Glycerol tribenzoate was prepared and recrystallized from ethanol, m.p. 74–75° (lit. 75°). A mixed melting point with the tribenzoate prepared from C.P. glycerol was not depressed.

A small aliquot of the C^{14}-glycerol was dissolved in absolute ethanol, and inactive glycerol was added. The ethyl alcohol was removed *in vacuo* at room temperature, and the resulting diluted C^{14}-glycerol was degraded according to the procedure outlined elsewhere.[13] The C^{14} content of each of its carbons was determined; this confirmed that the synthesized glycerol was evenly labeled with C^{14}. The results are recorded in Table I.

TABLE I

C^{14}-GLYCEROL PREPARED FROM 24-HOUR PHOTOSYNTHETIC C^{14}-GLUCOSE

Compound	Reaction	Glycerol carbons converted to CO_2	Specific activity expressed as $BaCO_3$, c.p.m. per mg.
Glycerol	Periodate oxidation[a]	C-1 + 3	18.6
Glycerol	Lead tetraacetate oxidation	C-2	18.4
Glycerol	Combustion	C-1 + 2 + 3	18.5

[a] The HCHO formed was oxidized to CO_2 with $KMnO_4$.

Acknowledgment.—The author wishes to thank Dr. I. L. Chaikoff of the Division of Physiology of the School of Medicine, and Dr. W. G. Dauben of the Department of Chemistry, University of California, for their many helpful suggestions in this work.

(13) D. Kritchevsky and S. Abraham, *Arch. Biochem. Biophys.*, **39**, 305 (1952).

DIVISION OF PHYSIOLOGY
SCHOOL OF MEDICINE
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

Occurrence of Cinnamic Acid in Sugar Pine (*Pinus lambertiana* Dougl.)

BY ARTHUR B. ANDERSON

RECEIVED JULY 14, 1952

Cinnamic acid (*trans*-isomer) is a common constituent of many plants and is the chief component of the oil of *Storax*.[1] In addition to being in the free form, it is likewise found as esters in various plant oils and resins.

While investigating the distribution and amount of pinitol present in sugar pine heartwood, on occasions a sublimate in the form of small white crystalline flakes would appear on the walls of the evaporating dish, as the aqueous extract was being concentrated to a sirup.[2] This substance melted at 131–133°, was insoluble in cold water, dissolved readily in dilute sodium bicarbonate solution, took up bromine, decolorized potassium permanganate, and has been identified as *trans*-cinnamic acid. While this acid was found in various heartwood sections from the bole of the tree, the largest quantity was obtained from the stumpwood area (*i.e.*, 3.20 g. from 400 g. of heartwood). This is believed to be the first report of the isolation of cinnamic acid from pine wood.

(1) G. Klein, "Handbuch der Pflanzenanalyse," Vol. 2, Springer, Wien, 1932, pp. 537–538.

(2) Arthur B. Anderson, *Tappi*, **35**, No. 5, 198 (1952).

Experimental[3]

Four hundred grams of air-dried sugar pine heartwood sawdust was extracted four times with hot water in a 4-liter glass percolator. The aqueous extracts were combined, neutralized with sodium bicarbonate, and the solution concentrated to about 400 ml. This was cooled and centrifuged to remove insoluble material. The decanted solution was then extracted several times with ethyl ether. The extracted, slightly alkaline, solution was poured slowly, with stirring, into an excess of dilute hydrochloric acid, resulting in the precipitation of a light-tan crystalline material. This precipitate was filtered, washed with water and recrystallized several times from hot dilute ethanol (charcoal) to constant melting point 134–135°; yield 3.2 g. (0.8%).

Anal. Calcd. for $C_9H_8O_2$: C, 72.95; H, 5.44; neut. equiv., 148.15. Found: C, 72.70; H, 5.54; neut. equiv., 147.5.

The *p*-nitrobenzyl- and phenacyl esters of the acid melted at 116–117° and 142–143°, respectively, mixed melting point with corresponding authentic derivatives of cinnamic acid were unchanged.

