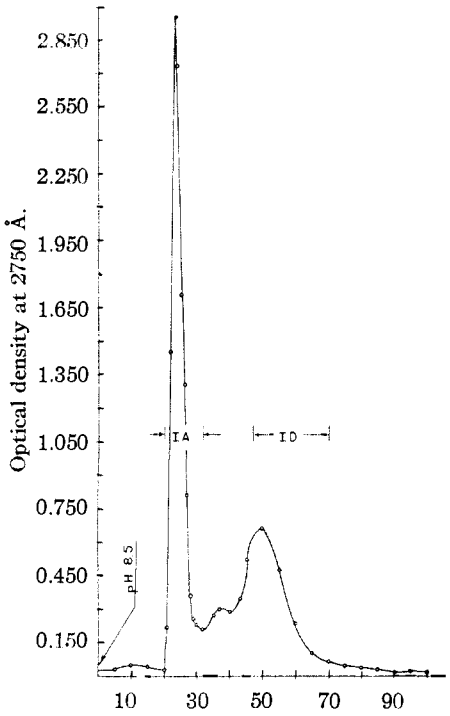

Fig. 2.—Chromatography of an oxycellulose eluate ACTH preparation on Amberlite XE-97 resin. The sample (430 mg. at 40 u./mg.) in 100 ml., *p*H 8.5 buffer was applied to a column 70 cm. high in a tube 5.4 cm. in diameter. Rate of flow was 3.5 ml./min, Volume collected per tube was 17 ml. The distribution of ultraviolet absorption was: IA, 54%; ID, 20%. The distribution of activity was: IA, < 2%; ID, 70%.

XE-97 column improved the resolution at *p*H 8.5. Figure 2 shows a run using a 70-cm. bed.

TABLE I

EFFECT OF VARIOUS HYDROLYTIC TREATMENTS ON THE PERFORMANCE OF AN OXYCELLULOSE ELUATE[a] ON XE-97 RESIN

Type of hydrolytic treatment	*p*H 8.5 (Type ID) O.D.[b]	*p*H 8.5 (Type ID) Activity[c]	*p*H 9.25 (Type II) O.D.	*p*H 9.25 (Type II) Activity	*p*H 11.25 (Type III) O.D.	*p*H 11.25 (Type III) Activity
None	31	70	5	<5	17	<5
Pepsin[d]: 2 hours	None	<5	21	100	17	<5
Pepsin: 24 hours	None	<5	44	80	12	<5
Pepsin: 4 hours followed by acid (1 hour at 100° in 0.01 *N* HCl)	None	<5	27	75	21	25

[a] The starting material in the experiments summarized in this table was a typical oxycellulose eluate made by the Astwood process and having a potency of about 25 μ/mg. [b] Optical densities are given as percentages of the total optical density recovered in all fractions. The difference between the totals given in the three fractions listed and 100 is the amount appearing before fraction ID. [c] Given as percentages of the amount of activity put on the column. Where the totals do not add up to 100, it is assumed that the remainder was destroyed in handling. [d] In these experiments, the amount of pepsin was 1% of the ACTH fraction and the digestion was done at 37° in 0.01 *N* HCl (*p*H 2.1–2.3). In other experiments, a considerable variation (0.6–4%) in the percentage of pepsin was without appreciable effect on the results.

In studying the relationships between the three types of ACTH, an oxycellulose eluate has been treated with pepsin and with pepsin and acid. Table I shows the fractionation of these materials on XE-97 resin. The percentages of ultraviolet absorption and of activity going to the various positions are shown in each case. As seen in the table, treatment with pepsin for as little as two hours converts all the type ID activity to type II, while even 24 hours does not produce an appreciable amount of type III. However, subsequent treatment with acid converts at least part of type II into type III.

In view of the fact that the variations in the conditions of pepsin treatment in the experiments of Table I and of other experiments (*cf.* footnote *d*, Table I) include those used by Brink, *et al.*,[7] it would appear likely that Type II activity predominated in the concentrate from which Corticotropin-B was isolated. Our experiments, using XE-97 resin on material processed by successive pepsin and acid treatment, appears to be the first in which two hydrolyzed types of ACTH are clearly differentiated. Further work directed toward the isolation in pure form of the three active types is under way.

