Anal. Calcd. for $C_{21}H_{14}O_3$: C, 80.26; H, 4.50. Found: C, 80.16; H, 4.78.

Carbonaphthoxy-L-tyrosine Ethyl Ester (VI).—Carbonaphthoxy chloride (0.5 g.) was added to a solution of L-tyrosine ethyl ester (0.5 g.) in anhydrous pyridine (10 cc.). The solution was kept at room temperature for 2 hours, treated with dilute hydrochloric acid and extracted with ether. The ether extract was washed with sodium carbonate solution, water, dried and evaporated. The residue was dissolved in hot alcohol. Upon cooling dinaphthyl carbonate crystallized and was collected by filtration. Carbonaphthoxy-L-tyrosine ethyl ester could be obtained on concentration of the filtrate. It was recrystallized from ethyl acetate by the addition of ligroin and then from ether by the addition of ligroin, 0.39 g. (41%), m.p. (with decomposition) 137°.

Anal. Calcd. for $C_{22}H_{21}O_5N$: C, 69.66; H, 5.57. Found: C, 69.86; H, 5.86.

Ethyl N-Carbonaphthoxy-2-pyrrolidone-5-carboxylate (VII).—To a solution of ethyl 2-pyrrolidone-5-carboxylate[18] (0.8 g.) in anhydrous pyridine (5 cc.) was added carbonaphthoxy chloride (1 g.). The mixture was warmed on the steam-bath for 30 minutes, treated with dilute hydrochloric acid and extracted with ether. The ether extract was washed with dilute sodium carbonate solution, water, dried and evaporated. The residue was dissolved in hot alcohol. Upon cooling, dinaphthyl carbonate crystallized. It was collected by filtration. After evaporation of some alcohol, the pyrrolidone carboxylate crystallized from the filtrate. It appeared in long, colorless needles, 0.7 g. (44%) m.p. 119°, on recrystallization from alcohol.

Anal. Calcd. for $C_{18}H_{17}O_5N$: C, 66.12; H, 5.23. Found: C, 66.28; H, 5.24.

(18) E. Fischer and R. Boehner, *Ber.*, **44**, 1332 (1911).

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

Synthesis of Ketomethyldihydronaphthoic Acid Derivatives as Possible Chromogenic Substrates[1]

BY GEORGE WOLF AND ARNOLD M. SELIGMAN

The syntheses of 1-keto-methyl-1,2-dihydro-2-naphthoyl-DL-phenylalanine (XIV) and of methyl 1-keto-2-methyl-1,2-dihydro-2-naphthoate (I) are described. Hydrolysis of the peptide or ester link is followed by decarboxylation and aromatization to 2-methyl-1-naphthol which can be converted to highly colored azo dyes. However, these substrates are not hydrolyzed by carboxypeptidase or esterase and lipase, respectively.

In a previous publication[2] reasons for expecting carbonaphthoxyphenylalanine to be a suitable chromogenic substrate for carboxypeptidase[3] were given. In that case enzymatic hydrolysis of the peptide linkage resulted in the formation of the unstable naphthyl carbonate, with subsequent loss of CO_2 and formation of naphthol. Because of the possibility of hydrolysis of the carbonaphthoxy ester linkage at alkaline *p*H, another acyl derivative of phenylalanine was sought which, upon enzymatic hydrolysis, would produce a carboxylic acid unstable enough to decompose spontaneously and immediately to a naphthol, which then could be converted to an azo dye by coupling with an appropriate diazonium salt. It was expected that these requirements might be fulfilled by 1-keto-2-methyl-1,2-dihydro-2-naphthoylphenylalanine (XIV). Following hydrolysis to the β-keto acid, 1-keto-2-methyl-1,2-dihydro-2-naphthoic acid, decarboxylation to the unstable 1-keto-2-methyl-1,2-dihydro naphthalene (II) would result in rearrangement to 2-methyl-1-naphthol (III). The aromatization of the intermediate ketone (II) would be an additional driving force in the decarboxylation of the β-keto acid formed from XIV. That this conversion does indeed take place was readily demonstrated by the hydrolysis of the β-keto ester (I) in dilute alkali, which led to an instantaneous conversion to the naphthol (III).

