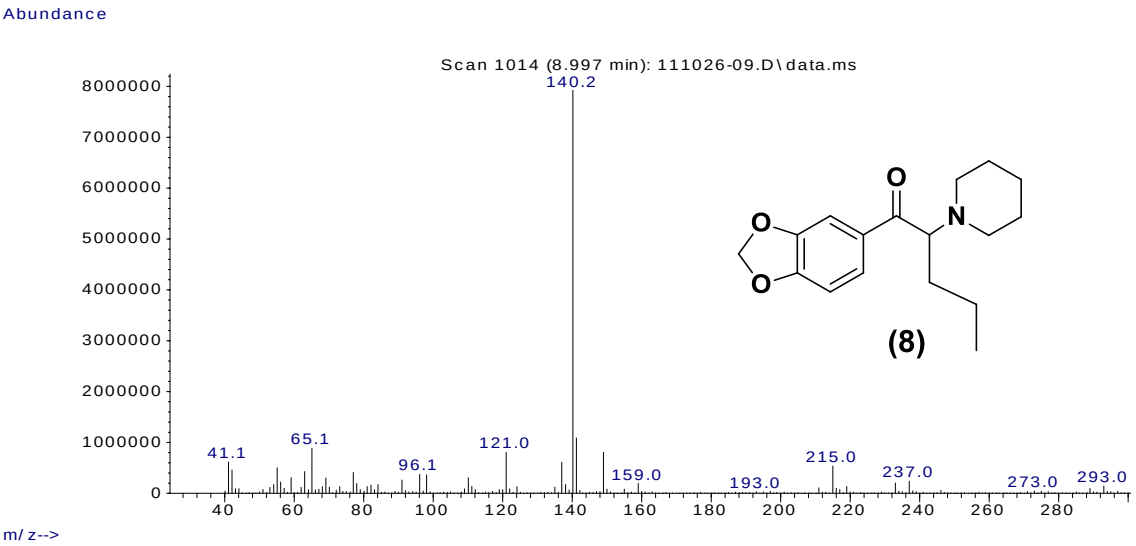


Scheme 7: The fragmentation pathways for MDPV.

The ion at *m/z* 80 is an even electron ion resulting from an even electron ion rearrangement reaction of the ion *m/z* 126 through unsaturated six membered ring transition state intermediate. Additionally, the ion at *m/z* 69 is an odd electron ion resulting from 1-6 hydrogen rearrangement reaction through an unsaturated six membered ring transition state intermediate. This last mechanism that gives the ion at *m/z* 69 is in contrast to what was described by (Westphal *et al*) mechanism to get this ion through a hydrogen rearrangement reaction of the *m/z* 126. The reason for this is an even electron ion never ends up into an odd electron ion (in this case *m/z* 126 into *m/z* 69). The proposed mechanism for the formation of *m/z* 69 by Westphal *et al* is shown in Scheme 8 [Westphal *et al*, 2009].



Scheme 8: The proposed mechanism for the formation of *m/z* 69 by Westphal *et al* [Westphal *et al*, 2009].



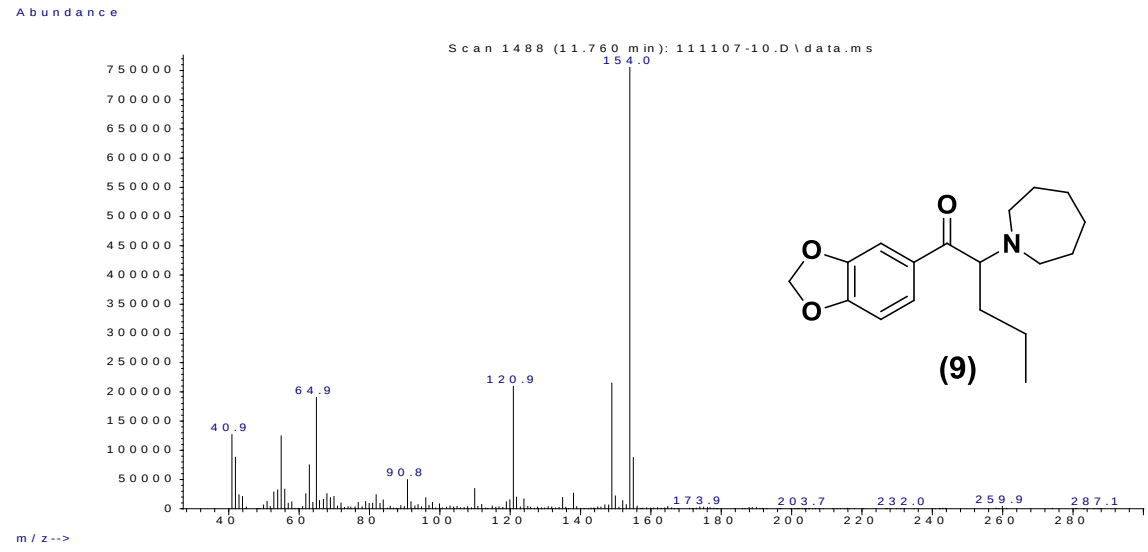
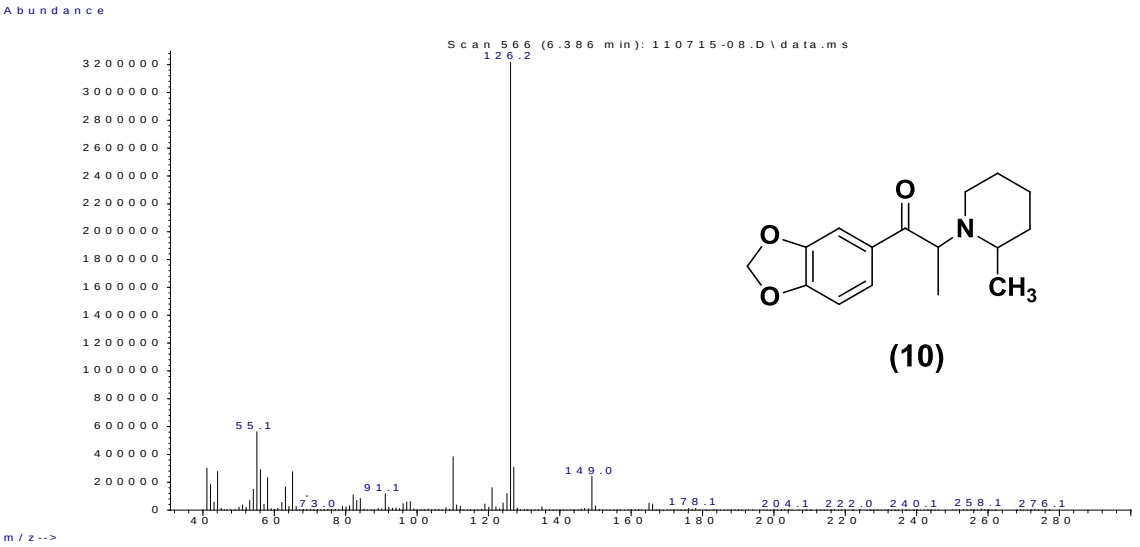


Figure 10: Mass spectra of 13 regioisomeric and homologous aminoketones prepared in this study.



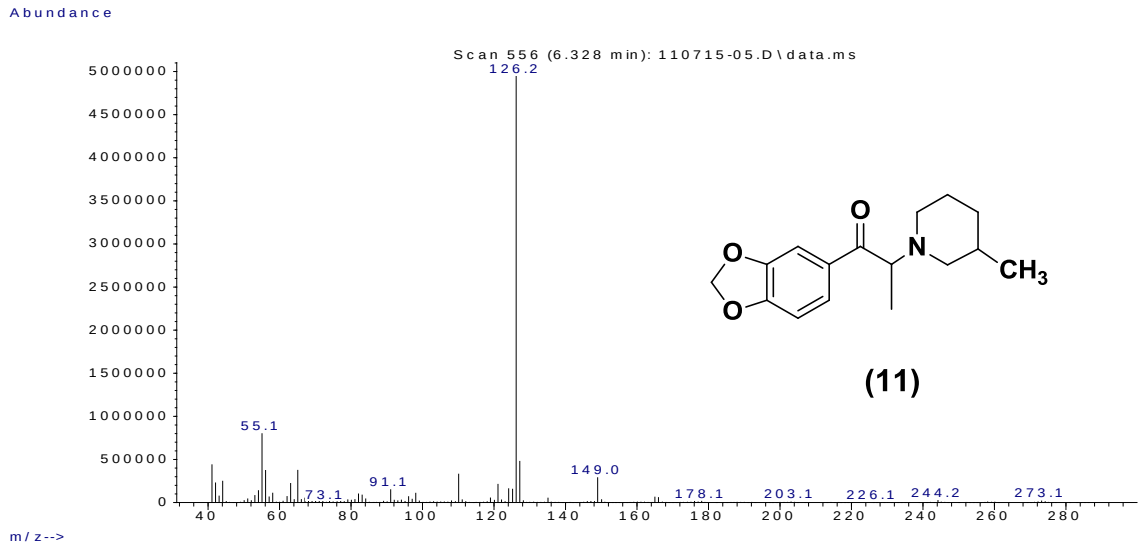
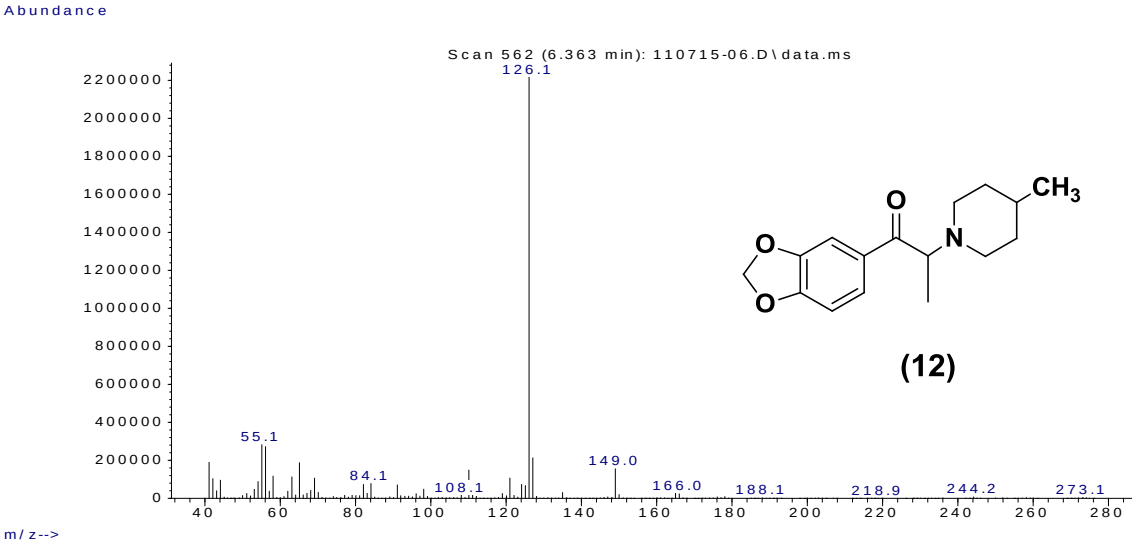


Figure 10: Mass spectra of 13 regioisomeric and homologous aminoketones prepared in this study.



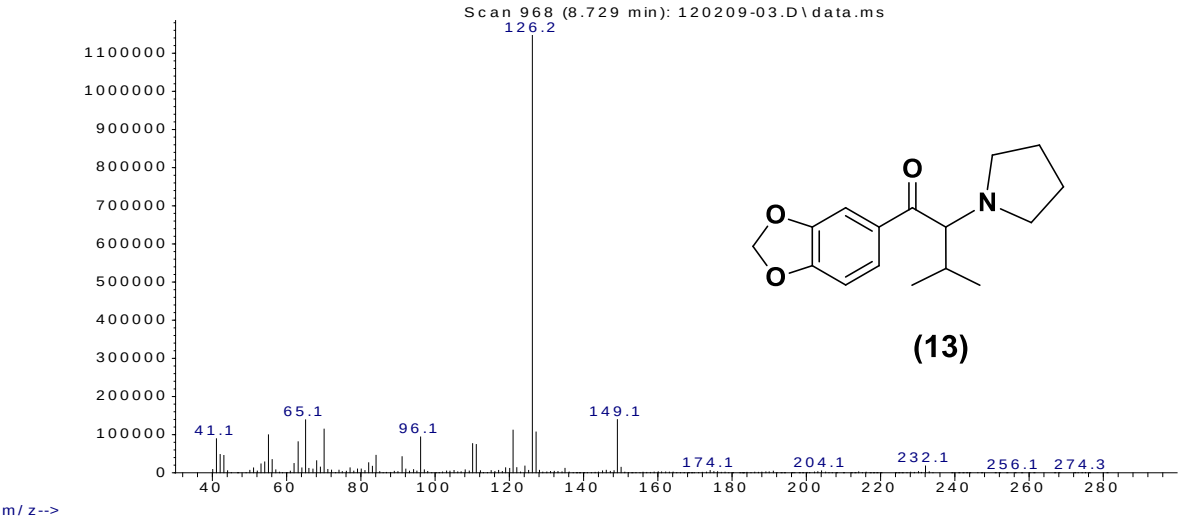


Figure 10: Mass spectra of 13 regioisomeric and homologous aminoketones prepared in this study (continued).

It is interesting that all regioisomeric compounds in this group produce the same base peak at *m/z* 126 with the same formula; the difference between the base peak fragment produced by MDPV and the base peak fragments of the other regioisomers is the arrangement of the atoms (direct regioisomers). This is a very important issue in terms of drugs of abuse since one regioisomer could be misidentified as the drug of abuse itself if the mass spectra profiles are the same and co-elution under reasonable chromatographic conditions is possible. Figure 11 shows the structures for the major fragments of the regioisomers in this group.

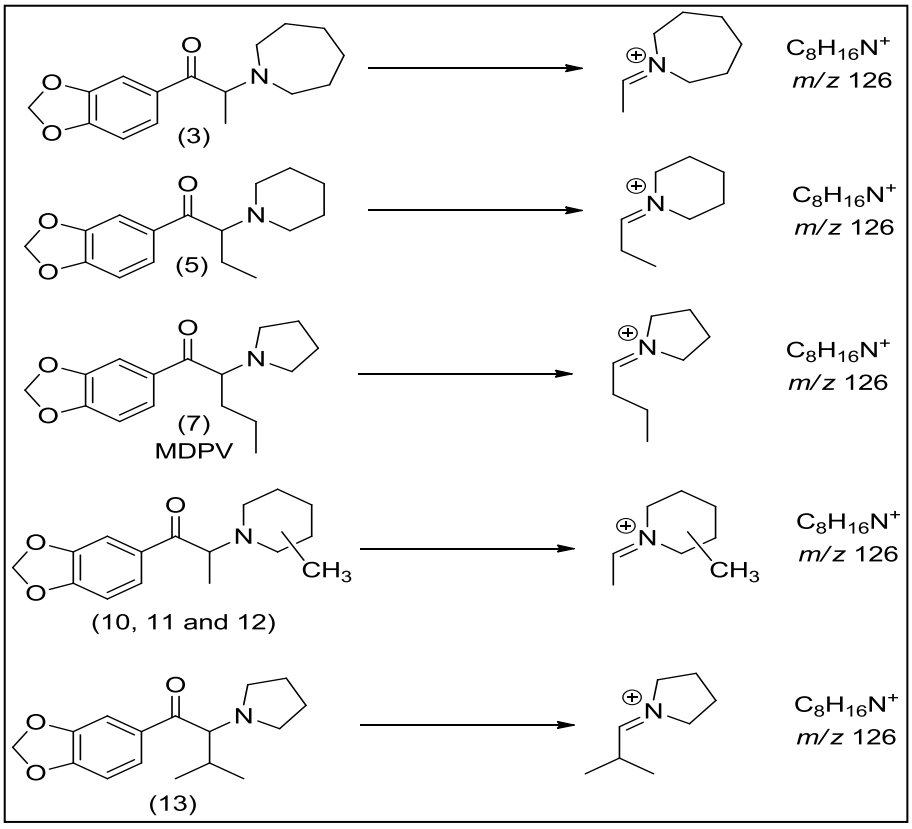


Figure 11: The major fragments of the regioisomeric aminoketones in this study.

