Characterization of 2β-(1,2,4-Oxadiazol-5-methyl)-3β-phenyltropane ("RTI-126")

John F. Casale* and Patrick A. Hays
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email address withheld at corresponding author's request]

ABSTRACT: Spectroscopic and chromatographic data are provided for 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane (commonly referred to as RTI-126), its 2α-epimer, and their respective synthetic intermediates. Direct comparisons of the analytical data are made to assist forensic chemists in correctly differentiating these epimeric isomers in suspected drug exhibits.

KEYWORDS: 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane, RTI-126, designer drugs, chemical analysis, forensic science

Businesses advertising and executing the sale of "legal highs" have recently flourished on the Internet. In conjunction, several Internet "chemical companies" have been publicizing the recent synthesis of many CNS-active compounds for sale that are "*not for human use.*" In 2010, for the first time, a cocaine-like derivative was being openly touted as a non-controlled stimulant, with an activity five-times greater than that of cocaine. The compound (Figure 1) is generically referred to as "RTI-126," or specifically, 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane.   3β-Phenyltropane-2-carboxylic acid esters and analogs were first synthesized and studied in 1973 for their biological activity [1]. RTI-126 was first synthesized in the early 1990's to study the binding affinities of several cocaine analogs in the dopamine, serotonin, and norepinephrine transport systems [2].

This paper presents the analytical profile of RTI-126, its 2α-epimer, and their respective synthetic intermediates (Figure 2) to assist forensic chemists who may encounter these substances in casework.

Experimental

Chemicals, Reagents, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). All other chemicals were of reagent-grade quality and products of Sigma-Aldrich Chemical (Milwaukee, WI). Additionally, a 5 mg sample of "RTI-126" was obtained from an undisclosed source.

Gas Chromatography/Mass Spectrometry (GC/MS)

Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and at a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm 100% dimethylpolysiloxane, DB-1, (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: initial temperature, 100°C; initial hold, 0.0 min; program rate, 6°C/min; final temperature, 300°C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) at 280°C. The MSD source was operated at 230°C.
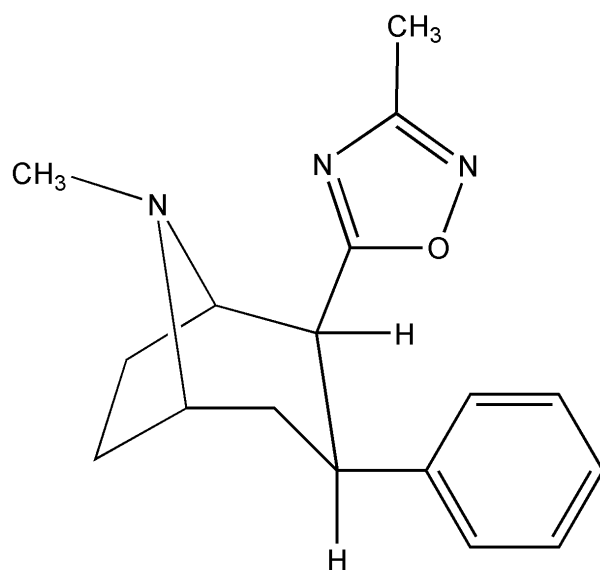

Figure 1 - Structural formula of 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane (RTI-126).

