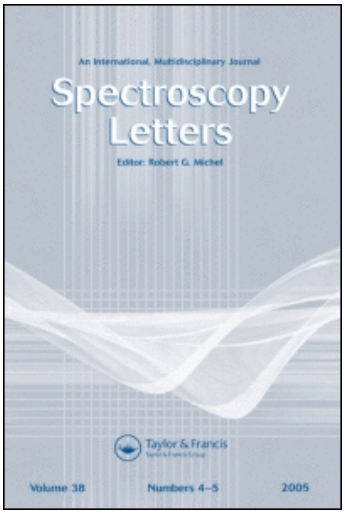

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^{13}C NMR of Some Malic Acid Derivatives

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^{13}C NMR OF SOME MALIC ACID DERIVATIVES

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ABSTRACT

The ^{13}C nmr for a number of malic acid esters and amide-esters have been recorded. These data confirm that alcoholysis of malic anhydride provides the α-monoester as the only isolable product.

INTRODUCTION

The structural similarity of malic acid (1) to aspartic acid suggests that peptides incorporating a malic acid residue might possess interesting biological activities[1]. The efficient construction of such peptides would depend on methodology for regioselective condensation of the two carboxyl groups of malic

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acid. To this end we were able to isolate only one monoesterified product following alcoholysis of the corresponding anhydride 2 with benzyl alcohol. From the results of a series of reactions with this monoester,[1] and by analogy with the findings of an earlier study with acidic amino acids,[2] the α-carboxylate structure 3 for the monoester was inferred.

In this paper the ^{13}C nmr spectra of a number of malic acid derivatives which may serve as useful synthetic intermediates are reported. The data presented here confirm the structure 3 in an independent, and relatively straightforward, manner. The synthesis of those derivatives is also reported in detail.

EXPERIMENTAL

^{13}C nmr spectra were obtained at ambient temperature on a Varian CFT-20 spectrometer with a spectral width of 4000 Hz and 8K data points (^{1}H-decoupled and off resonance) or 2000 Hz and 16K (^{1}H-coupled). TMS was the internal standard for the $CDCl_3$ solutions and dioxane (67.4 ppm from TMS) was used with the D_2O solutions. $^{1}HNMR$ spectra were obtained on a Varian T-60 spectrometer.

Compounds 1 and 4 were available from commercial sources (Sigma Chemical Co., St. Louis, MO, USA).

Malic anhydride (2) was prepared from malic acid as follows: to a cold stirred ammoniac solution (10 ml, 4N) of malic acid (2.68 g, 20 mM), a solution of $AgNO_3$ (6.80 g, 40 mM) was added. After 30 min, the precipitated dissilver salt of malic acid was

filtered, washed repeatedly with H_2O, and dried over P_2O_5 in the dark (yield: 75%). To a suspension of the dissilver salt (3.6 g, excess) in ether (30 mL), thionyl chloride (1.19 g, 10 mM)was added. The mixture was shaken thoroughly for 15 min, filtered and the filtrate was evaporated, to afford malic anhydride (0.82 g, 70%).

The monoester of malic acid (3) was prepared by addition of benzyl alcohol (2.16 g, 20 mM) in a solution of malic anhydride (1.16 g, 10 mM) in ether (20 mL). The solution was stirred at room temperature for 24 h, refluxed for 10 h,then evaporated and the remaining residue was dissolved in a mixture of H_2O/CH_2Cl_2 (50 ml, 1/1). The organic phase was washed with H_2O (25 mL), dried (Na_2SO_4) and evaporated. The residue (1.72 g) was redissolved in ether, then dicyclohexylamine (1 mL) was added dropwise and the precipitated dicyclohexylamine salt of the monoester was collected by filtration. Recrystallization from ethyl acetate, afforded 1.25 g (30%), m.p.(ℓ) = 153 - 4°C, m.p.(dℓ) = 140 - 1°C. The salt was treated with 10% citric acid to afford the free monoester of malic acid (3), which was extracted from the H_2O phase, with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and evaporated to yield 3 in a pure state.