2.3.2.2. Vapor phase infrared spectroscopy

The absorption of IR radiation is also considered one of the non-destructive techniques that can be used for the identification of organic molecules. The region from 1250 to 600 cm^{-1} is generally classified as the "fingerprint region" and is usually a result of bending and rotational energy changes of the molecule as a whole. However since the clandestine samples are usually impure, overlapping absorptions of different molecules present in the sample becomes a possibility. Hence, this region is not useful for identifying functional groups, but can be useful for determining whether or not samples are chemically identical.

Infrared spectroscopy is often used as a confirmatory method for compound identification in forensic drug analysis. Gas chromatography coupled with infrared detection (GC-IRD) was evaluated for differentiation among the thirteen aminoketones. Infrared analysis should provide compound specificity without the need for chemical modification of the parent molecule. The vapor phase infrared spectra for the thirteen aminoketones are shown in Figure 12. The spectra were generated in the vapor phase following sample injection into the gas chromatograph. Each compound shows a vapor phase IR spectrum with bands in the regions 650 –1700 cm^{-1} and 2700 – 3100 $cm^{-}1$ which represent the carbonyl and the amine groups, respectively.

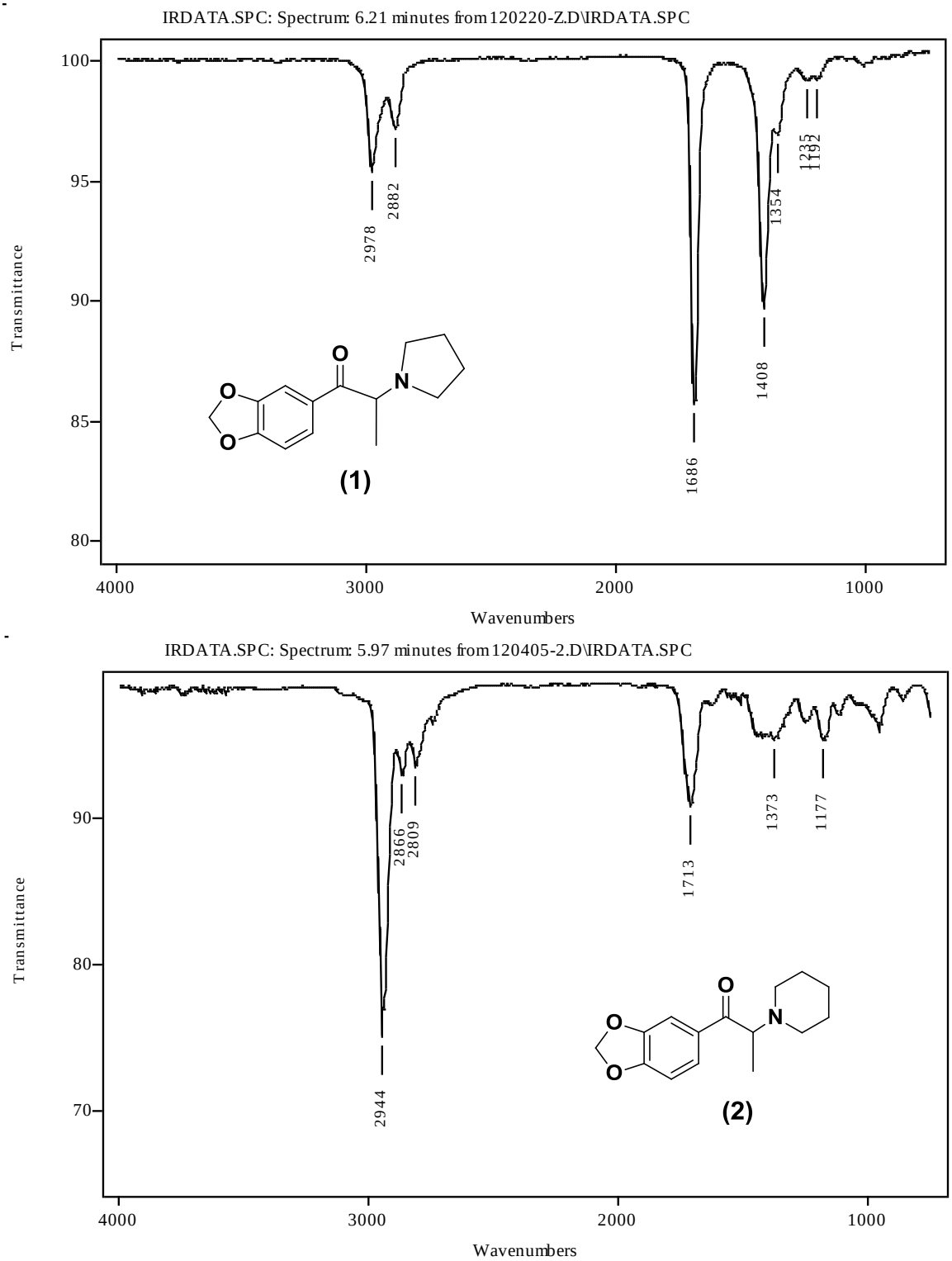


Figure 12: Vapor phase IR spectra of the 13 proposed homologous and regioisomeric aminoketones.

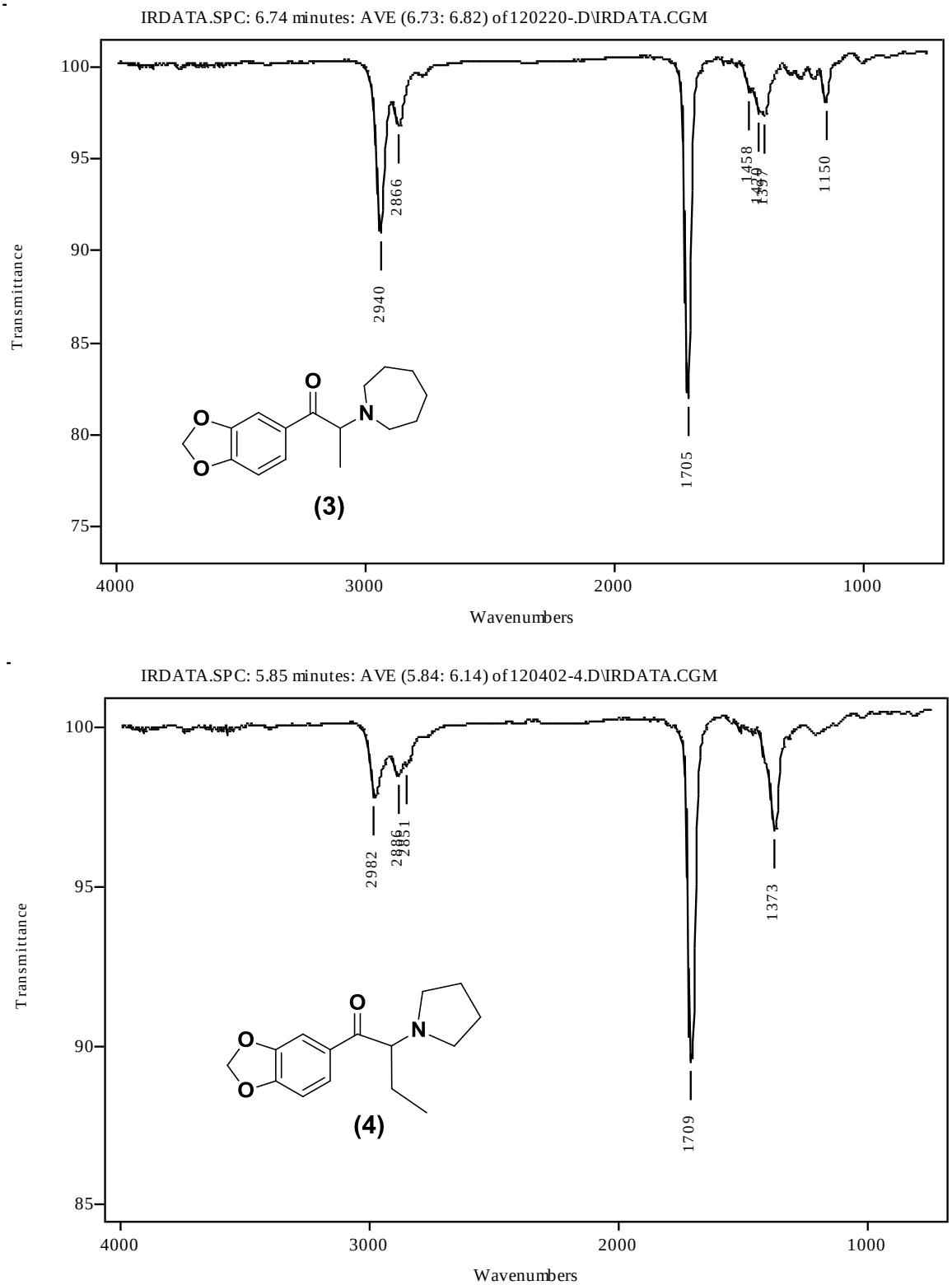


Figure 12: Vapor phase IR spectra of the 13 proposed homologous and regioisomeric aminoketones.

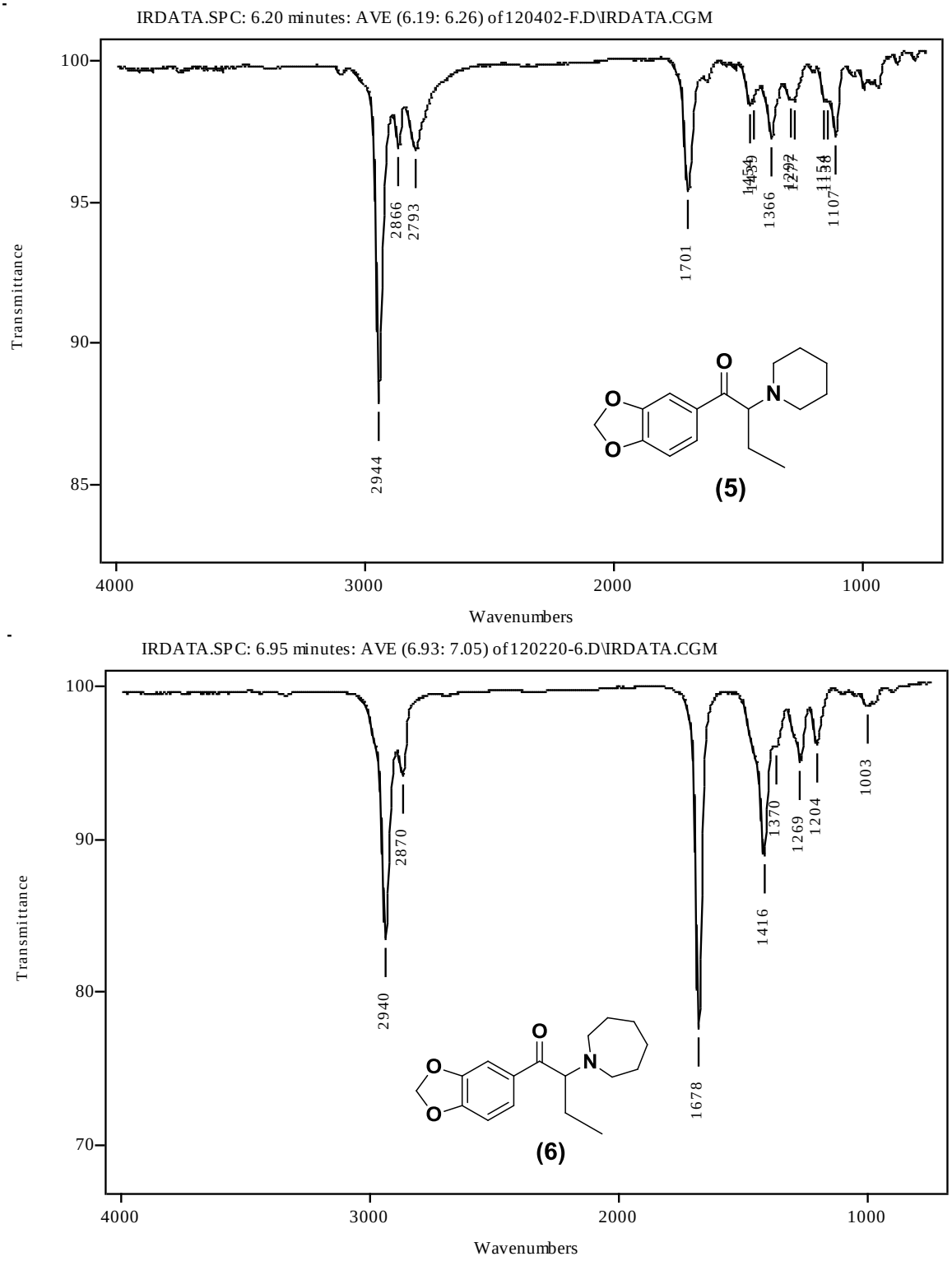


Figure 12: Vapor phase IR spectra of the 13 proposed homologous and regioisomeric aminoketones.

