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Aplicação da Espectroscopia de Ressonância Magnética Nuclear (RMN) à toxicologia forense

Recife, 2006

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Tese que o aluno apresenta ao Doutorado em Ciências Biológicas do Centro de Ciências Biológicas da Universidade Federal de Pernambuco, como um dos requisitos para obtenção do título de Doutor em Ciências Biológicas.

Profa. Luana Cassandra Breitenbach Barroso Coelho, M.Sc., Ph.D. (Orientadora)

Prof. Manfred Oswald Erwin Schwartz, M.Sc., Ph.D. (Co-orientador)

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Palavras...

...sobre diversidade:

Enquanto você na arquibancada eu na geral

Enquanto eu além de tudo você afinal

Enquanto eu rondó você madrigal

Enquanto eu paro e penso você avança o sinal

Enquanto você carta marcada eu canastra real

Enquanto eu lugar comum você especial

Enquanto eu na cozinha você no quintal

Zeca Baleiro

...sobre medidas:

Amar sem medidas.

Sorrir sem medidas.

Ajudar sem medidas.

Aperfeiçoar sem medidas.

Consciência sem medidas

para viver sem medidas.

...sobre drogas:

Me chamam de desaparecido

Que quando chega já tem ido

Vagando venho, vagando vou

Ao sabor da brisa pro desconhecido

Se me procuram nunca estou

Se me encontram eu não sou

Com a dor eu vivo, com a dor estou

Dizem que consumo, mas sou consumido.

Adaptado de Manu Chao

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CFC - Clorofluorocarbonos.

CP - Polarização Cruzada (do inglês, *Cross-Polarization*).

DD - Defasamento Dipolar (do inglês, *Dipolar Dephasing*).

DEPT - Intensificação sem Distorção por Transferência de Polarização (do inglês, *Distortionless Enhancement by Polarization Transfer*).

GC - Cromatografia em fase gasosa (do inglês, *Gas Chromatography*).

HCFC - Clorofluorohidrocarbonos.

HETCOR - Correlação Heteronuclear (do inglês, *Heteronuclear Correlation*).

HMBC - Correlação Heteronuclear em Ligações Múltiplas (do inglês, *Heteronuclear Multiple Bond Correlation*).

MAS - Rotação no Ângulo Mágico (do inglês, *Magic Angle Spinning*).

MDMA - 3,4-metilenodioxi-N-metilanfetamina (*ecstasy*).

MS - Espectrometria de massas (do inglês, *Mass Spectrometry*).

NMR - *Nuclear Magnetic Resonance*.

RMN - Ressonância Magnética Nuclear.

THC - Tetrahidrocanabinol.

RESUMO

A Ressonância Magnética Nuclear (RMN) e a Cromatografia em fase gasosa acoplada à espectrometria de massas (GC/MS) fornecem informações sobre a estrutura química de substâncias, bem como, sobre a constituição qualitativa e quantitativa de amostras sem a necessidade de padrões de análise ou pré-tratamento de amostras. Uma jovem estudante de 19 anos morreu de forma súbita após inalar um aerossol cujo componente principal, o 1-1-dicloro-1-fluoretano (HCFC-141b), foi identificado por meio de RMN e GC/MS. Nas vísceras e no sangue da vítima não foram detectados venenos, álcool ou outras drogas. Os resultados dos exames necroscópico, toxicológico e microscópico não excluem a hipótese de morte por inalação do HCFC-141b e estabelecem dois mecanismos de morte como viáveis: asfixia e arritmia cardíaca. As análises realizadas e os dados do inquérito policial suportam probabilisticamente a hipótese de morte por arritmia cardíaca fatal que pode ter ocorrido de forma associada com asfixia. Algumas técnicas de RMN como ^{1}H, ^{13}C, Distortionless Enhancement by Polarization Transfer (DEPT), Cross-Polarization and Magic Angle Spinning (CP/MAS) and Dipolar Dephasing (DD) foram usadas para caracterizar amostras de cocaína. Os deslocamentos químicos dos espectros obtidos foram atribuídos por meio de Heteronuclear Correlation (HETCOR) e Heteronuclear Multiple Bond Correlation (HMBC). As amostras foram primariamente caracterizadas por espectrometria de massas cujos espectros foram comparados com dados obtidos na literatura. Os resultados obtidos indicam que ^{1}H, ^{13}C e DEPT são técnicas bastante eficientes para caracterização de amostras ilícitas de cocaína no estado líquido e que por meio de CP/MAS e CP/MAS/DD RMN é possível distinguir o crack do cloridrato de cocaína de uma maneira não destrutiva. Portanto, a RMN é uma excelente ferramenta para caracterização de amostras ilícitas de cocaína.

Palavras-chave: inalantes, HCFC-141b, cocaína, crack, RMN, DEPT, CP/MAS, Dipolar Dephasing.

ABSTRACT

Nuclear magnetic resonance (NMR) and gas chromatography/mass spectrometry (GC/MS) techniques provide information about the chemical structure and also qualitative and quantitative composition of samples even without standard or reagents addition. A 19 year-old woman died suddenly after inhalation of an aerosol whose main component, 1-1-dichloro-1-fluoroethane (HCFC-141b), was identified by NMR and GC/MS. No poisons, alcohol or other drugs were detected in the victim's blood or viscera. Necropsy, toxicological and microscopic findings did not exclude the hypothesis of death due to HCFC-141b inhalation, so, two mechanisms of death were acceptable: asphyxia and fatal cardiac arrhythmia. The analysis and police inquest data supported probabilistically as cause of death either a fatal cardiac arrhythmia or a combination of arrhythmia and asphyxia. Some NMR techniques like ^{1}H, ^{13}C, Distortionless Enhancement by Polarization Transfer (DEPT), Cross-Polarization and Magic Angle Spinning (CP/MAS) and Dipolar Dephasing (DD) NMR spectroscopy were used to characterize cocaine samples. Making use of Heteronuclear Correlation (HETCOR) and Heteronuclear Multiple Bond Correlation (HMBC), the chemical shifts could be attributed. The samples were primarily characterized by mass spectroscopy whose spectra were compared with the ones in literature. The findings indicate that ^{1}H, ^{13}C NMR and DEPT are efficient techniques to characterize illicit cocaine samples in liquid state and CP/MAS and CP/MAS/DD allow distinguish between of crack and cocaine hydrochloride by a non-destructive way. So, NMR is an excellent tool for fast characterization of cocaine samples in a non-destructive and reproducible way with forensic objectives.

Keywords: inhalants, HCFC-141b, arrhythmia, cocaine, crack, NMR, DEPT, CP/MAS, Dipolar Dephasing.

1 INTRODUÇÃO

1.1 CIÊNCIA FORENSE

A ciência forense estabelece a aplicação dos diversos campos do conhecimento humano no estudo, apreciação, descrição, investigação, interpretação e identificação de fatos e vestígios relativos a litígios criminais ou cíveis. O advento de novas tecnologias contribui sistematicamente para que a ciência forense seja uma das áreas do conhecimento que mais cresce. Cientistas de todas as áreas, desde a genética até a sismologia, estão cada vez mais envolvidos em objetos de pesquisa relacionados com a interpretação de vestígios encontrados em local de crime. A toxicologia forense, um dos mais tradicionais ramos da ciência forense, também tem seu potencial analítico aumentado em função dos avanços tecnológicos. No presente trabalho é feito um estudo das vantagens, limitações e das possibilidades de utilização da Espectroscopia de Ressonância Magnética Nuclear (RMN) à toxicologia forense como técnica não destrutiva na análise de drogas de abuso, com enfoque em uma substância que causou a morte de uma jovem estudante, o 1-1-dicloro-1-fluoretano, e em amostras de cocaína.

1.2 TÉCNICAS NÃO DESTRUTIVAS

Técnicas não destrutivas são métodos de análise nos quais o analito não sofre quaisquer alterações, portanto, seu emprego é preferencial em qualquer análise na qual seja necessária a preservação da amostra. No universo da Ciência Forense estas técnicas são ferramentas bastante úteis, uma vez que a quantidade da substância a ser analisada geralmente é limitada e, em cada procedimento de análise, é necessário o armazenamento da mesma quantidade de analito utilizada no primeiro exame para viabilizar exames de contraprova a serem realizados em qualquer tempo. Técnicas não destrutivas baseadas em Ressonância Magnética Nuclear (RMN) já foram utilizadas para determinação do 3,4-metilenodioxi-N-metilanfetamina (MDMA) em comprimidos de *ecstasy* [Lee *et al.*, 1999] e

podem ser empregadas na análise das mais variadas substâncias, inclusive drogas de abuso como tetrahidrocanabinol (THC), cocaína, heroína e inalantes.

1.3 RMN

Em um campo magnético e sob condições adequadas uma amostra pode absorver radiação eletromagnética na região de radiofreqüências (rf) em uma freqüência regida por características estruturais da molécula. Um espectro de RMN é um registro gráfico das freqüências dos picos de absorção e de suas intensidades [Silverstein & Webster, 2000]. As análises de RMN podem ser feitas em amostras líquidas ou sólidas. Nas análises de líquidos algumas técnicas de espectroscopia de RMN empregadas são: ^{1}H, ^{13}C, *Distortionless Enhancement by Polarization Transfer* (DEPT), *Heteronuclear Correlation* (HETCOR) e *Heteronuclear Multiple Bond Correlation* (HMBC). Nas análises de sólidos são empregadas, entre outras, as técnicas *Magic Angle Spinning* (MAS), *Cross Polarization/Magic Angle Spinning* (CP/MAS) e *Dipolar Dephasing* (DD). Substâncias sólidas também podem ser analisadas em solução com o emprego de um solvente deuterado.

1.3.1 DEPT

O espectro DEPT fundamenta-se na transferência de polarização de ^{1}H acoplados (para o ^{13}C. Por meio desta técnica é possível a distinção entre grupos CH, CH_2 e CH_3. Carbonos quaternários não aparecem no espectro DEPT porque não estão ligados a hidrogênios [Silverstein & Webster, 2000].

1.3.2 HETCOR E HMBC

O espectro HETCOR correlaciona núcleos ^{13}C e ^{1}H diretamente ligados, ou seja acoplamentos de uma ligação ($^{1}J_{CH}$) enquanto o espectro HMBC correlaciona núcleos ^{13}C e ^{1}H a uma distância de duas ($^{2}J_{CH}$) ou três ($^{3}J_{CH}$) ligações. Com estes experimentos é possível assinalar deslocamentos químicos de carbonos (δ_C) através de deslocamentos químicos de hidrogênios (δ_H) ou o inverso [Reynolds & Enríquez, 2002; Kaiser, 2000; Silverstein &

Webster, 2000].

1.3.3 ^{13} CP/MAS E DD RMN

No experimento CP-MAS ocorre uma transferência de polarização do núcleo mais abundante, o ^{1}H, para o menos abundante, o ^{13}C, o que diminui o tempo de obtenção dos espectros. No espectro DD RMN aparecem apenas carbonos não protonados e grupos metilas [Adhyaru *et al.,* 2003].

1.4 INALANTES

Os inalantes são substâncias voláteis que produzem vapores químicos e podem ser inalados para induzir um efeito psicoativo. Embora outras drogas de abuso possam ser inaladas como o tabaco, a maconha e o *crack*, o termo "inalante" é usado para descrever uma variedade de substâncias cujo uso não envolva uma outra via de administração e que são levados à inalação sem que sejam submetidos a elevadas temperaturas. Este conceito abrange uma grande quantidade de substâncias químicas com diferentes efeitos farmacológicos.

Teoricamente, qualquer produto químico que contenha um ou mais componentes voláteis pode ser utilizado para inalação, mas a escolha quase sempre recai sobre aqueles que contêm alta concentração dessas substâncias, seja de baixo custo, fácil aquisição e uso. As colas são os produtos com maior popularidade em todo o mundo. Segundo pesquisas disponíveis no Brasil, os produtos mais utilizados são lança-perfume (cloreto de etila), cola-de-sapateiro e cheirinho-da-loló (versão caseira alternativa do lança-perfume, contendo duas ou mais das seguintes substâncias: éter etílico, clorofórmio e etanol) [Oga, 1995].

1.4.1 CLASSIFICAÇÃO

A classificação dos inalantes pode corresponder a vários critérios o que a torna bastante complexa. Um dos tipos de classificação, baseado na forma pela qual os inalantes são freqüentemente encontrados em produtos domésticos, industriais e médicos, Oga (1995) divide os inalantes em quatro categorias gerais:

1.4.1.1 Solventes voláteis

São líquidos que vaporizam em temperatura ambiente. Essa categoria engloba grande número de produtos; a maioria deles apresenta mistura de vários compostos voláteis. Incluem, dentre outros, tíner e removedores de tintas, fluidos de limpeza a seco, desengordurantes, gasolina, colas, fluidos corretivos, esmaltes, éter, clorofórmio e halotano.

1.4.1.2 Aerossóis

São líquidos ou sólidos em suspensão contidos em recipientes pressurizados. A fonte propelente é usualmente um gás liquefeito, e é esse o componente procurado pelo usuário. Incluem tintas, desodorantes e produtos para cabelos em spray.

1.4.1.3 Gases

Nesta categoria incluem anestésicos de uso médico, como o óxido nitroso, e outros gases usados em produtos comerciais ou caseiros como butano e propano presentes em gases combustíveis e fluidos de isqueiros.

O óxido nitroso, conhecido como "gás hilariante", é usado por profissionais como médicos e dentistas, para alívio do estresse. Alguns usuários relatam experiências similares àquelas obtidas com as drogas psicodélicas, alucinações auditivas são freqüentes. Estudos recentes têm demonstrado sua utilidade no tratamento da síndrome de abstinência acarretada pelo etanol, THC, nicotina e opiáceos.

1.4.1.4 Nitritos orgânicos voláteis

São freqüentemente considerados uma classe especial de inalantes. Foram originalmente utilizados para aliviar dores no peito associadas com angina pectoris, devido às suas propriedades vasodilatadoras. Hoje, todavia, são usados como drogas de abuso por adolescentes ou homossexuais para intensificar o desempenho sexual e o prazer. Incluem os nitritos de amila, isoamila, butila e isobutila.

1.4.2 VIAS DE ADMINISTRAÇÃO

1.4.2.1 SNIFFING

Os vapores são inalados pelo nariz diretamente de seu recipiente de origem. Resulta na inalação de uma baixa concentração de vapor significativamente dissipado no ar circunvizinho.

1.4.2.2 HUFFING

A substância a ser inalada é utilizada para saturar um material qualquer (geralmente tecido de algodão) que é pressionada na boca ou no nariz. Em casos extremos o aerossol é borrifado diretamente na boca para ser inalado.

1.4.2.3 BAGGING

Os vapores são inalados diretamente de uma embalagem de plástico ou papel que é pressionada firmemente sobre a boca e o nariz.

Usuários crônicos de inalantes começam praticando o *sniffing* e progridem para o *huffing* e *bagging* para aumentar a concentração de vapor inalado, diminuindo o tempo de obtenção do efeito e aumentando a eficiência da sua manutenção [Kurtzman *et al.*, 2001].

1.5 HCFC-141B (1,1-DICLORO-1-FLUOROETANO)

O *HCFC-141b*, também conhecido como CFC 141b, freon 141b, Isotron 141b, R 141b, é um líquido volátil incolor e com fraco odor etéreo. Seu vapor é mais pesado que o ar e pode deslocá-lo em ambientes confinados. Algumas características do *HCFC-141b* estão descritas na Tabela 1.

Tabela 1. Algumas propriedades químicas e físicas do HCFC-141b.

