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Synthesis and Properties of 3-(3-Carboxyphenyl)pyruvic Acid and 3-(3-Carboxy-4-hydroxyphenyl)pyruvic Acid

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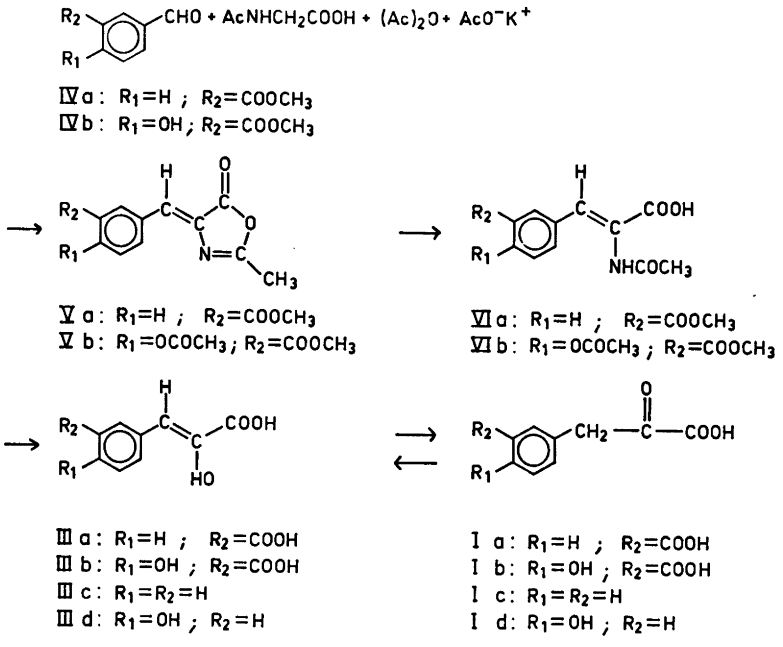
3-(3-Carboxyphenyl)pyruvic acid and 3-(3-carboxy-4-hydroxyphenyl)pyruvic acid have been synthesized from the corresponding benzaldehydes *via* azlactones and α-acetaminocinnamic acids. The equilibrium and equilibration rate between the two keto acids above, phenylpyruvic acid, and *p*-hydroxyphenylpyruvic acid and the corresponding enolic acids have been studied by PMR and UV spectroscopy. The configuration of the enolic acids and of the azlactones and α-acetamino acids have been determined.

3-(3-Carboxyphenyl)pyruvic acid (Ia) and 3-(3-carboxy-4-hydroxyphenyl)pyruvic acid (Ib) are the keto acids corresponding to 3-(3-carboxyphenyl)alanine (IIa) and 3-(3-carboxy-4-hydroxyphenyl)alanine (IIb). IIa and IIb occur free in various higher plants.[1–4] Biosynthesis of IIa and IIb has been proposed to proceed from chorismic acid *via* isochorismic acid [2-(3-carboxy-1,2-dihydro-2-hydroxyphenoxy)acrylic acid], isoprephenic



acid [3-(3-carboxy-4-hydroxycyclohexa-2,5-dienyl)pyruvic acid] and Ia and Ib on basis of incorporation studies with stereospecifically tritium-labeled shikimic acids.[5] Ia has been identified on spectroscopic evidence as a result of chemical rearrangement of isochorismic acid,[6] whereas Ib was unknown. For these reasons the synthesis and characterization of Ia and Ib were undertaken. The equilibrium of the keto forms Ia and Ib with the corresponding enol forms IIIa and IIIb was studied by PMR and UV spectroscopy and the results were compared with those obtained for the keto forms (Ic and Id) and the corresponding enol forms (IIIc and IIId) of phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid.