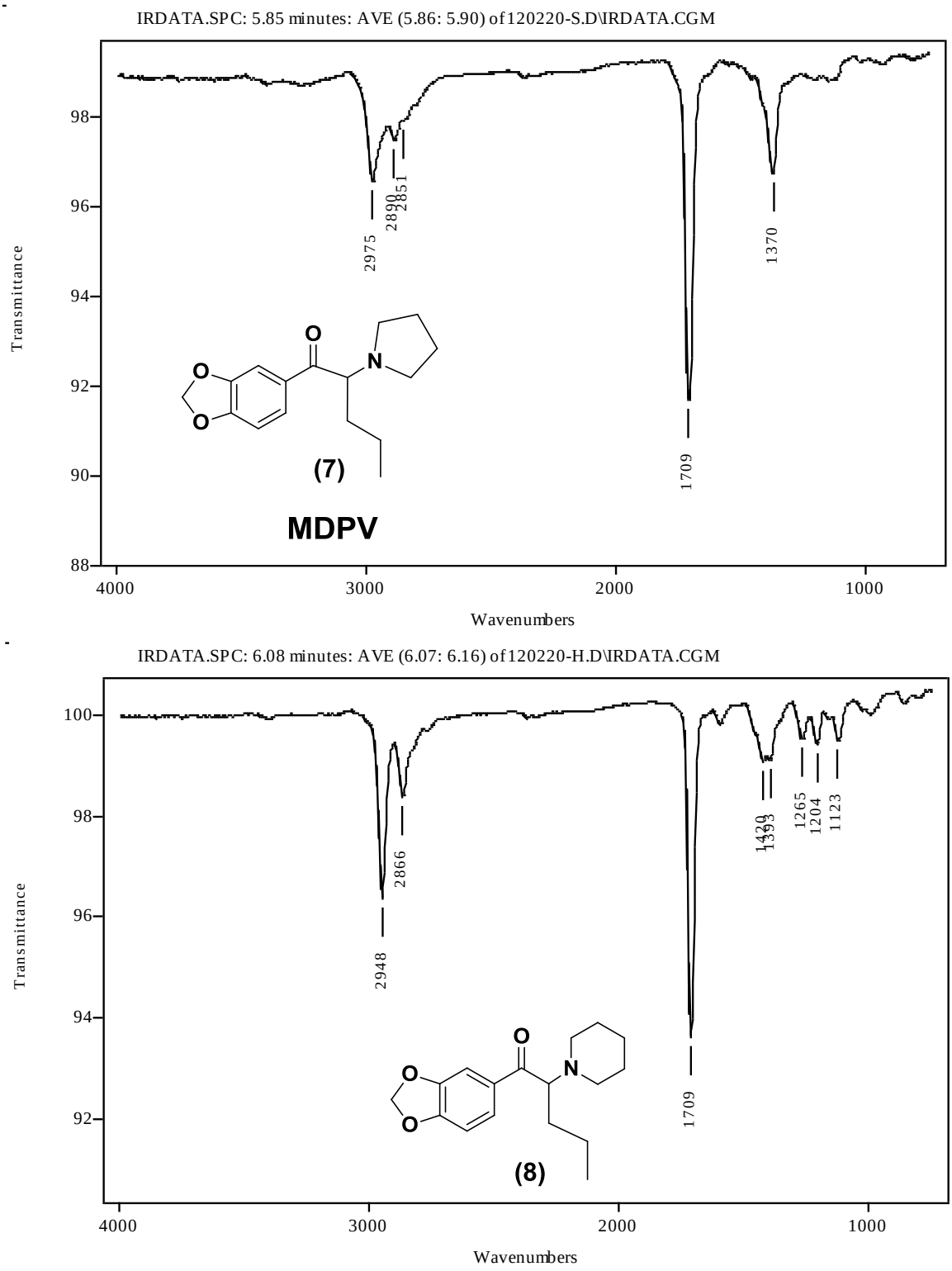


Figure 12: Vapor phase IR spectra of the 13 proposed homologous and regioisomeric aminoketones.

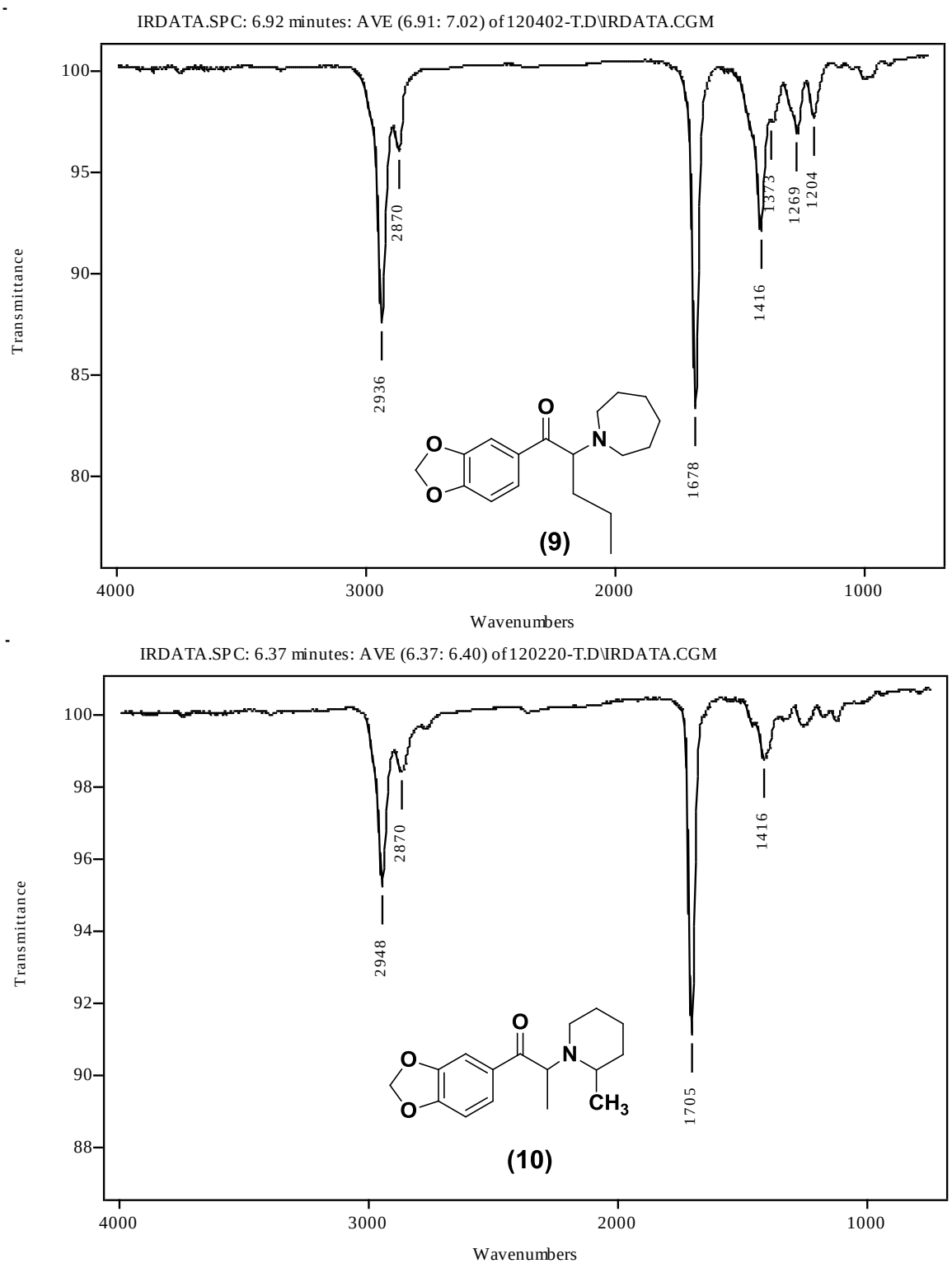


Figure 12: Vapor phase IR spectra of the 13 proposed homologous and regioisomeric aminoketones.

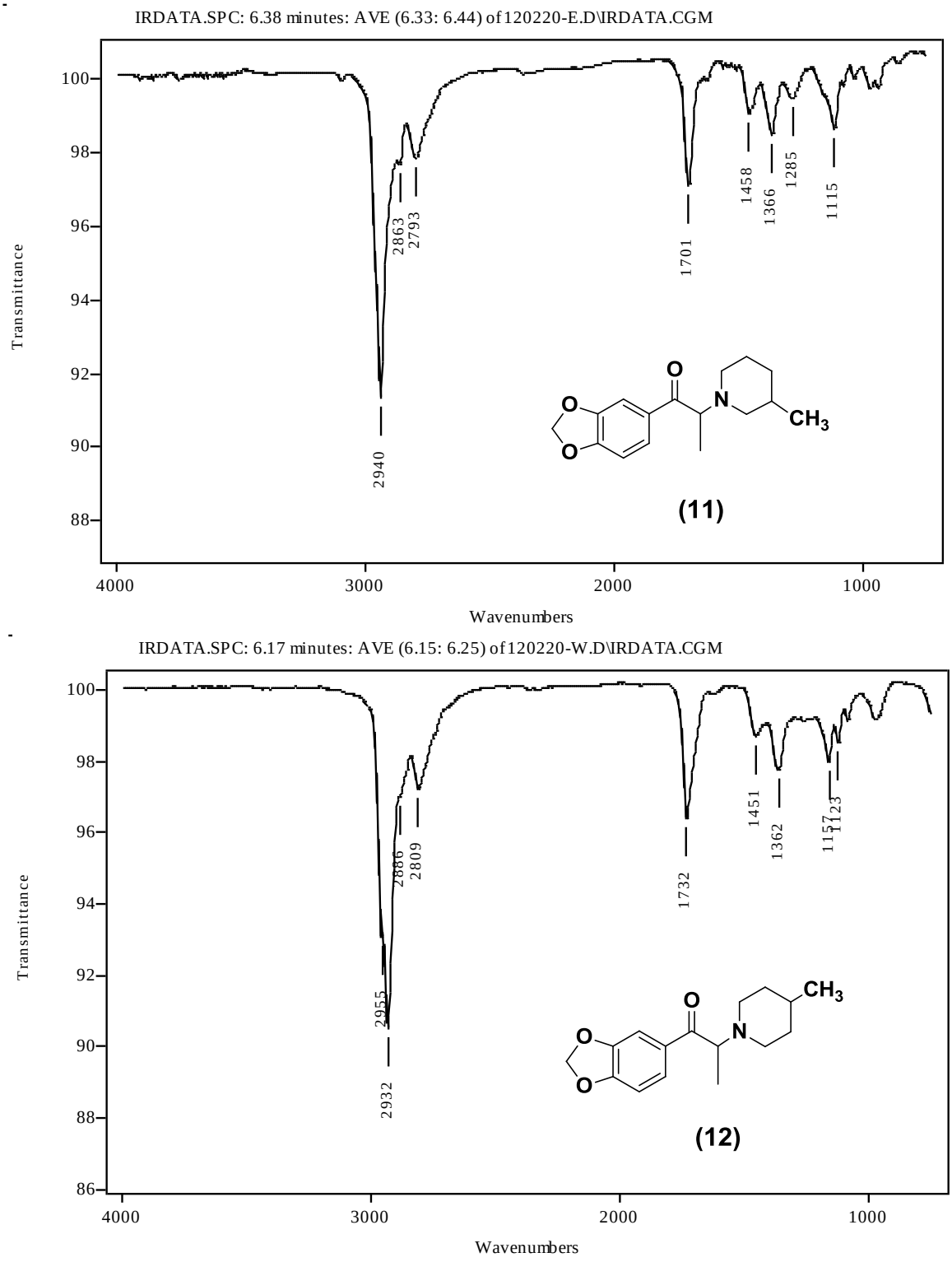


Figure 12: Vapor phase IR spectra of the 13 proposed homologous and regioisomeric aminoketones.

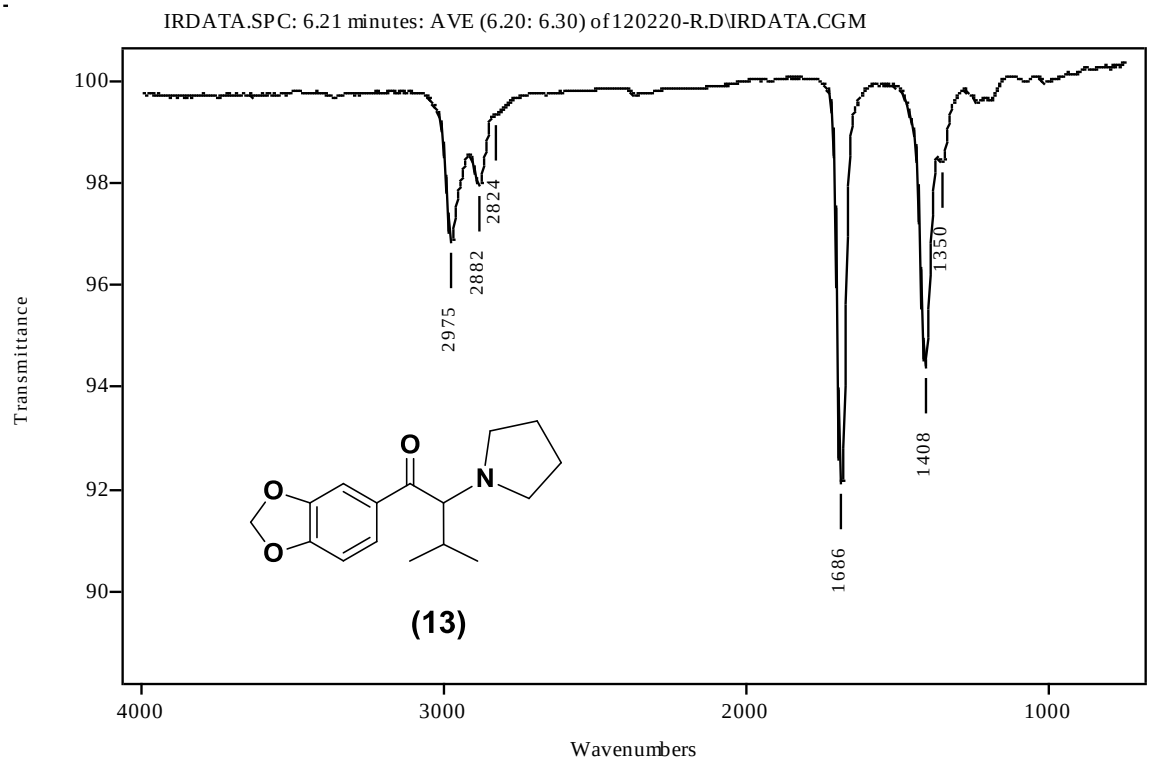


Figure 12: Vapor phase IR spectra of the 13 proposed homologous and regioisomeric aminoketones (continued).

In general, since all thirteen aminoketones have the same aromatic ring, share the same degree of nitrogen substitution, i.e. the same side chain; they have almost identical IR bands in the 650 –1700 cm^{-1} and 2700 – 3100 cm^{-1} regions. However, there is a slight difference between the regioisomers 3, 5 and 7 series and 7 and 13 series in terms of bands intensity (Figures 13 and 14).

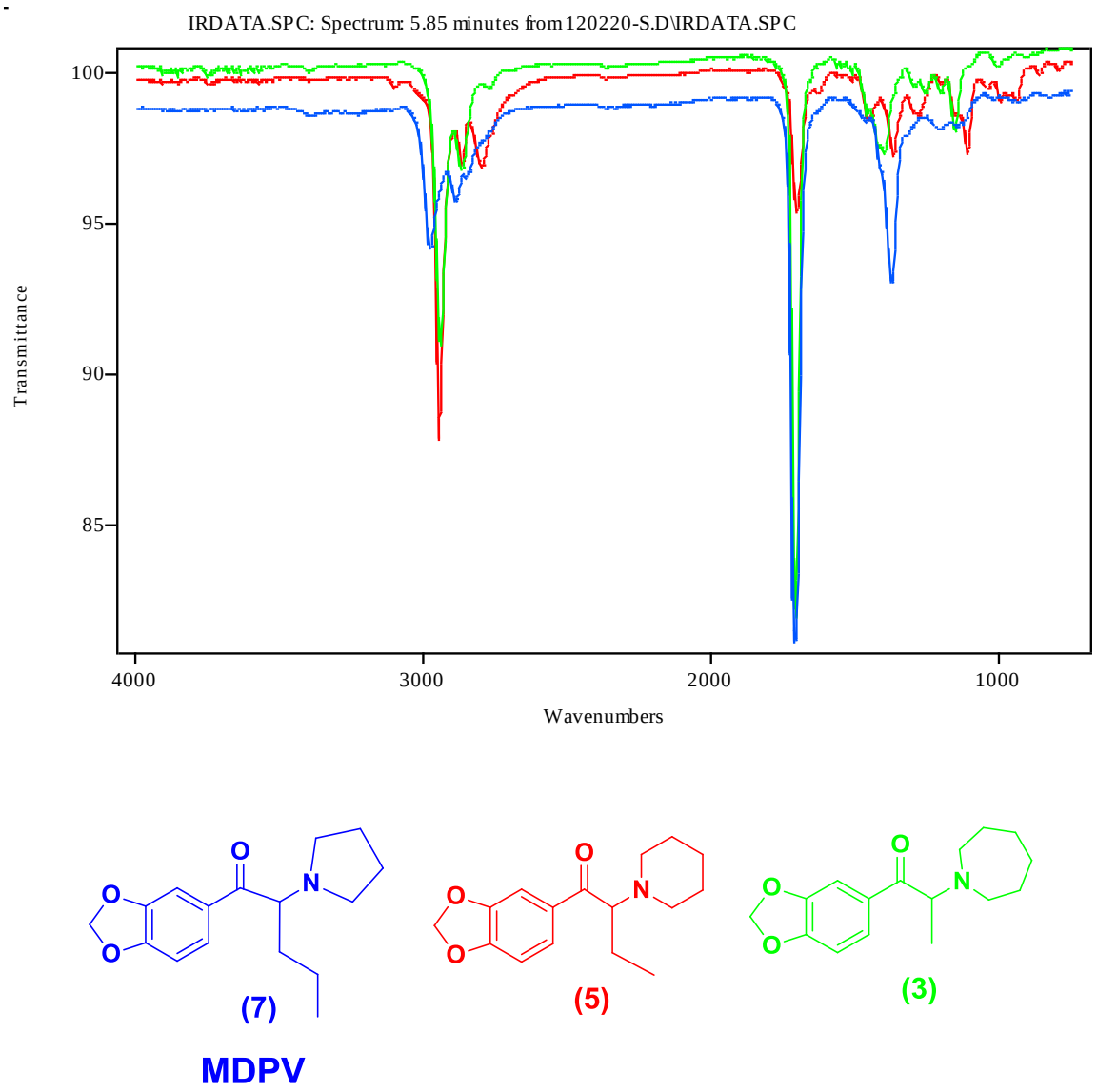


Figure 13: Overlayed vapor phase IR spectra of the regioisomers 3, 5 and 7.

