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Nuclear Magnetic Resonance and Molecular Orbital Study of Some Cocaine Analogues

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Abstract: ^{1}H NMR spectra of (-)-cocaine and some of its derivatives (α-CPT, β-CPT, nor-β-CIT, cocaine-HCl and ecgonine-HCl) were analysed and the spectral parameters were used for conformational analysis of the compounds in conjunction with theoretical HF/6-31G*, MMP2, AM1 and molecular dynamics calculations. Comparison of the experimental and theoretical data reveals that the compounds are predominantly in a rigid chair conformation, which is rather similar for all compounds. No large differences were found in the dynamical behaviour of the molecules. The performance of the Haasnoot and Altona equations is discussed. © 1999 Elsevier Science Ltd. All rights reserved.

Key words: cocaine analogues, conformation, molecular modelling, NMR.

INTRODUCTION

Computational methods and NMR are common tools used in the conformational analysis of small compounds. Molecular orbital and molecular mechanical methods normally yield good estimates of bond lengths and angles, at least with respect to biological function. Molecular dynamics gives estimates for the magnitude of dynamic motions, whose relationship with biological activity is of great interest.[1] However, these methods are less reliable in the estimation of the dihedral angles and conformational energetics, especially in the presence of solvent. In this case NMR is the only method which can produce reliable results.

The most important coupling constants in structure determinations are $^3J_{HH}$-couplings, although occasionally long-range couplings can also provide useful information. However, for large spin-systems, the complexity of spectra may cause serious problems in the coupling constant analysis. In recent years, powerful tools for the analysis of NMR spectra have been developed in our laboratory[2] and the full analysis of spectra has become feasible also for large spin-systems. One aim of this work was to test a strategy based on a combination of NMR spectral analysis and computational tools to achieve a complete conformational characterisation of cocaine and its analogues.

(-)-Cocaine binds with high affinity to monoamine transporters in the brain. These transporters have been linked to Alzheimer's disease,[3] alcoholism[4] and Parkinson's disease.[5, 6] Diagnostics, disease evolution and

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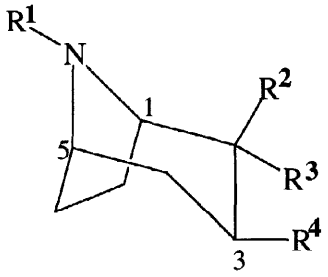
therapeutic effects of treatments can be followed *in vivo* by brain scanning methods using cocaine analogues as radioligands. There are different types of neurotransmitter transporters in the brain and for diagnostic purposes a radioligand with high selectivity for the individual transporters would be desirable. The selectivity can be influenced by substitution on the tropane ring.[7] Another aim of this work was to study the conformation of the tropane ring of cocaine and its analogues and the possible effects, including dynamic effects, of different substituents on the peripheral parts of the ring that are usually assumed to be unaffected by the substitution. In this work we characterised the structural properties of α-CPT (2α-carbomethoxy-3β-phenyltropane), β-CPT (2β-carbomethoxy-3β-phenyltropane), nor-β-CIT (2β-carbomethoxy-3β-(iodophenyl)tropane), (-)-cocaine, (-)-cocaine-HCl and ecgonine-HCl. The chemical shifts and coupling data provide a useful database for ^{1}H NMR analysis of other tropane ring derivatives.



	R^1	R^2	R^3	R^4
α-CPT	$-CH_3$	-H	$-COOCH_3$	$-C_6H_5$
β-CPT	$-CH_3$	$-COOCH_3$	-H	$-C_6H_5$
Nor-β-CIT	-H	$-COOCH_3$	-H	$-C_6H_4I$
(-)-Cocaine	$-CH_3$	$-COOCH_3$	-H	$-OC(O)C_6H_5$
Ecgonine	$-CH_3$	-COOH	-H	-OH

EXPERIMENTAL

Spectral analysis

(-)-Cocaine and cocaine-HCl were purchased from commercial suppliers. α-CPT, β-CPT and nor-β-CIT were synthesised using an established method[8] and purified by TLC. Ecgonine was synthesised as its hydrochloride.[9] Samples were dissolved in benzene-d_6, except ecgonine-HCl and (-)-cocaine-HCl which were dissolved in CD_3OD (concentration of cocaine, β-CPT and nor-β-CIT approx. 5 mM, concentration of the rest of the samples approx. 20 mM). Samples were filtered and degassed using a freeze-pump-thaw technique. ^{1}H NMR spectra were measured at 303 K by a Bruker AM 400 WB-spectrometer using TMS as the internal reference.

Preparation and analysis of the spectra were made with PERCH software.[2] FIDs were multiplied with sin*exp window function, Fourier transformed, base line corrected, and minor impurity and solvent signals were removed. The spectra were solved first by the integral transform method,[2] after which the solutions were refined by the total-line shape procedure. Dihedral angles were estimated with the Haasnoot[10] and Altona[11] equations using the graphical interface of the PERCH software

Computational methods

MO calculations were performed at the semi-empirical AM1[12] and *ab initio* HF/6-31G* and HF/3-21G levels[13] (the latter basis set was used for nor-β-CIT, since 6-31G* parameterisation for iodine is missing) employing the AMPAC (QCPE No. 506, ver. 2.1) and GAUSSIAN94 (version RevD.3) program packages[13] running on an IBM RISC/6000 320 workstation. All the geometric variables were completely optimised for each compound. Molecular dynamics were calculated by HyperChem™ software.

