

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 C¹⁴-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 C¹⁴-glycerol was degraded according to the procedure outlined elsewhere.¹³ The C¹⁴ content of each of its carbons was determined; this confirmed that the synthesized glycerol was evenly labeled with C¹⁴. The results are recorded in Table I.

TABLE I
C¹⁴-GLYCEROL PREPARED FROM 24-HOUR PHOTOSYNTHETIC
C¹⁴-GLUCOSE

Com- pound	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 CO₂ with KMnO₄.

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(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

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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).

Experimental³

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 C₉H₈O₂: 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 System¹

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.

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(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).