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: resolution = 4 cm^{-1}, gain = 8, optical velocity = 0.4747, aperture = 150, and scans/sample = 32.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Proton (^{1}H), carbon (^{13}C), and 2-dimensional NMR spectra were obtained on a Agilent (formerly Varian) 400MR 400 MHz NMR using a 5 mm Protune indirect detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). All compounds were dissolved in deuterochloroform (CDCl$_3$) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound. The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to acquire ^{1}H, proton-decoupled ^{13}C, and gradient versions of COSY, NOESY, HSQC, and HMBC spectra. Data processing was performed using software from Agilent and Applied Chemistry Development
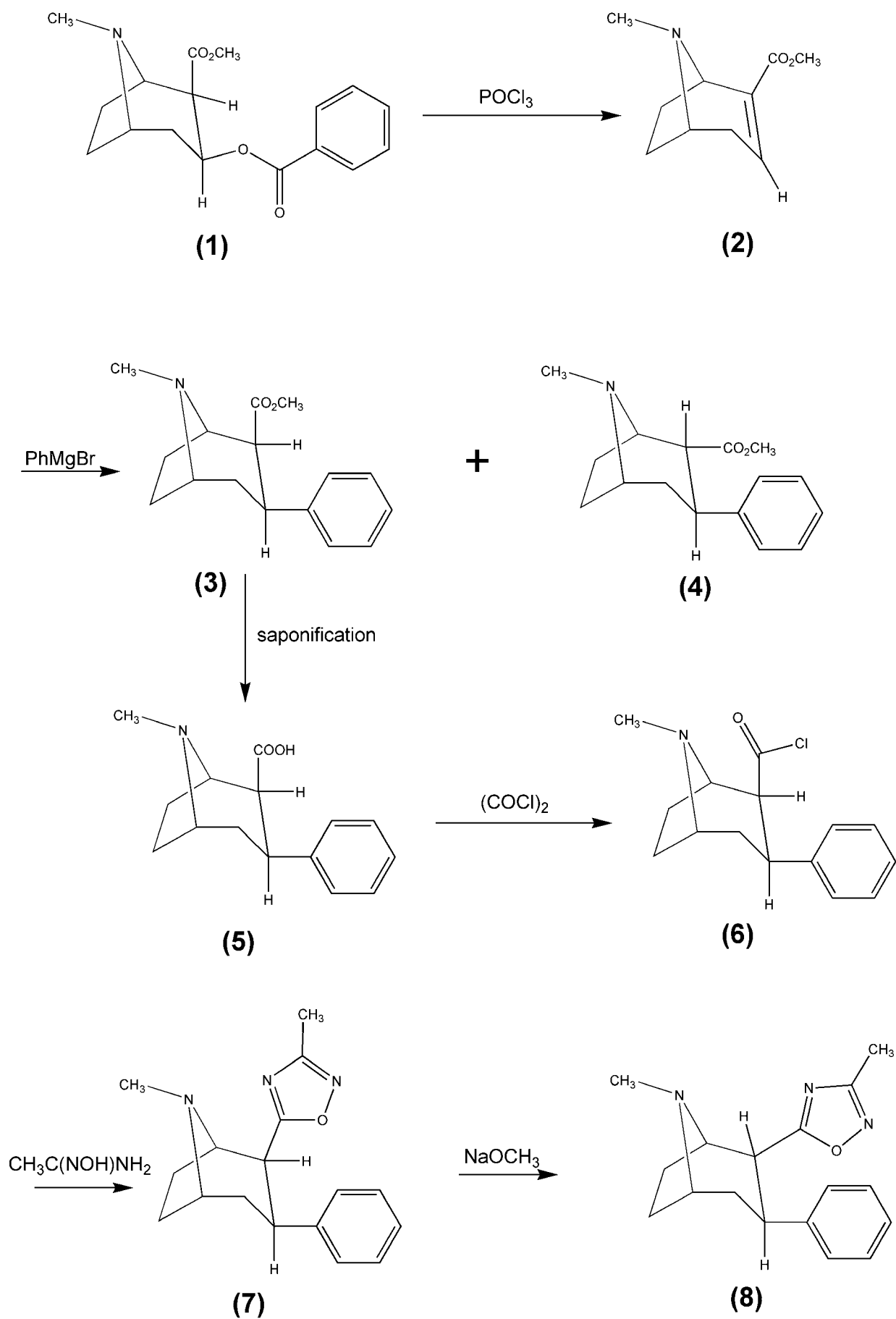
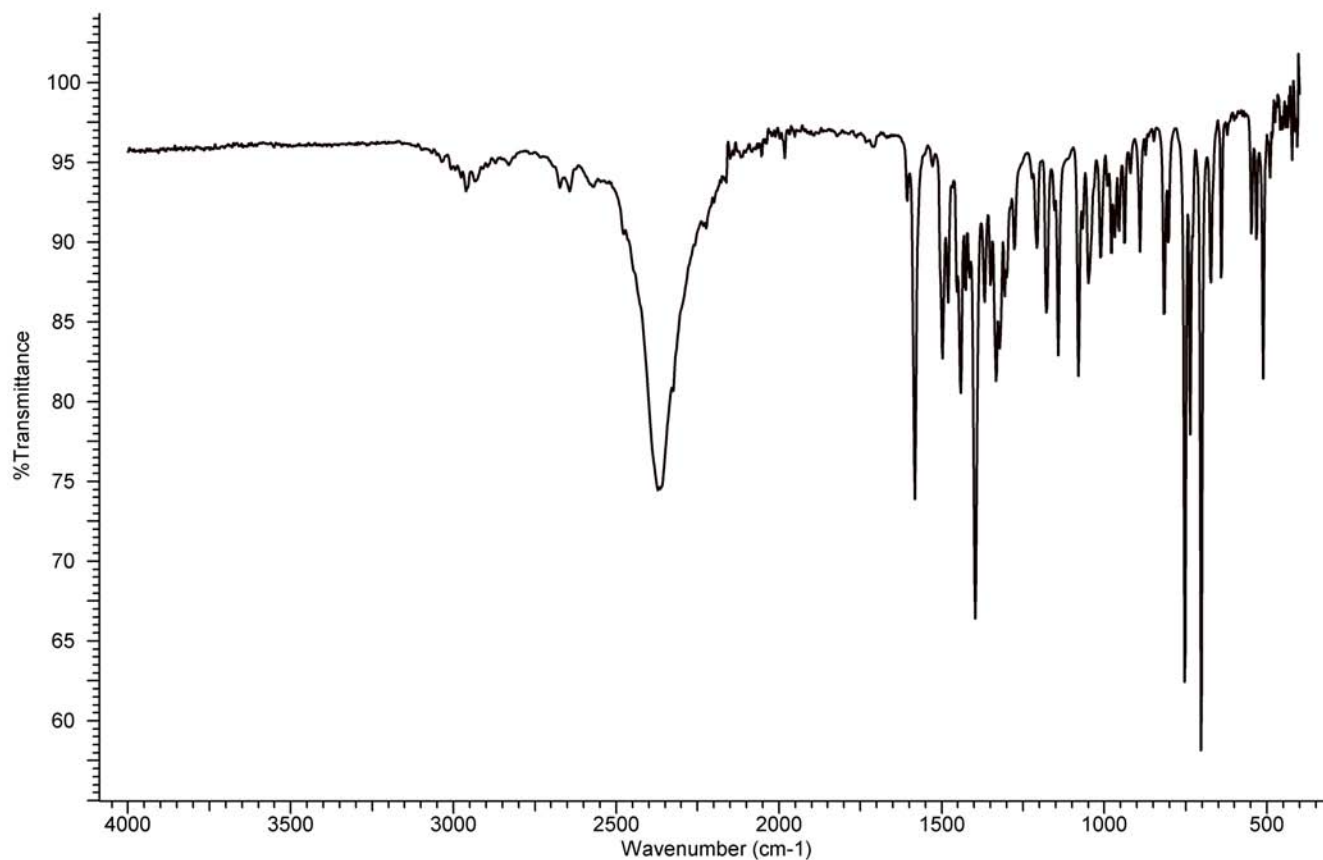Figure 2 - Synthetic route to 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane **7** (RTI-126).
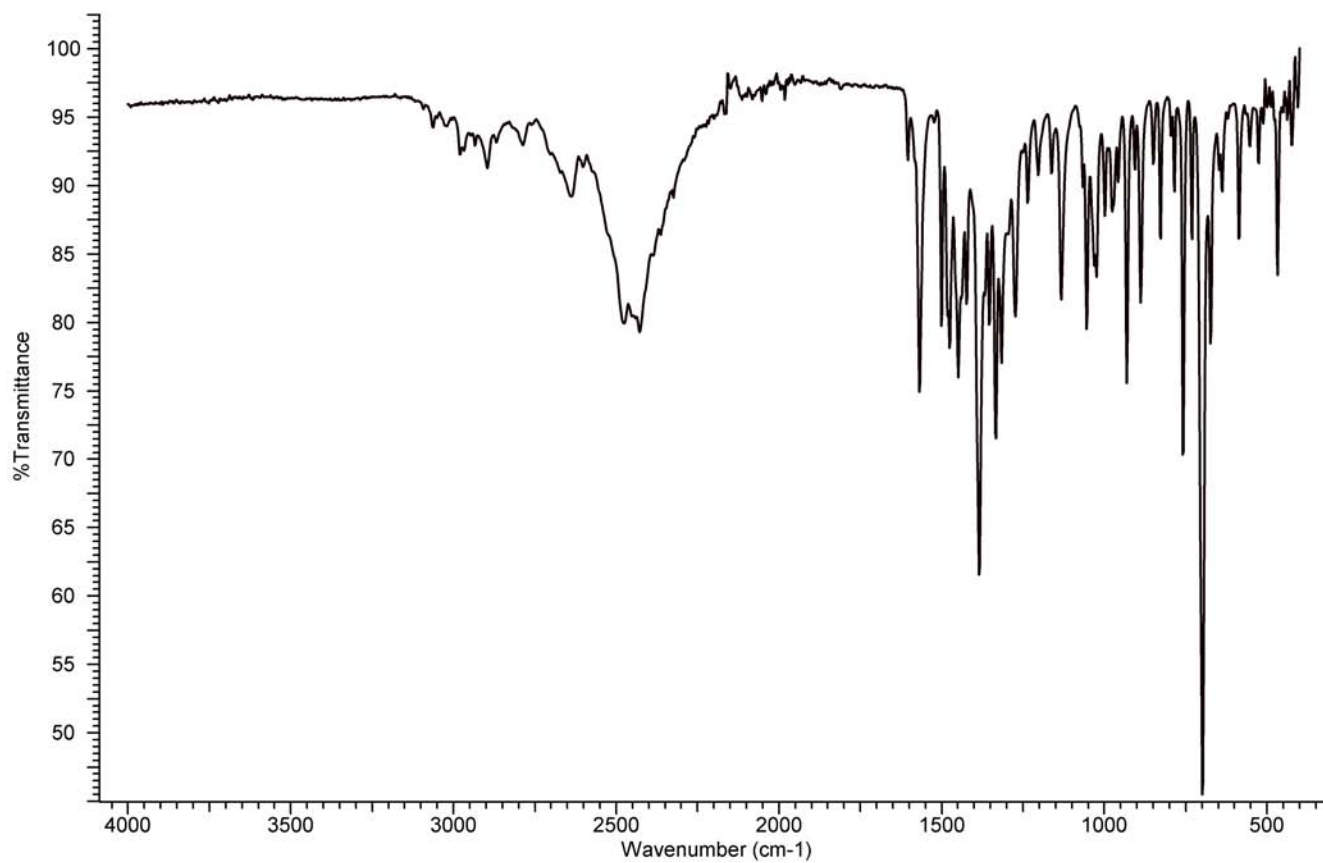