Parâmetro	Resultado
Fórmula molecular	$C_2H_3Cl_2F$
Peso molecular	116,95
Estado físico	Líquido
Cor	Incolor
Odor	Fracamente etéreo
Solubilidade em água	Aproximadamente 4 g/L
Pressão de vapor	412 mm Hg a 25°C
Densidade (g/cm³) a 20°C	1,24
Ponto de fusão	- 103,5 °C
Ponto de ebulição	32 °C

1.5.1 Uso

O *HCFC-141b* vem substituindo os clorofluorocarbonos (CFCs) totalmente halogenados como refrigerante, propelente e como solvente na limpeza de equipamentos eletrônicos (limpeza a seco), etc. Em relação aos CFCs, os clorofluorohidrocarbonos (HCFCs) possuem um maior número de ligações C-H o que os torna mais suscetíveis à oxidação na troposfera reduzindo sua migração para a estratosfera e seu efeito sobre a camada de ozônio [Tong *et al.*, 1998; Dekant, 1996].

Apesar de possuir todos os requisitos necessários para sua caracterização como inalante, o uso do *HCFC-141b* como droga de abuso não está explicitamente descrito.

1.5.2 Toxicologia

A inalação é a rota primária de absorção do gás *HCFC-141b*. O contato prolongado ou repetido remove óleos da pele causando desidratação e conseqüente irritação, vermelhidão e erupção cutânea. Altas concentrações de vapor causam irritações nos olhos e aparelho respiratório e podem resultar em efeitos no sistema nervoso central, como dor de cabeça, vertigem, sonolência e, em exposição severa, inconsciência, depressão respiratória e morte. O vapor denso deste material reduz a disponibilidade de oxigênio em áreas fechadas ou sem ventilação onde uma exposição prolongada pode ser fatal. Inalação pode causar um aumento na sensibilidade do coração para adrenalina resultando em taquicardia ou arritmia cardíaca [Brock *et al.*, 1995].

Apenas um caso fatal atribuído ao uso do *HCFC-141b* está descrito na literatura. Um homem de 40 anos de idade foi encontrado morto no interior de um tanque no qual o *HCFC-141b* estava sendo usado como solvente. O homem não usava roupas de proteção e não havia mais líquido no interior do tanque no momento em que o corpo foi encontrado. [Johansson *et al.*, 1998; Astier & Paraire, 1997].

1.5.3 Metabolismo

O *HCFC-141b* é convertido a 2,2-dicloro-2-fluoroetanol, que se conjuga com o ácido glicurônico e é excretado na urina na forma do 2,2-dicloro-2-fluoroetil glicuronídeo e ácido diclorofluoracético, produzido pela oxidação do 2,2-dicloro-2-fluoroetanol [Tong *et*

al., 1998; Dekant, 1996; Harris & Anders, 1991].

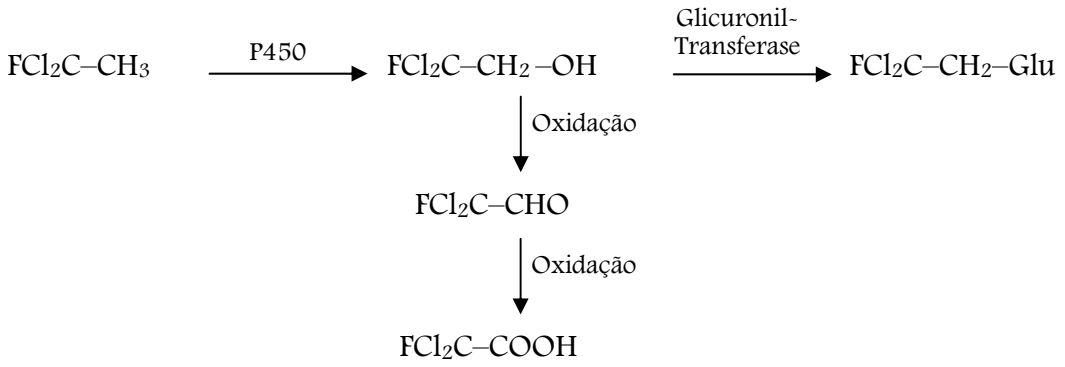

Figura 1. Via metabólica do HCFC-141b.

Análises através de *RMN* de ^{19}F de proteínas microssomais e citosólicas isoladas de fígados de ratos expostos ao HCFC 141b não mostraram evidências de ligação covalente com metabólitos do HCFC-141b [Harris & Anders, 1991].

1.5.4 MÉTODOS ANALÍTICOS

A detecção do *HCFC-141b* e seus metabólitos pode ser feita através de técnicas fundamentadas em processos cromatográficos como a GC-MS (*Gas Chromatography-Mass Spectrometry*) [Tong *et al.*, 1998], ou através da espectroscopia de RMN [Tong *et al.*, 1998; Harris & Anders, 1991].

1.6 COCAÍNA

A cocaína é um dos alcalóides encontrados em plantas do gênero *Erytroxylum*, vulgarmente denominado coca e com cerca de duzentas espécies, entre as quais, apenas duas apresentam quantidades significativas de cocaína, a *E. coca,* a principal fonte de cocaína para uso ilícito como droga de abuso, e a *E. novogranatense*, geralmente, cultivada para uso da cocaína como anestésico local na indústria farmacêutica ou como constituinte de chás na indústria alimentícia [Casale & Klein,1993; Warner, 1993].

1.6.1 HISTÓRICO

Dados arqueológicos datados de 3000 a.C. são a mais antiga prova do uso da folha de coca pelo homem. A folha de coca continuou sendo mascada pelos Incas (900 d.C.) até

chegar a Europa onde começou a ser comercializada em 1580. Em 1860, a cocaína foi isolada por *Albert Niemann*. As folhas de coca passaram a ser maceradas em chás e incorporadas em outras bebidas como a *Coca-Cola,* que até algum tempo atrás apresentava a cocaína entre os seus ingredientes. Entre 1880 e 1890, o uso da cocaína era bastante popular, chegando a conquistar muitos usuários ilustres. Em 1921, no Brasil, o Congresso Nacional aprovou o Decreto-lei 4.292 que, entre outras coisas, estabeleceu: a) penalidades (multa e prisão) para as contravenções na venda de cocaína e outras drogas; b) criação de estabelecimento especial para tratamento de dependentes com duas seções, uma para internados judiciários e outra para internados voluntários [Bahls & Bahls, 2002]. Em 1970, a fabricação, a distribuição e a posse da cocaína foram definitivamente proibidas nos EUA, entretanto, nos anos 70 e 80 o consumo da cocaína foi revolucionado com a criação de formas de cocaína que poderiam ser inaladas, a base livre e o *crack* [Warner, 1993].

1.6.2 FORMAS DE APRESENTAÇÃO

A cocaína é encontrada com mais freqüência na forma de pasta de coca, cloridrato de cocaína ou na sua forma alcalóide, como base livre ou *crack*. A pasta de coca corresponde a um extrato de folhas de coca misturadas com água, querosene e ácido sulfúrico; seu uso é mais comum na América do Sul onde também é conhecido como *bazuca* [Warner, 1993; Oga, 1995].

O cloridrato de cocaína é um pó branco, cristalino e solúvel em água que, por causa do alto ponto de ebulição, se decompõe quando submetido a altas temperaturas, portanto, não pode ser fumado. Amostras de cloridrato de cocaína são freqüentemente adulteradas com manitol e lactose para dar volume ao pó, com cafeína para simular o efeito estimulante da cocaína e/ou com procaína e lidocaína para imitar o efeito de anestesia local [Warner, 1993; Oga, 1995].

Na forma alcalóide, a cocaína possui um menor ponto de ebulição, portanto, pode ser fumada. A literatura médica é bastante ambígua quando se refere a definições da base livre e do *crack*. A base livre e o *crack* representam a mesma forma química da cocaína, o alcalóide, entretanto, possuem diferentes métodos de obtenção. A base livre é produzida pela

dissolução do cloridrato de cocaína em água, adicionando uma base como a amônia e um solvente apolar como o éter; a cocaína na forma de alcalóide dissolve-se na fração apolar da qual pode ser extraída por evaporação. Este método de obtenção permite a eliminação de alguns contaminantes polares. O *crack* é obtido através de um processo mais simples, no qual o cloridrato de cocaína é dissolvido em água, misturado com soda cáustica e aquecido. A cocaína na sua forma alcalóide apresenta-se como um precipitado que endurece ao secar. A terminologia *crack* foi inspirada pelo som que os seus cristais fazem durante o preparo para o uso [Warner, 1993; Oga, 1995].

1.6.3 Uso

O cloridrato de cocaína é freqüentemente utilizado por via intranasal ou por via intravenosa. Em função do seu alto ponto de ebulição (180°C), o cloridrato de cocaína não pode ser inalado, em conseqüência disto, o cloridrato de cocaína vem sendo gradativamente substituído pelo *crack*.

O *crack* e a base livre podem ser fumados em cachimbos ou cigarros e misturados com maconha ou tabaco. A presença residual de éter em amostras de base livre provoca um alto risco de queimadura em usuários desta forma de cocaína. Com a disponibilidade do *crack* o uso de base livre vem decaindo continuadamente.

As folhas de coca continuam sendo mascadas na América do Sul. Entretanto, não produzem os efeitos gerados pelo consumo das formas purificadas em função da limitada absorção gastrointestinal e da, relativamente, pequena quantidade de cocaína presente nas folhas de coca [Warner, 1993].

1.6.4 Toxicocinética

1.6.4.1 Absorção

A absorção da cocaína depende da via de auto-administração utilizada. Na utilização da droga pela via intranasal (aspiração nasal) ou pela mucosa bucal a absorção ocorre através das membranas nasoorofaríngeas, com a velocidade e a quantidade de droga absorvida limitada pela propriedade vasoconstrictora da cocaína. Há referências que pela aspiração nasal os efeitos intensos da droga aparecem entre 3 e 5 min, com níveis máximos

de cocaína alcançados entre 30 e 60 min [Jatlow, 1987; Oga, 1995].

Experimentos com voluntários mostraram que fumantes de cocaína apresentaram níveis sanguíneos equivalentes aos obtidos através de injeção intravenosa [Cook & Jeffcoat, 1990]. O ato de fumar cocaína é a rota mais rápida de penetração da droga na corrente sanguínea através da absorção pelos alvéolos pulmonares (6 a 8 s, aproximadamente). Isso resulta em uma maior rapidez de aparecimento e maior intensidade de efeitos experimentados pelos usuários, quando comparados aos propiciados pelo uso por via intravenosa. Há referências de que efeitos intensos aparecem entre 1 e 2 min após a administração por via intravenosa e respiratória. Entretanto, a duração dos efeitos é curta para as duas vias; os efeitos obtidos com uso da droga por via respiratória são mais pronunciados e rápidos que os obtidos pela via intravenosa. A cocaína também é absorvida por via gastrointestinal. Por esta via, a cocaína começa a penetrar na corrente sanguínea 30 min após sua administração e o pico de concentração plasmática é atingido entre 45 e 90 min [Oga, 1995; Jones, 1990].

1.6.4.2 DISTRIBUIÇÃO E BIODISPONIBILIDADE

A cocaína liga-se às proteínas plasmáticas apresentando alta afinidade pela α-1-glicoproteína ácida, e baixa, porém significativa, pela albumina. A fração livre situa-se entre 67 e 68% da quantidade absorvida, mas, varia de acordo com alterações de pH sanguíneo. O acúmulo verificado no fígado é compatível com a suposição de que há receptores hepáticos com alta afinidade pela cocaína [Oga, 1995].

A incorporação da cocaína no cabelo se dá por mecanismos ainda não totalmente determinados. Alguns autores sugerem que esta incorporação ocorra por difusão passiva para o folículo piloso [Oga, 1995], enquanto outros autores afirmam que este mecanismo é excessivamente simples e sugerem que outros fatores devem estar envolvidos [Henderson *et al.* 1996]. O caráter lipofílico da cocaína faz com que a substância atravesse prontamente a barreira hematoencefálica. A transferência placentária e a secreção láctea estão bem estabelecidas. Foi também reportada a presença de cocaína no sêmen de usuários.

A biodisponibilidade da cocaína está relacionada com sua via de administração.

Quando fumada a biodisponibilidade da droga é de aproximadamente 70%, e quando inalada é da ordem de 60 a 80% [Oga, 1995]. Entretanto, no experimento de Cone (1995), a biodisponibilidade foi de aproximadamente 94%.

1.6.4.3 METABOLISMO

A benzoilecgonina e o éster metilecgonina são os principais metabólitos e representam mais de 80% dos produtos da biotransformação da cocaína. A benzoilecgonina é originada através de hidrólise espontânea ou por reação catalisada pelas carboxilesterases. O éster metilecgonina resulta da hidrólise do grupo benzoato da cocaína e ocorre por ação de colinesterases plasmática e hepática. A colinesterase plasmática que catalisa esta reação é a *EC 3.1.1.8*, também denominada pseudocolinesterase, cujo substrato principal é a benzoilcolina. Outra via de biotransformação da cocaína é a que resulta na norcocaína, produto farmacologicamente ativo. Essa via é mediada pelo sistema citocromo P-450 por N-desmetilação direta ou seguida à oxidação da cocaína pelo sistema de monoxigenases FAD-dependente. A norcocaína é então convertida por ação dos dois sistemas enzimáticos à N-hidroxinorcocaína, com posterior oxidação ao radical nitróxido de norcocaína, e eventualmente ao íon nitrosônio, comprovadamente eletrofílico [Oga, 1995].

1.6.4.4 ELIMINAÇÃO

Independentemente da via de administração, a excreção urinária da cocaína e seus metabólitos ocorre nas primeiras 24 h. A meia vida biológica da cocaína no sangue é de aproximadamente 1 h e menos de 5% de cocaína inalterada é eliminada na urina. O éster metilecgonina constitui de 32 a 49% da excreção urinária da cocaína e a benzoilecgonina de 29 a 45%. Outros produtos de biotransformação como a ecgonina, norcocaína e benzoilnorecgonina, se apresentam em menor quantidade (Oga, 1995; Warner, 1993).

1.6.5 MECANISMO DE AÇÃO

A cocaína bloqueia a reabsorção de neurotransmissores (noradrenalina, dopamina e serotonina), produzindo um aumento na concentração dos neurotransmissores nas junções sinápticas (figura 2) [Warner, 1993].

