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Poly(β-3-methylmalic acid): A new degradable functional polyester with two stereogenic centers in the main chain

S. Cammas[1], I. Renard[1], J. P. Girault[2], and P. Guérin[1,*]

[1]Laboratoire de Chimie Biologique et Macromoléculaire URA CNRS 1467, ENSCR, Avenue du Général Leclerc, F-35700 Rennes, France
[2]Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, URA CNRS 400, Université René Descartes, 45 rue des Saints Pères, F-75270 Paris Cedex 06, France

Summary

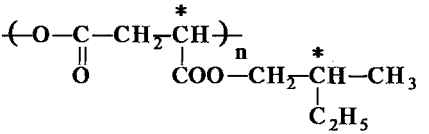
Racemic ((3S,4R)-(3R,4S))-3-methyl-4-benzyloxycarbonyl-2-oxetanone has been prepared by a simple and reproductible method starting from a racemic mixture of threo-((2S,3S)-(2R,3R))-3-methylaspartic acid as chiral precursor. This α, β substituted β-lactone has been polymerized anionically, using tetraethylammonium benzoate as initiator, to yield high molecular weight and amorphous racemic threo-poly(β-benzyl-3-methylmalate). The catalytic cleavage of protecting benzyl ester groups has been conducted in different solvents and racemic threo-poly(β-3-methylmalic acid) has been obtained in N-methylpyrrolidone at room temperature. Racemic threo-poly(β-3-methylmalic acid-co-benzyl-β-3-methylmalate) has been prepared by heterogeneous H_2 / Pd charchoal catalyzed partial hydrogenolysis of the polymer precursor. Solubility of these different materials has been considered. Hydrolysis of threo-poly(β-3-methylmalic acid) has conducted to racemic threo-3-methylmalic acid. High resolution ^{13}C NMR and selective INEPT NMR have been used for resonances assignment of polymers and copolymers. This new poly(β-hydroxy acid) type polyester expands the family of poly(β-malic acid) derivatives by opening the route for tailor making functional polystereoisomers with two stereogenic centers in the main chain and with the presence of an hydrophobic alkyl group and an hydrophilic carboxylic acid group in the macromolecule.

Introduction

The benefit of introducing stereogenic centers in the macromolecular chain of synthetic polymers has been recognized many years ago. However, investigations were primarily focused on studying chirality, conformation relationships and chiroptical properties (1). More recently, people started looking at the possibility for taking advantage of chirality to modify and adjust physical and mechanical properties of synthetic polymers. For example, in the field of bioresorbable polyesters aimed at drug delivery or surgery, VERT and coworkers have exemplified the effects of configurational structure on physical, mechanical and biological properties and consequently on the degradation rate in the case of a lactic acid stereocopolymers series (2). Results have been applied in several temporary therapeutic applications (3). For example, gentamycin / poly(lactic acid) blends aimed at sustained release local antibiotic therapy administered per-

*Corresponding author, present address: LPCB, UMR 27, Université Paris XII, CNRS, 2 à 8 rue Henri Dunant, F-94320 Thiais, France

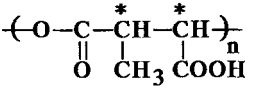
operatively have been recently studied (4). In the case of poly(β-hydroxyalkanoates), which can be prepared by chemical or biological synthesis routes, the presence of a chiral center in the main chain is essential in regard to the morphology of these materials, and to physical and thermal properties which are ensured by the repetition of (R)-hydroxyalkanoate monomer units in the macromolecular chain (5). Poly(β-malic acid) which is the parent compound of a large family of functional and reactive polymers and copolymers (6) includes also a chiral center in the monomer unit besides an cleavable bond. Optically active stereocopolymers are accessible starting from L - or D - aspartic acid enantiomers (7) or L- or D-malic acid enantiomers (8) as chiral precursors. Pure optically active poly(β-malic acid) can be produced by *"Physarum polycephalum"* (9), a myxomycete, or *Aureobasidium sp-A91* (10), a yeast. Moreover, poly(β-malic acid) derivatives containing an asymmetric carbon in the lateral ester groups have been prepared using 2-methyl-1-butyl malolactonate as monomer (11). The structure / property relationship of the different polystereoisomers (12) has shown, among other characteristics, that materials morphology was strictly depending on the enantiomeric composition of the main chain and of the ester pendant group.



