TABLE OF RESULTS

Molecule	273.16°K.	Q_{14}/Q_{12} 293.16°K.	313.16°K.	Spectral data reference[a]	Remarks
HCHO	1.574	1.515	1.467	1, p. 300	
HCO_3^-	1.509	1.451	1.403	2	Freq. assgt. made on basis of VFF calcn. as $(OH)CO_2$
$CO_3^=$	1.478	1.424	1.378	1, p. 178	
CO_2	1.444	1.397	1.358	1, p. 276	Anharmonicity corrections applied
$CO(NH_2)_2$	1.418	1.372	1.333	3	ν_6 estd. by VFF calcn. and analogy to $COCl_2$
HCO_2^-	1.360	1.322	1.289	4	ν_6 estd. by VFF calcn. with k_Δ as for CH_2O
COS	1.355	1.317	1.286	1, p. 174	
$(C_2N_2)^{1/2}$	1.322	1.289	1.261	1, p. 294	
CNO^-	1.315	1.283	1.255	5	
ClCN	1.299	1.269	1.244	1, p. 174	
$COCl_2$	1.295	1.263	1.237	6	Frequency assgt. checked by VFF calculation
SCN^-	1.292	1.262	1.237	1, p. 174	
BrCN	1.282	1.254	1.230	1, p. 174	
HCN	1.281	1.256	1.233	1, p. 280	Anharmonicity corrections applied
ICN	1.265	1.239	1.216	1, p. 174	
CS_2	1.255	1.227	1.204	1, p. 173	
CO	1.216	1.196	1.179	7	Anharmonicity corrections applied
CN^-	1.192	1.174	1.159	1, p. 195	

[a] References: (1) G. Herzberg, "Infrared and Raman Spectra of Polyatomic Molecules," D. Van Nostrand Co., Inc., New York, N. Y., 1945. (2) R. Duval, C. Duval and J. Lecomte, *Bull. soc. chim. France*, **5**, 517 (1943). (3) K. W. F. Kohlrausch and A. Pongratz, *Z. physik. Chem.*, **B27**, 176 (1934). (4) J. Lecomte, *et al.*, *Cahiers de Physique*, 17 (1943); *Ann. phys.*, **17**, 5 (1942); *Compt. rend.*, **208**, 1401 (1939). (5) J. Goubeau, *Ber.*, **68**, 912 (1935). (6) C. R. Bailey and J. B. Hale, *Phil. Mag.*, **25**, 98 (1938). (7) G. Herzberg, "Molecular Spectra and Molecular Structure. I. Diatomic Molecules." D. Van Nostrand Co., Inc., New York, N. Y., 1950, p. 522.

by the division of the required pair of partition function ratios.

Reactions involving species of great chemical similarity and which undergo rapid exchange show the expected small enrichment effects. Thus, in the case of the important biological equilibrium

$$HC^{12}O_3^- + C^{14}O_3^= \rightleftharpoons HC^{14}O_3^- + C^{12}O_3^=$$

at 293.16°K., $K = 1.451/1.424 = 1.019$. This represents but a 1.9% enrichment of carbon-14 in the bicarbonate. On the other hand, a number of probable exchanges, so far for the most part uninvestigated experimentally, should exhibit appreciable enrichments. There is the series involving cyanide ion—in addition to the CN^-/HCN system (well-known in carbon-13 studies) with a predicted 6.8% effect at 293.16°K., there are those comprising CN^- and CNO^- (9.2%), CNS^- (7.5%), C_2N_2 (9.8%), ClCN (8.1%), BrCN (6.8%) and ICN (5.5%). Additional particularly intriguing possibilities are the low-temperature catalytic equilibration (say on active MnO_2) of CO and CO_2 with a predicted enrichment of no less than 16.8% at 293.16°K., the photochemical exchange of CO with $COCl_2$ (6.5%), the very probable CO_2/COS exchange (6.1%), and the significant $CO(NH_2)_2/CNO^-$ system (6.9%).

