analytical chemistry

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Analytical Differentiation of 1-Alkyl-3-acylindoles and 1-Acyl-3-alkylindoles: Isomeric Synthetic Cannabinoids

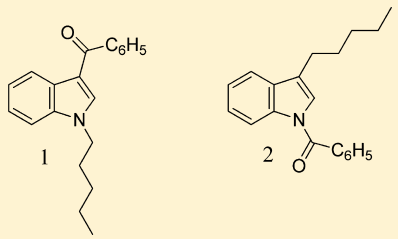
Jack DeRuiter,[†] Forrest T. Smith,[†] Karim Abdel-Hay,[†,‡] and C. Randall Clark*[,†]

[†]Department of Drug Discovery and Development Harrison, School of Pharmacy, Auburn University, Auburn, AL 36849, United States

[‡]Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt

Supporting Information

ABSTRACT: The 1-alkyl-3-acylindoles and the inverse regioisomeric 1-acyl-3-alkylindoles can be prepared directly from a common set of precursor materials and using similar synthetic strategies. The EI mass spectra for these isomers show a number of unique ions which allow for the differentiation of the 1-alkyl-3-acylindole compounds from the inverse regioisomeric 1-acyl-3-alkylindoles. The base peak at *m*/*z* 214 in the 1-*n*-pentyl-3-benzoylindole represents the M-77 cation fragment resulting from the loss of the phenyl group, and this ion is not observed in the inverse isomer. The 1-benzoyl-3-*n*-pentylindole inverse regioisomer shows a base peak at *m*/*z* 105 for the benzoyl cation. Thus, these two base peaks are the result of fragmentation initiated at the carbonyl-oxygen for both isomers. The 1-pentyl-3-benzoylindole is characterized by the strong intensity carbonyl band at 1703 cm^{-1}, while the amide carbonyl appears as a strong band of equal intensity at 1681 cm^{-1} in the 1-benzoyl-3-pentyl regioisomer.



A number of synthetic cannabinoids including direct analogues of delta-9-tetrahydrocannabinol (THC), 5-(dimethylheptyl)-2-[3-hydroxycyclohexyl]-phenols (CP-47,497), and 1-alkyl-3-acylindoles (JWH compounds) have been developed[1,2] over the past several decades to investigate the cannabinoid receptors and the pharmacology of the cannabinoid receptors. The 1-alkyl-3-acylindoles compounds act as full agonists at both the CB_1 and CB_2 cannabinoid receptors, with some selectivity for CB_2.[3] CB_1 is found predominantly in the brain and is responsible for most of the overt pharmacological effects of cannabinoids. The CB_2 receptor is primarily present in peripheral tissues. In spite of this CB_2 selectivity, many of the 1-alkyl-3-acylindoles are more potent as agonists than THC at CB_1 receptors. Some 1-alkyl-3-acylindoles have cannabinoid receptor affinity (CB_1) five times greater than that of THC and produce psychoactive effects in animals similar to those of THC.[4]

The reported structure−activity relationships have led, in recent years, to the emergence of a variety of 1-alkyl-3-acylindoles and other synthetic cannabinoids in the clandestine drug market. These compounds were added to plant material and marketed under brand names such as "Spice" or "K2" as legal natural products often described as "herbal incense" or "herbal smoking blends".[5,6] Subsequent analysis revealed, however, that these products were in fact synthetic compounds. The frequency and variety of these products and the types of synthetic cannabinoids in them have increased in recent years as these products have become better known and more popular.[7,8] Thus the emergence of these synthetic cannabinoids represents a recent phenomenon in the designer drug market, focusing primarily on those indole derivatives with structures known to produce the desired CNS effects.

A number of structure−activity studies have been published with respect to the activity of 3-acyl-1-alkylindole derivatives at the cannabinoid-1 (CB-1) receptor, the receptor that mediates the cannabis-like psychologic effects of these drugs.[4,9−11] These studies have focused primarily on modification of the substituents on positions 1, 2, and 3 on the indole nucleus. Varying the substituent at the 3-position of the indole ring has been most extensively investigated in this series of compounds. The 1,3-substitution pattern on the indole ring has been studied extensively for cannabinoid receptor affinity and pharmacological activity as well as analytical evaluation using modern techniques such as nuclear magnetic resonance (NMR) and gas chromatographic mass spectrometric (GC−MS) analysis.[12−14]

Simple indoles typically undergo substitution reactions at their two most electron rich centers: position 1 (indole nitrogen) or position 3. The most reactive position on the indole ring toward electrophilic substitution is C-3, which is 10^{13} times more reactive than benzene toward electrophilic aromatic substitution. Thus under electrophilic substitution conditions, indole will tend to react at this position, yielding a 3-substituted product. The indole nitrogen has relatively low nucleophilicity and is essentially unreactive due to delocalization of its nonbonded pair of electrons into the aromatic system. However, in the presence of very strong bases, the indole nitrogen can be deprotonated to yield an ionic salt which is significantly more nucleophilic and can react with electrophiles to form 1-substituted