(3) All melting points uncorrected taken on Fisher melting-point block; microanalysis by Microchemical Laboratory, University of California.

FOREST PRODUCTS LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIF.

Solvent Effects in the α-Chymotrypsin–Hydrocinnamic Ester System[1]

BY M. LUCETTA BARNARD AND KEITH J. LAIDLER

RECEIVED MAY 16, 1952

Consideration of the entropies of activation associated with the formation and breakdown of enzyme–substrate complexes has suggested the possibility that specific solvent or structural effects occur during these processes.[2,3] The available data indicate that complex formation is associated with a negative entropy of activation when the substrate is uncharged, and with a positive one when the substrate is charged. This can be explained if charge separation occurs in the former case, with binding of water molecules, and charge neutralization in the latter case, with release of water molecules.

In the present note we describe an approach which is designed as a check on the plausibility of this type of hypothesis. The entropy terms associated with the electrostriction of solvent molecules have been evaluated by measuring rates in mixed solvents, the work being done on the α-chymotrypsin–hydrocinnamic ester system, in which the substrate is uncharged. It is emphasized that in view of the complications of enzyme systems a rigorous application of the theoretical treatment is not possible; consequently a detailed experimental study of solvent effects has not been thought worth while, although a rough application of the general method to other systems may well be useful and is being carried out in this Laboratory.

(1) Abstracted from a dissertation submitted by Sister M. Lucetta Barnard, C.S.C., to the Graduate School of the Catholic University of America in partial fulfillment of the requirements for the degree of Master of Science. The work was carried out in part under Contract N8onr-05300 with the Office of Naval Research, Biochemistry Branch.

(2) K. J. Laidler, "Symposium on Biochemical Kinetics," Diamond Jubilee Meeting of the American Chemical Society, September 6, 1951.

(3) E. J. Casey and K. J. Laidler, THIS JOURNAL, **72**, 2159 (1950).

It is known from simple electrostatic theory[4] that the rate constant k of a reaction in which charges of valence z_A and z_B are produced in the activated state at a distance r apart is given by

$$\ln k = \ln k_0 - \frac{\epsilon^2 z_A z_B}{\mathbf{k}Tr}\left(1 - \frac{1}{D}\right) \qquad (1)$$

Here D is the dielectric constant, k_0 is the rate at unit D, ϵ is the electronic charge, $\mathbf{k}$ is Boltzmann's constant and T the absolute temperature. This equation may be written in the general form

$$\ln k = \ln k_0 - \frac{A}{T}\left(1 - \frac{1}{D}\right) \qquad (2)$$

and A may be determined experimentally by measuring k at different values of D.

The magnitude of the entropy changes associated with electrostatic effects may then be determined as follows. The free energy is equal to $\ln (\mathbf{k}T/h) - RT \ln k$, and that part of it, $(\Delta F^*)_{e.s.}$, that is associated with electrostatic interactions is thus

$$(\Delta F^*)_{e.s.} = RA\left(1 - \frac{1}{D}\right) \qquad (3)$$

The electrostatic entropy of activation is given by

$$(\Delta S^*)_{e.s.} = -\left(\frac{\partial(\Delta F^*)_{e.s.}}{\partial T}\right)_P \qquad (4)$$

and differentiation of eq. (3) with respect to T, allowing for the temperature dependence of D, gives

$$(\Delta S^*)_{e.s.} = -\frac{RA}{D^2}\left(\frac{\partial D}{\partial T}\right)_P \qquad (5)$$

In an aqueous solution D is about 80 and $\partial \ln D/\partial T$ is about -0.0046; it thus follows that

$$(\Delta S^*)_{e.s.} \simeq 1.13 \times 10^{-4} A \qquad (6)$$

We are applying this treatment to a number of enzyme reactions by measuring rates at different substrate concentrations and in mixed methanol–water solvents. Methanol is one of the few solvents suitable for this purpose, since it has no specific effect on proteins; however, only dilute solutions may be used, as enzymes are deactivated at higher concentrations. The present work was carried out in mixtures up to 25% in methanol.