The syntheses were accomplished by use of the classical Erlenmeyer method,[7] starting from isophthalaldehydic acid methyl ester (IVa)[8] or 5-formylsalicylic acid methyl ester (IVb)[9] and proceeding *via* 4-(3-carbomethoxybenzylidene)-2-methyl-2-oxazolin-5-one (the azlactone of α-acetamino-3-carbomethoxycinnamic acid) (Va) or 4-(3-carbomethoxy-4-acetyloxybenzylidene)-2-methyl-2-oxazolin-5-one (the azlactone of α-acetamino-3-carbomethoxy-4-acetyloxycinnamic acid) (Vb) and α-acetamino-3-carbomethoxycinnamic acid (VIa) or α-acetamino-3-carbomethoxy-4-acetoxycinnamic acid (VIb), respectively. Isolation of the intermediates VIa and VIb was not necessary. V and VI are tentatively assigned *Z*-configuration since the δ-values (see Experimental) for the olefinic protons in these compounds are similar to those reported (in $CDCl_3$) for *Z*-4-benzylidene-2-phenyl-2-oxazolin-5-one and *Z*-α-benzaminocinnamic acid methyl ester and differ from those of the corresponding *E*-isomers.[10–12] A reported δ-value (in D_2O) of 7.28 ppm for the vinylic proton in *Z*-α-(methylaminoacetyl)aminocinnamic acid[13] also supports this assignment. The syntheses were performed under acidic conditions to avoid base induced degradation. Phenylpyruvic acid and derivatives thereof substituted in the *o*- or *p*-position with substituents with negative mesomeric effects are decomposed by base to the corresponding substituted toluene and oxalate.[14] *p*-Hydroxyphenylpyruvic acid and other phenylpyruvic acids substituted in the *o*- or *p*-position with substituents with a positive mesomeric effect are decomposed by base to the corresponding substituted benzaldehydes.[14–16] Phenylpyruvic acids can also be synthesized in high yields from aromatic aldehydes and hydantoin.[14,16] The azlactone method was chosen in the present case, however, since a method was sought which subsequently could be adopted for synthesis of ^{14}C-labeled material, and since ^{14}C-glycine is easily available.

Phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid exist in the crystalline state solely as the enols IIIc and IIId, whereas the salts exist mainly as ketones, as established by chemical studies and ultraviolet spectroscopy.[17] As expected, therefore, the PMR spectra in DMSO of the freshly dissolved free acids only show the presence of singlets for the olefinic protons in IIIa (δ 6.50 ppm), IIIb (δ 6.41 ppm), IIIc (δ 6.41 ppm), and IIId (δ 6.31 ppm) besides the protons in the aromatic nuclei. The δ-values found are in close agreement with those previously reported for IIIa, IIIc, and IIId.[6] III is tentatively assigned the *Z*-configuration partly on the basis of the δ-values for the olefinic protons and partly on the basis of the UV-spectra (see below). The δ-values for the proton at C_3 with *E*- or *Z*-configuration can be calculated as 5.88 ppm and 6.40 ppm, respectively, assuming additivity of substituent effects on chemical shifts of olefinic protons[18] and using the same values for the effect of the hydroxy group as those reported[18] for alkoxy groups. The spectra of the lithium salts of the four acids in D_2O exhibited beside signals for the aromatic protons only benzylic protons as singlets, indicating that in the salts the anions corresponding to I are present. The signals for the benzylic protons slowly disappear as a result of exchange with D_2O.

In aqueous acid an equilibrium is established between I and III, as previously established for Ic – IIIc and Id – IIId.[17] The tautomerization of IIId follows reversible first-order kinetics, increasing in speed with increase in pH.[17] This was confirmed by UV measurements for all four acids as demonstrated in Table 1. Half times and rate constants for establishing the equilibrium at 25° both in 1 M HCl and in phosphate buffer at pH 6.35 are given. Determination of the amount of enol present at equilibrium in 1 M HCl permitted the calculation of the individual rate constants for the keto-enol tautomerization.

Table 1. Keto-enol tautomerization of phenylpyruvic acids at 25°. For designation of compounds see formula chart.

Compound	1 M HCl					Phosphate buffer pH 6.35	
	$t_{½}$[a] (min)	k[a] (min^{-1})	% enol at equilibrium	$k_{I→III}$ (min^{-1})	$k_{III→I}$ (min^{-1})	$t_{½}$[a] (min)	k[a] (min^{-1})
Ia – IIIa	10	6.9×10^{-2}	7	6.4×10^{-3}	6.3×10^{-2}	5	1.3×10^{-1}
Ib – IIIb	29	2.4×10^{-2}	12	2.9×10^{-3}	2.1×10^{-2}	16.5	4.2×10^{-2}
Ic – IIIc	16	4.4×10^{-2}	10	4.5×10^{-3}	4.0×10^{-2}	3.5	2.0×10^{-1}
Id – IIId	51	1.4×10^{-2}	17	2.4×10^{-3}	1.1×10^{-2}	11.5	6.0×10^{-2}

[a] For equilibration.

The values found in 1 M HCl for Id – IIId are in good agreement with those reported for this compound at pH 1.[17] For Ia – IIIa it has been stated that keto to enol conversion in strong acid does not occur readily,[6] but this is contradicted by the present results.

