Gas Chromatography–Mass Spectrometry Analysis of Regioisomeric Ring Substituted Methoxy Methyl Phenylacetones

Tamer Awad, Jack DeRuiter, and **C. Randall Clark***
Department of Pharmacal Sciences, School of Pharmacy, Auburn University, Auburn, AL 36849

Abstract

The methoxy methyl phenylacetones share an isobaric relationship (equivalent mass but different elemental composition) to the controlled precursor substance 3,4-methylenedioxyphenylacetone. The 10 methoxy methyl phenylacetones as well as the methylenedioxyphenylacetones show essentially equivalent mass spectra with major fragment ions at *m/z* 135 and 43. Those methoxy methyl phenylacetones with the methoxy group substituted ortho to the benzylic cation in the *m/z* 135 ion show a further fragmentation to lose formaldehyde (CH_2O) and yield a significant ion at *m/z* 105. The loss of formaldehyde from the ortho methoxy benzyl cation was confirmed using commercially available regioisomeric 2-, 3-, and 4-methoxyphenylacetones. The 10 regioisomeric methoxy methyl phenylacetones were prepared from the appropriately substituted benzaldehydes. Complete gas chromatographic resolution of all ten regioisomeric ketones was obtained on a stationary phase containing modified β-cyclodextrin. Using the cyclodextrin containing phase, the ortho methoxy-substituted ketones (K1–K4) eluted before the meta-methoxy-substituted ketones (K5–K8) and the para-methoxy-substituted ketones (K9–K10) showed the greatest affinity for the stationary liquid phase and eluted last. Complete separation of the 10 ketones was not obtained on Rtx-1 and Rtx-200 columns.

Introduction

Regioisomeric and isobaric substances are considered a significant challenge for the analytical techniques used to identify specific substances. This is extremely important when some of these molecules are legally controlled drugs of abuse or controlled precursor substances (1–4). While the mass spectrum is often considered a specific "fingerprint" for an individual compound, there are other substances that produce very similar or almost identical mass spectra. Many of these compounds that yield the same mass spectrum are positional isomers of side-chain or aromatic ring substituents. Such compounds having mass spectral equivalency and similar elution properties, perhaps coelution, represent a serious analytical challenge. The mass spectrum is often the confirmatory piece of evidence in the identification of drugs in drug testing and forensic laboratories.

When other compounds have the potential to produce the same or nearly identical mass spectrum as the substance of interest, identification by gas chromatography (GC)–mass spectrometry (MS) must be based primarily upon the chromatographic system's ability to separate the entire set of substances. Those substances coeluting in the chromatographic system could be misidentified. A complete set of standards must be available for a thorough method validation study and to exclude the possibility of coelution of combinations of the regioisomeric molecules. Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target molecules.

Regioisomer differentiation is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories (5–7). Regioisomer issues in drug identification occur in both the identification of ethical pharmaceutical products and in the analysis of drugs of abuse. Phentermine and methamphetamine are regioisomeric substances and both have ethical pharmaceutical uses. Additionally, ephedrine and pseudoephedrine have legitimate therapeutic uses, and their isomeric relationship limits MS alone as a specific method of identification. Issues of regioisomerism in the analysis of drugs of abuse have occurred in studies to evaluate the analytical specificity for the diethyl amide of lysergic acid versus its methyl-propyl amide, 2,3- versus 3,4-methylenedioxymethamphetamine (3,4-MDMA), methylendioxyphenylbutanamine (MBDB) versus 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA) and many others. The continued designer exploration of some drug categories will likely produce even larger numbers of regioisomeric substances especially among the phenethylamines. These increasing numbers of regioisomers will continue to provide a challenge for chromatographic separations and their forensic identification.

In the present study, the 10 aromatic ring-substituted regioisomeric methoxy methyl phenylacetones are prepared for MS

*Author to whom correspondence should be addressed: email clarkcr@auburn.edu.

and chromatographic evaluation. These compounds share an isobaric relationship (equivalent mass but different elemental composition) to the controlled precursor substance 3,4-methylenedioxyphenylacetone. These regioisomeric ketones are likely to produce major mass spectral fragment ions of equivalent mass to 3,4-methylenedioxyphenylacetone and provide a significant challenge for chromatographic resolution.

Experimental

Analytical

GC–MS analysis was performed with an HP-5890 GC coupled with a HP-5970 mass selective detector (Hewlett Packard, Palo Alto, CA). The MS was operated on the electron impact mode using ionization voltage of 70 eV and a source of temperature of 230°C, except when the β-cyclodextrin column was in use, the source of temperature was 200°C. Samples were dissolved in high-performance liquid chromatography-grade acetonitrile (Fisher Scientific, Fair Lawn, NJ) and manually introduced (1 µL), individually and in physical mixture using a 10-µL Hamilton syringe (Hamilton Co., Reno, Nevada). The GC was operated in splitless mode with a flow rate of 1.37 mL/min and a column head pressure of 10 psi.

All separations and collected retention data were obtained on one of the following columns: a 30 m × 0.25-mm i.d. column coated with 0.25 µm 100% dimethyl polysiloxane (Rtx-1), and a 30 m × 0.25-mm i.d. column coated with 0.25 µm trifluoropropyl methyl polysiloxane (Rtx-200) purchased from Restek Corporation (Bellefonte, PA). A 30 m × 0.25 mm-i.d. column coated with 0.25 µm 14% cyanopropyl phenyl–86% dimethylpolysiloxane doped with a proprietary cyclodextrin material (RtβDEXcst-TM) was kindly supplied from Restek Corporation. The temperature program consisted of an initial column temperature at 70°C for 2 min then ramped up to 150°C at a rate of 2.5°C/min, held at 150°C for 3 min, and finally ramped up to 200°C at a rate of 15°C/min and held for 5 min. Proton NMR spectra were recorded in C_6D_6 at 25°C on a Brucker DRX 250 MHz spectrometer (Bellerica, MA).