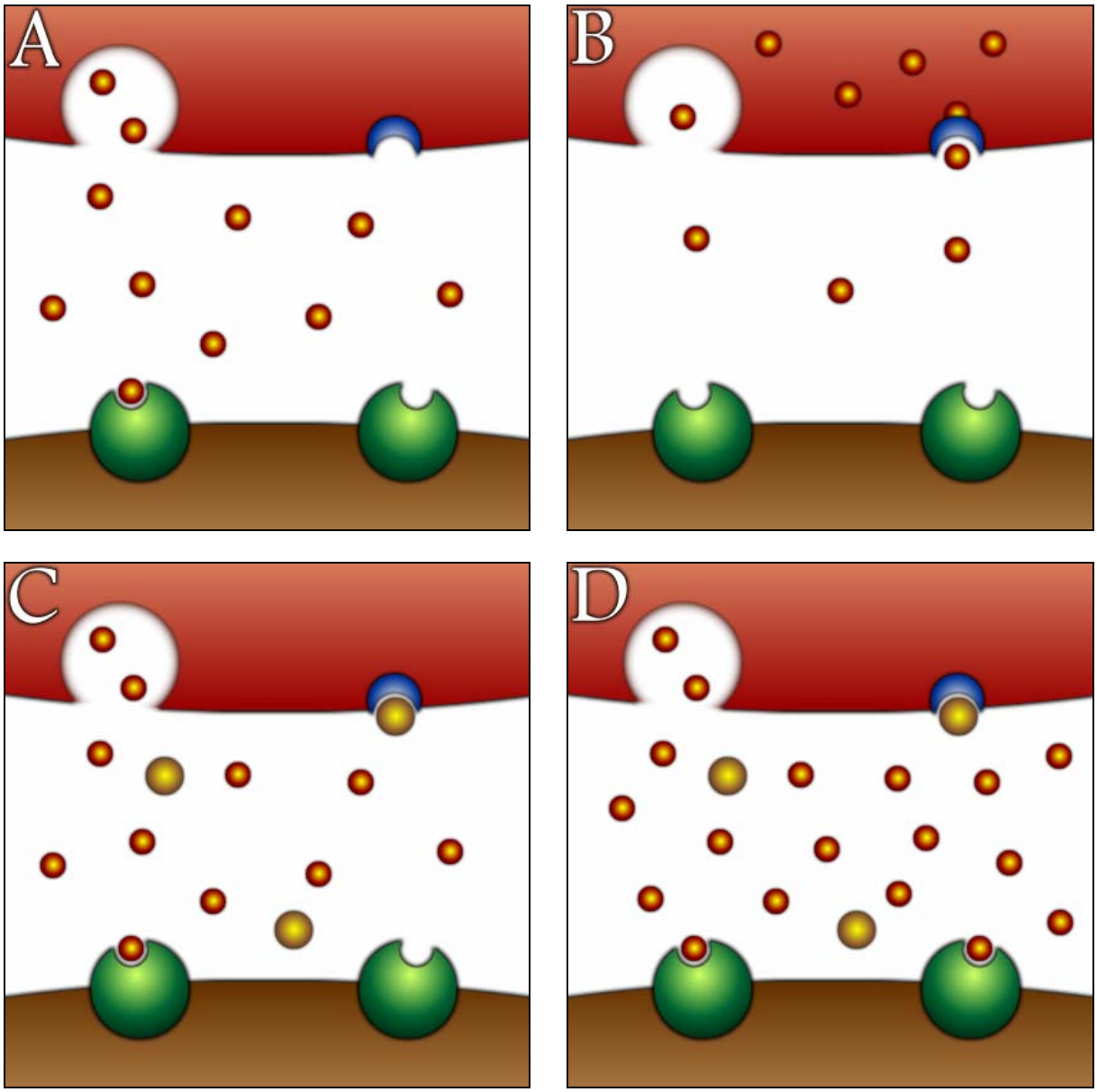

Figura 2. Mecanismo de ação da cocaína. A e B sem cocaína; C e D com cocaína. Legenda: ( ) cocaína, ( ) neurotransmissor, ( ) receptor, ( ) transportador.

1.6.6 EFEITOS FISIOLÓGICOS

O aumento da concentração de noradrenalina nas junções sinápticas produz uma estimulação do sistema nervoso simpático causando vasoconstricção, taquicardia, midríase e hipertermia [Lathers *et al.*, 1988]. A estimulação do sistema nervoso central causa sinais como bem-estar, comportamento repetitivo, verbosidade, aceleração do pensamento e do curso das idéias, redução da fadiga e da fome, irritabilidade, impulsividade e alterações no comportamento sexual. O estímulo psicológico produz uma intensa euforia que muitas vezes é comparada ao orgasmo. A cocaína também funciona como anestésico local, um efeito resultante da sua propriedade de bloquear os canais de sódio [Warner, 1993; Oga,

1995].

1.6.7 MÉTODOS ANALÍTICOS

A análise de cocaína inclui testes de orientação e de confirmação. Os testes de orientação são relativamente simples, rápidos e baratos; possuem uma alta sensibilidade e baixa especificidade analítica. Imunoensaios são exemplos de testes de orientação.

Os testes de confirmação, geralmente caros, são viabilizados pelo seu alto grau de especificidade. A cromatografia em camada delgada é um método bastante econômico, mas seu emprego é limitado em função da sua baixa sensibilidade. A caracterização definitiva de amostras de cocaína pode ser feita através de técnicas fundamentadas em processos cromatográficos como *High Performance Liquid Chromatography* (HPLC) [Bujan *et al.*, 2001; Tagliaro *et al.*, 1993; Gill *et al.*, 1984] e *Gas Chromatography-Mass Spectrometry* (GC-MS) [Lewis *et al.*, 2004], ou através da espectrofotometria no ultravioleta/visível (UV/VIS) [Cruz *et al.*, 1994]. Todas essas técnicas modificam ou destroem a amostra analisada. A GC-MS apresenta a vantagem de fornecer dados sobre a estrutura química do composto, enquanto que nas outras técnicas a caracterização é feita de forma indireta, com a utilização de padrões e/ou reações químicas.

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3 OBJETIVOS

3.1 GERAL

Viabilizar a aplicação da Espectroscopia de Ressonância Magnética Nuclear (RMN) em toxicologia forense.

3.2 ESPECÍFICOS

Descrever as características da intoxicação letal por uso do HCFC-141b como droga inalante.

Estabelecer a utilização da RMN como técnica de rotina na caracterização não destrutiva de amostras ilícitas de cocaína no estado líquido ou sólido.

Viabilizar a utilização da RMN de ^{1}H, e DEPT como procedimento de rotina para caracterização de amostras ilícitas de cocaína com finalidades forenses.

Estudo das vantagens e desvantagens do emprego da RMN na análise de drogas de abuso utilizando como referência a técnica GC/MS

4 RELEVÂNCIA DO PROJETO

Este trabalho abrange dois tópicos distintos: o estudo de um caso de morte atribuída ao uso do HCFC-141b e a análise de amostras de cocaína através da RMN, ambos relativos à Toxicologia Forense.

Apenas um caso de morte atribuída à exposição acidental ao gás HCFC-141b foi relatado na literatura. Sua utilização como droga inalante ainda não está amplamente difundida tornando imprescindível a descrição dos perigos relacionados com o uso do HCFC-141b como droga inalante, dos eventos que podem causar a morte e das características *post mortem* do usuário.

A RMN é capaz de fornecer dados sobre a estrutura molecular do composto, com a

vantagem de ser uma técnica não destrutiva. Nas análises forenses, a quantidade de amostra a ser analisada geralmente pequena e a necessidade de armazenar a mesma quantidade de analito utilizada no exame primário para contraprova por tempo indeterminado torna essencial a otimização de processos de análise que não destruam ou modifiquem a amostra, o que estabelece a RMN como uma excelente opção para caracterização de amostras de cocaína e outras drogas de abuso com finalidades forenses.

Produção Científica

5 SUDDEN DEATH AFTER USE OF HCFC-141B AS INHALANT DRUG

Vanduir S. A. Filho[1], Verônica C. M. L. Santos[1], Ronivaldo O. Barros[1], Ricardo O. Silva[2], Manfred O. E. Schwartz[2], Marcos A. P. L. Lima[1] and Luana C. B. B. Coelho[3]

1 – Instituto de Polícia Científica, Av. Antônio Teotônio, s/n, Cristo Redentor, João Pessoa – PB, Brasil – CEP 58071-620.

2 – Departamento de Química Fundamental – UFPE, Av. Prof. Luiz Freire, s/nº Cidade Universitária, Recife – PE, Brasil – CEP 50670-901.

3 – Departamento de Bioquímica, CCB, Universidade Federal de Pernambuco (UFPE), Av. Moraes Rego, s/n, Cidade Universitária, Recife – PE, Brazil, CEP: 50670-420.

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ABSTRACT

A 19 year-old woman died suddenly after inhalation of an aerosol. 1-1-dichloro-1-fluoroethane (HCFC-141b) was identified as the main component of the aerosol by nuclear magnetic resonance (NMR) and gas chromatography/mass spectrometry (GC/MS). No poisons, alcohol or other drugs were detected in the victim's blood or viscera. According to police inquest the cause of death was the aerosol inhalation. In the necropsy, toxicological and microscopic findings did not exclude the hypothesis of death due to HCFC-141b inhalation, so, two mechanisms of death were acceptable: asphyxia and fatal cardiac arrhythmia. The analysis and police inquest data supported probabilistically as the cause of death either a fatal cardiac arrhythmia or a combination of arrhythmia and asphyxia.

Keywords: hydrochlorofluorocarbon, HCFC-141b, asphyxia, arrhythmia.

5.1 INTRODUCTION

Inhalants are volatile substances that vaporize at room temperature and can be inhaled to induce a psychoactive effect. 1-1-dicloro-1-fluoroetano (HCFC-141b), a hydrochlorofluorocarbon (HCFC), is a colorless volatile liquid with weak ethereal odor and a relatively high vapor pressure. It is being used as a replacement for chlorofluorocarbons (CFCs) as a refrigerant, propellant and dry-cleaning agent. HCFC-141b has a narcotic effect [1] and other necessary requirements to characterize it as an inhalant drug. However, its use as a recreational drug is not explicitly reported in literature. The human metabolism of HCFC-141b was well established. It is metabolized to 2,2-dichloro-2-fluoroethanol, which is conjugated with glucuronic acid and excreted in the urine; dichlorofluoroacetic acid is a minor metabolite of HCFC-141b [2,3,4,5].

There are four possible mechanisms that cause sudden death due to inhalant abuse: asphyxia (anoxia), vagal inhibition, respiratory depression and cardiac arrhythmia [6]. Cases of death for asphyxia or fatal cardiac arrhythmia due to the inhalation of inert or low toxicity gases are frequently reported [7,8,9,10,11]. However, only one case of death involving exposition to HCFC-141b was reported in the literature [12]. In the present work, we report the findings in the body of a young woman who died after using HCFC-141b as an inhalant.

5.2 CASE REPORT

5.2.1 CASE HISTORY

A 19 year-old female was at a nightclub at a beach resort when a substance in an aerosol flask was offered her to inhale. Hesitant, she went to the toilet and took her panty hose off. Immediately, she returned and soaked up the substance inside the flask with the panty hose. At a certain moment, she took the panty hose to her face, inhaling for some time the impregnated substance. Suddenly, she complained of pains in the chest and fainted. The

people in that place took her to a hospital, where she arrived apparently dead, with mydriasis, cyanosis and cardio respiratory failure. In some minutes, the death was diagnosed. In the parking of the hospital, a policeman found an aerosol container, identified by all eyewitnesses as the one used by the dead girl.

5.2.2 TOXICOLOGICAL ANALYSIS

5.2.3 MATERIALS AND METHODS

Toxicological screening of blood and viscera was performed using solvent extraction and chromatographic techniques.

The analysis of the composition of the aerosol used by the victim analysis was carried out by Nuclear Magnetic Resonance (NMR) of the nucleus of ^{19}F, in a *Varian Unity Plus 300 MHz NMR* instrument, and gas chromatography/mass spectrometry (GC/MS), in a *Shimadzu GCQ 5050* GC-MS instrument, by using the manufacturers' equipments standard procedures. A sample of HCFC-141b, kindly given by the *Brazilian Federal Police*, was used as standard.

Blood-alcohol analysis was undertaken utilizing 2-propanol as internal standard by *Headspace* gas chromatography. One milliliter or 1.0 g of the samples was put into a 10 ml vial. The vial was sealed immediately with Teflon cap and kept warm at 55 °C for 30 min. After balancing with room temperature, 1.0 ml of the headspace sample from the vial was injected into a *Shimadzu GCQ 5050* GC-MS instrument. The Headspace parameters for analysis of HCFC-141b in blood, urine, stomach contents, lung and liver samples were similar to the parameters for that of ethanol [13]. These analyses were performed 16 days after victim's death.

5.2.3.1 FINDINGS

In the analyses mentioned above, no alcohol, cannabinoids, opiates, cocaine, amphetamines, barbiturates, benzodiazepines or poisons were detected in the victim's samples. HCFC-141b was the major component of the aerosol used by the victim. However,

it was not found in the victim's blood, lung and liver samples in an analysis undertaken 16 days after her death.

5.2.4 Histological findings

Histological examinations revealed lungs, kidney, liver and cerebrum congestion, pulmonary edema and pancreatic autolysis.

5.3 Discussion

The necropsy findings revealed some signs found in cases of death by asphyxia, however, these signs are not specific [14] so, other death diagnoses cannot be excluded. Petechial hemorrhages were observed in both ocular conjunctivas and lungs. Petechial hemorrhages are small extravasations of blood with size up to 2 mm in diameter and are found often in association with larger “purpuric” lesions, some of which may have arisen by the coalition of petechiae [15] as in the present case. The mechanism for petechiae formation was not yet established, but elevated venous pressure, venous stasis, hypoxia, tissue acidosis were associated with injury to endothelial cells generating petechiae [16,1718,19]. Though they are most easily seen on surfaces (skin, mucous and serous membranes, organ capsules, conjunctiva, sciera, and retina) and against the light and homogenous background of brain tissue, they occur in all organs. Petechial hemorrhages are common in asphyxia victims but they also appear in nonasphyxial deaths as, for instance, in sudden cardiovascular deaths, particularly those with acute right heart failure, and instances in which individuals die with their faces prone [19], however, in a case of death for heart arrhythmia associated to the inhalation of the butane, petechiae were not found [20].

Visceral congestion and pulmonary edema also was reported in sudden deaths related to inhalation of butane [21], Ethyl Chloride [22], 1,1-difluoroethane [23] and accidental exposition to HCFC-141b [12]. The normal aspect of the victim’s trachea shows

that HCFC-141b did not cause respiratory injuries to the victim. It suggests nonasphyxial death. The histological findings confirm the necropsy results. The microscopic exams did not reveal inflammatory lesions or natural diseases justifying the victim's sudden death.

In researches with mice dead by asphyxia with butane [20] and chlorodifluoromethane (Freon-22) [8], soon after death, those gases concentrations in the bodies decreased rapidly, thus, the long period elapsed between the death and HCFC-141b analysis in the victim's viscera might have made unfeasible its detection.

HCFC-141b has low toxicity if administered orally; it does not produce irritation in contact with the skin and causes moderate irritation in contact with the eyes [1]. Just one case of death attributed to the inhalation of HCFC-141b steam was reported. A 40-year-old man, with a history of cardiac and respiratory diseases, died while degreasing a tank by using HCFC-141b as solvent. For this case, two causes of death were proposed. One of them suggests that HCFC-141b sensitizes the heart to the arrhythmogenic effect of endogenous beta-agonists and may induce sudden death [12]; this effect was also observed in dogs and monkeys [1]; the other proposal suggests that the HCFC-141b steam displaced the oxygen of the air, leading to respiratory and cardiac failure in the man cleaning the tank [24]. It means deaths by inhalant use may be caused by either cardiac arrhythmia or the combination of arrhythmia and asphyxia [6]. Therefore, both proposals are viable and they might have coexisted as the causes of the man's death.

The necropsy, histological and toxicological findings, as well as the police inquest lead to the conclusion that the victim was killed by HCFC-141b inhalation. Asphyxia, vagal inhibition, respiratory depression and heart arrhythmia are the mechanisms associated to the sudden death by inhalant addiction [6]. The differential diagnosis among these mechanisms is not easy. However, the method used by the victim to inhale the steams of the aerosol, the normal aspect of the trachea and the absence of strange bodies in the trachea and lungs represent probabilistic factors that may have caused death either by cardiac arrhythmia or by the combination of arrhythmia and asphyxia.

