^{13}C- AND D- LABELLED 3-PHENYLPROPIONIC ACIDS; SYNTHESIS AND CHARACTERIZATION BY NMR AND MS SPECTRA

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Lucrarea prezintă sinteza şi caracterizarea structurală prin metode spectrale moderne (rezonanţă magnetică nucleară, spectrometrie de masă) a compuşilor marcaţi izotopic, acid 2-^{13}C-3-fenilpropionic şi respectiv 2.2-D-3-fenilpropionic necesari investigării mecanismelor de oxidare electrochimică ale acizilor carboxilici.

This paper presents the synthesis and characterization using modern spectral methods (nuclear magnetic resonance, mass spectrometry) of labelled compounds 2-^{13}C-3-phenylpropionic acid and 2,2-D_2-3-phenylpropionic acid, respectively, useful tools for the investigation of the mechanisms involved in the electrochemical oxidation of carboxylic acids.

Keywords: 2-^{13}C-3-phenylpropionic acid, 2,2-D_2-3-phenylpropionic acid, NMR spectra, mass spectra, synthesis

1. Introduction

Deuterium-, ^{13}C- and ^{14}C- labelled compounds have been extensively used in order to elucidate reaction mechanisms or biochemical pathways. We were interested in electrochemical oxidation of carboxilic acids and esters [1-3] especialy 3-phenylpropionic acid [1,2]. In order to elucidate the mechanism of one-electron and two-electron oxidation respectively, we had to synthesize several deuterium and ^{13}C- labelled 3-phenylpropionic acids: 2-^{13}C-3-phenylpropionic acid, 2,2-D_2-3-phenylpropionic acid

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Deuterium labelled 3-phenylpropionic acids may be obtained by reduction of the appropriate substrates (esters, aldehydes) with $LiAlD_4$ [1, 4] or by hydrogenation of the double bond ofin the cinnamic acid with catalysts and deuterium containing species: $Zn/NiCl_2/ D_2O$ [5], $Ru(OAc)_2$-BINAP/D_2 [6] deuterated Ni-Raney [7] or $NaBD_4$-Cu_2Cl_2/CD_3OD [8]. Another method is kinetic deuteration of 3-phenylpropionic acid with D_2O/HCl in the presence of homogenous K_2PtCl_4 [9]. The methods employed to prepare ^{13}C- and ^{14}C- labelled 3-phenylpropionic acids are mainly based on the carbonation of suitable Grignard reagents with $^{13}CO_2$ or $^{14}CO_2$ respectively or by substitution and addition reactions involving $K^{14}CN$ or $Na^{13}CN$ [10,11].

2. Experimental

^1H- and ^{13}C-NMR spectra were recorded on a Bruker Avance DRX spectrometer, aproximatively 0.2 M (for^1H-NMR spectra) and 0.5 M (for^{13}C-NMR spectra) solution of substrates in $CDCl_3$, TMS as internal standard were used.

GC-MS analyses were performed using a Varian 3400 gas chromatograph coupled with Saturn II mass spectrometer provided with ion trap.

FT-IR spectra were recorded on a Bruker Equinox 55 spectrometer in KBr.

Melting points are determined using a Boetius type microscope with electric plate and are uncorrected.

Solvents are purified according to procedures described in literature. ^{13}C labeling was made using a source of $Ba^{13}CO_3$ of 98% purity. D-labeling was accomplished using $LiAlD_4$ of 99.9 % purity.

2-^{13}C-3-phenylpropionic acid, **1** was prepared by alkaline hydrolysis of 2-^{13}C-3-phenylpropionitrile, **6**. A mixture of 15 mL 0.5M solution of **6** in ethanol and 4 mL of 12.5M solution of NaOH in water was heated on reflux for 20 hrs. After low pressure evaporation of the solvent the residue was dissolved in water and purified by boiling with charcoal and subsequent filtration of the hot solution. The cooled filtrate was acidified at pH=1 with 6N H_2SO_4 and the resulted product was suction filtered. Thus 1 g (88% yield) of **1** was obtained as a creamy white solid (m.p.= 49^{o}C).

