

separation had not occurred. It is the specific activity originally in the HCN solution multiplied by the amount of total cyanide in each fraction after the run and is therefore identical in shape with the total cyanide band contour. The constant errors shown are those involved in fraction collection. Figure 1b illustrates the ratio of C^{14} to total cyanide in each fraction with the pre-run ratio normalized to 1.00.

As can be seen from the figure, C^{14} -enriched cyanide appeared at the front of the band, the enrichment factor being of the order of 3.5. Toward the rear of the band, C^{14} was depleted by a factor of about 10. There was slight tailing of total cyanide at the band rear. It is evident that areas under curves 1 and 2 in Fig. 1a are not equal. This definitely indicates that the ratio of C^{14}/C^{12} in the cyanide lost in the run is greater than in the cyanide originally utilized in the run. It further indicates that the successful solution of the problem of cyanide depletion in the band will lead to an even more successful isotope separation.

The degree of C^{14} enrichment as a result of this relatively short experiment demonstrates the practicality of this approach to carbon isotope fractionation. We are presently investigating the C^{12} - C^{13} separation which must have occurred in this experiment and the cyanide and resin decomposition described above and expect to make a detailed report at a later date.

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DEPARTMENT OF CHEMISTRY
FLORIDA STATE UNIVERSITY
TALLAHASSEE, FLORIDA

CHARLES N. DAVIDSON
CHARLES K. MANN
RAYMOND K. SHELIN

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RUTHENIUM HEXAFLUORIDE¹

Sir:

The preparation of ruthenium hexafluoride, which we now report, brings to three the number of volatile hexafluorides known for the second transition group elements. Molybdenum hexafluoride has been known for some time and technetium hexafluoride recently has been prepared.² In contrast, hexafluorides are known for all the third transition group elements that might be expected to form them, namely, tungsten through platinum.³ Prior to the present work the only known binary compounds of ruthenium and fluorine were the trifluoride⁴ and the pentafluoride.^{5,6}

This first preparation of ruthenium hexafluoride was made by heating ruthenium metal powder in a fluorine atmosphere at 300 mm. pressure. The

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

(2) H. Selig, C. L. Chernick and J. G. Malm, *J. Inorg. & Nuclear Chem.*, in press.

(3) M. Stacey, J. C. Tatlow and A. G. Sharpe, eds., "Advances in Fluorine Chemistry," Vol. I, Chap. 2, Academic Press, Inc., New York, N. Y., 1960.

(4) E. E. Aynsley, R. D. Peacock and P. L. Robinson, *Chem. & Ind.*, 1002 (1952).

(5) O. Ruff and E. Vidic, *Z. anorg. Chem.*, **72**, 329 (1911).

(6) M. A. Hepworth, R. D. Peacock and P. L. Robinson, *J. Chem. Soc.*, 1197 (1954).

reaction was carried out in a cylindrical quartz reactor (2.9 cm. o.d.) provided with an internal, cold finger that ended about 3 mm. above a shallow, annular, nickel trough which contained the ruthenium. This crucible was supported on three nickel legs and was heated with an induction coil until the ruthenium began to react. The burning metal sustained a reddish incandescence without further heating. The volatile reaction products were condensed rapidly by keeping the walls of the reactor and the cold finger at liquid nitrogen temperature. The main product was a dark-brown solid that collected on the cold finger and distilled under vacuum at room temperature. A light-green material, possibly RuF_5 , that was not volatile at room temperature was obtained in lesser yield. The volatile product was handled and purified by techniques described elsewhere.⁷ Ruthenium metal (1.0 g.) was converted into RuF_6 in 49% yield.

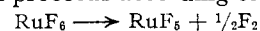
Vapor density determinations indicate a molecular formula RuF_6 (mol. wt. found, 214.3; calcd. for RuF_6 , 215.1). Chemical analyses give the calculated value for fluoride, based on the formula RuF_6 , but give results light in ruthenium possibly owing to volatilization of RuO_4 during analysis (Found: Ru, 39.3; F, 48.1. Calcd. for RuF_6 : Ru, 43.0; F, 48.4). The solid is dark brown in color, has a transformation at 2.5° , and a fusion point at 54° . X-Ray powder patterns show each solid form to be isostructural with the corresponding form of the other transition metal hexafluorides.⁸ The vapor is brownish-red in color, resembling bromine. Preliminary vapor pressure measurements with the solid give the results:

t , ($^\circ C.$)	0.0	4.6	8.6	11.1	14.4	17.6
p , (mm.)	23	32	40	46	56	67

Ruthenium hexafluoride is the least volatile of the three known 4d transition series hexafluorides. Although the volatility of these hexafluorides decreases with increasing atomic number within each transition group, the 4d hexafluorides are less volatile than their 5d counterparts despite being 90 units lighter in molecular weight.