Figure 3 - Infrared spectra of 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane **7** (RTI-126) HCl (upper) and 2α-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane **8** HCl (lower).
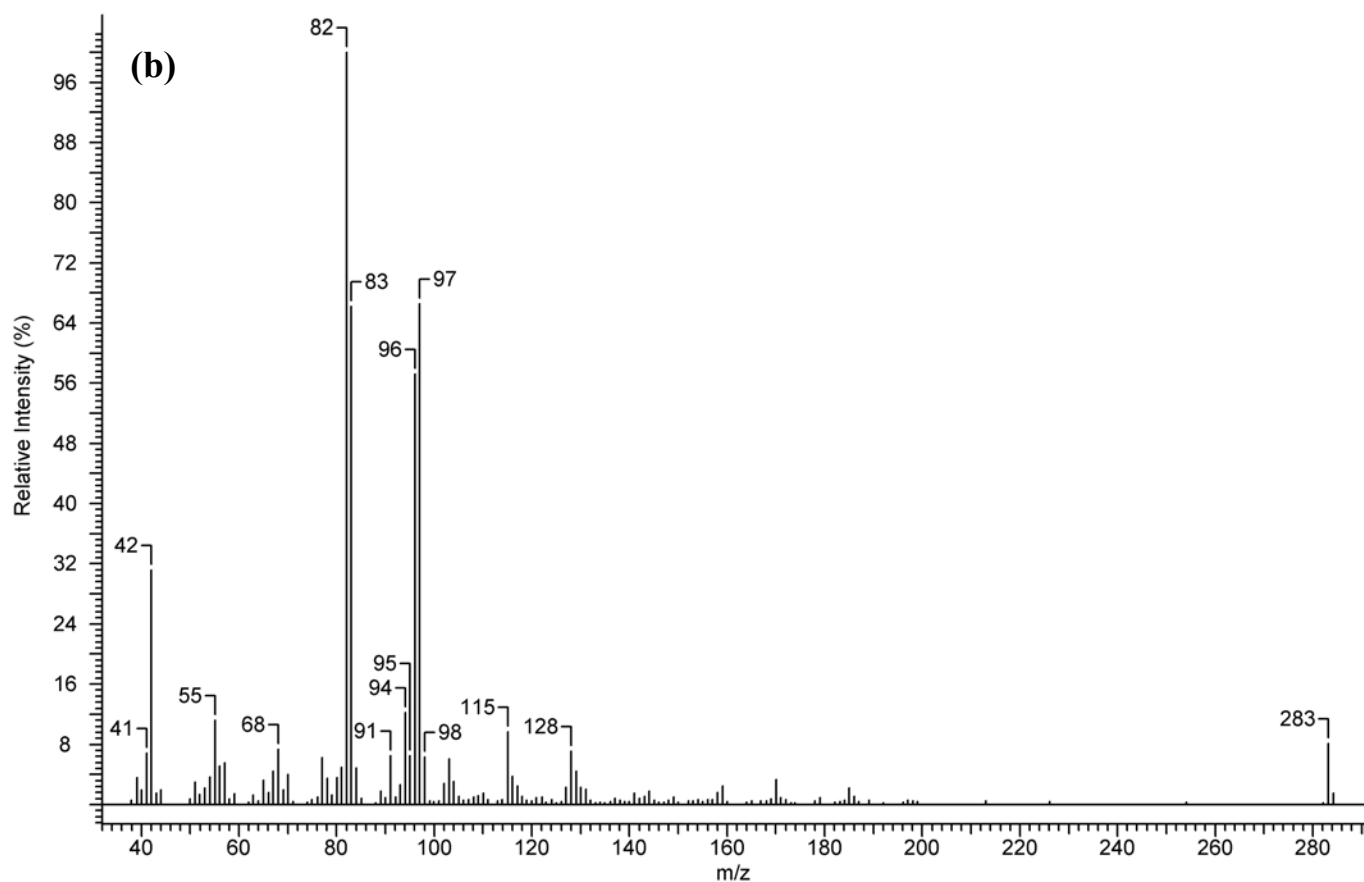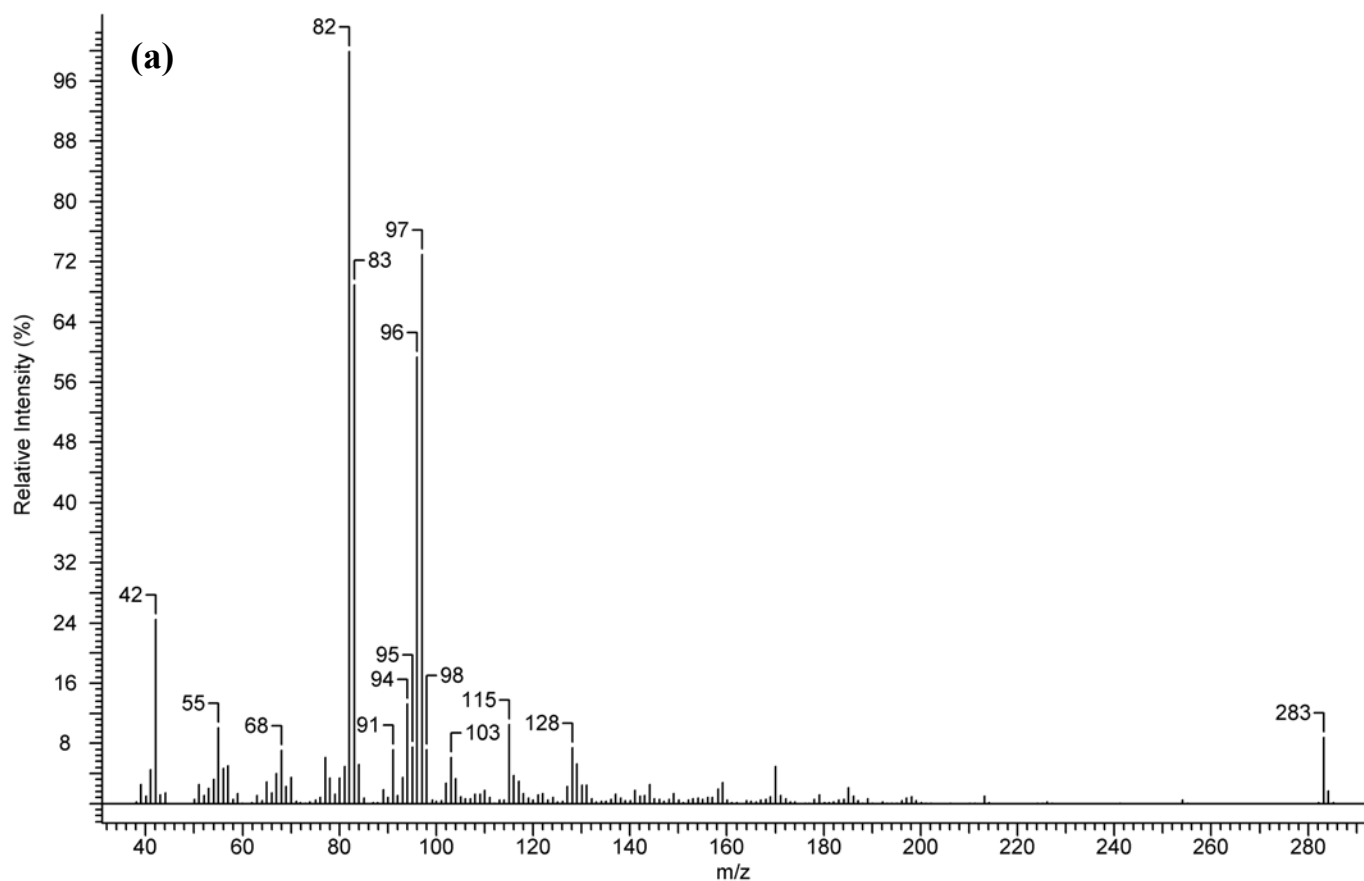
Figure 4 - Electron ionization mass spectra of (a) 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane **7** (RTI-126) and (b) 2α-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane **8**.

