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The Naphyrone Story: The Alpha or Beta-naphthyl Isomer?

Simon D. Brandt,[a]* Robert C. R. Wootton,[a] Giorgia De Paoli[b] and Sally Freeman[c]

Naphyrone (naphthylpyrovalerone, O-2482) has been recently advertised for purchase on a number of websites. This compound has been viewed as a so-called 'legal high' and was classified as a controlled drug under the UK Misuse of Drugs Act 1971 in mid-July 2010. So far, naphyrone is commonly equated with 1-naphthalen-2-yl-2-pyrrolidin-1-yl-pentan-1-one (β-naphyrone) but analytical characterization of two naphyrone samples revealed the existence of a novel isomer consistent with 1-naphthalen-1-yl-2-pyrrolidin-1-yl-pentan-1-one (α-naphyrone). Analyses of both α- and β-naphyrone were carried out using gas chromatography ion trap (EI/CI) mass spectrometry and 1D/2D nuclear magnetic resonance spectroscopy. This provides the first report of α-naphyrone in the scientific literature and the ability to differentiate it from the β-isomer should be of interest to forensic and clinical communities.

Keywords: naphyrone; legal highs; NRG-1; nuclear magnetic resonance spectroscopy; mass spectrometry; forensic; clinical

The current interest in the identification of cathinone derivatives and other 'legal highs' stems from their wide range of biological activities.[1–3] This, together with their availability on the Internet and in high street shops,[4,5] makes them an attractive option for recreational use.[6] The cathinone class has been subject to intense research activities within the medicinal field and continues to do so.[7–9] The collection of detailed clinical data about the currently circulating cathinones used recreationally are needed. Dedicated websites such as *Erowid* [10] host a wide range of information relating to many aspects of recreational drug use, considering both legal and illegal products and drugs. Misidentification and the presence of wrongly labelled products, on the other hand, can make the assessment of user-oriented forms of harm reduction very difficult or perhaps redundant.

One of the products that received considerable exposure and interest was *Energy 1* (NRG-1). This has been commonly advertised as naphyrone (naphthylpyrovalerone, O-2482) that is structurally associated with 1-naphthalen-**2**-yl-2-pyrrolidin-1-yl-pentan-1-one (β-naphyrone; Figure 1A). Since mid-July 2010, a number of pyrovalerone analogues have been classified as Class B drugs under the UK Misuse of Drugs Act 1971.[11] Increased perception about the availability of NRG-1 and related products on the Internet followed as a response to the legal ban on mephedrone and related cathinones in mid-April 2010, in the attempt to offer legal replacements.

As part of an initial study, a total number of 13 NRG-1 products were purchased over the Internet over a period of six weeks following the ban on mephedrone in mid-April 2010. Only one sample appeared to show analytical data consistent with naphyrone whereas a large number of the remaining products contained a variety of drugs including illegal cathinones just carrying a new label.[12]

The identification of novel derivatives has to be provided within a fast-paced environment in order to serve healthcare providers, legislators, and law enforcement. This is by no means a straightforward endeavor, particularly when dealing with large sample numbers and the absence of synthesized standards. The present study describes the first identification of a naphyrone isomer, 1-naphthalen-**1**-yl-2-pyrrolidin-1-yl-pentan-1-one (α-naphyrone; Figure 1B), when investigating two samples sold as NRG-1/naphyrone.

Experimental

Naphyrone samples

One NRG-1 sample that appeared to show analytical data consistent with naphyrone was available from a previous study[12] and a second similarly labelled NRG-1/naphyrone product was obtained from an additional website. Analyses of the two naphyrone samples were carried out by gas chromatography ion trap mass spectrometry (GC-IT-MS) using both electron and chemical ionization. 1D and 2D nuclear magnetic resonance spectroscopy (NMR) was also employed. For GC-IT-MS analyses, samples were dissolved in methanol at a concentration of 0.50 mg/mL. For NMR analyses ~25 mg were dissolved directly in d_6-DMSO.