It will be noted that the predicted enrichments for certain of the systems studied here are somewhat greater than twice the values previously given in the literature for the corresponding carbon-13/carbon-12 systems. For example, Urey[1] reports a carbon-13 enrichment of 3.4% for the HCN/CN^- system at 273°K., while the carbon-14 value deduced from our table is 7.5% at the same temperature. Close examination shows that this apparent disagreement with theory stems from the vibrational frequencies used for the isotopic HCN molecules. Our method of calculation (using the VFF equations given by Herzberg[5]) gives for $HC^{13}N$ the frequencies 1969, 723 and 3431 cm.$^{-1}$, respectively, as compared to Urey's values of 1967, 723 and 3435 cm.$^{-1}$.

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Heats of Hydrolysis of Amide and Peptide Bonds[1]

BY JULIAN M. STURTEVANT

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In recent publications,[2,3] a procedure for the calorimetric determination of the heats of hydrolysis of amide and peptide bonds has been described. The present note reports the application of the method to the hydrolysis of the amide bond of glycyl-L-phenylalanylamide and the peptide bond of carbobenzoxyglycyl-L-leucine.

Experimental

Glycyl-L-phenylalanylamide acetate, kindly supplied by Dr. J. S. Fruton of Yale University, was hydrolyzed by cathepsin C, also supplied by Dr. Fruton. Cysteine was added as activator. Kjeldahl analysis gave 14.79% nitrogen, as compared with the theoretical value of 14.94%. Carbobenzoxyglycyl-L-leucine, for which the author is indebted to Dr. R. Lumry of the University of Utah, was hydrolyzed by carboxypeptidase, purchased from the Worthington Biochemical Laboratory. Both reactions were carried out in buffered aqueous solution at 25°, and were accompanied by heat evolution which accurately followed in each case a first-order rate law to more than 90% completion. The former reaction was observed in the *p*H range 4.69 to 5.65; in this range no correction[3] for ionization heats is necessary, since the products are practically completely

(1) This research was aided by a grant from the Rockefeller Foundation. Presented at the Atlantic City Meeting of the American Chemical Society, September, 1952.

(2) A. Dobry and J. M. Sturtevant, *J. Biol. Chem.*, **195**, 141 (1952).

(3) A. Dobry, J. S. Fruton and J. M. Sturtevant, *ibid.*, **195**, 148 (1952).

ionized. The latter reaction was investigated in the pH range 6.46 to 7.45; the small ionization corrections were estimated using +800 and +10,800 cal. per mole for the second ionization heats of phosphoric acid[4] and leucine,[5] respectively, and pK_2' 9.60 for the second ionization of leucine.[5]

Results

The experimental results are summarized in Tables I and II. The notation adopted in previous work[2,3] has been followed. The rate constants given for each reaction have no absolute significance since it is known that the enzymes used were not highly purified preparations.

TABLE I

THE HYDROLYSIS OF GLYCYL-L-PHENYLALANYLAMIDE BY CATHEPSIN C AT 25°

Buffer concentration, 0.05 M; ionic strength, 0.3 M; P, sodium phosphate, A, sodium acetate

pH	Initial substrate concn., mole per l., × 10³	Enzyme concn., mg. prot. nitrogen per ml.	Cysteine concn., moles per l., × 10³	k, min.⁻¹	$-\Delta H_h$, cal. per mole	Buffer
4.69	0.587	0.0387	1.90	0.072	5900	P + A
4.69	.587	.0387	1.90	.066	5880	P + A
4.85	.453	.116	1.92	.088	6285	A
4.85	.439	.116	1.92	.086	6285	A
5.09	.453	.116	1.93	.107	6300	P
5.11	.694	.116	2.75	.137	6780	P
5.11	.453	.116	1.93	.115	6300	P
5.65	.342	.116	1.90	.095	5970	P
5.65	.342	.116	1.90	.093	6260	P
				Mean	6220 ± 150	