Received: November 21, 2013
Accepted: March 10, 2014
Published: March 10, 2014

   dx.doi.org/10.1021/ac500316x | *Anal. Chem.* 2014, 86, 3801−3808

regioisomeric products. Typically, simple monovalent sodium or potassium indole nitrogen salts tend to react with electrophiles at nitrogen to give 1-substitution products. However, multivalent indole nitrogen salts such as magnesium or zinc tend to react to substitute the electrophilic 3-carbon to give the other regioisomer. The solvents for these reactions also play a role in the regiochemistry of reaction. For example, polar aprotic solvents such as DMF and DMSO tend to favor substitution at the indole nitrogen, while nonpolar solvents such as toluene generally yield 3-substitution. Finally, for indoles that already contain a substitutent at the 1-position, electrophilic substitution occurs exclusively at the 3-position. These reactivity trends demonstrate that different reaction conditions can produce different regioisomeric indole-substitution products (even from the same precursor compounds), either by design as we describe in this paper or by accident as may occur in a clandestine laboratory. This paper directly compares a 1-alkyl-3-acylindole with its specific inverse 1-acyl-3-alkylindole regioisomer, two substances directly available from indole using identical precursor reagents and similar reaction conditions. The structures for the model compounds in this study are shown in Figure 1.

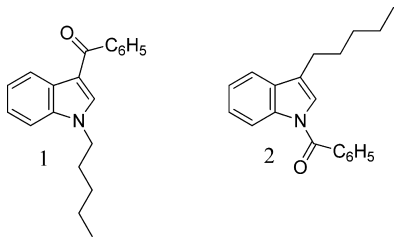


Figure 1. Structures of the regioisomeric indoles in this study, where 1 = 1-*n*-pentyl-3-benzoylindole and 2 = 1-benzoyl-3-*n*-pentylindole.

EXPERIMENTAL SECTION

GC−MS System 1 consisted of an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 240 Agilent Ion Trap mass spectrometer. The mass spectral scan rate was 2.86 scans/s. The GC was operated in splitless mode with a helium (grade 5) flow rate of 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 temperature of 230 °C. The GC injector was maintained at 250 °C and the transfer line at 280 °C. The GC studies were performed on a column (30 m × 0.25 mm i.d.) coated with 0.5 μm 5% phenylmethyl polysiloxane (Agilent, J&W VF-5 ms) purchased from Agilent technologies (Santa Clara, CA). The mass spectra were collected using a GC temperature program consisting of an initial hold at 70 °C for 1.0 min, ramped up to 250 °C at a rate of 30 °C/min, held at 250 °C for 25 min. Samples were dissolved and diluted in high-performance liquid chromatography-grade acetonitrile (Fisher Scientific, Fairlawn, NJ) and introduced via the auto injector using an injection volume of 1 μL.

GC−MS System 2 consisted of an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 5975C VL Agilent mass selective detector. The mass spectral scan rate was 2.86 scans/s. The GC was operated in splitless mode with a helium (grade 5) flow rate of 0.7 mL/min and a column head pressure of 10 psi. The MS was operated in the electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230 °C. The GC injector was maintained at 300 °C and the transfer line at 325 °C. Chromatographic separations were carried out on a column (30 m × 0.25 mm i.d.) coated with 0.5 μm 100% trifluoropropyl methyl polysiloxane (Rtx-200) purchased from Restek Corporation (Bellefonte, PA). The separations were obtained using a temperature program consisting of an initial hold at 80 °C for 1.0 min, ramped up to 300 °C at a rate of 30 °C/min, held at 300 °C for 0.5 min, and then ramped to 340 °C at a rate of 5 °C/min and held at 340 °C for 25.0 min. Samples were dissolved and diluted in high-performance liquid chromatography-grade acetonitrile (Fisher Scientific, Fairlawn, NJ) and introduced via the auto injector using an injection volume of 1 μL.

Attenuated total reflection-Fourier transform-infrared (ATR FT-IR) spectra were obtained on a Shimadzu IRAffinity-1 FT-IR Spectrophotometer (Kyoto, Japan) equipped with a DLATGS detector with temperature control system at a resolution of 4 cm^{-1} with an aperture of 3.5 mm and scan rate of 10 scans per second. The FT-IR spectrophotometer was equipped with MIRacle Single Reflection Horizontal ATR Accessory (Pike Technologies, WI). The single-reflection sampling plate of the accessory has a 1.8 mm round crystal surface allowing reliable analysis of small samples. FT-IR spectra were recorded in the range of 4000−520 cm^{-1}. The samples were prepared by dissolving the solid or oily compounds in acetonitrile and introducing the resulting solutions in small volumes to the center of the single-reflection sampling plate.

RESULTS AND DISCUSSION

The goal of this project was to compare the analytical properties (especially GC-MS) of a 1-alkyl-3-acylindole with an inverse isomer 1-acyl-3-alkylindole. Differentiation of isomers can be a critical issue in forensic drug chemistry when only one isomeric form is explicitly listed as a controlled drug, requiring other isomeric forms to be ruled out as the identity of the unknown. The isomeric 1,3-substituted alkylacylindoles are likely to share a number of equivalent analytical properties yet have different affinity at the cannabinoid receptors and variable biological activity.

