Investigation of the phosphinyl analog of α-ketoglutaric acid

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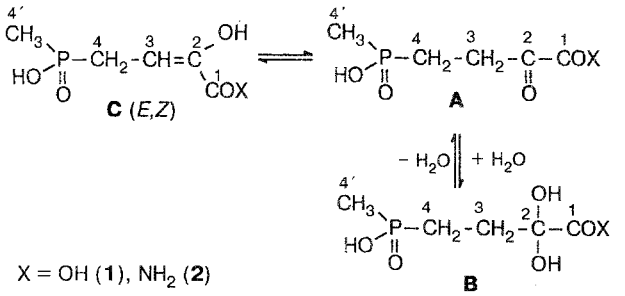
It was shown by ^{31}P and ^{13}C NMR spectroscopy that methyl(3-carboxy-3-oxopropyl)phosphinic acid (4-methylhydroxyphosphinyl-2-oxobutyric acid) (**1**) and the amide (**2**) of the latter exist in keto forms in non-aqueous solutions. In aqueous solutions an equilibrium between the keto, *gem*-diol, and enol forms has been observed. The proportions of the diol and enol forms increase as the acidity of the media increases. Silylation of acid **1** with hexamethyldisilazane gives the tris(trimethylsilyl) derivative of enol form (**3**) (*Z*- and *E*-isomers).

Key words: phosphinyl analog of α-ketoglutaric acid, α-ketocarboxylic acids, Phosphinotricin, keto-enol tautomerism, *gem*-diols, NMR spectroscopy; *Z,E*-isomers.

The phosphinyl analog of α-ketoglutaric acid, (me-thyl(3-carboxy-3-oxopropyl)phosphinic (or 4-(methylhydroxyphosphinyl)-2-oxobutanoic) acid $Me(HO)P(O)CH_2CH_2C(O)COOH$ (**1**), is a biosynthetic intermediate of the highly effective herbicide Phosphinotricin, which is an active inhibitor of plant glutamine-synthetase.[1] The acid **1** does not have an antimetabolic effect on purified glutamine-synthetase, however, it possesses high herbicide activity.[2,3] This phenomenon can be explained by the *in vivo* transformation of the α-carbonyl group into an α-amino group with the participation of pyridoxal phosphate-dependent enzymes or glutamate dehydrogenase. It is known that in the case of α-ketoglutaric acid only one of its tautomeric forms,[4,5] *i.e.*, the keto form, can serve as a substrate for these enzymes.[5] Thus, the investigation of the forms of acid **1** and the corresponding amide **2** existing in solutions is of interest. The synthesis of α-keto acid **1** was described in patents,[2,3,6,7] but the problem of its tautomeric forms has not previously been formulated. However, the derivatives of the enol as well as the ketone form of acid **1** were obtained.[3] This fact allows one to assume that keto-enol tautomerism is a property of acid **1** in contrast to non-enolizable α-ketoglutaric acid.[5] It is known also that the β-cyano substituted analog of acid **1** *i.e.*, 4-(methylhydroxyphosphinyl)-3-cyano-2-oxobutanoic acid $Me(HO)P(O)CH_2CH(CN)C(O)COOH$ exists in the enol form in the crystal state as well as in solution.[8]

In the present paper acid **1** and amide **2** are investigated in solution by ^{31}P and ^{13}C NMR spectroscopy (Tables 1 and 2). It appeared that in non-aqueous media (acetone-d_6, DMSO-d_6) only the keto forms (**A**) of these compounds were registered (Scheme 1).

Scheme 1



Singlets at 55.3 and 49.5 ppm, respectively, are observed in the ^{31}P NMR spectra (Table 1). The signal systems in the ^{13}C NMR spectra are similar to those of α-ketoglutaric acid, which exists in the keto form.[5] Of course, this comparison takes in account the effect of the phosphorous atom on the values of the chemical shifts (δ) and the multiplicities of the signals. The spectrum possesses doublets at 14.10 ppm (**1**) and 15.15 ppm (**2**), which correspond to the C(4′) atoms of the CH_3-groups, and in the 23 ppm region, which correspond to the C(4) atom of the CH_2P-fragment, and they have spin coupling constants $^1J_{PC}$ of 93—97 Hz. The singlets of the C(3) atoms of the methylene fragments bonded to the keto group have δ 31.82 ppm (**1**) and 30.20 ppm (**2**), respectively. The signals of the α-carbons C(2) exhibit doublets at 194.0 ppm (**1**) and 198.2 ppm (amide **2**) with the characteristic spin coupling constant $^3J_{PC}$ 13.0—13.8 Hz.[9] The signals of the carboxyl carbon atoms are of singlet character and they

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are shifted upfield *ca.* 30 ppm relative to the signal of the carbonyl carbon to 161.7—163.2 ppm region.