^1H-NMR: 10.70, s, 1H (COOH); 7.24, t, 2H (H^3 and H^5); 7.17, t, 1H (H^4); 7.16, d, 2H (H^2 and H^6); 2.91, t, 2H, $^3J_{(H,H)}$=7.9Hz, (CH_2-psn 3); 2.69, dt, $^1J_{(c,H)}$=151.0Hz, $^3J_{(H,H)}$=7.9Hz (CH_2- psn 2). 2.67, t, (CH_2- psn 2- unlabelled).

^{13}C-NMR: 179.04, d, $^1J_{(C,C)}$=218.7Hz, (CO); 140.18 (C^{ipso}); 128.24, 128.56, 126.27 (phenyl); 35.59 (C^2); 30.49, d, $^1J_{(C,C)}$=73.4Hz (C^3).

MS: 152 (3) M+1; 151 (30) $C_6H_5CH_2{}^{13}CH_2COOH]^{+\bullet}$ M; 150 (5) M-1;

134 (2) $C_6H_5CH_2{}^{13}CH_2CO]^{+\bullet}$; 106 (16) $C_6H_5CH_2{}^{13}CH_2]^{+\bullet}$; 105 (52) $C_6H_5CH_2 =^{13}CH_2]^{+\bullet}$; 91 (100) $C_7H_7^{+\bullet}$; 79 (11) $C_6H_7^{+\bullet}$; 78 (14) $C_6H_6^{+\bullet}$; 77 (11) $C_6H_5^{+\bullet}$; 65 (10) $C_5H_5^{+\bullet}$; 51 (10) $C_4H_3^{+\bullet}$; 45 (11) $COOH^{+\bullet}$; 39 (8) $C_3H_3^{+\bullet}$.

2,2-D$_2$-3-phenylpropionic acid, **2** was prepared by acid hydrolysis of 2,2-D_2-3-phenylpropionitrile, **10**. A mixture of 2.1 g (16 mmoles) **10** dissolved in 4 mL CH_3COOH and 4 mL (74 mmoles) H_2SO_4 dissolved in 4mL H_2O was heated on reflux for 4hrs. The cooled reaction mixture was diluted with 20 mL H_2O and extracted with 3x10 mL ether. The organic layer was washed with water then extracted with concentrated NaOH solution. The alkaline solution was acidified at pH=1 with 6N H_2SO_4 and the resulted product was suction filtered. Thus 2.2 g (92% yield) of **2** was obtained as a white solid (m.p.= 49^{o}C).

^{1}H-NMR: 10.72, s, 1H (COOH); 7.27, t, 2H (H^3 and H^5); 7.19, t, 1H (H^4); 7.18, d, 2H (H^2 and H^6); 2.93, s, 2H (CH_2-psn 3); the signal from 2.97, (CH_2- psn 2) has an intensity of about 1%, showing practically complete deuteration.

^{13}C-NMR: 179.27, d, (CO); 140.13 (C^{ipso}); 128.33, 128.21, 126.34 (phenyl); 35.45, cv (C^2); 30.45, d, (C^3).

MS: 153 (3) M+1; 152 (31) $C_6H_5CH_2CD_2COOH]^{+\bullet}$ M; 151 (5) M-1; 135 (2) $C_6H_5CH_2CD_2CO]^{+\bullet}$; 107 (16) $C_6H_5CH_2]^{+\bullet}$; 106 (53) $C_6H_5CH_2 =CD_2]^{+\bullet}$; 91 (100) $C_7H_7^{+\bullet}$; 81 (5) $C_6H_5D_2^{+\bullet}$; 80 (8) $C_6H_6D^{+\bullet}$; 79 (10) $C_6H_7^{+\bullet}$; 78 (12) $C_6H_6^{+\bullet}$; 77 (6) $C_6H_5^{+\bullet}$; 65 (11) $C_5H_5^{+\bullet}$; 51 (10) $C_4H_3^{+\bullet}$; 45 (11) $COOH^{+\bullet}$; 39 (9) $C_3H_3^{+\bullet}$.