The use of HCFC-141b and other HCFC's as inhalants has been increasing

significantly among Brazilian youths, because of its easy acquisition compared to other inhalant such as the ether spray. Considering the potential risks that involve the use of inhalants, the control by the authorities of the acquisition of these substances has become necessary.

5.4 ACKNOWLEDGEMENT

The authors would like to thank the Directors Antônio Albuquerque Toscano (Institute of Scientific Police – IPC/PB) and Maria do Socorro Dantas Araújo (Forensic Medicine Department – DML/IPC/PB), for the permit to publish details of this case.

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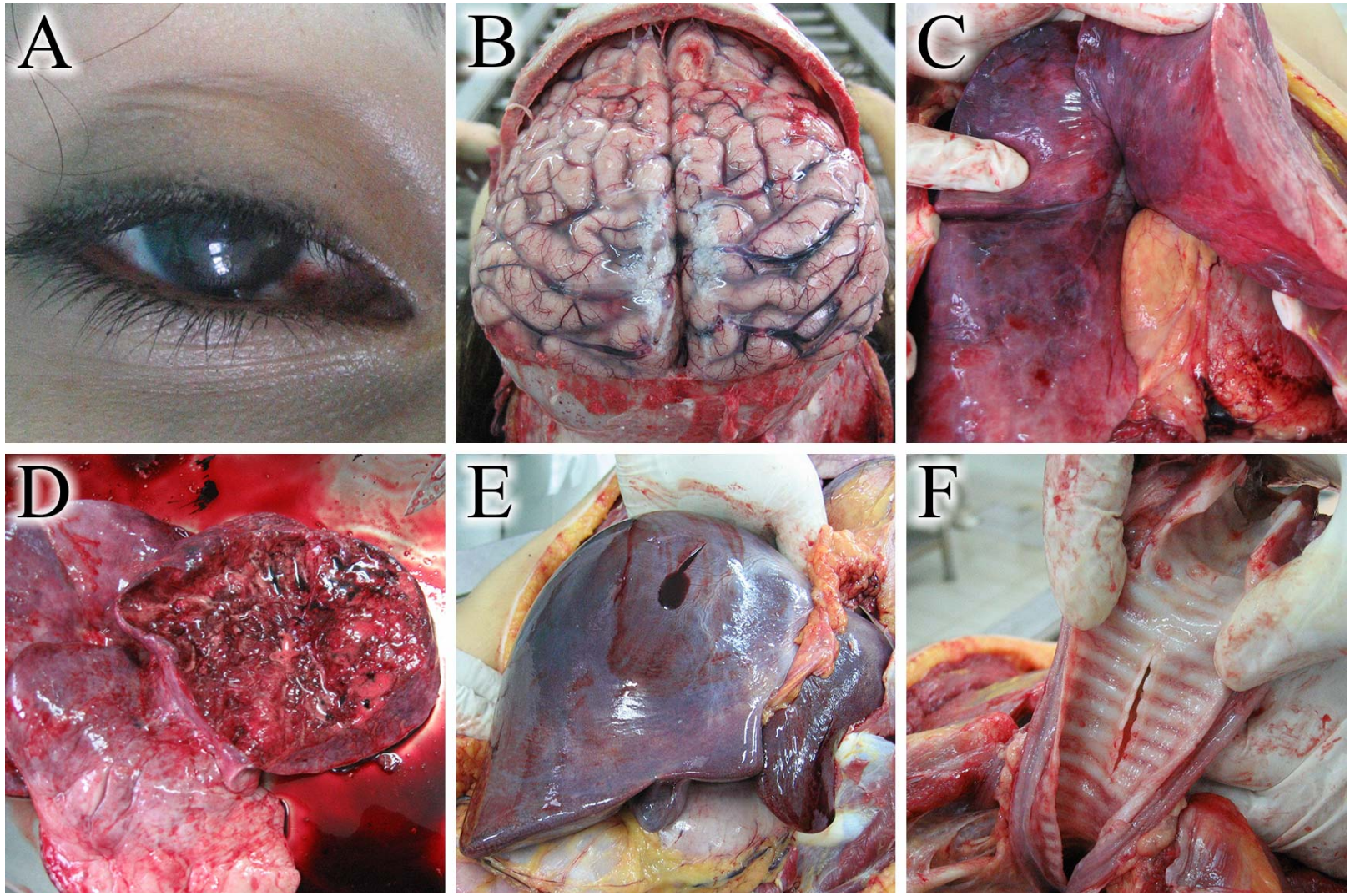

Figure 1. Necropsy findings: (A) Petechial hemorrhages in both eyes; (B) vascular cerebral congestion; (C and D) lungs showing marked swelling and congestion, with violet areas and foaming serum hematic secretion; (E) liver showing congestion; (F) trachea mucous with normal aspect, with no secretion.

6 QUALITATIVE ANALYSIS OF ILLICIT COCAINE SAMPLES BY NMR

Vanduir S. A. Filho[†1], Ricardo O. Silva[‡], Manfred O. E. Schwartz[‡] and Luana C. B. B. Coelho[§].

[†]*Instituto de Polícia Científica, Av. Antônio Teotônio, s/n, Cristo Redentor, João Pessoa – PB, Brasil – CEP 58071-620.*

[‡]*Departamento de Química Fundamental – UFPE, Av. Prof. Luiz Freire, s/nº Cidade Universitária, Recife – PE, Brasil – CEP 50670-901.*

[§]*Departamento de Bioquímica, CCB, Universidade Federal de Pernambuco (UFPE), Av. Moraes Rego, s/n, Cidade Universitária, Recife – PE, Brazil, CEP: 50670-420*

Trabalho a ser submetido a o periódico *Forensic Science International*

[1] Corresponding author: vanduir@msn.com

ABSTRACT

NMR techniques deliver data about the molecular structure of a sample in a short time. In the present work some techniques of Nuclear Magnetic Ressonance (NMR), ^{1}H, ^{13}C and Distortionless Enhancement by Polarization Transfer (DEPT), were used to characterize cocaine samples. Making use of Heteronuclear Correlation (HETCOR) and Heteronuclear Multiple Bond Correlation (HMBC) the signals can be attributed. The samples were primarily characterized by mass spectroscopy whose spectra were compared with the ones in literature. The findings indicate that ^{1}H, ^{13}C NMR and DEPT are quite efficient techniques to characterize illicit cocaine samples.

Keywords: Cocaine, Crack, NMR, DEPT.

6.1 INTRODUCTION

Cocaine is an alkaloid found in about two hundred plants of the family *Erytroxylum*, popularly called *coca*. Only two of them show significant quantities of cocaine, *E. coca*, major cocaine source for illicit use as drug, and *E. novogranatense*, in general cultivated for cocaine use in local pharmaceutical industry or as a constituent in teas for food industry [1,2].

Mass Spectroscopy (MS) and Nuclear Magnetic Resonance (NMR) are excellent tools to elucidate structure of organic compounds [3]. MS is frequently used for cocaine and other drugs analysis, Normally MS is used in "on-line" combination with a chromatographic method like High Performance Liquid Chromatography (HPLC) or Gas Chromatography (GC), resulting in techniques named HPLC-MS and GC-MS, respectively [4,5]. Even being a technique quite often used for structure analysis, NMR is not a routine technique for drug analysis. In the present work, the use of ^{13}C NMR and Distortionless Enhancement by Polarization Transfer (DEPT) is proposed as a routine procedure for characterization of illicit cocaine samples for forensic aims, as well as a comparison of advantages and disadvantages of the NMR technique against the standard procedure GC/MS.

6.2 MATERIALS AND METHODS

6.2.1 SAMPLES

All cocaine samples were obtained from Institute of Scientific Police and from Department of Federal Police of Paraíba State. 10 mg of each crack cocaine and cocaine hydrochloride samples were dissolved in 600 μl of $CDCl_3$ and D_2O, respectively. The samples used in the GC/MS tests were the same as in NMR analyses.

6.2.2 GC/MS

All the spectra were performed using Finnigan Mat GCQ Ion Trap spectrometer equipped with DB-5 column with 30 m of length and 0.25 mm i.d. The carrier gas used was helium with linear velocity equal to 40 cm s^{-1} and split ratio of 1:60. The ionization energy used was of 70 eV. The GC conditions were as follows: the oven temperature varied from 60°C to 275°C with a heating rate of 10°C min^{-1}; the injector temperature was maintained at 250°C; the ion source temperature was maintained at 175°C.

6.2.3 NMR

All the NMR spectra were performed using VARIAN Unity Plus 300 spectrometer operating at 300 MHz and 75 MHz for ^{1}H and ^{13}C nucleus, respectively, at 293K.

Solution NMR spectra were performed using the follows parameters: spectral width of 5 kHz, acquisition time of 3.7 s and 16 transients for ^{1}H NMR; spectral width of 16.5 kHz, acquisition time of 2.5 s, relaxation delay of 2 s and 512 transients for ^{13}C and DEPT NMR. ^{1}H and ^{13}C NMR spectra were performed with sample spinning of 20 Hz, while the two-dimensional spectra Heteronuclear Correlation (HETCOR) and Heteronuclear Multiple Bond Correlation (HMBC) were performed without sample spinning.

6.3 RESULTS AND DISCUSSION

6.3.1 GC/MS

Mass spectra of cocaine shows all the ions described in the very detailed analysis made by Smith (1997) [6], including the molecular ion at m/z 303 and the base peak at m/z 82.

6.3.2 SAMPLES

In the present work, were studied two cocaine forms: cocaine hydrochloride, crystalline form, a white powder water-soluble and crack cocaine amorphous alkaloid form, water insoluble but soluble in organic solvents [2].

6.3.3 NMR SPECTROSCOPY

HETCOR, HMBC and DEPT spectra were made for assignments in ^{1}H and ^{13}C NMR. HETCOR spectrum shows heteronuclear correlations between carbon and hydrogen nuclei directly bonded (fig. 2) [7], while HMBC spectrum shows, generally, heteronuclear correlations between carbon and hydrogen nuclei separated by three bonds (fig. 3) [8,9,10]. In DEPT NMR spectra (fig. 4) the signals of ^{13}C NMR spectrum are separate according to the number of hydrogen atoms bonded [11]. Figure 1 shows the chemical structure of the cocaine (alkaloid form) and the carbon number used to assign the NMR spectra. Small alterations were observed between crack and cocaine hydrochloride samples δ_H and δ_C due to pH change and used solvent (see Table 1).

6.3.4 DEPT AND ^{13}C NMR ASSIGNMENTS

6.3.4.1 QUATERNARY CARBONS

Chemical shifts at 130.1, 166.1 and 170.7 ppm did not appear in DEPT spectrum (fig. 4), so they can be assigned to quaternary carbons. Peak at δ_C 130.1 ppm was assigned to carbon 13 because it appears in aromatic region of ^{13}C NMR spectra. Peaks at δ_C 166.1 and 170.7 ppm were assigned to the carbonyl groups. In HMBC spectrum the signal at δ_C 166.1 ppm shows correlation with the signal at δ_H 8.0 ppm assigned to aromatic protons 14 and 14' (fig. 3). So, chemical shift at 166.1 ppm was assigned to the carbon 12 and δ_C 170.7 ppm, by exclusion, was assigned to carbon 10.

6.3.4.2 CH_3 GROUPS

According to DEPT spectrum chemical shifts at 41.1 and 51.5 ppm, belongs to two methyl groups of cocaine (fig. 4), in HETCOR, peaks at shows correlation with chemical shifts at 2.3 and 3.7 ppm, respectively (fig. 2). In HMBC, the peak at δ_H 3.7 ppm shows a correlation with the signal attributed at carbon 10 (fig. 3), thereby, chemical shift at 51.5 ppm was assigned to carbon 11 and peak at δ_C 41.1 ppm, by exclusion, was attributed to

carbon 9.

6.3.4.3 AROMATIC CH GROUPS

In DEPT spectrum (fig. 4), chemical shifts at 128.3, 129.7 and 132.9 ppm was attributed to aromatic CH groups. In HMBC, the previously assigned to carbon 13 peak at δ_C 130.1 ppm shows correlation with the 15 and 15' protons peak at δ_H 7.4 ppm (fig. 3) which shows, in HETCOR, a correlation with the chemical shift at 128.3 ppm thus assigned to the carbons 15 and 15' (fig. 2). Chemical shift at 129.7 ppm can be assigned to carbons 14 and 14' on the basis of its correlation, in the HETCOR, with the signal at δ_H 8.0 ppm already attributed to of 14 and 14' carbons protons (fig. 2). The peak at δ_C 132.9 ppm was assigned to carbon 16 because of its correlation with carbon 14 proton chemical shift at 8.0 ppm in HMBC (fig. 3), and with the peak at δ_H 7.6 ppm in HETCOR (fig. 2).

6.3.4.4 ALIPHATIC CH GROUPS

According to DEPT spectrum (fig. 4), chemical shifts at 50.0, 61.6, 64.7 and 66.7 ppm were assigned to aliphatic CH groups. The signal at δ_H 5.2 ppm belongs to carbon 3 protons because it shows correlations with the peak assigned to carbon 12 at δ_C 166.1 ppm in HMBC (fig. 3); so, chemical shift at 66.7 ppm was assigned to carbon 3 on the basis of its correlation with the peak at δ_H 5.2 ppm in HETCOR (fig. 2). In HMBC spectrum (fig. 3), peak at δ_H 2.3 ppm attributed to proton 9 shows correlation with the signals at δ_C 61.6 and 64.7 ppm, thus belonging to carbons 1 and 5. Carbon 5 is more shielded than carbon 1 due to proximity of carbon 1 to the carbonyl group, so, peaks at δ_C 61.6 and 64.7 ppm was assigned to carbon 5 and carbon 1, respectively. By exclusion, the chemical shift at 50.0 ppm was assigned to carbon 2.

6.3.4.5 CH_2 GROUPS

In cocaine structure (fig. 1), carbons 4, 6 and 7 are CH_2 groups whose protons are magnetically different (diasterotopic) and, therefore, have different chemical shifts. In DEPT

spectrum (fig. 4) three chemical shifts at 25.1, 25.4 and 35.3 ppm are assigned to CH_2 groups; since carbon 6 and carbon 7 are chemically and magnetically similar, chemical shift at 35.3 ppm was assigned to carbon 4. Carbon 7 is discreetly more shielded than carbon 6, so, peaks at δ_C 25.1 and 25.4 ppm was attributed to carbon 7 and carbon 6, respectively.

6.3.5 ^1H NMR ASSIGNMENTS

Protons not mentioned were assigned by HETCOR spectrum (see table 1).

6.3.6 GC-MS OR NMR?

MS gives information about the molecular mass and structural formula of a sample by its fragmentation products employing very small amount of samples, it makes this tool very useful in forensic toxicology. Only 0,005 mg of the sample were used to obtain mass spectra, while 10 mg were necessary to get NMR spectra. Even being not as sensitive as GC/MS, the big advantage of NMR is the fact that the samples used for analysis is not destroyed, so with one and the same sample, all the NMR spectra can be made and the sample can be kept for further investigation. Cocaine is a potent stimulating agent, doses between 10 and 100 mg are sufficient to achieve these effects. However, by use, the tolerance development rapidly conducts doses higher than 1 g [12]. The minimum quantity to obtain the effect of cocaine (10 mg) is sufficient for the analysis by GC/MS and NMR, both efficient tools for determination and quantification of drugs.

NMR is the most powerful and non destructive technique for structure determination of unknown compounds [13] where the most diversified pulse sequences like one-dimensional, e.g. APT (Attached Proton Test), DEPT, NOEDIF (Nuclear Overhauser Effect Difference), or two-dimensional, e.g. COSY (Correlated Spectroscopy), HETCOR and HMBC, amongst others, can be used [14]. For structure elucidation MS and NMR are normally used in association.