RESULTS AND DISCUSSION

Previous computations for cocaine and its diastereomers indicate that even the semi-empirical AM1[14] or molecular mechanical MMP2[15] descriptions are in good accordance with the X-ray structures. In this study, the geometries were optimised at the intermediate level of *ab initio* MO theory. The different methods give very similar results with the X-ray analysis of (-)-cocaine: no distance in the tropane ring has a deviation larger than 0.02 Å, and the largest deviation observed for the angles is only 2° (Table 1). However, for the substituents, in particular C-O distances and O-C=O angles, deviations are clearly larger up to 0.06 Å and 6°, respectively. For a detailed analysis of the conformational behaviour of the parent compound cocaine, see refs. (14-17).

Table 1. Geometrical Features of Cocaine as Calculated from the X-ray[a], MM2[a], AM1 and HF/6-31G* Data.

Bond	X-ray[a]	MM2[a]	AM1	HF/6-31G*	**Bond**	X-ray[a]	MM2[a]	AM1	HF/6-31G*
C(1)-C(2)	1.554	1.549	1.549	1.547	C(5)-N	1.487	1.467	1.484	1.464
C(1)-C(7)	1.562	1.542	1.554	1.543	C(6)-C(7)	1.556	1.541	1.532	1.549
C(1)-N	1.503	1.466	1.482	1.460	C(R^2)-O	1.291	1.361	1.374	1.355
C(2)-C(3)	1.558	1.547	1.539	1.535	C(R^2)=O	1.250	1.210	1.230	1.204
C(2)-C(R^2)	1.508	1.530	1.503	1.519	O-CH_3	1.432	1.418	1.426	1.452
C(3)-C(4)	1.524	1.540	1.531	1.525	O(R^4)-C	1.392	1.366	1.371	1.322
C(3)-O(R^4)	1.385	1.423	1.435	1.421	C-C(Ph)	1.494	1.365	1.470	1.490
C(4)-C(5)	1.554	1.542	1.537	1.535	C(R^4)=O	1.172	1.212	1.235	1.194
C(5)-C(6)	1.525	1.541	1.555	1.550	Av.dev.[b]	0.000	0.031	0.024	0.026
Dihedral[c]		MM2[a]	AM1	HF/6-31G*	**Dihedral**[c]		MM2[a]	AM1	HF/6-31G*
C(1)-C(2)-C(3)-C(4)		-45.6	-42.8	-47.4	C(3)-C(2)-C(1)-N		63.2	60.0	63.1
C(1)-C(7)-C(6)-C(5)		-0.7	-0.3	-1.9	C(3)-C(4)-C(5)-C(6)		54.5	54.7	54.1
C(2)-C(1)-C(7)-C(6)		90.1	90.8	91.2	C(3)-C(4)-C(5)-N		-59.5	-60.7	-60.6
C(2)-C(3)-C(4)-C(5)		44.1	43.2	46.4	C(2)-C(1)-N-CH_3		162.5	161.5	159.0
C(3)-C(2)-C(1)-C(7)		-51.5	-55.1	-52.2					
Angle	X-ray[a]	MM2[a]	AM1	HF/6-31G*	**Angle**	X-ray[a]	MM2[a]	AM1	HF/6-31G*
C(2)-C(1)-C(7)	112.5	112.6	109.0	112.0	C(6)-C(7)- C(1)	104.0	104.0	104.2	103.7
C(2)-C(1)-N	109.1	107.9	107.6	106.7	C(1)-N-C(5)	103.6	102.0	101.0	102.2
C(7)-C(1)-N	101.1	104.3	106.5	105.7	C(1)-N-CH_3	112.4	113.0	113.9	114.4
C(1)-C(2)-C(3)	109.1	110.1	109.7	108.8	C(5)-N-CH_3	112.5	113.0	114.0	114.5
C(1)-C(2)-C(R^2)	109.3	109.9	109.5	108.9	C(2)-C(R^2)=O	122.3	125.4	130.8	127.0
C(3)-C(2)-C(R^2)	114.7	113.9	113.0	113.8	O-C(R^2)=O	121.4	123.8	117.6	122.7
C(2)-C(3)-C(4)	112.8	110.6	113.7	112.1	C(2)-C(R^2)-O	115.9	110.7	111.7	110.3
C(2)-C(3)-O(R^4)	114.5	113.0	110.2	112.7	C(R^2)-O-CH3	118.1	116.3	116.6	116.9
C(4)-C(3)-O(R^4)	108.3	111.1	105.7	108.3	C(3)-O(R^4)-C	117.4	117.9	118.2	119.0
C(3)-C(4)-C(5)	110.4	111.7	110.0	110.0	O(R^4)-C-C(Ph)	111.9	113.5	113.1	112.6
C(4)-C(5)-C(6)	112.1	111.3	109.6	111.9	O(R^4)-C=O	122.7	122.1	118.6	123.6
C(4)-C(5)-N	108.0	108.4	107.7	107.6	C(Ph)-C=O	125.4	124.4	128.4	123.8
C(6)-C(5)-N	102.3	104.5	106.4	104.7	Av.dev.[b]	0.000	1.508	2.412	1.562
C(5)-C(6)-C(7)	105.8	103.6	104.0	103.5					

[a] X-ray and MM2/MMP2 values taken from ref. 17. [b] Average deviation = $\sum_{i=1}^{n} \left| x_i - x_i(X\text{-}ray) \right| / n$. [c] No X-ray data.