Synthetic methods

All laboratory reagents and chemicals were obtained from Aldrich Chemical Company (Milwaukee, WI), TCI America (Portland, OR), Transworld Chemical (Rockville, MD), or Thermo Fisher Scientific (Fair Lawn, NJ). The following general procedures were applied in various combinations as needed to prepare the necessary substituted methoxy methyl benzaldehydes and the desired final ketones.

Methylation

O-Methylation of carboxylic acids and phenols was performed as described. Excess methyl iodide was added, along with potassium carbonate, to a solution of the corresponding hydroxyl methyl benzoic acids in dry acetone, and the mixture was stirred at room temperature. Solids were removed by filtration, and the organic layer was evaporated under reduced pressure to yield the corresponding methoxy methyl benzoic acid methyl ester.

Ester reduction

Red-Al was added to a solution of the corresponding methoxy methyl benzoic acid methyl ester or ethyl ester in benzene under a nitrogen atmosphere. The mixture was refluxed then terminated by the addition of ethanol and water. The residue was dissolved in methylene chloride, and the organic layer was washed with water. The methylene chloride layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to yield the crude methoxy methyl benzyl alcohol.

Alcohol oxidation

Pyridinum chlorochromate and celite were added to a solution of the methoxy methylbenzyl alcohol in methylene chloride and the resulting mixture stirred at room temperature. The mixture was diluted with ether then filtered over a pad of silica gel. The combined organic filtrate was evaporated under reduced pressure to afford the corresponding methoxy methyl benzaldehydes, which were purified by Kugelrohr distillation.

Nitropropene reduction/hydrolysis

A mixture of individual methoxy methylbenzaldehydes and *n*-butylamine in benzene was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature, and the solvent was evaporated under reduced pressure to yield the crude imine. The imine was dissolved in glacial acetic acid, nitroethane was added dropwise, and the resulting mixture was refluxed. The mixture was cooled to room temperature, poured over crushed ice, and acidified to pH 1 using concentrated hydrochloric acid. The substituted phenyl-2-nitropropene was extracted into methylene chloride, washed with water, and dried over anhydrous sodium sulfate. The organic layer was filtered and evaporated under reduced pressure to yield ring substituted 1-(methoxymethyl phenyl)-2-nitropropene.

The resulting nitropropene in toluene was mixed with powdered iron, ferric chloride, water, and concentrated hydrochloride acid. The mixture was stirred vigorously and refluxed overnight, cooled, filtered, and the residue washed with toluene and water. The organic layer was separated and washed with 5N hydrochloric acid solution, water, and saturated sodium bicarbonate solution. The organic layer was dried, and the solvent was evaporated under reduced pressure to afford the corresponding ring-substituted methoxy methyl phenyl acetones, which were purified by Kugelrohr distillation.

2-Methoxy-6-methylbenzaldehyde

A solution of copper sulfate pentahydrate and potassium persulfate in water was added dropwise to a solution of 2,3-dimethyl anisole in acetonitrile, and the resulting mixture was refluxed. The reaction mixture was cooled to room temperature, and the solvent volume was reduced under vacuum and extracted using methylene chloride. The combined organic extract was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford crude 2-methoxy-6-methylbenzaldehyde, which was purified by Kugelrohr distillation.

3-Methoxy-5-methylbenzoic acid

Sodium metal was added in small portions to absolute ethanol

in an ice-cooled, dry, three-neck flask under a nitrogen atmosphere, and the mixture was stirred overnight. Acetone and diethyl oxalate were then added dropwise, and the resulting thick yellow mixture was stirred, and the resulting ethyl sodium acetopyrovate was collected by vacuum filtration and dried overnight.

Ethyl sodium acetopyrovate was dissolved in water followed by the addition of glacial acetic acid, and the mixture was stirred at room temperature, and the reaction mixture was then poured on ice followed by the addition of concentrated sulfuric acid. 3-Acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydrofuran-2-carboxylic acid ethyl ester was formed as a yellow solid and collected by vacuum filtration. Magnesium oxide was added in three portions to a suspension of 3-acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester in water and refluxed. The reaction mixture was then filtered under vacuum, and the residue was washed with hot water. The filtrate volume was reduced under vacuum and cooled to room temperature. Addition of hydrochloric acid gas gave 3-hydroxy-5-methyl benzoic acid.

Ethyl-5-hydroxy-2-methylbenzoate

A solution of 2-methylfuran in methylene chloride was added dropwise to a solution of ethyl propiolate and anhydrous aluminum chloride in methylene chloride, and the resulting mixture was stirred at room temperature. The organic layer was isolated and extracted with sodium hydroxide solution, and the combined aqueous basic layer was acidified with concentrated hydrochloric acid. The acidified aqueous layer was extracted with ethyl acetate, and the organic phase dried over anhydrous sodium sulfate, filtered, and evaporated to afford ethyl 5-hydroxy-2-methylbenzoate.