The dibenzyl ester of malic acid (5) was prepared by adding benzyl bromide (13,68 g, 80 mM) in a solution of malic acid (5.36 g, 40 mM) and triethylamine (8.08 g, 80 mM) in THF (100 mL). The mixture was stirred for 24 h at room temperature and refluxed for another 10 h. The solvent was then evaporated, H_2O was added

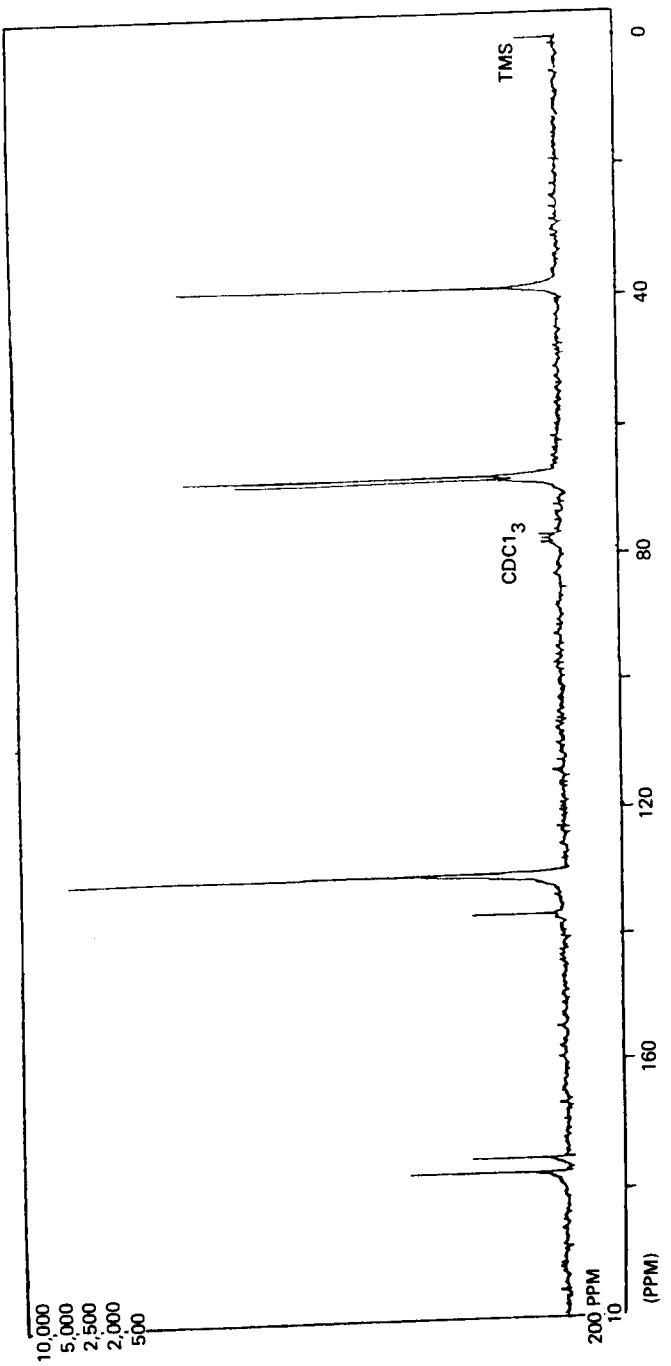


Fig. 1 ^{13}C NMR spectrum of malic acid, α-benzyl ester, in $CDCl_3$ and TMS.