The procedure is to determine, by the usual extrapolation procedures,[5] the values of k_2K and k_2 at various dielectric constants. The logarithms of these constants are then plotted against $1/D$, and the value of A determined. The electrostatic entropies calculated using eq. (6) are denoted as $(\Delta S^*)_{e.s.}$ and $(\Delta S_2^*)_{e.s.}$, respectively, and refer to the formation of the enzyme–substrate complex and to its breakdown. If the over-all entropies of activation are also known, the corresponding non-electrostatic terms $(\Delta S^*)_{n.e.s.}$ and $(\Delta S_2^*)_{n.e.s.}$ are obtained by subtraction.

The results for the α-chymotrypsin–hydrocinnamic ester system are as follows, the over-all entropies, which we have confirmed, being those of Snoke and Neurath.[6]

Complex formation:

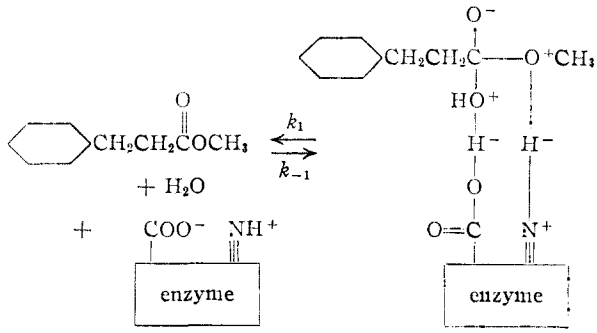
$\Delta S^* = 23$ $\quad (\Delta S^*)_{e.s.} = -38$ $\quad (\Delta S^*)_{n.e.s.} = 15$

Breakdown of complex:

$\Delta S_2^* = -12$ $\quad (\Delta S_2^*)_{e.s.} = -20$ $\quad (\Delta S_2^*)_{n.e.s.} = 8$

The value of -38 e.u. for $(\Delta S^*)_{e.s.}$ is of special interest in suggesting that there is considerable charge separation during the formation of the complex, and the value of -20 for $(\Delta S_2^*)_{e.s.}$ indicates further charge separation during the subsequent reaction. The value $(\Delta S_2^*)_{n.e.s.}$ is a normal one for a unimolecular reaction, but the value of 15 e.u. for $(\Delta S^*)_{n.e.s.}$ implies some structural change (perhaps unfolding) during complex formation, as discussed previously[7] for other reactions on the basis of the pressure data.

The charge separations occurring during reaction are capable of explanation in terms of a model which also explains other features of the reaction. The position of the pH optimum (7.8) and the shape of the rate–pH curve for the reaction are consistent with the hypothesis that the active site on the enzyme consists of a $-COO^-$ group and an $\equiv NH^+$ group, the latter belonging to the imidazole ring in a histidine residue. Formation of the complex can thus be represented as



which clearly involves considerable charge separation. Such a formulation of the complex satisfactorily explains the catalytic action (which is of the ordinary acid–base type) and explains the stability of the complex.

Data are also available in the literature for making a similar, but less complete, analysis of the α-chymotrypsin-catalyzed hydrolysis of benzoyl-L-tyrosine ethyl ester, a substrate that is also uncharged. The data are given by Kaufman, Neurath and Schwert[8] in the form of k_2K at three different methanol concentrations. The results obtained are

$\Delta S^* = -38$ $\quad (\Delta S^*)_{e.s.} = -27$ $\quad (\Delta S^*)_{n.e.s.} = -11$

The value of -11 is reasonable for bimolecular interaction, and it is not necessary to suggest structural change. The negative electrostatic term again implies charge separation.