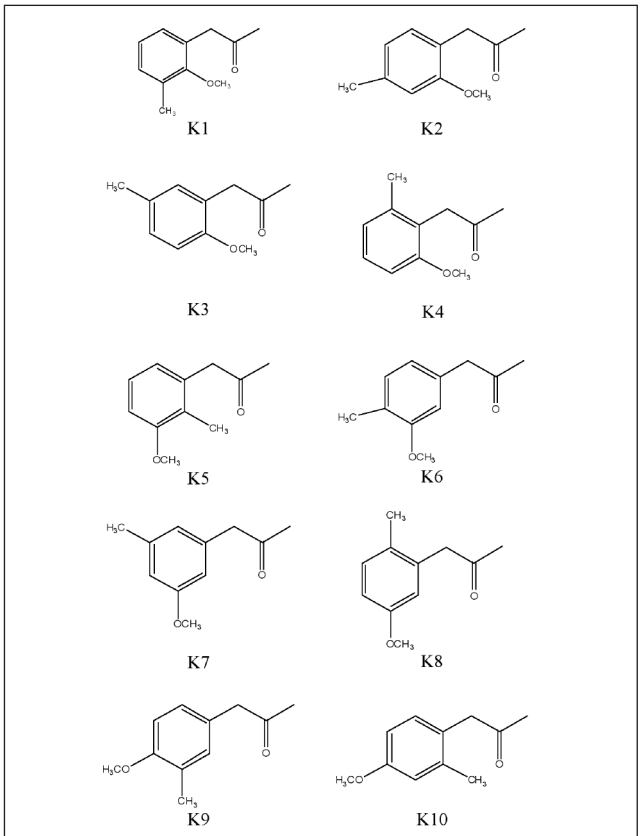

Figure 1. Structures of the ring-substituted methoxy methyl phenylacetones.

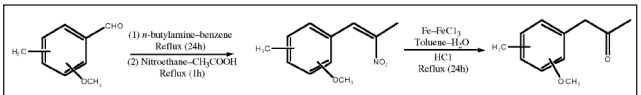

Figure 2. General procedure for the preparation of the 10 regioisomeric methoxy-methylphenyacetones.

Results and Discussion

Chemistry

This report describes the synthesis and analytical properties of the ring-substituted methoxy-methyl-phenyl-2-propanones (methoxy-methyl-phenylacetones) (Figure 1). The general procedure for the preparation of the 10 regioisomeric methoxy-methylphenyl-2-propanones is outlined in Figure 2. The appropriately substituted methoxy-methylbenzaldehyde was converted to the corresponding *N*-butylimine, and the imine was dissolved in glacial acetic acid and allowed to react with nitroethane to form the desired (methoxymethylphenyl)-2-nitropropenes.

The corresponding ketones were obtained by reduction of the nitropropenes with iron and hydrochloric acid in the presence of a catalytic amount of ferric chloride. During the reaction, the nitro group was first reduced to yield the corresponding 2-aminopropenes, which tautomerizes to the imine followed by hydrolysis to

Table I. Proton NMR Data for the 10 Regioisomeric Methoxy Methyl Phenylacetones.

Functional Group	Ar-OCH_3		Ar-CH_3		Ar-CH_2-CO-	-$COCH_3$	Ar-H
	Position	Chemical Shift	Position	Chemical Shift	Chemical Shift	Chemical Shift	Chemical Shift
Chemical Shift (δ ppm)	2	3.763	3	2.297	3.674	2.147	(6.907,2H) (7.042,1H)
	2	3.909	4	3.345	3.790	2.120	6.698–6.818
	2	3.856	5	2.550	3.61	2.114	6.734–6.919
	2	3.663	6	2.269	3.379	2.136	(6.654–6.850,2) (7.124–7.337,1)
	3	3.74	2	2.085	3.656	2.075	7.092–6.706,3H
	3	3.818	4	2.091	3.596	2.051	6.62–7.05,3H
	3	3.758	5	2.295	3.386	2.123	6.544–6.610,3H
	5	3.759	2	2.155	3.640	2.119	6.679–7.089,3H
	4	3.810	3	2.217	3.593	2.135	(7.725,2H) (7.007,1H)
	4	3.819	2	2.191	3.612	2.098	(6.722,2H) (7.00,1H)

give the corresponding methoxy-methyl-phenylacetones. The H^1NMR chemical shift data for all 10 of the methoxy-methyl-phenylacetones synthesized in this study were determined and are consistent with structural assignments (Table I).

Three of the methoxy methyl benzaldehydes are commercially available: 2-methoxy-5-methylbenzaldehyde, 4-methoxy-3-methylbenzaldehyde, and 4-methoxy-2-methylbenzaldehyde. These were used to prepare phenylacetones K3, K9, and K10, respectively. Three other substitution patterns were obtained from commercially available hydroxy-methyl benzoic acids, 3-methyl salicylic acid (precursor for K1), 4-methyl salicylic acid (precursor for K2), and 3-hydroxy-2-methyl benzoic acid (precursor for K5).

The hydroxy acids were individually dimethylated with excess methyl iodide according to the procedure outlined in Figure 3. The resulting methyl esters of the methoxy methylbenzoic acids were reduced to the corresponding benzyl alcohols followed by selective oxidation to the corresponding benzaldehydes. The methyl ester of 3-methoxy-4-methyl benzoic acid was obtained from a commercial source and converted to the corresponding aldehyde (precursor for K6) as outlined in Figure 3.

Selective oxidation of the 2-methyl group in 2,3-dimethyl-anisole (Figure 5) using potassium persulfate and cupper sulfate pentahydrate gave 2-methoxy-6-methylbenzaldehyde (precursor

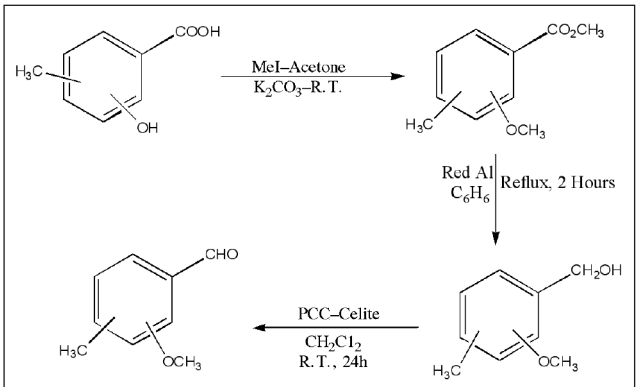

Figure 3. Synthesis of ring-substituted methoxy methyl benzaldehydes from the corresponding hydroxyl methyl benzoic acids.