Acknowledgment.—The authors wish to acknowledge the technical assistance of Mr. R. L. Peters.

(7) N. G. Brink, F. A. Kuehl, Jr., J. W. Richter, A. W. Bazemore, M. A. P. Meisinger, D. E. Ayer and K. Folkers, THIS JOURNAL, **74**, 2120 (1952).

THE ARMOUR LABORATORIES
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Rates of Solvolysis of Some Alkyl Fluorides and Chlorides[1]

BY C. GARDNER SWAIN AND CARLETON B. SCOTT

RECEIVED MAY 14, 1952

Table I shows that the RCl/RF rate ratio for hydrolysis in neutral or slightly acidic solutions varies from 10^6 for triphenylmethyl (trityl) halides to less than 10^2 for benzoyl halides. This reflects the tendency of C–X rupture to be more complete than O–C formation at the transition state of trityl halide hydrolysis, and the opposite tendency with benzoyl halides.[2] The change from Cl to F hinders the C–X break, but facilitates O–C formation by making the carbon more electron-deficient and positive.

The ratio is further reduced in basic solution (*cf.* Table II). Toward hydroxide ion, benzoyl fluoride actually reacts faster (by 40%) than benzoyl chloride.

Experimental

Reagents.—Benzoyl fluoride was prepared from 140 g. (1 mole) of benzoyl chloride in a polyethylene bottle, fitted with copper entrance and exit tubes in a 2-hole rubber stopper, by passing in anhydrous hydrogen fluoride until the exit gas gave no precipitate with silver nitrate solution. Best results were obtained when the polyethylene bottle rested in an ice-bath and the hydrogen fluoride was con-

(1) This work was supported by the Office of Naval Research.

(2) The same factor is responsible for the negative ρ-values[3] and small s-values[4] generally observed with trityl halides in contrast to the positive ρ-values and large s-values with benzoyl halides.

(3) C. G. Swain and W. P. Langsdorf, Jr., THIS JOURNAL, **73**, 2813 (1951).

(4) C. G. Swain and C. B. Scott, *ibid.*, **75**, 141 (1953).

TABLE I

RELATIVE RATES OF SOLVOLYSIS OF ORGANIC CHLORIDES AND FLUORIDES

Compound	Solvent	Temp., °C.	k_1, sec.$^{-1}$	ΔS^*, cal. deg.$^{-1}$	ΔE^*, kcal.	k_{RCl}/k_{RF}
Trityl fluoride[a]	15% H_2O 85% acetone	25	2.7×10^{-6}	−10	22.6	
Trityl chloride[b]	15% H_2O 85% acetone	25	2.7	−17	12.5	1×10^6
t-Butyl fluoride[c]	20% H_2O 80% EtOH	25	1×10^{-10}			
t-Butyl chloride[c]	20% H_2O 80% EtOH	25	9.1×10^{-6}			1×10^5
Acetyl fluoride	25% H_2O 75% acetone	25	1.1×10^{-4}			
Acetyl chloride[d]	25% H_2O 75% acetone	25	8.6×10^{-1}	−14	13.9	7.8×10^3
Benzenesulfonyl fluoride	50% H_2O 50% acetone	25	$<5 \times 10^{-8}$			
Benzenesulfonyl chloride	50% H_2O 50% acetone	25	2.4×10^{-4}	−29	14.3	$>4.8 \times 10^3$
Benzoyl fluoride	50% H_2O 50% acetone	0.5	1.1×10^{-5}			
Benzoyl fluoride[e]	25% H_2O 75% acetone	25	8.2×10^{-6}			
Benzoyl chloride[f]	50% H_2O 50% acetone	0	4.3×10^{-4}	−7.1	18.8	39
Benzoyl chloride[e]	25% H_2O 75% acetone	25	7.2×10^{-4}			88

[a] Calculated from runs in 30% water–70% acetone by Mr. R. B. Mosely. [b] Calculated from runs at −34 and −14°. [c] K. A. Cooper and E. D. Hughes, *J. Chem. Soc.*, 1183 (1937). [d] Calculated from runs at −30 and −11°. [e] Data supplied by Mr. D. E. Bown. [f] G. Berger and S. C. J. Olivier, *Rec. trav. chim.*, **46**, 516 (1927).