In the tropane system almost all of the protons are coupled with each other. However, most of the couplings are smaller than the line-width, about 0.25 Hz for β-CPT and 0.4 -1.4 Hz for the others. In general, if there are many couplings of the order of line-width, the couplings only broaden the spectral signals and also the fine structure arising from larger couplings may disappear. This means that the coupling information correlates with

the line width information: a good fit can be obtained with many parameter combinations, especially if the line widths are optimised independently for each proton, as done here. However, our experience suggests that in using the total-line-shape fitting, the values of the couplings yielding well-defined splittings are accurate even if some long-range couplings may have incorrect values.

A detailed long-range coupling analysis was performed only for β-CPT. The trial signs of the couplings were adapted from the analysis of tropinone[18] (this analysis also yields, due to symmetry, the relative signs of the couplings) or presumed on the basis of general rules. For example, the five-bond couplings were assumed to be positive. The effects of the signs were then tested by total-line-shape fitting. The values of β-CPT were used as starting values for the other compounds. For the above reasons, the values and confidence limits of the couplings which are smaller than the line width should be viewed with some caution. Fortunately, all the values of conformationally useful couplings are accurate and do not depend significantly on those of the small long-range couplings.

The dihedral angles calculated by *ab initio* HF/6-31G*-method and those obtained by the Altona and Haasnoot equations are compared in Figure 1 and Table 2 (for cocaine-HCl only dihedral angles from empirical equations are given). In general, the fits are good: standard deviations are 6.6° and 11.9° (r = 0.994, r = 0.984) for the Haasnoot and Altona equations, respectively. The fits are poor when the absolute value of the dihedral angle is less than 30°. This observation can be accounted by the nature of the coupling function: the value of the function is not sensitive to the dihedral angle around 0°. Also the couplings for the protons in the vicinity of the substituents give angles deviating clearly from the calculational values and from each other in different compounds (for example angles 1- R^2/R^3); the finding implies that the empirical equations fail to predict small conformational variations between compounds with different substituents. On the other hand, the couplings of protons 4, 5, 6 and 7 with each other vary to a much lesser degree (Table 3) and their trends agree with the calculated trends.

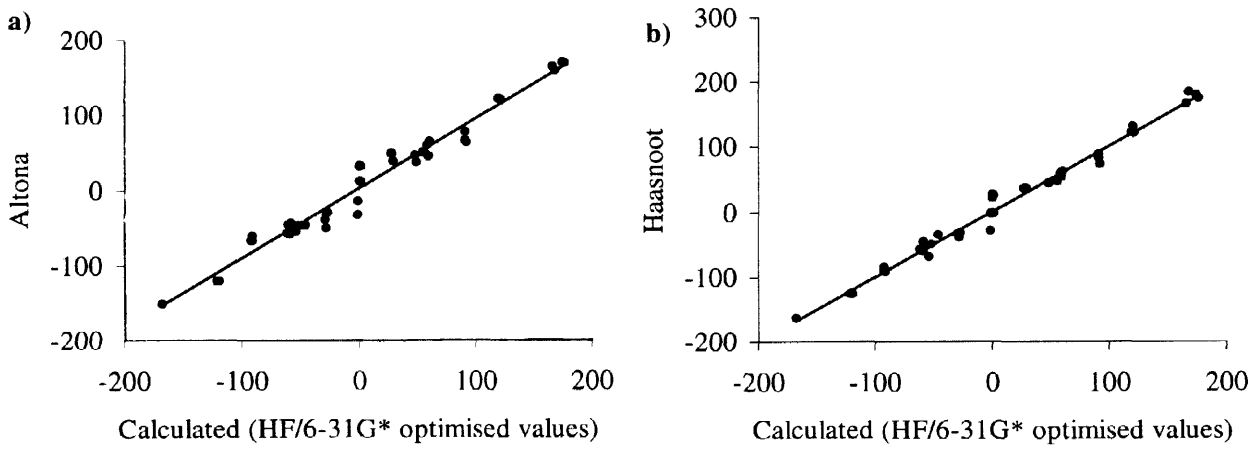


Figure 1. Dihedral angles obtained by the Altona and Haasnoot equations vs. HF/6-31G* optimised angles.

In general, the results indicate that the substituents do not perturb in any significant manner the remote parts of the system. There is one conformationally important observation, the values of $^3J(3,4a)$ and $^3J(3,4b)$ are close to the values predicted by the equations: which means that there is virtually no any other conformation present in the system, at least not within a free energy of 9 -10 kJ/mol (assuming $N_{boat} / N_{chair} = e^{-\Delta G/RT}$).

The dihedral angles indicate that variations in the tropane ring are rather small, with the exception of ecgonine. The only noticeable variation is in the tropane ring chair angle, i.e. the angle between planes C(2)-C(3)-

C(4) and C(1)-C(5)-C(2)-C(4) (Figure 2). Nor-β-CIT has the smallest angle and ecgonine has the largest, as indicated by the dihedral angles of proton 3. For ecgonine and nor-β-CIT, the C(7)C(1)C(2) moiety is slightly bent, as indicated by the 1-R^3 and 4b-5 dihedral angles. The clear bending of ecgonine is obviously due to the effect of a hydrogen bond type interaction between the hydroxyl and carboxylic acid groups. The lack of methyl group on the nitrogen enables the bending of nor-β-CIT, but the direction is opposite to ecgonine. On the other hand, the C(6)-C(7) bridge is highly symmetrical for all of the compounds.