Poly(β-2-methyl-1-butyl malate)

The second possibility for introducing a second stereogenic center in the polymer consists in the use of a chiral precursor with two asymmetric carbons conducting to main chains combining enantiomers and / or diastereoisomers.

In this paper, we want to present the preparation of a new degradable polyester, poly(β-3-methylmalic acid) PMMLA under threo racemic form (threo-PMMLA$^{50}H_{100}$) starting from DL-threo-3-methylaspartic acid. This amino acid is accessible by chemical synthesis and one stereoisomer is present in living organisms and appears in the metabolism of glutamate and citramalate in the presence of mesaconate. The choice of this new polymer is based on the concern to make compatible degradable polymeric materials with the living species during using time up to the degradation ultimate stage, by taking advantage of the biomass (13).



PMMLA

Moreover, the need for expanding the spectrum of available functional degradable polyesters is evident. It is important to achieve the required material properties for a specific temporary application by tailor-making biomedical and environmental polymers.

Experimental part

All chemicals were purchased from SIGMA and JANSSEN chemical.

DL-threo-2-bromo-3-methylsuccinic acid (2) : 20 g (0.136 mol) of DL-threo-3-methylaspartic acid were introduced in a round-bottomed three necked flask with 282 cm^3 of H_2SO_4 2N and 77 g (5.5 eq) of NaBr ; the mixture was cooled in an ice bath. 11.3 g (1.2 eq) of $NaNO_2$ was smothly added, the suspension was stirred magnetically at 0°C for 30 min. Then, 1.41 g (1.2 eq) of urea were added. The mixture was extracted with three portions of ethyl acetate. The combined organic solutions were dried over $MgSO_4$ and charcoal. After 2 hours, the suspension was filtered over celite and evaporated to give crude product. 2 was again crystallized in acetonitrile, filtered and washed with dichloromethane to give pure as a white solid. $HOOCCHBrCHCH_3COOH$: yield=73% (20.95 g) ; mp=124°C ; ^1H NMR (90 MHz ; CD_3COCD_3 ; δ ppm) : 1.36-1.44 (d, 3H) ; 2.92-3.26 (m, 1H) ; 4.51-4.61 (d, 1H) ; 10.97 (s, 2H). ^{13}C NMR (22.5 MHz ; CD_3COCD_3 ; δ ppm) : 14.48 ($\underline{C}H_3$) ; 45.62 ($\underline{C}H$) ; 50.86 ($\underline{C}H$) ; 185.00 ($\underline{C}O$) ; 189.44 ($\underline{C}O$).

DL-threo-2-bromo-3-methylsuccinic acid anhydride (3) : 20 g (0.095 mol) of DL-threo-2-bromo-3-methylbromosuccinic acid 2, dried under vacuum at 40°C, were dissolved in dried THF (35.5 ml), placed in a round-bottomed flask (0°C). Trifluoroacetic anhydride (TFAA) 14.07 ml (1.2 eq) were added, the suspension was stirred magnetically (1 hour at 0°C and 3 hours at room temperature). Trifluoroacetic acid and TFAA excess were removed by vacuum distillation to give 3.