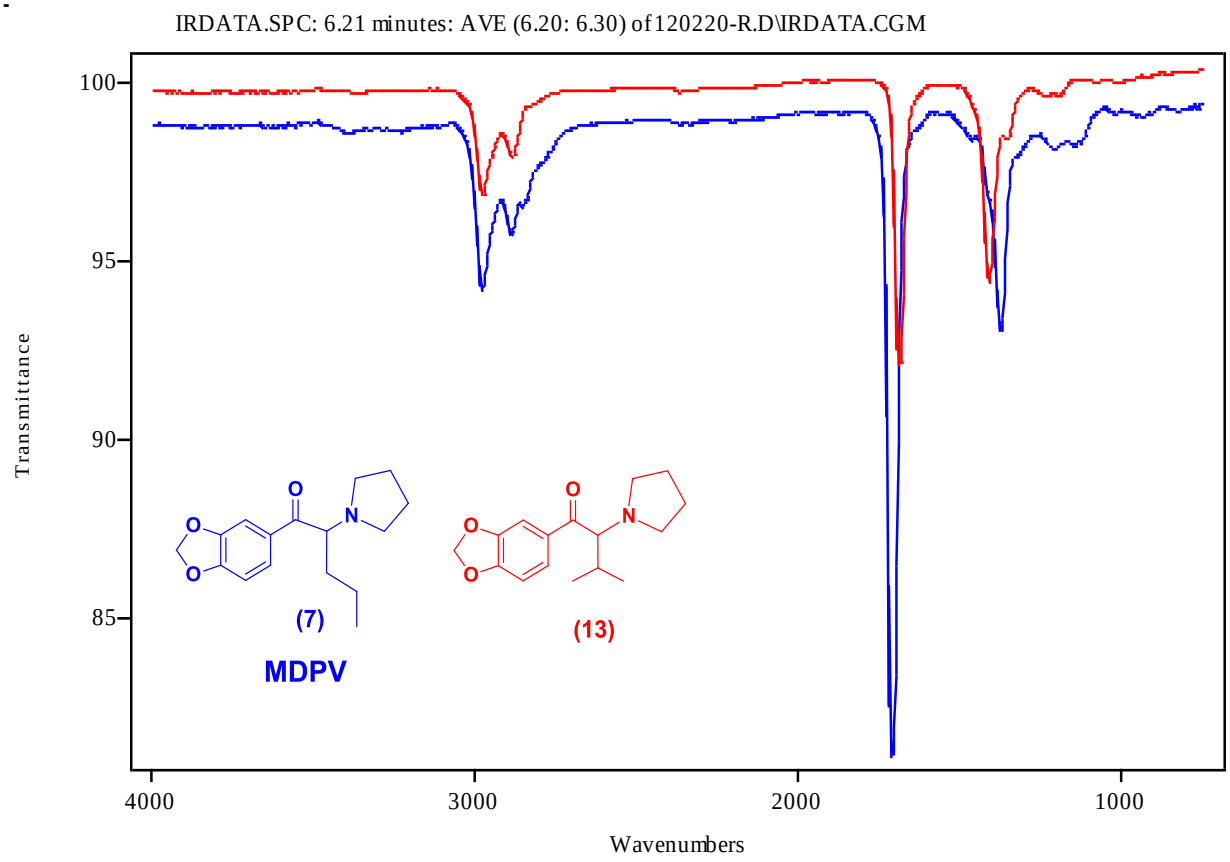


Figure 14: Overlayed vapor phase IR spectra of the regioisomers 7 and 13.

2.3.2.3. Gas chromatographic separation

2.3.2.3.1. Gas chromatographic separation of homologous aminoketones

Gas chromatographic separation of the homologous aminoketones was carried out using two stationary phases. Column one was a 30 m × 0.25 mm i.d. capillary coated with a 0.50 µm film of 5% phenyl– 95% methyl polysiloxane (Rtx-5 Sil). The separation was performed using a temperature program consisted of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes. Column two was a 30 m × 0.25 mm i.d. capillary coated with 0.50 µm film of 50% phenyl – 50% methyl polysiloxane (Rxi-50). The temperature program consisted of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

These studies were done using only the ring and side-chain homologous compounds 1-9 in this portion of the project. In this group of nine compounds the greatest number of homologous series is five compounds using both side-chain and ring size variables. There are several subsets of five compounds depending on the emphasis placed on side-chain or ring homologation. For example, compounds 1, 2, 3, 6, and 9 represent one subset of five while compounds 1, 4, 7, 8, and 9 represent another. Figure 15 shows all four possible subsets of five homologs each that can be obtained from compounds 1-9.

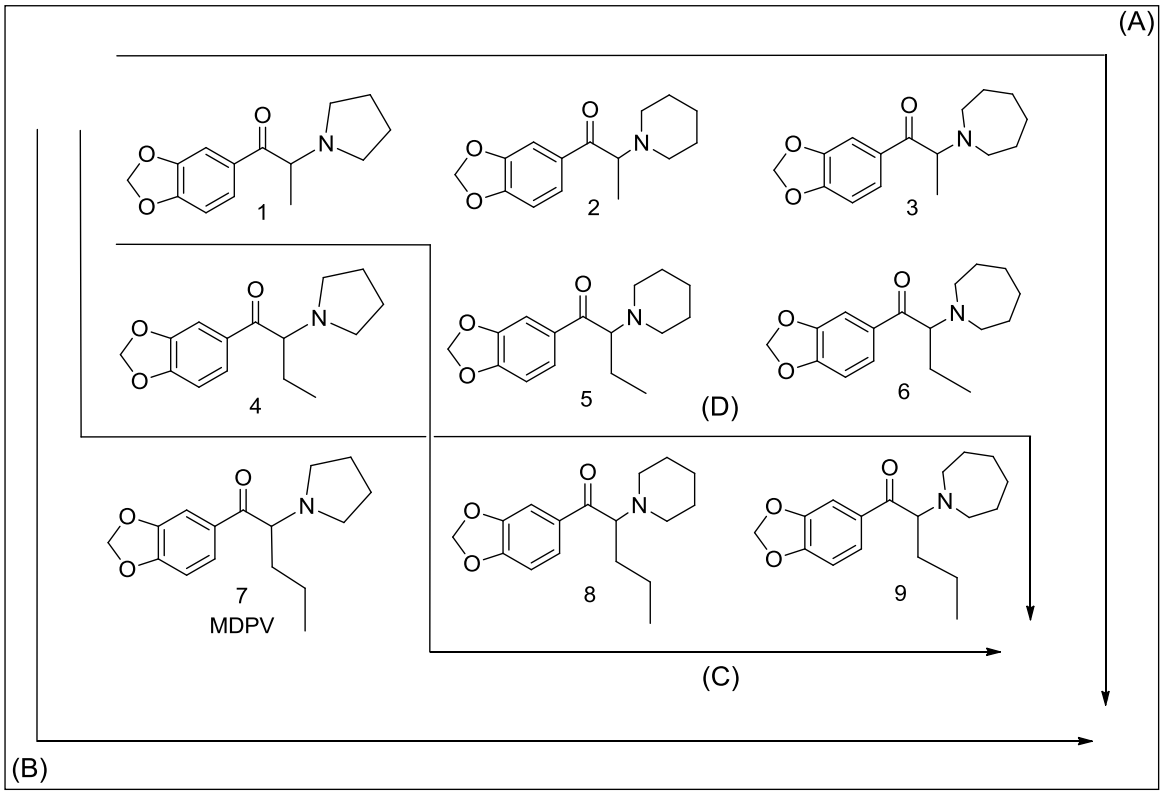


Figure 15: Four possible subsets of five homologs each from compounds 1-9. A: 1, 2, 3, 6, 9; B: 1, 4, 7, 8, 9; C: 1, 2, 5, 8, 9; D: 1, 4, 5, 6, 9.

Several temperature programs were evaluated and the chromatogram in Figure 16 is a representative of the result obtained for the first homologous subset (A) shown in Figure 15 which consists of (1, 2, 3, 6 and 9) compounds. This subset of compounds was resolved on the (Rtx-5 Sil) column. The first compound (1) and the last compound (9) represent the least and the highest masses in terms of homologs, respectively. Compound (1) gives the base peak at *m/z* 98 and eluted first, and compound (9) gives the base peak at *m/z* 154 and eluted last. This subset is a combination of homologs obtained by increasing the ring size (1, 2 and 3) and the alkyl side chain (3, 6 and 9). The separation

was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

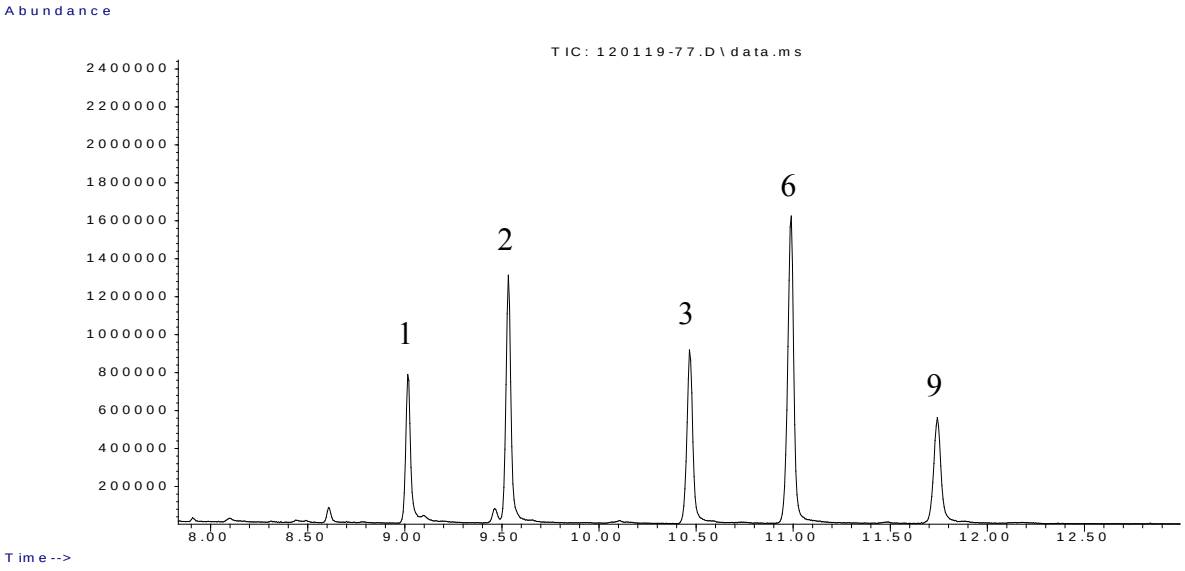


Figure 16: Gas chromatographic separation of the homologous aminoketones (subset A), 1, 2, 3, 6 and 9 on Rtx-5 Sil stationary phase.

The chromatogram in Figure 17 is a representative of the result obtained for the second homologous subset (B) shown in Figure 15 which consists of (1, 4, 7, 8 and 9) compounds. This subset of compounds was resolved on the (Rtx-5 Sil) column. The first compound (1) and the last compound (9) represent the least and the highest masses in terms of homologs, respectively. Compound (1) gives the base peak at *m/z* 98 and eluted first, and compound (9) gives the base peak at *m/z* 154 and eluted last. This is the only homologus subset that contains compound 7 (MDPV). MDPV is eluted in the middle according to its position in terms of homologous properties. This subset is a combination

of homologs obtained by increasing the alkyl side chain (1, 4 and 7) and the ring size (7, 8 and 9). The separation was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

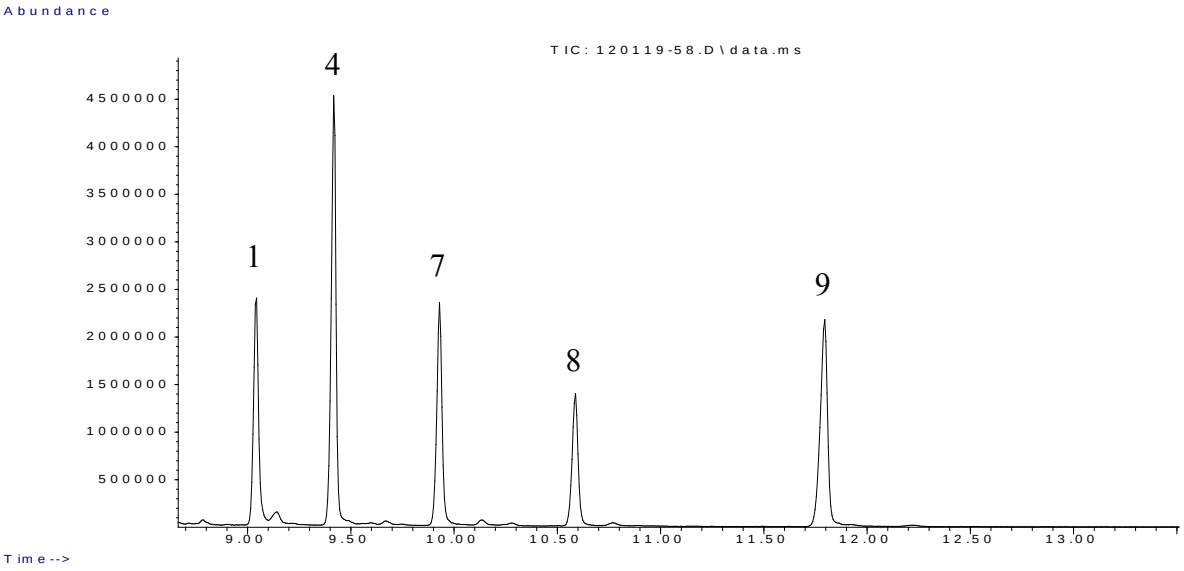


Figure 17: Gas chromatographic separation of the homologous aminoketones (subset B), 1, 4, 7, 8 and 9 on Rtx-5 Sil stationary phase.