The infrared spectrum has characteristics similar to the other hexafluoride spectra. However, the band $\sigma_3 + \sigma_2$ is even more diffuse than that observed for osmium hexafluoride,⁹ probably because of a stronger Jahn-Teller vibronic coupling in this transition series. The infrared active fundamental, σ_3 , has been observed at 735 cm.^{-1} , compared with 745 cm.^{-1} for TcF_6 and 742 cm.^{-1} for MoF_6 .

Although ruthenium hexafluoride is unstable, it can be stored for weeks at room temperature in a nickel can with only slight decomposition. Ruthenium hexafluoride attacks Pyrex glass rapidly at room temperature but is less reactive in quartz. At 200° RuF_6 is decomposed rapidly and completely into a non-volatile fluoride and fluorine. Measurement of the amount of fluorine produced per mole of RuF_6 decomposed indicates that the decomposition proceeds according to the reaction



(7) B. Weinstock and J. G. Malm, *J. Inorg. Nuclear Chem.*, **2**, 380 (1956).

(8) S. Siegel and D. Northrup, ANL Memorandum, March 24, 1961.

(9) B. Weinstock, H. H. Claassen and J. G. Malm, *J. Chem. Phys.*, **32**, 181 (1960).

The great reactivity may well account for the fact that previous attempts to prepare and identify ruthenium hexafluoride have been unsuccessful.

HOWARD H. CLAASSEN
 ARGONNE NATIONAL LABORATORY
 ARGONNE, ILLINOIS

HENRY SELIG
 JOHN G. MALM
 CEDRIC L. CHERNICK
 BERNARD WEINSTOCK

FORD MOTOR COMPANY
 SCIENTIFIC RESEARCH LABORATORY
 DEARBORN, MICHIGAN

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THE FORMATION OF THE ACYL-ENZYME INTERMEDIATE, TRANS-CINNAMOYL- α -CHYMOTRYPSIN, IN THE HYDROLYSES OF NON-LABILE TRANS-CINNAMIC ACID ESTERS^{1,2}

Sir:

The hydrolysis of labile acyl derivatives (e.g., nitrophenyl esters) is catalyzed by α -chymotrypsin in a three-step process: (i) adsorption of the substrate on the enzyme; (ii) acylation of the enzyme with the release of the phenol; and (iii) deacylation of the acyl-enzyme giving the carboxylic acid product and regenerating the enzyme.³ However, it has been maintained on the basis of kinetic arguments that the α -chymotrypsin-catalyzed hydrolysis of methyl hippurate^{4,5} does not proceed through a hippuryl-enzyme intermediate and, by implication, that the α -chymotrypsin-catalyzed hydrolyses of other non-labile acyl derivatives also do not involve acyl-enzyme formation.

We have investigated the possible existence of *trans*-cinnamoyl- α -chymotrypsin (I) in the enzyme-catalyzed hydrolyses of a series of substrates, both labile and non-labile, derived from *trans*-cinnamic acid (see Table I). In each case, unequivocal evidence has been found for the involvement of I, both from rate measurements and absorption spectral characteristics. Therefore, we conclude

TABLE I
 RATES OF α -CHYMOTRYPSIN CATALYZED HYDROLYSES

Substrate	pH	$k_2 \times 10^3$ (sec. ⁻¹) acylation	$k_3 \times 10^3$ (sec. ⁻¹) deacylation
1 N-Cinnamoylimidazole ^a	9.0	...	13
2 <i>p</i> -Nitrophenyl cinnamate ^a	9.0	...	13
3 <i>m</i> -Nitrophenyl cinnamate ^a	9.0	...	13
4 <i>o</i> -Nitrophenyl cinnamate ^a	9.0	...	13
5 <i>p</i> -Cresyl cinnamate ^a	9.0	...	13
6 Methyl cinnamate ^b	7.80	2.66	(11.1)
7 Benzyl cinnamate ^c	6.95	...	5.31
8 N-Cinnamoylimidazole ^c	6.95	...	5.24

^a $[E]_0 \cong 1.05[S]_0^d$; $[S]_0 = 2-4 \times 10^{-6}M$; 25.6°; 1.6 to 3% CH₃CN. ^b For exact experimental conditions, see Fig. 1. ^c $[E]_0 = 8.68 \times 10^{-4}M$; $[S]_0 = 7.12 \times 10^{-6}M$; 25.0°; 3.2% CH₃CN. ^d $[E]_0$ = initial enzyme concentration; $[S]_0$ = initial substrate concentration.