Mixture of DL-threo-2-methyl-3-benzyloxycarbonyl-3-bromo propanoic acid (4) and DL-threo-2-bromo-3-benzyloxycarbonyl-3-methylpropanoic acid (5) 3 was immediately kept under nitrogen atmosphere. Then, 3 was dissolved in 1 eq of anhydrous benzyl alcohol (9.76 ml). The mixture was stirred overnigth at 70°C. The oil was dissolved in diethylether and washed with three portions of water and 1 portion of brine. The organic layer was dried over $MgSO_4$, filtered and evaporated to give crude $C_6H_5CH_2CO_2CHBrCHCH_3CO_2H$ 4 (75%)+ $HOOCCHBrCHCH_3CO_2CH_2C_6H_5$ 5 (25%) (oil) : yield=96% (27.6 g) ; ^1H NMR (90 MHz ; CD_3COCD_3 ; δ ppm) : 1.22-1.30 (d, 3H) ; 2.83-3.16 (m, 1H) ; 4.44-4.54 (d, 1H) ; 5.02 (s, 2H 5) ; 5.07 (s, 2H 4) ; 7.24 (s, 5H 4+5). ^{13}C NMR (22.5 MHz, CD_3COCD_3, δ ppm) : 16.00 ($\underline{C}H_3$) ; 44.30 ($\underline{C}H$) ; 49.02 ($\underline{C}H$) ; 67.68 ($\underline{C}H_2$ 5) ; 68.41 ($\underline{C}H_2$ 4) ; 129.25-137.35 ($\underline{C}_6H_5$) ; 169.99-174.62 ($\underline{C}O$).

DL-threo-3-methyl-4-benzyloxycarbonyl-2-oxetanone 6 : 20 g (0.066 mol) of mixture of esters 4 and 5 were dissolved in diethylether and 70 cm^3 of water were introduced in a beaker. The suspension was strongly stirred magnetically and a solution of sodium hydroxyde 2N was added up to pH=7.2. Then, the mixture was decanted. The aqueous layer was placed in a round-bottomed flask with 140 cm^3 of dichloromethane. The mixture was strongly stirred at 37°C during 4 hours. After decantation, the aqueous layer was washed with 3 portions of CH_2Cl_2. The combined organic solutions were washed with 3 portions of water and 1 portion of brine, dried over $MgSO_4$ filtered and evaporated to give crude as an yellow oil 8.4 g (82% yield). Chromatography on silica gel with 90% CH_2Cl_2 in petroleum ether

gave 6.82 g of this β-lactone β-substitued. Then 6 was distilled under vacuum in a short pass-still column to give pure monomer 6 as an clear oil : yield=20 % (2.04 g) ; ^{1}H NMR (90 MHz, CD_3COCD_3, δ ppm) : 1.43-1.51 (d, 3H) ; 3.81-4.45 (m, 1H) ; 4.80-4.87 (d, 1H) ; 5.26 (s, 2H) ; 7.40 (s, 5H) ; ^{13}C NMR (22.5 MHz, CD_3COCD_3, δ ppm) : 12.96 ($\underline{C}H_3$) ; 53.11 ($\underline{C}H$); 68.33 ($\underline{C}H2$) ; 73.77 ($\underline{C}H$) ; 129.49-136.78 ($\underline{C}_6H_5$) ; 169.23-171.21 ($\underline{C}O$).

Threo-poly(β-benzyl-3-methylmalate) : $PMMLA^{50}Be_{100}$ was synthesized by polymerizing 2 g of monomer in the presence of initiator (10^{-3} mole per mole of monomer) under nitrogen at 37°C during 3 days. The end of polymerization was controled by I.R. After cooling, crude resulting material was dissolved in acetone and precipited by addition of ethanol. After separation, polymer was dried under vacuum ; ^{1}H NMR (300 MHz, CD_3COCD_3, δ ppm) : 1.00-1.20 (m, 3H) ; 3.30 (m, 1H) ; 5.05-5.20 (m, 2H) ; 5.50-5.63 (2m, 1H) ; 5.75 (m, 1H) ; 7.32 (s, 5H) ; ^{13}C NMR (75 MHz, CD_3COCD_3, δ ppm) : 10.43-11.07 ($\underline{C}H_3$) ; 41.00 ($\underline{C}H$) ; 67.96 ($\underline{C}H_2$) ; 72.81 ($\underline{C}H$) ; 129.32-136.42 ($\underline{C}_6H_5$) ; 168.43 ($\underline{C}O$: side chain) ; 171.14 ($\underline{C}O$: main chain).

The selective INEPT experiments have been carried out by using conditions described by GUERIN and col. (14) : the polymer was dissolved in CD_3COCD_3, ^{1}H and ^{13}C NMR spectra were recorded at 305 K, on Bruker WM-250-MHz or AM-300-MHz spectrometers using 5-mm sample tubes.