For analysis of mixtures normally a chromatographic method like HPLC of GC is used on-line with MS, giving the well known HPLC/MS and GC/MS techniques [4,5].

Actually, GC/MS is one of the most used tools to analyze cocaine and other drugs. Systems constituted of NMR coupled with HPLC (HPLC/NMR) are at a technologically satisfying level, but are not as widely used as GC/MS [13]. Lidocaine, procaine and caffeine are frequently used as adulterants in cocaine samples [2]. ^{1}H NMR (fig. 5) and DEPT (fig. 6) was capable tools for analysis of a cocaine sample containing lidocaine. ^{1}H NMR has been used to quantify components in mixtures containing cocaine [15,16].

6.4 Conclusions

The presented results show that ^{1}H NMR can be a very useful tool for characterization of cocaine containing samples. Isolated analysis of primary, secondary and tertiary carbon atoms by DEPT pulse sequence, as well as of quaternary carbon atoms by comparative studies of ^{13}C NMR and DEPT, reduces the superposition of signals probability. It makes analysis of cocaine samples by NMR faster, relatively easy, practical and reproducible.

6.5 Acknowledgements

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Table 1 - Assignments of the peaks in NMR ^{1}H and ^{13}C of cocaine.

Numeration (figure 1)	Alkaloid in $CDCl_3$		Cocaine hydrochloride in D_2O	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
1	3,6	64,7	4,1	63,9
2	3,0	50,0	3,5	46,1
3	5,2	66,7	5,4	64,5
4	1,9 / 2,5	35,3	2,1 /2,4	32,7
5	3,4	61,6	4,0	63,2
6	1,7 / 2,2	25,4	2,0 / 2,4	23,8
7	1,7 / 2,2	25,1	2,3	22,7
9	2,3	41,1	2,8	39,0
10	-	170,7	-	173,3
11	3,7	51,5	3,5	53,4
12	-	166,1	-	167,2
13	-	130,1	-	128,5
14	8,0	129,7	7,8	129,6
15	7,4	128,3	7,4	129,0
16	7,6	132,9	7,6	134,5

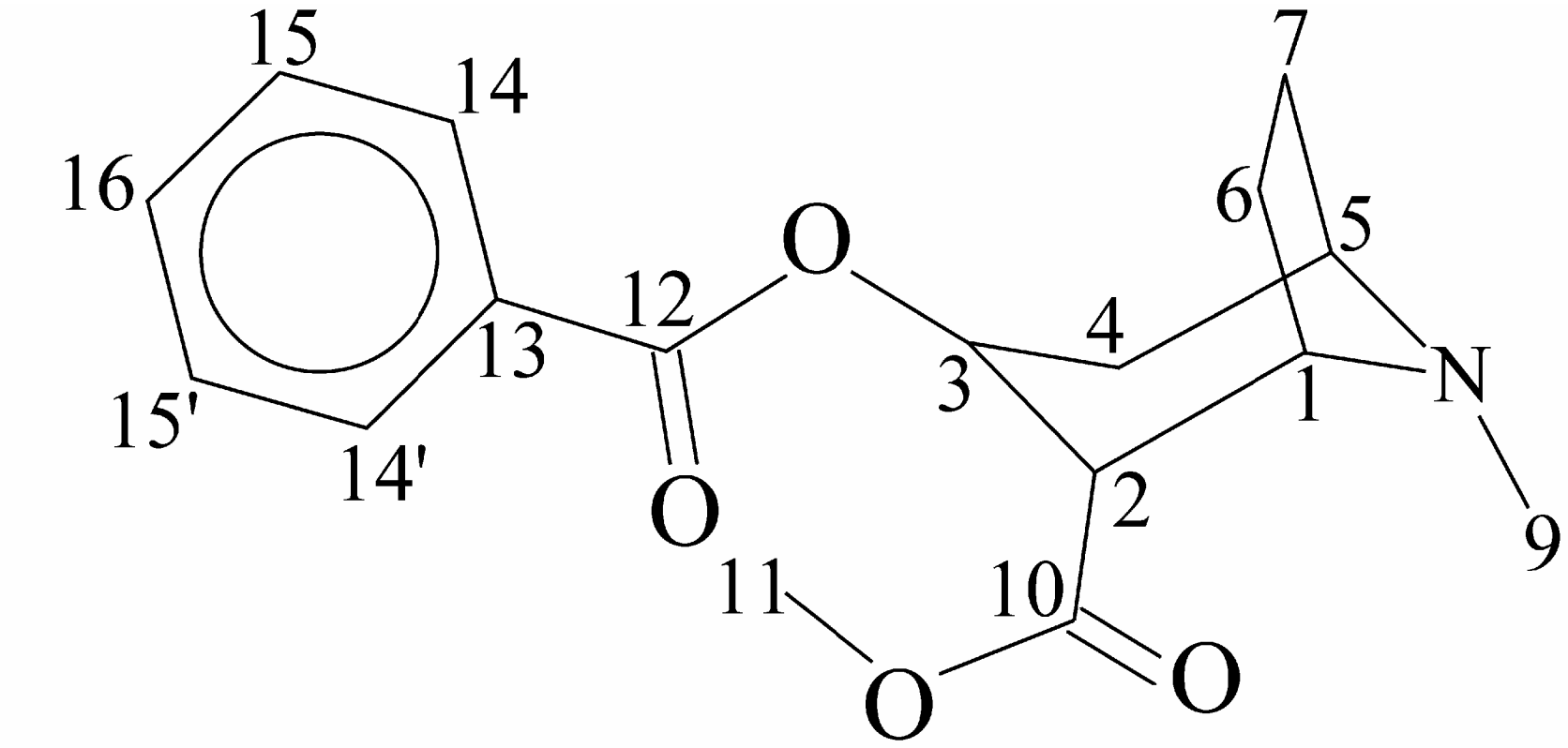

Figure 1 - Cocaine alkaloid form structure and numbering used in assignments.

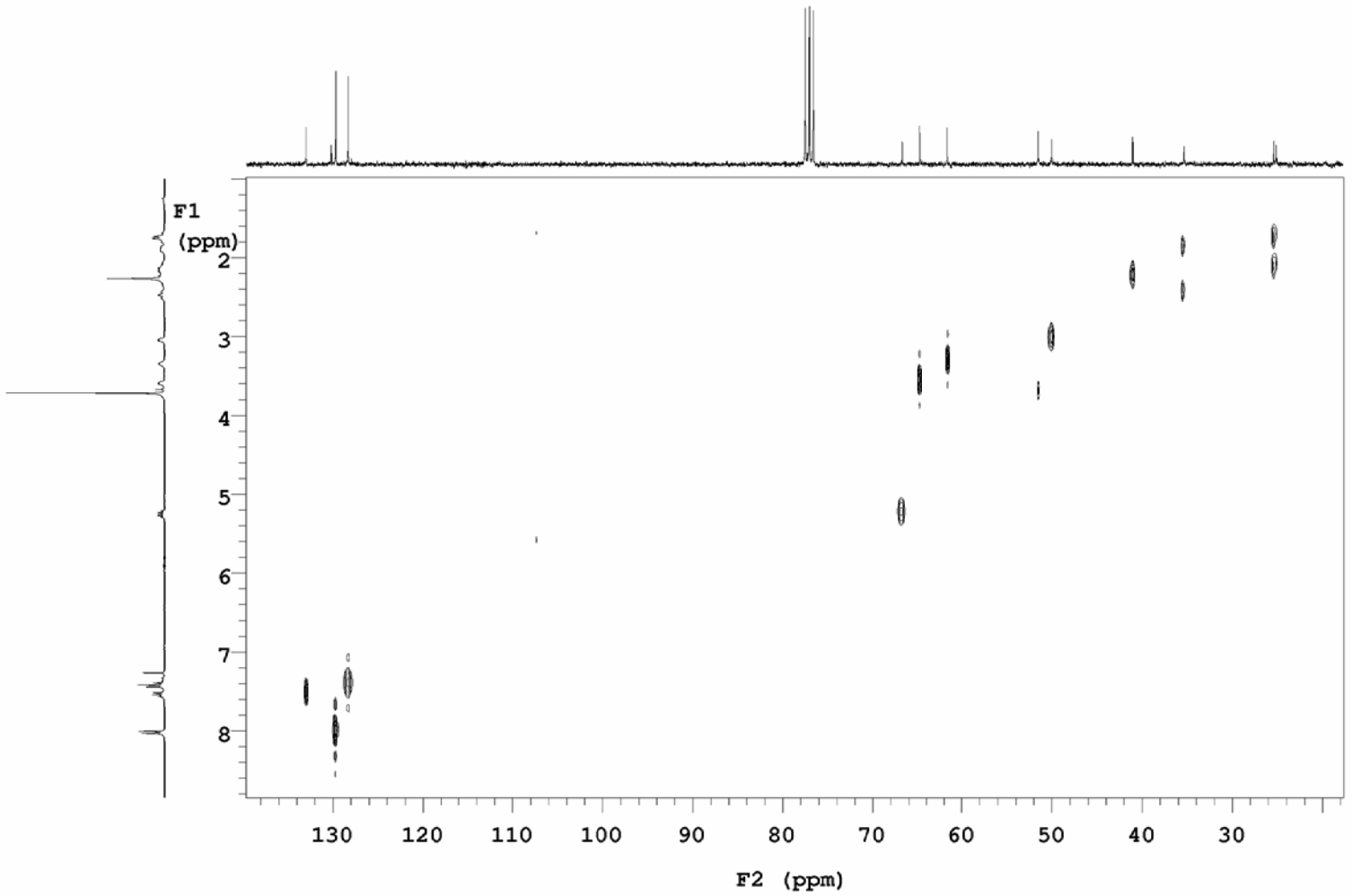

Figure 2 - HETCOR spectrum of cocaine in $CDCl_3$, 20°C, ^{13}C – 75 MHz and ^{1}H – 300 MHz.

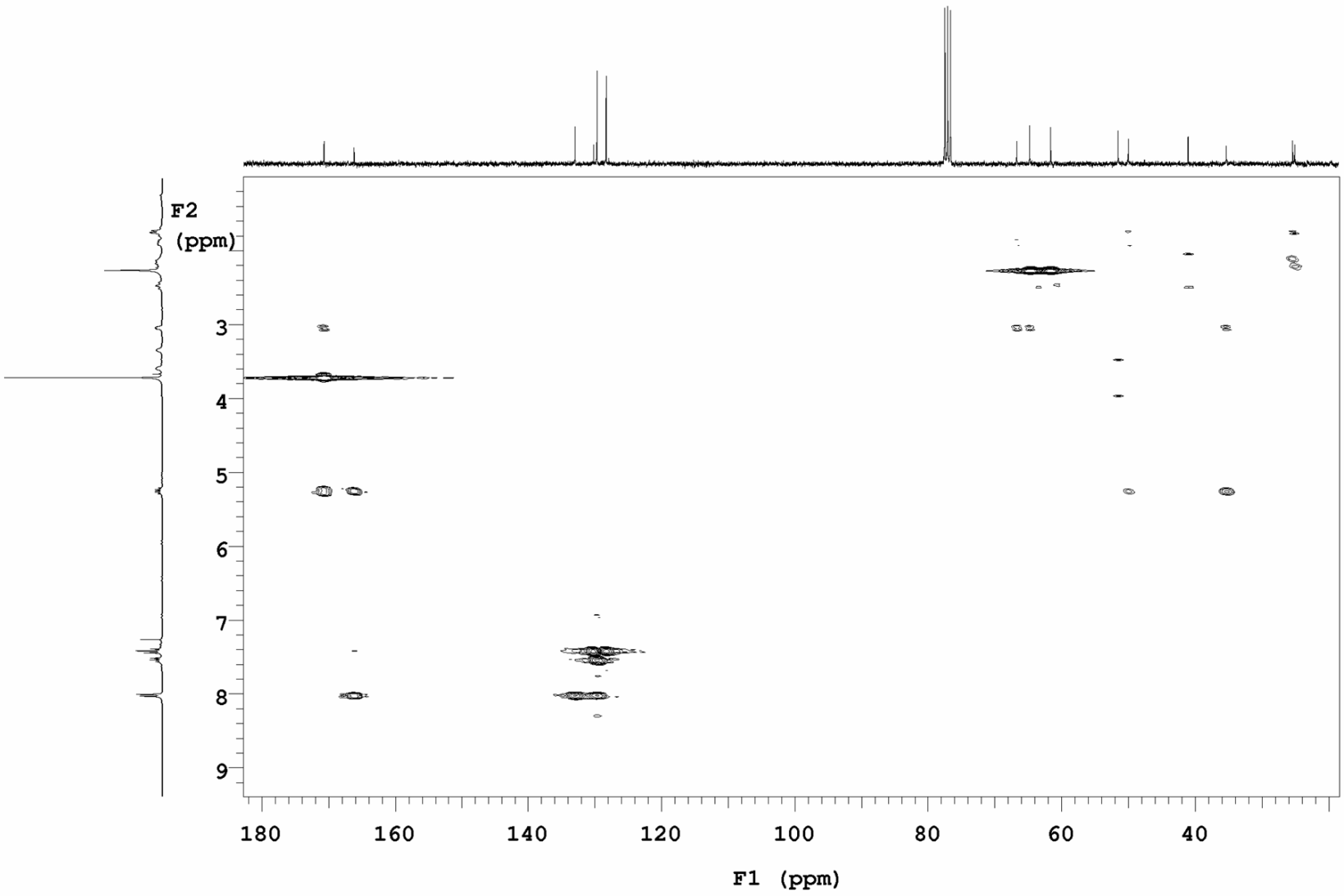

Figure 3 - HMBC spectrum of cocaine in $CDCl_3$, 20°C, ^{13}C – 75 MHz and ^{1}H – 300 MHz.

