[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Abnormal Esters of Cholesterol

BY LOUIS F. FIESER AND WILLIAM P. SCHNEIDER[1]

Demonstration of low-order carcinogenicity of cholesterol injected into mice in lard solution (Hieger) and of high carcinogenic potency of the crude Spielman–Meyer progesterone preparation derived from cholesterol (Bischoff and Rupp) suggests the possible existence of a non-aromatic steroid carcinogen derived from or related to cholesterol. One of three hypotheses under investigation is that the substance is an ester of cholesterol and a fatty acid abnormal to the tissues of the injection site. The ester of cholesterol with isoheptylic acid, which is abnormal with respect to both carbon content and branching, was synthesized for bioassay. Esters of cholesterol with preparations rich in arachidonic acid also may represent abnormal types. The relative rates of enzymatic hydrolysis of the β-naphthyl esters of a series of normal and abnormal fatty acids were determined by the colorimetric method of Nachlas and Seligman.

Hieger[2] observed that subcutaneous injection of cholesterol in lard solution into several hundred mice resulted in 4–6% incidence[3] of slowly developing (18 months) tumors at the site of injection. Bischoff and Rupp[4] injected into mice Spielman and Meyer's[5] crude progesterone preparation, derived from cholesterol by bromination, oxidation, and debromination, and obtained slowly developing tumors in 32% of the animals treated, whereas they found pure progesterone to be non-carcinogenic. The results suggest the existence of a steroid carcinogen derived from cholesterol or a companion substance by a low-temperature process that might, under a coincidence of circumstances, occur in the body.

The possibility, suggested by the demonstrated carcinogenicity of methylcholanthrene[6] and by the pyrolytic conversion of bile acids[7] and of cholesterol[8] into this substance, that neoplastic growth can be initiated by abnormal metabolism of a body steroid to an aromatic hydrocarbon cannot be definitely excluded, but the evidence that has accumulated in the 17 years that this initially inviting hypothesis has been under investigation strongly discounts the possibility. The property of initiating malignant growth is now known not to be specific to polycyclic aromatic hydrocarbons of the methylcholanthrene and 1,2-benzpyrene types but to be exhibited, in varying degree and kind, by such structurally distinct compounds as *o*-aminoazotoluene,[9] 2-acetylaminofluorene[10] and vinylcyclohexene diepoxide.[11] There is, then, no *a priori* reason to exclude the possibility of the existence of a non-aromatic steroid carcinogen related to cholesterol.

This paper is concerned with one of three hypotheses that are under investigation.[12] The idea was suggested by the fact that, in early control experiments, Bischoff[4,13] observed no tumor production from cholesterol injected into mice in sesame oil or in colloidal aqueous dispersion, whereas the tumors observed by Hieger[2,3] resulted from injection of cholesterol in 20% solution in lard. The long period of incubation of the implanted cholesterol–lard mixture could afford opportunity for a transesterification that might produce an ester of cholesterol with a fatty acid abnormal to the particular tissues of the injection site. The hypothesis is weakened by subsequent experiments by Bischoff[13] in which a batch of commercial cholesterol administered in sesame oil produced 6–19% incidence of tumors. However, differences have been noted in the amounts of ester cholesterol and phospholipid in the cell nuclei of normal and tumorous rat livers,[14] and Leary[15] has expressed the view that crystalline ester cholesterol deposited focally is the stimulating agent responsible for the growth of benign cortical adenomas in man.

That the Spielman–Meyer process affords both cholestenone and progesterone shows that the side chain is in part retained and in part subjected to oxidative fission. Since methyl isohexyl ketone is a known product of oxidative fission of the side chain,[16] isoheptylic acid might have been produced in the oxidation step; the final partition of the reaction mixture between petroleum ether and concentrated hydrochloric acid could have effected esterification of unchanged cholesterol with such an acid. As far as is known, cholesteryl isoheptylate would be abnormal to subcutaneous tissue in respect both to the odd-carbon content and the branching of the fatty acid component.[17]

Cholesteryl isoheptylate was synthesized and is being tested for possible carcinogenic activity by Dr. Fritz Bischoff. Arachidonic acid also seemed of interest as an acid component, since this tetraunsaturated C_{20}-acid is present in the fatty acids of human depot fat to the extent of only 0.6%[18] but accounts for 22% of the fatty acids

(1) Postdoctoral Research Fellow on an Institutional grant from the American Cancer Society.