Threo-poly(β-3-methylmalic acid) : $PMMLA^{50}H_{100}$ was obtained by the catalytic hydrogenolysis of $PMMLA^{50}Be_{100}$. After dissolution of the polymer in NMP with 20 % of palladium, the hydrogenolysis was conducted with hydrogen at room temperature during 24 hours. $PMMLA^{50}H_{100}$ was obtained after filtration over celite, evaporation of NMP and dried under vacuum ; ^{1}H NMR (90 MHz, CD_3COCD_3, δ ppm) : 1.18-1.37 (m, 3H) ; 3.33-3.40 (m, 1H) ; 5.59-5.75 (d, 1H) ; 6.55 (s, 1H) ; ^{13}C NMR (22.5 MHz, CD_3COCD_3, δ ppm) : 11.15 ($\underline{C}H_3$) ; 41.89 ($\underline{C}H$) ; 73.59 ($\underline{C}H$) ; 170.34 ($\underline{C}O$: main chain) ; 172.18 ($\underline{C}OOH$).

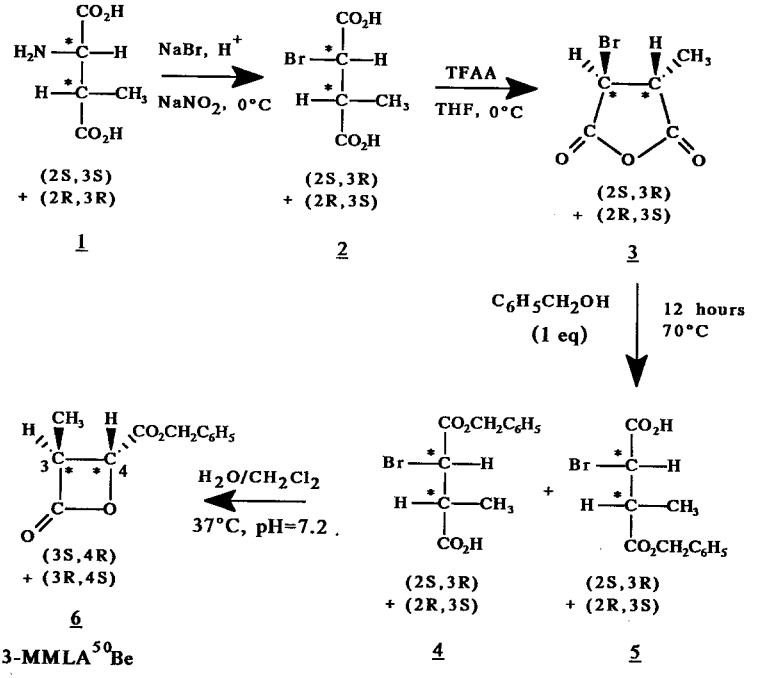
Threo-poly(β-3-methylmalic acid-co-benzyl-β-3-methylmalate) : Different compositions of copolymers were obtained by partial catalytic hydrogenolysis of $PMMLA^{50}Be_{100}$. The polymers were dissolved in dioxane or dimethylformamide, and 20% of palladium were added. The hydrogenolysis was conducted with hydrogene at different temperatures. Copolymers were obtained after filtration over celite, evaporation of solvent and dried under vacuum ; ^{1}H NMR : (90 MHz ; CD_3COCD_3 ; δ ppm) : 1.10 (m, 3H) ; 3.32 (m, 1H) ; 5.16 (s, 2H) ; 5.57-5.74 (d, 1H) ; 7.31 (s, 5H), for a copolymer 50/50.