***1-^{13}C-phenylacetic acid,* 3** was prepared in several batches by reaction of benzyl magnesium chloride with $^{13}CO_2$ generated from $Ba^{13}CO_3$ and H_2SO_4 according to an original procedure and apparatus described in literature [9]. In a typical procedure, a solution of benzyl magnesium chloride prepared from 5 g (39.5 mmoles) and 1 g (42 mmoles) dry magnesium in 30 mL anhydrous ether was cooled at -18^{o}C and connected to vacuum pump (20 mmHg) until all the air was replaced by ether vapors. The vacuum pump was disconnected and $^{13}CO_2$ generated from 1.8 g (9 mmoles) $Ba^{13}CO_3$ (98%) and sufficient H_2SO_4 was introduced in the system. The reaction mixture was frozen with liquid nitrogen and then heated using hot air until melted and then stirred using a magnetic stirrer. The procedure freeze/heat was repeated three times and then the reaction mixture was allowed to come back at room temperature. The white reaction mass was acidified at pH=1 with 6N H_2SO_4 and extracted with 3x20 mL ether. The organic layer was then extracted with 3x20 mL 1N NaOH and the alkaline layer was filtrated and acidified at pH=1 with concentrated HCl to precipitate the labelled phenylacetic acid. Thus 0.9 g (73% yield) of **3** were obtained as a white solid (m.p. =79^{o}C).

^{1}H-NMR: 11.05, s, 1H (COOH); 7.30, t, 2H (H^3 and H^5); 7.27, t, 1H (H^4); 7.24, d, 2H (H^2 and H^6);3.64, d, 2H (CH_2).

^{13}C-NMR: 177.76, , (CO); 133.28 (C^{ipso}); 128.38, 128.65, 127.35 (phenyl); 41.05, d, $^1J_{(C,C)}$=73.4Hz (C^2), 41.066, s (C^2 unlabelled).

1-^{13}C-2-phenylethanol, **4** was prepared by direct reduction of **3** with excess $LiAlH_4$. From 4.3 g (31.4 mmoles) **3** and 3 g (340 mmoles) $LiAlH_4$ in 60 mL anhydrous ether, 3.5 g (90% yield) of **4** was obtained as a colorless liquid (b.p.= 93^{o}C/8mmHg).

IR (cm^{-1}): 3200-3500 (ν_{O-H}); 3109 (ν_{Car-H}); 2944 and 2877 (ν_{Csat-H}); 1476 (δ_{CH_2}); 1046 (ν_{C-O}).

1-^{13}C-2-phenylethyl bromide, **5** was prepared from alcohol **4** and PBr_3 in dry benzene. A stirred mixture of 3.5 g (28 mmoles) alcohol **4** and 7 mL (20.1 g, 74 mmoles) PBr_3 in 50 mL dry benzene were heated under reflux for 9 hrs. The excess bromide was decomposed with 30 mL H_2O and the resulted organic layer was separated, washed with H_2O, 10% Na_2CO_3 and H_2O, dried over $CaCl_2$ and concentrated. The residue was distilled in vacuum. Thus 4 g (78% yield) of labelled bromide **5** was obtained as a colorless liquid (b.p. = 90^{o}C/7mmHg).

IR (cm^{-1}): 3029 (ν_{Car-H}); 2946 and 2863 (ν_{Csat-H}); 1476 (δ_{CH_2}); 1031 and 699 (ν_{C-Br}).

2-^{13}C-3-phenylpropionitrile, **6** was prepared from the bromide **5** and NaCN in acetone. A mixture of 4 g (21 mmoles) bromide **5** and 1.27 g NaCN in 25 mL dry acetone were heated under reflux for 20 hrs. The cooled reaction mixture was diluted with H_2O and extracted with 2x15mL CH_2Cl_2. The organic layer was washed with H_2O, dried over $CaCl_2$ and concentrated. The resulted crude product, 2.6 g (96%yield), was used for hydrolysis without further purification.

IR (cm^{-1}): 3030 (ν_{Car-H}); 2934 and 2868 (ν_{Csat-H}); 2247 ($\nu_{C\equiv N}$); 1455 (δ_{CH_2}).

