Bull. Soc. Chim. Belg. vol.91/n° 7/1982

CARBON-13 CHEMICAL SHIFTS OF THREE OXO-, SIX MONOHYDROXY-, AND SIX DIHYDROXYCYCLOHEXYL DERIVATIVES OF PROCYCLIDINE

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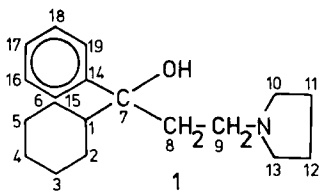
Received : 1/02/1982 - Accepted : 5/05/1982

ABSTRACT

In order to elucidate the metabolic pattern of procyclidine in the rat, a variety of oxo-, monohydroxy-, and dihydroxycyclohexyl derivatives of this drug were synthetized. All of them have been identified by ^{13}C-NMR spectroscopy.

INTRODUCTION

Procyclidine (KEMADRIN^{R}), 1-cyclohexyl-1-phenyl-3-(1-pyrrolidinyl)-1-propanol (1), is a synthetic anticholinergic drug, used for many years in the treatment of Parkinson's disease and in the long-term therapy of as well as in acute neuroleptic induced Parkinson syndrome[2,3]. Mono- and dihydroxylation as well as ketone formation on the cyclohexyl part of the molecule are major metabolic pathways in the rat. To identify a number of isolated metabolites, several oxo-, monohydroxy-, and dihydroxycyclohexyl derivatives of procyclidine were synthetized as follows.



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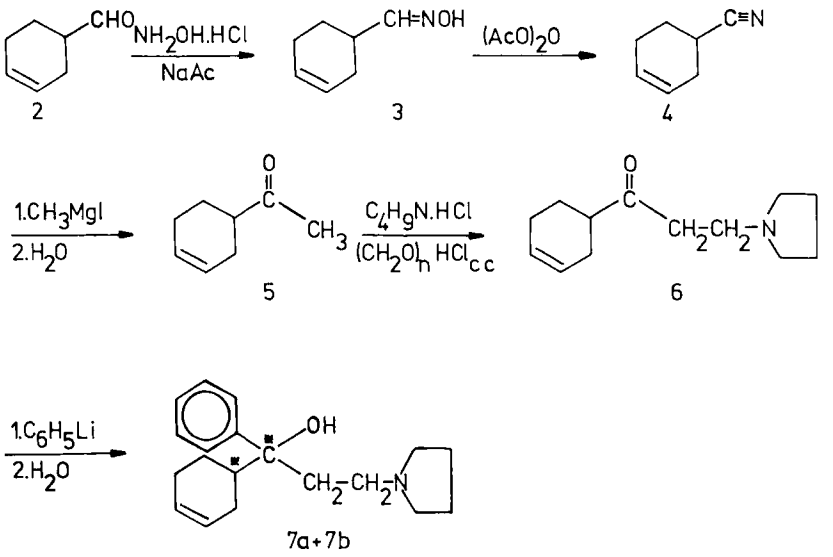
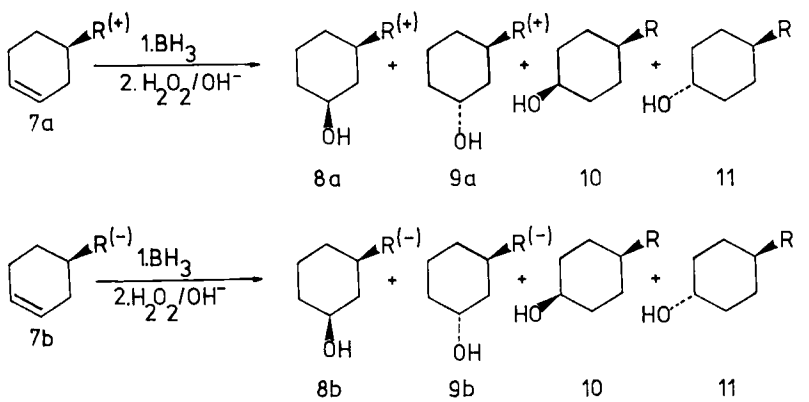
1,2,3,6-Tetrahydrobenzaldehyde (2, scheme 1) was converted to its oxime (3), which was dehydrated to 1,2,3,6-tetrahydrobenzonitrile (4). 3-Cyclohexenyl methyl ketone (5) was prepared by a Grignard reaction on 4, and 1-(3-cyclohexenyl)-3-(1-pyrrolidinyl)-1-propanone (6) by a Mannich reaction on 5. Reaction of phenyllithium on 6 gave two diastereoisomers, (1R*,7R*)- and (1R*,7S*)-1-(3-cyclohexenyl)-1-phenyl-3-(1-pyrrolidinyl)-1-propanol (7a,7b).