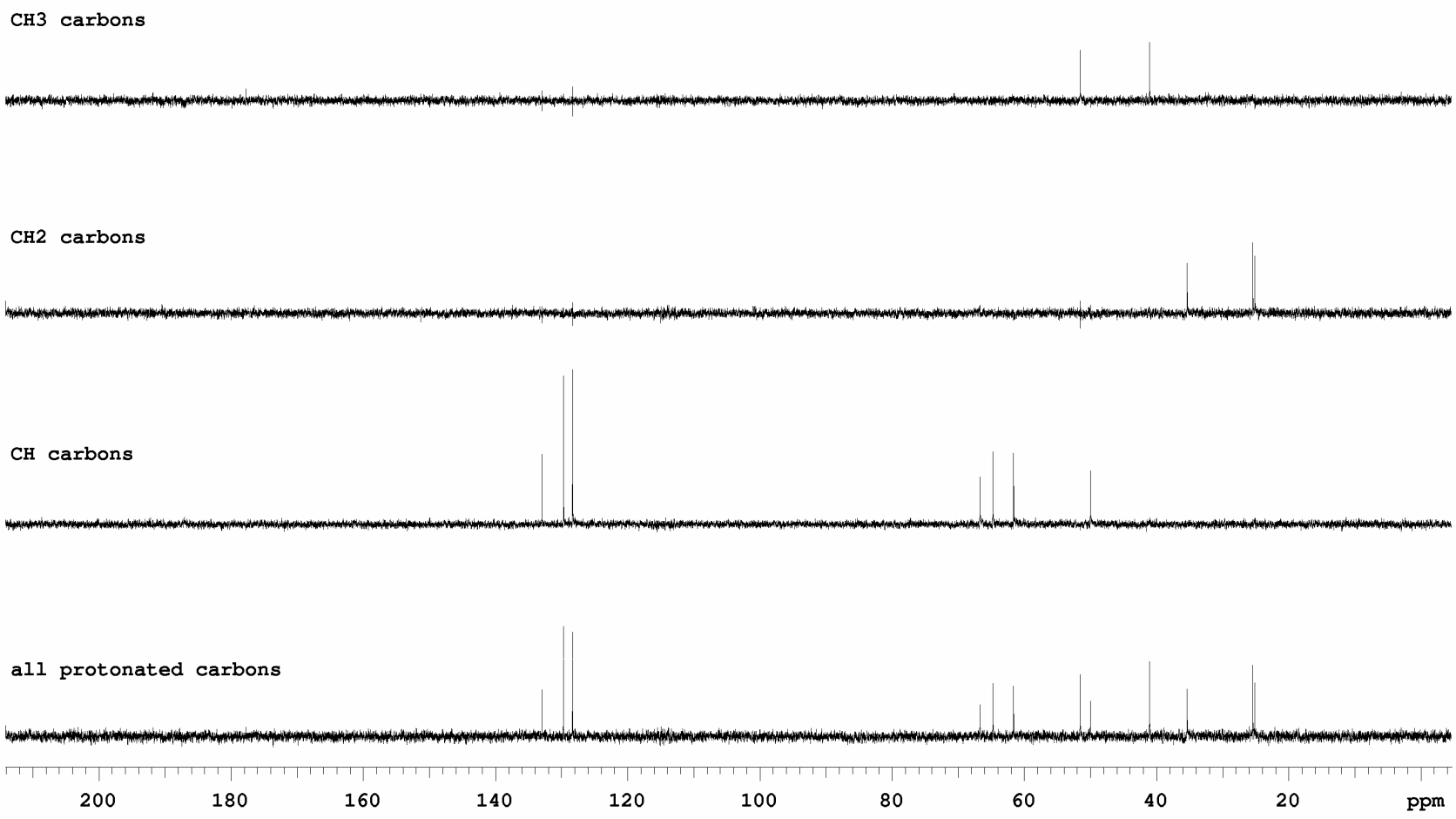

Figure 4 - DEPT spectra of illicit cocaine sample in CDCl3, 20°C, 75 MHz.

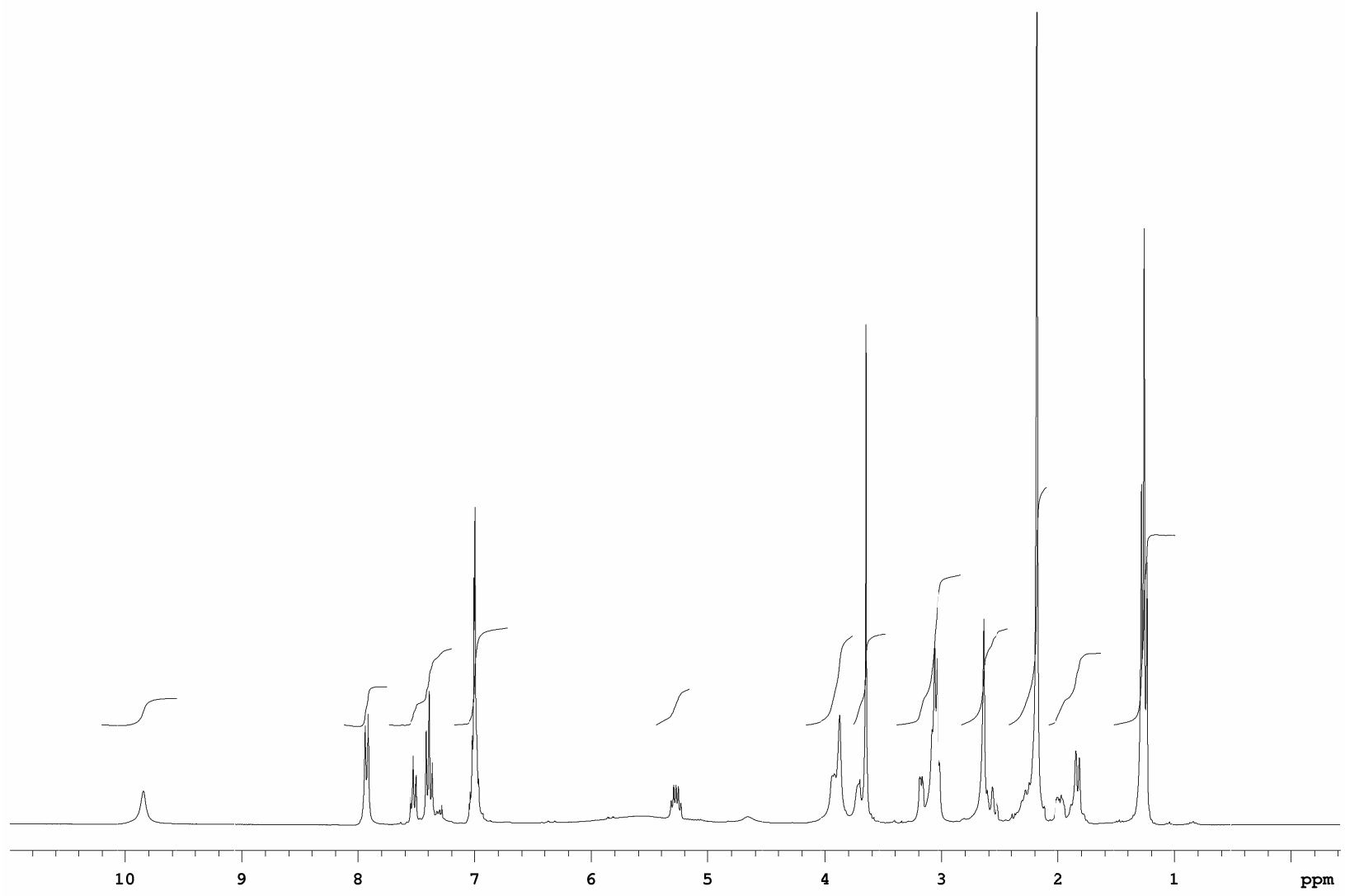

Figure 5 - ^{1}H NMR spectra of illicit cocaine sample containing lidocaine dissolved in $CDCl_3$, 20°C, 300 MHz.

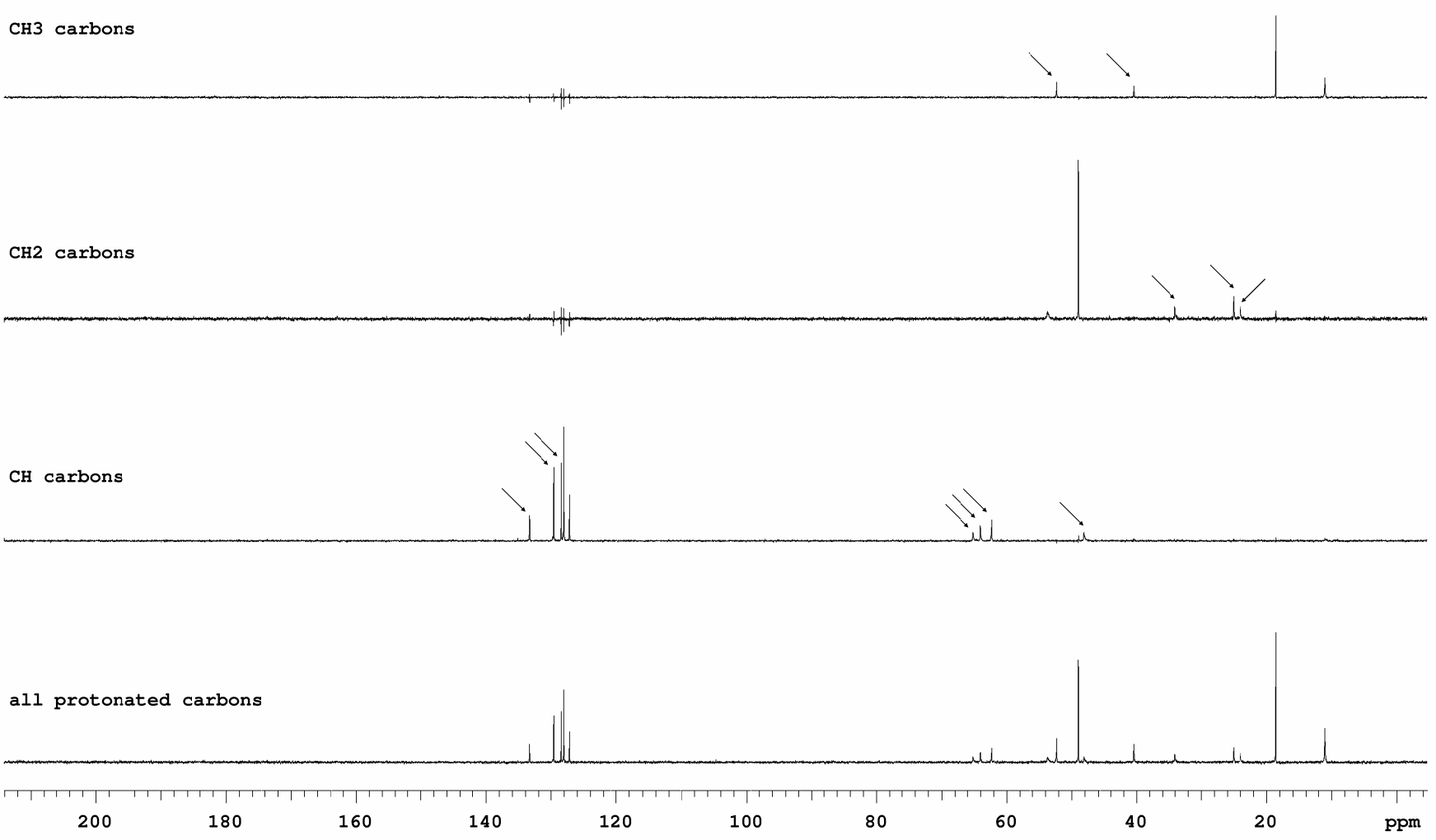

Figure 6 - DEPT spectra of illicit cocaine sample containing lidocaine dissolved in $CDCl_3$, 20°C, 75 MHz. Arrows indicate peaks assigned to cocaine.

7 Distinction between Crack and Cocaine Hydrochloride by CP/MAS and DD NMR

Ricardo O. Silva[†*], Fernando Hallwass[†], Marcos A. P. L. Lima[‡], Manfred O. E. Schwartz[†], Luana C. B. B. Coelho[§] and Vanduir S. A. Filho[‡].

[†]*Departamento de Química Fundamental – UFPE, Av. Prof. Luiz Freire, s/nº Cidade Universitária, Recife – PE, Brasil – CEP 50670-901.*

[‡]*Instituto de Polícia Científica, Av. Antônio Teotônio, s/n, Cristo Redentor, João Pessoa – PB, Brasil – CEP 58071-620.*

[§]*Departamento de Bioquímica, CCB, Universidade Federal de Pernambuco (UFPE), Av. Moraes Rego, s/n, Cidade Universitária, Recife – PE, Brazil, CEP: 50670-420*

*Corresponding author: Universidade Federal de Pernambuco – Departamento de Química Fundamental, Av. Prof. Luiz Freire, s/nº Cidade Universitária, Recife – PE, Brasil – Phone/FAX: +55 081 3453 0171 – CEP 50670-901 ros@ufpe.br or rosilva31@aol.com

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ABSTRACT

Neste trabalho, pela primeira vez, técnicas de RMN no estado sólido foram usadas para caracterizar amostras de cocaína na forma de alcalóide e de cloridrato. As seqüências *Cross-Polarization - Magic Angle Spinning* CP-MAS e *Dipolar Dephasing* (DD) RMN foram utilizadas na análise de amostras de *crack* e cloridrato de cocaína apreendidas pela polícia. Um estudo comparativo entre espectros de ^{13}C RMN obtidos em solução e no estado sólido foi realizado. Os resultados obtidos indicam que as seqüências CP-MAS e DD RMN são excelentes ferramentas para caracterizar, de forma rápida, reprodutível e não-destrutiva, amostras de cocaína com finalidades forenses.

The present work makes use for the first time of solid-state NMR techniques to characterize cocaine samples collected in the alkaloid or hydrochloride forms. Cross-Polarization - Magic Angle Spinning (CP/MAS) and Dipolar Dephasing (DD) NMR spectroscopy were used for analysis of the cocaine samples received from Brazilian police. The samples were analyzed in solution and solid state, a comparison of their ^{13}C NMR resonances was given. The observed results show that CP/MAS and CP/MAS/DD NMR are excellent tools for fast characterization of cocaine samples in a non-destructive and reproducible way with forensic objectives.

KEYWORDS: Drug Analysis, Cocaine, Crack, Nuclear Magnetic Resonance, Cross Polarization/Magic Angle Spinning, Dipolar Dephasing.

7.1 INTRODUCTION

Cocaine is an alkaloid extracted from the native plant of South America popularly called coca *(Erytroxylum coca).* The cocaine use occurs in the hydrochloride or alkaloid forms. The last form is found as free base or crack; both forms have the same chemical structure, however, different production ways.[1]

The use of cocaine as drug has increased in the last years, therefore it is necessary to develop new analytical methods for its characterization. Currently cocaine analyses are made by HPLC – High Performance Liquid Chromatographic, GC/MS – Gas Chromatographic and Mass Spectrometry or UV/VIS – Ultraviolet Visible spectroscopy techniques.[2,3] All these techniques alter or destroy the samples which is a disadvantage of these analytical methods. GC/MS has the advantage of supplying information about the chemical structure of the sample, while the characterization in the other techniques happens in an indirect way, using the standards and/or chemical reactions.

NMR spectroscopy techniques provide information about the chemical structure and also qualitative and quantitative composition of the sample even without standard or reagents addition, being therefore a non-destructive technique (NDT). NMR analysis can be performed in liquid or solid samples. Solid samples are packaged into a rotor and the analyses are performed using MAS – Magic Angle Spinning, CP/MAS – Cross-Polarization/Magic Angle Spinning, DD – Dipolar Dephasing sequences and others. In the CP/MAS NMR sequence the polarization of the most abundant nuclei (^{1}H) is transferred to those which are less abundant as ^{13}C nuclei, reducing the experimental time.[4] The CP/MAS/DD NMR sequence identifies the quaternary carbon and methyl groups signals.[5] Solid-state NMR is sensitive to study changes in the crystalline structure, allowing differentiate between crystalline and amorphous forms.[6]

In Forensic Science it is necessary to keep an aliquot for reanalysis and sometimes the sample quantity is small, so it is preferable to use NDT. Therefore, solid-state NMR techniques are an excellent option for characterization of solid samples. Lee *et al.* used solid

state NMR spectroscopy to analyze ecstasy samples.[7] In the present paper we characterize and distinguish crack and cocaine hydrochloride, using CP/MAS and CP/MAS/DD NMR sequences.

7.2 EXPERIMENTAL

7.2.1 COCAINE SAMPLES

The Scientific Police Institute and the Brazilian Federal Police Department of the State of Paraíba supplied cocaine hydrochloride and crack samples. All the samples were seized in police operations.

7.2.2 NMR EXPERIMENTS

All the NMR spectra were performed using VARIAN Unity Plus 300 spectrometer operating at 300 and 75 MHz for ^{1}H and ^{13}C nucleus, respectively, at 293K.