* *Correspondence to: Dr Simon D. Brandt, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, UK. E-mail: s.brandt@ljmu.ac.uk*

a *School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, UK*

b *Centre for Forensic and Legal Medicine, University of Dundee, Small's Wynd, Dundee, DD1 4HN, UK*

c *School of Pharmacy and Pharmaceutical Sciences, The University of Manchester, Oxford Road, Manchester, M13 9PT, UK*

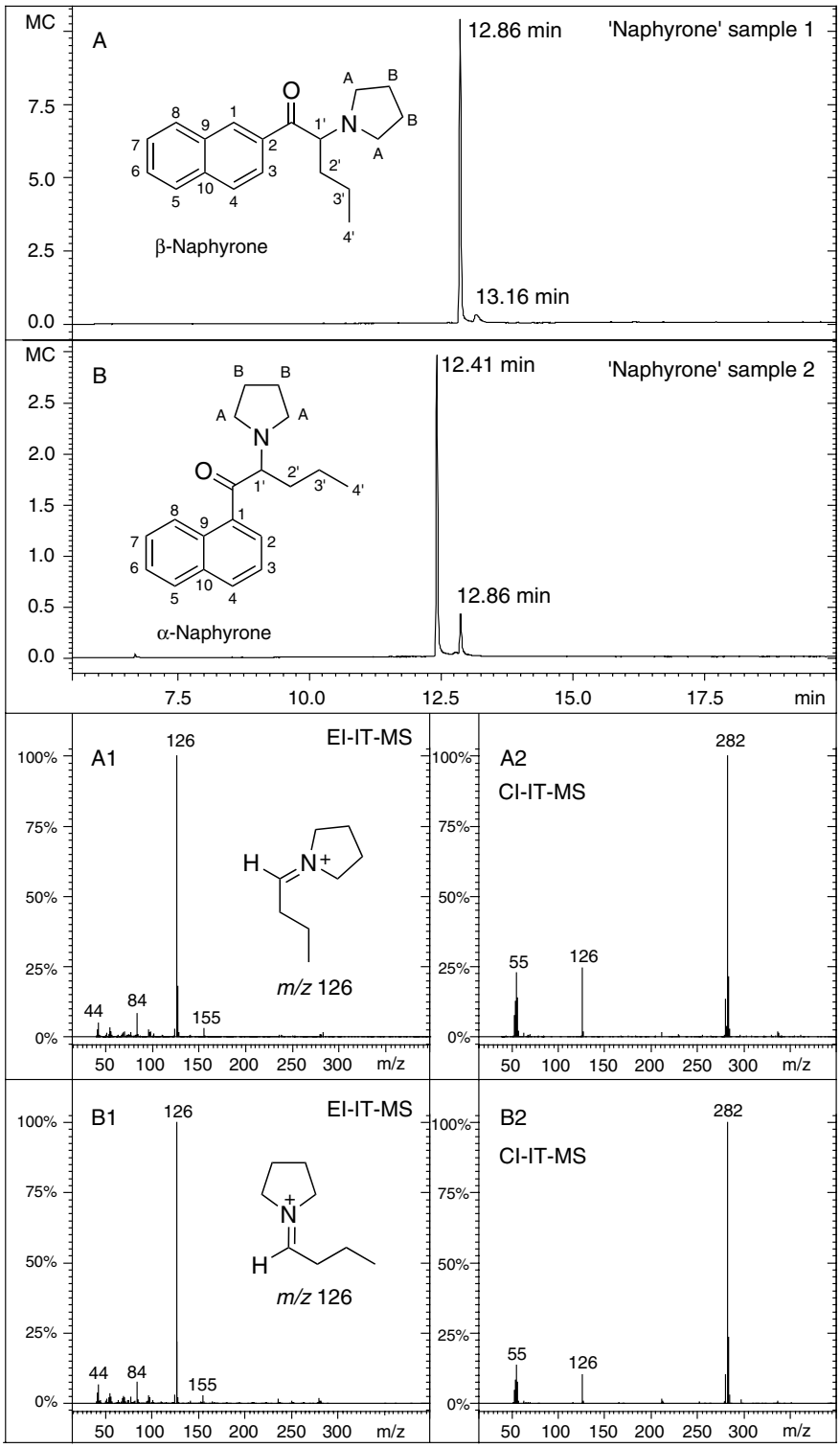

Figure 1. A and B: GC-IT-MS traces of β- and α-naphyrone obtained from the Internet. A1–B2: Electron ionization and chemical ionization mass spectra of both isomers.

