cells were used in a concentration of 24 mg. (dry weight) per ml. of dist. water.

For the preparation of a cell-free extract of crushed *E. coli* cells, 35 cc. of a chilled bacterial suspension (24 hr. growth, 3.3 g. dry weight) in distd. water was passed for 3 hr. through an ice-cooled wet crushing mill for bacteria,[20] constructed by Unicam Instruments, Ltd., Cambridge, England. All subsequent operations also were carried out in the cold. The opalescent supernatant from a centrifugation at 8000 × *g* for 30 minutes was divided into two fractions by centrifugation at 31,000 × *g* for 2 hours: a sediment, retaining a negligible deaminating activity for cytosine (about 2% deamination in 2 hr.), and a very active, clear yellowish supernatant (95% deamination in 2 hr. at 36°), containing 21 mg. of dry matter per ml. The latter was used in the enzyme experiments.

The preparation of a cell-free extract of baker's yeast (6 g. of fresh yeast per ml. of dist. water) followed similar procedures. Here again, the sediment obtained by centrifugation at 31,000 × *g*, similar to the lipoprotein particles described previously,[21] displayed little activity, in contrast to the very active supernatant, containing 62 mg. of dry matter per ml. (91% deamination in 60 minutes at 36°).

All assays were carried out at 36° and *p*H 7.0; the proportions of substrate and enzyme were the same as for adenase and guanase. When incubation was performed *in vitro*, aliquots were acidified prior to their analysis for uracil, cytosine and ammonia. The separation of the reaction mixtures was achieved with *n*-butanol saturated with water as the chromatography solvent.[3] The paper strips were equilibrated with gaseous NH_3 before chromatography. The deamination of cytosine to uracil by *E. coli*, followed by the technique described as "on paper-moist," is illustrated in Fig. 1. Several *in vitro* experiments are shown in Fig. 2. Between 90 and 100% of the pyrimidine substrate could be accounted for in all experiments. The uracil formed by deamination was identified by chromatography and determination of the complete ultraviolet absorption spectrum. Experiments with cell-free yeast extracts, including some carried out in a nitrogen atmosphere, are assembled in Table IV. The yeast enzyme preparations were inactive toward adenine and guanine.

TABLE IV

DEAMINATION OF CYTOSINE BY CELL-FREE YEAST EXTRACTS[a]

Preparation	Incubation time, min.	Extent of deamination From uracil formed, %	Extent of deamination From ammonia liberated, %
1900 × *g* supernatant	30	65	
	60	81	
31,000 × *g* supernatant, prepn. 1	60		91
31,000 × *g* supernatant, prepn. 2	120	98	
31,000 × *g* supernatant, prepn. 3	60	89	
	60[b]	100	
31,000 × *g* supernatant, prepn. 4	60		70
	60[c]		72

[a] The incubations were carried out *in vitro*. [b] Nitrogen atmosphere. [c] Evacuated Thunberg tube, after replacement of air by nitrogen.

NEW YORK, N. Y.

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[21] M. A. Nyman and E. Chargaff, *J. Biol. Chem.*, **180**, 741 (1949).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF DENISON UNIVERSITY]

Stereochemistry of Formation and Hydrolysis of Esters of Perfluoro Acids

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High yields of esters have been obtained by direct esterification of (–)2-octanol with trifluoroacetic acid and with perfluorobutyric acid. Both the formation and hydrolysis of the esters have been shown to proceed without inversion or racemization.

Esterification of secondary alcohols with acetic acid and saponification of the esters have been shown to occur with complete retention of configuration[1]; *i.e.*, only the acyl–oxygen bond is broken. The same course has been established even for the stronger carboxylic acid, trichloroacetic acid; formation and saponification of neopentyl trichloroacetate occurs without rearrangement of the neopentyl skeleton.[2] However, when small amounts of sulfuric acid are used as catalysts for the esterification reaction, esters are formed from acetic acid and optically active alcohols with a small but measurable degree of racemization.[1] It is well known that with the strong acids HCl and HBr, halide ion displaces –OH in the alcohol to give the inorganic ester with inversion of configuration.[3] The hydrolysis of esters of the strong acid *p*-toluenesulfonic acid also proceeds with inversion.[4]

It was of interest then to ascertain the course of similar reactions involving very strong carboxylic acids such as trifluoroacetic acid and perfluorobutyric acid. Each of the three paths, *i.e.*, retention of configuration (normal esterification with un-ionized acid), partial racemization (strong acid catalysis), or inversion (displacement by perfluoroalkanoate anion), while not equally probable, at least seemed plausible.

From both trifluoroacetic acid and perfluorobutyric acid, the esters were prepared in high yields by simply refluxing (–)2-octanol with a slight excess of the acid. The optical rotations of these esters were identical both in sign and magnitude with the corresponding esters prepared from the acid chlorides, whereby no attack is made on the asymmetric center and therefore no change in configuration can occur. Similarly hydrolysis of the esters with aqueous alkali led to recovery of the

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[1] E. D. Hughes, C. K. Ingold and S. Masterman, *J. Chem. Soc.*, 840 (1939).