In preliminary experiments[4] with the peptide (XIV), no enzymatic hydrolysis by the carboxypeptidase in tryptically activated pancreatic homogenate was obtained at *p*H 7.8 and 37° in 2 hours. The β-keto ester (I) was not hydrolyzed by esterase[5] or lipase[5] at *p*H 7.8 at 37° in 2 hours with rat liver and pancreas.

The ketomethyldihydronaphthoylphenylalanine (XIV) was prepared from methyl 1-keto-2-methyl-1,2,3,4-tetrahydronaphthoate (VI), readily obtainable from 1-tetralone by the method of Bachmann and Thomas.[6] The ester (VI) was hydrolyzed to ketomethyltetrahydronaphthoic acid (VII) by cold, dilute alkali within 24 hours in good yield. This reaction is remarkable in that generally a tertiary ester group as in VI is more resistant to hydrolysis. The acid (VII) is stable at room temperature for periods of several days. It was converted into ketomethyltetrahydronaphthoyl chloride (VIII), which reacted readily with ammonia to give the amide (IX), and with glycine ester and phenylalanine ester to afford (after saponification) the respective ketomethyltetrahydronaphthoyl amino acid derivatives (X and XI). Upon treatment with N-bromosuccinimide, the acid chloride (VIII) gave presumably the bromo-acid chloride (XII) which, at once and without isolation, was made to react with phenylalanine ethyl ester in the presence of an excess of pyridine to form ketomethyldihydronaphthoylphenylalanine ethyl ester. The ester was readily

(1) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service, (in part) by a grant from the American Cancer Society (Massachusetts Division), and (in part) by an institutional grant to Harvard University from the American Cancer Society.

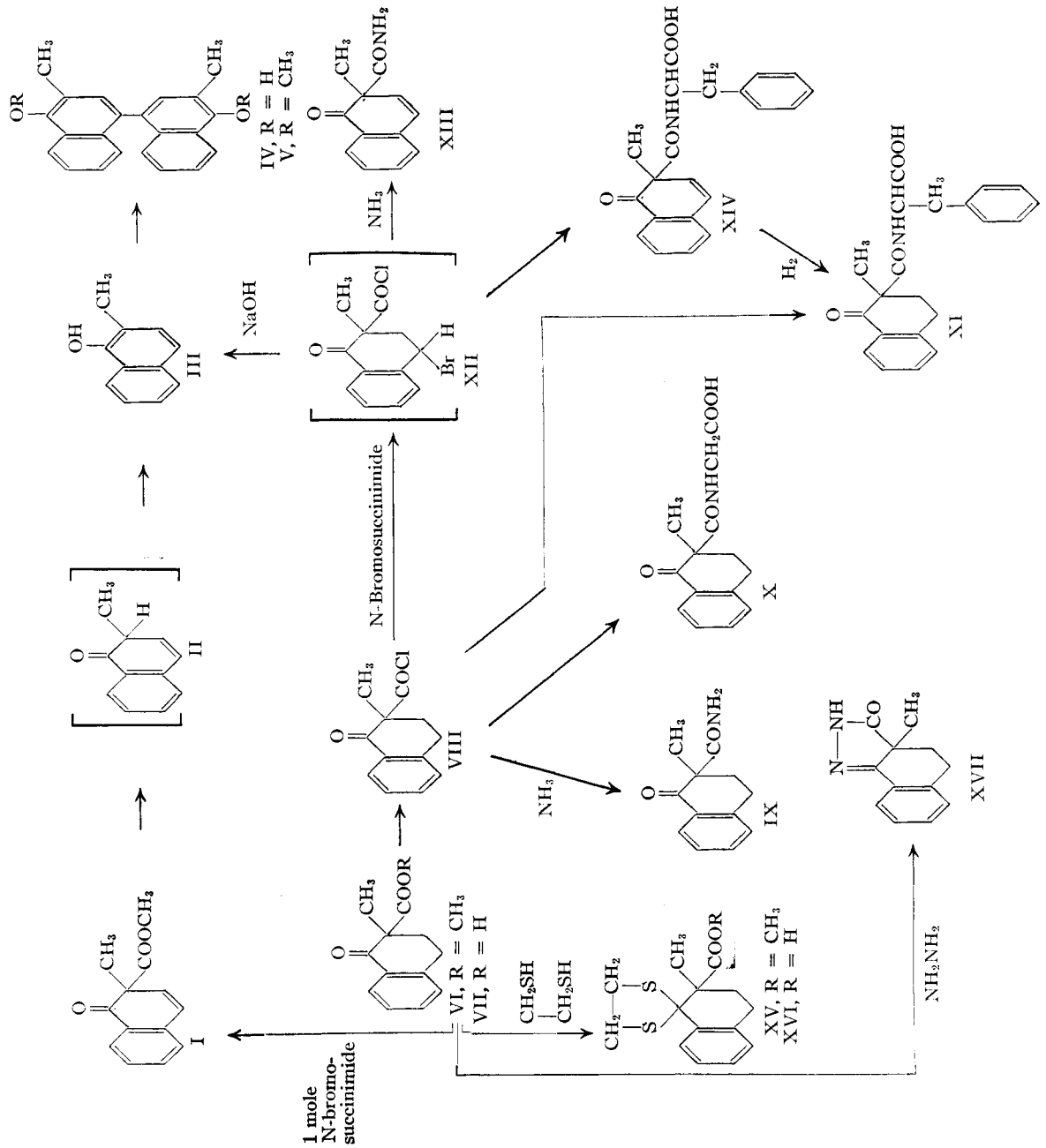
(2) G. Wolf and A. M. Seligman, THIS JOURNAL, **73**, 2080 (1951).