(2) L. Hieger, *Cancer Research*, **6**, 657 (1946); *Nature*, **160**, 270 (1947); *Brit. J. Cancer*, **3**, 123 (1949).

(3) Supplementary data personally communicated by Dr. I. Hieger.

(4) F. Bischoff and J. J. Rupp, *Cancer Research*, **6**, 403 (1946).

(5) M. A. Spielman and R. K. Meyer, THIS JOURNAL, **61**, 893 (1939).

(6) J. W. Cook and G. A. D. Haslewood, *J. Chem. Soc.*, 428 (1934).

(7) H. Wieland and E. Dane, *Z. physiol. Chem.*, **219**, 240 (1933); L. F. Fieser and M. S. Newman, THIS JOURNAL, **57**, 961 (1935).

(8) W. Rossner, *Z. physiol. Chem.*, **249**, 267 (1937).

(9) T. Yoshida, *Trans. Soc. Path. Japan*, **22**, 193, 934 (1932); **23**, 636 (1933); **24**, 523 (1934).

(10) R. H. Wilson, F. DeEds and A. G. Cox, *Cancer Research*, **1**, 595 (1941).

(11) J. A. Hendry, F. F. Homer, F. L. Rose and A. L. Walpole, *Brit. J. Pharmacol. and Chemotherap.*, **6**, 235 (1951).

(12) The other two are stated in a communication, L. F. Fieser, THIS JOURNAL, **73**, 5007 (1951).

(13) Supplementary information personally communicated by Dr. Fritz Bischoff.

(14) H. H. Williams, M. Kaucher, A. J. Richards, E. Z. Mayer and G. R. Sharples, *J. Biol. Chem.*, **160**, 227 (1945).

(15) T. Leary, *Arch. Path.*, **50**, 151 (1950).

(16) A. Windaus and C. Resau, *Ber.*, **46**, 1246 (1913).

(17) A. W. Weitkamp, A. M. Smiljanic and S. Rothman, THIS JOURNAL, **69**, 1936 (1947), have isolated normal odd-carbon acids from human hair fat, and A. W. Weitkamp, *ibid.*, **67**, 447 (1945), has shown that wool fat contains esters of cholesterol with a series of even-carbon iso-acids.

(18) D. L. Cramer and J. B. Brown, *J. Biol. Chem.*, **151**, 427 (1943).

of the phosphatid fraction of beef adrenal glands.[19] We are indebted to Dr. J. B. Brown for advice on the preparation of arachidonic acid concentrates from adrenal phospholipid and from menhaden oil and to Parke Davis and Co. for a supply of the former material. For comparison, we prepared the esters of cholesterol with *n*-heptylic, lauric, isolauric and stearic acid.

The acids were converted to the acid chlorides by reaction with oxalyl chloride in benzene solution at 0–20° with a trace of pyridine as catalyst, and the acid chlorides were allowed to react with cholesterol in pyridine solution at room temperature. This process was not attended by appreciable loss in unsaturation. Thus a preparation of menhaden oil fatty acid of iodine number 321 (calculated for arachidonic acid, 333) gave an oily cholesteryl ester of iodine number 189 (calculated for cholesteryl arachidonate, 188).

Exploratory experiments on the relative rates of enzymatic hydrolysis of normal and abnormal esters were made by an adaptation of the method of Nachlas and Seligman[20,21] for demonstration of esterase in tissues. The β-naphthyl esters of the same series of abnormal and normal fatty acids were incubated with esterase–lipase isolated from pancreatic tissue in an alcohol–water mixture, aliquots were withdrawn at various intervals and the amount of free β-naphthol established by coupling with diazotized sulfanilic acid and determining spectrophotometrically the Orange II formed. The amount of unhydrolyzed β-naphthyl ester was estimated from the difference between the extinction coefficient of the solution (E) at the wave length of maximum absorption (485 mμ) and the extinction coefficient of pure Orange II (E_0). If the concentration of enzyme remains constant during the hydrolysis and water is present in large excess, the reaction should be first order and the rate dependent only upon the concentration of ester: $-\log C = (k/2.303)t + K$. The graphs of Figs. 1 and 2, in which the log of the concentration of unhydrolyzed ester is plotted against elapsed time, show the expected linear relationship, at least up to the half-life period of the reaction. The reaction velocity constants, obtained by multiplying the slopes estimated from the graphs by 2.303 are given in Table I.