NMR data for α-naphyrone (salt form unknown)

^{1}H NMR (300 MHz, d_6-DMSO): δ 10.89 (br, NH), 8.51 (dd, J_{ortho} = 9.0 Hz, J_{meta} = 1.5 Hz, 1H, H-8), 8.46 (dd, J_{ortho} = 7.5 Hz, J_{meta} = 0.9 Hz, 1H, H-2), 8.30 (br d, J_{ortho} = 8.4 Hz, 1H, H-4), 8.09 (dd, J_{ortho} = 7.8 Hz, J_{meta} = 1.5 Hz, 1H, H-5), 7.75-7.63 (m, 3H, H-3/6/7), 5.66 (br q, J = 6.2 Hz, 1H, 1′), 3.75-3.55 (m, 2H, A), 3.40-3.20 (m, 2H, A), 2.15-1.85 (m, 6H, B+2′), 1.38-1.20 (m, 1H, 3′-C*Ha*Hb), 1.15-0.97 (m, 1H, 3′-CHa*Hb*), 0.70 (t, J = 7.2 Hz, 3H, 4′-Me). ^{13}C NMR (75.5 MHz, d_6-DMSO): δ 199.1 (C═O), 134.7 (C-4), 133.6 (C-1), 132.0 (C-10), 130.4 (C-2), 129.5 (C-9), 128.9 (C-5), 128.8 (C-7), 127.0 (C-6), 124.7 (C-3), 124.5 (C-8), 69.2 (C-1′), 53.5 (CA), 51.9 (CA), 31.7 (C-2′), 23.0 (2 × CB), 17.7 (C-3′), 13.6 (C-4′).

