thermometer and a glycerol-sealed stirrer. Seventeen grams of anhydrous aluminum chloride was added in small portions by shaking from a small Erlenmeyer flask equipped with a long neck made of glass tubing. The reaction was carried out at 25–30° and the temperature kept constant by use of a water-bath, when necessary. After standing overnight the mixture was decomposed by pouring on ice and hydrochloric acid. The condensate was extracted from the water solution with ether and the phenols isolated from this ether extract by fractionation.

The 3,5-dinitrobenzoyl esters were prepared by the method of Shriner and Fuson.[15]

The use of pyridine as a catalyst resulted in high yields of the esters which were recrystallized from 60% alcohol.

The method of French and Wirtel[16] was employed in the preparation of α-naphthylurethans. Addition of a few drops of a solution of trimethylamine in ether caused an immediate reaction. Recrystallization was accomplished from warm petroleum ether.

(15) Shriner and Fuson, "Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1940, p. 138.

(16) French and Wirtel, THIS JOURNAL, **48**, 1736 (1926).

Summary

1. The methyldipropyl carbinols have been condensed with phenol in the presence of aluminum chloride to give good yields of the corresponding *p*-*t*-octylphenols.

2. The 3,5-dinitrobenzoyl esters and the α-naphthylurethans of these *p*-*t*-octylphenols have been prepared.

3. The structures have been established by synthesis.

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[CONTRIBUTION FROM THE LEDERLE LABORATORIES]

Water-soluble Compounds with Antihemorrhagic Activity

BY B. R. BAKER AND G. H. CARLSON

In a search for water-soluble compounds with antihemorrhagic activity, a number of derivatives of 2-methyl-1,4-naphthohydroquinone and of the quinone have been prepared and, to minimize duplication of efforts, the experience of this Laboratory is reported at this time.

The highly active sodium salts of 1-acetoxy-2-methyl-4-naphthyl hydrogen succinate[1] (a summary of bioassays is given in Table III) and of the corresponding hydrogen glutarate were sufficiently soluble for parenteral use but were rapidly decomposed in solution even at ordinary temperature.[2] Both the mono- and the disodium salts of the hydroquinone *bis*-hydrogen glutarate were readily hydrolyzed. Likewise the salt of the hydrogen succinate of 3-methyl-1-naphthol,[3] though somewhat more stable than that of 2-methyl-1,4-naphthohydroquinone, gradually decomposed in aqueous solution and further work with partially esterified polycarboxylic acids was discontinued.

Chloroacetyl chloride readily converted 1-acetoxy-2-methyl-4-naphthol to the acetoxy-monochloroacetoxy derivative and the latter, with trimethylamine, yielded the readily soluble quaternary ammonium salt which, like the corresponding diammonium salt prepared from the 1,4-*bis*-chloroacetoxy-2-methylnaphthalene as well as the hydrochloride of 1-acetoxy-4-(β-aminopropionoxy)-2-methylnaphthalene, hydrolyzed in warm, aqueous solution. Accordingly, the purely organic esters proved impractical whether employed in alkaline or acidic media and were not further considered.

Partial esterification of phosphoric, thiophosphoric and sulfuric acids with 1-acetoxy-2-methyl-4-naphthol gave compounds which, as sodium salts, were stable in aqueous solution and could be sterilized by autoclaving. Whereas the phosphate and the sulfate showed antihemorrhagic activity equal to 50 and 35%, respectively, of that of an equimolecular amount of 2-methyl-1,4-naphthoquinone, the thiophosphate was less than

(1) Orientation of the substituents is established by the following reactions. Partial deacetylation of 1,4-diacetoxy-2-methylnaphthalene, prepared by reductive acetylation of 2-methyl-1,4-naphthoquinone [for the method see THIS JOURNAL, **64**, 1096 (1942)], gave an acetoxy-2-methylnaphthol, and its methyl ether, after deacetylation and treatment with ammonium sulfite at 180°, yielded an amine which, as the acetate, was identical with that prepared by reducing the coupling product of 3-methyl-1-naphthol and diazotized sulfanilic acid, acetylating the resulting 2-methyl-aminonaphthol (the hydrochloride of which was readily oxidized to 2-methyl-1,4-naphthoquinone) and converting the acetaminonaphthol to the corresponding methyl ether. By this series of reactions the methoxyl in the acetamino derivative and the acetoxyl group in the acetoxy-2-methylnaphthol are fixed in the 4- and 1-positions, respectively.