The GC separation of the 1- and 3-pentylindole intermediate reaction products are shown in Figure 2 along with the mass spectra for these two regioisomeric compounds. The two isomers are well-resolved using the GC-MS system 2 with the 1-pentyl isomer eluting about 0.5 min ahead of the regioisomeric 3-pentylindole. The mass spectra for these two monosubstituted indoles are quite similar, as shown in Figure 2 (panels B and C). The base peak in both spectra occurs at m/z 130 and represents the regioisomeric methylene indole cation resulting from loss of mass 57 (C_4H_9) from the molecular ion for both compounds. The isomeric m/z 130 ions form following initial radical cation ionization of the parent molecule on the nitrogen (2B) or the pi electron system of the 5-membered pyrrole ring (2C). This fragmentation process is illustrated via the mechanistic insets in the spectra in Figure 2 (panels B and C).

The GC separation of the target compounds 1-*n*-pentyl-3-benzoylindole and 1-benzoyl-3-*n*-pentylindole is shown in Figure 3. Peak 1 in the chromatogram is the 1-benzoyl-3-*n*-pentylindole, and peak 2 is the inverse isomer 1-*n*-pentyl-3-benzoylindole. The separation was obtained using GC-MS system 1 consisting of a 5% phenyl methylpolysiloxane stationary phase in a 30 m capillary column under temperature programming conditions.

The mass spectra for the 1-*n*-pentyl-3-benzoylindole and the inverse isomer 1-benzoyl-3-*n*-pentylindole are shown in Figure 4 (panels A and B). The spectra for the substitution pattern of the