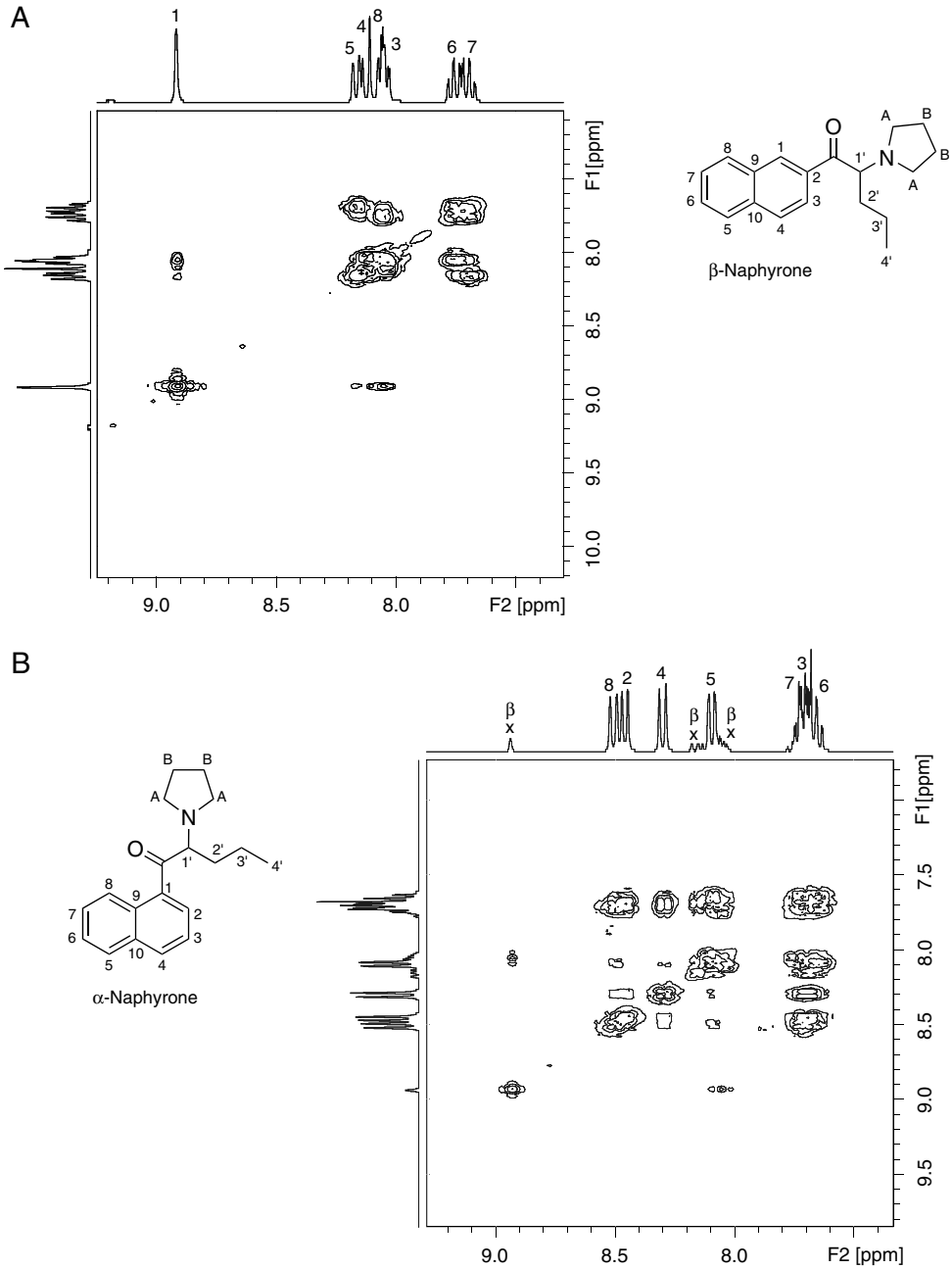

Figure 2. Two-dimensional COSY NMR spectra. A: Aromatic region of β-naphyrone. B: Aromatic region of α-naphyrone.

NMR data for β-naphyrone (salt form unknown)

^{1}H NMR (300 MHz, d_6-DMSO): δ 10.60 (br, NH), 8.92 (s, 1H, H-1), 8.17 (br d, J_{ortho} = 8.1 Hz, 1H, H-5), 8.12 (d, J_{ortho} = 8.7 Hz, 1H, H-4), 8.06 (br d, J_{ortho} = 7.5 Hz, 1H, H-8), 8.04 (dd, J_{ortho} = 8.5 Hz, J_{meta} = 1.6 Hz, 1H, H-3), 7.76 (td, J_{ortho} = 7.5 Hz, J_{meta} = 1.5 Hz, 1H, H-6), 7.69 (td, J_{ortho} = 7.5 Hz, J_{meta} = 1.2 Hz, 1H, H-7), 5.73 (ddd∼q, J = 6.6 Hz), 3.75-3.05 (m, 4H, A), 2.15-1.90 (m, 6H, B+2′), 1.35-1.00 (m, 2H, 3′-C*Ha*Hb), 0.78 (t, J = 7.3 Hz, 3H, 4′-Me). ^{13}C NMR (75.5 MHz, d_6-DMSO): δ 196.6 (C═O), 135.7 (C-2), 132.0 (C-9/C-10), 131.8 (C-10/C-9), 131.7 (C-1), 129.8 (C-5), 129.7 (C-6), 129.0 (C-4), 127.8 (C-8), 127.5 (C-7), 123.4 (C-3), 67.3 (C-1′), 53.5 (CA), 51.9 (CA), 31.7 (C-2′), 23.0 (2 × CB), 17.7 (C-3′), 13.6 (C-4′).