The hydrolysis of acetyl-L-tyrosinamide has also been studied at different methanol concentrations,[9] and analysis of the results gives -30 e.u. for $(\Delta S^*)_{e.s.}$ and 0 e.u. for $(\Delta S_2^*)_{e.s.}$ For the closely analogous substrate benzoyl-L-tyrosinamide the

(4) *Cf.* K. J. Laidler, "Chemical Kinetics," McGraw-Hill Book Co., Inc., New York, N. Y., 1950, p. 133.

(5) *E.g.*, G. S. Eadie, *J. Biol. Chem.*, **146**, 85 (1942).

(6) J. E. Snoke and H. Neurath, *Arch. Biochem.*, **21**, 351 (1949); *J. Biol. Chem.*, **182**, 577 (1950).

(7) K. J. Laidler, *Arch. Biochem.*, **30**, 226 (1951).

(8) S. Kaufman, H. Neurath and G. W. Schwert, *J. Biol. Chem.*, **177**, 793 (1949).

(9) S. Kaufman and H. Neurath, *ibid.*, **180**, 181 (1949).

entropy values[8] are $\Delta S^* = -43.0$ and $\Delta S_2^* = -13.0$. If these values can be accepted tentatively for the acetyl compound the non-electrostatic contributions are found to be $(\Delta S^*)_{n.e.s.} = 13$ and $(\Delta S_2^*)_{n.e.s.} = -13$. The value of -30 for $(\Delta S^*)_{e.s.}$ suggest that, as with hydrocinnamic ester, there is considerable charge separation during complex formation: this is rather to be expected for an uncharged substrate. There appears to be little further separation during the reaction of the complex. The non-electrostatic entropy values indicate some structural change in this system, the enzyme unfolding during complex formation and folding during the subsequent process.

Experimental

The enzyme, salt-free α-chymotrypsin, was used in solution at a concentration of 8.41×10^{-6} M, calculated on the basis of a molecular weight of 27,000.[10] The solvents used were methanol–water mixtures of various concentrations. All water used was triply distilled in an all-glass apparatus. The kinetic procedure was very similar to that employed by Snoke and Neurath,[6] the main difference being that the buffer concentration used was very much lower in the present experiments, a 0.0045 M $NaOH$–NaH_2PO_4 buffer being used. The pH optimum was found to be 7.8, and all work was done at this pH.

Rates were measured in 15, 20 and 25% methanol–water, and at the two extreme substrate concentrations, 0.00075 M and 0.01 M. The values of k_2 and k_2K obtained from the intercepts on the two axes are given in Table I. The values of A calculated from the plots of log k_2 and log k_2K *vs.* $1/D$, and the corresponding entropies, are also shown in Table I. The dielectric constants were taken from the work of Davies and Jones.[11]

TABLE I

VALUES OF k_2 AND k_2K

% Methanol (by weight)	D	$k_2 \times 10^2$	k_2K
15	72.48	1.84	17.1
20	70.25	1.38	12.5
25	68.01	1.07	6.2
		$A = -1.77 \times 10^5$	-3.38×10^5
		$(\Delta S_2^*)_{e.s.} = -20$ e.u.	$(\Delta S^*)_{e.s.} = -38$ e.u.

(10) E. Jansen, M.D. Fellows-Nutting, R. Jang and A. K. Balls, *J. Biol. Chem.*, **179**, 189 (1949); E. Jansen, M.D. Fellows-Nutting and A. K. Balls, *ibid.*, **179**, 201 (1949).

(11) R. Davies and T. Jones, *Phil. Mag.*, **28**, 307 (1939).

DEPARTMENT OF CHEMISTRY
THE CATHOLIC UNIVERSITY OF AMERICA
WASHINGTON, D. C.

Solubility of Cerium(IV) Pyrophosphate

BY J. C. BRANTLEY[1a] AND J. R. HUIZENGA[1b]

RECEIVED JULY 14, 1952

The solubility of cerium(IV) pyrophosphate was measured in water and in four sulfuric acid solutions ranging from 0.114 to 0.684 N. Phosphorus-32[2] was used to trace the cerium(IV) pyrophosphate. The results are given in Table I.