TABLE I

HYDROLYSIS BY PANCREATIC ESTERASE–LIPASE IN ALCOHOL–WATER

β-Naphthyl ester	k
Adrenal phosphatid acid	0.48
Isoheptylate	.150
Menhaden oil acid	.147
Isolaurate	.108
n-Heptylate	.092
Laurate	.088
Stearate	.042

The action of three other enzyme systems in tissue homogenates on some of the same substrates in a buffered aqueous system was kindly explored by Dr. Herbert A. Ravin of the Beth Israel Hos-

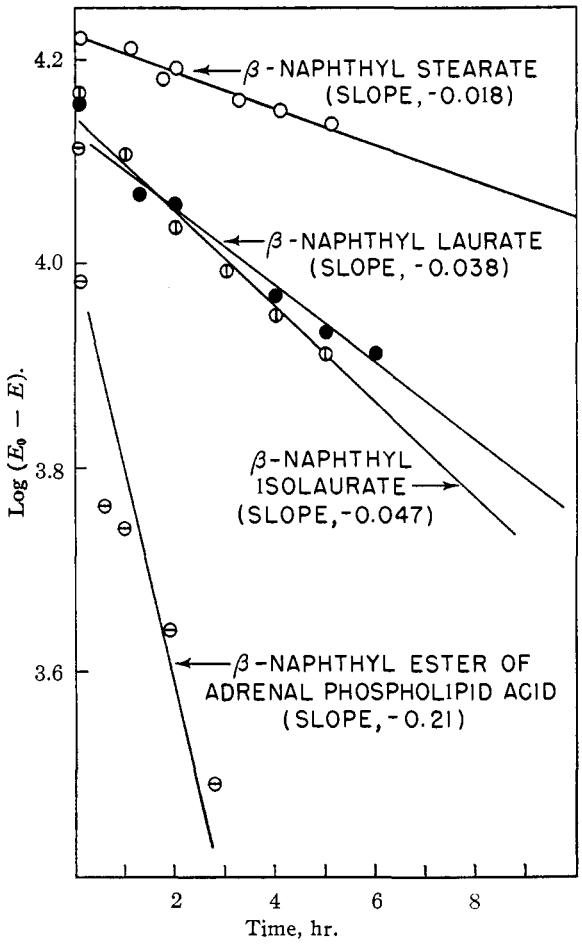

Fig. 1.—Enzymatic hydrolysis of esters (concentration of unhydrolyzed ester plotted against time).

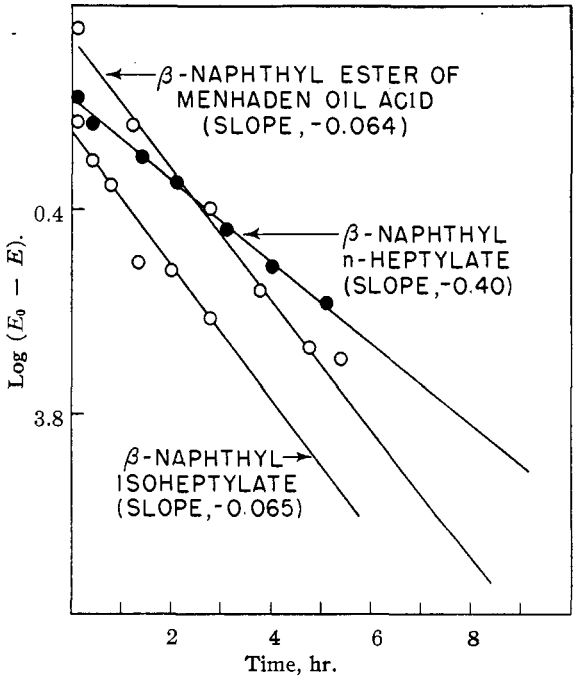

Fig. 2.—Enzymatic hydrolysis of esters.

