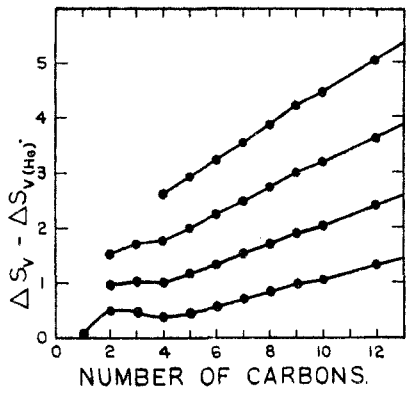

Fig. 3.

entropies are a linear function of the number of carbon atoms from 4 to 12 carbon atoms.

The increase in excess entropy of vaporization with increasing molal volume of vapor (which corresponds to decreasing temperature) is evidence for the freezing in of degrees of freedom in the liquid state as the molecules are crowded more closely together in the contracting liquid. Halford[7] suggested that hindered rotation could be the cause of such departures, but investigated this only for the case of log $(P/T) = 0.1$.

TABLE III

$(\Delta S_v - \Delta S_{v(He)})$ for normal hydrocarbons (Cal. Mole^{-1} Deg.$^{-1}$)

	$\log_{10}(P_{mm}/T)$			
	0.1	−0.4	−0.9	−1.4
Methane	0.06			
Ethane	.49	0.95	1.51	
Propane	.46	1.02	1.69	
Butane	.36	1.00	1.76	2.60
Pentane	.43 (0.6)	1.15	1.99	2.92
Hexane	.54 (1.4)	1.32	2.23	3.24
Heptane	.67 (1.4)	1.51	2.48	3.55
Octane	.81 (1.1)	1.69	2.73	3.87
Nonane	.95	1.90	3.00	4.22
Decane	1.04	2.03	3.18	4.45
Dodecane	1.31	2.40	3.66	5.04
$\Delta S_{v(He)}$	22.46	25.11	27.70	30.28

(7) R. S. Halford, *J. Chem. Phys.*, **8**, 496 (1940).

SYRACUSE, N. Y.

NOTES

Preparation of Glycerol Evenly Labeled with C^{14} [1]

BY S. ABRAHAM

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Various methods for the preparation of glycerol singly labeled with C^{14} have already been reported.[2-4] Although certain of these procedures could be adapted to the preparation of this compound with all of its carbons uniformly labeled with C^{14}, I should like to report here an alternate method utilizing uniformly labeled glucose[5] as starting material.[6]

Evenly labeled glucose with a specific activity of 3×10^5 c.p.m. per mg.[7] was prepared photosynthetically.[5] It was converted in 84% yield to the methyl glucopyranoside with methanol and hydrochloric acid.[8] No attempt was made to isolate the isomers of methylglucopyranoside.

The methyl glucoside was treated with sodium periodate at 2° for 24 hours. The reaction was quantitative as determined by titration of the resulting formic acid. The solution was treated with barium chloride, and the resulting precipitate was filtered and washed. The filtrate was concentrated[9] under reduced pressure at a temperature below 60°.

The dialdehyde remaining in solution was then hydrogenated with Raney nickel at 2,700 p.s.i. and 140° for 18 hours. The dialdehyde in the filtered solution was hydrolyzed with H_2SO_4. Addition of 2,4-dinitrophenylhydrazine solution precipitated the glycolaldehyde as its hydrazone. Excess 2,4-dinitrophenolhydrazine was removed by the addition of formaldehyde.

The solution containing the radioactive glycerol was neutralized with solid $BaCO_3$. The filtrate was clarified with charcoal and concentrated *in vacuo*, at 40°, to a thick, viscous mass. Extraction with boiling, anhydrous acetone (C.P.) and final evaporation of the acetone at room temperature yielded the C^{14}-glycerol. The over-all yield based on glucose was 64%.[10] The refractive index of this glycerol was 1.4398 at 25°. This represents 77.6% glycerol in water.[11] The specific activity of the resulting glycerol was 3×10^5 c.p.m. per mg. $BaCO_3$.

Two-dimensional paper chromatography using phenol:water and butanol:acetic acid:water[12] revealed only one radioactive spot, and this spot was identical with the color spot obtained with inactive glycerol, using a solution of lead tetraacetate in benzene as the color spray.

Two similar experiments with inactive glucose yielded glycerol (65%) which was identified in the following manner: (a) by its refractive index, as given above, (b) by the preparation of the crystalline glycerol tribenzoate, and (c) two-dimensional paper chromatography.

(1) Aided by a grant from the American Cancer Society as recommended by the Committee on Growth of the National Research Council.

(2) A. P. Doerschuk, THIS JOURNAL, **73**, 821 (1951).

(3) H. Schlenk and B. W. DeHaas, *ibid.*, **73**, 2921 (1951).

(4) M. L. Karnovsky and L. I. Gidez, *Federation Proc.*, **10**, 205 (1951).

(5) E. W. Putman, W. Z. Hassid, G. Krotkov and H. A. Barker, *J. Biol. Chem.*, **173**, 785 (1948).

(6) For complete details order Document 3658 from American Documentation Institute, 1719 N Street, N.W., Washington 6, D. C., remitting $1.00 for microfilm (images 1 inch high on standard 35 mm. motion picture film) or $1.00 for photocopies (6 × 8 inches) readable without optical aid.

(7) All compounds were oxidized, and assayed as $BaCO_3$, with an end-window counter.

(8) E. Fischer, *Ber.*, **28**, 1145 (1895).

(9) The formic acid distilled assayed at 3×10^5 c.p.m. per mg.

(10) Corrected for 78% glycerol in water as judged by refractive index.

(11) "International Critical Tables," Vol. 7, McGraw-Hill Book Co., Inc., New York, N. Y., 1930, p. 68.

(12) S. M. Partridge, "Partition Chromatography," Vol. 3, Biochemical Society Symposia, Cambridge, 1950, p. 52.

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).

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

BY ARTHUR B. ANDERSON

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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.

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

BY M. LUCETTA BARNARD AND KEITH J. LAIDLER

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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 Socicty, September 6, 1951.

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