dx.doi.org/10.1021/ac500316x | Anal. Chem. 2014, 86, 3801−3808

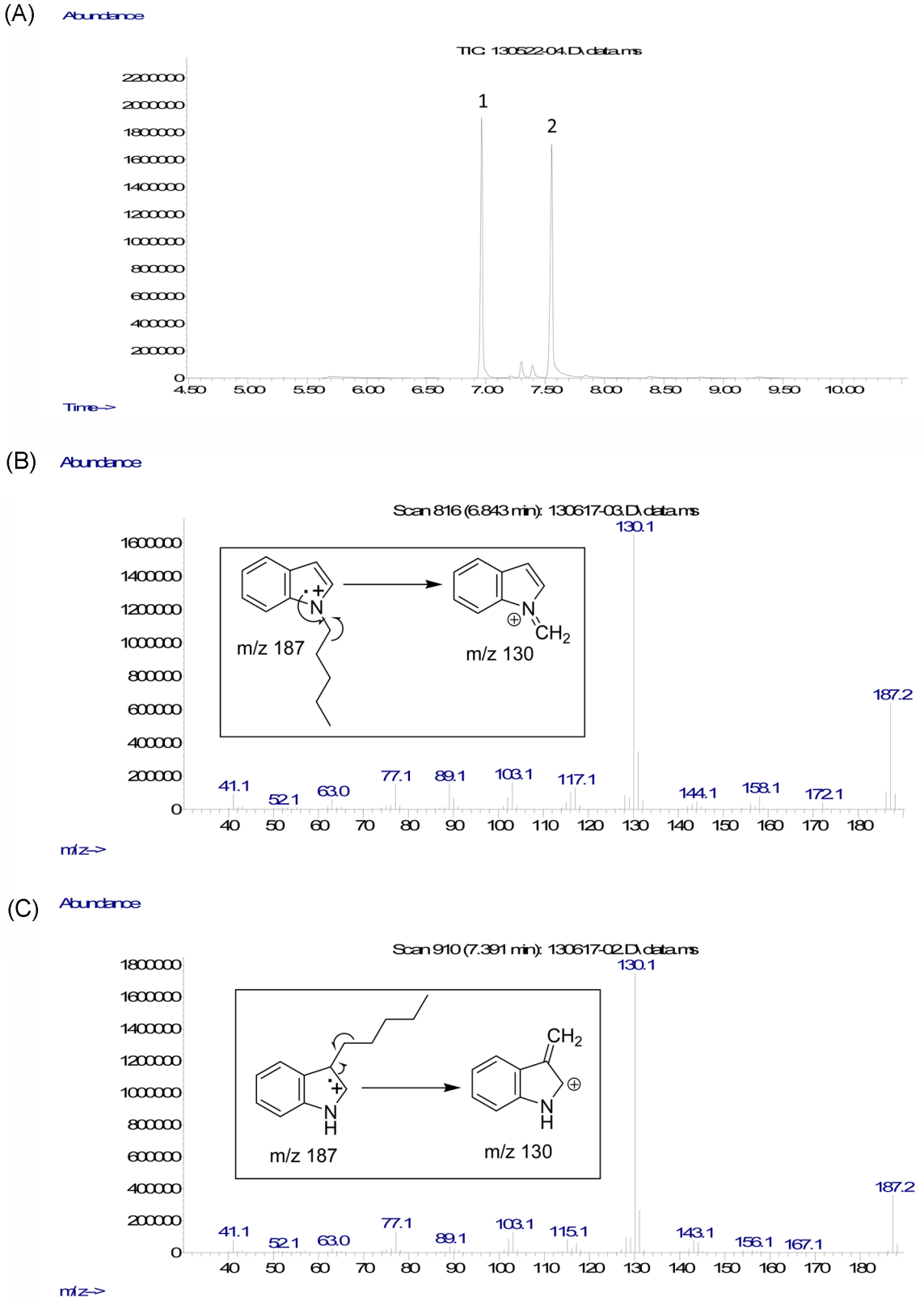


Figure 2. (A) GC of monosubstituted *n*-pentylindoles, where peak 1 = 1-*n*-pentylindole and peak 2 = 3-*n*-pentylindole. (B) Mass spectrum of 1-*n*-pentylindole. (C) Mass spectrum of 3-*n*-pentylindole.

traditional synthetic cannabinoid isomer, 1-*n*-pentyl-3-benzoylindole, is shown in Figure 4A, and the spectrum for the inverse isomer is in Figure 4B. The spectrum in Figure 4C for the 1-*n*-pentyl-3-d_5-benzoylindole provides significant assistance in elucidating the structures for the major fragment ions in the mass spectrum of the traditional synthetic cannabinoid compound shown in Figure 4A. The mass spectrum in Figure 4A for the 1-*n*-pentyl-3-benzoylindole shows a number of fragment ions

dx.doi.org/10.1021/ac500316x | *Anal. Chem.* 2014, 86, 3801−3808

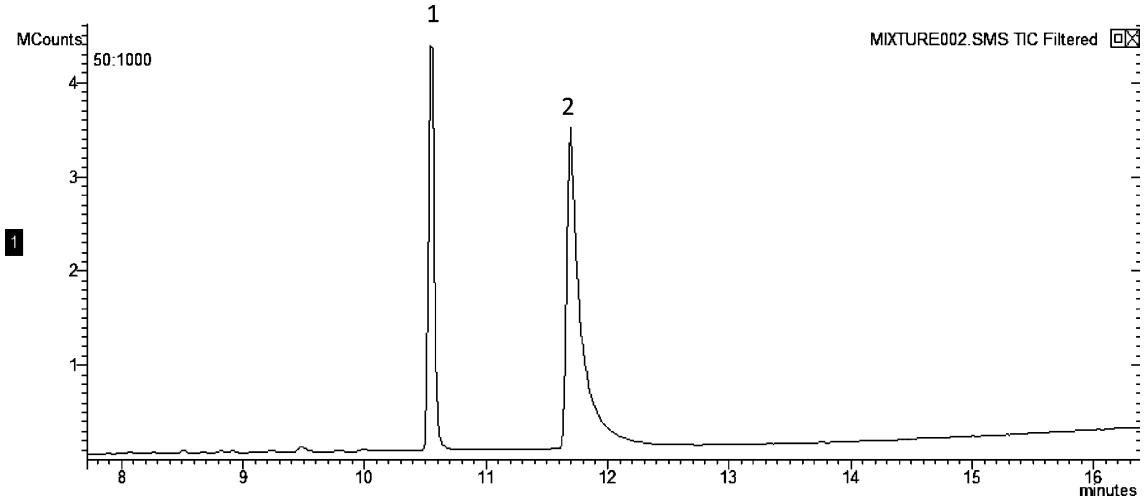


Figure 3. GC separation of the regioisomeric *n*-pentylbenzoylindoles, where peak 1 = 1-benzoyl-3-*n*-pentylindole and peak 2 = 1-*n*-pentyl-3-benzoylindole.

characteristic of the 1-alkyl-3-acylindoles. A comparison of the spectra in 4 (panels A and C) shows a mass shift of +5 Da for the fragments containing the phenyl group. The +5 Da shift for the molecular ion as well as the phenyl and benzoyl fragments confirm the structure for this model compound obtained by acylation of 1-pentylindole with d_5-benzoylchloride.

The structures for the major fragment ions in the spectrum for 1-*n*-pentyl-3-benzoyl-indole are shown in Figure 5. These ions primarily form as a result of fragmentation of the substituents attached to the indole ring with the base peak occurring at m/z 214. This fragment ion represents the loss of mass 77 from the molecular ion at m/z 291 (see Figure 4A) and does not undergo a mass shift in the spectrum shown in Figure 4C, confirming the m/z 214 base peak as the loss of the phenyl group from the molecular ion as shown in the fragmentation scheme in Figure 5. A comparison of the spectra in Figure 4 (panels A and C) shows a +5 Da shift in the ions at m/z 77 and 105 in Figure 4C confirms the deuterium labels in the phenyl portion of the benzoyl group attached at the 3-position of indole. The ion at m/z 144 occurs via hydrogen rearrangement from the positively charged nitrogen resonance form of the base peak at m/z 214. This process results in the loss of 1-pentene from the base peak cationic species. The high mass ions at m/z 234 and m/z 276 both show the expected +5 Da mass shift in the spectrum of the d_5-labeled compound in Figure 4C.