Table 1 - Gas chromatographic retention times (R_t) and Relative Retention Times (RR_t) for the 3β-Phenyltropane Related Compounds[a].

Compound	R_t (min)	RR_t
Anhydroecgonine methyl ester **(2)**	7.70	0.36
2α-Carbomethoxy-3β-phenyltropane **(4)**	16.40	0.77
2β-Carbomethoxy-3β-phenyltropane **(3)**	17.02	0.80
3β-Phenyltropane-2β-carboxylic acid-TMS **(5)**	18.30	0.86
2α-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane **(8)**	19.49	0.92
2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane[b] **(7)**	19.89	0.94
2β-Carbomethoxy-3β-benzoyloxytropane **(1)**	21.25	1.00
3β-Phenyltropane-2β-carboxylic acid **(5)**	22.57	1.06

[a]Conditions given in Experimental Section
[b]RTI-126

(ACD/Labs, Toronto, Canada). Structure elucidation and the prediction of ^{1}H and ^{13}C spectra was accomplished using ACD/Labs software.

Synthesis

The procedures of Carroll *et al*. [2, 3] were followed for the preparation of RTI-126 and its intermediates. Synthetic details and yield values are not reported.

Results and Discussion

The synthetic procedure (Figure 2) to give RTI-126 begins with cocaine **1**, but the first intermediate compound (anhydroecgonine methyl ester) **2** can also be produced from other cocaine derivatives such as ecgonine, ecgonine methyl ester, benzoylecgonine, or anhydroecgonine (all controlled substances). It is unlikely that a Mannich-type condensation reaction would be utilized to eventually give **2**, because of extremely low yields and it results in an enantiomeric (racemic) mixture. Reactions in some steps require temperatures from -45 to -78°C, thus limiting this synthesis to sophisticated laboratories equipped to operate at this range. The synthesis of 2β-carbomethoxy-3β-phenyltropane **3** gives significant amounts of the 2α-epimer **4** and other by-products, which must be separated through chromatographic means. Saponification of **3** gives the carboxylic acid **5**, which is then converted to the acid chloride **6**. Formation of the oxadiazol group in **7** is very low yielding. Epimerization of **7** to **8** is accomplished via treatment with sodium methoxide.

GC retention time data for the respective compounds (Figure 2) are presented in Table 1; a mixture of **7** and **8** was baseline resolved.

The FTIR spectra for **7** and **8** as their HCl ion-pairs are illustrated in Figure 3. Comparison of the hydrochloride ion pairs reveals somewhat similar absorption patterns, however prominent differences in the C-H out-of-plane bending frequencies between 500-900 cm^{-1} easily distinguish the two compounds.

Mass spectra for **7** and **8** are presented in Figure 4. Both compounds have a base peak at *m/z* 82, indicative of the N-methyl-pyrrolidinium ion [4]. In addition, both have multiple fragment ions with ion abundances in the same pattern, including a molecular ion at *m/z* 283. The two spectra are virtually identical and indistinguishable. Since the spectra are identical, additional or supplementary spectroscopic methods must be utilized for identification. Spectra of known intermediate products are included (Figures 5 and 6), in case they reside as impurities in a sample of suspected **7**. The intermediates **3** and **4** (Figure 5) also gave virtually identical mass spectra, including a base peak at *m/z* 82, multiple fragment ions with ion abundances in the same pattern, and a molecular ion at *m/z* 259. However, GC retention times differentiate these compounds. Finally, the spectrum of carboxylic acid **5** is shown both underivatized and as its TMS derivative (Figure 6).

The ^{1}H and ^{13}C chemical shifts and splitting patterns for **7** and **8** are presented in Tables 2 and 3, respectively. Assignments were based on proton and carbon chemical shift values, proton splitting patterns and coupling constants, and correlations between protons using COSY (coupled protons) and NOESY (protons spatially near each other) experiments, as well as proton and carbon using the HSQC (directly bonded carbon to proton) and HMBC (2, 3, or 4 bond correlation between carbon and proton) experiments. Structure elucidation was performed on NMR data manually and by using the ACD/Labs Structure Elucidator program.

The key diagnostic feature of the proton spectrum, for differentiating compounds of this type, is the splitting pattern and coupling constants of H-3 (found at 3.3 ppm). This axial proton is coupled to three protons: H-2 (either equatorial as in structure 7 or axial as in structure 8) and the two H-4 protons. An axial-axial coupling constant is typically twice the size (about 12 Hz) of an axial-equatorial coupling (about 6 Hz). In the case of structure 7, H-2 is equatorial (H3ax-H2eq=5.7 Hz) giving H-3 a doublet of triplets peak pattern (13.1, 5.7, 5.7 Hz in a 1:2:2:2:1 pattern), while in the case of structure 8, H-2 is axial (H3ax-H2ax=12.1 Hz) giving H-3 a triplet of doublets pattern (12.1, 12.1, 5.7 Hz in a 1:1:2:2:1:1 pattern).