Hydroboration of 7a and 7b (scheme 2) gave six monohydroxycyclohexyl derivatives in all : (1R*,3S*,7R*)- and (1R*,3S*,7S*)-1-(cis-3-, (1R*,3R*,7R*)- and (1R*,3R*,7S*)-1-(trans-3-, 1-(cis-4-, and 1-(trans-4-hydroxycyclohexyl)-1-phenyl-3-(1-pyrrolidinyl)-1-propanol (8a, 8b, 9a, 9b, 10, 11).

(1R*,7R*)- and (1R*,7S*)-1-(3-, and 1-(4-Oxocyclohexyl)-1-phenyl-3-(1-pyrrolidinyl)-1 propanol (12a,12b,13) were prepared by oxidation of the different monohydroxycyclohexyl derivatives by means of chromic anhydride in acetic acid (scheme 3).



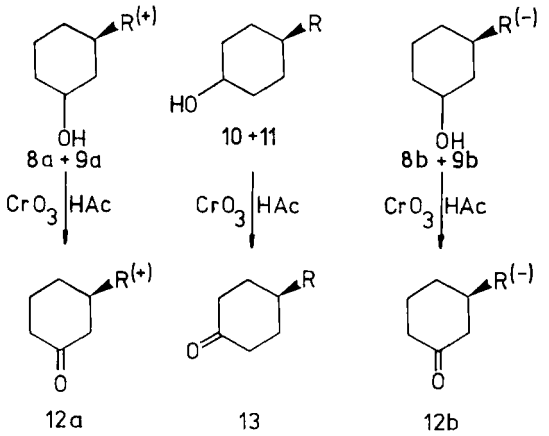
Scheme 1. Synthesis of ($1R^{*},7R^{*}$)- and ($1R^{*},7S^{*}$)-1-(3-Cyclohexenyl)-1-Phenyl-3-(1-Pyrrolidinyl)-1-Propanol.



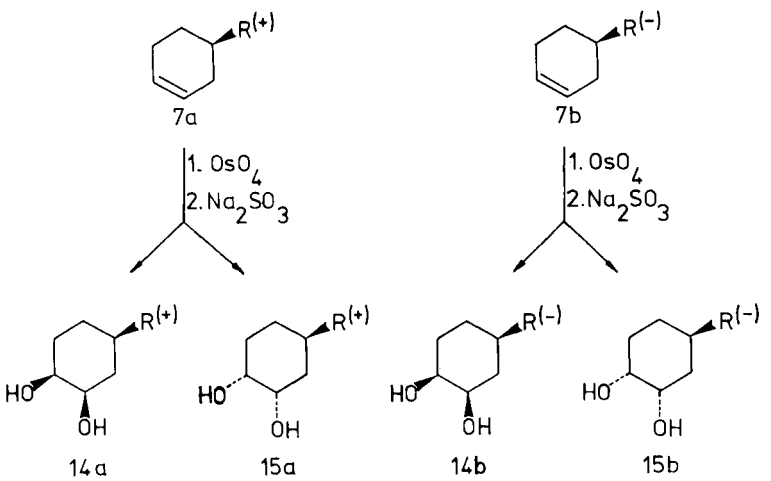
Scheme 2. Synthesis of the Monohydroxycyclohexyl Derivatives (R = Procyclidine Rest).

Reaction of osmium tetroxide on 7a and 7b (scheme 4) gave four cis-dihydroxycyclohexyl derivatives in all : ($1R^{*},3R^{*},4S^{*},7R^{*}$)- and ($1R^{*},3R^{*},4S^{*},7S^{*}$)-1-(cis-3,cis-4-, and ($1R^{*},3S^{*}$, $4R^{*},7R^{*}$)- and ($1R^{*},3S^{*}$, $4R^{*},7S^{*}$)-1-(trans-3,trans-4-dihydroxycyclohexyl)-1-phenyl-3-(1-pyrrolidinyl)-1-propanol (14a,14b,15a,15b).