(3) H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 69 (1950).

(4) H. A. Ravin and A. M. Seligman, *J. Biol. Chem.*, in press.

(5) M. M. Nachlas and A. M. Seligman, *ibid.*, **181**, 343 (1949).

(6) W. E. Bachmann and D. G. Thomas, THIS JOURNAL, **63**, 598 (1941).



hydrolyzed to the desired 1-keto-2-methyl-1,2-dihydro-2-naphthoyl-DL-phenylalanine (XIV). Pyridine served to dehydrobrominate as well as to take up the acid formed in the reaction between the acid chloride and the amino-acid ester. The known 2-methyl-1-naphthol[7] (III) was isolated (in the form of a picrate) as a by-product of this reaction. In order to demonstrate the presence of the essential 3,4-double bond in compound (XIV), it was hydrogenated under very mild conditions to ketomethyltetrahydronaphthoyl-phenylalanine (XI), which proved to be identical with XI obtained by the reaction of the acid chloride (VIII) with phenylalanine ester followed by saponification.

(7) R. Lesser, *Ann.*, **402**, 1 (1914); M. Tishler, L. F. Fieser and N. L. Wendler, *ibid.*, **62**, 2866 (1940).

The bromo-acid chloride (XII) also reacted with ammonia to give the unsaturated amide (XIII). With dilute alkali, the bromo-acid chloride (XII) gave 2-methyl-1-naphthol (III), obtained as the picrate.

Exploratory brominations of methyl ketomethyltetrahydronaphthoate (VI) with N-bromosuccinimide were carried out. With an excess of the reagent and a long reaction time, an intermediate methyl ketomethyldibromotetrahydronaphthoate was presumably formed, though not isolated, and dehydrobrominated by pyridine to methyl 1-keto-2-methyl-1,2-dihydro-7-bromonaphthoate which, upon saponification, gave 2-methyl-7-bromo-1-naphthol. The position of the bromine atom was tentatively assigned to 7 in the naphthalene nucleus since this position is meta to the

keto group and para to the methylene group in the ester (VI) which was brominated. 2-Methyl-5-bromo-1-naphthol is known[8] and differs from the compound obtained in this reaction.