(19) W. C. Ault and J. B. Brown, *J. Biol. Chem.*, **107**, 607, 615 (1934).
(20) M. M. Nachlas and A. M. Seligman, *J. Nat. Cancer Inst.*, **9**, 415 (1949).
(21) M. M. Nachlas and A. M. Seligman, *J. Biol. Chem.*, **181**, 343 (1949).

pital with the results recorded in Table II. The small differences between the normal and iso acids suggested by the experiments in alcohol–water (Table I) are not confirmed by the experiments conducted under more nearly physiological conditions (Table II); here the most striking difference is that the esters of the C_8-acids are hydrolyzed much more rapidly than are the esters of the C_{12}-acids. The apparent great reactivity of the adrenal phosphatid ester in alcohol–water indicated in Table I seems of questionable significance in view of the failure of the other highly unsaturated ester from menhaden oil to exhibit comparable properties.

TABLE II

HYDROLYSIS IN EMULSIONS OF SUBSTRATE IN A VERONAL BUFFER AT *p*H 7.4

0.1 mg./cc., incubated for 1 hr. at 37° except as noted.

β-Naphthyl ester	Micrograms of β-naphthol released[a] on incubation with Human serum esterase[b]	Rat liver esterase[c]	Rat pancreatic esterase-lipase[c]
n-Heptylate	139.4	286	264
Isoheptylate	118.4	164.5	172
Laurate	3.8 (27.6; 19.0)[d]	15.3 (13.4; 30.2)[e]	55 (182; 225)[e]
Isolaurate	3.2 (27.4)[d]	16.5[e]	(1923)[e]

[a] Coupled with tetrazotized di-*o*-anisidine; micrograms of β-naphthol calculated from the calibration curve reported by A. M. Seligman and M. M. Nachlas, *J. Clin. Investig.*, 29, 31 (1950). [b] From pooled human serum (0.2 cc. per test tube). [c] 0.25 mg. per test-tube. [d] Repeat experiment with a 3-hour period of incubation. [e] Repeat experiment with a preparation from another rat.

Experimental

Isoheptylic acid, prepared from 155 g. (1.13 moles) of isoamyl bromide, 160 g. (1 mole) of diethyl malonate and 23 g. (1 g. atom) of sodium in 500 cc. of dry butyl alcohol by the procedure outlined for preparation of pelargonic acid,[22] was obtained as a colorless oil, b.p. 132–134° (20 mm.), $n^{23}D$ 1.4210; yield 89.6 g. (69%).

***n*-Heptylic** and **lauric acid** (m.p. 42–45°) were used as supplied by Eastman Kodak Co. U.S.P. stearic acid was recrystallized from methanol, m.p. 67–69°.

Isolauric acid was prepared essentially by the method of C. Heidelberger.[23] The Grignard reagent from 31.4 g. of isoheptyl bromide and 4.25 g. of magnesium in 100 cc. of ether was stirred with 16.85 g. of anhydrous cadmium chloride for one-half hour, the ether was removed by aspirator; 80 cc. of benzene was added and removed as before, and an additional 80 cc. of benzene was added. The mixture was refluxed to dissolve the gray, pasty mass, cooled in an ice-bath, and treated with a solution of 23.1 g. of the half-acid chloride methyl ester of glutaric acid (freshly distilled, b.p. 125° (20 mm.)) in 50 cc. of benzene, added over 15 minutes. After refluxing for one-half hour, ice was added, then 10% sulfuric acid, and the mixture was extracted with ether. The extract was washed with water, bicarbonate solution, water again, saturated salt solution and dried. Distillation through a 30-cm. Vigreux column with a heated jacket was conducted at aspirator pressure until the ether and benzene were removed; then, at 2 mm., a forerun of 3.3 g. was collected up to 130°. Then 25.9 g. (81% based on the acid chloride) of methyl **γ-ketoisolaurate** was collected at 130–135° (1–2 mm.). Wolff–Kishner reduction[24] was conducted in 125 cc. of triethylene glycol with 12 cc. of 94% hydrazine and 17 g. of potassium hydroxide (¾ hr. at 135°, 3.5 hr. at 196°). The oily suspension obtained on pouring the mixture onto 250 cc. of water and 55 cc. of 36% hydrochloric acid was stirred in an ice-bath until solid; the yield of isolauric acid, m.p. 38–42°, was 21.6 g. (96.5%). Crystallization from petroleum ether raised the m.p. to 42–43°.