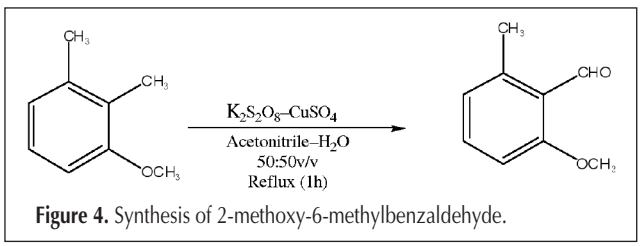

Figure 4. Synthesis of 2-methoxy-6-methylbenzaldehyde.

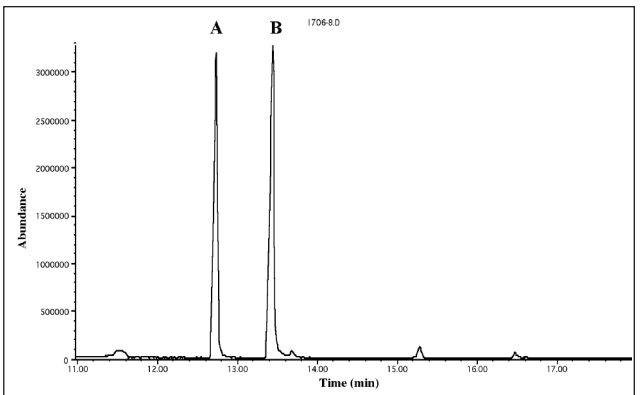

Figure 5. GC separation between 2-methoxy-6-methyl-phenylacetone (A) and 3-methoxy-2-methyl-phenylacetone (B).

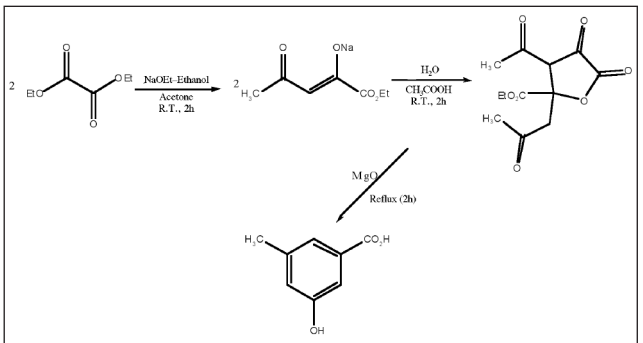

Figure 6. Synthesis of 3-hydroxy-5-methyl benzoic acid.

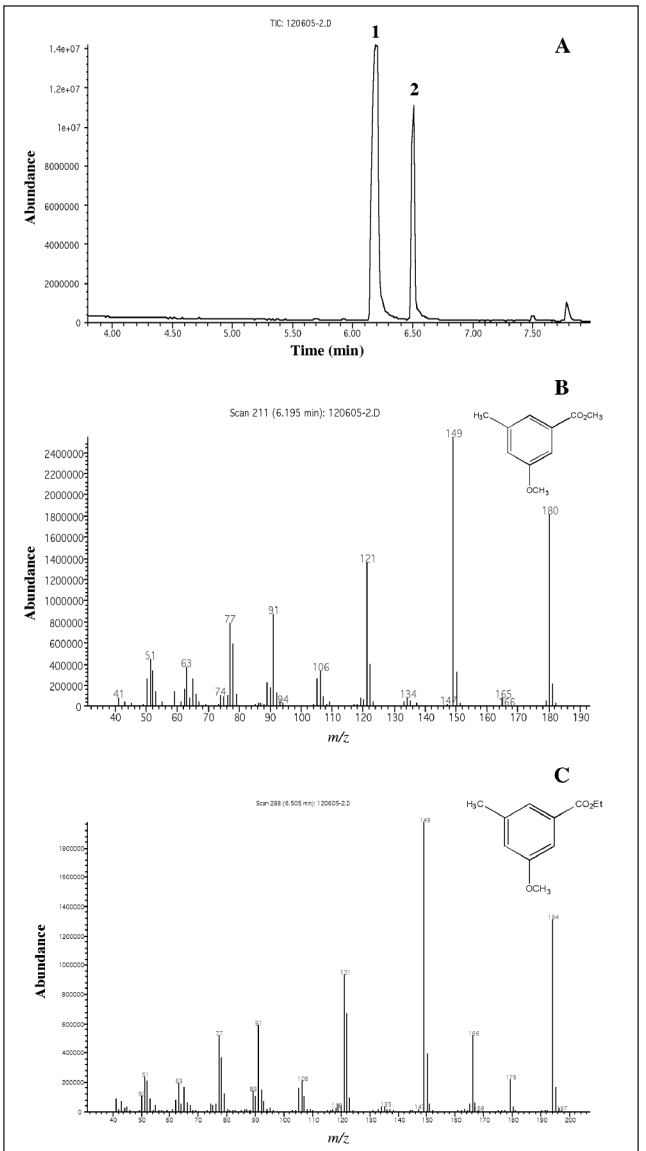

Figure 7. GC separation and mass spectra of methyl-3-methoxy-5-methyl benzoate (A) and ethyl-3-methoxy-5-methyl benzoate (B).

for K4). This aldehyde is reported (8) to be the only product obtained from the regioselective oxidation of 2,3-dimethylanisole using copper (II) and peroxydisulfate. GC–MS studies were employed to investigate the regioselectivity of this reaction. Oxidation of the 3-methyl group in this reaction would yield 3-methoxy-2-methylbenzaldehyde, the precursor for K5 as described earlier. Figure 5 shows the GC separation of phenylacetones K4 and K5. The two ketones are well resolved, indicating that a product mixture in the oxidation would have been observed by GC analysis. GC analysis of the phenylacetone product obtained via the selective oxidation of 2,3-dimethylanisole (Figure 4) showed only phenylacetone K4, indicating the regioselectivity of the persulfate/copper oxidation.