Bromination of the ester (VI) with one mole of N-bromosuccinimide and subsequent dehydrobromination, resulted in a mixture of a methyl ketomethyldihydrobromonaphthoate and methyl ketomethyldihydronaphthoate (I). The latter compound was isolated in small yield by fractional distillation.

Attempts to obtain the 2-methyl-1-naphthol, frequently isolated as a by-product in the form of the picrate, failed owing to rapid oxidation in air, as already observed by Lesser.[7] The product of oxidation, dihydroxydimethyldinaphthyl (IV) was isolated and characterized as the corresponding dimethoxy derivative (V).

Attempts were made to protect the keto group of the ester (VI) by a group which could again be easily removed. The ester (VI) failed to react with ethylene glycol, ethyl mercaptan or 2-thioethanol, but was readily converted into the ethylene mercaptol (XV) by treatment with 1,2-ethanedithiol[9] and zinc chloride. The mercaptol of the ester was hydrolyzed to the mercaptol of the acid (XVI), although with great difficulty. Removal of the mercaptol group with recovery of the keto ester (VI) was not achieved. Treatment of the ester (VI) with hydrazine resulted in the formation of 2-methyl-1,2,3,4-tetrahydronaphtho-1,2-pyrazolone (XVII).

Experimental[10]

1-Keto-2-methyl-1,2,3,4-tetrahydro-2-naphthoic Acid (VII).—Methyl 1-keto-2-methyl-1,2,3,4-tetrahydro-2-naphthoate[6] (VI) (2 g.), suspended in *N* sodium hydroxide solution (9.2 cc.), was shaken for 24 hours. The resulting mixture was filtered, washed with ether and acidified with dilute acid. The precipitated product was extracted with ether, the ether solution was washed with water, dried and evaporated under reduced pressure at room temperature. The residue, 1.4 g. (76%) crystallized from ether by addition of ligroin as colorless prisms, m.p. (with decomposition) 102°.

Anal. Calcd. for $C_{12}H_{12}O_3$: C, 70.58; H, 5.94. Found: C, 70.65; H, 5.92.

The compound was stable at room temperature for several days. It was prepared more rapidly, though in smaller yield, by heating the ester (VI) (1 g.) in 5% potassium hydroxide solution (10 cc.) and ethanol (10 cc.) with shaking at 60° for 30 minutes. The alcohol was evaporated under reduced pressure at room temperature, the solution filtered, washed with ether, acidified and further worked up in the same way as described above; yield 0.45 g. (48%).

1-Keto-2-methyl-1,2,3,4-tetrahydro-2-naphthoyl Chloride (VIII).—To ketomethyltetrahydronaphthoic acid (VII) (4 g.) dissolved in anhydrous benzene (200 cc.) was added thionyl chloride (4.8 cc.) and the solution kept at room temperature for about 18 hours. The solvents were removed under reduced pressure at 50° and three portions of anhydrous benzene (100 cc.) were added and removed successively in the same manner, to ensure complete removal of thionyl chloride. The oily residue consisted of the acid chloride.

1-Keto-2-methyl-1,2,3,4-tetrahydro-2-naphthoylamide (IX).—A solution of the above acid chloride (0.5 g.) in benzene (20 cc.) was saturated with dry ammonia. The solvent was then evaporated and upon concentration and addition of ligroin colorless prisms, 0.34 g. (74%), were obtained; crystallized from benzene–ligroin, m.p. 144°.

Anal. Calcd. for $C_{12}H_{13}O_2N$: C, 71.01; H, 6.46. Found: C, 71.25; H, 6.40.