Discussion

The preparation of racemic ((3S,4R)-(3R,4S))-3-methyl-4-benzyl-oxycarbonyl-2-oxetanone, or benzyl 3-methylmalolactonate (3-MMLABe), was based on the chemical synthesis route which was adjusted for optically active benzyl malolactonate. The presence of the methyl group in β-position does not change the reactivity of the intermediate compounds in the preparation of the α, β-

substitued β-lactone, which was obtained with 20% yield in relation to the lactonizable 2-bromo-3-methylsuccinate of benzyl 4. More important is to consider the stereochemical development of the synthesis (scheme 1). In the first step, racemic threo-3-methylaspartic acid was transformed by substitution of the amino group with the bromine atom, with configuration retention (double configuration inversion (15)) of the carbon 2 conducting to threo form of 2-bromo-3-methylsuccinic acid. The ring closure of 4 occurs with inversion of configuration (3) and starting from threo-L-(2S,3S)-3-methylaspartic acid, (3S,4R)-3-MMLABe is obtained.The two groups : methyl and benzyloxycarbonyl are in trans position in relation to the average plan of the lactone. In our case, the precursor being a racemic mixture of threo-DL-3-methylaspartic acid, 3-methylmalolactonate of benzyl is composed of (3S,4R) and (3R,4S) enantiomers in equal quantities : racemic (threo-3-MMLABe). This compound has been compared with erythro-3-MMLABe obtained in very low quantity from malic acid (16). ^1H NMR spectra display peaks at different chemical shifts for all protons, except benzyl protons. Moreover coupling constants for CH signal (threo : ^3J=4.3 Hz, erythro : ^3J=7.1 Hz) (C_3 and C_4) are in agreement with the present stereochemical structures. The α, β-substitued β-lactone has been polymerized in bulk at 37°C with benzoate tetraethylammonium as initiator. The presence of bulky methyl groups in the main chain does not modify the polymerization which is complete in 3 days.

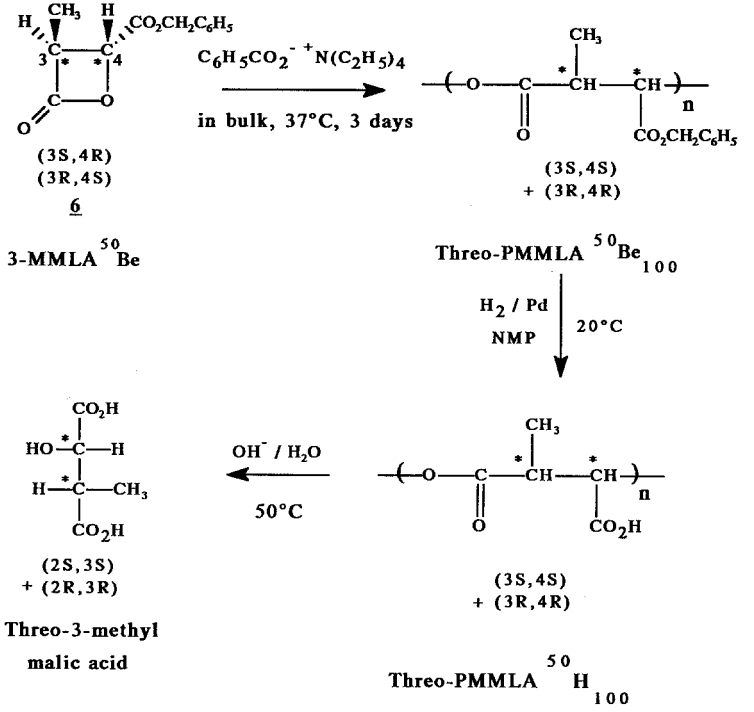
SCHEME 1 : Synthesis of 3-MMLABe from 3-methylaspartic acid



Very high molecular weight (Msec=785 000) racemic polymer (threo-PMMLA^{50}Be$_{100}$) are obtained ; this polymeric material is amorphous (Tg = 42.5°C) and soluble in acetone, CH_2Cl_2, $CHCl_3$, CCl_4, benzene, dioxane, ethyl acetate, N-methyl pyrrolidone (NMP) and dimethylformamide (DMF).

