[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY, AND THE DEPARTMENT OF SURGERY, BETH ISRAEL HOSPITAL, AND HARVARD MEDICAL SCHOOL]

Preparation of Alkyl 2-Naphthyl Carbonates as Chromogenic Substrates for Esterases[1]

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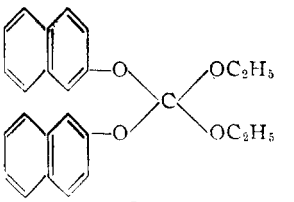
To explore the effect of varying the alcoholic moiety of chromogenic substrates for esterases the following alkyl 2-naphthyl carbonates were prepared: ethyl, butyl, *n*-hexyl, *n*-octyl, lauryl, cetyl and stearyl. The pyrolysis of ethyl 2-naphthyl carbonate to form 2-naphthol and ethylene was studied, and its mechanism was correlated to that of the Chugaev-type thermal elimination. From a study of the enzymatic hydrolysis of these substrates by pancreas and liver homogenates, it was found that the long chain alkyl 2-naphthyl carbonates are preferentially hydrolyzed by pancreatic lipase similar to the long chain fatty acid esters.

Many esterases are characterized by a low relative specificity. As an approach to the development of chromogenic substrates with greater specificity for lipase and for aliesterase, a number of 2-naphthylcarboxylic acid esters were prepared in these laboratories.[2] The study of these substrates confirmed the relation of chain length to the specificity for lipase. A sensitive colorimetric method for lipase was only evolved by taking advantage of the activation by taurocholate of lipase in distinction to esterase.[3] Since these substrates differ only in the acid moiety, it was important to explore the effect of chromogenic esters with different alcohols. Balls and Matlack[4] had previously shown that the rate of hydrolysis of stearic acid esters of various alcohols by pancreatic lipase differed appreciably.

2-Carbonaphthoxyphenylalanine,[5] and 2-carbonaphthoxycholine iodide[6] have been used, respectively, for the colorimetric demonstration of carboxypeptidase and serum cholinesterase. In the present investigation a number of alkyl 2-naphthyl carbonates as shown in the accompanying general formula were prepared. Such compounds upon enzymatic hydrolysis liberate carbon dioxide, an alcohol, and 2-naphthol.[7] This in turn is converted to an azo dye for colorimetric estimation. A preliminary report of the enzymatic hydrolysis of some of these substrates has been published elsewhere.[8] While further study is still in progress, this paper concerns the preparation and properties of these compounds.

Ethyl 2-naphthyl carbonate (I) was made by treatment of 2-naphthol with ethyl chloroformate in ether solution in the presence of pyridine. It has been prepared before by Bender,[9] and later by Harfenist and Baltzly[10] by a different method in low yield. *n*-Butyl 2-naphthyl carbonate (II) and *n*-hexyl 2-naphthyl carbonate (III) were prepared by the reaction of 2-naphthol with the approoriate chlorocarbonate in ether in the presence of tri-*n*-ethylamine. *n*-Octyl 2-naphthyl carbonate (IV), lauryl 2-naphthyl carbonate (V), cetyl 2-naphthyl carbonate (VI) and stearyl 2-naphthyl carbonate (VII) were all prepared by the reaction of 2-naphthyl chlorocarbonate[6] with the corresponding alcohols. The conditions varied somewhat in each case. The physical constants are summarized in Table I.

The identity of I, however, had been thrown into doubt in the literature. From its analytical value, Bender[9] contended that his product was dinaphthoxy orthoethylester (Ia). Recently, Harfenist and Baltzly[10] obtained I with a correct analysis.



Ia

Based on the observation that methyl 2-naphthylcarbonate on refluxing gave mainly methyl 2-naphthyl ether and carbon dioxide, Einhorn and Rothlauf[11] had speculated that ethyl 2-naphthylcarbonate prepared by Bender might be a mixture of ethyl 2-naphthyl carbonate and 2-naphthyl ethyl ether. The infrared spectrum of our sample showed a carbonyl band at 5.70 μ which is demonstrable with all the naphthyl carbonates that we have prepared. Therefore the structure of I is not in doubt.

When ethyl 2-naphthyl carbonate was refluxed for ten hours at 300°, carbon dioxide and ethylene were evolved and the residue obtained on cooling failed to show a carbonyl band in its infrared spectrum. The products were proved to be mainly 2-naphthol (m.p. 120°), an unidentified naphthyl derivative (m.p. 196–197°), and a trace of 2-naphthyl ethyl ether (m.p. 35°). It thus appeared that the course of pyrolysis of ethyl 2-naphthyl carbonate followed a Chugaev-type reaction as shown in (1), whereas that of methyl 2-naphthyl-

(1) This investigation was supported by a research grant from the National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare, and by the Slosberg Fund for Research in Diabetes.