Presumably the conversion of keto to enol takes place without acid catalysis [19] and the rate is determined by the kinetic acidity of the protons on C_3. The order of the rate constants for I→III is that expected from the inductive and mesomeric effects of the substituents in the aromatic ring in I. The changed order of half-times and corresponding rate constants at pH 6.35 supposedly reflects the change of sign of the inductive effect of the aromatic carboxyl group by ionization.

In these considerations no regard is made to the hydration of I. It can, however, be expected that the degree of hydration is nearly the same for all four compounds and the effect on the order of the rate constants would be opposite to the effect described above.[20]

UV-data for the enols in 1 M HCl and for the anions of the enols at pH 6.35 are recorded in Table 2. The amount of enol at equilibrium at pH 6.35 supposedly is negligible. Therefore the UV-absorption recorded at equilibrium pre-

Table 2. UV-data for enol and keto forms of phenylpyruvic acids at 25°. For designation of compounds see formula chart.

Compound	1 M HCl		Phosphate buffer pH 6.35			
	Enol form		Anion of enol form		Anion of keto form	
	λ_{max} (nm)	ε_{max}	λ_{max} (nm)	ε_{max}	λ_{max} (nm)	ε_{max}
Ia – IIIa	286	23 000	284	23 000	275	1 600
	234	13 000	225	shoulder	225	shoulder
	204	6 000	199		199	29 000
Ib – IIIb	295	23 000	291	23 000	299	4 800
	233	15 000	227	shoulder	227	shoulder
	208	17 000	204	19 000	204	33 000
Ic – IIIc	289	25 000	285	25 000		
	208		197	14 000	197	13 000
Id – IIId	302	25 000	290	26 000	275	2 800
	224	7 400	220	shoulder	220	shoulder
			197	14 500	197	22 000

sumably represents the anions of the ketones as given in Table 2. The high ε-values for the high wavelength absorption band for the enols support the *Z*-configuration for these compounds since ε_{max} for the corresponding band for the similar configuration of cinnamic acid (*E*-cinnamic acid) is 20 000 whereas ε_{max} for the opposite configuration (*Z*-cinnamic acid) is only 9000.[21] The intensity and location of these bands are only changed very little in the corresponding anions of the cinnamic acids.[21] The spectral data for IIIa, IIIc, and IIId are in agreement with those reported in the literature.[6,16,22,23] In the spectrum of the anion of Ib the salicylic acid grouping is clearly visible.

EXPERIMENTAL

PMR spectra were measured on a JEOL-C-60 HL instrument. Chemical shifts are given in ppm downfield from TMS in deuterio-DMSO and from sodium 2,2,3,3,-tetradeuterio-3-(trimethylsilyl)propionate in D_2O. UV-spectra were measured on a Zeiss DMR-21 instrument at 25°. Transformations of enol to keto forms in 1 M HCl and in 0.06 M phosphate buffer pH 6.35 were observed at λ_{max} for the highest positioned absorption band of the enol form (see Table 1) in freshly prepared solutions and half-times for equilibration were determined as described in the literature.[17] Transformations of keto to enol forms in 1 M HCl were observed by acidification of neutral solutions. Extrapolations to $t=0$ permitted the determination of ε-values in acid for both keto and enol forms and the combined data permitted calculation of the equilibrium composition. Melting points are uncorrected. Microanalyses were performed by Mr. G. Cornali and his staff.

4-(3-Carbomethoxybenzylidene)-2-methyl-2-oxazolin-5-one (Va). A mixture of isophthalaldehydic acid methyl ester (IVa)[8] (5.01 g), acetylglycine (3.16 g), and potassium acetate (2.5 g) in acetic anhydride (5.5 ml) was kept with stirring at 100° for 3 h. After cooling overnight crystals of Va were collected by filtration and washed with methylene chloride. Yield 2.93 g (45 %), m.p. 185–187°. Recrystallization from benzene-benzine (80–110°) produced an analytical sample of yellow needles, m.p. 195–197°. (Found: C 59.64; H 4.33; N 5.33. Calc. for $C_{13}H_{11}O_5N$: C 59.77; H 4.24; N 5.36). PMR-spectrum in deuterio-DMSO: δ 2.41 (3 H) singlet, methyl; δ 3.95 (3 H) singlet, methoxyl; δ 7.36 (1 H) singlet, olefinic; δ 7.75–8.87 (4 H) multiplet, aromatic.