An attempt was made to synthetize the trans-dihydroxycyclohexyl derivatives by reaction

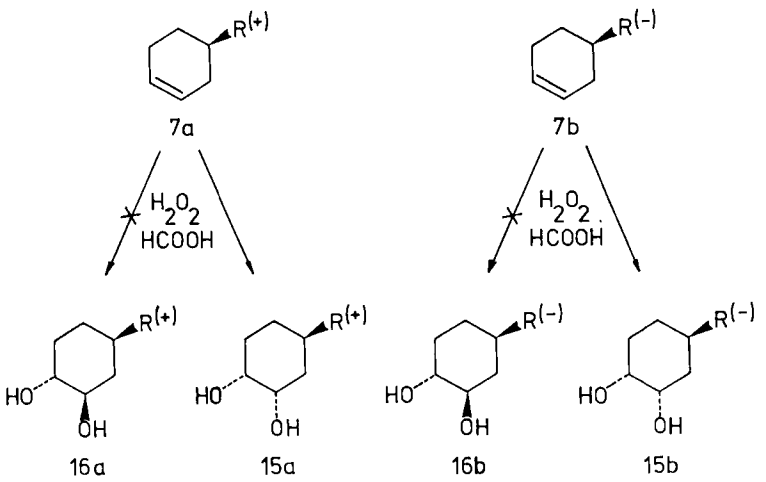


Scheme 3. Synthesis of the Oxocyclohexyl Derivatives (R = Procyclidine Rest).



Scheme 4. Synthesis of the cis-Dihydroxycyclohexyl Derivatives (R = Procyclidine Rest).

of 7a and 7b with hydrogen peroxide in formic acid (scheme 5). Unfortunately no trans- but only a cis-dihydroxycyclohexyl derivative was formed in both cases.



Scheme 5. Reaction of the Dehydrocyclohexyl Isomers with Hydrogen Peroxide in Formic Acid (R = Procyclidine Rest).

MATERIALS AND METHODS

1,2,3,6-Tetrahydrobenzaldoxime (3). *1,2,3,6-Tetrahydrobenzaldehyde (0.5 mol) was added dropwise to a solution of hydroxylamine hydrochloride (1 mol) and sodium acetate (1.4 mol) in 500 ml water. The reaction mixture was heated at 45°C for 2 hours and was further stirred overnight. The organic layer was separated, and the water layer was extracted twice with chloroform. The extracts were added to the organic layer, which was then dried, evaporated, and distilled to give 1,2,3,6-tetrahydrobenzaldoxime (94 %) as a colourless liquid, b.p. 116°/21 mm.*

1,2,3,6-Tetrahydrobenzonitrile (4). *1,2,3,6-Tetrahydrobenzaldoxime (0.4 mol) was added dropwise to acetic acid anhydride (0.6 mol). The reaction mixture was stirred for 1 hour at room temperature, and was then distilled to give 1,2,3,6-tetrahydrobenzonitrile (77 %) as a colourless liquid, b.p. 83°/21 mm.*

3-Cyclohexenyl methyl ketone (5). *An ethereal solution of methylmagnesium iodide (0.375 mol) was added dropwise to a refrigerated solution of 1,2,3,6-tetrahydrobenzonitrile (0.25 mol) in ether. The mixture was then stirred at room temperature for 1/2 hour, boiled under reflux for 3 1/2 hours, and finally stirred at room temperature for 18 more hours. The resulting pasty mass was decomposed with an ice-cold 10 % ammonium chloride solution. The organic layer was separated, and the water layer was extracted twice with ether. The extracts were added to the organic layer, which was then dried, evaporated, and distilled to give 3-cyclohexenyl methyl ketone (71 %) as a colourless liquid, b.p. 79°/21 mm.*

1-(3-Cyclohexenyl)-3-(1-pyrrolidinyl)-1-propanone (6). *A mixture of pyrrolidine hydrochloride (0.1 mol), paraformaldehyde (0.15 mol), 3-cyclohexenyl methyl ketone (0.1 mol), absolute ethanol (30 ml), and concentrated hydrochloric acid (0.25 ml) was boiled under reflux. After 1 hour paraformaldehyde (0.1 mol) was added, and the mixture was allowed to reflux for 2 more hours. Water was then added, and the ethanol was evaporated. After an*

ether clean-up, the water solution was alkalified and extracted three times with ether. The combined extracts were dried, evaporated and distilled. The fraction, b.p. 97-102°/5 mm, was taken as 1-(3-cyclohexenyl)-3-(1-pyrrolidinyl)-1-propanone (59%).