Instrumentation

Samples were subjected to both electron ionization (EI) and chemical ionization (CI) modes. Both EI and CI mass spectra (scan range *m/z* 40 – *m/z* 500) were obtained on a Varian 220-MS ion trap MS equipped with a Varian 450-GC gas chromatograph and a Varian 8400 autosampler. Data handling was carried out with the Workstation, Version 6.91 software. The carrier gas was helium at a flow rate of 1 mL/min using the EFC constant flow mode. A CP-1177 injector (275 °C) was used in split mode (1 : 20). Transfer line, manifold and ion trap temperatures were set at 280, 80 and 220 °C, respectively. HPLC grade methanol was used as the liquid CI reagent. CI ionization parameters (0.5 s/scan): CI storage level 19.0 *m/z*; ejection amplitude 15.0 *m/z*; background mass 55 *m/z*; maximum ionization

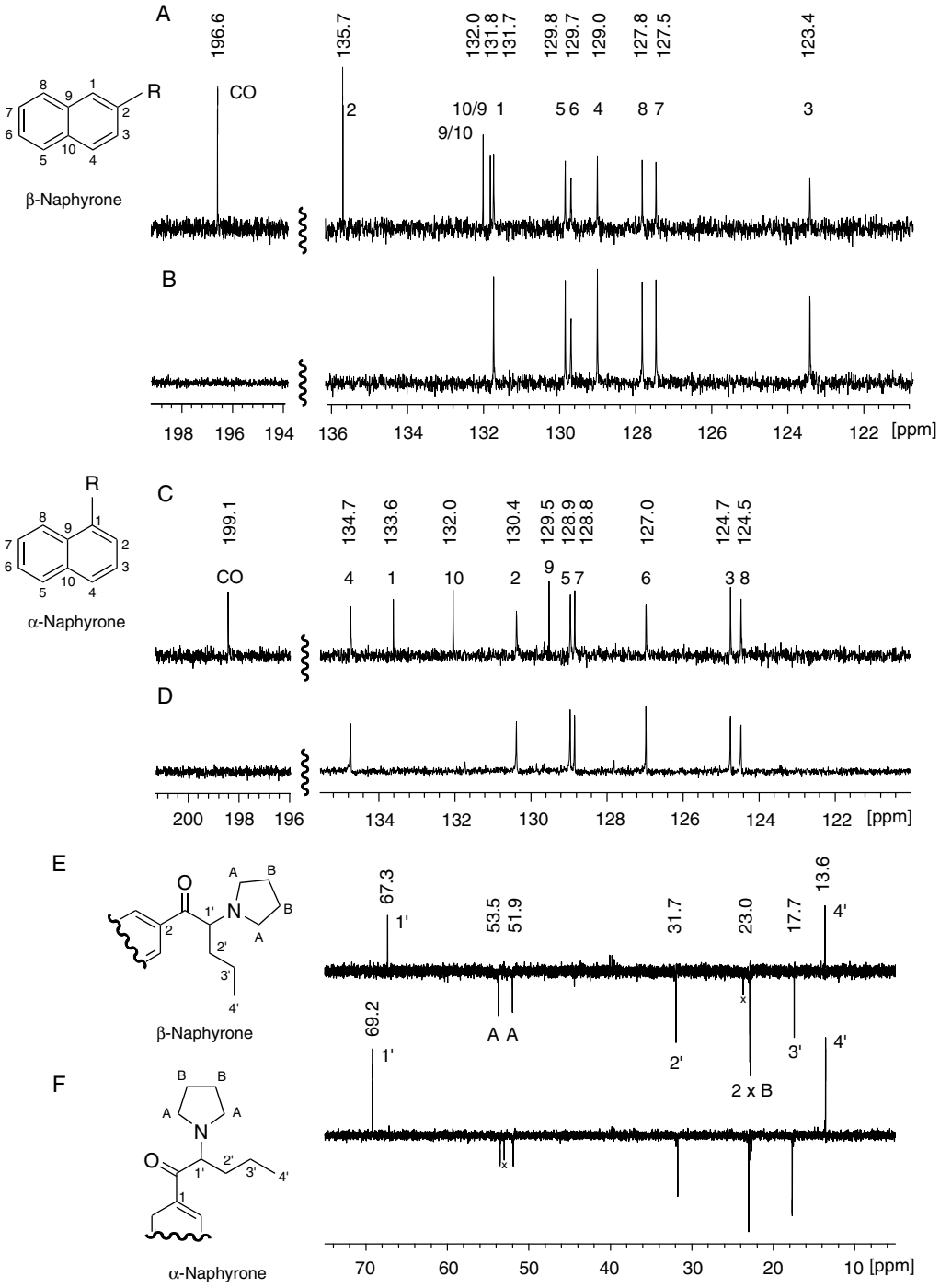

Figure 3. A and C: 13C NMR spectra of the aromatic region of β- and α-naphyrone. B and D: Aromatic DEPT-135 traces. E and F: Side-chain related DEPT-135 spectra of β- and α-naphyrone.