4-(3-Carbomethoxy-4-acetyloxybenzylidene)-2-methyl-2-oxazolin-5-one (Vb). A mixture of 5-formylsalicylic acid methyl ester sodium salt (IVb)[9] (406 mg), acetylglycine (234 mg), and potassium acetate (200 mg) in acetic anhydride (2 ml) was kept with stirring under nitrogen at 100–110° for 2 h. After cooling overnight crystals of Vb were collected by filtration and washed with ethanol. Yield 366 mg (60 %), m.p. 140–141°. Recrystallization from benzene produced an analytical sample of yellow needles, m.p. 140–141°. (Found: C 59.47; H 4.46; N 4.42. Calc. for $C_{15}H_{13}O_6N$: C 59.40; H 4.36; N 4.62.) PMR-spectrum in deuterio-DMSO: δ 2.34 (3 H) singlet, acetoxy; δ 2.42 (3 H) singlet, methyl; δ 3.87 (3 H) singlet, methoxyl; δ 7.33 (1 H) singlet, olefinic; δ 7.41–8.82 (3 H) multiplet, aromatic.

α-Acetamino-3-carbomethoxycinnamic acid (VIa). A suspension of Va (123 mg) in water (5 ml) and ethanol (5 ml) was refluxed for 2 h. After removal of part of the solvents by evaporation and cooling overnight crystals of VIa were collected by filtration. Yield 68 mg (52 %), m.p. 217–219° (decomp.). Recrystallization from methanol produced an analytical sample, m.p. 222° (decomp.). (Found: C 59.15; H 5.15; N 5.32. Calc. for $C_{13}H_{13}O_5N$: C 59.31; H 4.97; N 5.32.) PMR-spectrum in deuterio-DMSO: δ 2.05 (3 H) singlet, acetyl; δ 3.91 (3 H), singlet, methoxyl; δ 7.36 (1 H) singlet, olefinic; δ 7.42–8.3 (4 H) multiplet, aromatic.

α-Acetamino-3-carbomethoxy-4-acetyloxycinnamic acid (VIb). Vb (121 mg) in water (10 ml) and methanol (5 ml) was refluxed for 4 h. After removal of part of the solvent, filtration and cooling crystals of VIb were collected by filtration. Yield 94 mg (73 %). Recrystallization from ethyl acetate produced an analytical sample. m.p. 180°. (Found: C 55.69; H 4.83; N 4.28. Calc. for $C_{15}H_{15}NO_7$: C 56.08; H 4.70; N 4.35.) PMR-spectrum in deuterio-DMSO: δ 2.05 (3 H) singlet, acetyl; δ 2.34 (3 H) singlet, acetoxy; δ 3.87 (3 H) singlet, methoxyl; δ 7.34 (1 H) singlet, olefinic; δ 7.47–8.28 (3 H) multiplet, aromatic.

3-(3-Carboxyphenyl)pyruvic acid in enol form (IIIa). A solution of Va (980 mg) in ethanol (20 ml) and 2 M HCl (40 ml) was refluxed under nitrogen for 2.5 h. The solution was decolourised with charcoal and after cooling overnight colourless crystals of IIIa were collected, m.p. 222–224° (decomp.). Yield 670 mg (80 %). Two recrystallizations from 2 M HCl produced an analytical sample, m.p. 219–222° (decomp.). (Found: C 57.46; H 4.04. Calc. for $C_{10}H_8O_5$: C 57.69; H 3.87.) PMR-spectrum in deuterio-DMSO: δ 6.50 (1 H) singlet, olefinic; δ 7.34–8.48 (4 H) multiplet, aromatic. PMR-spectrum of lithium salt in D_2O: δ 4.18 (2 H) singlet, benzyl; δ 7.28–7.82 (4 H) multiplet, aromatic. For PMR-data, *cf.* Ref. 6.

3-(3-Carboxy-4-hydroxyphenyl)pyruvic acid in enol form (IIIb). A solution of Vb (366 mg) in ethanol (10 ml) and 4 M HCl (35 ml) was refluxed under nitrogen for 14 h. After cooling overnight and filtration the solution was left in the refrigerator for three days. Crystals of IIIb were collected by filtration. Yield 169

mg (68 %), m.p. 230 – 235° (decomp.). Recrystallization from 2 M HCl produced an analytical sample, m.p. 234 – 237° (decomp.). (Found: C 53.64; H 3.77. Calc. for $C_{10}H_8O_6$: C 53.58; H 3.59.) PMR-spectrum in deuterio-DMSO: δ 6.41 (1 H) singlet, olefinic; δ 6.9 – 8.45 (3 H) multiplet, aromatic. PMR-spectrum of lithium salt in D_2O: δ 4.08 (2 H), singlet, benzyl; δ 6.8 – 7.8 (3 H) multiplet, aromatic.

Ib – IIIb is rapidly decomposed at pH > 8 at room temperature. The corresponding aldehyde has been identified in the decomposition mixture by UV-spectroscopy.

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