(1R*,7R*)- and (1R*,7S*)-1-(3-cyclohexenyl)-1-phenyl-3-(1-pyrrolidinyl)-1-propanol (7a, 7b). An ethereal solution of phenyllithium (0.075 mol) was added dropwise to an ethereal solution of 1-(3-cyclohexenyl)-3-(1-pyrrolidinyl)-1-propanone (0.05 mol) cooled at -80°C. The mixture was then stirred at -80°C for 1 hour, was further stirred overnight at room temperature and was finally decomposed with ice-cold water. The organic layer was separated, and the water layer was extracted twice with ether. The extracts were added to the organic layer, which was then dried and evaporated. (1R*,7R*)- and (1R*,7S*)-1-(3-cyclohexenyl)-1-phenyl-3-(1-pyrrolidinyl)-1-propanol were separated by PLC[4] on silica gel TLC[5] plates with solvent system A. 7a (Rf = 0.64) was defined as the (1R*,7R*)- and 7b (Rf = 0.72) as the (1R*,7S*)-3,4-dehydrocyclohexyl isomer, since none of them was identified definitely as the one or the other. The essential m/e values in both the mass spectra of the dehydroxycyclohexyl derivatives were : 77, 84 (100 %), 105, 204, 205, 208, 267, 268, 285 (M^+).

(1R*,3S*,7R*)- and (1R*,3S*,7S*)-1-(cis-3-, (1R*,3R*,7R*)- and (1R*,3R*, 7S*)-1-trans-3-, 1-(cis-4-, and 1-(trans-4-hydroxycyclohexyl)-1-phenyl-3-(1-pyrrolidinyl)-1-propanol (8a,8b, 9a,9b,10,11). An ethereal solution of 7a (0.2mmol) was added to a refrigerated solution of borane-tetrahydrofuran complex (0.6 mmol) in tetrahydrofuran. The mixture was stirred at room temperature for 2 hours, and was then decomposed with a solution of hydrogen peroxide in 10 % sodium hydroxide. The organic layer was separated, and the water layer was extracted twice with ether. The extracts were added to the organic layer, which was then dried and evaporated. The residue was dissolved in methanol and submitted to a double PLC, giving the (1R*,3S*,7R*)-cis-3- (8a), the (1R*,3R*,7R*)-trans-3- (9a), the cis-4- (10), and the trans-4-hydroxycyclohexyl isomer (11). The same procedure was repeated after reaction of 7b (0.2 mmol), giving the (1R*,3S*,7S*)-cis-3- (8b), the (1R*,3R*,7S*)-trans-3- (9b), the cis-4- (10) and the trans-4-hydroxycyclohexyl derivative (11). The first PLC was performed on silica gel TLC plates with solvent system B, the second on aluminum oxide TLC plates with solvent system C. The respective Rf-values were : 8a:0.32-0.65; 9a:0.27-0.75; 10:0.22-0.46; 11:0.25-0.63; 8b:0.17-0.50; 9b:0.17-0.62. The essential m/e values in the mass spectra of the six hydroxycyclohexyl isomers were : 77, 84 (100 %), 105, 204, 205, 226, 285, 286, 303.(M^+).