time 2000 µs; maximum reaction time 40 ms; target TIC 5000 counts. The number of ions in the trap was controlled by an automatic gain control function. Separations were carried out using 30 m × 0.25 mm (0.25 µm film thickness) Factor Four capillary column (VF-5 ms, Varian). The column temperature was programmed as follows: 100 °C held for 1 min, then heated at 20 °C/min to 280 °C and held constant for 10 min; total run time was 20 min.

NMR spectra were recorded using a Bruker Avance 300 spectrometer at 300.1 MHz (^{1}H NMR) or 75.5 MHz (^{13}C NMR). NMR spectra were recorded in d_6-DMSO and obtained by ^{1}H, proton decoupled ^{13}C, DEPT-135, COSY, HSQC and HMBC experiments. Chemical shifts were determined relative to the residual solvent peak at $\delta = 2.51$ (^{1}H NMR) and $\delta = 39.6$ ppm (^{13}C NMR), respectively.

Results and Discussion

A direct comparison between the GC-IT-MS traces obtained from two naphyrone samples revealed a detection of peaks at

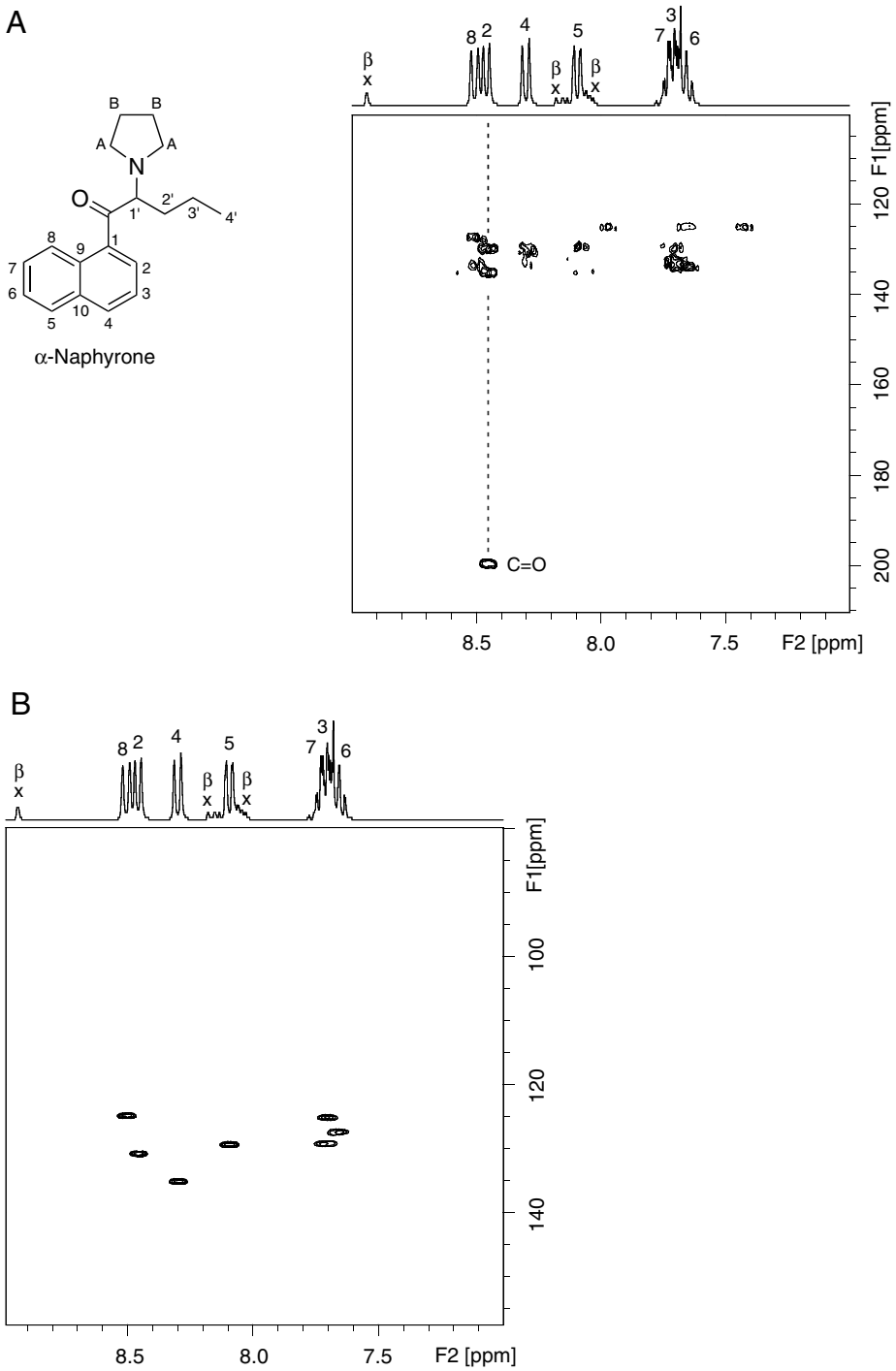

Figure 4. Two-dimensional NMR spectra of the aromatic region of α-naphyrone. A: HMBC. B: HSQC.

two different retention times: 12.86 (Figure 1A) and 12.41 min (Figure 1B). The corresponding EI and CI mass spectra shown in Figures 1A1 and 1A2 and 1B1 and 1B2, respectively, and are consistent with the structural features of the analytes. Suggested key features might include base peak formation at *m/z* 126, presumably with neutral loss of propene to give a secondary iminium ion at *m/z* 84. This would be in agreement with reported mass spectra obtained from 3,4-methylenedioxypyrovalerone (MDPV) that shares an identical side chain.[13] The protonated molecules (Figures 1A2 and 1B2) show the expected *m/z* 282 ion.