Methylphenylacetate, **7** was prepared by direct esterification of phenylacetic acid and methanol in acid medium. From 10 g (74 mmols) phenylacetic acid, 30 mL (740 mmoles) methanol and 6.5mL (11.6g, 120mmoles) H_2SO_4 7.8 g (70%yield) of **7** were obtained as a colorless liquid (b.p.= 110/15 mm Hg)

^{1}H-NMR: 7.36-7.17, m, 5H (phenyl); 3.65, s 3H (CH_3); 3.60, s, 2H (CH_2).

IR: 3032 (ν_{Car-H}); 2952 and 2843 (ν_{Csat-H}); 1740 ($\nu_{C=O}$); 1257 (ν_{C-O} acetate); 1160 (ν_{C-O} methyl ester).

1,1-D$_2$-2-phenylethanol, **8** was prepared by reduction of ester **7** with $LiAlD_4$, (99.9% D) in dry ether. From 5 g (33 mmoles) ester **7** and 1 g (24 mmoles) $LiAlD_4$ in 50 mL dry ether 3.5g (86% yield) labelled alcohol **8** were obtained as a colorless liquid (b.p. =104^{o}C/14 mm Hg).

^{1}H-NMR: 7.24, t, 2H (H^3 and H^5); 7.16, t, 1H (H^4); 7.15, d, 2H (H^2 and H^6); 2.83, s, 1H (OH); 2.74, s, 2H (CH_2- psn 2).

^{13}C-NMR: 138.48 (C^{ipso}); 128.78, 128.24 and 126.09 (phenyl); 62.47, cv (C^1); 38.74 (C^2).

IR (cm^{-1}): 3200-3500 (ν_{O-H}); 3064. ν_{Car-H}); 2947 and 2880 (ν_{Csat-H}); 1044 (ν_{C-O}).

1,1-D$_2$-2-phenylethylbromide, **9** was prepared according to the procedure described for the compound **5**. From 3.5 g (28.7 mmoles) labelled alcohol **8** and 7mL (20.1g, 74 mmoles) PBr_3 in 45 mL dry benzene 3.6 g (69% yield) of **9** were obtained as a colorless liquid (b.p. 94^{o}C/8 mm Hg).

^{1}H-NMR: 7.34-7.15, m, 5H (phenyl); 3.15, s, 2H (CH_2- psn 2).

IR (cm^{-1}): 3030 (ν_{Car-H}); 2948 and 2865 (ν_{Csat-H}); 1032 and 698 (ν_{C-Br}).

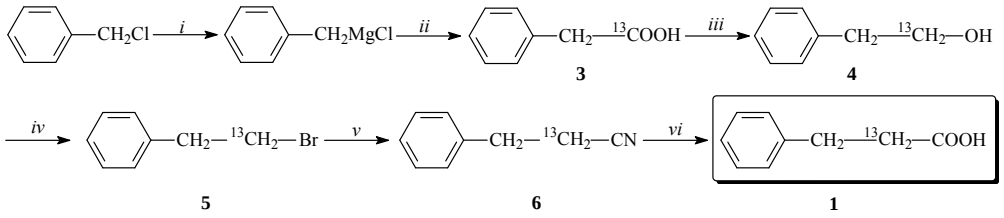
***2,2-D$_2$-3-phenylpropionitrile,* 10** was obtained according to the procedure described for the compound **6**. From 3.6 g (19 mmoles) bromide **9**, 1.23 g (26 mmoles) NaCN , 30 mL acetone and 1 mL H_2O, 2.1 g (87%yield) were obtained as a colorless liquid (b.p. =113/9mmHg).

^{1}H-NMR: 7.47-7.10, m, 5H (phenyl);2.19, s, 2H (CH_2- psn 2).

IR (cm^{-1}): 3032 (ν_{Car-H}); 2937 and 2870 (ν_{Csat-H}); 2250 ($\nu_{C\equiv N}$).

3. Results and discussion

2-^{13}C-3-Phenylpropionic acid, **1**, was synthesized by the 6 steps reaction sequence presented in *Scheme 1* using, as source of ^{13}C, a sample of $Ba^{13}CO_3$ of 98% purity. The total yield was 49% from the first labelled intermediate 1-^{13}C-phenylacetic acid, **3**.