1-Keto-2-methyl-1,2,3,4-tetrahydro-2-naphthoylglycine (X).—To a solution of the acid chloride (VIII) (0.5 g.) in benzene (20 cc.) was added a solution of glycine methyl ester (0.2 g.) and anhydrous pyridine (0.2 cc.) in anhydrous benzene (5 cc.). The solution was warmed on the steam-bath for 10 minutes, cooled and extracted with dilute hydrochloric acid. The benzene was then replaced by methanol (30 cc.) and the ester was saponified by the addition of a *N* solution of potassium hydroxide (5 cc.) and water (10 cc.). The methanol was evaporated on the steam-bath. The residual solution was then washed with ether and acidified with dilute acid. The precipitated product was extracted with ether and crystallized by addition of ligroin. After recrystallization, the yield was 0.28 g. (48%), m.p. 127°.

Anal. Calcd. for $C_{14}H_{15}O_4N$: C, 64.30; H, 5.76. Found: C, 64.01; H, 5.54.

1-Keto-2-methyl-1,2,3,4-tetrahydro-2-naphthoyl-DL-phenylalanine (XI).—To a solution of the acid chloride (VIII) (0.5 g.) in benzene (20 cc.) was added a solution of DL-phenylalanine ethyl ester (0.44 g.) and anhydrous pyridine (0.2 cc.) in benzene (5 cc.). The solution was warmed on the steam-bath for 10 minutes, cooled and extracted with dilute hydrochloric acid. The ketomethyltetrahydronaphthoylphenylalanine ethyl ester was then saponified in the same manner as the glycine ethyl ester above. It first appeared as an oil, but was crystallized by solution in hot benzene followed by the addition of ligroin until cloudy. Benzene was added dropwise until the hot solution was just clear. Upon cooling, crystals of the phenylalanine derivative appeared, 0.24 g. (31%); after recrystallization, m.p. 109°.

Anal. Calcd. for $C_{21}H_{21}O_4N$: C, 71.74; H, 6.04. Found: C, 71.28; H, 6.57.

1-Keto-2-methyl-1,2-dihydro-2-naphthoyl-DL-phenylalanine (XIV).—A solution of the acid chloride (VIII) (2 g.), N-bromosuccinimide (1.5 g.) and benzoyl peroxide (0.05 g.) was dissolved in carbon tetrachloride (100 cc.) and refluxed for 1 hour with illumination by a 300-watt bulb. When the reaction was complete, the succinimide floated on the surface of the solvent. The reaction mixture containing the 1-keto-2-methyl-4-bromo-1,2,3,4-tetrahydro-2-naphthoyl chloride (XII) was then cooled in ice and filtered into a solution of DL-phenylalanine ethyl ester (1.76 g.) in anhydrous pyridine (10 cc.). The residue consisted of succinimide. The filtrate was heated on the steam-bath until most of the carbon tetrachloride had evaporated and was refluxed for 30 minutes. The solution was then poured into dilute hydrochloric acid and extracted with ether. The ether extract was washed with dilute sodium carbonate solution to remove traces of succinimide. The ether was then replaced by methanol and the ketomethyldihydronaphthoylphenylalanine ethyl ester was saponified by addition of a *N* potassium hydroxide (20 cc.) and water (10 cc.) and the methanol was evaporated on the steam-bath. The residue was washed with ether, acidified and extracted with ether. The ether extract was re-extracted with a dilute solution of sodium carbonate, in which the ketomethyldihydronaphthoylphenylalanine dissolved, leaving behind some 2-methyl-1-naphthol (III). The product was precipitated from the carbonate solution by addition of dilute acid and extracted with ether; the ether extract was washed with water and dried. The product was crystallized by addition of ligroin to the concentrated ether solution, 0.9 g. (26%), and crystallized from chloroform–ligroin in long, colorless needles, m.p. 228°. It was soluble in alcohol and acetone and was sparingly soluble in ether, chloroform, ligroin and water (0.3 mg./cc.). Very little hydrolysis was observed at *p*H 7.8 at 37° in 2 hours.

Anal. Calcd. for $C_{21}H_{19}O_4N$: C, 72.19; H, 5.49. Found: C, 72.29; H, 5.54.