(1R*,7R*)- and (1R*,7S*)-1-(3-, and 1-(4-oxocyclohexyl)-1-phenyl-3-(1-pyrrolidinyl)-1-propanol (12a,12b,13). An equimolecular mixture of 8a and 9a (0.1 mmol) was dissolved in a solution of chromic anhydride (0.5 mmol) in 80 % acetic acid. The mixture was allowed to stand overnight at 50°C, and was then alkalified and extracted three times with ether. The combined extracts were dried and evaporated. The (1R*,7R*)-3-oxocyclohexyl derivative (12a) was purified by PLC on silica gel TLC plates with solvent system B (Rf = 0.56). The same procedure was repeated after reaction of equimolecular mixtures of 10 and 11 resp. 8b and 9b (0.1 mmol), giving the 4- (13) (Rf : 0.43) resp. the (1R*,7S*)-3-oxocyclohexyl derivative (12b) (Rf = 0.42). The essential m/e values in the mass spectra of the three oxocyclohexyl isomers were : 77, 84 (100 %), 105, 204, 205, 224, 284, 301 (M^+).

(1R*,3R*, 4S*, 7R*)- and (1R*,3R*,4S*,7S*)-1-(cis-3,cis-4-, and (1R*,3S*,4R*,7R*)- and (1R*,3S*,4R*,7S*)-1-(trans-3,trans-4-dihydroxycyclohexyl)-1-phenyl-3-(1-pyrrolidinyl)-1-propanol (14a,14b,15a,15b). A water solution of osmium tetroxide (0.15 mmol) was added to a solution of 7a (0.1 mmol) in 1 % sulfuric acid, and the mixture was stirred at room temperature for 1/2 hour. Sodium sulfite (1 mmol) was then added. After a time the mixture

was alkalified and extracted three times with ether. The combined extracts were dried and evaporated. The residue was dissolved in methanol and submitted to PLC on aluminum oxide TLC plates with solvent system D, giving the (1R,3R*,4S*,7R*)-cis-3,cis-4- (14a) (Rf = 0.52') and the (1R*,3S*,4R*,7R*)-trans-3, trans-4-dihydroxycyclohexyl isomer (15a) (Rf = 0.62). The same procedure was repeated after reaction of 7b (0.1 mmol), giving the (1R*,3R*,4S*,7S*)-cis-3,cis-4- (14b) (Rf = 0.43) and the (1R*,3S*,4R*,7S*)-trans-3,trans-4-dihydroxycyclohexyl isomer (15b) (Rf = 0.58). The essential m/e values in the mass spectra of the four cis-dihydroxycyclohexyl derivatives were : 77, 84 (100 %), 105, 204, 205, 242, 301, 302, 319 (M^+).*

(1R,3R*,4R*,7R*)-, and (1R*,3R*,4R*,7S*)-1-(cis-3,trans-4-dihydroxycyclohexyl)-1-phenyl-3-(1-pyrrolidinyl)-1-propanol (16a,16b). Hydrogen peroxide (1 mmol) was added to a solution of 7 a (0.1 mmol) in formic acid, and the mixture was allowed to stand overnight. Water was then added, and the mixture was boiled under reflux for 3 hours. After cooling the mixture was alkalified and extracted three times with ether. The combined extracts were dried and evaporated. The residue was dissolved in methanol and submitted to PLC on aluminum oxide TLC plates with solvent system D. Unfortunately not the (1R*,3R*,4R*,7R*)-cis-3,trans-4-(16a) but the (1R*,3S*,4R*,7R*)-trans-3,trans-4-dihydroxycyclohexyl isomer (15a) was recovered. When the same procedure was repeated after reaction of 7b, only the (1R*,3S*,4R*,7S*)-trans-3,trans-4-dihydroxycyclohexyl derivative (15b) was recovered as well.*

PLC was performed on silica gel and aluminum oxide (type E)-precoated glass plates (Merck 60 F_{254}, 20cm x 20cm x 0.25 mm). Solvent systems were : A, chloroform/acetone/25% ammonium hydroxide, 75:25:1; B, chloroform/acetone/25% ammonium hydroxide, 25:75:1; C, chloroform/diethylamine, 90:10; D, chloroform/methanol/diethylamine, 80:10:10. Acidified iodoplatinate was used as location reagent, and methanol was used as eluent.

The mass spectra were recorded with a Hewlett-Packard model 5992A gas chromatograph-mass spectrometer system equipped with a glass column (2m x 3mm i.d.) packed with 2 % OV 101 on Gas-Chrom Q 80-100 mesh. The injection port temperature was 275°C, and the column temperature 235 or 250°C.

