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GC–MS and FTIR evaluation of the six benzoyl-substituted-1-pentylindoles: Isomeric synthetic cannabinoids

Forrest T. Smith [a], Jack DeRuiter [a], Karim Abdel-Hay [a,b], C. Randall Clark [a,*]

[a] Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University, Auburn, AL 36849, United States
[b] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt

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ABSTRACT

This report compares the GC–MS and FTIR properties of all 6 regioisomeric benzoyl substituted-1-*n*-pentylindoles. These compounds have the benzoyl-group attached at each of the possible ring substituent positions of the indole ring. The six compounds have the same elemental composition $C_{20}H_{21}NO$ yielding identical nominal and exact masses. Additionally, the substituents attached to the indole ring, benzoyl- and 1-*n*-pentyl-groups, are identical for all six isomers. The electron ionization mass spectra show equivalent regioisomeric major fragments resulting from cleavage of the groups attached to the central indole nucleus. Fragment ions occur at *m*/*z* 77 and 105 for the phenyl and benzoyl cations common to all six regioisomeric substances. Fragmentation of the benzoyl and/or pentyl groups yields the cations at *m*/*z* 234, 220, 214, 186 and 144. While the relative abundance of the ions varies among the six regioisomeric substances the 1-*n*-pentyl-3-benzoylindole and 1-*n*-pentyl-5-benzoylindole share very similar relative abundances for the major fragment ions.

Chromatographic separations on a capillary column containing a 0.5 μm film of 100% trifluoropropyl methyl polysiloxane (Rtx-200) provided excellent resolution of these six compounds. The elution order appears related to the relative distance between the two indole substituted groups. The latest eluting compounds (highest retention time) have the two substituents on opposite sides of the indole nucleus. Infrared absorption spectral data show the carbonyl absorption band for each of the benzoylindoles and provide distinguishing and characteristic information to individualize each of the regioisomers in this set of compounds.

1. Introduction

A number of new designer substances have appeared in forensic drug samples during the past decade. The synthetic cannabinoid series of designer drugs include direct analogues of delta-9-tetrahydrocannabinol (THC) and 5-(dimethylalkyl)-2-[3-hydroxycyclohexyl]-phenol (CP-47,497) as well as the 1-alkyl-3-acylindoles (JWH compounds) [1,2]. These compounds were initially used to investigate the cannabinoid receptors and their pharmacology. The "JWH" 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 the cannabinoids while the CB_2 receptor is primarily present in peripheral tissues. In spite of any CB_2 selectivity, many of the synthetic cannabinoids are more potent as agonists than THC at CB_1 receptors. Some 1-alkyl-3-acylindoles have affinity for the cannabinoid brain (CB_1) receptor five times greater than that of THC and have been shown to produces 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 originally referred to as "Spice" or "K2" and marketed as legal natural products described as "herbal incense" or "herbal smoking blends" [5,6]. Subsequent analysis revealed, however, that these products were in fact synthetic compounds. Many synthetic cannabinoids with psychotropic effects have been identified in clandestine drug samples in recent years [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 CB_1 receptor, the receptor that mediates the cannabis-like psychologic effects of these drugs [4,9,10,11]. 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

* Corresponding author. Tel.: +1 334 844 8326; fax: +1 334 844 8331.
E-mail address: clarkcr@auburn.edu (C. Randall Clark).

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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 [12,13,14].

The regioisomer issue in forensic drug analysis is extremely important when some molecules are legally controlled drugs or controlled precursor substances. Such regioisomeric compounds often possess mass spectral equivalency and similar chromatographic elution properties. Those substances co-eluting in the chromatographic system and having common mass spectra could be misidentified. Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target molecules. This issue is made even more critical when numerous regioisomeric precursor substances are commercially available in those drug categories produced by totally synthetic methods.

This paper directly compares a series of all six regioisomeric 1-*n*-pentylbenzoylindoles having the benzoyl-group at each of the possible ring substituent positions of the indole ring. The structures for the model compounds in this study are shown in Fig. 1. The 1,3-substitution pattern as shown for Compound 2 in Fig. 1 is the classic pattern for the indole derivatives often described as synthetic cannabinoids. While the 1,3-substitution pattern is directly available from indole as a synthetic starting point all five of the other possible regioisomeric 1-*n*-pentyl-benzoylindoles are available from other commercially available synthetic precursor materials.

2. Experimental

2.1. Instrumentation

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 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 then ramped to 340 °C at a rate of 5 °C/min and held at

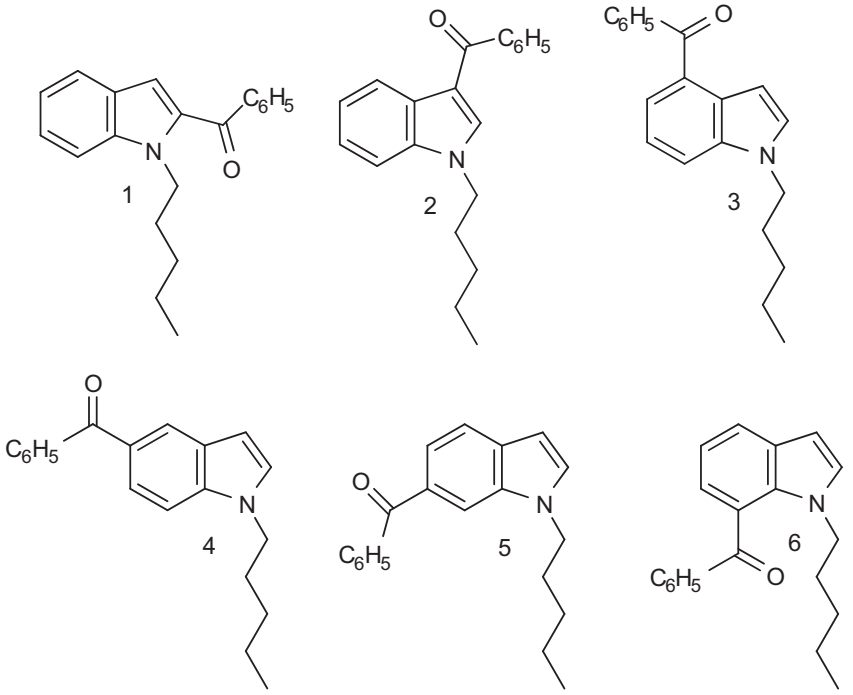

Fig. 1. Structures of the regioisomeric 2-, 3-, 4-, 5-, 6-, and 7-benzoyl-1-*n*-pentylindoles in this study.

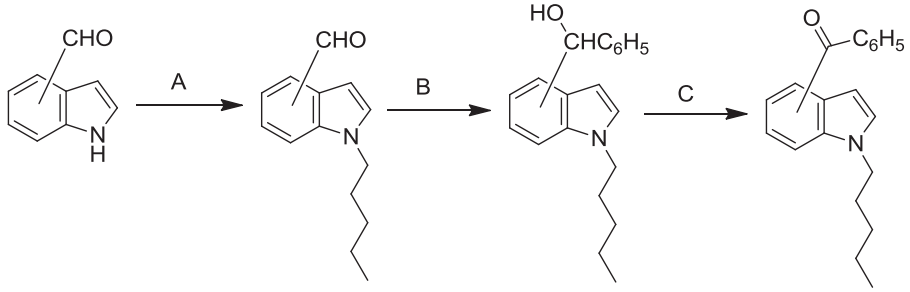

Fig. 2. General synthetic scheme for the compounds in this study. *A*=sodium hydroxide and 1-bromo-*n*-pentane; *B*=phenylmagnesium bromide; *C*=pyridinium dichromate.