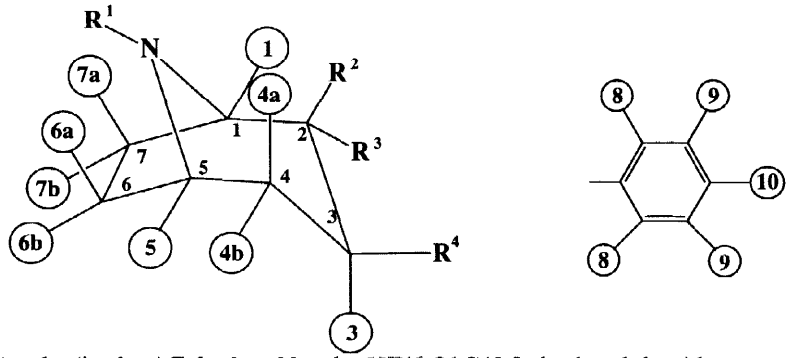


Table 2. Dihedral Angles(in deg.)Calculated by the HF/6-31G*Method and the Altona and Haasnoot Equations.

Dihedral	α-CPT[a]			β-CPT[a]			Nor-β-CIT[a]		
$Angle_{HH}$	HF/6-31G	Altona	Haas-noot	HF/6-31G	Altona	Haas-noot	HF/6-31G	Altona	Haas-noot
1-R^2/R^3	60	65	64	-59	-58	-59	-54	-54	-68
1-7a	-28	-50	-38	-28	-30	-33	-29	-39	-32
1-7b	91	67	90	91	66	85	91	78	84
R^2/R^3-3	-168	-151	-163	-52	-47	-48	-58	-43	-45
3-4a	166	165	168	174	170	181	176	169	176
3-4b	48	47	46	55	51	50	56	50	48
4a-5	-58	-46	-57	-59	-46	-57	-61	-56	-57
4b-5	59	46	57	59	45	55	58	60	60
5-6a	27	49	37	28	49	38	29	39	38
5-6b	-92	-67	-90	-91	-67	-90	-91	-60	-91
6a-7a	1	12	0	-1	14	0	0	12	0
6a-7b	-120	-120	-124	-120	-120	-125	-121	-120	-125
6b-7a	120	122	133	119	122	124	120	121	123
6b-7b	0	31	23	-1	32	28	0	33	28
Dihedral	Ecgonine[b]			Cocaine[a]			Cocaine-HCl[b]		
$Angle_{HH}$	HF/6-31G	Altona	Haas-noot	HF/6-31G	Altona	Haas-noot	HF/6-31G[c]	Altona	Haas-noot
1-R^2/R^3	-66	-55	-64	-59	-58	-58	-	-55	-64
1-7a	-27	-35	-31	-27	-29	-31	-	-35	-30
1-7b	92	78	79	92	64	75	-	77	77
R^2/R^3-3	-38	-32	-25	-46	-46	-34	-	-38	-25
3-4a	164	160	182	168	159	186	-	158	186
3-4b	47	48	47	49	37	46	-	36	45
4a-5	-60	-52	-57	-60	-45	-57	-	-52	-58
4b-5	59	52	56	59	45	55	-	51	55
5-6a	28	42	32	27	49	37	-	42	35
5-6b	-92	-58	-82	-92	-66	-84	-	-60	-90
6a-7a	0	15	0	1	12	0	-	15	0
6a-7b	-120	-120	-123	-119	-120	-125	-	-120	-125
6b-7a	120	122	124	121	121	123	-	123	124
6b-7b	0	28	22	1	32	27	-	28	22

[a] Angles from Altona and Haasnoot equations calculated in $CDCl_3$. [b] Angles from Altona and Haasnoot equations calculated in D_2O. [c] *Ab initio* HF/6-31G* data not available.

Table 3. Coupling Constants[a] (Hz) of α-CPT, β-CPT[b, c], Nor-β-CIT, (-)-Cocaine, (-)-Cocaine-HCl and Ecgonine-HCl.[d]