The chromatogram in Figure 18 is a representative of the result obtained for the third homologous subset (C) shown in Figure 15 which consists of (1, 2, 5, 8 and 9) compounds. This subset of compounds was resolved on the (Rtx-5 Sil) column. The first compound (1) and the last compound (9) represent the least and the highest masses in terms of homologs, respectively. Compound (1) gives the base peak at *m/z* 98 and eluted first, and compound (9) gives the base peak at *m/z* 154 and eluted last, This subset is a

combination of homologs obtained by increasing the ring size (1 and 2) and the alkyl side chain (2, 5 and 8) and the ring size (8 and 9). The separation was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

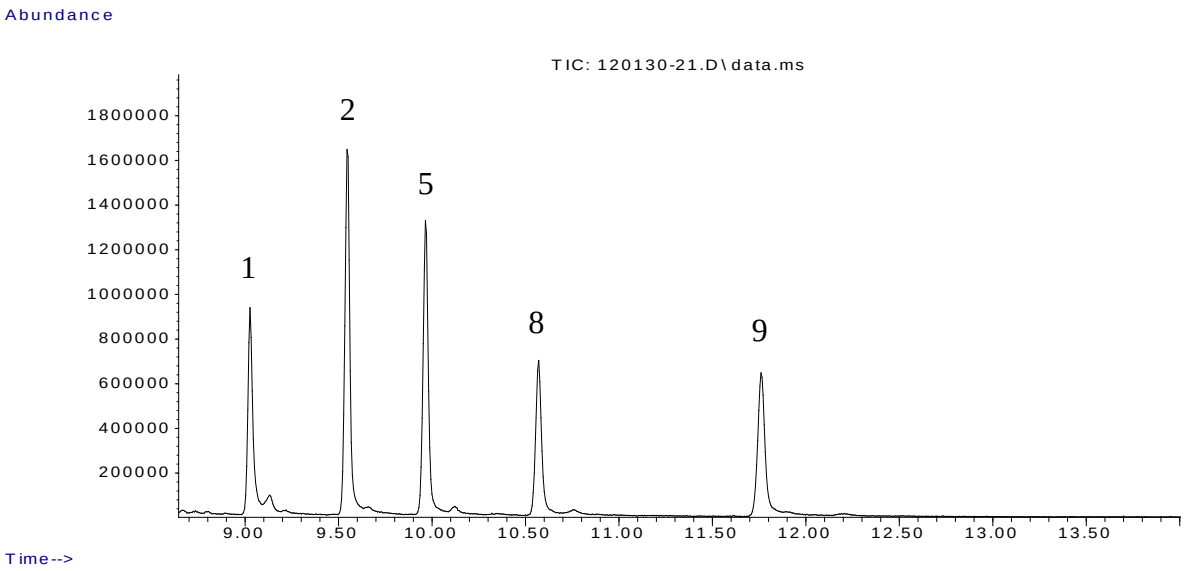


Figure 18: Gas chromatographic separation of the homologous aminoketones (subset C), 1, 2, 5, 8 and 9 on Rtx-5 Sil stationary phase.

The chromatogram in Figure 19 is a representative of the result obtained for the last homologous subset (D) shown in Figure 15 which consists of (1, 4, 5, 6 and 9) compounds. This subset of compounds was resolved on the (Rxi-50) column. The first compound (1) and the last compound (9) represent the least and the highest masses in terms of homologs, respectively. Compound (1) gives the base peak at *m/z* 98 and eluted first, and compound (9) gives the base peak at *m/z* 154 and eluted last, This subset is a combination of homologs obtained by increasing the alkyl side chain (1 and 4) and the

ring size (4, 5 and 6) and the alkyl side chain (6 and 9). The separation was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

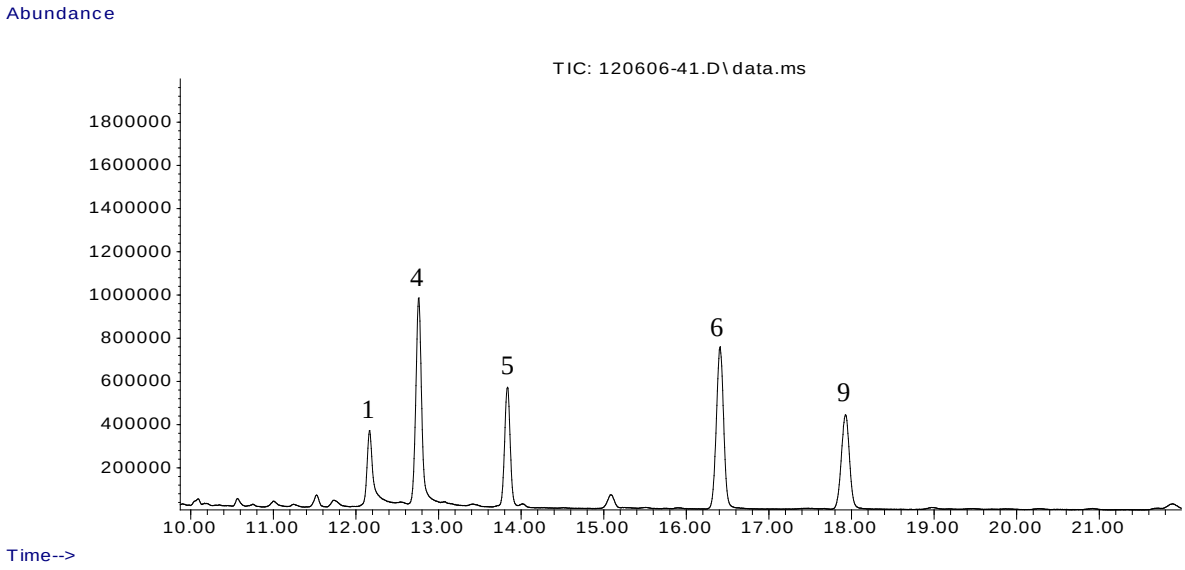


Figure 19: Gas chromatographic separation of the homologous aminoketones (subset D), 1, 4, 5, 6 and 9 on Rxi-50 stationary phase.

The chromatogram in Figure 20 shows the results obtained for the attempted separation of all nine side-chain and ring size homologs (1-9) and this sample mixture also contains compound 13, the isobutyl side-chain regioisomer of MDPV (compound 7), this subset of compounds was resolved on the (Rxi-50) column. The separation was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

Essentially base line resolution was obtained in this chromatogram for each adjacent set of peaks with one exception (compounds 5 and 7). This one exception is a set of two compounds (5 and 7) representing regioisomeric equivalents. It is interesting that compound 7, MDPV is one of the members of this set and its regioisomeric equivalent (compound 5) represent two molecules of equivalent mass spectra and equivalent mass spectral base peaks at *m/z* 126. Additionally, compound (1) represents the least one in terms of homologous properties and eluted first, while compound (9) represents the highest one and eluted last. Gas chromatographic separation of all homologous aminoketones (1-9) as well as compound 13 is shown in Figure 20.

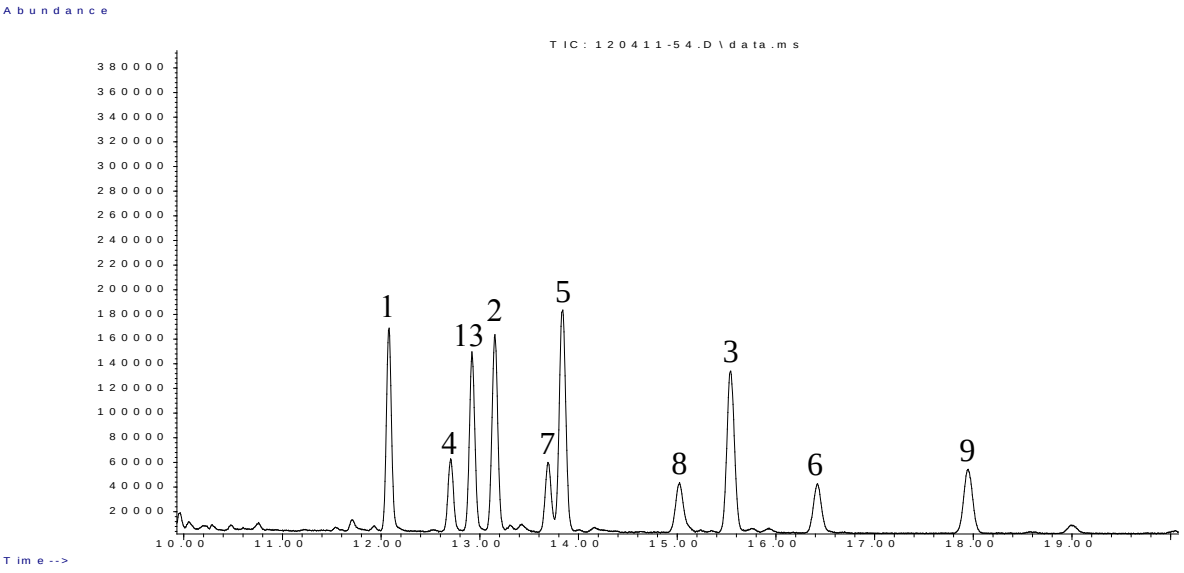


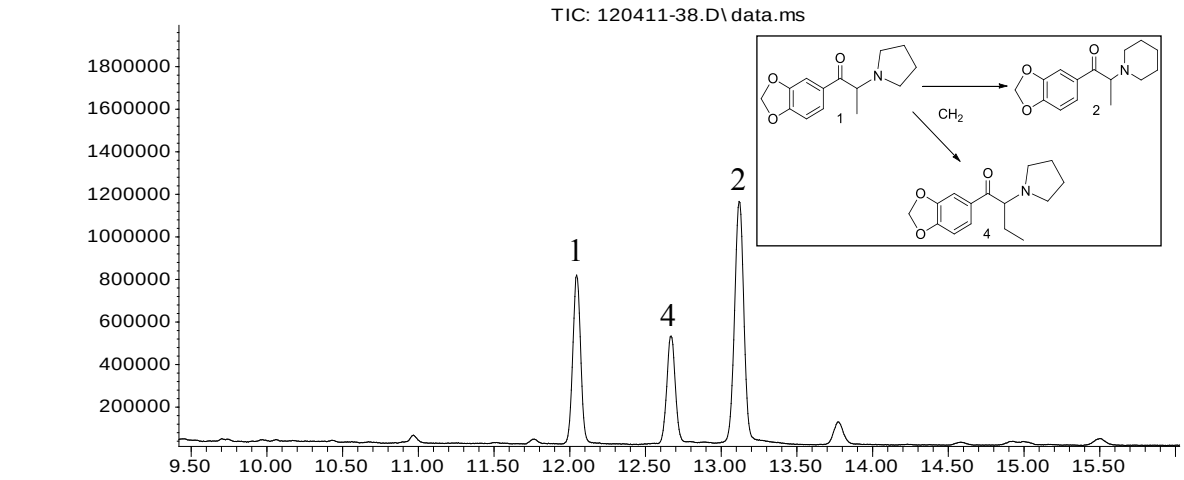
Figure 20: Gas chromatographic separation of all homologous aminoketones (1-9) and 13 on Rxi-50 stationary phase.

The thirteen aminoketones show similar mass spectra pattern with the same fragmentation process producing ions at *m/z* 149 and 121 and a base peak ranging from *m/z* 98 (compound 1) and *m/z* 154 (compound 9). The regioisomers (3, 5, 7, 10, 11, 12, and 13) have the same fragmentation pattern producing a base peak at *m/z* (126). The molecular ion peak is absent from each spectrum because of the very active radical site alpha-cleavage reaction initiated by the nitrogen and fragments the compound completely. Each compound shows a vapor phase IR spectrum with bands in the regions 650 –1700 cm^{-1} and 2700 – 3100 cm-1 which represent the carbonyl and the amine groups respectively. Each compound shows a vapor phase IR spectrum with bands in the regions 650 –1700 cm^{-1} and 2700 – 3100 cm-1 which represent the carbonyl and the amine groups respectively. The four subsets that consist of five homologs are well resolved on the Rtx-5 Sil and Rxi-50 stationary phases. They are eluted according to their homologous order from lower to higher. All nine side-chain and ring size homologs in addition to compound 13, the isobutyl side-chain regioisomer of compound 7 are well resolved on Rxi-50 stationary phase.

2.3.2.3.2. Comparison of the chromatographic properties of the homologous aminoketones

In this study, we made a comparison between three homologous compounds, we fixed the first one as a standard, and the other two are homologs to the first, one by increasing the ring size by adding a methylene group (CH_2, 14 mass units) and the other by making the side chain longer by adding a methylene group (CH_2, 14 mass units). After running these four different subsets of three homologs each, we confirmed that the increase in ring size has the greater effect on retention (increases retention) than the increase in side chain. Essentially base line resolution was obtained in this chromatogram for each adjacent subset of peaks with one exception (compounds 5 and 7). This one exception is a subset of two compounds (5 and 7) representing regioisomeric equivalents having equivalent mass spectra and equivalent mass spectral base peaks at *m/z* 126.

Gas chromatographic separation of these homologous aminoketones was carried out using a stationary phase which has 30 m × 0.25 mm i.d. capillary coated with 0.50 µm film of 50% phenyl – 50% methyl polysiloxane (Rxi-50). The temperature program consisting of an initial temperature of 70^{o}C for 1 minute ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes. Gas chromatographic separation of all four subsets of three homologous aminoketones each (1-8) is shown in Figure 21.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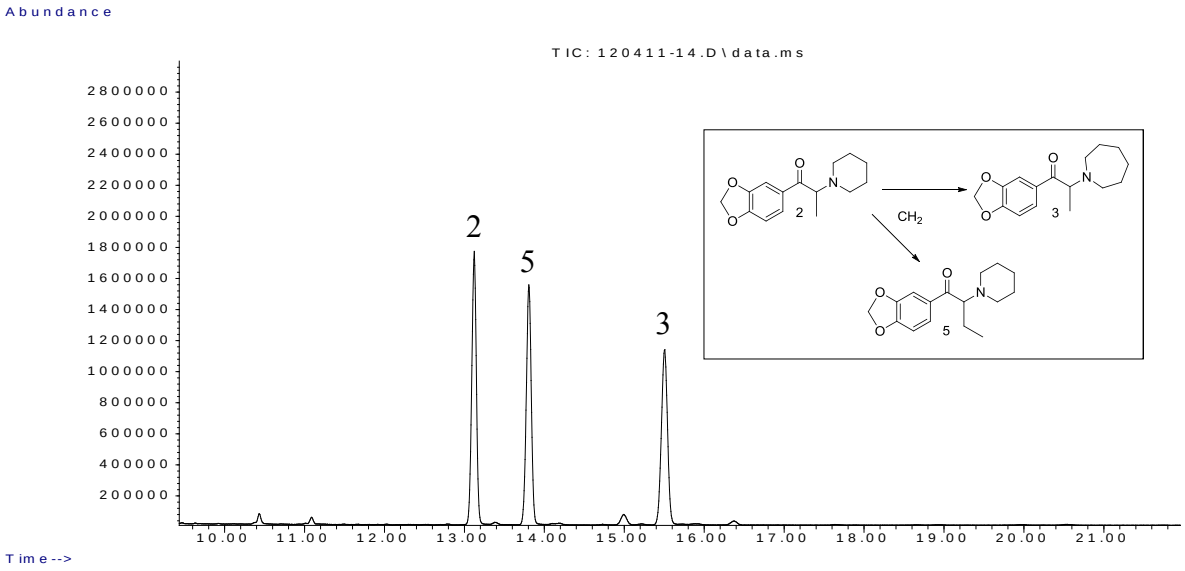
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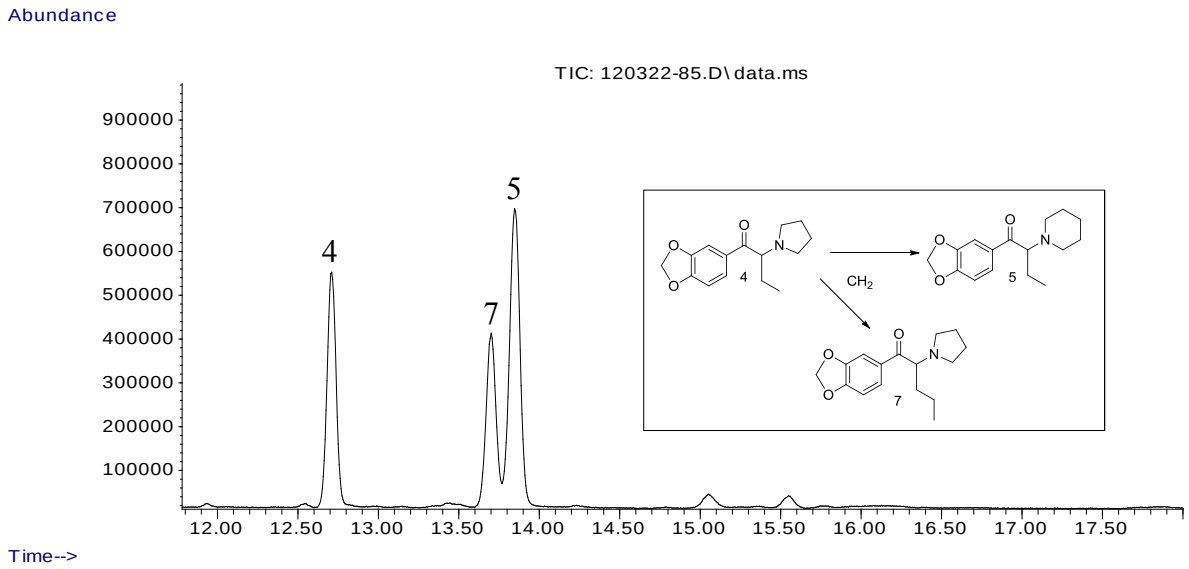