(2) M. M. Nachlas and A. M. Seligman, *J. Biol. Chem.*, **181**, 343 (1949).

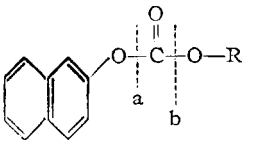
(3) A. M. Seligman and M. M. Nachlas, *J. Clin. Invest.*, **29**, 31 (1950).

(4) A. K. Balls and M. B. Matlack, *J. Biol. Chem.*, **123**, 679 (1938).

(5) H. A. Ravin and A. M. Seligman, *ibid.*, **190**, 391 (1951); G. Wolf and A. M. Seligman, THIS JOURNAL, **73**, 2080 (1951).

(6) H. A. Ravin, K.-C. Tsou and A. M. Seligman, *J. Biol. Chem.*, **191**, 843 (1951).

(7) At the initiation of this work we assumed that hydrolysis took place at b rather than at a, because carbonaphthoxycholine was specific for serum cholinestearase[6] and carbonaphthoxyphenylalanine was specific for carboxypeptidase.[5]



(8) H. A. Ravin and A. M. Seligman, *Arch. Biochem. Biophys.*, **42**, 337 (1953).

(9) B. Bender, *Ber.*, **13**, 696 (1880).

(10) M. Harfenist and R. Baltzly, THIS JOURNAL, **69**, 362 (1947).

(11) A. Einhorn and L. Rothlauf, *Ann.*, **382**, 237 (1911).

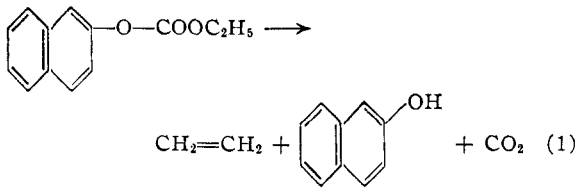
TABLE I

ALKYL 2-NAPHTHYL CARBONATES

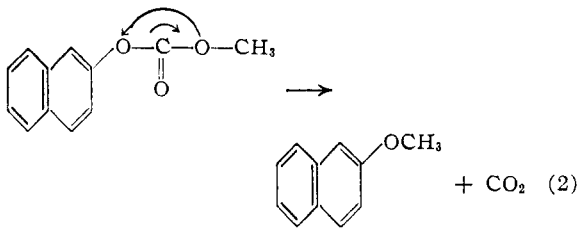
Compound	Yield, %	B.p. or m.p.,[a] °C.	Formula	Mol. wt.	Analyses, %[d] Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
Ethyl- (I)	77	33–34[b]	$C_{13}H_{12}O_3$	221.23				
n-Butyl- (II)	50	180–181 (5.5 mm.)	$C_{15}H_{16}O_3$	244.28	73.75	73.82	6.60	6.61
n-Hexyl- (III)	47	196–197 (3.2 mm.)	$C_{17}H_{20}O_3$	272.33	74.97	75.05	7.40	7.46
n-Octyl- (IV)	52	209–210 (3.0 mm.)	$C_{19}H_{24}O_3$	300.38	75.97	75.98	8.05	7.93
Lauryl- (V)	63	42–44[b]	$C_{23}H_{32}O_3$	356.49	77.49	77.80	9.05	8.77
Cetyl- (VI)	68	57–58[b,c]	$C_{27}H_{40}O_3$	412.59	78.59	78.75	9.77	9.52
Stearyl- (VII)	67	64–65[b,c]	$C_{29}H_{44}O_3$	440.64	79.04	78.99	10.07	10.06

[a] All melting points are corrected. [b] Recrystallized from ether. [c] Colorless flakes which turned slightly pink on exposure to light. [d] Microanalyses by Mrs. Shirley Golden and Dr. S. M. Nagy and Associates, Microchemical Laboratory, M.I.T.

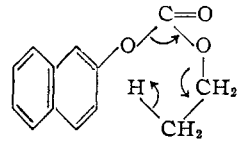
carbonate probably proceeded by a homolytic 1,3-migration of the methyl radical with simultaneous



elimination of carbon dioxide as in (2).[12] When possible, path (1) is to be preferred over path (2),



since decarboxylation *via* (1) has a lower energy and is readily explained by an analogous quasi-six-membered transition state in the Chugaev reaction, originally suggested by Barton and Cram.[13a,b]



Therefore Einhorn and Rothlauf were not justified in predicting the results of pyrolysis of ethyl naphthyl carbonate from the results with methyl naphthyl carbonate.