$^{n}J(i,j)$	α-CPT	β-CPT[b, c]	Nor-β-CIT	Cocaine	Cocaine-HCl	Ecgonine-HCl
$^{4}J(1,3)$	-0.54	-0.42 [1]	-0.47	-0.47	0.00	-0.31
$^{5}J(1,4a)$	0.06	(+)0.17 [1]	0.07	0.44	0.23	0.25
$^{5}J(1,4b)$	0.28	0.41 [1]	0.58	0.01	0.61	0.31
$^{4}J(1,5)$	0.98	1.44 [1]	0.71	1.71	1.37	1.29
$^{4}J(1,6a)$	-0.03	(-)0.01 [1]	-0.59	-0.15	0.00	0.00
$^{4}J(1,6b)$	-0.46	-0.45 [1]	-0.65	-0.48	-0.51	-0.56
$^{3}J(1,7a)$	6.87	7.10 [0]	7.08	7.46	7.60	7.55
$^{3}J(1,7b)$	0.68	0.73 [1]	0.48	0.84	0.82	0.74
$^{3}J(1,R^{2}/R^{3})$	2.78	3.33 [0]	2.24	3.44	2.60	2.58
$^{3}J(3,4a)$	12.43	12.90 [1]	12.85	11.75	11.77	11.45
$^{3}J(3,4b)$	5.87	4.87 [1]	5.07	6.03	6.25	6.08
$^{4}J(3,5)$	-0.60	-0.71 [1]	-0.81	-0.57	-0.59	-0.48
$^{5}J(3,6a)$	0.15	(+)0.10 [1]	0.67	0.00	0.00	0.67
$^{5}J(3,6b)$	0.16	(+)0.33 [1]	0.41	0.14	0.48	0.00
$^{5}J(3,7a)$	0.28	(+)0.30 [2]	0.37	0.36	0.21	0.05
$^{5}J(3,7b)$	-0.15	(-)0.28 [2]	-0.04	-0.37	-0.17	-0.43
$^{4}J(3,8)$	-0.34	-0.88 [0]	-0.68	-	-	-
$^{5}J(3,9)$	< 0.30	0.29 [0]	0.26	-	-	-
$^{6}J(3,10)$	>-0.20	-0.56 [0]	-	-	-	-
$^{3}J(3,R^{2}/R^{3})$	11.71	5.17 [1]	5.84	5.82	7.28	7.05
$^{2}J(4a,4b)$	-13.08	-12.04 [1]	-13.10	-11.68	-14.29	-14.37
$^{3}J(4a,5)$	3.02	3.05 [1]	3.13	3.08	2.91	3.03
$^{4}J(4a,6a)$	1.20	1.04 [1]	1.04	1.22	1.26	1.15
$^{4}J(4a,6b)$	0.53	(+)0.11 [2]	0.56	0.64	0.04	0.10
$^{5}J(4a,7a)$	0.17	(+)0.19 [2]	0.42	0.14	0.00	0.00
$^{5}J(4a,7b)$	-0.24	(-)0.11 [2]	-0.01	-0.04	-0.01	0.00
$^{4}J(4a,R^{2}/R^{3})$	-0.36	-0.56 [1]	-0.46	-0.33	-0.57	-0.32
$^{3}J(4b,5)$	2.95	3.34 [0]	2.56	3.33	3.38	3.18
$^{4}J(4b,6a)$	-0.39	-0.28 [1]	-0.90	-0.36	-0.61	-1.15
$^{4}J(4b,6b)$	-0.27	(-)0.18 [1]	-0.27	-0.27	-0.02	-0.58
$^{5}J(4b,7a)$	-0.18	(-)0.11 [1]	-0.43	-0.12	-0.32	-0.46
$^{5}J(4b,7b)$	0.21	(+)0.25 [1]	0.12	0.23	0.01	0.00
$^{4}J(4b,R^{2}/R^{3})$	-0.50	1.32 [0]	0.91	1.27	0.98	0.48
$^{5J}(5,R^{2}/R^{3})$	-0.28	0.41 [1]	0.67	0.41	0.40	0.87
$^{3}J(5,6a)$	6.91	6.76 [1]	6.92	6.91	7.31	7.40
$^{3}J(5,6b)$	0.62	0.64 [1]	0.44	0.63	0.41	0.91
$^{4}J(5,7a)$	0.12	(+)0.09 [1]	0.30	0.07	0.01	0.07
$^{4}J(5,7b)$	-0.56	-0.50 [1]	-0.33	-0.60	-0.44	-0.33
$^{2}J(6a,6b)$	-12.82	-13.03 [0]	-12.49	-13.12	-14.45	-14.35
$^{3}J(6a,7a)$	12.64	12.50 [0]	12.66	12.66	12.37	12.32
$^{3}J(6a,7b)$	4.66	4.75 [0]	4.74	4.79	4.71	4.71
$^{5}J(6a,R^{2}/R^{3})$	0.15	(+)0.05 [2]	0.15	0.07	0.04	0.42
$^{3}J(6b,7a)$	4.71	4.59 [0]	4.51	4.44	4.70	4.64
$^{3}J(6b,7b)$	9.75	9.52 [0]	9.39	9.57	10.31	10.36
$^{5}J(6b,R^{2}/R^{3})$	0.19	(+)0.10 [3]	0.50	0.60	0.40	0.71
$^{2}J(7a,7b)$	-13.32	-13.44 [0]	-12.91	-13.61	-14.85	-14.66
$^{5}J(7a,R^{2}/R^{3})$	0.57	(-)0.00 [3]	-0.38	-0.22	-0.01	-0.20
$^{5}J(7b,R^{2}/R^{3})$	-0.37	(-)0.27 [1]	-0.01	-0.17	-0.29	0.00

[a] Coupling constants of aromatic protons are available from authors. [b] Optimised signs without closures. [c] 90% Confidence limits in brackets.
[d] For rms values see table 5.