At the same time four closely located signals are observed in the ^{31}P NMR of aqueous solutions of acid **1** and amide **2**; their integral intensities vary with variation in the pH of the solutions (Table 2, Fig. 1). On the basis of the ^{13}C NMR spectra of these solutions and the literature data[5,8] the observed signals were assigned to the keto form (**A**), the hydrate *gem*-diol form (**B**) and the *Z,E*-isomers of the enol form (**C**). The **A** and **B** forms predominate, and the percentage of the enol form is 15 % for acid **1** and 6 % for amide **2**, respectively.

In the ^{13}C NMR spectra there was a corresponding increase in the number of doublets of the carbon atoms of the CH_3P- and CH_2P-groups and singlets of the C(3) carbon atoms were observed.* Along with the signal of the ketone function, slightly shifted downfield (acid **1**) or upfield (amide **2**) due to the solvent effect, the signal in 94 ppm region, characteristic of the *gem*-diol C atom of the hydrate form,[5] exists, and its spin coupling constant $^3J_{PC}$ is 18.4—18.9 Hz. The signals of the olefinic carbon atoms of the enol form C of the acid **1** (two pairs of doublets at 104.5—106.8 ppm and in the 139.7—142.7 ppm regions) were assigned to two geometrical isomers on the basis of previously published data[8,9].

* The signals of the C atom of the enol form (C) of amide **2** do not exceed noise level as a result of the low concentration of the tautomer.

Table 1. The ^{31}P and ^{13}C NMR parameters of the compounds **1—4**

Compound	Form	Content (%)	Solvent	^{31}P NMR δ, ppm	^{13}C NMR (δ, *J*/Hz)				
					δ C(4′) ($^1J_{PC}$)	δ C(4) ($^1J_{PC}$)	δ C(3) ($^2J_{PC}$)	δ C(2) ($^3J_{PC}$)**	δ C(1)
1*	**A**	100	$(CD_3)_2CO$	55.3	14.10 (94.2)	23.26 (97.0)	31.82 —	194.00 (13.8)	161.73 —
1	**A**	26	D_2O (pH 1.7)	56.4	13.80 (93.7)	23.07 (94.9)	31.25 —	195.62 (8.0)	162.75 —
	B	60		56.9	13.70 (93.5)	24.04 (93.6)	31.17 —	94.31 (18.4)	173.40 —
	C (*Z*)	6		56.1	14.15 (94.0)	27.01 (94.5)	106.80 (10.1)	139.74 (12.2)	175.50 —
	C (*E*)	8		54.3	14.06 (94.0)	27.12 (94.4)	104.57 (9.7)	142.66 (12.2)	175.1 —
1	**A**	80	D_2O (pH 6.4)	42.7	14.79 (93.0)	24.28 (92.6)	32.75 —	204.59 (14.5)	169.34 —
	B	14		43.6	14.56 (92.6)	23.25 (94.2)	32.51 —	96.12 (18.7)	175.40 —
	C (*E, Z*)	4		41.4	***	***	***	***	***
		2		41.2	***	***	***	***	***
2*	**A**	100	DMSO-d_6	49.5	15.15 (93.0)	23.89 (94.5)	30.20 —	198.18 (13.0)	163.16 —
2	**A**	46	D_2O (pH 1.7)	57.1	13.35 (93.0)	23.58 (94.5)	30.90 —	196.80 (13.1)	162.42 —
	B	48		56.9	13.10 (91.5)	22.51 (94.4)	29.46 —	94.14 (18.9)	175.15. —
	C (*Z, E*)	2		56.4	***	***	***	***	***
		4		56.2	***	***	***	***	***
3	**C** (*E*)	46	C_6D_6	42.9	15.94 (94.3)	31.45 (92.7)	99.88 (11.6)	137.74 (10.0)	165.24 —
	C (*Z*)	54	C_6D_6	42.2	16.03 (94.0)	31.75 (92.3)	103.62 (12.1)	138.98 (10.0)	166.34 —
4	**B**	18	C_6D_6	42.9	16.36 (94.8)	27.15 (96.6)	28.91 —	113.46 (9.6)	172.68 —

* The ^1H spectra (δ, *J*/Hz), **1**: 1.47 (d, $^2J_{PH}$ = 14.2, 3 H, CH_3), 1.95 (d.t, $^3J_{HH}$ = 7.2, $^2J_{PH}$ = 14.0, 2 H, PCH_2), 3.11 (d.t, $^3J_{PH}$ = 10.2, 2 H, $CH_2C(O)$); **2**: 1.34 (d, $^2J_{PH}$ = 14.0, 3 H, CH_3), 1.81 (d.t, $^3J_{HH}$ = 7.5, $^2J_{PH}$ = 14.0, 2 H, PCH_2), 2.99 (d.t, $^3J_{PH}$ = 10.5, 2 H, $CH_2C(O)$), 7.78 (d, $J15_{NH}$ = 41, 2 H, NH_2).