TABLE II

RATES WITH HYDROXIDE ION IN 50% WATER–50% ACETONE AT 0.5°

Compound	k_2, M^{-1} sec.$^{-1}$	Relative to solvolysis[a]
Acetyl fluoride	250	3.1×10^7
Benzoyl fluoride	21	5.4×10^7
Benzoyl chloride	15	1.0×10^6
Benzenesulfonyl fluoride	0.11	$>6 \times 10^7$
Benzenesulfonyl chloride	0.68	7.3×10^5

[a] Values of k/k° where $k = k_{OH^-} = k_2$, $k^\circ[H_2O] = k_w = k_1$.

densed by cooling the entrance tube. Frequent swirling of the contents was necessary since the reaction was very vigorous. After the addition, the ice-bath was replaced by a steam-bath until hydrogen fluoride evolution ceased. The dark liquid residue was dissolved in benzene and rapidly extracted with ice-water to remove more hydrogen fluoride. The benzene solution was dried over sodium sulfate and the benzoyl fluoride distilled, yielding 74 g. (60%), b.p. 155–157°, $n^{15}D$ 1.4988.[5]

Acetyl fluoride was prepared from 150 g. (2.1 moles) of acetyl chloride added dropwise from a dropping funnel to 100 g. (0.96 mole) of zinc fluoride (Harshaw technical, dried at 100° for 10 hours under oil-pump vacuum) in a 500-ml. flask at 0° fitted with vertical condenser, sealed stirrer and calcium chloride tubes.[6] After the addition, the water-bath was warmed to 40°. The vapors passed through a 50-cm. vertical condenser held at 20°, then into a condensing head and well-cooled receiver. The distillate was mixed with 3 g. of anhydrous sodium fluoride, redistilled through a small Vigreux column and stored in a polyethylene bottle containing a small test-tube of sodium fluoride to remove hydrogen fluoride; yield 76 g. (1.2 moles, 63%), b.p. 19.5–20.0°.

Benzoyl bromide from Eastman Kodak Co. was redistilled, b.p. 80° (7 mm.), $n^{25}D$ 1.5864.

Benzenesulfonyl fluoride from the Pennsalt Co. was redistilled, b.p. 83° (3 mm.), $n^{25}D$ 1.4897.

TABLE III

SAMPLE KINETIC DATA

Substrate	Concn., $M \times 10^3$	Water, % by volume	Temp., °C.	Added reagent	Concn., $M \times 10^3$	k_1, sec.$^{-1}$	Run no.
Acetyl chloride	6.8	25	−30	.....	...	4.1×10^{-3}	254
	7.6	25	−11	.....	...	3.3×10^{-2}	255
Acetyl fluoride	3.3	25	25	.....	...	1.1×10^{-4}	267
	4.7	50	0.5	.....	...	2.2×10^{-4}	248
	8.6	50	.5	$LiClO_4$	100	2.0×10^{-4}	251
	4.9	50	.5	$HClO_4$	100	3.1×10^{-4}	252
	2.8	50	.5	H_3BO_3[a]	20	4.1×10^{-2}	244
				NaH_2BO_3	20		

(5) A. I. Mashentsev, *J. Gen. Chem., U.S.S.R.*, **15**, 915 [1945]; *C. A.*, **40**, 6443 (1946), reported $n^{15}D$ 1.4988 from a different synthesis.

(6) M. Meslans, *Ann. chim. phys.*, [7] **1**, 411 (1894).

TABLE III (*Continued*)

Substrate	Concn., $M \times 10^3$	Water, % by volume	Temp., °C.	Added reagent	Concn., $M \times 10^3$	k_1, sec.$^{-1}$	Run no.
Benzoyl fluoride	4.4	50	.5	.....	...	1.1×10^{-5}	156
	1.6	50	.5	H_3BO_3[a]	20	3.6×10^{-3}	149
				NaH_2BO_3	20		
	2.1	50	.5	H_3BO_3[a]	10	3.2×10^{-3}	151
				NaH_2BO_3	10		
Benzoyl bromide	3.7	50	.5	.....	...	6.3×10^{-2}	265
	4.8	50	.5	H_3BO_3[a]	20	5.3×10^{-2}	263
				NaH_2BO_3	20		
Benzenesulfonyl fluoride	5.1	50	25.1	.....	...	$<5 \times 10^{-8}$	257
	4.9	50	25.1	$HClO_4$	100	$<5 \times 10^{-8}$	260
	6.9	50	0.5	H_3BO_3[a]	20	1.8×10^{-5}	242
				NaH_2BO_3	20		

[a] Hydroxide ion concentration = 1.6×10^{-4} *N*.