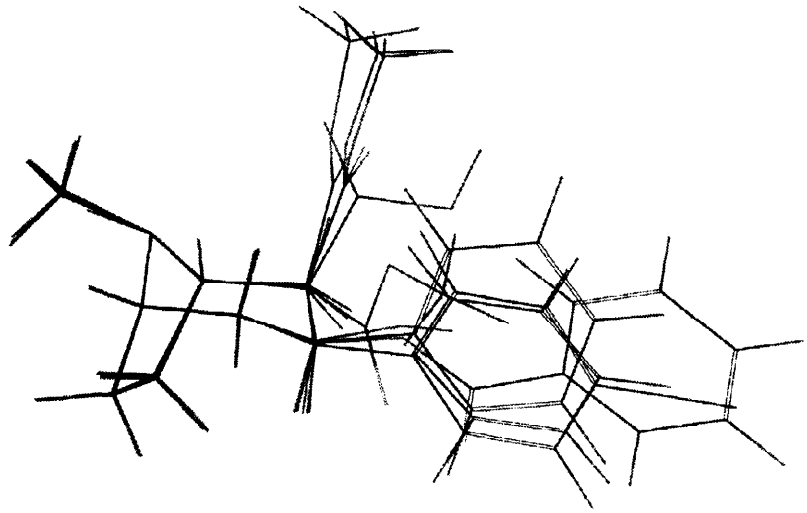


Figure 2. Superposition of HF/6-31G* optimised structures of nor-β-CIT, β-CPT, (-)-cocaine, α-CPT and ecgonine. The figure is adapted from a computer-generated image produced by the SYBYL (Tripos. Associates, Inc.) program package.

Although the minimum energy structures may be similar to each other, there might be some differences when their dynamic behaviour is taken into consideration; for example due to the steric interactions with the substituents. As the first result of our MD simulations, the motions in the C(6)–C(7) bridge were observed to be surprisingly large (Table 4): the amplitude of the motions (2 × rms) was approx. 14 degrees. On the other hand, the variation between the compounds for the dihedral angles 6a-7a and 6b-7b varied only from 6.5° to 7.1°. Furthermore there was no significant difference in the correlation of the motions; the 6a-7a and 6b-7b torsions were, as expected, rather highly correlated.

Table 4. Molecular Dynamics Results: the Average Angles and Their Rms Values[a] (in parenthesis).

Angle	α-CPT	β-CPT	Nor-β-CIT	Cocaine	Ecgonine
H(1)-H(7a)	-28.66 (5.78)	-29.33 (5.74)	-29.12 (5.90)	-29.26 (5.81)	-29.46 (5.93)
H(1)-(7b)	93.04 (5.80)	92.24 (5.67)	92.67 (6.01)	92.39 (5.71)	92.26 (5.94)
H(1)-C(5)	162.34 (3.72)	162.29 (3.68)	163.23 (3.67)	162.26 (3.69)	163.11 (3.87)
H(5)-H(6a)	29.64 (5.76)	29.43 (5.85)	28.92 (6.05)	29.56 (5.86)	29.35 (5.85)
H(5)-H(6b)	-92.10 (5.80)	-92.24 (5.81)	-92.92 (6.11)	-92.20 (5.81)	-92.30 (5.89)
H(5)-C(1)	-163.13 (3.72)	-163.04 (3.88)	-163.95 (3.75)	-163.09 (3.77)	-163.11 (3.87)
H(6a)-H(7a)	0.05 (6.57)	0.32 (6.67)	0.45 (7.08)	0.17 (6.72)	0.27 (6.86)
H(6a)-H(7b)	-120.83 (6.57)	-120.94 (6.54)	-121.06 (6.87)	-121.13 (6.59)	-121.72 (6.72)
H(6b)-H(7a)	121.52 (6.51)	121.73 (6.49)	122.12 (7.00)	121.61 (6.67)	120.99 (6.77)
H(6b)-H(7b)	0.65 (6.61)	0.47 (6.58)	0.62 (6.99)	0.31 (6.72)	0.46 (6.85)
H(4a)-H(4b)	108.07 (3.21)	108.21 (3.21)	108.24 (3.21)	107.97 (3.26)	108.01 (3.23)
H(6a)-H(6b)	109.22 (3.14)	109.18 (3.24)	109.38 (3.27)	109.23 (3.20)	109.20 (3.27)
H(7a)-H(7b)	109.02 (3.06)	109.11 (3.13)	109.32 (3.29)	109.16 (3.21)	109.15 (3.39)
C(3217)	-53.55 (4.34)	-57.22 (3.23)	-57.22 (3.23)	-57.41 (4.43)	-57.42 (4.56)
C(3456)	56.67 (4.44)	55.16 (4.39)	54.99 (4.49)	56.14 (4.53)	56.03 (4.48)
$Angle_{HH}/Angle_{HH}$			Correlation[b]		
6a-7a/6b-7b	0.64	0.62	0.65	0.63	0.62
1-7a/5-6a	0.27	0.27	0.31	0.31	0.29

[a] $\text{Rms} = \sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2 \Big/ n-1}$, [b] $\text{Correlation} = \Sigma(\varphi_i - \bar{\varphi}_i)(\varphi_j - \bar{\varphi}_j) \Big/ \sqrt{\Sigma(\varphi_i - \bar{\varphi}_i)^2 \Sigma(\varphi_j - \bar{\varphi}_j)^2}$

The long-range couplings between the proton 3 and phenyl protons provide information about the torsional freedom of the phenyl ring: the smaller values of the couplings of α-CPT indicate that the phenyl ring-C(3)-H(3) angle is small while in β-CPT, the angle is larger or the ring can rotate rather freely[19]; this latter possibility was supported also by MD calculations. The phenyl ring plays a role in biological functions[7] and offers an opportunity for adjustment of the biological properties via substitution at the phenyl ring.