Menhaden oil fatty acid, kindly supplied by Dr. J. B. Brown of The Ohio State University, had an iodine number of 321.

Adrenal Phospholipid Fatty Acid.—The Parke–Davis Co. kindly processed 20 lb. of beef adrenals by grinding, extraction with alcohol at room temperature, distillation of the alcohol, extraction of the aqueous residue with petroleum ether, and separation of the petroleum ether soluble material into an acetone-soluble portion and an acetone-insoluble phospholipid fraction consisting of a dark brown wax (115 g.). This wax was subjected to methanolysis by an adaptation of the method of Shinowara and Brown[25]: 20 g. was refluxed in a solution of 10 g. of dry hydrogen chloride in 200 cc. of methanol in an atmosphere of nitrogen for six hours and let stand overnight at 25°. The solution was poured into water and the mixture extracted twice with ether; the extract was washed twice with water, then with bicarbonate solution, 5% potassium hydroxide, saturated salt solution, dried and evaporated. Distillation through a short Vigreux column with the capillary supplied with nitrogen gave 10.18 g. of a methyl ester fraction that distilled as a pale yellow oil, b.p. 160–180° (0.3 mm.). This was dissolved in 100 cc. of dry acetone and the solution cooled in Dry Ice–acetone for one hour, and the colorless solid that precipitated was collected on a Buchner funnel surrounded by a Dry Ice–acetone-bath. The yellow filtrate was concentrated in a stream of nitrogen to about 20 cc., cooled in Dry Ice–acetone, and the resulting solid removed as before. Distillation of the material from the filtrate gave 2.99 g. of a fraction collected at 0.3 mm. at a bath temperature of 180–200°; iodine no.[26] 213 (calculated for methyl arachidonate, 308).

This methyl ester fraction was saponified by heating it with 0.6 g. of potassium hydroxide in 10 cc. of methanol for 15 minutes under nitrogen. After addition of water and 2 cc. of 36% hydrochloric acid, the mixture was extracted with ether and the washed and dried extract was evaporated at reduced pressure without heating. Then 10 cc. of benzene was added and evaporated at reduced pressure. The resulting brown, oily residue of unsaturated fatty acids was used immediately for preparation of esters as described in the next section.

Cholesteryl and β-Naphthyl Esters.—The esters were all prepared by the same procedure, illustrated as follows for the preparation of **cholesteryl isoheptylate**. A solution of 2.0 g. (0.015 mole) of isoheptylic acid in 10 cc. of dry benzene containing 2–3 drops of pyridine was cooled in an ice-bath and stirred while 2.0 cc. of oxalyl chloride was added dropwise. Gas evolution began at once and, when the solution was allowed to warm to room temperature, continued for about 20 minutes. After another half-hour the solution was evaporated at room temperature at aspirator pressure; two successive portions of 10 cc. and 5 cc. of benzene were then added and evaporated. A solution of the oily acid chloride in 10 cc. of benzene was cooled in an ice-bath during the addition, during 15 minutes, of a solution of 4.0 g. (0.0103 mole) of cholesterol (U.S.P. Wilson, crystallized twice from methanol, m.p. 148.4–149.2°) in 20 cc. of benzene and 5 cc. of dry pyridine. The mixture, from which a large amount of pyridine hydrochloride separated, was allowed to come to room temperature and stand for 2 hr. Water was added and the product collected in an ether–benzene extract, and washed with water, acid and alkali, dried and evaporated. The residue consisted of 4.78 g. (93%) of solid cholesteryl isoheptylate, m.p. 97–99°. Crystallization from acetone gave colorless slender rods, m.p. 100–101°.

Anal. Calcd. for $C_{34}H_{58}O_2$ (498.80): C, 81.86; H, 11.72. Found: C, 81.89; H, 11.81.