Other reagents were analytical reagent grade or previously described.[4]

Procedure.—Most of the procedure has been described.[4] The rate of hydrolysis of acetyl fluoride in 25% water–75% acetone was determined by allowing a mixture of 150 ml. of acetone and 50 ml. of water to come to 25° in a 250-ml. polyethylene bottle and adding acetyl fluoride directly from a pipet. Aliquots (10 ml.) were shaken with 20 ml. of benzene, the aqueous layer removed, and the benzene extracted twice with 5 ml. of water. The water solutions were combined and titrated for fluoride ion.

The hydrolysis of acetyl fluoride in 50% water–50% acetone was accomplished by cooling a mixture of 45 ml. of acetone and 50 ml. of water at 0.5° in the 100-ml. round-bottomed reaction cell and adding the acetyl fluoride in 5 ml. of cold acetone. The 10-ml. aliquots were shaken with 20 ml. of chloroform and titrated for fluoride ion. When an inert salt or an acid was present, 5 ml. of 2 *N* lithium perchlorate or perchloric acid replaced 5 ml. of water in the solvent.

The hydrolyses of benzoyl fluoride and benzenesulfonyl fluoride were followed in a similar manner. The reaction cell was a 250-ml. polyethylene bottle and the solvent was 100 ml. of acetone and 100 ml. of water. The aliquots for benzoyl fluoride were 20 ml., those for benzenesulfonyl fluoride were 10 ml. Since benzenesulfonyl fluoride hydrolyzed at an extremely slow rate, if at all, the 100% point was found by hydrolyzing a 10-ml. aliquot with sodium hydroxide and titrating for fluoride ion. The reaction proceeded to less than 10% in 2.2×10^6 seconds (26 days). The presence of 0.1 *N* lithium perchlorate or perchloric acid had no apparent effect on the rate.

Table III gives supporting kinetic data in addition to those previously reported.[4]

DEPARTMENT OF CHEMISTRY
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COMMUNICATIONS TO THE EDITOR

THE FRACTIONATION OF HYDROGEN ISOTOPES IN BIOLOGICAL SYSTEMS[1]

Sir:

Although deuterium has been extensively used as an isotopic tracer in studies of intermediary metabolism,[2] relatively little is known about the H:D fractionation that occurred and its effect on the quantitative interpretation of the metabolic data. Although this factor can be measured in chemical reactions it is inherently difficult to measure in metabolic (*in vivo*) studies utilizing only protium (H) and deuterium. However, the use of precursor compounds labeled with both deuterium and tritium can yield precise values for D:T fractionation effects in such studies and the latter can then be used to estimate these effects for D relative to H.[3] We have administered water containing D and T to rats by intraperitoneal injection in order to bring the deuterium body water level up to about two per cent. and then supplied drinking water having the same T/D ratio for several days to maintain this level. Analysis of the glycogen and fatty acid fractions from the livers of these animals shows a preferential incorporation of the deuterium by approximately 8 and 18 per cent., respectively (Table I). The results for the fatty acids are in qualitative agreement with those recently reported by Glascock and Dunscombe.[4] In the latter experiments, the body fluid isotope

(1) This work was supported in part by grants-in-aid from the Atomic Energy Commission No. AT(30-1)-910.

(2) R. Schoenheimer, Dynamic State of Body Constituents, Harvard University Press, 1946; M. Kamen, Radioactive Tracers in Biology, Chap. VII, Academic Press, N. Y., 1951.

(3) W. G. Verley, J. R. Rachele, V. du Vigneaud, M. L. Eidinoff and J. E. Knoll, THIS JOURNAL, **74**, 5941 (1952). When methanol containing CD_3OH, CHD_2OH, CH_2DOH and CH_2TOH was administered to rats, the (T/D) ratio in the methyl groups of choline and creatine was greater than the corresponding ratio in the administered methanol.

(4) R. F. Glascock and W. G. Dunscombe, *Biochem. J.*, **51**, August, (1952), xl, Communication to Proceedings of the Biochemical Society.