The m/z 234 ion in Figure 5 is the nitrogen-initiated alpha-cleavage fragmentation process resulting in the loss of C_4H_9 from the molecular ion, an equivalent pathway to that described for the formation of the base peak in the monosubstituted 1-pentylindole precursor substances previously described (see Figure 2B). The m/z 276 results from the loss of a methyl group (M-15) from the molecular ion and the structure shown in Figure 5 is a possible stable form of this cationic species. The m/z 186 ion does not undergo a mass shift in the spectrum of the d_5-labeled compound and represents the loss of the benzoyl-group (M-105) from the molecular ion.

The remaining m/z 130 ion represents the loss of the benzoyl group as well as four of the five carbons in the pentyl side chain (likely as 1-butene). The formation of the m/z 130 ion likely occurs via a bicyclic intermediate formed following a hydrogen rearrangement from the pentyl side chain to the indole ring (see Figure 6). A six-membered ring transition for this migration yields a radical species which directly eliminates the 1-butene portion from the alkyl side chain of the molecule. The bicyclic intermediate species forms by the direct elimination of the benzoyl group, and this carbocation species can further rearrange to yield the *N*-methylindole cation. The possible mechanism for this m/z 130 ion formation is shown in Figure 6. The lack of a mass shift for this ion in the spectrum of the d_5-labeled compound in Figure 4C further supports the source of the migrating hydrogen as the alkyl (*n*-pentyl) side chain. Since the m/z 130 ion is also one of the few ions of significance in the spectrum for the inverse isomer 1-benzoyl-3-*n*-pentylindole, its formation likely occurs by an analogous process.

These mass spectral results clearly show a number of unique ions which allow for the differentiation of the 1-alkyl-3-acylindole compounds from the inverse regioisomeric 1-acyl-3-alkylindoles. The base peak at m/z 214 in the 1-*n*-pentyl-3-benzoylindole represents the cationic fragment resulting from the loss of the phenyl radical from the molecular ion, and this ion is not observed in the inverse isomer. The 1-benzoyl-3-*n*-pentylindole inverse regioisomer shows a base peak at m/z 105 for the benzoyl cation. This fragmentation of the amide bond to yield the m/z 105 is the dominate process for this isomer. This dominate process yields fewer other ions of significant abundance in the mass spectrum for the 1-benzoyl-3-*n*-pentylindole. Thus, even though these two unique isomers can be prepared from identical precursor materials via similar synthetic procedures, standard forensic analytical techniques can differentiate these materials without the specific need for reference standards of both compounds.

ATR-FT-IR was evaluated for differentiation among the two regioisomeric compounds. This method has the possibility of yielding compound specificity without the need for chemical modification of the drug molecule. The IRs for the two compounds are shown in Figure 7.

The 1-pentyl-3-benzoylindole is characterized by the strong intensity band at 1703 cm^{-1}, which is shifted into a singlet of strong and equal intensity at 1681 cm^{-1} in the 1-benzoyl-3-pentyl regioisomer. Also the IR spectrum of the 1-pentyl-3-benzoylindole shows strong doublet peaks at 1519 and 1558 cm^{-1}, which shifted to a singlet at 1450 cm^{-1} for the 1-benzoyl-3-pentyl regioisomer. The 1-benzoyl-3-pentyl regioisomer has a relatively