TABLE II

THE HYDROLYSIS OF CARBOBENZOXYGLYCYL-L-LEUCINE BY CARBOXYPEPTIDASE AT 25°

Sodium phosphate buffer, 0.05 M; ionic strength, 0.4 M

pH	Init. substrate concn., moles per l., × 10³	Enzyme concn., mg. prot. nitrogen per ml.	$-\Delta H_{obsd.}$ cal. per mole	$-\alpha(\Delta H_i - \Delta H_p)$, cal. per mole	$-\Delta H_h$, cal. per mole	k_3/K_m
6.46	1.63	0.0321	2015	7	2022	0.89
6.46	1.63	.0321	2115	7	2122	1.01
6.77	1.49	.0391	2090	15	2105	1.03
6.77	1.49	.0391	2060	15	2075	0.99
6.81	2.35	.0414	2040	16	2056	1.34
6.81	2.35	.0414	2030	16	2046	1.27
7.24	1.87	.0413	2180	44	2224	1.92
7.24	1.87	.0413	2110	44	2154	1.85
7.44	1.14	.0413	2100	69	2169	2.21
7.45	1.14	.0321	2060	70	2130	2.55
				Mean	2110 ± 50	

Table III lists the heats of hydrolysis for the five compounds studied by our method. These values refer in each case to the formation of fully charged products, according to the equation

$$RCONHR' + H_2O = RCOO^- + {}^+NH_3R'$$

The data are as yet not sufficiently extensive to permit tracing in detail the relation between peptide structure and heat of hydrolysis. However, it is

TABLE III

HEATS OF HYDROLYSIS OF SYNTHETIC PEPTIDES AT 25°

Substrate	Bond hydrolyzed	$-\Delta H_h$, cal. per mole
Benzoyl-L-tyrosinamide	Amide	5840 ± 220
Benzoyl-L-tyrosylglycinamide	Tyrosine–glycine	1550 ± 100
Carbobenzoxyglycyl-L-leucine	Glycine–leucine	2110 ± 50
Carbobenzoxyglycyl-L-phenylalanine	Glycine–phenylalanine	2550 ± 50
Glycyl-L-phenylalanylamide	Amide	6220 ± 150

evident that the amide hydrolyses are considerably more exothermic than the peptide hydrolyses. It should be noted that, since the heat of ionization of the ammonium ion is about 2000 cal. per mole more positive than the heat of the second ionization of most amino acids, the heat of hydrolysis of an amide bond to give *uncharged* products would be more nearly equal to that of the peptide bond to give *uncharged* products. A small structural change, such as replacement of the isobutyl side chain of leucine by the benzyl side chain of phenylalanine, produces a small but definite change in the heat of hydrolysis. It is perhaps significant that the smallest heat is observed in the hydrolysis of the neutral molecule benzoyltyrosylglycinamide; presumably this value will be found to be approximately characteristic of the hydrolysis of the majority of the peptide bonds in a protein which are located at considerable distances from charged groups.

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(5) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides." Reinhold Publ. Corp., New York, N. Y., 1943, pp. 80 and 84.

Antimetabolites of Uridine with Two Structural Alterations[1]

BY DONALD W. VISSER, GERALD BARRON AND RICHARD BELTZ

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A number of 5-substituted uridine derivatives competitively inhibit the growth response of *Neurospora* 1298 produced by uracil, uridine or cytidine[2,3,4] and several of these compounds also inhibit the propagation of Theilers GD VII encephalomyelitis virus *in vitro*.[5] Substitution of the hydrogen in the 3-position of uridine produces a similar antimetabolite, 3-methyluridine.[3,4] It was of interest, therefore, to prepare nucleosides which are substituted in both the 3- and 5-positions and compare the biological activity of these compounds with the corresponding derivatives having a single structural alteration. The new compounds are of interest not only because of their structural similarity to uridine and cytidine, which are utilized for nucleic acid biosynthesis, but also may be of value

(1) This work was aided by a grant from Research Corporation. Presented, in part, before the Biochemistry Division, at the April, 1952, Meeting of the American Chemical Society at Milwaukee, Wisconsin.

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