The precursor aldehyde for the synthesis of K7 was obtained by condensation of acetone and diethyl oxalate (9) in presence of sodium ethoxide according to Figure 6. The product ethyl sodium acetopyrovate was converted to 3-acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester by stirring in water and glacial acetic acid. The furan carboxylic acid was converted to 3-hydroxy-5-methyl benzoic acid by refluxing with magnesium oxide in water. Methylation of the 3-hydroxy-5-methyl benzoic acid using the procedure outlined in Figure 3 produced a mixture of two esters. GC–MS analysis of the mixture (Figure 7) showed the expected methyl ester of 3-methoxy-5-methyl benzoic acid and a second later eluting component identified by its mass spectrum as the ethyl ester of the described acid. The ethyl ester is likely the result of incomplete hydrolysis of the product in Figure 6. The mixture of methyl and ethyl esters was subjected to Red-Al reduction according to step 2 in Figure 3, and the GC–MS analysis of that product is shown in Figure 8. As expected, both the methyl and ethyl esters were converted to the same benzyl alcohol and the alcohol was used to prepare the desired aldehyde, 3-methoxy-5-methylbenzaldehyde (precursor for K7).

The remaining aldehyde, 5-methoxy-2-methylbenzaldehyde (precursor for K8), was prepared by the cycloaddition of 2-methylfuran and ethyl propiolate (Figure 9). The cycloaddition reaction (10) is complete in 30 min at room temperature, yielding the ethyl ester of 5-hydroxy-2-methyl benzoic acid. This product was converted to the desired aldehyde according to the steps outlined in Figure 3. The initial cycloaddition reaction is reported (10) to be regiospecific, yielding only the 1-methyl-2-carbethoxy-7-oxobicyclo[2.2.1]heptadiene intermediate shown in Figure 9. If the other regioisomeric intermediate had formed (i.e., 1-methyl-3-carbethoxy-7-oxobicyclo[2.2.1]heptadiene), the resulting aldehyde would be 2-methoxy-5-methyl benzaldehyde and yield phenylacetone 3 in Figure 1. The chromatogram in Figure 10 shows the separation of phenylacetones K3 and K8, which allowed the successful monitoring of the above reaction sequence. GC–MS analysis of the product obtained from the reaction sequence represented by Figure 9 followed by Figure 3 showed only one product, K8. Furthermore, the absence of the *m/z* 105 ion (characteristic of K3 and all the ortho-methoxy substituted ketones) provided further conformation of the identity of K8.

Mass spectra

The mass spectrum of 3,4-methylenedioxyphenyl-2-propanone (the controlled precursor for the synthesis of 3,4-MDMA) shows a molecular ion at *m/z* 178 and major fragment ions at *m/z* 135/136 and 43 for the acetyl $(CH_3CO)^+$ fragment. The various isomeric forms of the methoxy methyl phenylacetone have the potential to produce mass spectra essentially equivalent to 3,4-methylenedioxyphenylacetone, all have molecular weight of 178 and can yield major fragment ions in their electron ionization mass spectra at *m/z* 135/136 and 43. These regioisomeric methoxy methyl phenylacetone have an isobaric relationship with 3, 4-methylenedioxyphenylacetone. For these individual regioisomers, the methoxy methyl benzyl $(C_9H_{11}O)^+$ fragments have the same mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at *m/z* 135. Furthermore, the *m/z* 43 acetyl cation is common to all the ketones and is produced from the alpha cleavage between the carbonyl carbon and benzylic carbon (Figure 11). The mass spectra of the 10 substituted methoxy methyl phenylacetones are shown

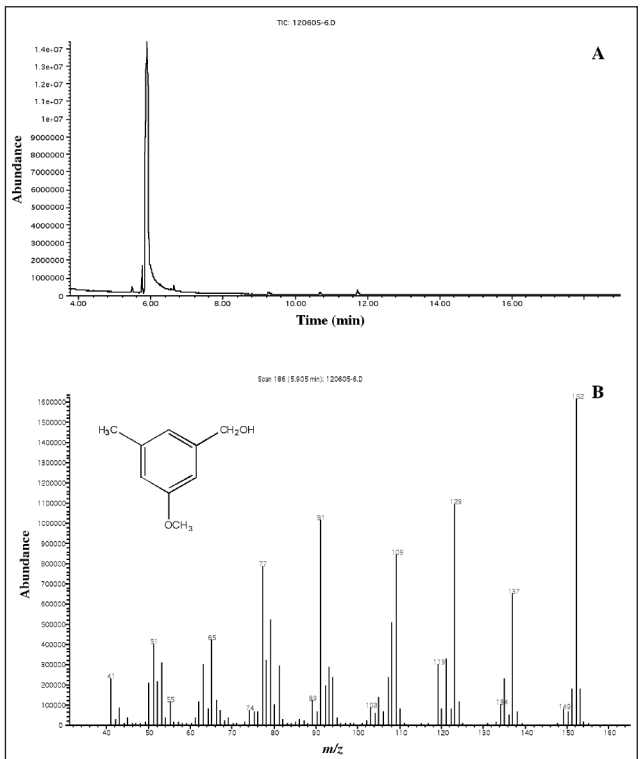

Figure 8. GC analysis (A) and mass spectrum (B) of 3-methoxy-5-methyl benzyl alcohol.

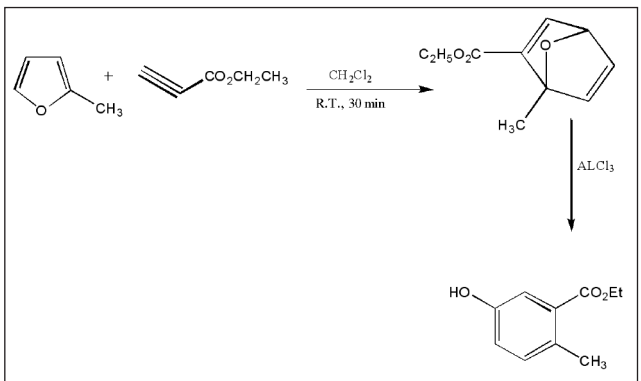

Figure 9. Synthesis of ethyl-5-hydroxy-2-methylbenzoate.

in Figure 12. All 10 compounds show a significant molecular ion peak at *m/z* 178, and the substituted benzyl cation (*m/z* 135) is the base peak in most of the spectra.