The 2-naphthyl carbonates were incubated with homogenates of liver (mainly aliesterase) and pancrease (mainly lipase) and the amount of free 2-naphthol enzymatically formed was determined spectrophotometrically after coupling with tetrazotized diorthoanisidine.[14] The rate of hydrolysis was compared with a typical aliesterase substrate, 2-naphthyl acetate and a typical lipase substrate, 2,7-naphthyl dicaprylate[9] (Table II). The ratio of hydrolysis by pancreas to that by liver was less than one when due to esterase and greater than one when due to lipase. Although the cetyl and the stearyl derivatives were hydrolyzed very slowly under the conditions we studied, they did show relative susceptibility and specificity for pancreatic lipase, whereas 2,2'-dinaphthyl carbonate showed a specificity for aliesterase resembling that of 2-naphthyl acetate.[15]

Experimental

Ethyl 2-Naphthyl Carbonate (I).—To an ether solution of 2-naphthol (54.3 g., 0.5 mole) and dry pyridine (39.5 g., 0.5 mole) was added dropwise 54.3 g. (0.5 mole) of ethyl chloroformate with stirring and occasional cooling. The reaction mixture was allowed to stand at room temperature for six hours and filtered. The filtrate was washed with water, ice-cold 2% sodium hydroxide solution, again with water, and dried over anhydrous calcium sulfate. The dried solution, on evaporation under reduced pressure, gave a low melting white solid; 87.0 g., m.p. 32–33.5°. Recrystallization from absolute ether raised the melting point to 33–34° (lit.[9,10] 34–35°).

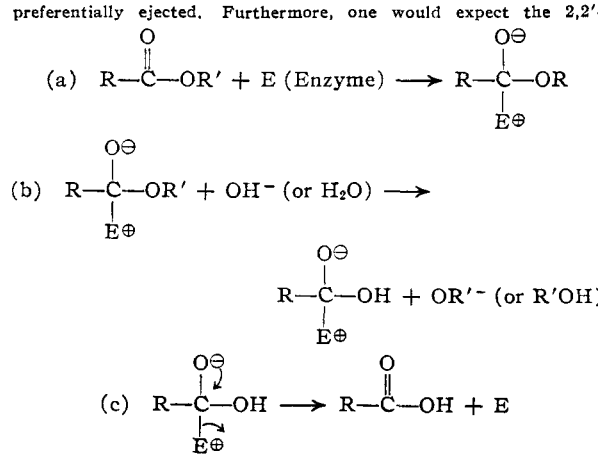
A suspension of I in cold sodium bicarbonate solution

(12) While this manuscript was in preparation, migration of the methyl group in methyl benzoate at high temperature was demonstrated by means of O^{18} (K. B. Wiberg, THIS JOURNAL, **75**, 2665 (1953)). It is very probable that in the present proposed path a similar mechanism operates.

(13) (a) D. H. R. Barton, *J. Chem. Soc.*, 2174 (1949); D. J. Cram, THIS JOURNAL, **71**, 3883 (1949). (b) It is interesting to note in a recent paper (G. L. O'Connor and R. R. Nace, *ibid.*, **75**, 2118 (1953), that a similar mechanism for the pyrolysis of cholesteryl ethyl carbonate has been proved by kinetic study. We have also studied cholesteryl naphthyl carbonate. Our results will be published in a subsequent paper.

(14) The experiments on enzymatic hydrolysis were performed with the collaboration of Dr. H. A. Ravin.

(15) Enzymatic hydrolysis may be explained by the following speculation. The first step of the enzymatic hydrolysis may be the formation of an esterase–substrate complex as illustrated in (a). This step is followed by an attack of an hydroxy ion or water molecule upon the carbonyl carbon with the ejection of an alkoxide ion, *i.e.*, *via* a displacement mechanism (b). The resulting product would then lose the enzyme again by a process as indicated in (c) to give the acid. In the present case, since the napthoxide anion (R) is more stable than the alkoxide ion (OR'), the former ion would be, according to theory, preferentially ejected. Furthermore, one would expect the 2,2'-



dinaphthyl carbonate to be hydrolyzed enzymatically faster than either the cetyl or the stearyl derivative. Since this was found to be true with liver but not with pancreas (Table II), further evidence is provided that esterase and lipase are distinct enzymes.