The occurrence of identical mass spectral data and detection at different retention times, however, indicated the presence of two distinct structural isomers which prompted further investigations. Examination of the ^{1}H and ^{13}C NMR spectra revealed that sample 1 was consistent with the expected 1-naphthalen-**2**-yl-2-pyrrolidin-1-yl-pentan-1-one (β-naphyrone), whereas data for sample 2 was supportive of its isomer 1-naphthalen-**1**-yl-2-pyrrolidin-1-yl-pentan-1-one (α-naphyrone). A comparison with a synthesized β-naphyrone standard confirmed a retention time of 12.86 min (not shown).

Although the splitting patterns in the ^{1}H NMR spectra for naphthyl systems can be complicated by long range couplings, in β-naphyrone there was an apparent singlet at δ 8.89 ppm for the isolated H-1 (Figure 2A). This singlet was absent from sample 2 confirming that there were no isolated aromatic hydrogens present in this structure (Figure 2B). Further indication for the presence of α-naphyrone derived from the splitting pattern of four apparent dd systems (J_{ortho}, J_{meta}) each integrating to 1H at 8.51, 8.46, 8.30, and 8.09 ppm for H-8, H-2, H-4, and H-5, respectively. In addition, there was a complex multiplet between δ 7.5–7.85 ppm integrating to 3H for H-3, H-6 and H-7 (Fig. 2B). The structure and assignment of the aromatic protons for both β- and α-naphyrone was further supported by a 2D COSY NMR (Figure 2B).

The presence of a mono-substituted naphthalene ring in both α- and β-naphyrone agreed with the detection of 7 aromatic CH signals in the ^{13}C NMR for both samples (Figures 3A and 3C). The DEPT-135 data for the aromatic region are summarized in Figures 3B and 3D where disappearance of three quaternary carbon resonances for each sample are visible. As anticipated, the ^{13}C NMR chemical shifts for the aromatic carbons are different for each sample, and the assignments (shown in Figures 3A and 3C) are consistent with the structure of each isomer.