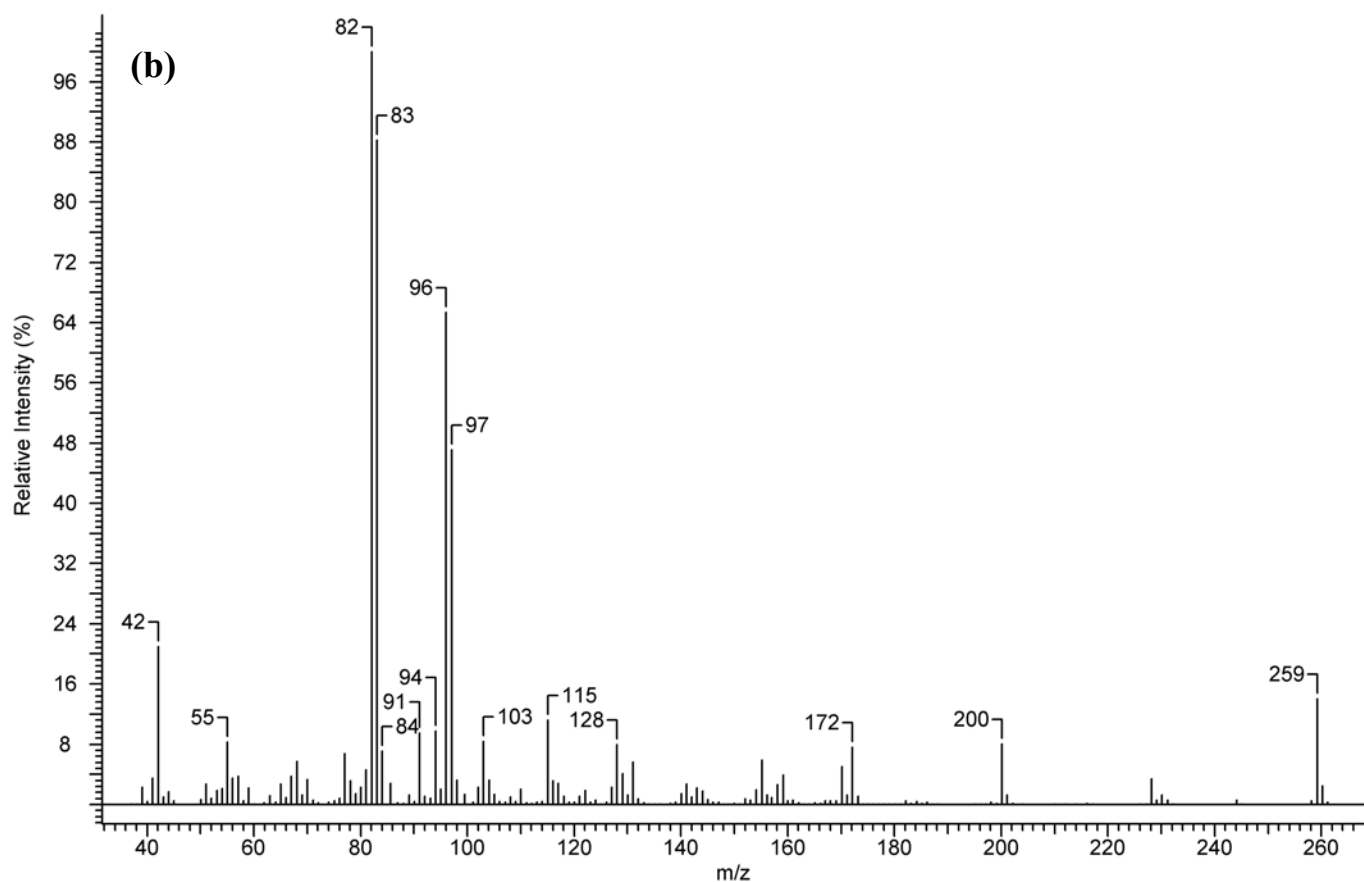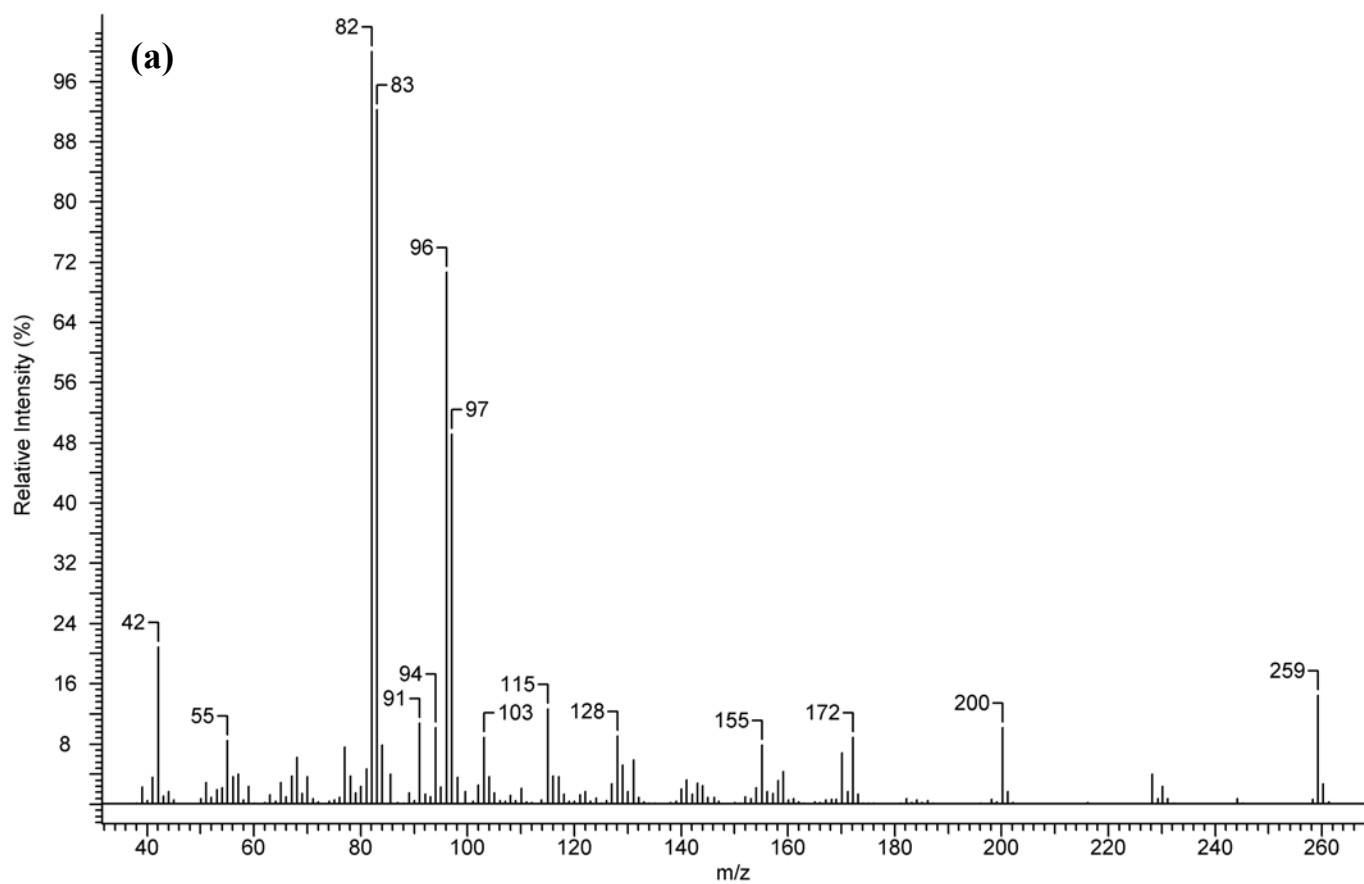A 5 mg sample of RTI-126 was acquired from an undisclosed