dx.doi.org/10.1021/ac500316x | *Anal. Chem.* 2014, 86, 3801−3808

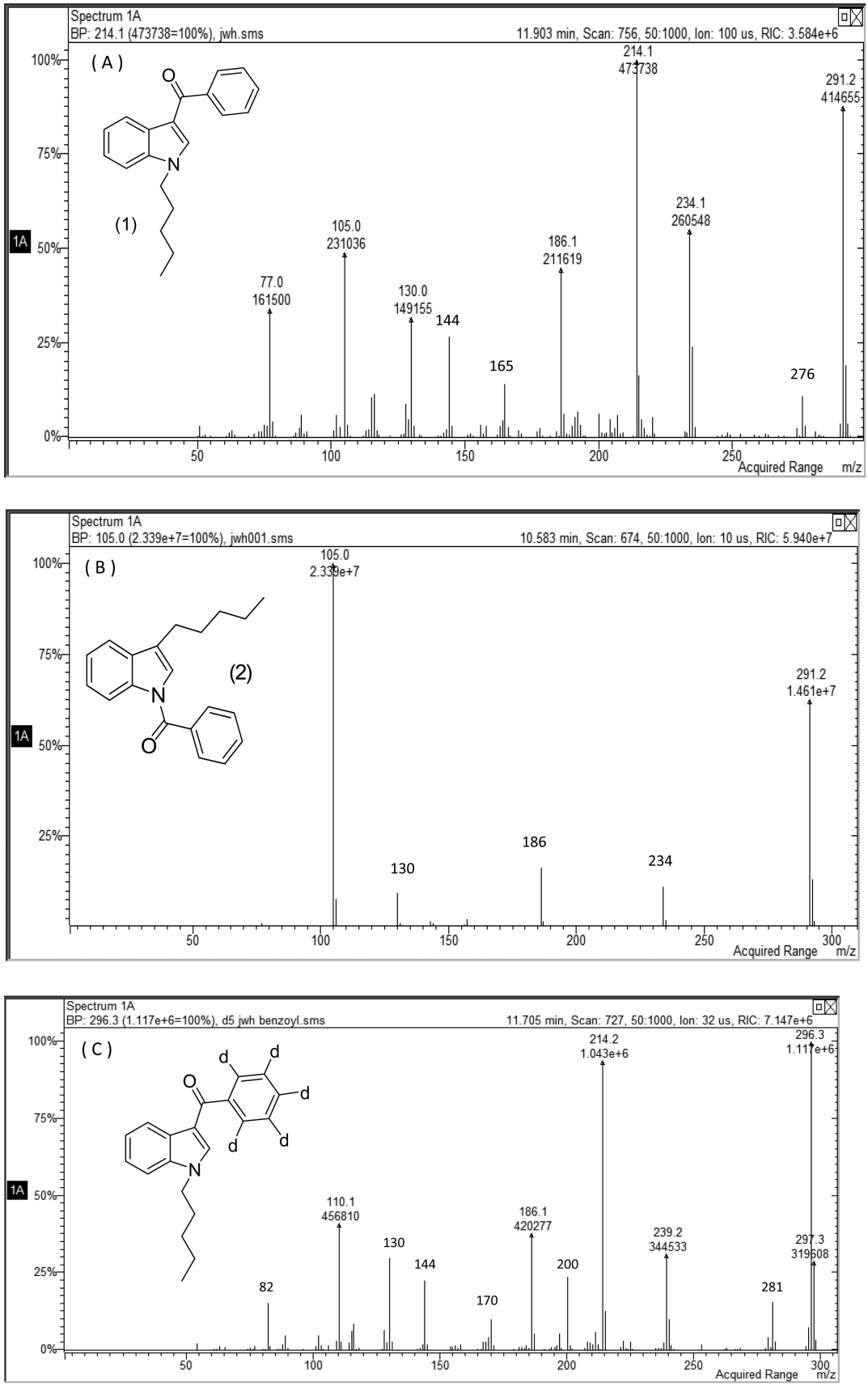


Figure 4. (A) Mass spectra of 1-*n*-pentyl-3-benzoylindole. (B) Mass spectrum of 1-benzoy-3-*n*-pentylindole. (C) Mass spectra of d_5-1-*n*-pentyl-3-benzoylindole.

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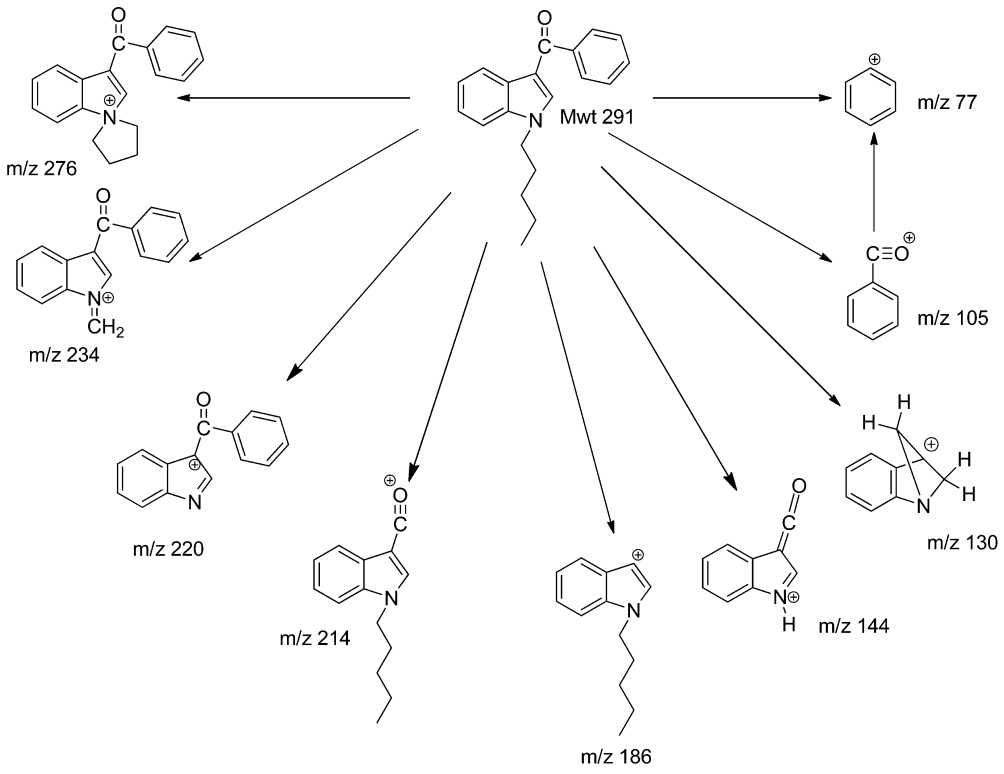

Figure 5. Structures for the major fragment ions in the spectrum for 1-*n*-pentyl-3-benzoyl-indole.