The solubility increases linearly with sulfuric acid concentration in the region 0.114 to 0.342 N acid. Cerium(IV) pyrophosphate has a greater solubility in sulfuric acid than the analogous thorium pyrophosphate[3] has in hydrochloric acid solutions of similar normalities. An examination of our data plus data of Moeller and Schweitzer[3] also indicates that cerium(IV) pyrophosphate is more soluble in hydrochloric acid solutions than in the same normality sulfuric acid solutions by about a factor of two.

(1) (a) Linde Air Products Company, Tonawanda, New York; (b) Argonne National Laboratory, Chicago, Illinois.

(2) Tracer supplied by Oak Ridge National Laboratory.

TABLE I

Acid concn., N	Average c./min.	CeP_2O_7, g./liter	CeP_2O_7, moles/l. $\times 10^4$	ThP_2O_7,[4] moles/l. $\times 10^4$
H_2O	4.5	0.00007 ± 0.00005	0.002	...
0.114	1,740	$.026 \pm .005$	0.8	0.2
.228	3,090	$.046 \pm .005$	1.5	.4
.342	4,950	$.073 \pm .005$	2.3	.6
.684	14,000	$.207 \pm .010$	6.6	...
Standard	9,610			

Experimental

Labeled cerium(IV) pyrophosphate was prepared by reaction of a solution of cerium(IV) ammonium sulfate with an excess of labeled sodium pyrophosphate solution, allowing the precipitate to digest overnight and washing the filtered precipitate thoroughly with warm water and hot ethyl alcohol. The resulting precipitate was a light yellow powder of very fine grain. The labeled sodium pyrophosphate was prepared by heating 4 g. of disodium phosphate with a trace of phosphoric acid containing 400 microcuries of phosphorus-32 in a furnace at 450° for two hours.

The labeled sodium pyrophosphate solution was standardized[3] by treating an excess with a standard solution of thorium nitrate. Thorium pyrophosphate is quantitatively precipitated[3,4] and the activity of the supernatant liquid was measured; 2.521 milligrams of Th^{+4} in 50 ml. precipitated 48.1% of the original pyrophosphate activity. From the decrease in the activity of the sodium pyrophosphate solution and the known amount of thorium nitrate added, the pyrophosphate concentration of the standard solution is calculated to be 3.927 mg. of $P_2O_7^{-4}$ per 50 ml.

The labeled cerium(IV) pyrophosphate was divided among five 50-ml. volumetric flasks containing various amounts of sulfuric acid (Table I). The flasks were shaken regularly for 72 hours in a bath maintained at 25.0°. The supernatant liquids were filtered and the radioactivity of each determined in a glass-jacketed Geiger counter tube with a conventional amplifying and scaling circuit. The standard pyrophosphate solution was counted in a similar manner.

Calculation

$$CeP_2O_7 \text{ (g./liter)} = \text{concn. of std. } (78.54 \times 10^{-3} \text{ g. of } P_2O_7^{-4}\text{/liter})(1.81)\left(\frac{\text{c./min. of unknown}}{\text{c./min. of std.}}\right)$$

1.81 = factor converting pyrophosphate into CeP_2O_7

Acknowledgment.—The experimental work was done as part of a laboratory course in radiochemistry taught by Prof. R. B. Duffield.

(3) From data of Moeller and Schweitzer, *Anal. Chem.*, **20**, 1201 (1948); their solubility data were determined in HCl.

(4) R. J. Carney and E. D. Campbell, THIS JOURNAL, **36**, 1134 (1914).

UNIVERSITY OF ILLINOIS AND
ARGONNE NATIONAL LABORATORY
CHICAGO, ILLINOIS

On the Production of Electronically Excited Molecules in the Oxidation Products of Graphite

BY R. H. BUSSO

RECEIVED JULY 14, 1952

The determination of hydrocarbon and carbon monoxide flame temperatures has revealed the existence of a latent energy in the combustion products. These products are also the center of an after-burning.