The NMR data can also be used in the characterisation of the solvent and protonation effects. First, there are surprisingly large variations in the geminal couplings. The largest effects are between the protonated and non-protonated structures, up to 2.7 Hz. The smaller, up to 1.4 Hz (for 4a, 4b), differences between the neutral compounds, reflect the effects of N-methylation and, in the case of cocaine, substituent effects at position 3. Smaller changes caused by solvent and substituent effects are found on ^{3}J(1,7a), ^{3}J(1,7b), ^{3}J(5,6a), ^{3}J(5,6b), ^{3}J(6b, 7a) and ^{3}J(6b, 7b). The protonation effect seems to be of diagnostic value.

Protonation also has conformational effects on the ring. This is apparent in several ways: the calculated-observed differences are not so good for ecgonine. The negative chloride-ion at the positively charged nitrogen causes repulsion towards the carbonyl oxygen of the R^2 group and thus forces it away from the charge. This enlarges the dihedral angle, reducing ^{3}J(1,R^3) and also reduces the dihedral angle and enlarges ^{3}J(3,R^3). The conformational change destroys the W-route between protons 4b and R^3. Since ^{4}J couplings are very sensitive to the planarity of the pathway, a deviation from a W-type planar pathway causes a decrease in the coupling.[20] This is apparent for neutral cocaine, β-CPT and nor-β-CIT and is useful in distinguishing the α and β isomers.

With respect to long-range couplings, there are some other significant variations between the compounds. The most informative are the ^{4}J couplings. Significant differences are seen for ^{4}J(4a, 6a), ^{4}J(7a, R^2), ^{4}J(1, 3), ^{4}J(3, 5) and ^{4}J(1,5). The last of these reflects geometric effects and substituent effects on the nitrogen, the others can be explained by the variation of the geometry.

Table 5. Chemical Shifts (ppm) of the Tropane Ring Protons and Chemical Shifts of Aromatic Protons of α-CPT, β-CPT and Nor-β-CIT.

Protons	α-CPT[a]	β-CPT[a]	Nor-β-CIT[a]	Cocaine[a]	Cocaine-HCl[b]	Ecgonine-HCl[b]
1	3.36	3.49	3.57	3.37	4.25	4.10
3	3.27	2.78	2.74	5.26	5.59	4.34
4a	1.90	2.80	2.24	2.68	2.45	2.18
4b	1.45	1.47	1.20	1.73	2.41	2.12
5	2.91	3.04	3.47	2.83	4.06	3.90
6a	1.82	1.75	1.74	1.61	2.45	2.40
6b	1.46	1.24	1.23	1.31	2.25	2.06
7a	1.76	1.84	1.83	1.65	2.53	2.34
7b	2.11	1.35	1.29	1.32	2.22	2.08
R^2/R^3	2.31	2.83	2.49	2.99	3.60	3.14
8	7.30	7.26	6.66	-	-	-
9	7.13	7.20	7.47	-	-	-
10	7.02	7.08	-[c]	-	-	-
Rms[d]	0.86	0.47	0.97	0.83	0.54	1.79

[a] Solvent C_6D_6. [b] Solvent CD_3OD. [c] Iodine at position 10. [d] Maximum intensity of the spectra = 100.

The ^{1}H chemical shifts of the compounds are given in Table 5. For (-)-cocaine, the proton 3 shift is 2-3 ppm downfield compared to the corresponding proton of α-CPT, β-CPT and nor-β-CIT, which all have the benzene ring directly attached to the tropane ring. For the β-configuration compounds, proton 4a signals are downfield in

comparison to α-CPT, which is due to the magnetic anisotropy effect[20] of the carbonyl oxygen at carbon 2. A similar effect is seen for proton 7b of α-CPT (Figure 3). Ecgonine-HCl and cocaine-HCl are measured in CD_3OD, consequently their chemical shifts are comparable only to each other.