** *Cf.* Ref. 9.

*** The signals do not exceed noise level.

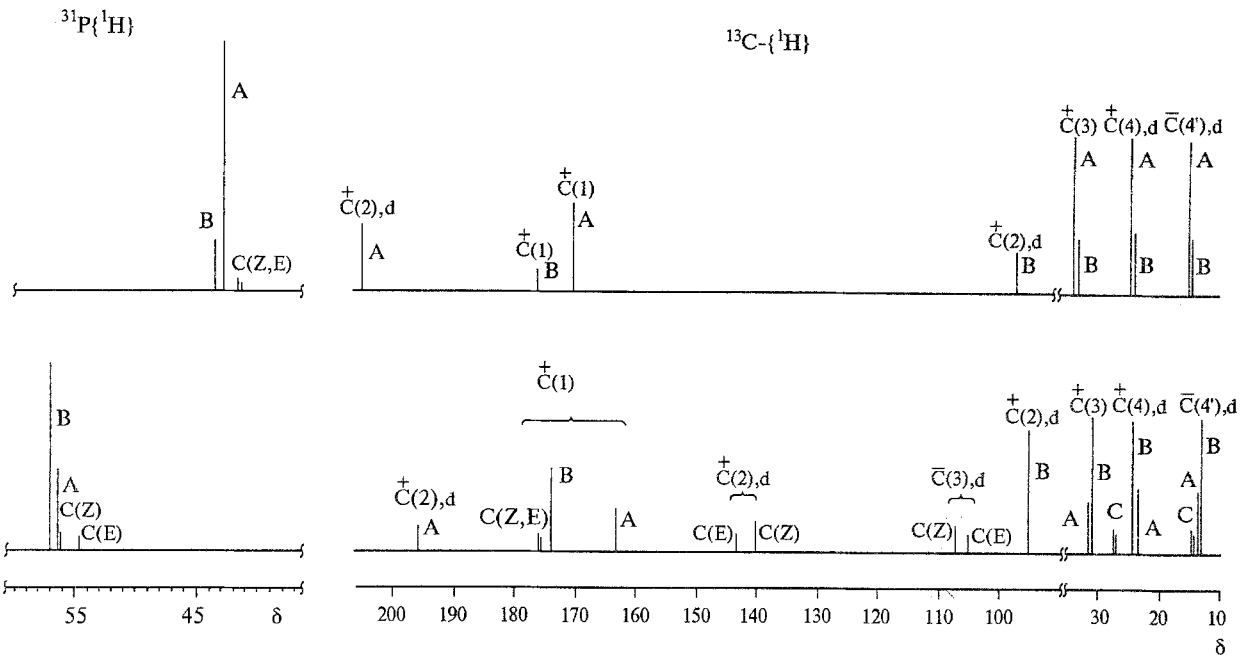

Fig. 1. The $^{31}P-\{^1H\}$ and $^{13}C-\{^1H\}$ NMR spectra of acid **1** in D_2O at pH = 1.7 (*a*), 6.4 (*b*); the ^{13}C MNR signals of the opposite polarity are signed as (+) and (−) respectively; d doublet, *J* see in Table 1.

The ratio of the **A**, **B** and **C** forms of compounds **1** and **2** in aqueous solutions depends on pH. Thus, a solution of **1** at pH 1 contains 64 % of *gem*-diol **B**, 21 % of the ketone form **A**, and 15 % of the enol forms **C**. Decreasing the acidity of the medium decreases the content of the *gem*-diol and enol forms strikingly, and the percentage of the ketone form inctreases up to 80 % (pH = 6.4) (Table 2), whereas in the case of amide **2** the content of the **B** and **C** forms is always lower than that of acid **1**. Like all functionally substituted phosphoric acids,[10] acid **1** possesses a characteristic dependence of δ in the ^{31}P NMR spectra on pH in aqueous solutions (Tables 1 and 2); a significant upfield shift is observed when the medium turns from acidic to neutral.