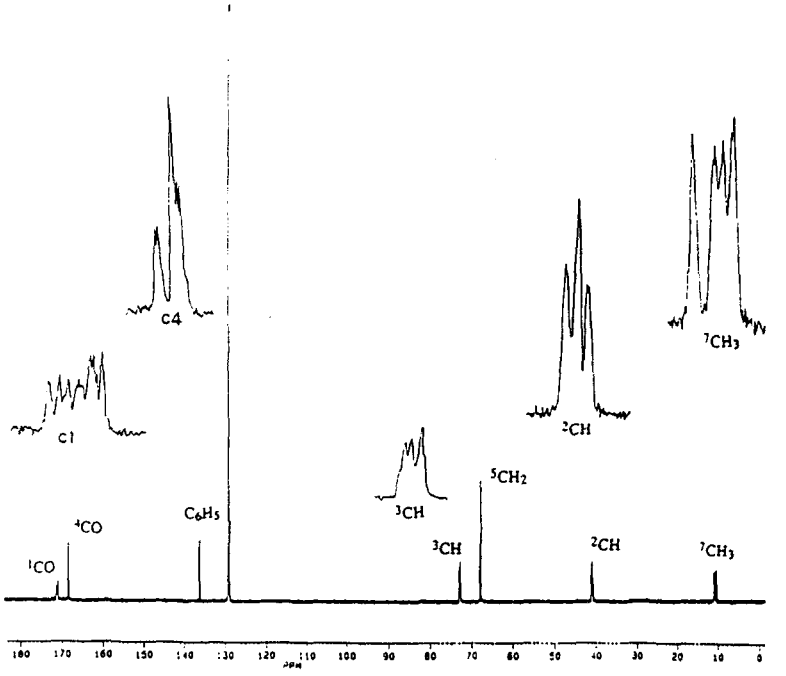
Ring opening polymerization proceeds with configuration inversion of the 3-MMLABe C_4 carbon atom as in the case of poly(β-malic acid esters); the ultimate degradation product of the polymer is definitively racemic threo-3-methylmalic acid as determined by ^{1}H NMR (scheme 2).

Scheme 2 : Preparation of threo-PMMLA^{50}H$_{100}$ and its degradation



^{1}H NMR spectrum of threo-PMMLA^{50}Be$_{100}$, in CD_3COCD_3, displays stereosensitivity, diastereotopic and conformational effects : three signals in the ratio (2/1/1) correspond to ^{3}C<u>H</u> (5.75, 5,62, 5,50 ppm) and signals assigned to ^{7}C<u>H</u>$_3$ and ^{5}C<u>H</u>$_2$ are very complex. An similar complexity concerns the threo-PMMLA^{50}Be$_{100}$ ^{13}C NMR spectrum as shown on figure 1. Assignment of the different signals has been conducted using DEPT sequence. Line narrowing by the enhanced resolution technique and modification of the field scale have revealed the presence of fine structures which can be explained by the same different effects found in the ^{1}H NMR study. For example, C_7 corresponding to the lateral methyl carbon atom displays one peak at 11,07 ppm and multiple peaks centered at 10,43 ppm.

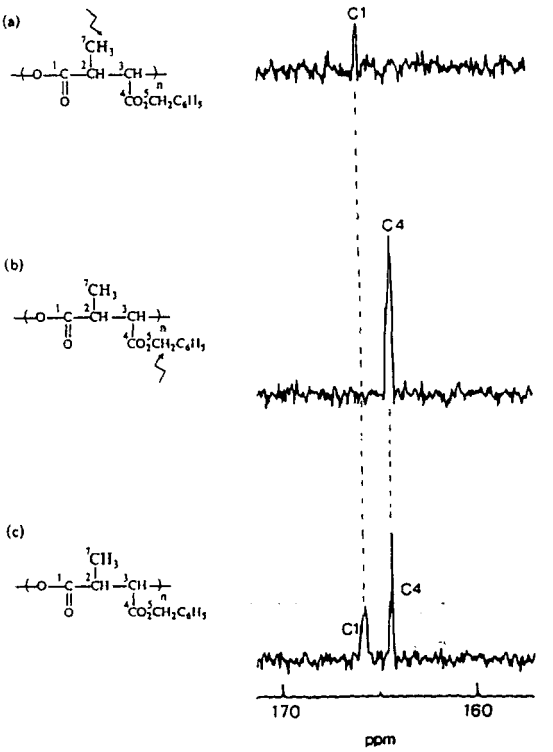
Figure 1 : ^{13}C NMR spectrum of threo-PMMLA$^{50}Be_{100}$ at 75.47 MHz in CD_3COCD_3.