Those methoxy methyl ring-substituted phenylacetones with the methoxy group in the ortho position are characterized by a significant *m/z* 105 peak; in fact, *m/z* 105 is the base peak in the mass spectra of 2-methoxy-3-methyl phenylacetone (Figure 5, K1). This ion likely arises from the loss of mass 30 (CH_2O) from the initial benzylic cation at *m/z* 135. The *m/z* 105 ion is a significant fragment only when the methoxy-group in the ketone is ortho to the acetone side-chain (K1–K4) and therefore the site of initial benzylic cation formation. This *m/z* 105 ion can be formed by 1,6-hydride shift (ortho-effect) from a hydrogen atom of the methoxy group to the benzyl cation followed by loss of formaldehyde (Figure 13). This ion at *m/z* 105 also appears in the mass spectra of 3-methoxy-2-methyl phenyl acetone (K5), and may be attributed to rearrangement of the initial benzylic cation to the adjacent methyl group, which is then ortho to the methoxy group for the elimination of formaldehyde.

The suggested mechanism for the loss of CH_2O from the ortho-methoxy benzyl cations was supported by screening the mass spectra of the commercially available 2-, 3-, and 4-methoxy phenyl acetones. The mass spectra of all three methoxy ring-substituted phenylacetones show *m/z* 121, which is analogous to *m/z* 135 for K1–10, and *m/z* 91, which is analogous to *m/z* 105, for the K1–K10 series. However, the *m/z* 91 ion is the base peak for 2-methoxy phenylacetone only, and this ion is less significant in the 3- and 4-methoxy isomers. The *m/z* 91 ion would also be the loss of mass 30 from the initial benzylic cation at *m/z* 121 for this model system. The 3-methoxyphenylacetone shows *m/z* 43 as the base peak and the 4-methoxy-isomer shows *m/z* 121 as the base peak.

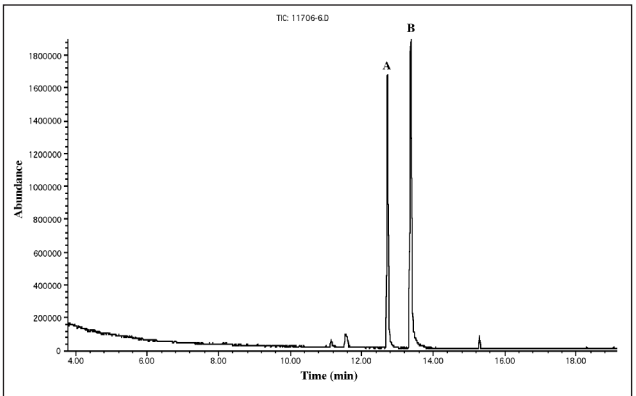

Figure 10. GC separation of 2-methoxy-5-methylphenylacetone (A) and 5-methoxy-2-methylphenylacetone (B).

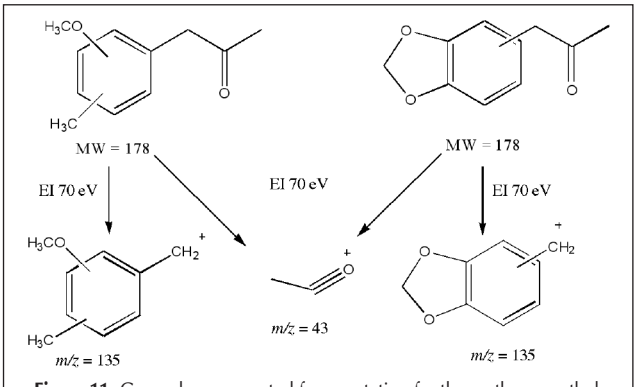

Figure 11. General mass spectral fragmentation for the methoxy methyl phenylacetones and methylenedioxyphenylacetones.

GC

The methoxy methyl ring-substituted phenylacetones were compared on three stationary phases using capillary columns of the same dimensions, 30 m × 0.25 mm and 0.25 µm depth of film. Several temperature programs were evaluated and used to collect retention data; however, only one column (Rt-β-DEXcst-TM) and only one temperature program (experimental section) yielded 10 well-resolved peaks for the 10 ketones (Figure 14). A direct comparison of Rtx-1 and Rtx-200 phases showed increasing retention on the more polar Rtx-200 phase and fewer coeluting compounds using an identical temperature program. The Rtx-1 dimethyl polysiloxane phase gave very similar retention properties for ketones K2, K4, and K6 with the most retained compound K10 eluting in 8.857 min. The Rtx-200 column using the same temperature program produced a retention time of 13.221 min for K10 and showed ketones K5 and K7 to have similar retention properties.

The chromatogram in Figure 14 shows the results obtained on a polysiloxane polymer containing a modified β-cyclodextrin. Cyclodextrins have been used extensively in separation science because they have shown the ability to discriminate between positional isomers, functional groups, homologues, and enantiomers. The unusual properties of cyclodextrins are due to their unique structure. Cyclodextrins are water-soluble oligosaccharides with a hydrophilic surface and a hydrophobic interior cavity. They are capable of forming inclusion compounds with a wide range of hydrophobic molecules, including organic moieties, inorganic ions, and organo-metallic species. Entrapment inclusion occurs without the formation of formal chemical bonds (11).