[2] O. R. Quayle and H. M. Norton, THIS JOURNAL, **62**, 1170 (1940).

[3] For summary see H. Gilman, ed., "Organic Chemistry: An Advanced Treatise," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1943, pp. 278, 279.

[4] F. C. Fuson, "Advanced Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 584.

alcohol with complete retention of configuration. Since the direct esterification is most probably an acid-catalyzed equilibrium reaction,[5] acid hydrolysis would be expected to give the same result. Thus it is established that esterifications involving these perfluoro acids and hydrolysis of their esters proceed without any change of configuration of the alcohol. Also it seems apparent that the strength of the acid involved (considering the sulfonic and perfluoro acids to be of comparable strength) plays an insignificant role, if any, in determining the courses of the reactions. Ease of addition to the polarized >C=O group of the carboxylic acid is probably the determining factor; the results reported here are consistent with the mechanism accepted for formation and hydrolysis of esters involving secondary alcohols and other carboxylic acids.[5]

It may be mentioned that the esters prepared in this study are believed to be new compounds, described here for the first time. The unusually low optical rotation of the *s*-octyl trifluoroacetate coupled with its low refractive index is of interest.

Acknowledgment.—The author is grateful to Professor Harold Shechter of The Ohio State University for the gift of a generous sample of partially resolved (−)2-octanol. The infrared data were obtained with the coöperation of the Department of Chemistry, The Ohio State University, Columbus. This study was supported in part by a grant from the Faculty Research Fund of Denison University.

Experimental

Boiling points reported are uncorrected. Optical rotations were observed with homogeneous compounds in one decimeter tubes and experimental error is believed to be within the limits ±0.03°. Research samples of trifluoroacetic acid and perfluorobutyric acid, supplied by Minnesota Mining and Manufacturing Company, Minneapolis, were redistilled before use.

(−) *s*-**Octyl Trifluoroacetate. A. From Trifluoroacetyl Chloride.**—In a three-necked, 200-ml. flask fitted with a mercury-sealed stirrer, a dropping funnel, and a condenser was placed 15.5 g. (0.11 mole) of benzoyl chloride.[6] A glass delivery tube from the top of the condenser passed into 6.5 g. (0.05 mole) of (−)2-octanol (b.p. 75° (11 mm.) $n^{25}\mathrm{D}$ 1.4241, $d^{25}{}_4$ 0.817, $\alpha^{25}\mathrm{D}$ −3.85°) in a small flask; all exits were protected with drying tubes. During 20 minutes, 9.1 g. (0.08 mole) of trifluoroacetic acid (b.p. 69.8° (726 mm.)) was added dropwise with stirring to the benzoyl chloride. This mixture was heated to gentle reflux. Bubbles of gas appeared in the alcohol and the mixture became quite warm. After 75 minutes the evolution of gaseous CF_3-COCl from the initial reaction flask appeared virtually to have ceased.

The ester was dissolved in petroleum ether (b.p. 60–70°) and this solution was washed once with water, twice with 5% sodium bicarbonate solution, and again with water. After being dried with Drierite, the material was distilled. Two distillations at reduced pressure gave 8.0 g. (71%) of (−)*s*-octyl trifluoroacetate boiling at 78.5–79.0° (20 mm.); $n^{25}\mathrm{D}$ 1.3744, $d^{25}{}_4$ 1.000, $\alpha^{25}\mathrm{D}$ −0.10°. Although the carbon analysis below is high, possibly suggesting contamination by 2-octanol, the lack of the characteristic -OH absorption band (at 3.0 microns) in the infrared absorption pattern excluded the presence of any significant amount of alcohol in the sample.

Anal.[7] Calcd. for $C_{10}H_{17}O_2F_3$: C, 53.09; H, 7.58. Found: C, 53.98; H, 7.88.

B. **From Trifluoroacetic Acid.**—A mixture of 13.0 g. (0.10 mole) of (−)2-octanol ($\alpha^{25}\mathrm{D}$ −3.85°) and 13.7 g. (0.12 mole) of trifluoroacetic acid was refluxed for 12 hours. During the heating period the top of the condenser was protected with a drying tube. The solution was dissolved in petroleum ether (b.p. 60–70°) and treated as described previously. On distillation two fractions were collected: I, 14.9 g. (66%), b.p. 78.8–79.8° (20 mm.), $n^{25}\mathrm{D}$ 1.3746, $d^{25}{}_4$ 1.001, $\alpha^{25}\mathrm{D}$ −0.13°; and II, 5.4 g. (24%), b.p. 79.8–80.8° (20 mm.), $n^{25}\mathrm{D}$ 1.3732.