Figure 5 - Electron ionization mass spectra of (a) 2β-carbomethoxy-3β-phenyltropane **3** and (b) 2α-carbomethoxy-3β-phenyl-tropane **4**.
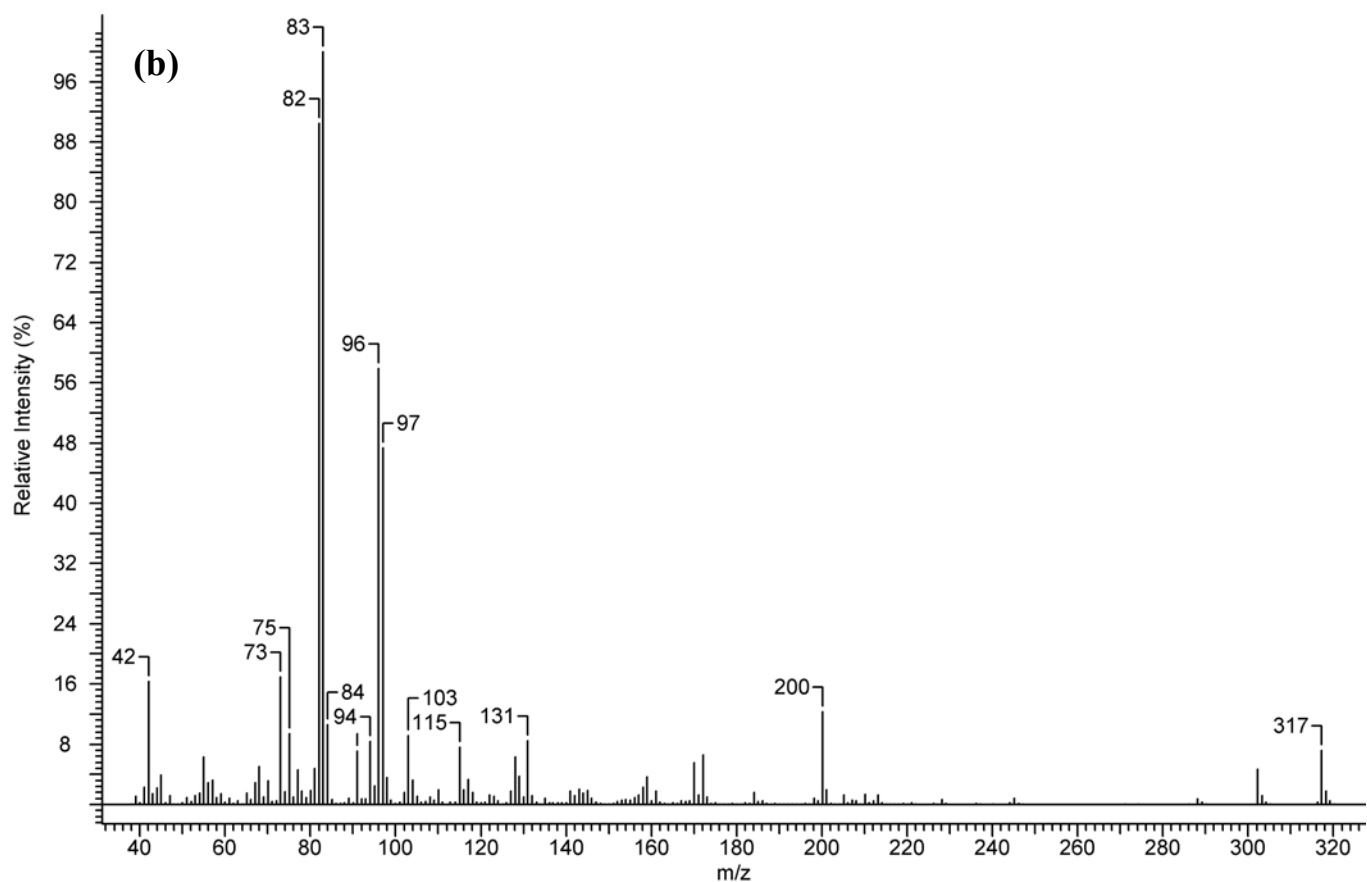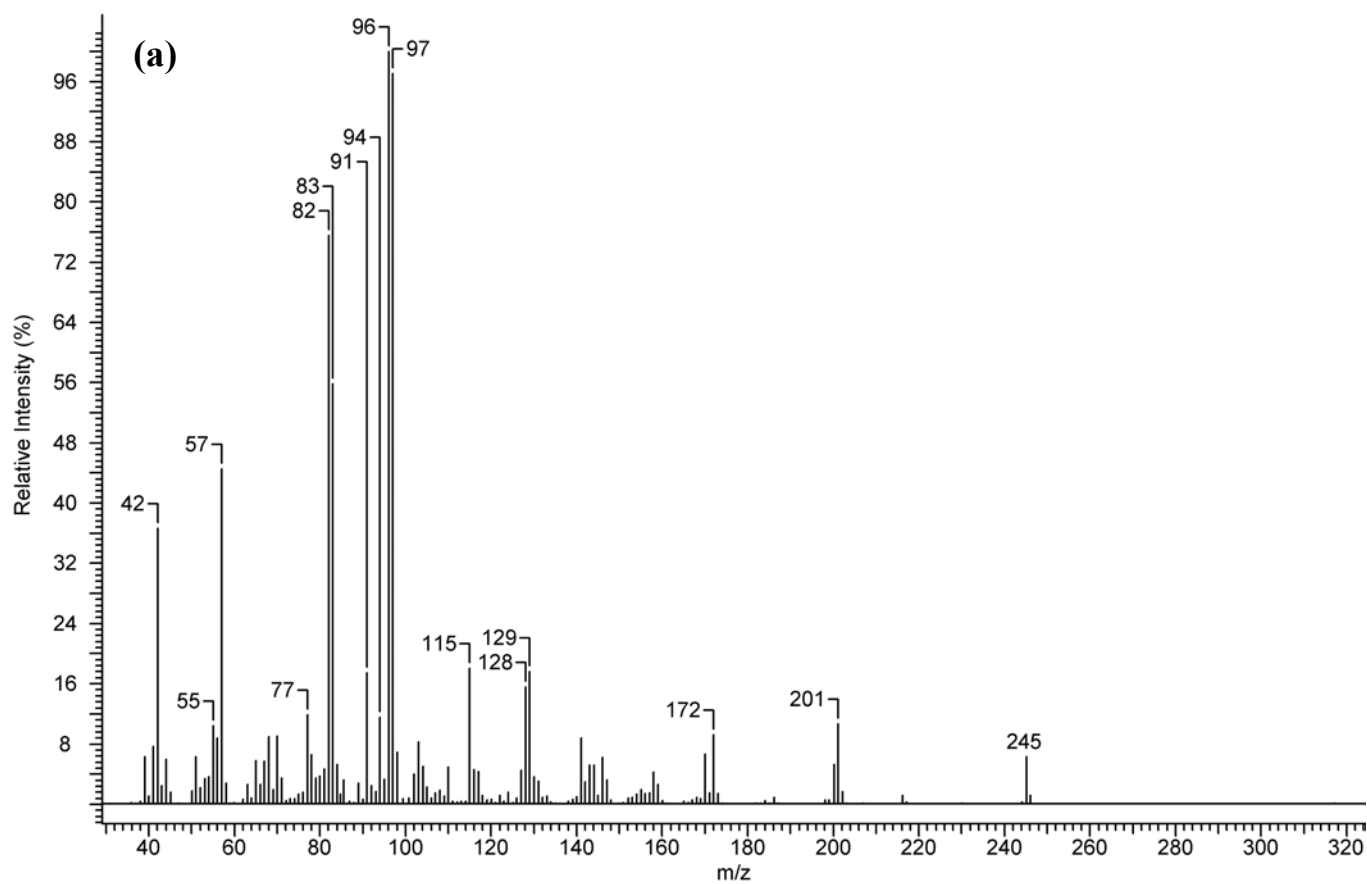
Figure 6 - Electron ionization mass spectra of (a) 3β-phenyltropane-2β-carboxylic acid **5** and (b) 3β-phenyltropane-2β-carboxylic acid TMS derivative.