Formation of inclusion complexes between the ligand and cyclodextrin was thought to be a result of hydrophobic interactions between the ligand and the relatively hydrophobic cavity of the cyclodextrin coupled with polar interactions between appropriate substituents on the ligand and the polar rim of cyclodextrin. The unmodified cyclodextrin rim is lined with primary hydroxyl groups on one side and secondary hydroxyls on the other side of the cavity. All the hydroxyl groups may be functionalized with hydrophobic or hydrophilic groups to enhance complex forming ability and selectivity towards certain analytes (12).

Three major types of cyclodextrins are known: α-, β-, and γ-cyclodextrin. The α-cyclodextrin contains six, β-cyclodextrin seven, and γ-cyclodextrin eight glucose units. The utility of underivatized cyclodextrins in GC applications was limited because of their high crystallinity, and the insolubility in most organic solvents made them difficult to be formulated into GC stationary phases. However, some functionalized cyclodextrins form viscous oils suitable for GC stationary-phase coatings and have been used either neat or diluted in polysiloxane polymer as chiral stationary phases for GC applications (13).

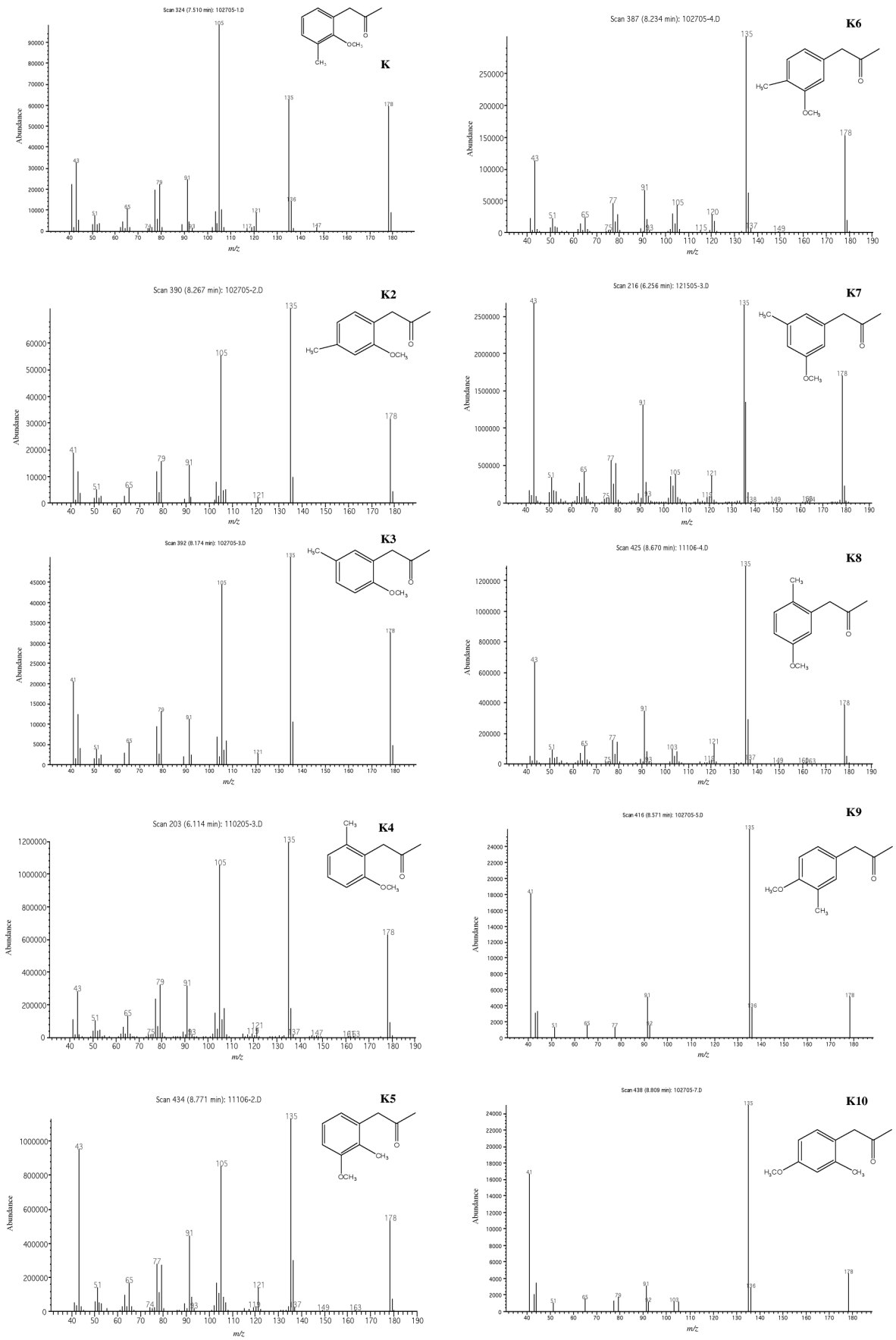

Figure 12. Mass spectra of the 10 methoxy methyl phenylacetones.

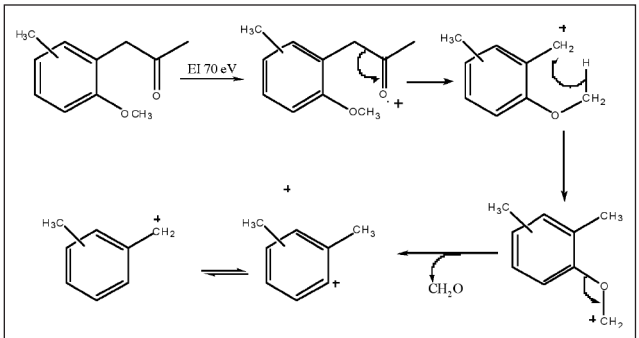

Figure 13. Formation of *m/z* 105 ion in the ortho-methoxy substituted benzyl cations.