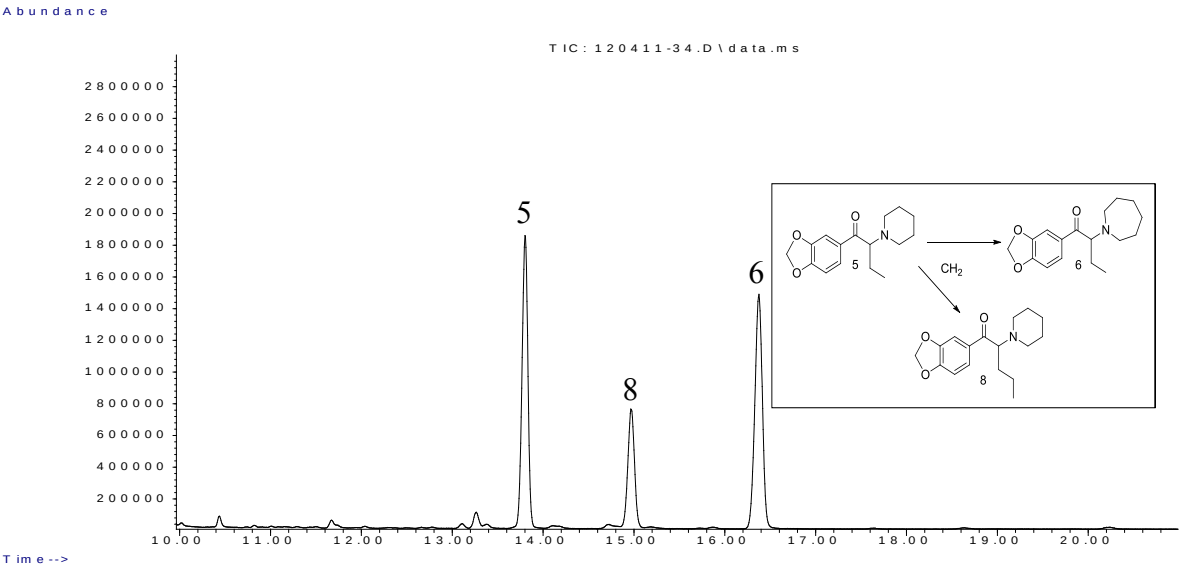

Figure 21: Comparison of chromatographic properties of homologous aminoketones (1-8) on Rxi-50 stationary phase.

Comparison of the homologous properties of four different subsets of three homologs each is accomplished by increasing the ring size for a particular compound, and making the alkyl side chain longer for the same compound. This comparison was consistent for all four subsets and indicates that the ring size has the greater effect than the alkyl side chain on the retention (the bigger the ring size the longer the retention).

2.3.2.3.3. Gas chromatographic separation of regioisomeric aminoketones

Gas chromatographic separation of the regioisomeric aminoketones was carried out using two stationary phases. Column one was a 30 m ×0.25 mm i.d. capillary coated with 0.5 μm film of 100% dimethyl polysiloxane (Rtx-1). The separation was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes. The second column was a 30 m × 0.25 mm i.d. capillary coated with 0.50 μm film of 50% phenyl – 50% methyl polysiloxane (Rxi-50). Two temperature programs were used; the first temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes, while the second temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 150^{o}C at a rate of 5^{o}C per minute followed by a hold at 150^{o}C for 2 minutes, and then ramped up to 250^{o}C at a rate of 10^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

The chromatogram in Figure 22 shows the results obtained for the attempted separation of compound 7, MDPV and compound 13, the isobutyl side-chain regioisomer of MDPV. It is clear that the branched side chain eluted before the straight side chain and this has been proved through the precursor ketones (D and C) which represent precursor ketones for aminoketones (13 and 7), respectively. Gas chromatographic separation of these two regioisomers was carried out using Rtx-1 stationary phase. The separation was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

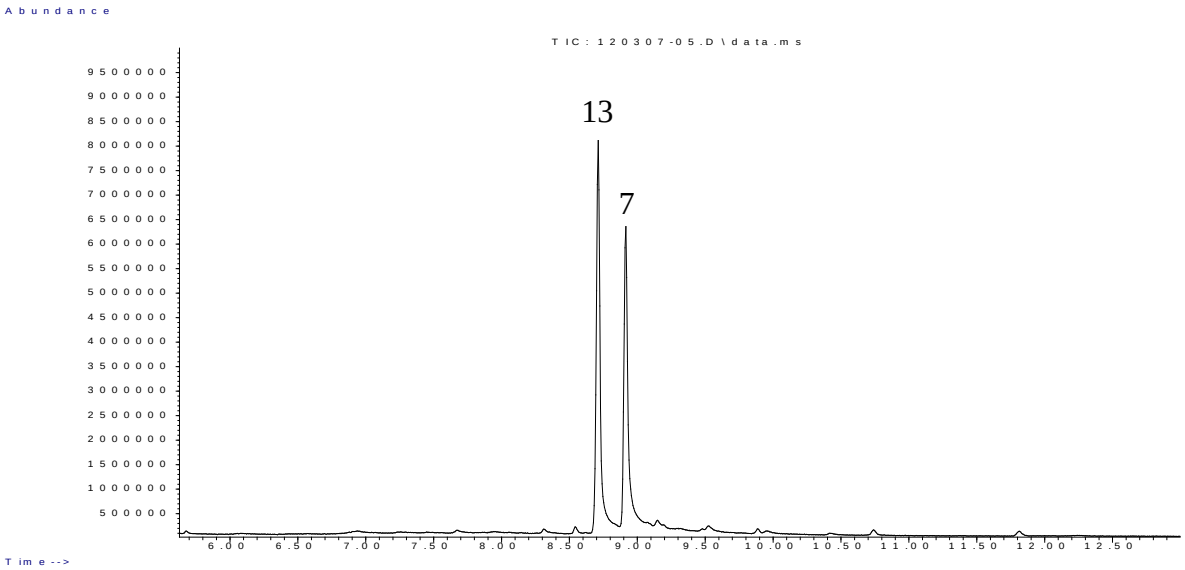

Figure 22: Gas chromatographic separation of the regioisomeric aminoketones (7 and 13) on Rtx-1 stationary phase.

The chromatogram in Figure 23 shows the results obtained for separation the regioisomers 3, 5, 7, and 13. Compound 13, the isobutyl side-chain regioisomer of MDPV eluted first and this is the same expected result from the previous chromatogram (Figure 21). Additionally, by comparing the elution order of the regioisomers 3, 5 and 7, it is obvious that the ring size has an effect on the elution order, consequently, the compound with the five membered pyrrolidine ring (compound 7) eluted before the one with seven membered perhydroazepine ring (compound 3). Gas chromatographic separation of these regioisomers was carried out using Rxi-50 stationary phase. The separation was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

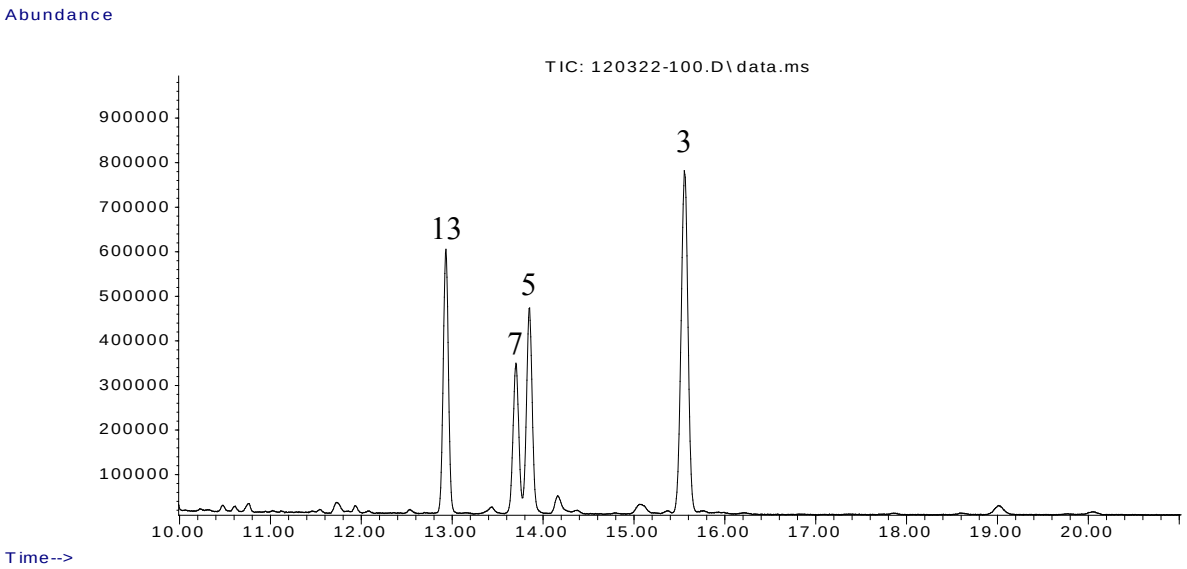

Figure 23: Gas chromatographic separation of the regioisomeric aminoketones (3, 5, 7 and13) on Rxi-50 stationary phase.

The analysis of drugs of abuse must depend on chromatographic resolution in addition to mass spectrometry. When other compounds with nearly identical mass spectra exist with the drug of interest, the identification by (GC-MS) must focus on the ability of the chromatographic system to separate the non-drug (imposter molecules) from the drug of abuse. In the absence of the appropriate reference standard compounds and with the possibility of co elution of the regioisomers with the drug (s) of interest in chromatographic separations, these related compounds could be mistaken for the drug of abuse itself. The chromatogram in Figure 24 shows that compound 5 is co-eluted with MDPV under reasonable chromatographic conditions and the misidentification is possible in this case.

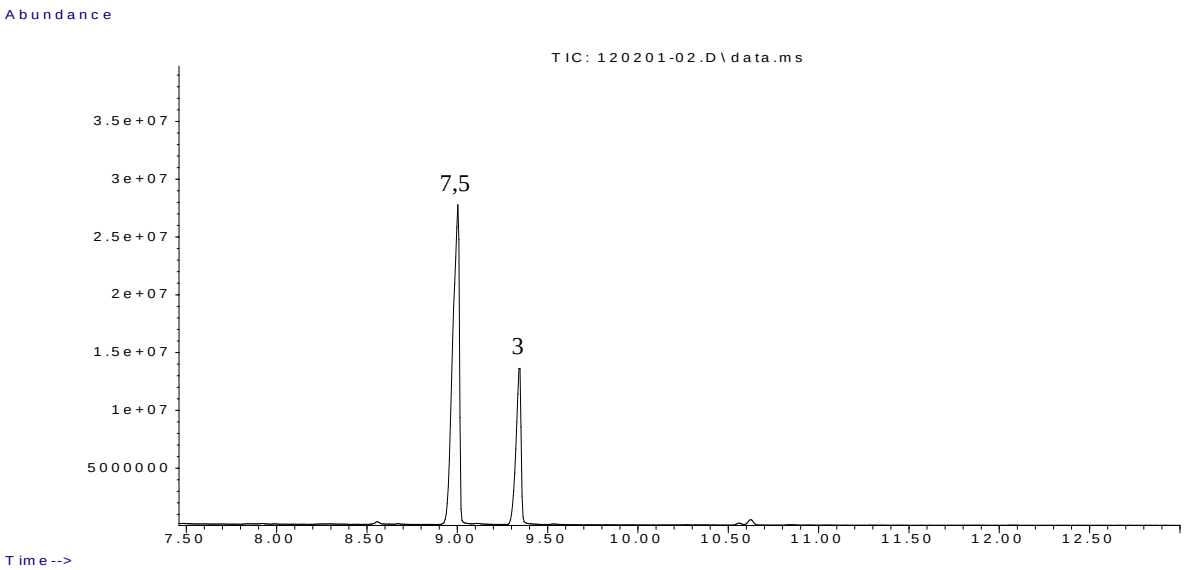

Figure 24: Gas chromatographic separation of the regioisomeric aminoketones (3, 5 and 7) showing co-elution of compound 5 and MDPV on Rtx-1 stationary phase.