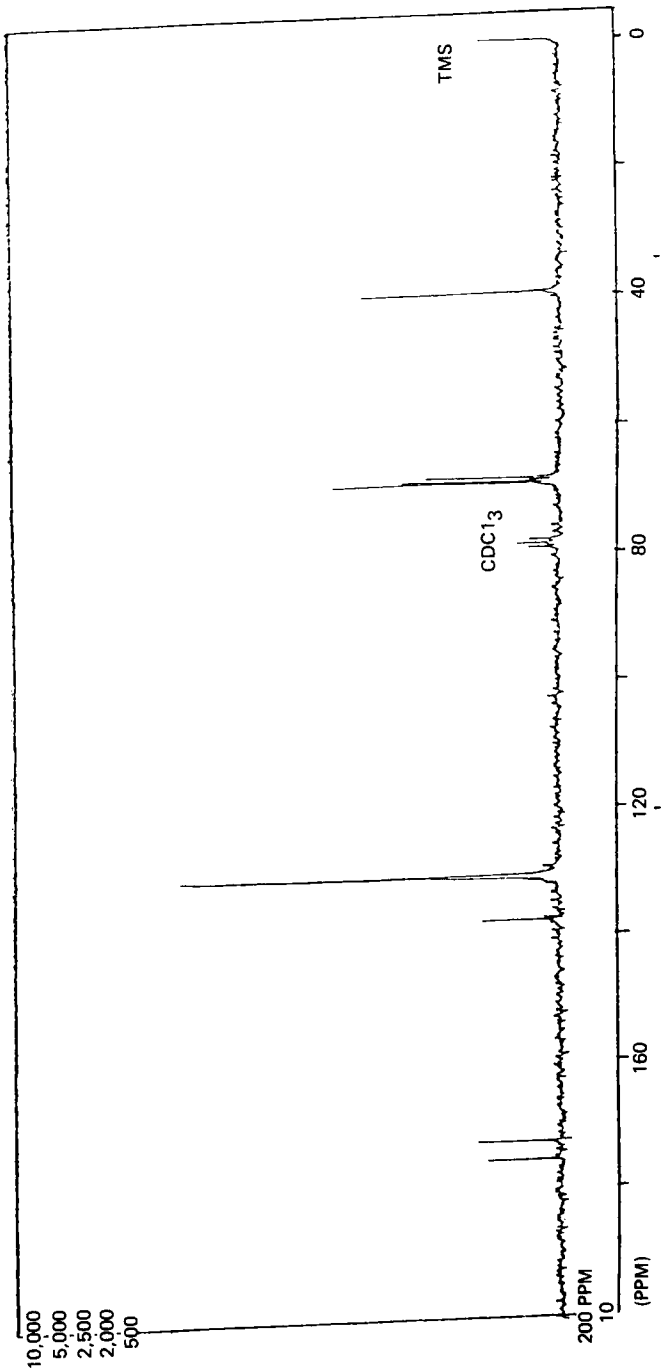


Fig. 2 ^{13}C NMR spectrum of malic acid, dibenzyl ester, in $CDCl_3$ and TMS.

and the dibenzyl ester was taken by extraction with CH_2Cl_2. The organic phase was washed with H_2O, 5% $NaHCO_3$, dried (Na_2SO_4) and evaporated. The remaining residue (12.5 g) was subjected to silica gel column chromatography and the product was purified by elution with Hexane/Chloroform (7/3) (Yield: 65%).

The β-amide 6 was obtained by condensation of the mixed anhydride[1,4,5] derived from the monoester 3, with ammonia. Thus, to a cold (-10°C) solution of 3 (0.224 g, 1 mM) and triethylamine (1 mM) in THF (15 mL), ethylchloroformate, $ClCOOC_2H_5$, (1 mM) was added. After 10 min, ammonia was introduced to the solution for a 15 min period. The reaction flask remained sealed for two hours and then the solution was evaporated to dryness. The residue was partitioned between ethyl acetate (50 mL) and water (50 mL), and the organic phase was washed with 10% $NaHCO_3$ (50 mL), H_2O (50 mL), dried over Na_2SO_4 and evaporated. The product was crystallized from ether (Yield: 35%), m.p.(ℓ) = 78 - 79°C, m.p.(dℓ) = 92 - 93°C.

The β-amide 7 was obtained by DCC coupling of 3 with glycine benzylester. Thus, to a cold (-10°C) and stirred solution of 3 (0.224 g, 1 mM) and 1-hydroxybenzotriazole (0.135 g, 1 mM) in DMF (3 mL), dicyclohexylcarbodiimide (0.206 g, 1 mM) was added. After 20 min, a solution of glycine benzylester, p-toluene sulfonate (0.337 g, 1 mM) and triethylamine (1 mM) in DMF (2 mL) was added. The mixture was stirred for 1 h at 0°C and for 24 h at 25°C, then it was filtered, the filtrate was evaporated, and the residue was partitioned between H_2O (30 mL) and ethyl acetate (30 mL). The organic phase was washed with 5% $NaHCO_3$, H_2O, 1% HCl,

H_2O, dried (Na_2SO_4) and evaporated. To the remaining oily residue, ethyl acetate (1 mL) was added and the precipitated salt was removed by filtration. Evaporation of the solvent and addition of ethyl acetate (1 mL) was repeated until no more salt was formed. The oily residue was crystallized from ethyl acetate/P. ether (Yield: 70%), m.p.(ℓ) = 82 - 83°C, m.p.(dℓ) = 73 - 74°C.

O-Trityl-Dibenzyl-Malate (8) was prepared by refluxing a solution of 5 (3.14 g, 10 mM) and trityl chloride (4.17 g, 15 mM) in pyridine (10 mL) for 4 h. The solvent was evaporated and the residue was treated with 10% citric acid and extracted with CH_2Cl_2. The organic phase was washed with 10% citric acid, H_2O dried (Na_2SO_4) and evaporated. The oily trityl derivative was purified by gel filtration chromatography (Sephadex LH-20) and ethyl acetate as the eluant (Yield: 40%).

RESULTS AND DISCUSSION

Table 1 summarizes the chemical shift data. The effect of replacement of a C-2 hydrogen in succinic acid (4) with an hydroxyl (→ malic acid 1) obliges the β-carboxyl group (C-4) to experience at least one γ-gauche interaction in all three staggered conformers; therefore, the β-carboxyl resonance is found upfield of the α-carboxyl (C-1) resonance in the spectrum of malic acid (1). Similarly, in the spectrum of the dibenzyl ester 5 the C-4 signal is found upfield of the C-1 signal. With the chemical shifts of malic acid (1) and its diester 5 in hand, the shifts of the two carboxyl resonances in the spectrum of the monoester suggested the structure 3.