β-Naphthyl isoheptylate, prepared in the same way, was obtained as an oil, which was distilled from a small Claisen flask with a short Vigreux side arm; the main fraction distilled at 168–170° (0.5 mm.) as a pale yellow oil.

Anal. Calcd. for $C_{17}H_{20}O_2$ (256.33): C, 79.65; H, 7.86. Found: C, 79.06; H, 7.89.

(22) E. E. Reid and J. R. Ruhoff, *Organic Syntheses*, Coll. Vol. II, 474 (1943).

(23) L. F. Fieser, M. T. Leffler and co-workers, THIS JOURNAL, **70**, 3174 (1948).

(24) Huang-Minlon, *ibid.*, **68**, 2487 (1946).

(25) G. Y. Shinowara and J. B. Brown, *Oil and Soap*, **15**, 151 (1938).

(26) Hanus' method, 4 hr. reaction period, see G. S. Jamieson, "Vegetable Fats and Oils," 2nd Ed., Reinhold Publishing Corp., 1943, p. 393.

Cholesteryl *n*-heptylate was obtained after three crystallizations from acetone as long, slender rods, m.p. 111.4–112.2°.

Anal. Calcd. for $C_{34}H_{58}O_2$ (498.80): C, 81.86; H, 11.72. Found: C, 82.12; H, 12.09.

β-Naphthyl *n*-heptylate distilled in a short-path apparatus at 0.3 mm. and a bath temperature of 200° as an oil that solidified, m.p. 37.5–39°. Crystallization from petroleum ether gave material of m.p. 38–39°.

Anal. Calcd. for $C_{17}H_{20}O_2$ (256.33): C, 79.65; H, 7.86. Found: C, 79.91; H, 7.70.

Cholesteryl laurate was obtained from acetone as colorless flat rods, m.p. 94–95° (reported,[27] 91°).

Anal. Calcd. for $C_{39}H_{68}O_2$ (568.93): C, 82.33; H, 12.05. Found: C, 82.16; H, 12.09.

β-Naphthyl laurate,[21] crystallized twice from acetone, formed small prisms, m.p. 62.5–64°.

Anal. Calcd. for $C_{22}H_{30}O_2$ (326.46): C, 80.94; H, 9.26. Found: C, 80.71; H, 9.21.

Cholesteryl isolaurate crystallized from acetone as small rods, m.p. 105.6–106.6°.

Anal. Calcd. for $C_{39}H_{68}O_2$ (568.93): C, 82.33; H, 12.05. Found: C, 82.40; H, 12.07.

β-Naphthyl isolaurate distilled in a short-path apparatus to give a solid, m.p. 43.5–44.5°, which crystallized from acetone in short rods, m.p. 44–45°.

Anal. Calcd. for $C_{22}H_{30}O_2$ (326.46): C, 80.94; H, 9.26. Found: C, 81.09; H, 9.36.

Cholesteryl stearate was crystallized from acetone, m.p. 74–78° (reported,[26] 78°).

β-Naphthyl stearate,[21] after two crystallizations from acetone, was obtained as a microcrystalline powder, m.p. 73.5–75° (reported,[28] 73–75°).

Cholesteryl Ester of Menhaden Oil Fatty Acid.—The crude ester (prepared under nitrogen) remaining after evaporation of solvents for several hours at 0.4 mm. was a brown oil of iodine number[25] 170. A 1.46-g. portion of ester was chromatographed on 40 g. of acid-washed alumina; elution with benzene removed the main portion as a pale yellow oil. After evacuation for several hours at 0.4 mm., the ester weighed 1.31 g. and had an iodine number of 189 (calculated for cholesteryl arachidonate, 188). Analyses indicated a carbon content 1–2% lower than that calculated for cholesteryl arachidonate. On storage, the ester darkened and became more viscous.

β-Naphthyl Ester of Menhaden Oil Fatty Acid.—Processing as described for the cholesteryl ester gave a pale yellow oil that was used at once for determination of the rate of enzymatic hydrolysis. The material darkened on storage, and an analysis conducted a few days after preparation indicated a carbon content over 3% below that required for the arachidonate.

Cholesteryl Ester of Adrenal Phospholipid Fatty Acid.—The crude ester, a pale brown oil, was eluted from alumina with benzene, the solvent was evaporated at the aspirator, and the residue was heated at 110° (0.2 mm.) for one hour before analysis. The results are close to those required for cholesteryl arachidonate.