The carbonyl carbon atoms region displays two groups of peaks corresponding to C_1 and C_4 (figure 2, (a)) ; assignment of these two carbonyl carbon atom resonances has been completed by using selective INEPT (selective insensitive nuclear enhancement by polarization transfer) (14). Selective radiofrequency pulses were applied to CH_2 of benzyl ester groups or to CH_3 of methyl ester groups.

Under these conditions and by setting in the pulse sequences $\Delta 1$ and $\Delta 2$ delays related to long range C--H heteronuclear coupling constants of c.a 5 Hz, the pulse sequence can transfer proton magnetization to ^{13}C nuclei which have a significant long range scalar interaction with the selected protons. The detectable selective INEPT signal corresponded to C4 determining three bands connectivity by bridging one oxygen nucleus, CH_2 groups of the benzyl substituent being pulsed (figure 2 (b)). After selective INEPT sequence with ^1H selective pulse on CH_3, only one signal corresponding to C_1, the carbonyl carbon atom of the main chain is present (figure 2 (c)). Spectra simplification and study of microstructures will be carried out on pure polystereoisomers (17).

Figure 2 : ^{13}C NMR spectra (carbonyl atom region) of threo-PMMLA$^{50}Be_{100}$ in deuterated acetone (a) after selective INEPT sequence with ^{1}H selective pulse on CH_3 ; (b) after selective INEPT sequence with ^{1}H selective pulse on CH_2 of benzyl group ; (c) normal conditions.



Conversion of threo-PMMLA$^{50}Be_{100}$ to racemic threo-poly(β-3-methylmalic acid), threo-PMMLA$^{50}H_{100}$, was carried out by catalytic hydrogenolysis in N-methylpyrrolidone at room-temperature, using 20% palladium-charcoal catalyst. ^{1}H NMR spectrum of deprotected compound has shown the absence of residual benzyl groups. Threo-PMMLA$^{50}H_{100}$ is soluble in : acetone, ethanol, methanol, water, NMP, and can be turned to sodium salt by addition of suitable amount of NaOH 1M to be stored. ^{13}C NMR spectrum in D_2O has displayed too stereosensitive carbons.

Copolymers containing benzyl ester groups and carboxylic acid pendant groups in different proportions can be prepared by partial hydrogenolysis of threo-

PMML$A^{50}Be_{100}$ (PMML$A^{50}Be_xH_{100-x}$) in dioxane, dimethylformamide (DMF) or acetone, at different temperatures (20°C<T<50°C).

These compounds have presented versatile solubility properties which could be explained by the presence of different repeat unit distribution (long blocks and random copolymers) as shown in the case of partially hydrogenolized PML$A^{50}Be_{100}$ (20). Moreover, catalytic hydrogenolysis seems to preferably operate along a macromolecular chain : at 20°C in dioxane, the hydrogenolysis has conducted to two types of polymers : one was soluble in acetone and corresponding to PML$A^{50}Be_{95}H_5$ and the other soluble in D_2O corresponding to PML$A^{50}H_{100}$. More investigations are under study.

Conclusion

By analogy with living species which are able to achieve suitable structural materials properties by retaining the macromolecular skeleton and the pendant groups being fitted (protein, polysaccharides), it seems desirable to apply this concern to biodegradable polymers and for tailor making biomedical an environmental polymeric materials. We have previously shown that using poly(β-malic acid) as skeleton, it was possible not only to vary the nature of the functional pendant groups, but also to introduce an lateral alkyl group which can expand the spectrum of properties. Moreover, the synthesis of new α, β substitued β-lactones with two chiral centers and the possibility of ring opening polymerization conducts to uncommun polymers with two stereogenic centers. The next step will be the preparation and the characterization of the different PMMLA polystereoisomers by using chemical and biological routes for accessing to the optically active corresponding 3-methylmalolactonate and by changing the nature chemical structure of the lateral alkyl group. The tool for building versatile polymeric temporary devices will be therefore in place.

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