TABLE 1. Chemical shifts of malic acid derivatives

Compound	Shift (δ)[a] C-1	C-2	C-3	C-4	Solvent
$\underset{\sim}{1}$	177.1	67.7	39.2	175.2	D_2O
$\underset{\sim}{3}$	173.0	67.1	38.4	175.6	$CDCl_3$
$\underset{\sim}{4}$	177.6	29.6	29.6	177.6	D_2O
$\underset{\sim}{5}$	173.1	67.4	38.8	170.2	$CDCl_3$
$\underset{\sim}{6}$	173.3	67.2	39.6	173.6	$CDCl_3$
$\underset{\sim}{7}$	173.2	67.8	39.8	170.1[b]	$CDCl_3$
$\underset{\sim}{8}$	169.5[c]	70.5	39.1	171.2[c]	$CDCl_3$

a. Additional signals (δ) (multiplicity in the off-resonance spectra):

$\underset{\sim}{3}$: 134.9(s), 128.7(d, 3C), 128.4(d, 2C) and 67.9(t)

$\underset{\sim}{5}$: 135.6(s), 135.1(s), 128.6(d, 4C), 67.6(t), and 66.7(t)

$\underset{\sim}{6}$: 135.3(s), 128.6(d, 2C), 128.4(d), 128.2(d, 2C), & 67.8(t)

$\underset{\sim}{7}$: 169.7[b](s), 135.2(s, 2C), 128.6(d, 5C), 128.4(d, 5C), 67.6(t), 67.3(t), and 41.4(t)

$\underset{\sim}{8}$: 143.7(s, 3C), 135.7(s), 135.5(s), 127.3-129.1(many d)

b, c. Assignments may be interchanged.

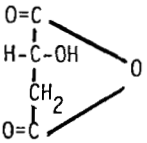
The ^{13}C-^{1}H coupling constants in malic acid ($\underset{\sim}{1}$) and the monoester were compared in order to unequivocally assign the carboxyl signals in the monoester. Gil[3] had reported that in the ^{1}H-coupled spectrum of malic acid C-4 appears as a triplet of doublets due essentially to equal coupling to both geminal protons; furthermore, both the $^{3}J_{C-H}$ and the $^{2}J_{C-H}$ couplings are approximately 6 Hz at

TABLE 2. ^{13}C-^{1}H coupling constants for malic acid 1 and the monoester 3.

Compound	^{13}C-^{1}H Coupling Constants (Hz)[a]					
	C-1	C-2		C-3		C-4
1	unresolved multiplet	^{1}J 146.6 ^{2}J 4.1		^{1}J 130.9 ^{2}J 4.1		^{2}J 6.5 ^{3}J 6.5
3	unresolved multiplet	^{1}J 147.9 ^{2}J 4.0		^{1}J 131.0 ^{2}J 3.5		^{2}J 6.8 ^{3}J 6.2

a. The signs were not determined.

TABLE 3. Malic Acid Derivatives.



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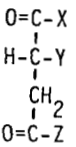

	X	Y	Z
1	OH	OH	OH
3	OBz	OH	OH
4	OH	H	OH
5	OBz	OH	OBz
6	OBz	OH	NH_2
7	OBz	OH	$NHCH_2CO_2Bz$
8	OBz	$OC(C_5H_5)_3$	OBz

lower pH. In contrast, $^{3}J_{C-H}$ couplings between C-1 and the two C-3 protons are significantly different in magnitude, resulting in a complex pattern with all coupling constants smaller than 5 Hz.

Our data for malic acid (1) (Table 2) were similar to those of Gil. On our 20 MHz instrument, the signal for C-4 appeared as a quartet (i.e. $^{2}J_{C-H} \simeq {}^{3}J_{C-H} \simeq 6.5$ Hz), and the signal for C-1 was a narrow, unresolved multiplet. In the spectrum of the monoester, the signal assigned to C-4 was a broadened quarter, whereas the upfield carboxyl signal (C-1) was a narrow multiplet.

The β-amides 6 and 7 were obtained by condensations of mixed anhydrides[1,4,5] derived from the monoester 3 with ammonia and glycine benzyl ester, respectively. We were unable to assign C-4 and the glycine carboxyl signals of 7 rigorously by examination of its ^{1}H-coupled spectrum due to the small amount of material available. As expected, addition of the bulky and anisotropic trityl group to the C-2 hydroxyl of the diester to form 8 resulted in significant changes in the chemical shifts of both groups.

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