Anal. Calcd. for $C_{47}H_{76}O_2$ (673.08): C, 83.86; H, 11.38. Found: C, 83.52; H, 11.70.

β-Naphthyl Ester of Adrenal Phospholipid Fatty Acid.—The composition found for material processed as above is not far from that required for β-naphthyl arachidonate.

Anal. Calcd. for $C_{30}H_{38}O_2$ (430.60): C, 83.67; H, 8.98. Found: C, 83.57; H, 9.55.

(27) I. H. Page and H. Rudy, *Biochem. Z.*, **220**, 304 (1930).
(28) F. Seidel and O. Engelfried, *Ber.*, **69**, 2567 (1936).

Rates of Hydrolysis of the β-Naphthyl Esters.—A 10–20 mg. sample of ester was weighed accurately into a 10-cc. volumetric flask, dissolved in absolute ethanol, and the solution was diluted with this solvent to the mark. A 1-cc. aliquot was transferred to a second 10-cc. flask, exactly 5 cc. of ethanol was added, and the solution was diluted to the mark with a solution of 80 mg. of esterase–lipase from pancreatic tissue (dry powder from Nutritional Biochemicals Co.) in 20 cc. of distilled water. Another 10-cc. volumetric flask containing 6 cc. of ethanol was also diluted to the mark with esterase–lipase solution. Both solutions were mixed well and let stand at room temperature, which in all cases was between 25 and 26.5°. At suitable intervals, a 1-cc. aliquot was removed from each flask and filtered into a separate 10-cc. volumetric flask. The filter was washed with about 3 cc. of distilled water, and the filtrate was treated with 2 cc. of ethanol and 2 cc. of a saturated solution of the inner salt of diazotized sulfanilic acid (freshly prepared for each run and washed with water until the wash liquor was neutral) and diluted to the mark with distilled water.

The extinction coefficient of the ester solution at 485 mμ was determined with a Beckman model DU quartz spectrophotometer (tungsten lamp) and compared with that of the reference solution prepared in the same way but containing no β-naphthyl ester. The absorption reached a maximum within 5 minutes after addition of the diazonium salt, remained nearly constant for 10–20 minutes, and then began to fade slowly. When no enzyme was added, no appreciable absorption developed in 18 hr., and hence the esters are stable in the medium used in the absence of lipase. When the ester–enzyme system was allowed to stand at room temperature for periods of 24–48 hr., the extinction coefficient in most cases approached closely that of pure Orange II.

The results obtained are exemplified by the data recorded in Table III for an experiment on the enzymatic hydrolysis of β-naphthyl isoheptylate. The first column gives the time in hours from the mixing of the ester and enzyme solutions. The second column gives the extinction coefficient of the solution of Orange II resulting from coupling with diazotized sulfanilic acid at time t. The third column gives the difference between this extinction coefficient and that found for Orange II ($E_0 = 1.7 \times 10^4$). The logarithm of the difference is given in the last column; these logarithmic values are plotted against time in the graph of Fig. 1. The slope reported is estimated from the graph; it is proportional to the reaction velocity constant for the hydrolysis. The results of the reaction velocity determinations of the other six esters were conducted in the same way with samples ranging from 10.0 to 21.9 mg. at temperatures of 25.5 to 26.5°; the results are recorded in the graphs of Figs. 1 and 2 and in the summary of relative reaction rates (Table I).

TABLE III

LIPASE HYDROLYSIS OF β-NAPHTHYL ISOHEPTYLATE

Sample, 13.3 mg., temperature, 26°

Time, hr.	$E \times 10^{-3}$	$(E_1 - E) \times 10^{-3}$	log $(E_0 - E)$
0.1	4.89	12.11	4.084
.4	5.89	11.11	4.046
.8	6.46	10.54	4.023
1.33	7.54	9.46	3.946
2.0	8.30	8.70	3.940
2.8	9.17	7.83	3.894
48.3	17.08	...	...

Slope = 0.065

CAMBRIDGE 38, MASSACHUSETTS
RECEIVED OCTOBER 19, 1951