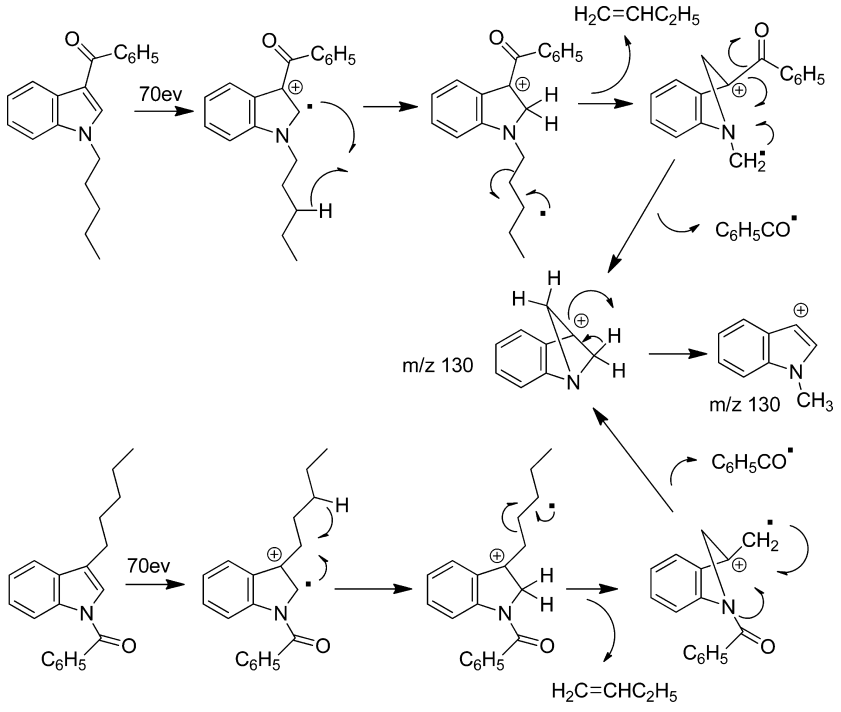

Figure 6. Mechanisms for the formation of the *m*/*z* 130 ions for the regioisomeric compounds in this study.

strong doublet at 1330 and 1359 cm^{-1}, which is shifted to a strong intensity peak at 1386 cm^{-1} in the 1-pentyl-3-benzoyl isomer. Finally the IR spectrum of the 1-benzoyl-3-pentylindole shows weak doublet peaks at 1213 and 1259 cm^{-1}, which shifted to a medium singlet at 1172 cm^{-1} for the 1-pentyl-3-benzoyl regioisomer. These results show that FT-IR spectra provide useful data for distinguishing and characterizing these uniquely regioisomeric compounds.

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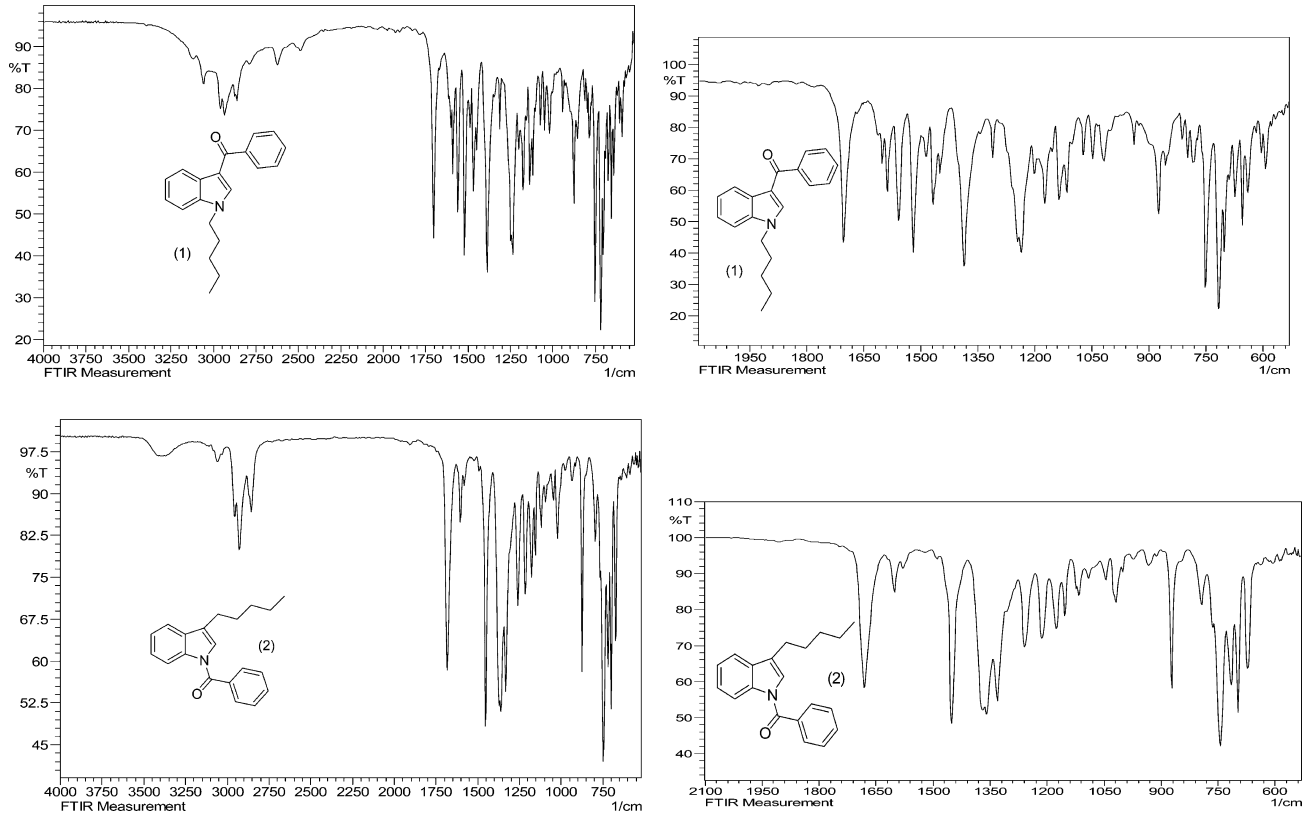