(2) The instability of apparently this same hydrogen succinate has been reported also by Buck and Ardis, THIS JOURNAL, **64**, 725 (1942).

(3) The naphthol and 3-methyl-1-tetralone [(a) Bachmann and Struve, THIS JOURNAL, **62**, 1618 (1940); (b) Tishler, Fieser and Wendler, *ibid.*, **62**, 2879 (1940)] were prepared by the improved methods given in the experimental section.

20% as active and was required in too large dosage to be generally applicable in practice. The response with both the phosphate and the sulfate, however, was rapid and these essentially neutral solutions are especially advantageous since administration may be either intravenous or intramuscular.

Whereas the diglucoside of 2-methyl-1,4-naphthohydroquinone is too insoluble for practical use of the aqueous solution,[4] about 3 mg. of the monoglucoside dissolves per milliliter and the solution, like that of the more soluble monomaltoside, showed approximately two-thirds the antihemorrhagic activity of an equimolecular amount of the parent quinone. Aqueous solutions of the glycosides are slowly oxidized by air but are stable in the absence of oxygen and under such conditions, or in the presence of reducing agents such as bisulfite, may be sterilized by autoclaving.

In contrast to the glycosides described above, those of 3-methyl-1-naphthol were less active and showed complete response at a minimum of 10 and 20 micrograms, respectively. Glycoside formation, apparently, had decreased the activity of the naphthol somewhat more than that of 2-methyl-1,4-naphthohydroquinone, but, whereas the naphthol has been reported as active at concentrations of 0.6 microgram,[5] complete response was obtained with no less than 5 micrograms and the relative decrease in the activities appears to be very nearly the same. Comparatively large doses of the naphthol derivatives are required for therapeutic uses, but these compounds have the advantage of being stable in aqueous solution without addition of stabilizers.

Complete response was obtained with 50 and 3 micrograms of the mono- and the dihydrochlorides, respectively, of 1-amino- and 1,4-diamino-2-methylnaphthalene,[6] but, on conversion to the monoacetates, these compounds were inactive at the high concentration. The N-(2-methyl-1-naphthyl)-gluconamide and the N-(1-amino-2-methyl-4-naphthyl)-succinamic acid[7] showed corresponding low activities and were inapplicable. Furthermore, because aqueous solutions of the hydrochlorides of these amines were photosensitive and oxidized very easily, commercial application, even of stabilized solutions, seemed inadvisable.

The decreased activity of 2-methyl-1,4-naphthoquinone-ω-potassium sulfonate,[8] of 2-piperidino-1-naphthol[9] and of 1-amino-2-naphthylacetic acid as compared with the parent compounds substantiates the now widely held opinion that, for high antihemorrhagic activity, the methyl group must remain intact in the 2-methylnaphthalene derivatives and the inactivity of 3-methyl-1,4-dihydroxyisoquinoline[10] indicates that alteration in the nuclear structure similarly results in inactivation.[11]

Experimental

1-Acetamino-2-methyl-4-methoxynaphthalene. (A) From 1,4-Diacetoxy-2-methylnaphthalene.—A mixture of 20 g. of 1-acetoxy-2-methyl-4-naphthol,[1] 20 cc. of dimethyl sulfate, 40 g. of anhydrous potassium carbonate and 200 cc. of acetone was boiled for fourteen hours, the filtered solution, diluted with benzene, was washed with water, cold 2% alkali containing a little sodium dithionite, again with water and solvent was distilled from the benzene solution *in vacuo*. The residue, crystallized from petroleum ether, gave 19 g. of the methyl ether, melting at 67–68° after crystallization from ethanol. *Anal.* Calcd. for $C_{14}H_{14}O_3$: C, 73.0; H, 6.1. Found: C, 72.7; H, 6.5.