As expected, the side-chain ^{1}H and ^{13}C NMR resonances were almost identical for the two samples, with the exception of the α-carbon (C-1′) of the side chain in the ^{13}C NMR (Figures 3E and 3F). These chemical shifts were also in agreement with the previously reported identical side chain of MDPV.[13]

The ^{1}H and ^{13}C NMR chemical shifts were in agreement with those estimated from the NMR predict tool in ChemDraw for both the α- and β-isomers. For α-naphyrone, the aromatic assignments were also in excellent agreement with the data reported by Perumal and coworkers for 1-acetylnapthalene.[14] The assignments of the aromatic signals for α-naphyrone were further confirmed by 2D ^{1}H-^{13}C HMBC (Figure 4A, showing 3 bond couplings) and HSQC (Figure 4B, showing 2 bond couplings) experiments. In the HMBC experiment (Figure 4A) there was a strong correlation between the C═O and H-2. A firm assignment of H-2 then allowed certain analysis of the other aromatic H and C resonances using the COSY (Figure 2B) and HSQC (Figure 4B) correlations.

This study highlighted a difficulty in over-reliance on one analytical tool. Although GC-MS is a versatile technique, it initially led to a misidentification of the naphyrone isomer. Although identification was difficult, the isomers were separable by GC. α-Naphyrone cannot be accidentally made from its β-counterpart. The manufacturers might have deliberately set out to create a naphyrone analogue, or, perhaps, an incorrect starting material was purchased. The GC-IT-MS trace of α-naphyrone (Figure 1B) showed the presence of β-naphyrone as well which provides some indication that an impure starting material might have been involved. Typical reactions might include Friedel-Crafts reactions starting from naphthalene or the use of Grignard reagents using the corresponding nitrile or aldehyde precursor. A number of examples have been reported for the Friedel-Crafts reaction between naphthalene and acid chlorides. The reaction conditions (temperature, solvent) were found to affect the ratio of the resulting α- or β-substituted ketones.[15] The GC-IT-MS trace of the β-naphyrone sample (Figure 1A) also showed an impurity at 13.16 min. Under CI-IT-MS conditions the protonated molecule was detected at *m/z* 280. The key fragments of the corresponding EI-IT-MS spectrum were as follows: *m/z* 279 ($M^{\bullet+}$, 65%), 250 (42%), 155 (21%), 127 (62%), 124 (100%), 70 (79%), 44 (70%). The exact identity of this contaminant is currently unclear but the presence of the base peak at *m/z* 124 indicated that a loss of H_2 might have occurred in the side chain of the molecule. Whether this species was formed artificially during GC-IT-MS analysis or formed during synthesis remains to be investigated. A similar species was not detected in the GC-IT-MS trace of α-naphyrone (Figure 1B). However, previous work in this laboratory on the synthesis of a variety of cathinones indicated that formation of the M-2 Da product was observed on a regular basis. Separation from the parent cathinone product was normally achieved under identical GC conditions although co-elution has been observed as well. This might be in agreement with a previous study on fluorinated methcathinone derivatives where a species at *m/z* 56 was detected under GC-MS conditions in addition to the *m/z* 58 base peak of the parent molecule.[16] In other cases, an M-4 Da species was detected albeit in trace amounts (unpublished).

Conclusion

The characterization of two naphyrone samples revealed that two structural isomers are circulating on the recreational drug market. It has now become apparent that the naphthylpyrovalerone derivative O-2482 is consistent with the β-naphyrone isomer. The α-naphyrone isomer (1-naphthalen-**1**-yl-2-pyrrolidin-1-yl-pentan-1-one) is absent from the literature. Further studies to evaluate its pharmacological properties are needed. The detection of both isomers illustrated one of the usual challenges faced by those who deal with frontline exposure to a variety of newly appearing compounds. The ability to differentiate between closely related analytes is not always straightforward by mass spectrometric analysis and this study highlights the importance of the use of other techniques, including GC and NMR spectroscopy.

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