Figure 7. FT-IR of 1-*n*-pentyl-3-benzoylindole and 1-benzoy-3-*n*-pentylindole.

CONCLUSIONS

The 1-alkyl-3-acylindoles and the inverse regioisomeric 1-acyl-3-alkylindoles can be prepared directly from a common set of precursor materials and using similar synthetic strategies. The EI mass spectra for these isomers show a number of unique ions which allow for the differentiation of 1-alkyl-3-acylindole compounds from the inverse regioisomeric 1-acyl-3-alkylindoles. The base peak at m/z 214 in the 1-*n*-pentyl-3-benzoylindole represents the immonium cation fragment, which is not observed in the inverse isomer. The 1-benzoyl-3-*n*-pentylindole inverse regioisomer shows a base peak at m/z 105 for the benzoyl cation. The 1-pentyl-3-benzoylindole is characterized by the strong intensity carbonyl band at 1703 cm^{-1}, while the amide carbonyl in the 1-benzoyl-3-*n*-pentylindole appears as a strong band of equal intensity at 1681 cm^{-1} allowing for additional differentiation in these regioisomers.

ASSOCIATED CONTENT

Supporting Information

Synthetic methods used to prepare the compounds in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: clarkcr@auburn.edu. Tel: 334-844-8326. Fax: 334-844-8331.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This project was supported by Award 2012-DN-BX-K026, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.

REFERENCES

(1) Huffman, J. W.; Mabon, R.; Wu, M. J.; Lu, J.; Hart, R.; Hurst, D. P.; Reggio, P. H.; Wiley, J. L.; Martin, B. R. *Bioorg. Med. Chem.* **2003**, *11*, 539−549.

(2) Huffman, J. W.; Duncan, S. G. *Bioorg. Med. Chem. Lett.* **1997**, *7* (21), 2799−2804.

(3) Huffman, J. W. *The Cannabinoid Receptors*; Humana Press: Totowa, NJ, 2009; pp 49−94.

(4) Aung, M. M.; Griffin, G.; Huffman, J. W.; Wu, M. J.; Keel, C.; Yang, B.; Showalter, V. M.; Abood, M. E.; Martin, B. R. *Drug Alcohol Depend.* **2000**, *60*, 133−140.

(5) Uchiyama, N.; Kikura-Hanajiri, R.; Ogata, J.; Goda, Y. *Forensic Sci. Int.* **2010**, *198* (1−3), 31−38.

(6) Schifano, F.; Corazza, O.; Deluca, P.; Davey, Z.; Di Furia, L.; Farre', M.; Flesland, L.; Mannonen, M. *International Journal of Culture and Mental Health* **2009**, *2*, 137.

(7) EMCDDA. *Thematic papers*; European Monitoring Centre for Drugs and Drug Addiction: Lisbon, 2009.

(8) Schedules of Controlled Substances: Temporary Placement of Five Synthetic Cannabinoids into Schedule I, A Proposed Rule by the Drug Enforcement Administration on 11/24/2010. *Federal Register*.

(9) Wiley, J. L.; Compton, D. R.; Dai, D.; Huffman, J. W.; Martin, B. R. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 994−1004.

(10) Compton, D. R.; Rice, K. C.; De Costa, B. R.; Razdan, R. K.; Melvin, L. S.; Johnson, M. R.; Martin, B. R. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 218−226.

(11) Poso, A.; Huffman, J. W. *Br. J. Pharmacol.* **2008**, *153* (2), 335−346.
(12) Westphal, F.; Sönnichsen, F. D.; Thiemt, S. *Forensic Sci. Int.* **2012**, *215*, 8−13.
(13) Roper, S.; England, C.; Fadness, L. Differentiation of Isomers of Synthetic Cannabinoids, In *Proceedings of the American Academy of Forensic Sciences*, Atlanta, GA, Feb 20−25, 2012, AAFS abstracts, Abstract #A186.
(14) Jankovics, P.; Váradi, A.; Tölgyesi, L.; Lohner, S. *Forensic Sci. Int.* **2012**, *214*, 27−32.