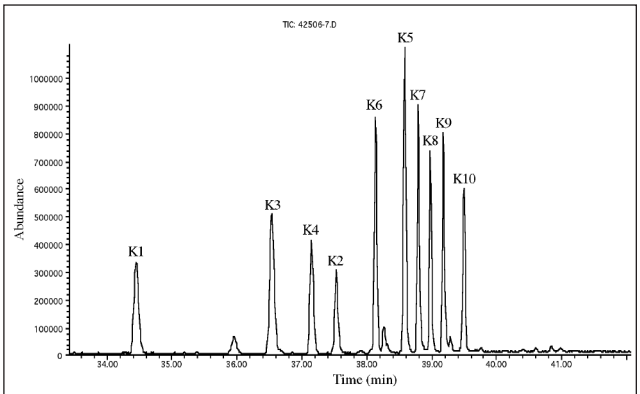

Figure 14. Capillary gas chromatographic separation of methoxy methyl phenylacetones (K1–K10) on β-cyclodextrin column (Rtβ–DEXcst-TM).

The derivative of β-cyclodextrin in cyanopropyl-dimethyl polysiloxane is one of these derivatives developed in the past few years for stereochemical separation purposes in GC. The column used during this project, Rt-βDEXcst (Figure 14), showed that the ring-substituted methoxy methyl phenylacetones with the methoxy group in the ortho position (K1–K4) elute first, followed by phenyl acetones where the methoxy group are in the meta postion (K5–K8), then last eluting were those ketones with the methoxy groups in the para postion (K9 and K10). The chromatogram in Figure 14 shows all the ketones well resolved; however, the elution of all ten regioisomeric ketones required an analysis time of almost 40 min.

Conclusion

The 10 regioisomeric methoxy methyl phenylacetones were prepared from the appropriately substituted benzaldehydes. Some of the required benzaldehydes were commercially available, and the others were prepared during the course of this study. The regioisomeric selectivity of the methods utilized in the synthesis of the substituted benzaldehydes was confirmed by GC–MS studies. The methoxy methyl phenylacetones have an isobaric relationship (equivalent mass but different elemental composition) to the controlled precursor substance 3,4-methylenedioxyphenylacetone. The 10 methoxy methyl phenylacetones as well as the methylenedioxyphenylacetones show essentially equivalent mass spectra with major fragment ions at *m/z* 135 and 43. Those ketones with the methoxy group substituted ortho to the benzylic cation in the *m/z* 135 fragment show a further fragmentation to lose formaldehyde (CH_2O) and yield a significant ion at *m/z* 105. The loss of formaldehyde from the ortho methoxy benzyl cation was confirmed using commercially available regioisomeric 2-, 3-, and 4-methoxyphenylacetones.

GC separation studies showed better resolution and greater retention on the more polar phase Rtx-200. However, complete resolution of all 10 regioisomeric ketones was only obtained on a stationary phase containing the shape selective host molecule β-cyclodextrin. Using the cyclodextrin-containing phase, the ortho substituted ketones (K1–K4) eluted before the meta substituted ketones (K5–K8), and the para substituted ketones (K9–K10) showed the greatest affinity for the stationary liquid phase and eluted last.

References

1. L. Aalberg, J. DeRuiter, F.T. Noggle, E. Sippola, and C.R. Clark. Chromatographic and mass spectral methods of mdentification for the side chain and ring regioisomers of methylenedioxymethamphetamine. *J. Chromatogr. Sci.* **38:** 329 (2000).
2. T. Awad, J. DeRuiter, and C.R. Clark. GC–MS analysis of acylated derivatives of the side chain and ring regioisomers of methylenedioxyamphetamine. *J. Chromatogr. Sci.* **43:** 296–303 (2005).
3. S. Borth, W. Hänsel, P. Rösner, and T. Junge. Synthesis of 2,3- and 3,4-methylenedioxy-phenalkylamines and their regioisomeric differentiation by mass spectral analysis using GC-MS-MS. *Forensic Sci. Int.* **114:** 139–53 (2000).
4. J.F. Casale, P.A. Hays, and R.F.X. Klein. Synthesis and characterization of the 2,3-methylenedioxyamphetamines. *J. Forensic Sci.* **40:** 391–400 (1995).
5. C.R. Clark, J. DeRuiter, A. Valaer, and F.T. Noggle. GC–MS analysis of acylated derivatives of methamphetamine and regioisomeric phenethylamines. *J. Chromatogr. Sci.* **33:** 485–92 (1995).
6. T. Awad, C.R. Clark and J. DeRuiter. Chromatographic and mass spectral studies on methoxy-methcathinones related to 3,4-methylenedioxymethamphetamine. *J. Chromatogr. Sci.* **44:** 155 (2006).
7. J. DeRuiter, C.R. Clark, and F.T. Noggle GC–MS and HPLC analysis of bromination products of the regioisomeric dimethoxyphenethylamines: differentiation of nexus from five positional isomers. *J. Chromatogr. Sci.* **36:** 23–27 (1998).
8. F. M. Hauser and S.R. Ellenberger. Specific oxidation of methyl groups in dimethylanisole. *Synth. Commun.* 723–724 (1987).
9. F.A. Turner and Gearien. Synthesis of reserpine analogs. J. Org. Chem. **24:** 1952–55 (1959).
10. R.S. Randid and J.W. Erickson, US patent 6066656 (2000).
11. S. Braga, F.A. Paz, M.Pillinger, J. Seixas, C. Romao, and I. Goncalves, Structural studies of β-cyclodextrin and permethylated β-cyclodextrin inclusion compounds of cyclopentadienyl metal carbonyl complexes. *Eur. J. Inorg. Chem.* **10:** 1662–69 (2006).
12. J. Szejtli and T. Osa. Comprehensive supramolecular chemistry. Cyclodextrins, vol 3, pergamon, Elsevier, Oxford, 1996.
13. E. Schneiderman and A. Stalcup. Cyclodextrins: a versatile tool in separation science. *J. Chromatogr. B.* **745:** 83–102 (2000).

Manuscript received July 14, 2006;
revision received February 28, 2007.