The chromatogram in Figure 25 shows the results obtained for separation of the regioisomers 7, 10, 11, 12, and 13. Compound 13, the isobutyl side-chain regioisomer of MDPV eluted first. Additionally, compound 7, MDPV, is co-eluted with compound 12. Compound 10 has two peaks which is most likely due to a diasteriomeric form. The reason that compounds 10, 11 and 12 are very close and there is no good resolution in the base line is that they are very similar and very close regioisomers and the only difference is the position of the methyl group attachment to the piperidine ring. Gas chromatographic separation of these regioisomers was carried out using an Rxi-50 stationary phase. The separation was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

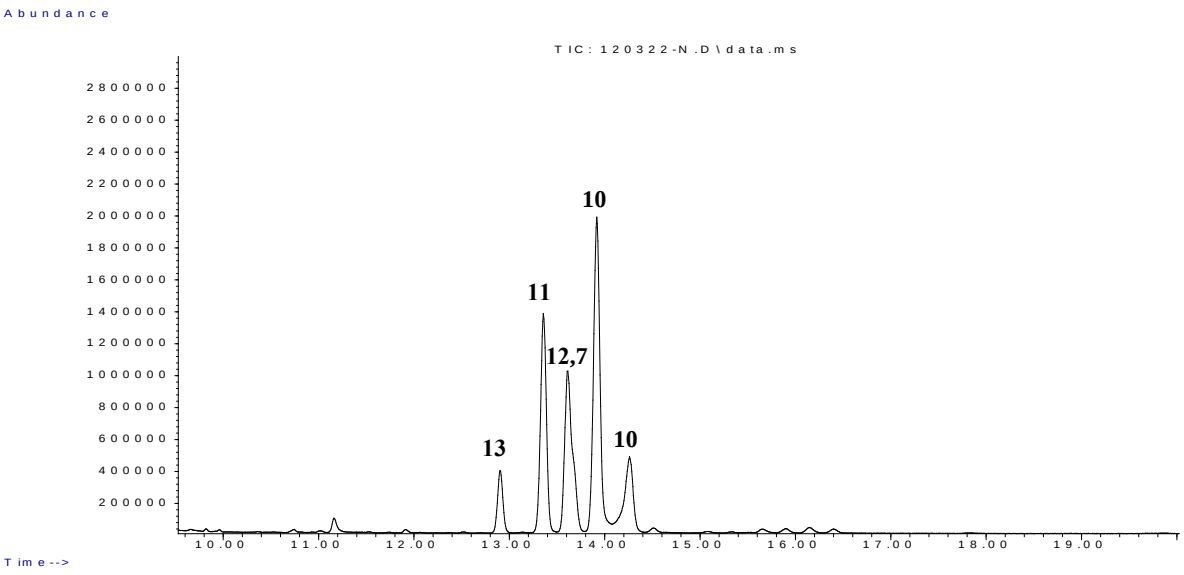

Figure 25: Gas chromatographic separation of the regioisomeric aminoketones (7, 10, 11, 12 and13) on Rxi-50 stationary phase.

The chromatogram in Figure 26 shows the results obtained for separation the regioisomers 7, 10, 11, 12, and 13. A temperature program with long retention time was used and compounds 7 and 12 were resolved although there was not a good resolution in the base line. Gas chromatographic separation of these regioisomers was carried out using Rxi-50 stationary phase. The separation was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 150^{o}C at a rate of 5^{o}C per minute followed by a hold at 150^{o}C for 2 minutes, and then ramped up to 250^{o}C at a rate of 10^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

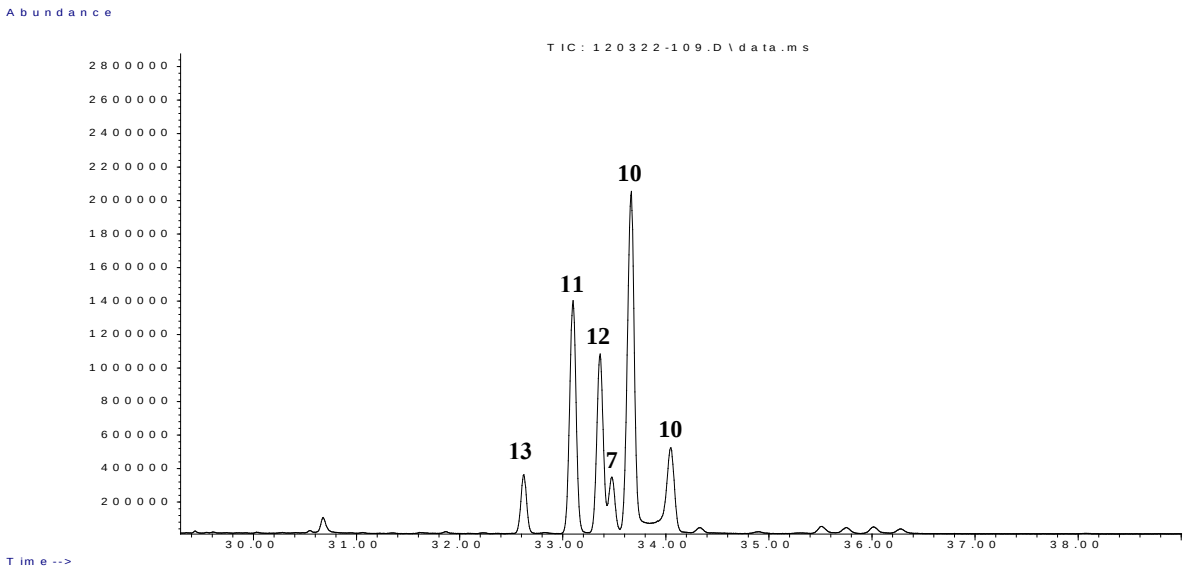

Figure 26: Gas chromatographic separation of the regioisomeric aminoketones (7, 10, 11, 12 and13 on Rxi-50 stationary phase.

The chromatogram in Figure 27 shows the results obtained for separation of the complete set of regioisomers in this project, compounds 3, 5, 7, 10, 11, 12 and 13. Compound 13, the isobutyl side-chain regioisomer of MDPV eluted first, compound 3 eluted last. Compounds 5 and 10 are co-eluted, and there is poor resolution in the base line between compounds 12, 7, 5, and 10. Gas chromatographic separation of these regioisomers was carried out using an Rxi-50 stationary phase. The separation was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 150^{o}C at a rate of 5^{o}C per minute followed by a hold at 150^{o}C for 2 minutes, and then ramped up to 250^{o}C at a rate of 10^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

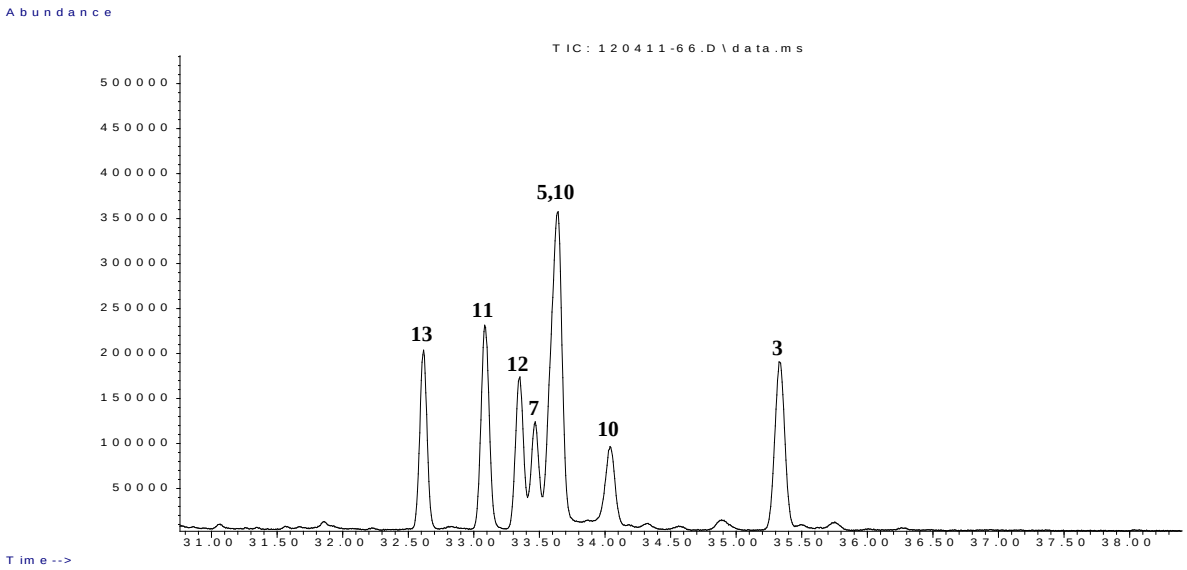

Figure 27: Gas chromatographic separation of all regioisomeric aminoketones (3, 5, 7, 10, 11, 12 and 13) on Rxi-50 stationary phase.

To prove that compound 10 in the previous three chromatograms has two peaks, an individual sample of compound 10 has been injected with the same temperature program and in the same stationary phase used to separate the regioisomeric series in the previous three figures (25, 26, and 27). The temperature program consisting of an initial temperature of 70^{o}C for 1 minute ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes. Two peaks have been obtained (10 A and 10 B) and this is shown in Figure 28.

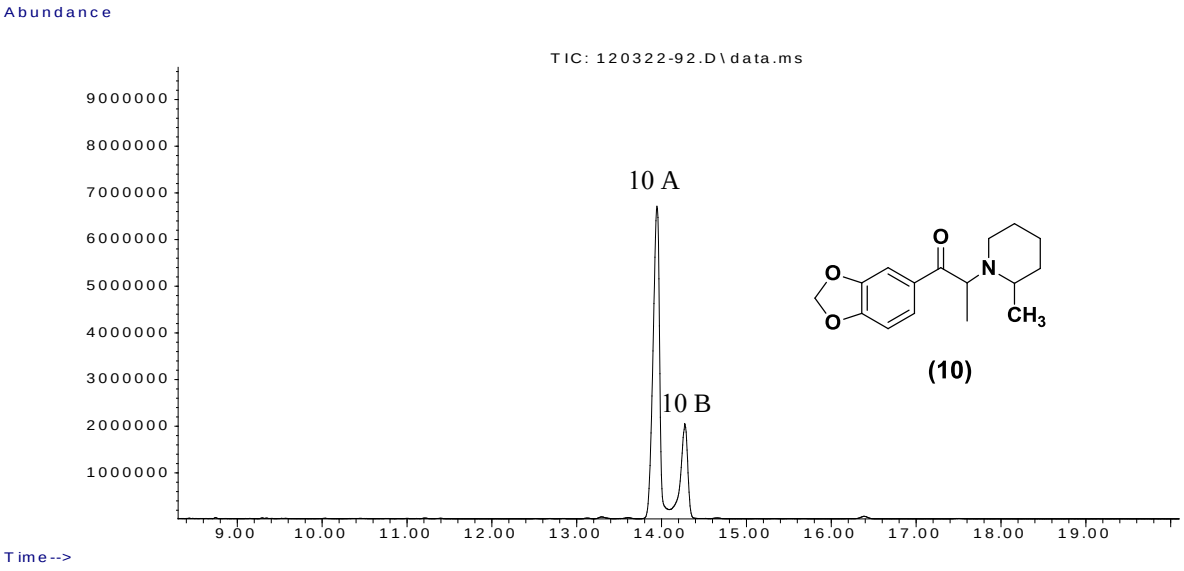

Figure 28: Gas chromatographic peak of compound 10 on Rxi-50 stationary phase.

Mass spectra of these two peaks confirmed that they have the same fragmentation pattern producing the same base peak at m/z 126; this clarification is shown in Figure 29.

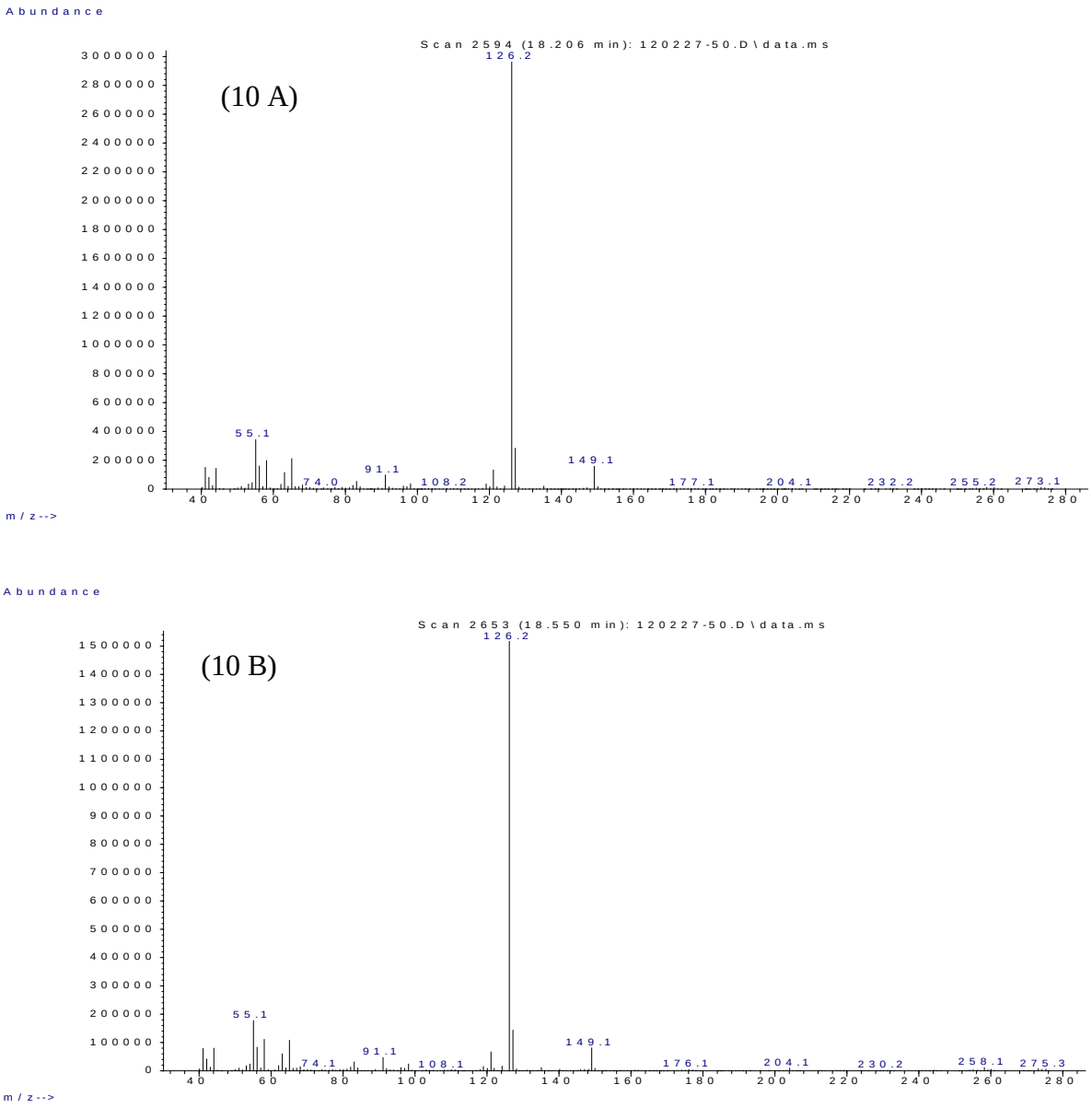

Figure 29: Mass spectra of the two peaks of compound 10.

2.4. Summary and conclusions

A series of 13 homologous and regioisomeric aminoketones related to the designer synthetic cathinone derivative MDPV were synthesized in this project. These compounds were all prepared from a common precursor chemical, piperonal (3,4 methylenedioxybenzaldehyde). The analytical properties of these compounds were compared by GC-MS and GC-IR techniques. These aminoketones show major fragments in their mass spectra corresponding to the regioisomeric and homologous immonium cation fragments primarily from the loss of 3,4-methylenedioxybenzoyl radical species. All these compounds in this project show equivalent EI MS fragments for the 3,4-methylenedioxybenzoyl fragments (*m/z* 149) and the methylenedioxybenzene fragment at *m/z* 121. The *m/z* 149 results from ionization of the carbonyl oxygen followed by an alpha-cleavage fragmentation. The loss of CO from this ion yields the *m/z* 121 fragment common to all spectra.

The regioisomeric aminoketones yield equivalent mass spectra including mass equivalent regioisomeric immonium cation base peak. Many subsets of these compounds have almost identical mass spectra to that of the designer drug MDPV, these regioisomers are of the same MW, base peak and other fragment ions as that found for MDPV. The vapor phase infrared spectra allow for the differentiation of the regioisomers of mass spectral equivalence.

An evaluation of the effects of homologation on gas chromatographic retention showed that addition of a methylene (CH_2) in the nitrogen-containing ring increases retention more than the equivalent group added to the alkyl side-chain.