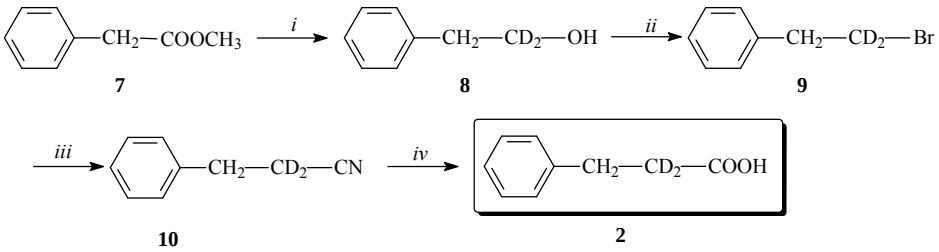


i: Mg/Et_2O; *ii*:$^{13}CO_2$; *iii*: $LiAlH_4$; *iv*: PBr_3; *v*:NaCN; *vi*:H_2O/HO^- (then H^+)

Scheme 1

The ^{13}C-content of 2-^{13}C-3-phenylpropionic acid was calculated from ^{1}H- and ^{13}C- NMR spectra and resulted 90±1%. The ^{1}H-NMR spectrum contains the signal from δ=2.67ppm (intensity 0.24) corresponding to CH_2 group from position 2 of unlabelled acid [1]; the signal from 2.45 ppm (intensity 1.10) consists of one side of the doublet of triplets of the same CH_2 from the ^{13}C-labelled acid. From the integral values an 89% ^{13}C-content was derived. Examining the peaks from ^{13}C-NMR spectrum a 91% ^{13}C-content resulted [2].

2,2-D_2-3-Phenylpropionic acid, **2**, was synthesized by the 5 steps reaction sequence presented in *Scheme 2* using, as source of deuterium, a sample of $LiAlD_2$ of 99.9% purity. The total yield was 47% from the first labelled intermediate 1,1-D_2-2-phenylethanol, **8**.

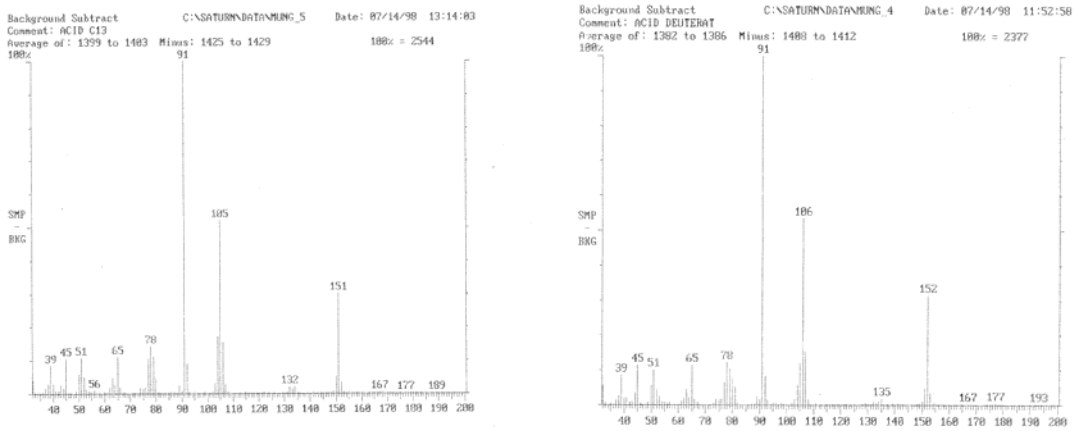


i: $LiAlD_4$; *ii*: PBr_3; *iii*: NaCN; *iv*: H_2O/ H^+

Scheme 2.

The deuteration degree of the 2,2-D_2-3-phenylpropionic acid, **2** resulted to be at least 99% because of the absence of the signal corresponding to CH_2 (psn2) in the ^{1}H-NMR spectrum as well as in the 1,1-D_2-2-phenylethanol intermediate [2]. The adopted reaction sequence proved to be more successful that direct deuteration with D_2O of the sodium salt of methyl-3-phenylpropionate which yielded only 86% D-content [1].