TABLE II

COMPARISON OF RATES OF HYDROLYSIS OF ALKYL 2-NAPHTHYL CARBONATES AND NAPHTHYL ESTERS BY ALIESTERASES AND PANCREATIC LIPASE

Enzyme source	pH	2-Naphthyl acetate	2,2'-Dinaphthyl carbonate	2,7-Naphthyl dicaprylate	Cetyl 2-naphthyl carbonate	Stearyl 2-naphthyl carbonate
Liver	7.4	11900[a,b,d]	51[a,c,e]	106[a,b,e]	10[a,c,e]	1[a,c,e]
Pancreas	7.4	4360	24	1450	63	71
Kidney	7.4	11900	58	49	8	0.4
Intestine	7.4	4160	3	57	27	7
Ratio of pancreas:liver	7.4	0.3	0.5	14	6	71

[a] Color density. [b] Incubation for 1 hour. [c] Incubation for 20 hours. [d] Homogenate of 0.1 mg. of tissue. [e] Homogenate of 1.0 mg. of tissue.

showed no color at first when coupled with tetrazotized dianisidine. On long standing, hydrolysis occurred as shown by the gradual development of a blue color.

n-Butyl 2-naphthyl carbonate (II) and *n*-hexyl 2-naphthyl carbonate (III) were prepared by a similar procedure from *n*-butyl chloroformate and *n*-hexyl chloroformate, using tri-N-ethylamine as condensing agent and distilling the resulting product under reduced pressure. They were more stable in sodium bicarbonate solution than the ethyl compound. The physical constants are summarized in Table I.

Cetyl 2-Naphthyl Carbonate (VI).—A dry ether solution of cetyl alcohol (2.42 g., 0.01 mole) and 8 cc. of pyridine was added to a dry ether solution of 2-naphthyl chloroformate[6] (2.07 g., 0.01 mole) in portions. The resulting mixture was stirred for five hours at room temperature. After filtering the precipitate, the ether solution was washed with water and dried. The dried solution was evaporated to dryness and the crude product recrystallized from ether.

The corresponding *n*-octyl (IV), lauryl (V) and stearyl derivatives (VII) were all prepared by using the appropriate alcohol in place of cetyl alcohol in the above procedure. Tri-N-ethylamine instead of pyridine was used as the condensing reagent. In the preparation of IV and V, the product was purified by distillation.

Pyrolysis of Ethyl 2-Naphthyl Carbonate.—A sample of 5.4 g. of (I) was refluxed in a Woods metal-bath at 300° under a CO_2-free nitrogen atmosphere. The exit gas was allowed first to pass a 1% solution of bromine in carbon tetrachloride and then a barium hydroxide solution. A positive test for ethylene and carbon dioxide was obtained after 15 minutes. The refluxing was stopped at the end of ten hours. Loss of weight was 1.6 g. (theoretical 1.8 g.). The brown residue showed no apparent carbonyl band in the 5.0–6.0 μ region. It was suspended in ether and the insoluble portion was removed by filtration (0.35 g.); m.p. 185–187° (dec.); no carbonyl band in 5.0–6.0 μ region of its infrared spectrum. Two recrystallizations from benzene changed its melting point to 196–197° dec. The structure of this by-product is yet unidentified.

The ether solution was washed with 8% sodium hydroxide solution until the washings showed no more free naphthol on coupling. From the combined alkali washings, 2.7 g. (theory 3.6 g.) of 2-naphthol, m.p. 119–120°, was recovered as lustrous flakes by acidification with concentrated hydrochloric acid. The ether solution was dried and concentrated to 0.2 g. of a brown oil from which 0.05–0.1 g. of pure 2-naphthyl ethyl ether, m.p. 35°, was obtained as plates by vacuum sublimation at 180° (5 mm.) twice. The sample had an identical infrared spectrum with an authentic sample of 2-naphthyl ethyl ether (no OH and no carbonyl band, ether band at 8.91 μ (s) and 9.10 μ).

Enzymatic Hydrolysis.[14]—Fresh organs of the rat were homogenized in distilled water (5 mg. per cc.) with a motor-driven ground glass homogenizer for 2 minutes and centrifuged for 2 minutes at 2500 r.p.m. Tissue homogenate (0.1 mg.) was used with 2-naphthyl acetate and 1.0 mg. of homogenate was used with the other substrates.

The period of incubation was one hour with naphthyl acetate and 2,7-naphthyl dicaprylate, 20 hours with all the other substrates. Each solution was made alkaline with 0.1 *M* veronal buffer (pH 7.4), coupled with 1 cc. of tetrazotized dianisidine (1 mg. per cc. dissolved immediately before use). The technique was essentially the same as that used in the study of glucosidase.[15] The results are given in Table II. The ratio of hydrolysis by pancreas to that by liver was less than one when hydrolysis was due mainly to aliesterase and was much greater than one when hydrolysis was due mainly to lipase.

(16) K.-C. Tsou and A. M. Seligman, THIS JOURNAL, **74**, 3066 (1952).

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