(1) This research was supported by Grant H-5726 of the National Institutes of Health.

(2) Paper VII in the series "The Mechanism of Action of Proteolytic Enzymes," previous paper, M. L. Bender, G. R. Schonbaum, G. A. Hamilton and B. Zerner, *J. Am. Chem. Soc.*, **83**, 1255 (1961).

(3) For references, see G. R. Schonbaum, K. Nakamura and M. L. Bender, *ibid.*, **81**, 4746 (1959).

(4) S. A. Bernhard, W. C. Coles and J. F. Nowell, *ibid.*, **82**, 3043 (1960).

(5) See also M. L. Bender and W. A. Glasson, *ibid.*, **82**, 3336 (1960), for kinetic results in the hydrolysis of N-acetyl-L-phenylalanine methyl ester which cannot be explained on the basis of acyl-enzyme formation.

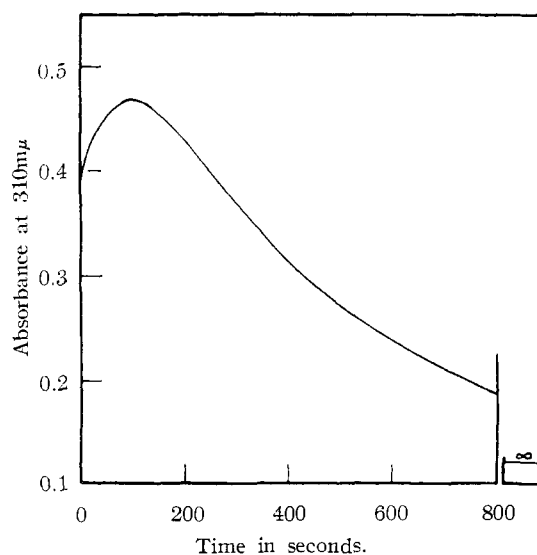


Fig. 1.—The α -chymotrypsin-catalyzed hydrolysis of methyl cinnamate: $[E]_0 = 1.33 \times 10^{-3}M$, $[S]_0 = 1.20 \times 10^{-4}M$, pH 7.80, 25.0°, 3.2% CH₃CN.

that the formation of an acyl-enzyme intermediate is not an artifact of α -chymotryptic catalyses resulting from the lability of the ester function, but is indeed part of the general mechanism of such catalyses.

The rates of enzyme-catalyzed hydrolysis of substrates 1 to 5, inclusive, are followed conveniently spectrophotometrically at 310 m μ ; in each case one observes a very rapid increase in absorbance followed by a slower, first-order decay whose rate constant is $13 \pm 0.5 \times 10^{-3} \text{ sec.}^{-1}$. Further, the appearance of cinnamate ion at 260 m μ has the same rate as the decrease of absorbance at 310 m μ (loss of acyl-enzyme). Since the hydrolysis of *o*-nitrophenyl cinnamate has been shown³ to proceed through I (whose deacylation is rate-controlling), the present data can best be interpreted in terms of the formation of the common intermediate, I, in the hydrolysis of all five substrates.⁶ In each case, acylation is very fast compared with deacylation for these labile derivatives.

However, if the intermediacy of I is general, reactions in which either acylation or deacylation is rate-controlling may be expected to occur,⁷ for example, in the hydrolysis of alkyl esters. Substrates 6 and 7 are two such esters. In order to make these systems experimentally accessible, high concentrations of the enzyme and ratios of $[E]/[S] > 1$ have been used. The hydrolysis of methyl cinnamate is shown in Fig. 1. The absorbance increases to a maximum in about 100 seconds and then decreases according to a strict first-order rate law (after approximately 450 sec.). The rate constant for this latter process is $2.66 \times 10^{-3} \text{ sec.}^{-1}$. Deacylation of I under identical conditions (see Fig. 1) has a rate constant of $11.1 \times 10^{-3} \text{ sec.}^{-1}$.⁸ Consequently the decay process for

(6) Substrates 1, 2 and 3 have different rates of acylation; unpublished work with G. R. Schonbaum.

(7) H. Gutfreund and B. R. Hammond, *Biochem. J.*, **73**, 526 (1959).

(8) Since these reactions were carried out at pH 7.8 instead of pH 9.0, the deacylation is somewhat slower here than with substrates 1-5.