The samples were packaged into a zirconium oxide 5 mm diameter rotor. CP/MAS and CP/MAS/DD NMR spectra were obtained using the following parameters: sample spinning at 4-5 kHz on magic angle (54.7°), spectral window of 50 kHz, contact-time of 800 ms, acquisition time of 50 ms, relaxation delay of 2 s and 512 transients. The spectra were processed with line broadening of 10 Hz. The methyl group of hexamethyl benzene (HMB) signal was used as external reference (δ 17.3 ppm).

Solution NMR spectra were performed using the follows parameters: for ^{1}H NMR spectral width of 5 kHz, acquisition time of 3.7 s and 16 transients; for ^{13}C NMR spectral width of 16.5 kHz, acquisition time of 2.5 s, relaxation delay of 2 s and 512 transients. ^{1}H and ^{13}C NMR spectra were performed with sample spinning of 20 Hz, while the two-dimensional spectra HETCOR (Heteronuclear Correlation) and HMBC (Heteronuclear Multiple Bond Correlation) were performed without sample spinning.

7.2.3 GC/MS EXPERIMENTS

All the spectra were performed using Finnigan Mat GCQ Ion Trap spectrometer

equipped with DB-5 column with 30 m of length and 0.25 mm i.d. The carrier gas used was helium with linear velocity equal to 40 cm s^{-1} and split ratio of 1:60. The ionization energy used was of 70 eV. The GC conditions were as follows: the oven temperature varied from 60°C to 275°C with a heating rate of 10°C min^{-1}; the injector temperature was maintained at 250°C; the ion source temperature was maintained at 175°C. The samples used in the GC/MS tests were the same as in NMR analyses.

8 RESULTS AND DISCUSSION

8.1.1 COCAINE GC/MS

GC/MS was used as a reference method to confirm that the samples were cocaine. In mass spectrum obtained was observed the molecular-ion at m/z 303, the base peak ion at m/z 82 and all the other ones described by Smith (1997).[8]

8.1.2 SOLUTION ^{13}C NMR SPECTRUM OF COCAINE

Assignments of resonances in the ^{13}C CPMAS and CP/MAS and CP/MAS/DD NMR solid state spectra of cocaine were made on the basis of ^{1}H, ^{13}C, HETCOR and HMBC solution spectra. HETCOR spectrum shows heteronuclear correlations between carbon and hydrogen nuclei directly bonded (fig. 2) while HMBC spectrum shows, generally, heteronuclear correlations between carbon and hydrogen nuclei separated by three bonds (fig. 3).[9,10,11] Figure 1 shows the chemical structure of the cocaine (alkaloid form) and the numeration used to assign the NMR spectra.

8.1.2.1 CARBONYL GROUPS

Peaks at δ_C 166.1 and 170.7 ppm were assigned to the carbonyl groups. In HMBC spectrum the signal at δ_C 166.1 ppm shows correlation with the signal at δ_H 8.0 ppm assigned to an aromatic proton (fig. 3). So, chemical shift at δ_C 166.1 ppm was assigned to the carbon 12, δ_H 8.0 ppm attributed to the protons of carbon 14 and 14' and δ_C 170.7

ppm, by exclusion, was assigned to carbon 10.

8.1.2.2 Aromatic carbons

In ^{13}C NMR spectra peaks at δ_C 128.3, 129.7, 130.1 and 132.9 ppm were attributed to aromatic carbons. The signal at δ_C 130.1 ppm was assigned to carbon 13 because it did not show correlations in HETCOR (fig. 2) and, in HMBC, it shows correlation with the aromatic proton peak at δ_H 7.4 ppm (fig. 3) which shows, in HETCOR, a correlation with the chemical shift at δ_C 128.3 ppm (fig. 2). So, signals at δ_H 7.4 ppm and δ_C 128.3 ppm was assigned to protons and carbons 15 (15'). The peak at δ_C 129.7 ppm can be assigned to 14 and 14' carbons on the basis of its correlation, in the HETCOR, with the signal at δ_H 8.0 ppm already attributed to 14 and 14' protons (fig. 2). The peak at δ_C 132.9 ppm was assigned to carbon 16 because of its correlation with carbon 14 proton chemical shift at δ_H 8.0 ppm in HMBC (fig. 3), and with the peak at δ_H 7.6 ppm in HETCOR (fig. 2).

8.1.2.3 Aliphatic carbons

Peaks at δ_C 25.1, 25.4, 35.3, 41.1, 50.0, 51.5, 61.6, 64.7 and 66.7 ppm were assigned to aliphatic carbons. Singlets at δ_H 2.3 and 3.7 ppm was assigned to methyl groups of cocaine. The peak at δ_H 3.7 ppm was assigned to protons of methyl group 11, because in HMBC it shows a correlation with the peak at δ_C 170.7 ppm attributed to carbon 10 (fig. 3), by exclusion, signal at δ_H 2.3 ppm was assigned to protons of methyl group 9. In HETCOR, peaks at δ_H 2.3 and 3.7 ppm show correlation with chemical shifts at δ_C 41.1 and 51.5 ppm, respectively (fig. 2); thereby, the signal at δ_C 41.1 ppm was attributed to carbon 9 and peak at δ_C 51.5 ppm was attributed to carbon 11.

The signal at δ_H 5.2 ppm was assigned to proton 3, because, in HMBC, it shows correlations with the peak assigned to carbon 12 at δ_C 166.1 ppm (fig. 3); so, peak at δ_C 66.7 ppm was assigned to carbon 3 on the basis of its correlation with the signal at δ_H 5.2

ppm in HETCOR (fig. 2). Proton 9 peak at δ_H 2.3 ppm shows correlation with signals belonging to carbons 1 and 5 at δ_C 61.6 and 64.7 ppm in HMBC (fig. 3). Carbon 5 is more shielded than carbon 1 due to proximity of carbon 1 to the carbonyl group, thus, peaks at δ_C 61.6 and 64.7 ppm were assigned to carbon 5 and carbon 1 respectively.

In cocaine structure (fig. 1), carbons 4, 6 and 7 are methylene groups whose protons are magnetically different (diasterotopic) and, therefore, have different chemical shifts. In HETCOR spectrum three signals at δ_C 25.1, 25.4 and 35.3 ppm show correlation with two peaks (fig. 2); since carbon 6 and carbon 7 are chemically and magnetically similar, chemical shift at δ_C 35.3 ppm was assigned to carbon 4. Carbon 7 is discreetly more shielded than carbon 6, so, peaks at δ_C 25.1 and 25.4 ppm were attributed to carbon 7 and carbon 6, respectively. By exclusion, the peak at δ_C 50 ppm was assigned to carbon 2.

8.1.3 ^{13}C CP/MAS AND DD NMR OF COCAINE SAMPLES

In CP/MAS pulse sequence, time to obtain spectra can be diminished by polarization transference from more abundant species (^{1}H) to less abundant (^{13}C).[4] CP/MAS/DD NMR was undertaken to confirm assignments of quaternary and methyl carbons in ^{13}C CP/MAS spectra. Chemical shifts in ^{13}C CP/MAS and CP/MAS/DD NMR spectra of crack (fig. 4) and cocaine hydrochloride (fig. 5) samples were assigned on the basis of ^{13}C NMR assignments.

^{13}C CP/MAS NMR spectra of cocaine hydrochloride and crack samples show three different regions: at low frequency (δ_C 10-70 ppm), attributed to aliphatic carbons; between 120 and 140 ppm, attributed to aromatic carbons and between 150 and 170 ppm, attributed to carbonyl groups (Table 1). Signals attributed to spinning sidebands (SSB)[12] can also be observed (fig. 4). Because of the spinning sidebands it is advisable that the spectra of the cocaine samples should be performed with spinning greater than 4 kHz to avoid the spinning sidebands overlap with the carbonyl signals.

In the ^{13}C CP/MAS NMR spectrum of the crack sample (fig. 4), we observed only two signals in the aromatic carbons region at δ_C 124.9 and 130.0 ppm, signals of carbons

13, 14 and 15 were overlapping at δ_C 124.9 ppm, while the signal at δ_C 130.0 ppm was assigned to carbon 16. In aliphatic carbons region there are three overlap regions: carbons 1 and 3 at δ_C 62.0 ppm; carbons 2 and 9 at δ_C 36.8 ppm; and signals of carbons 6 and 7 at δ_C 21.0 ppm. In ^{13}C CP/MAS NMR spectrum of the cocaine hydrochloride samples (fig. 5) was observed a better signals splitting as compared with the spectrum of the crack sample and only one overlap of carbons 6 and 7 at δ_C 22.5 ppm was observed.

^{13}C CP/MAS/DD spectra (figs. 4 and 5) allow attributing the signals of carbonyl groups (number 10 and 12 in figure 1), substituted aromatic carbon atoms (number 13 in figure 1) and carbon atoms in methyl groups (number 9 and 11 in figure 1). The chemical shift of these nuclei and others present in the chemical structure of the cocaine are shown in the Table 1.

Solid-state NMR spectroscopy supplies information about the crystalline structure of samples.[6] Cocaine hydrochloride and crack are different in their intermolecular interactions and solid-state configuration. The first is crystalline, while the second is amorphous,[1] it justifies the differences observed in the ^{13}C CP/MAS spectra and allow the distinction between crack and cocaine hydrochloride by a non-destructive technique that can be important in a investigative process.

8.2 Conclusion

The presented results show that CP/MAS and CP/MAS/DD can be used as a very powerful and non-destructive tool for characterization and distinction of crack and cocaine hydrochloride for forensic purposes, since the operation is fast, easy and reproducible.

8.3 ACKNOWLEDGMENTS

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Table 1 - Assignments of the peaks in ^{13}C NMR spectra of cocaine samples.

Carbon number (figure 1)	Crack		Cocaine Hydrochloride	
	^{13}C	^{13}C CP/MAS	^{13}C	^{13}C CP/MAS
1	64.7	62.0	63.9	62.0
2	50.0	45.5	46.1	44.6
3	66.7	62.0	64.5	63.9
4	35.3	32.0	32.7	29.9
5	61.6	58.0	63.2	59.9
6	25.4	21.0	23.8	22.5
7	25.1	21.0	22.7	22.5
9	41.1	36.8*	39.0	38.1*
10	170.7	167.5*	173.3	166.5*
11	51.5	45.5*	53.4	50.8*
12	166.1	161.4*	167.2	163.3*
13	130.1	124.9*	128.5	126.4*
14	129.7	124.9	129.6	128.8/129.7
15	128.3	124.9	129.0	127.7
16	132.9	130.0	134.5	131.1

**Confirmed by DD NMR spectrum*

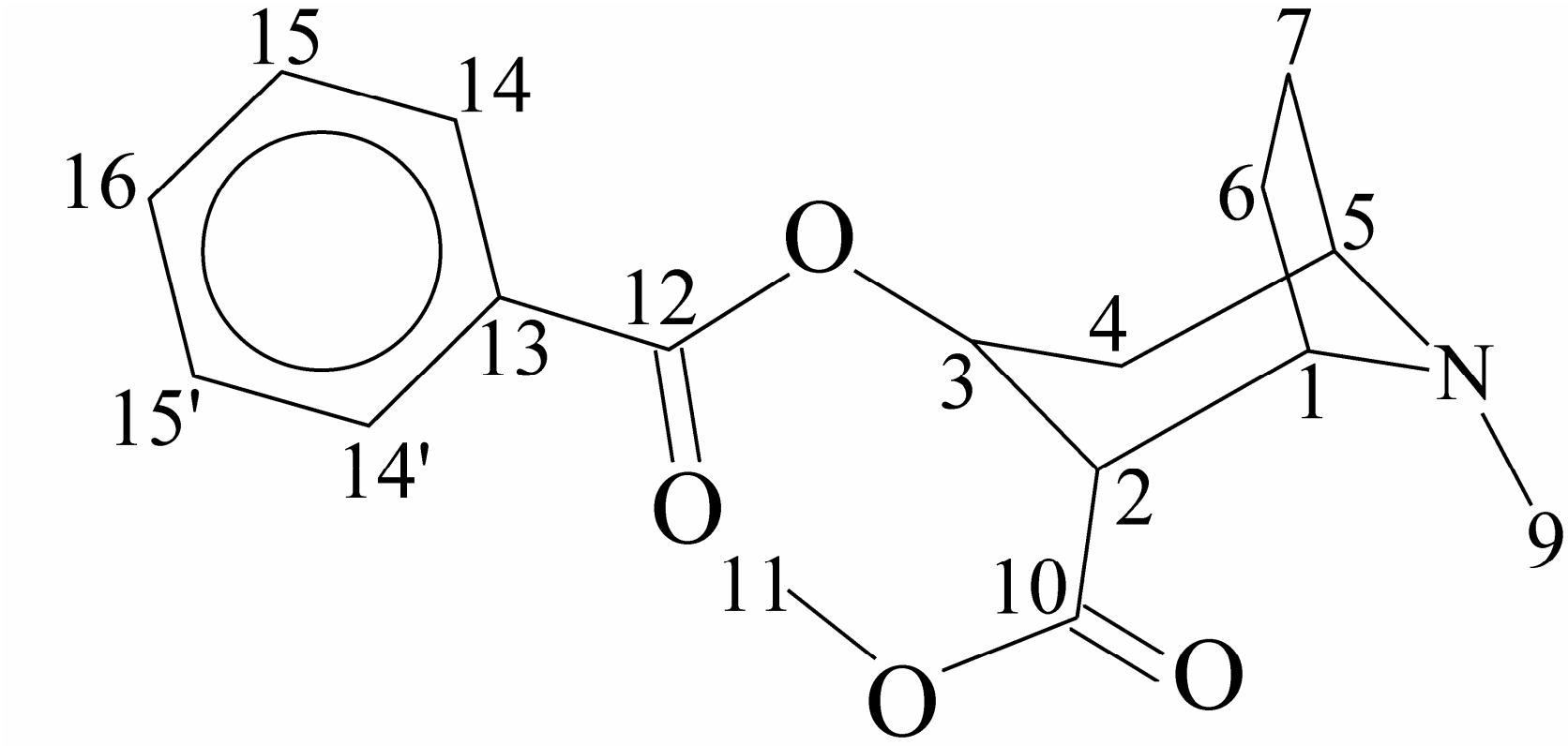

Figure 1 - Cocaine alkaloid form structure and numbering used in assignments.