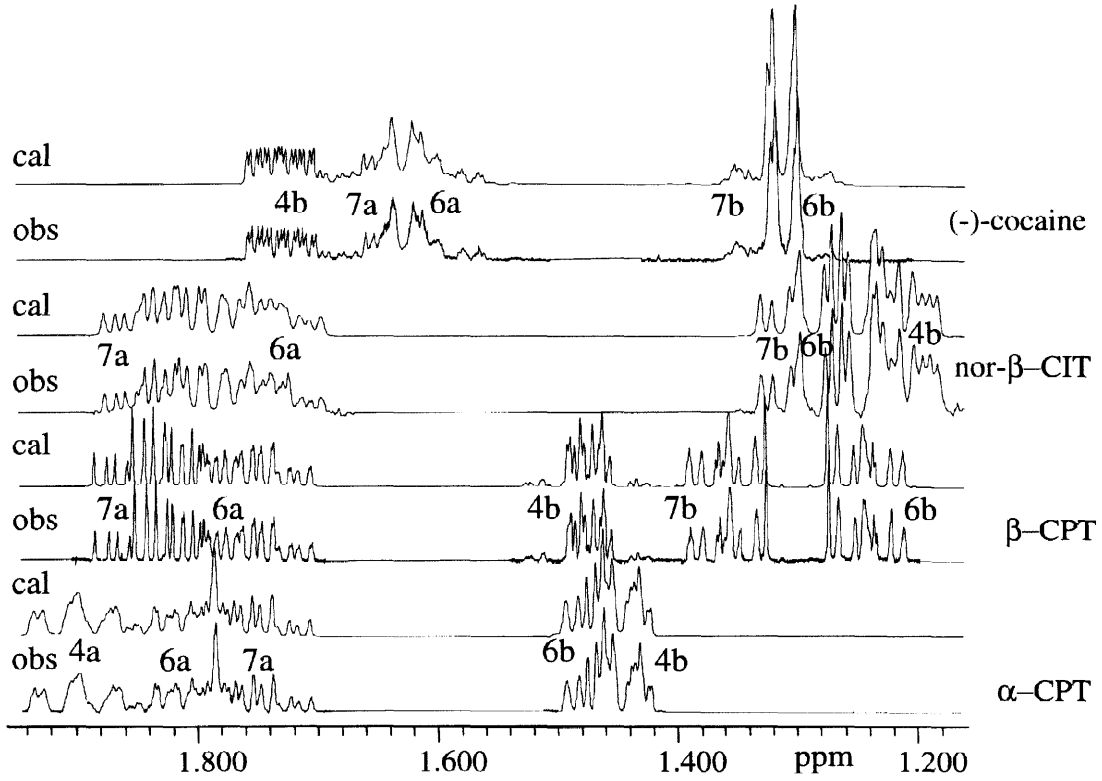


Figure 3. Calculated and observed spectra for some protons of α-CPT, β-CPT, nor-β-CIT and cocaine.

CONCLUSION

The modelling methods which were supplemented by the experimental data indicate that the substituents in (-)-cocaine, ecgonine, α-CPT, β-CPT and nor-β-CIT have only minor effects on the geometry of the tropane system. The only significant variations are seen around the substituents and the N-bridge, as indicated also by the ^4J couplings of the bridge protons. The NMR data indicates that the six membered ring conformations are close to the chair conformation predicted by the theoretical methods and that there does not seem to be any other conformation within 10 kJ/mol. The molecular dynamics calculations indicate that the ethylene bridge can make surprisingly large movements. The substituent and the solvent effects are rather large for the HCl salts. In particular the HH geminal couplings appear to be sensitive to protonation of the bridge nitrogen.

The geometries at different theoretical levels are very similar, indicating that all of the methods, as expected, give good estimates of geometries. It is interesting that the Haasnoot equation[10] provides a better fit between the NMR and HF/6-31G* data than the newer Altona equation.[11] The variation of the coupling constants can be mostly accounted for by direct substituent effects; the empirical Haasnoot and Altona equations are too inaccurate to permit one to make any conclusions about variation in the geometries in the vicinity of the substituents.

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REFERENCES

1. Turner, D. B.; Willett, P.; Ferguson, A.; Heritage, T. *J. Comput.-Aided Mol. Design* **1997**, 11, 409-422.
2. Laatikainen, R.; Niemitz, M.; Weber, U.; Sundelin, J.; Hassinen, T.; Vepsäläinen, J. *J. Magn. Reson.* **1996**, A120, 1-10.
3. Palmer, A. M.; Francis, P. T.; Benton, J. S.; Sims, N. R.; Mann, D. M.; Neary, D.; Snowden, J. S.; Bowen, D. M. *J. Neurochem.* **1987**, 48, 8-15.
4. Tiihonen, J.; Kuikka, J.; Bergström, K.; Hakola, P.; Karhu, J.; Ryynänen, O. P.; Föhr, J. *Nature Med.* **1995**, 1, 654-657.
5. Cash, R.; Raisman, R.; Ploska, A.; Agid, Y. *Eur. J. Pharmacol.* **1985**, 117, 71-80.
6. Kaufman, M . J.; Madras, B. K. *Synapse* **1991**, 9, 43-49.
7. Emond, P.; Garreau, L.; Chalon S.; Boazi, M.; Caillet, M.; Bricard, J.; Frangin, Y.; Mauclaire, L.; Besnard, J.-C.; Quilloteau, D. *J. Med. Chem.* **1997**, 40, 1366-1372.
8. Swahn, C.-G.; Halldin, C.; Günther, I.; Patt, J.; Ametamey, S. *J. Labelled Compd.Radiopharm.* **1996**, 7, 676-685.
9. Meltzer, P. C.; Liang, A. Y.; Brownell, A.-L.; Elmaleh, D. R.; Madras, B. K. *J. Med. Chem.* **1993**, 36, 855-862.
10. Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, 36, 2783-2792.
11. Altona, C.; Francke, R.; de Haan, R.; Ippel, J. H.; Daalmans, G. J.; Westra Hoekzema, A. J. A.; van Wijk, J. *Magn. Reson. Chem.* **1994**, 32, 670-678.
12. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F., Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, 107, 3902-3909.
13. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.;Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.;Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *GAUSSIAN 94*, Revision D.3, Gaussian, Inc., Pittsburgh PA, 1995.
14. Villar, H. O.; Loew, G. H. *J. Comput. Chem.* **1990**, 11, 1111-1118.
15. Froimowitz, M. *J. Comput. Chem.* **1993**, 14, 934-943.
16. Yang, B.; Wright, J.; Eldefrawi, M. E.; Pou, S.; MacKerell, A. D., Jr. *J. Am. Chem. Soc.* **1994**, 116, 8722-8732.
17. Mosquera, R. A.; Uriarte, E. *J. Mol. Struct.* **1989**, 195, 325-333.
18. Niemitz, M. unpublished work
19. Parr, W. J. E.; Schaefer, T. *Acc. Chem. Res.* **1980**, 13, 400-406.
20. Gunther, H. *NMR Spectroscopy, basic principles, concepts, and applications in chemistry*, John Wiley & Sons, Inc., Chichester, 1994, 2nd ed.