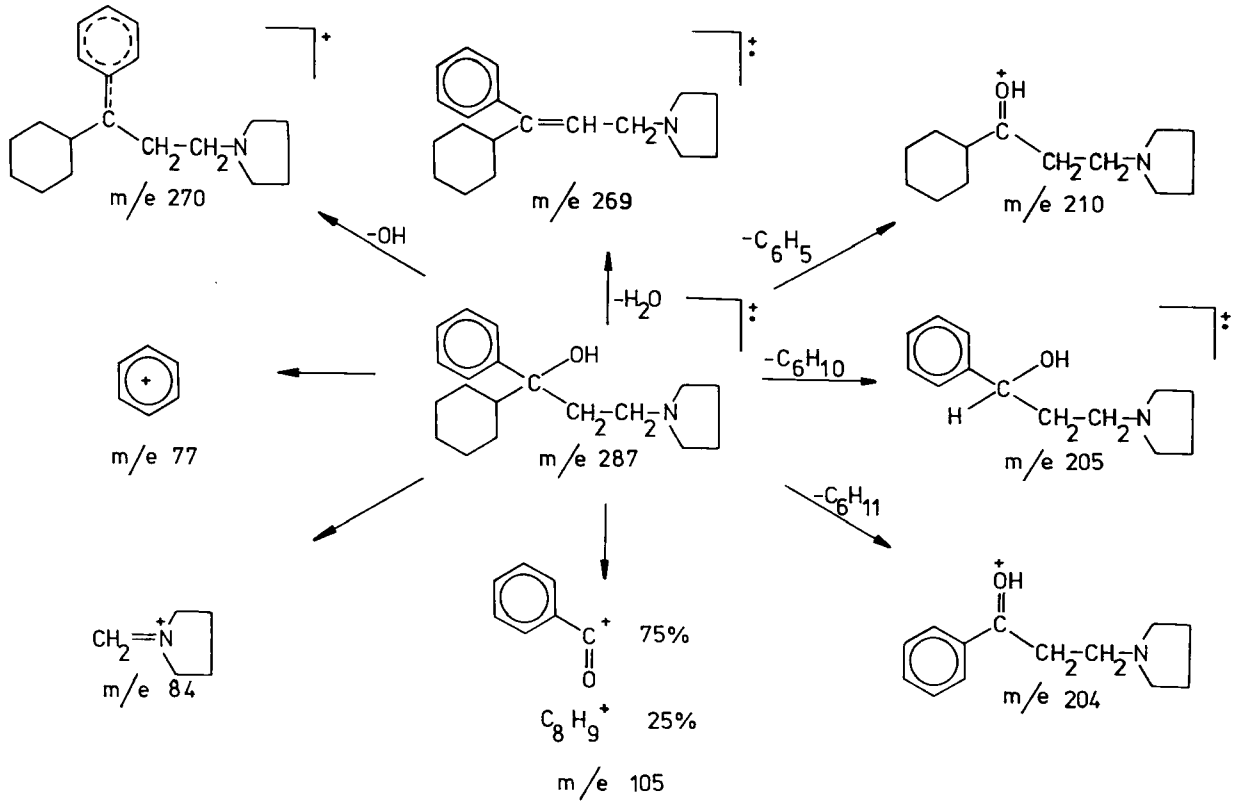
Peak-matching was performed on a AEI model 902 S mass spectrometer, at 70 eV. The ion source temperature was 200°C, and the samples were introduced via the direct insertion inlet.

^{13}C-NMR spectroscopy was performed on a JEOL model FX 100 pulsed spectrometer, operating at 25.0 MHz with 5 mm sample tubes and internal deuterium lock. The spectra were standardized against chloroform-d used as solvent. Proton noise decoupling, 45° pulses, and a repetition rate of 1.2 sec for a 5000 or 6000 Hz spectral width were employed.

RESULTS AND DISCUSSION

Peak-matching on the essential m/e values in the mass spectrum of procyclidine gave the mass fragmentation pattern outlined in sheme 6. The same fragmentation pattern can be applied to the dehydro-, the monohydroxy-, the dihydroxy- and the oxocyclohexyl derivatives, except that no (M^+-H_2O) was detected for the latter.

Table 1 shows the ^{13}C-chemical shifts of procyclidine, and those of the dehydro-, the monohydroxy-, the oxo- and the dihydroxycyclohexyl derivatives. Since no trans-dihydroxycyclohexyl derivatives could be synthetized, the chemical shifts listes for 16a and 16 b are those of isolated rat metabolites.