Table 2. The ratios of the **A**—**C** forms of compound **1** and their δ (^{31}P) in D_2O at various pH

pH	A		B		C	
	δ_P	content (%)	δ_P	content (%)	δ_P	content (%)
1.0	56.6	21	57.4	64	56.2	9
					54.4	6
1.7	56.4	26	56.9	60	56.1	8
					54.3	6
2.4	52.0	50	52.3	41	50.0	6
					49.9	3
4.5	43.0	76	44.2	16	42.4	5
					42.0	3
6.4	42.7	80	43.6	14	41.4	4
					41.2	2

It is known that in aqueous solutions of α-ketoglutaric acid the equilibrium of ketone, *gem*-diol and hydroxylactone forms exists, and the content of the latter achieves 30 %.[4,5] Though the ^1H and ^{13}C NMR signals of the hydroxylactone form of α-ketoglutaric acid are not exhibited as a result of the fast mutual transformation of the cyclic and keto forms in the NMR time scale, several indirect indications of its existence are present. The ^{13}C NMR and UV spectra of the methylphosphine analog of α-ketoglutaric acid give no indications of the existence of the corresponding hydroxylactone form. Thus, the line width of the carbonyl carbon signal remains constant (5—6 Hz) in a wide range of pH and is independent of the temperature. When pH is decreased, the chemical shift of this signal decreases gradually from 204.6 ppm (pH = 6.4) to 195.6 ppm (pH = 1.7), as was observed previously[5] for α-oxopentanoic acid, which does not occur in the cyclic form. The percentages of the keto form of acid **1** in the aqueous solutions at various pH, determined by UV and ^{13}C NMR spectroscopy are in agreement with each other within the range of experimental error. Therefore, the formation of the hydroxylactone form is not characteristic of acid **1** in contrast to its non-phosphorylated analog. Thus, the pH-dependent equilibrium of the three forms described (keto, *gem*-diol and enol (*Z,E*)) was observed for aqueous solutions of acid **1** and amide **2**.

The reaction of acid **1** with excess hexamethyldisilazane gives a mixture of *Z*- and *E*-isomers of the tris(trimethylsilyl) derivative of the enol (**3**) (*E* : *Z* =

46 : 54) in high yield. Upon the silylation of slightly moist acid **1** a small amount of the tetrasilyl derivative of the *gem*-diol form (**4**) is obtained along with the derivative **3**. The spectral parameters of compounds **3** and **4** are presented in Table1. In the case of **3** the assignment of the ^{13}C NMR signals of the *Z*- and *E*-isomers was based on the data from Ref. 8 on the position of the characteristic signal of the olefin carbon atom not bonded to siloxy group. The assignment of the signals in ^{31}P NMR spectra was performed according to the integral intensities of the signals of the isomers and corresponding signals in the ^{13}C NMR spectra. The composition of **3** was confirmed by elemental analysis. Upon hydrolysis of **3** or its mixture with **4**, acid **1** is formed; **1** was identified by its NMR spectra.

Experimental

The NMR spectra were registered with a Bruker WP-200-SY instrument for 0.25 *M* solutions in $CD_3C(O)CD_3$, DMSO-d_6, C_6D_6, or D_2O in relation to tetramethylsilane (^1H, ^{13}C) and 85% H_3PO_4 (^{31}P). The ^{13}C NMR spectra were registered in JMODECHO mode, so the signals of the carbon atoms bearing odd and even numbers of protons had opposite polarity. The UV-spectra were registered with a Specord UV VIS spectrometer. The starting compounds **1** and **2** were obtained using the procedure previously described.[9] The pH values were measured potentiometrically, and were achived by neutralization of the solution of the starting compound **1** or **2** with a 0.25 *M* Na_2CO_3 solution, or by the addition of these compounds to a 0.1 *M* potassium phosphate buffer.

Trimethylsilyl derivative of 2-trimethylsilyloxy-4-[methyltrimethylsilyloxy]phosphinyl-2-butenoic acid 3. A mixture of acid **1** (9.0 g, 0.05 mol) and hexamethyldisilazane (50 mL) was refluxed for 4 h in a flow of Ar. The excess of silazane was removed and the residue was distilled *in vacuo*. Tris(trimethylsilyl) derivative **3** (16.8 g, 85 %) was obtained as a mixture of *Z,E*-isomers in a ratio of 54 : 46, b.p. 146 °C (0.01 Torr). Found (%): C, 42.01; H, 8.65; P, 7.37; $C_{14}H_{33}O_5PSi_3$. Calculated (%): C, 42.39; H, 8.39; P, 7.81.

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