Saponification of (−)*s*-Octyl Trifluoroacetate.—A mixture of 14.0 g. (0.062 mole) of (−)*s*-octyl trifluoroacetate ($\alpha^{25}\mathrm{D}$ −0.13°) and 80 g. of 10% sodium hydroxide solution was refluxed for 2 hours and then steam distilled. The alcohol was recovered from the distillate by the usual procedure (salting out and extraction). Distillation gave 7.2 g. (89%) of (−)2-octanol; b.p. 79.2–79.4° (12 mm.), $n^{25}\mathrm{D}$ 1.4240, $d^{25}{}_4$ 0.816, $\alpha^{25}\mathrm{D}$ −3.87°.

(−)*s*-**Octyl Perfluorobutyrate. A. From Perfluorobutyryl Chloride.**—Perfluorobutyryl chloride was prepared by adding 21.4 g. (0.10 mole) of perfluorobutyric acid (b.p. 120°) dropwise with stirring in 50 minutes to 27.1 g. (0.13 mole) of PCl_5 in a three-necked, 200-ml. flask. A delivery tube from the top of the condenser led into a receiving flask immersed in an ice–salt-bath (−10°). All exits were protected with drying tubes. When the addition of the acid was complete, nearly all of the solid had dissolved. After 2.5 hours of heating with stirring, the distillation of the acid chloride through the condenser appeared to be complete. The yield was 17 g. (73%).

This acid chloride (0.073 mole) was added dropwise with stirring to a solution of 7.4 g. (0.055 mole) of (−)2-octanol ($\alpha^{25}\mathrm{D}$ −3.85°) and 10 ml. of petroleum ether (b.p. 35–60°) in a three-necked flask. Gentle refluxing occurred during the 20-minute addition period. The material was left standing for one hour before being washed with water and 5% sodium bicarbonate solution. On distillation, 16.6 g. (93%) of (−)*s*-octyl perfluorobutyrate was obtained. Redistillation gave material with the following properties: b.p. 80.4–81.6° (10 mm.), $n^{25}\mathrm{D}$ 1.3551, $d^{25}{}_4$ 1.171, $\alpha^{25}\mathrm{D}$ −2.23°.

Anal. Calcd. for $C_{12}H_{17}O_2F_7$: C, 44.17; H, 5.25. Found: C, 44.19; H, 5.28.

B. **From Perfluorobutyric Acid.**—A mixture of 13.0 g. (0.10 mole) of (−)2-octanol ($\alpha^{25}\mathrm{D}$ −3.85°) and 25.7 g. (0.12 mole) of perfluorobutyric acid was refluxed for 11 hours. After cooling overnight, the solution was dissolved in petroleum ether (b.p. 60–70°) and treated as described previously. Distillation yielded two fractions: I, 15.6 g. of alcohol–ester mixture collected until the temperature became constant; b.p. 70–82° (10 mm.), $n^{25}\mathrm{D}$ 1.3612; and II, 13.1 g. (40%) of ester boiling constantly at 79.4° (9 mm.); $n^{25}\mathrm{D}$ 1.3551, $d^{25}{}_4$ 1.173, $\alpha^{25}\mathrm{D}$ −2.23°. (On the basis of refractive indices, fraction I appears to be over 90% ester; precise fractionation probably would afford pure ester from this mixture. The total yield of ester then was about 83%.) The infrared absorption pattern, with no absorption band in the region of 3.0 microns, established the absence of any alcohol-contaminant in fraction II.

Saponification of (−)*s*-Octyl Perfluorobutyrate.—(−)*s*-Octyl perfluorobutyrate ($\alpha^{25}\mathrm{D}$ −2.23°) (19.0 g., 0.058 mole) was refluxed for 18 hours[8] with 100 g. of 10% sodium hydroxide solution and the mixture was steam distilled to give the alcohol. Customary separation and distillation gave 7.0 g. (82%) of (−)2-octanol; b.p. 79.9–80.2° (12 mm.), $n^{25}\mathrm{D}$ 1.4243, $d^{25}{}_4$ 0.817, $\alpha^{25}\mathrm{D}$ −3.85°.

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[5] (a) J. N. E. Day and C. K. Ingold, *Trans. Faraday Soc.*, **37**, 686 (1941); (b) E. R. Alexander, "Principles of Ionic Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1950, pp. 224-235.

[6] The preparation of CF_3COCl is based upon the procedure reported by A. L. Henne, R. M. Alm and M. Smook, THIS JOURNAL, **70**, 1968 (1948).

[7] Analyses performed at Elek Micro Analytical Laboratories, Los Angeles, California.

[8] When the mixture was refluxed for only 2 hours, the crude organic material obtained on steam distillation had a greater density than water and a refractive index $n^{22}\mathrm{D}$ 1.3700, indicating that only partial hydrolysis of the ester had taken place. This distillate was then refluxed overnight with 10% sodium hydroxide solution (prepared from the aqueous portion of the steam distillate to minimize losses of organic material) to ensure complete saponification. The slower rate of the perfluorobutyrate as compared with the trifluoroacetate in heterogeneous hydrolysis probably arises mainly from solubility differences.