Hydrogenation.—A solution of ketomethyldihydronaphthoylphenylalanine (XIV) (0.2 g.) in ethanol (50 cc.) was subjected to hydrogenation at room temperature and atmospheric pressure with platinum oxide (0.02 g.) as catalyst. The compound took up hydrogen (12.8 cc. or one mole) in 15 minutes. After removal of the catalyst by filtration and evaporation of the solvent, ketomethyltetra-

(8) V. Vesely and J. Kapp, *Chem. Zentr.*, **95**, II, 2750 (1924).

(9) Obtained through the courtesy of Dr. M. M. Brubaker, E. I. du Pont de Nemours & Co., Wilmington, Delaware.

(10) Microanalyses by Mrs. Shirley Golden. Melting points uncorrected.

hydronaphthoxylphenylalanine (XI) 0.19 g. (94%), m.p. 109°, was obtained, in every respect identical with the substance obtained from ketomethyltetrahydronaphthoyl chloride (VIII).

1-Keto-2-methyl-1,2-dihydro-2-naphthoyl-DL-phenylalanine Ethyl Ester.—Hydrogen chloride was passed through a solution of ketomethyldihydronaphthoylphenylalanine (XIV) (0.5 g.) in absolute alcohol (20 cc.) until saturated and the solution was refluxed for 1 hour. Upon concentration and addition of ligroin the ester precipitated, 0.3 g. (56%), and crystallized from petroleum ether (b.p. 30–60°), in long, colorless needles, m.p. 131°.

Anal. Calcd. for $C_{23}H_{23}O_4N$: C, 73.16; H, 6.14. Found: C, 73.01; H, 6.00.

1-Keto-2-methyl-1,2-dihydro-2-naphthoylamide (XIII).—A solution of 1-keto-2-methyl-4-bromo-1,2,3,4-tetrahydro-2-naphthoyl chloride (XII) in carbon tetrachloride was saturated with dry ammonia. After standing at room temperature for 30 minutes, the mixture was washed with dilute acid, then with dilute alkali and was refluxed in pyridine for 30 minutes, in order to effect dehydrobromination. The mixture was then poured into acid and was extracted with ether. The amide crystallized from ether–ligroin in small yield. It was recrystallized from ligroin, m.p. 133°. The melting point of a sample of this compound mixed with a sample of ketomethyltetrahydronaphthoylamide (IX) was greatly depressed.

Anal. Calcd. for $C_{12}H_{11}O_2N$: C, 71.70; H, 5.49. Found: C, 72.04; H, 5.88.

1,1′-Dimethoxy-2,2′-dimethyl-4,4′-dinaphthyl (V).—A solution of the acid chloride (XII) in carbon tetrachloride was heated with dilute alkali on the steam-bath. The aqueous solution obtained after evaporation of the carbon tetrachloride was washed with ether and treated, with stirring, with several portions of dimethyl sulfate while keeping the solution alkaline. The precipitated dimethoxy compound was extracted with ether and crystallized from alcohol in colorless flakes, m.p. 175°.

Anal. Calcd. for $C_{24}H_{22}O_2$: C, 83.98; H, 6.51; mol. wt., 344. Found: C, 83.56; H, 6.98; mol. wt., 332.

Some 2-methyl-1-naphthol (III) was obtained from the alkaline solution above by precipitation with acid and extraction with ether. The ether solution was washed with dilute sodium carbonate solution. On addition of picric acid in ether, the picrate of the methylnaphthol separated; m.p. 133° (reported[11] m.p. 133°). The compound was identical with that obtained by saponification of methyl ketomethyldihydronaphthoate (I).

Methyl 1-Keto-2-methyl-1,2-dihydro-7-bromo-2-naphthoate.—A solution of the ester (VI) (1 g.), N-bromosuccinimide (1.6 g.) and benzoyl peroxide (0.05 g.) in carbon tetrachloride (50 cc.) was refluxed for 3 hours under illumination by a 300-watt bulb. The solvent was then replaced by pyridine (25 cc.) and the solution refluxed for 30 minutes, cooled, poured into dilute acid and extracted with ether. The ether solution was washed with dilute sodium carbonate solution, water and dried. The ether was replaced by ligroin from which the bromo ester crystallized. It was recrystallized from ligroin, 0.87 g. (64%), m.p. 80°.