Mass spectra of the synthesized labelled 3-phenylpropionic acids are presented in Fig. 1.

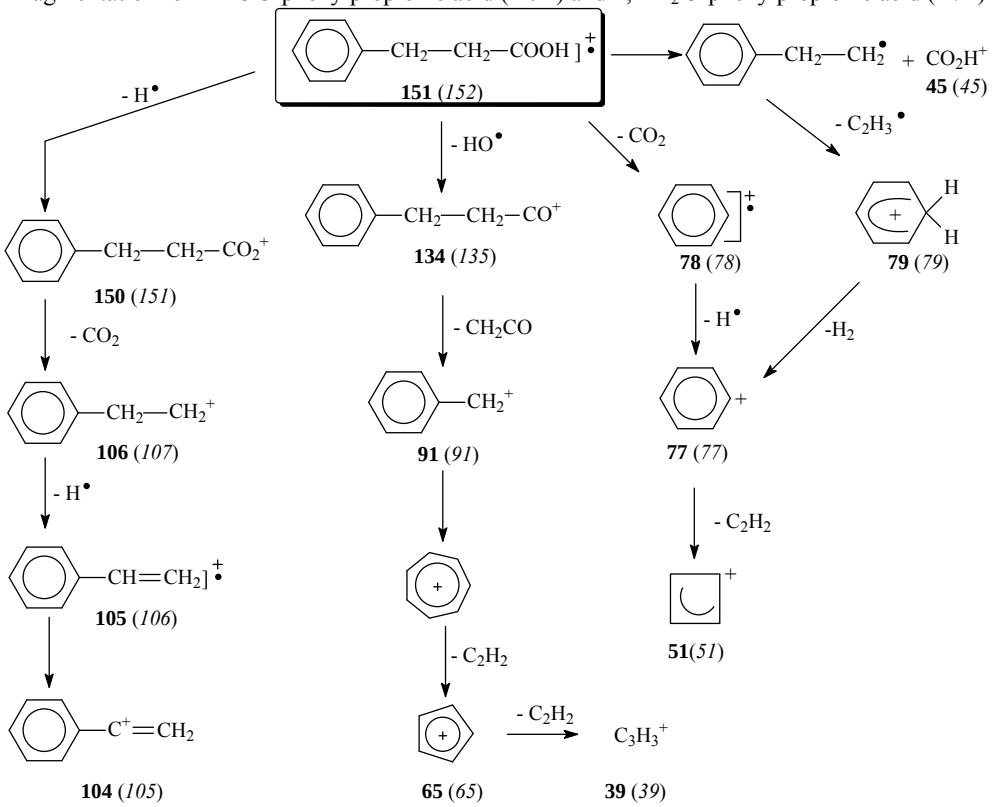


(a) (b)

Fig. 1. Mass spectra of 2-^{13}C-3-phenylpropionic acid (a) and 2,2-D_2-3-phenylpropionic acid (b)

All the peaks from the mass spectra of labelled 3-phenylpropionic acids were explained by the fragmentation pathway depicted in *Scheme 3*. In order to simplify the presentation unlabelled 3-phenylpropionic acid was taken as substrate and M/Z values are given separately for 2-^{13}C-3-phenylpropionic acid (**M/Z**) and 2,2-D_2-3-phenylpropionic acid (*M/Z*).

Fragmentation for 2-^{13}C-3-phenylpropionic acid (**M/Z**) and 2,2-D_2-3-phenylpropionic acid (*M/Z*)



Scheme 3

Mass spectra of trimethylsilyl derivatives of ^{13}C- and D-labelled 3-phenylpropionic acids obtained as intermediates in a biochemical process exhibits similar peaks [12].

4. Conclusions

We present the reasonable yields synthesis and characterization by NMR and mass spectra of 2-^{13}C-3-phenylpropionic acid and 2,2-D_2-3-phenylpropionic acid, respectively. A 90% ^{13}C-content and over than 99% deuteration degree were calculated from NMR spectra.

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