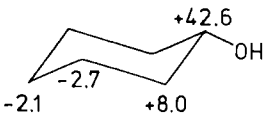


Scheme 6. Mass Fragmentation Pattern of Procyclidine.

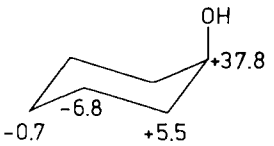
Table I. Carbon - 13 Chemical Shifts (ppm) of Procyclidine and the Dehydro-, Monohydroxy-, Oxo- and Dihydroxycyclohexyl Derivatives.

Carbon	1	2	3	4	5	6	7	8	9	10,13	11,12	14	15,19	16,18	17
1	49.0	26.8[a]	26.7	26.5	26.7	27.1[a]	80.0	34.5	52.4	53.7	23.3	147.0	127.4	126.1	125.5
7a	45.0	26.5[a]	127.3[b]	126.5[b]	26.3[a]	23.3	79.6	34.7	52.6	53.9	23.5	146.7	127.7	126.4	125.9
7b	45.0	26.3	127.1[a]	126.9[a]	26.3	23.3	80.2	34.6	52.6	53.9	23.5	147.3	127.8	126.3	125.9
8a	47.7	36.6	71.4	35.7	23.9	25.6	79.7	34.8	52.6	53.9	23.5	146.5	127.8	126.3	126.1
8b	47.6	36.3	71.3	35.7	23.9	25.9	79.5	34.8	52.5	53.9	23.5	146.5	127.8	126.2	126.1
9a	42.0	33.6	67.0	32.5	20.2	26.3	80.0	34.8	52.5	53.9	23.5	147.0	127.8	126.2	125.9
9b	42.1	33.4	67.3	32.7	20.3	26.7	80.0	34.8	52.6	53.9	23.5	147.0	127.8	126.3	125.9
10	48.7	20.4[a]	33.0[b]	66.0	33.1[b]	20.5[a]	80.0	34.5	52.6	53.9	23.5	147.0	127.8	126.4	126.0
11	48.0	24.9[a]	35.8[b]	71.0	35.9[b]	25.2[a]	79.8	35.0	52.6	53.9	23.5	147.0	127.8	126.3	126.0
12a	49.6	42.7[a]	213.1	41.4[a]	25.2[b]	25.3[b]	79.5	34.9	52.5	53.8	23.5	145.7	128.1	126.0	126.4
12b	49.4	42.9[a]	213.4	41.5[a]	25.2[b]	25.4[b]	79.6	34.6	52.4	53.8	23.5	146.6	128.1	126.0	126.3
13	47.3	26.4[a]	40.8[b]	212.4	41.0[b]	26.9[a]	79.5	34.9	52.6	53.9	23.5	146.0	128.0	126.1	126.4
14a	46.3	29.5	71.6	69.3	29.9	19.8	80.1	34.6	52.5	53.8	23.5	146.6	127.9	126.1	126.1
14b	46.1	29.8	71.5	69.6	29.8	20.4	80.2	34.7	52.6	53.9	23.5	146.7	127.9	126.1	126.1
15a	40.7	31.7	69.7	71.8	28.4	24.3	79.6	35.0	52.5	53.8	23.5	146.8	127.8	126.0	126.0
15b	40.8	31.3	69.8	71.9	28.5	24.6	79.7	35.1	52.5	53.8	23.5	146.9	127.8	126.0	126.0
16a	47.3	33.5	76.0	76.0	31.9	24.4	79.4	34.9	52.6	53.9	23.5	146.4	127.9	126.2	126.2
16b	47.1	33.2	76.0	76.0	32.0	24.7	79.4	34.9	52.5	53.9	23.5	146.4	127.9	126.1	126.1

[a,b]Assignments may be interchanged.



17



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The chemical shifts of the cyclohexyl carbon atoms of the monohydroxycyclohexyl derivatives were assigned by comparison of the measured and calculated values; the latter were obtained by adding the hydroxyl-induced shifts caused by hydroxylation of cyclohexane (17, 18)[6] to the chemical shifts of the corresponding carbon atoms of procyclidine (table II). The measured and calculated shifts are represented in table III.