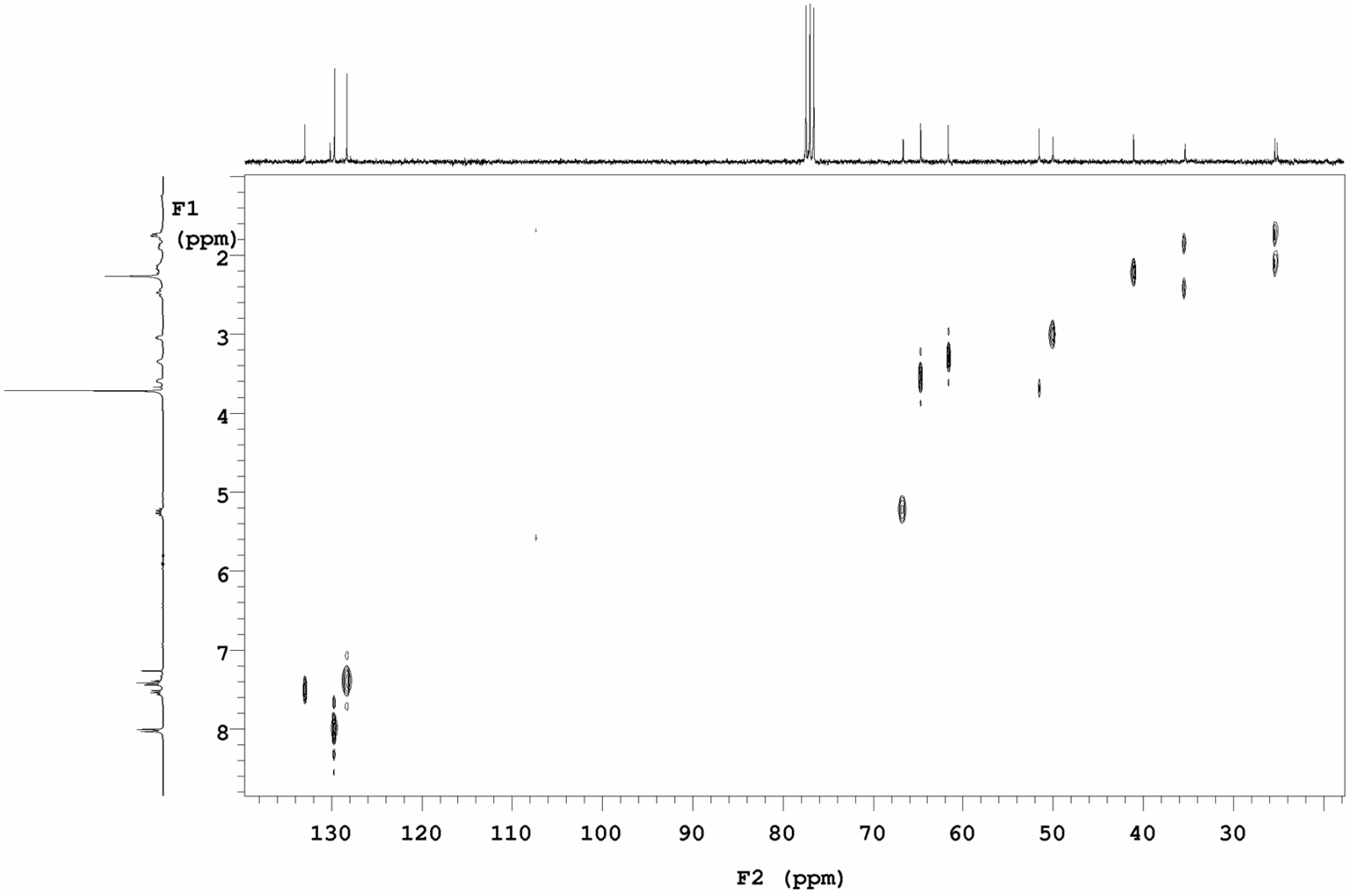

Figure 2 - HETCOR spectrum of cocaine in $CDCl_3$, 20°C, ^{13}C – 75 MHz and ^{1}H – 300 MHz.

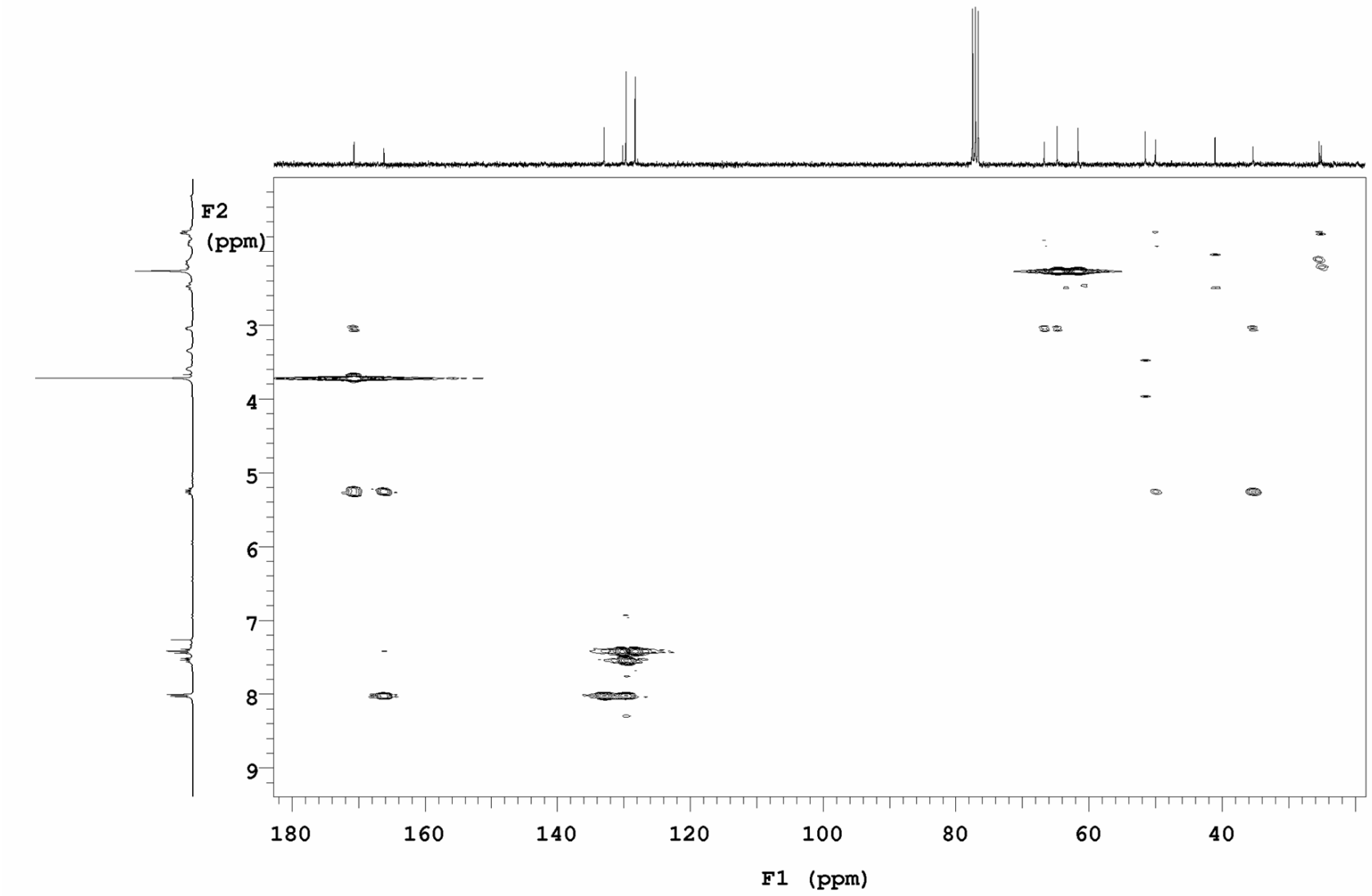

Figure 3 - HMBC spectrum of cocaine in $CDCl_3$, 20°C, ^{13}C – 75 MHz and ^{1}H – 300 MHz.

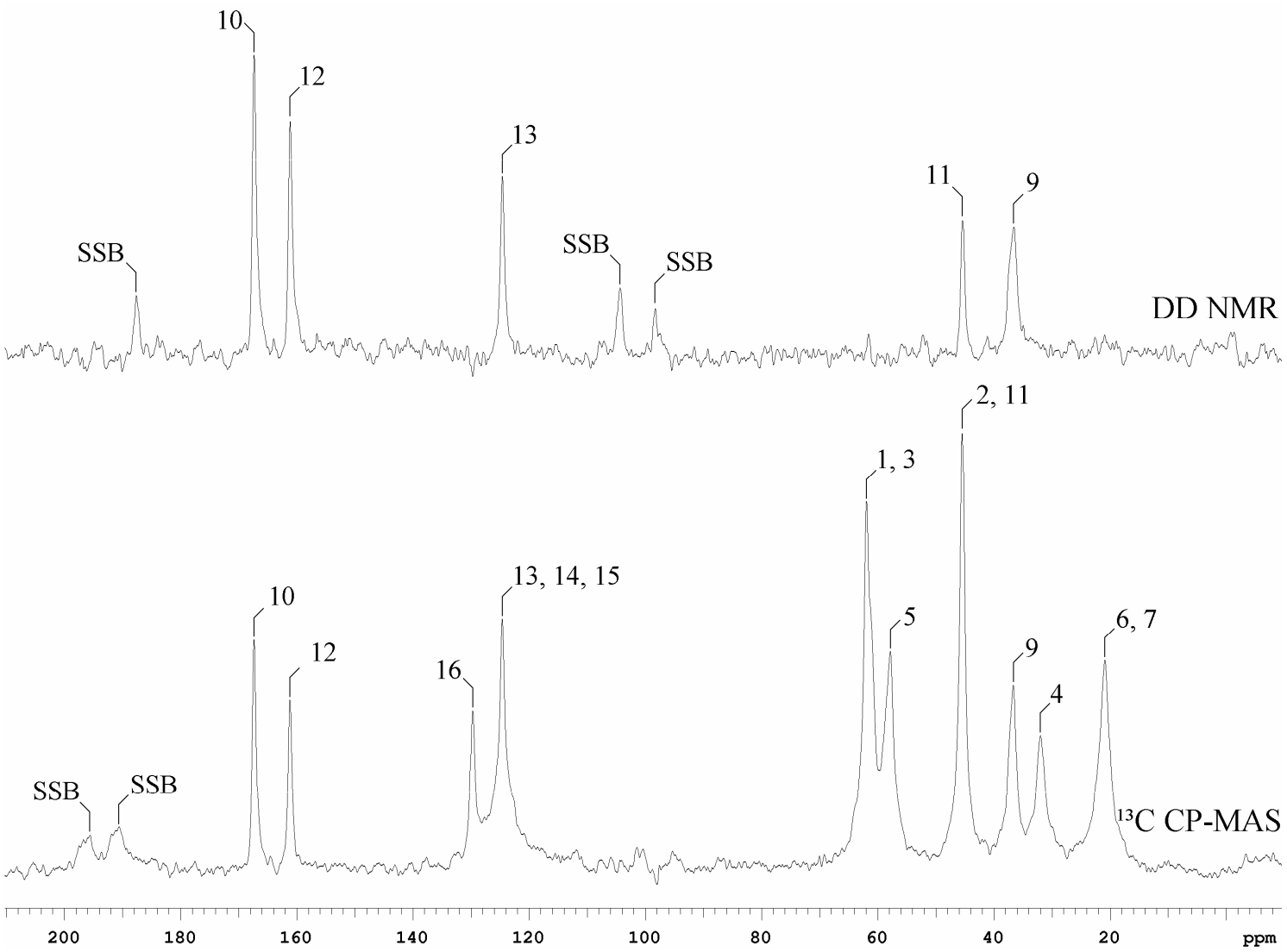

Figura 4 – ^{13}C CP-MAS and DDNMR spectra of crack, 20°C, spinning 4-5 KHz, 75 MHz.

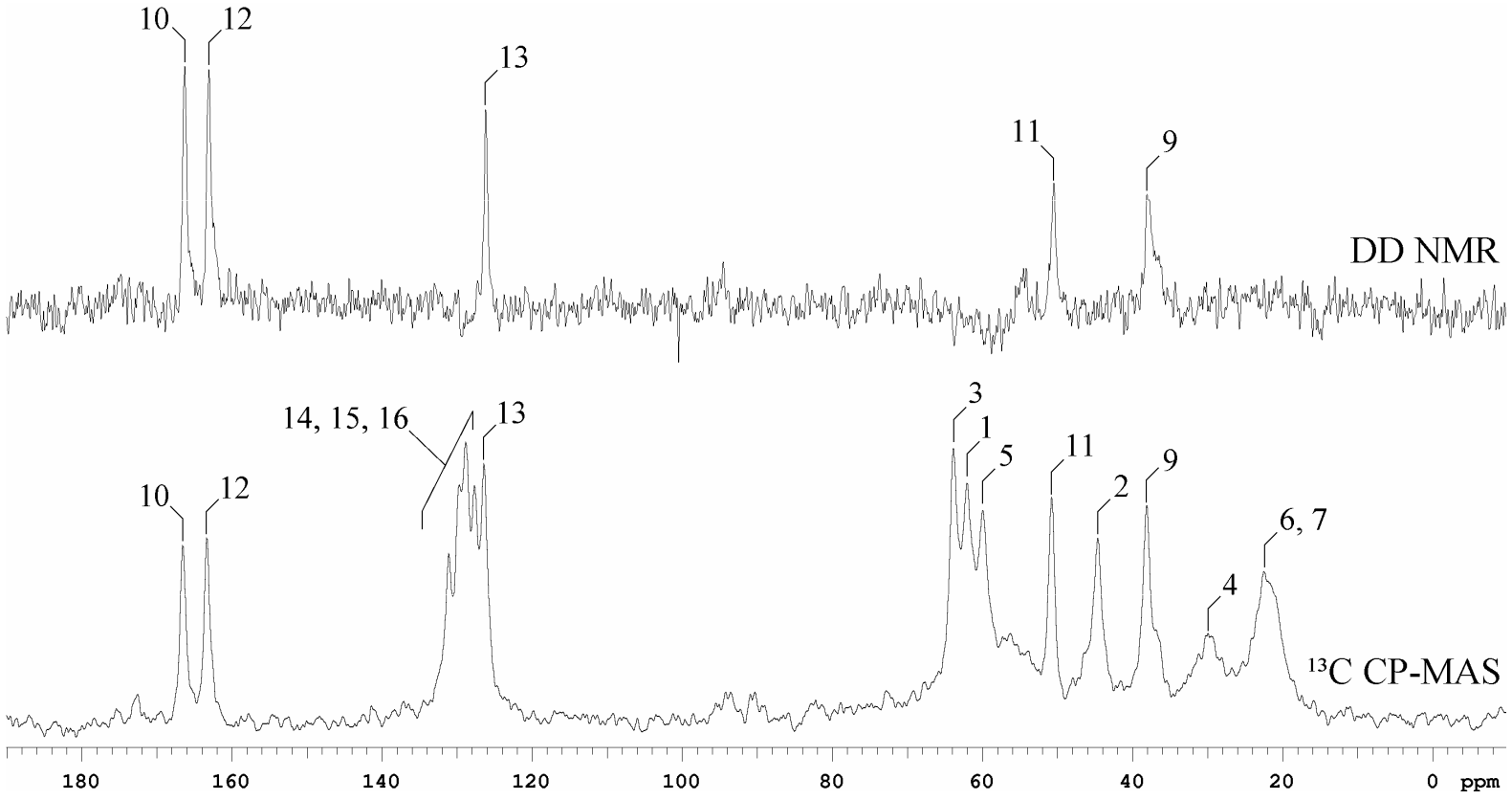

Figure 5 - ^{13}C CP-MAS and DD NMR spectra of cocaine hydrochloride, 20°C, spinning 4-5 KHz, 75 MHz.

Anexos

9 INSTRUÇÕES PARA AUTORES

9.1 FORENSIC SCIENCE INTERNATIONAL

FORENSIC SCIENCE INTERNATIONAL

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C. Cattaneo Osteology and Anthropology

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E-mail: cristina.cattaneo@unimi.it

P. Margot Questioned Documents (with the assistance of A. Khanmy aita.khanmy-vital@unil.ch and W. Mazzella williams-mazzella@unil.ch) and Physical Science: ballistics, tool marks, contact traces, drugs analysis, fingerprints and identification, etc.

Tel: +41 21 692 4605

Fax: +41 21 692 4605

E-mail: pierre.margot@unil.ch

O.H. Drummer Toxicology

Tel: +61 3 9684 4334

Fax: +61 3 9682 7353

E-mail: olaf@vifm.org

G. Willems Odontology

Tel: +32 16 33 24 59

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12. http://jbcs.sbq.org.br/jbcs/2002/v13_n5/09-supplementary.pdf, accessed in January 2004.

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