Anal. Calcd. for $C_{13}H_{11}O_3Br$: C, 52.96; H, 3.77. Found: C, 53.34; H, 4.01.

(11) J. W. Cornforth, R. H. Cornforth and R. Robinson, *J. Chem. Soc.*, 168 (1943).

2-Methyl-7-bromo-1-naphthol.—The above bromo ester was saponified by solution in methanol, followed by addition of potassium hydroxide. The methanol was removed at the steam-bath. The aqueous residue was washed with ether, acidified and the precipitated methylbromonaphthol extracted with ether. The ether solution was washed with dilute sodium carbonate solution and water, and concentrated. The product crystallized from 75% alcohol in long, colorless needles, m.p. 101°. The picrate crystallized in orange prisms, m.p. 135°.

Anal. Calcd. for $C_{11}H_9OBr \cdot C_6H_3O_7N_3$: C, 43.95; H, 2.73. Found: C, 44.55; H, 3.55.

Methyl 1-Keto-2-methyl-1,2-dihydro-2-naphthoate (I).—A solution of the ester (VI) (1 g.), N-bromosuccinimide (0.8 g.) and benzoyl peroxide (0.05 g.) in carbon tetrachloride (50 cc.) was refluxed for 1 hour under illumination by a 300-watt bulb. The resulting mixture was worked up in the same way as the bromo ester above. The resulting oil was distilled and the fraction boiling at 170–173° (1.5 mm.) was collected and redistilled. It consisted of methyl ketomethyldihydronaphthoate and gave methylnaphthol (III) (isolated as the picrate) upon saponification. At *p*H 7.8 and 37°, hydrolysis in 2 hours was very slight.

Anal. Calcd. for $C_{13}H_{12}O_3$: C, 72.32; H, 5.85. Found: C, 72.76; H, 6.45.

Ethylene Mercaptol of Methyl 1-Keto-2-methyl-1,2,3,4-tetrahydro-2-naphthoate (XV).—To a solution of the ester (VI) (1 g.) in ethanedithiol[9] (1.3 g.) was added freshly fused powdered zinc chloride (0.65 g.). The mixture was heated to the boiling point for 30 seconds, cooled, saturated with hydrogen chloride and kept at room temperature for 30 minutes. It was then poured into water and extracted with benzene. The benzene solution was washed with water, dilute sodium carbonate solution, water again, dried and evaporated. The solid residue was recrystallized from ligroin and obtained as clusters of colorless needles, 1.1 g. (82%), m.p. 109°.

Anal. Calcd. for $C_{15}H_{18}O_2S_2$: C, 61.19; H, 6.17. Found: C, 61.10; H, 6.39.

Ethylene Mercaptol of 1-Keto-2-methyl-1,2,3,4-tetrahydro-2-naphthoic Acid (XVI).—The above ester (0.5 g.) was refluxed in 10% alcoholic potassium hydroxide (25 cc.) and water (2.5 cc.) for 12 hours, and poured into water. The alcohol was removed under reduced pressure, the aqueous solution was washed with ether, acidified and extracted with ether. The ether solution was dried and evaporated. The residue was crystallized from ethyl acetate in colorless flakes, m.p. 150°, 0.3 g. (63%).

Anal. Calcd. for $C_{14}H_{16}O_2S_2$: C, 59.95; H, 5.76. Found: C, 59.61; H, 5.39.

2-Methyl-1,2,3,4-tetrahydronaphtho-1,2-pyrazolone (XVII).—The ester (VI) (0.5 g.) suspended in 85% hydrazine hydrate (10 cc.) was heated on the steam-bath for 30 minutes and then kept at room temperature overnight. The mixture was poured into water and extracted with ether. The ether solution was dried and evaporated, and the residue recrystallized from ligroin in colorless prisms, m.p. 154°.

Anal. Calcd. for $C_{12}H_{12}ON_2$: C, 71.92; H, 6.06. Found: C, 71.51; H, 5.95.

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