TABLE II

Calculation of the Carbon-13 Chemical Shifts (ppm) of the Monohydroxycyclohexyl Derivatives.

Atom	Procyclidine	Cis-3	Trans-3	Cis-4	Trans-4
1	49.0	-2.7	-6.8	-0.7	-2.1
2	26.8	+8.0	+5.5	-6.8	-2.7
3	26.7	+42.6	+37.8	+5.5	+8.0
4	26.5	+8.0	+5.5	+37.8	+42.6
5	26.7	-2.7	-6.8	+5.5	+8.0
6	27.1	-2.1	-0.7	-6.8	-2.7

The chemical shifts of the cyclohexyl carbon atoms of the dihydroxycyclohexyl derivatives were calculated as well. The real hydroxyl-induced shifts caused by hydroxylation of procyclidine in cis-4 and trans-4 position of the cyclohexyl ring were calculated by substracting the chemical shifts of the cyclohexyl carbon atoms of procyclidine form the chemical shifts of the corresponding carbon atoms of both the 4-hydroxycyclohexyl derivatives. The obtained values were to the chemical shifts of the corresponding carbon atoms of the different 3-hydroxycyclohexyl derivatives. The chemical shifts calculated for the hydroxylated carbon atoms were finally corrected for the mutual interaction of the vicinal hydroxyl groups, which is not accounted for when adding the individual hydroxyl effects. The cor-

TABLE III

Comparison between the Measured and Calculated Carbon-13 Chemical Shifts (ppm) of the Monohydroxycyclohexyl Derivatives

Atom	Cis-3			Trans-3			Cis-4		Trans-4	
	8a	Calc	8b	9a	Calc	9b	10	Calc	11	Calc
1	47.7	46.3	47.6	42.0	42.2	42.1	48.7	48.3	48.0	46.9
2	36.6	34.8	36.3	33.6	32.3	33.4	20.4	20.0	24.9	24.1
3	71.4	69.3	71.3	67.0	64.5	67.3	33.0	32.2	35.8	34.7
4	35.7	34.5	35.7	32.5	32.0	32.7	66.0	64.3	71.0	69.1
5	23.9	24.0	23.9	20.2	19.9	20.3	33.1	32.2	35.9	34.7
6	25.6	25.0	25.9	26.3	26.4	26.7	20.5	20.3	25.2	24.4

TABLE IV

Comparison between the Measured and Calculated Carbon-13 Chemical Shifts (ppm) of the Dihydroxycyclohexyl Derivatives

Atom	14a		14b		15a		15b		16a		16b	
	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.
1	47.4	46.3	47.3	46.1	41.0	40.7	41.1	40.8	46.7	47.3	46.6	47.1
2	30.2	29.5	29.9	29.8	31.7	31.7	31.5	31.3	34.7	33.5	34.4	33.2
3	72.0	71.6	71.9	71.5	69.3	69.7	69.6	69.8	76.3	76.0	76.2	76.0
4	68.4	69.3	68.4	69.6	71.3	71.8	71.5	71.9	76.0	76.0	76.0	76.0
5	30.3	29.9	30.3	29.8	29.4	28.4	29.5	28.5	33.1	31.9	33.1	32.0
6	19.0	19.8	19.3	20.4	24.4	24.3	24.8	24.6	23.7	24.4	24.0	24.7

rections were performed according to the observed deviations from addivity in (25R)-5α-spirostane-2,3-diol[7]. Depending on the orientation of the vicinal hydroxyl groups the applied corrections were : axial-equatorial : axially substituted carbon : -6.8 ppm; equatorially substituted carbon : -5.7 ppm; diequatorial : -4.2 ppm. The measured and calculated chemical shifts are represented in table IV.

Other localizations than 3,4-diequatorial localization of the hydroxyl groups with regard to the isolated metabolites (e.g. 3,4-diaxial, 3,5-diaxial, 3,5-diequatorial, 3,5-axial-equatorial, etc.) could be rejected because of the important differences between the experimental and the calculated chemical shifts.

ACKNOWLEDGMENTS

We thank Mr. F. Parmentier[1] for giving the opportunity of recording the ^{13}C-NMR spectra, and Mr. W. Sonck for technical assistance in GLC-MS.

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