3. Experimental

3.1. Chemicals, instrumentation, and methods

3.1.1. Chemicals

3,4-(methylenedioxy)propiophenone, 3,4-(methylenedioxy)butyrophenone, pyrrolidine, 2-methylpiperidine, 3-methylpiperidine, 4-methylpiperidine, hexamethyleneimine, bromine liquid, and piperonal were obtained from Alfa Aesar (Ward Hill, MA, USA). Sodium hydroxide and potassium hyroxide were obtained from Acros Organics (Morris Plains, New Jersey, USA). Butylmagnesium chloride, isobutylmagnesium bromide, and triethylamine were obtained from Sigma-Aldrich (St Louis, MO, USA). Pyridine and anhyrous sodium sulfate were obtained from EMD Millpore Chemicals (Merck KGaA, Darmstadt, Germany). HPLC grade acetonitrile, methylene chloride, diethyl ether, celite, sodium chloride and chloroform were obtained from Fisher Scientific (Fair Lawn, N.J., USA).

3.1.2. Instrumentation

GC–MS analysis was performed using a 7890A gas chromatograph with a 7683B auto injector coupled with a 5975C VL mass selective detector obtained from Agilent Technologies (Santa Clara, CA). The mass spectral scan rate was 2.86 scans /s. The GC was operated in splitless mode with a helium (grade 5) flow rate at 0.7 mL/min and the column head pressure was 10 psi. The MS was operated in the electron impact (EI) mode using an ionization voltage of 70 eV and a source of temperature of 230°C. The GC

injector was maintained at 250°C and the transfer line at 280°C.

GC-IRD studies were carried out using a Hewlett-Packard 5890 Series II gas chromatograph and a Hewlett-Packard 7673 auto-injector coupled with an IRD-II infrared detector (IRD-II) obtained from Analytical Solutions and Providers (ASAP), Covington, KY. The vapor phase infrared spectra were recorded in the range of 4000 – 650 cm^{-1} with a resolution of 8 cm^{-1} and a scan rate 1.5 scans per second. The IRD flow cell and transfer line temperatures were maintained at 280ºC and the GC was operated in the splitless mode with a carrier gas (helium grade 5) flow rate of 0.7 mL/min and a column head pressure of 10 psi.

3.1.3. GC-columns

Different capillary GC columns were evaluated throughout the course of this work, however only columns showing a good compromise between resolution and analysis time are listed in Table 1. All columns used were obtained from Restek Corporation (Bellefonte PA, USA) and have the same dimensions, 30m x 0.25mm-i.d. and coated with 0.25 µm film depth of polysiloxane.

Table 1: List of columns used and their composition

Column name	Column composition
Rtx-1	100% dimethyl polysiloxane
Rtx-5	95% dimethyl-5%diphenyl polysiloxane
Rtx-5 Sil	5% methyl-95% methyl polysiloxane
Rtx-200	100% trifluoropropyl methyl polysiloxane
Rxi-50	50% phenyl–50% methyl polysiloxane

3.1.4. Temperature programs

Table 2: List of temperature programs used

Temperature program name	Injector temperature °C	Detector temperature °C	Program setup
TP-1	250	280	Hold column temperature at 70°C for 1 minute then the temperature was ramped up to 250°C at a rate of 30°C / minute and set at 250°C for 5 min
TP-2	250	280	Hold column temperature at 100°C for 1 minute then the temperature was ramped up to 180°C at a rate of 12°C /minute. Column temperature was held at 180°C for 2 minutes then was ramped up to 200°C at a rate of 1°C / minute and set at 200°C for 5 minutes
TP-3	250	280	Hold column temperature at 100°C for 1 minute then the temperature was ramped up to 180°C at a rate of

			9°C /minute. Column temperature was held at 180°C for 2 minutes then was ramped up to 200°C at a rate of 10°C / minute and set at 200°C for 5 minutes
TP-4	250	250	Hold column temperature at 70°C for 1 minute then the temperature was ramped up to 150°C at a rate of 5°C /minute. Column temperature was held at 150°C for 2 minutes then was ramped up to 250°C at a rate of 10°C / minute and set at 250°C for 15 minutes

3.2. Synthesis of the regioisomeric and homologous aminoketones

3.2.1. Preparation of 3,4-(methylenedioxy)phenones

3.2.1.1. Preparation of 3,4-(methylenedioxyphenyl)pentan-1-one

A solution of piperonal (6g, 40 mmol) in 50 ml of dry diethyl ether was added to a flask and maintained under an atmosphere of dry nitrogen. Butylmagnesium chloride solution in diethyl ether (30ml, 60 mmol) was added with a syringe and the reaction mixture was stirred at -20 ^{o}C for two hours. The reaction mixture was quenched using 1N hydrochloric acid (25 ml) and the ether layer was separated, washed with water and dried over anhydrous sodium sulfate overnight. The ether layer was filtered and evaporated under reduced pressure to yield the 3,4-(methylenedioxyphenyl)pentan-1-ol (7.19g).

A solution of 3,4-(methylenedioxyphenyl)pentan-1-ol (7.19g) in methylene chloride (60 ml) were stirred overnight at room temperature with PCC (6.5 g, 30 mmol) and Celite (6.5 g). The reaction mixture was diluted with diethyl ether (150 ml), stirred for 30 minutes then, vacuum filtered on a pad of silica gel (200-400 mesh). The organic layer was evaporated under reduced pressure to yield 3,4-(methylenedioxyphenyl)pentan-1-one (5g) which was purified using Kugelrohr distillation.

3.2.1.2. Preparations of 3,4-(methylenedioxyphenyl)isopentan-1-one

A solution of piperonal (3g, 20 mmol) in 50 ml of dry diethyl ether was added to a flask and maintained under an atmosphere of dry nitrogen. Isobutylmagnesium bromide solution in diethyl ether (20ml, 40 mmol) was added with a syringe and the reaction mixture was stirred at -20 ^{o}C for two hours. The reaction was quenched using 1N hydrochloric acid (25 ml) and the ether layer was separated, washed with water and dried over anhydrous sodium sulfate overnight. The ether layer was filtered and evaporated under reduced pressure to yield the 3,4-(methylenedioxyphenyl)isopentan-1-ol (4.15g).

A solution of 3,4-(methylenedioxyphenyl)isopentan-1-ol (4.15g) in methylene chloride (60 ml) were stirred overnight at room temperature with PCC (6.5 g, 30 mmol) and celite (6.5 g). The reaction mixture was diluted with diethyl ether (150 ml), stirred for 30 minutes then, vacuum filtered on a pad of silica gel (200-400 mesh) under vacuum. The organic layer was evaporated under reduced pressure to yield 3,4-

(methylenedioxyphenyl)isopentan-1-one which was purified using Kugelrohr distillation.

3.2.2. Preparation of 3,4-(methylenedioxyphenyl)bromoketones

3.2.2.1. Preparation of 3,4-(methylenedioxy)bromopropiophenone

Commercially available 3,4-(methylenedioxy)propiophenone (2 g, 11.23 mmol) was dissolved in acetic acid in a round bottom flask. Bromine (2.13 g, 13.5 mmol) was dripped slowly into the solution and stirred for one hour. The reaction mixture was poured into cold water and extracted with dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield a yellow oil of the alpha brominated 3,4-(methylenedioxy)propiophenone.

3.2.2.2. Preparation of 3,4-(methylenedioxy)bromobutyrophenone

Commercially available 3,4-(methylenedioxy)butyrophenone (1g, 5.2 mmol) was dissolved in acetic acid in a round bottom flask. Bromine (1.1g, 6.76 mmol) was dripped slowly into the solution and stirred for one hour. The reaction mixture was poured into cold water and extracted with dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield a yellow oil of the alpha brominated 3,4-(methylenedioxy)butyrophenone.

3.2.2.3. Preparation of 3,4-(methylenedioxyphenyl)-2-bromopentan-1-one

3,4-(methylenedioxyphenyl)pentan-1-one (6g, 29 mmol) was dissolved in acetic acid in a round bottom flask. Bromine (6g, 37.7 mmol) was dripped slowly into the solution and stirred for one hour. The reaction mixture was poured into cold water and extracted with dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield a yellow oil of the alpha brominated 3,4-(methylenedioxyphenyl)pentan-1-one.

3.2.2.4. Preparation of 3,4-(methylenedioxyphenyl)-2-bromoisopentan-1-one

3,4-(methylenedioxyphenyl)isopentan-1-one (3g, 14.6 mmol) was dissolved in acetic acid in a round bottom flask. Bromine (3g, 18.98 mmol) was dripped slowly into the solution and stirred for one hour. The reaction mixture was poured into cold water and extracted with dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield a yellow oil of the alpha brominated 3,4-(methylenedioxyphenyl)isopentane-1-one.

3.2.3. Synthesis of 3,4-(methylenedioxy)aminoketones

3.2.3.1. Preparation of 3,4-(methylenedioxyphenyl)-2-pyrrolidinylpropan-1-one (compound 1)

A solution of alpha brominated 3,4-(methylenedioxy)propiophenone (0.5g, 1.95 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dichloromethane solution of pyrrolidine (0.3g, 3.9 mmol) in a round bottom flask and the reaction mixture

was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the dichloromethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxyphenyl)-2-pyrrolidinylpropan-1-one product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. Brown crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-pyrrolidinylpropan-1-one were obtained by recrystalization of the HCl salt susing a mixture of dry acetone, diethyl ether, and absolute ethanol. MS, m/z 98 [100%].

3.2.3.2. Preparation of 3,4-(methylenedioxyphenyl)-2-piperidinylpropan-1-one (compound 2)

A solution of alpha brominated 3,4-(methylenedioxy)propiophenone (0.4g, 1.56 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dichloromethane solution of piperidine (0.26g, 3.12 mmol) in a round bottom flask and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the dicholormethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxyphenyl)-2-piperidinylpropan-1-one product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-piperidinylpropan-1-one were obtained. MS, m/z 112 [100%].

3.2.3.3. Preparation of 3,4-(methylenedioxyphenyl)-2-perhydroazepinylpropan-1-one (compound 3)

A solution of alpha brominated 3,4-(methylenedioxy)propiophenone (0.4g, 1.56 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dicholoromethane solution of perhydroazepine (0.3g, 3.12 mmol) in a round bottom flask and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the dicholormethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxyphenyl)-2-perhydroazepinylpropan-1-one product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-perhydroazepinylpropan-1-one were obtained. MS, m/z 126 [100%].

3.2.3.4. Preparation of 3,4-(methylenedioxyphenyl)-2-methylpiperidinylpropan-1-one (compounds 10, 11 and 12)

A solution of alpha brominated 3,4-(Methylenedioxy)propiophenone (0.4g, 1.56 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dichloromethane solution of 2-methylpiperidine or 3-methylpiperidine or 4-methylpiperidine (0.3g, 3.12 mmol) in a round bottom flask and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the dichloromethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxyphenyl)-2-methylpiperidinylpropan-1-one product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-methylpiperidinylpropan-1-one

product were obtained by recrystalization of the HCl salts using a mixture of dry acetone, diethyl ether, and absolute ethanol.MS, m/z 126 [100%].

3.2.3.5. Preparation of 3,4-(methylenedioxyphenyl)-2-pyrrolidinylbutan-1-one (compound 4)

A solution of alpha brominated 3,4-(methylenedioxy)butyrophenone (1g, 3.7 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dichloromethane solution of pyrrolidine (0.3g, 4.4 mmol) in a round bottom flask and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the dichloromethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxypheyl)-2-pyrrolidinylbutan-1-one product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. Yellow crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-pyrrolidinylbutan-1-one were obtained. MS, m/z 112 [100%].

3.2.3.6. Preparation of 3,4-(methylenedioxyphenyl)-2-piperidinylbutan-1-one (compound 5)

A solution of alpha brominated 3,4-(methylenedioxy)butyrophenone (1.5g, 5.6 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dichloromethane solution of piperidne (0.72g, 8.4 mmol) in a round bottom flask and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the dichloromethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxyphenyl)-2-piperidinylbutan-1-one product. The oil was dissolved in

anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. Yellowish brown crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-piperidinylbutan-1-one were obtained. MS, m/z 126 [100%].

3.2.3.7. Preparation of 3,4-(methylenedioxyphenyl)-2-perhydroazepinylbutan-1-one (compound 6)

A solution of alpha brominated 3,4-(methylenedioxy)butyrophenone (1.5g, 5.6 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dichloromethane solution of perhydroazepine (0.83g, 8.4 mmol) in a round bottom flask and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the dichloromethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxyphenyl)-2-perhydroazepinylbutane-1-one product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. Yellowish white crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-perhydroazepinylbutane-1-one were obtained. MS, m/z 140 [100%].

3.2.3.8. Preparation of 3,4-(methylenedioxyphenyl)-2-pyrrolidinylpentan-1-one (compound 7, MDPV)

A solution of alpha brominated 3,4-(methylenedioxyphenyl)pentan-1-one (4g, 14 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dichloromethane solution of pyrrolidine (1.5g, 21 mmol) in a round bottom flask and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the

dichloromethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxyphenyl)-2-pyrrolidinylpentan-1-one product (MDPV). The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. Dark brown crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-pyrrolidinylpentan-1-one were obtained by solvent extraction and recrystalization of the HCl salts. MS, m/z 126 [100%].

3.2.3.9. Preparation of 3,4-(methylenedioxyphenyl)-2-piperidinylpentan-1-one (compound 8)

A solution of alpha brominated 3,4-(methylenedioxyphenyl)pentan-1-one (4g, 14 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dichloromethane solution of piperidine (1.8g, 21 mmol) in a round bottom flask and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the dichloromethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxyphenyl)-2-piperidinylpentan-1-one product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. Yellowish brown crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-piperidinylpentan-1-one were obtained. MS, m/z 140 [100%].

3.2.3.10. Preparation of 3,4-(methylenedioxyphenyl)-2-perhydroazepinylpentane-1-one (compound 9)

A solution of alpha brominated 3,4-(methylenedioxyphenyl)pentan-1-one (4g, 14 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dichloromethane

solution of perhydroazepine (2g, 21 mmol) in a round bottom flask and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the dichloromethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxyphenyl)-2-perhydroazeoinylpentan-1-one product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. Light brown crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-perhydroazepinylpentan-1-one were obtained. MS, m/z 154 [100%].

3.2.3.11. Preparation of 3,4-(methylenedioxyphenyl)-2-pyrrolidinylisopentan-1-one (compound 13)

A solution of alpha brominated 3,4-(methylenedioxyphenyl)isopentan-1-one (3g, 10.56 mmol) in (50 ml) dichloromethane was slowly added to a (50 ml) dichloromethane solution of pyrrolidine (1.12g, 15.84 mmol) in a round bottom flask and the mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the dichloromethane was evaporated under vacuum to yield the oily 3,4-(methylenedioxyphenyl)-2-pyrrolidinylisopentan-1-one product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. Dark brown crystals of the corresponding 3,4-(methylenedioxyphenyl)-2-pyrrolidinylisopentan-1-one were obtained by solvent extraction and recrystalization of the HCl salts. MS, m/z 126 [100%].

3.3. Preparation of pyridinium chlorochromate (PCC)

Chromium trioxide (100.0g, 1mol) was added rapidly with stirring to a 6M hydrochloric acid solution (184.0 ml). After 5 minutes, the homogenous solution was cooled to 0^{o}C and pyridine (79.1g, 1mol) was carefully added over 10 minutes. The mixture was cooled again to 0^{o}C to give a yellow orange solid which was collected on a sintered glass funnel and dried for one hour under vacuum [Corey and Suggs, 1975].

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