Table 2 - NMR proton/carbon chemical shifts (in ppm) and splitting patterns of 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane **7** (RTI-126). Samples run in CDCl₃ with TMS as the reference compound for 0 ppm.

No.	Proton	H's	Type	J (Hz)	Carbon
1	3.43	1	m	-	65.9
2 eq	3.51	1	dd	5.7, 2.8	47.1
3 ax	3.30	1	ddd	13.1, 5.7, 5.5	34.8
4 ax	2.67	1	td	13.1, 13.1, 2.6	34.4
4 eq	1.78	1	ddd	13.1, 5.5	34.4
5	3.45	1	m	-	62.0
6 endo	1.73	1	ddd	12.6, 9.6, 4.4	25.2
6 exo	2.16	1	dddd	12.6, 12.5, 6.8, 4.4	25.2
7 endo	1.86	1	ddd	12.9, 9.6, 4.4	26.4
7 exo	2.28	1	dddd	12.9, 12.5, 6.7, 4.4	26.4
N-methy	2.20	3	s	-	41.8
CH3 on oxadiazol	2.24	3	s	-	11.6
C-3 of oxadiazol	-	-	-	-	166.3
C-5 of oxadiazol	-	-	-	-	179.3
phenyl (ortho, meta)	7.19	4	m		127.4, 128.2
phenyl (para)	7.11	1	m		126.4
phenyl (quaternary)	-	-	-	-	141.2

Proton and carbon chemical shifts are in ppm referenced to TMS (0 ppm)
Type abbreviations: d = doublet, m = multiplet, s = singlet, t = triplet

Table 3 - NMR proton/carbon chemical shifts (in ppm) of 2α-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane **8**. Samples run in CDCl₃ with TMS as the reference compound for 0 ppm.

No.	Proton	H's	Type	J (Hz)	Carbon
1	3.41	1	dd	6.5, 2.2	64.8
2 ax	3.75	1	dd	12.1, 2.2	44.6
3 ax	3.32	1	td	12.1, 12.1, 5.7	38.1
4 ax	3.32	1	ddd	13.5, 12.1, ~2	39.2
4 eq	1.73	1	ddd	13.5, 5.7, 2.9	39.2
5	3.32	1	m	-	61.2
6 endo	1.79	1	dddd	12.2, 9.7, ~6.5,4.7	26.4
6 exo	2.16	1	m	-	26.4
7 endo	2.07	1	ddd	13.8, 9.7, 4.7	32.2
7 exo	1.92	1	dddd	13.8, 12.1, 6.5, 4.7	23.2
N-methy	2.44	3	S	-	39.7
CH3 on oxadiazol	2.27	3	s	-	11.6
C-3 of oxadiazol	-	-	-	-	166.7
C-5 of oxadiazol	-	-	-	-	179.5
phenyl (ortho, meta)	7.23	4	m		127.6, 128.5
phenyl (para)	7.14	1	m		126.7
phenyl (quaternary)	-	-	-	-	142.5

Proton and carbon chemical shifts are in ppm referenced to TMS (0 ppm)
Type abbreviations: d = doublet, m = multiplet, s = singlet, t = triplet

source and analyzed. GC/MS analysis of the sample revealed that **7** contributed to 96+% of the total ion chromatogram. Three impurities were detected, and were identified as **3** (~0.5%), **4** (~0.2%), and **8** (~2.7%).

Conclusions

Analytical data is presented to assist in characterizing suspected drug exhibits containing 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane (RTI-126), as well as some synthetic intermediates. Characterization of RTI-126 is best achieved by combined GC/MS and FTIR or NMR spectroscopy.

References

1. Clarke RL, Daum SJ, Gambino AJ, Aceto MD, Pearl J, Levitt M, Cumiskey WR, Bogado EF. Compounds affecting the central nervous system. 4. 3β-phenyltropane-2-carboxylic esters and analogs. J Med Chem 1973;16:1260-7.

2. Carroll FI, Gao Y, Rahman P, Parham K, Lewin A, Boja JW, Kuhar MJ. Synthesis, ligand binding, QSAR, and CoMFA study of 3β-(*p*-substituted phenyl)tropane-2β-carboxylic acid methyl esters. J Med Chem 1991;34:2719-25.

3. Carroll FI, Gray JL, Kuzemko MA, Lewin AH, Boja JW, Kuhar MJ. 3-Aryl-2-(3'-substituted-1',2',4'-oxadiazol-5'-yl)tropane analogues of cocaine: affinities at the cocaine binding site at the dopamine, serotonin, and norepinephrine transporters. J Med Chem 1993;36:2886-90.

4. Smith RM, Casale JF. The mass spectrum of cocaine: Deuterium labeling and MS/MS studies. Microgram J 2010;7(1):16-41.