Deacetylation of the methyl ether (15 g.) with sodium methylate gave 4-methoxy-2-methyl-1-naphthol (5.5 g.; m. p. 101–103° after crystallization from carbon tetrachloride. *Anal.* Calcd. for $C_{12}H_{12}O_2$: C, 76.7; H, 6.4. Found: C, 76.9; H, 6.6), but a better yield was obtained by adding, during ten minutes, 80 cc. of 10% sodium hydroxide containing 0.5 g. of sodium dithionite to a boiling solution of 19 g. of 1-acetoxy-2-methyl-4-methoxynaphthalene in 80 cc. of methanol and, after forty-five minutes, diluting the cooled solution with water. Extraction of the benzene solution of the product with 5% alkali and acidification of the alkaline extract yielded 13 g. of the methoxynaphthol, m. p. 101–103°.

In the conversion to the 1-amino- derivative, an agitated mixture of 2-methyl-4-methoxy-1-naphthol (15 g.), 25 g. of ammonium sulfite and 75 cc. of 9% ammonia water was heated at 175–180° for thirty hours, the oily product was dissolved in benzene, unchanged naphthol was removed by 10% alkali containing a little sodium dithionite, solvent was evaporated from the washed benzene solution and the residue was distilled at 1 mm. A solution of the crude 1-amino-2-methyl-4-methoxynaphthalene in 10 cc. of benzene was treated with 1 cc. of acetic anhydride and,

(4) Riegel, Smith and Schweitzer, THIS JOURNAL, **63**, 1231 (1941).

(5) Fieser, Tishler and Sampson, *J. Biol. Chem.*, **137**, 685 (1941).

(6) In contrast, 1-amino-3-methylnaphthalene was inactive in concentrations of 25 micrograms.

(7) Identity of the acetate of the succinamic acid prepared from 1,4-diamino-2-methylnaphthalene with that obtained by converting 1-acetamino-2-methyl-4-aminonaphthalene to the succinamic acid established the structure

(8) Attempts to prepare the ω-sulfonic acid by oxidation of bis-ω-(2-methyl-1,4-dimethoxynaphthalene)-disulfide led to the formation of 2-methyl-3-hydroxy-1,4-naphthoquinone-ω-sulfonic acid. See the experimental section for details.

(9) Prepared according to the method of Auwers, *Ann.*, **344**, 289 (1906).

(10) Prepared according to the method of Gabriel and Colman, *Ber.*, **33**, 989 (1900).

(11) Christiansen and Dolliver [THIS JOURNAL, **63**, 1470 (1941)], report that 6-methyl-5,8-quinolinequinone is inactive.

TABLE I

Expt.	Substance	G.	Anhydride, g.	Pyridine, cc.	Time, hr.	Yield, g.	M. p., °C.	Analyses: Calcd. for	Calcd. C	Calcd. H	Found C	Found H
I	1-Acetoxy-2-methyl-4-naphthol	4.3	8	30	24	2.2	136–138	$C_{17}H_{16}O_4$	64.6	5.1	65.4	5.5
											65.3	5.3
II	1-Acetoxy-2-methyl-4-naphthol	2.5	5	10	43	2.4	109–110	$C_{18}H_{18}O_6$	65.4	5.5	65.7	5.6
III	3-Methyl-1-naphthol	1	2.5	10	50	0.85	109–111	$C_{15}H_{14}O_4$	69.7	5.5	69.4	5.3
IV	2-Methyl-1,4-naphthohydroquinone	1.5	4.0		16	3.0	156–158	$C_{21}H_{22}O_8$	62.7	5.5	62.6	5.1

after two hours at room temperature, the amide (0.5 g.; m. p. 197–199° after crystallization from ethanol) was filtered off. *Anal.* Calcd. for $C_{14}H_{15}O_2N$: C, 73.3; H, 6.6; N, 6.1. Found: C, 73.5; H, 7.0; N, 6.3.

(B) From 3-Methyl-1-naphthol.—The naphthol (4 g.; preparation described below) was treated with a solution prepared by diazotizing 5.3 g. of sulfanilic acid, the azo-derivative was reduced with 16 g. of sodium dithionite and the precipitated aminonaphthol, purified by recrystallization from hydrochloric acid containing stannous chloride, yielded 3.4 g. of pure 1-amino-2-methyl-4-naphthol hydrochloride (charred at 270°). *Anal.* Calcd. for $C_{11}H_{12}NOCl$: C, 63.1; H, 5.8; N, 6.7. Found: C, 63.2; H, 5.7; N, 6.9.

Oxidation of 1 g. of the aminonaphthol with acidified potassium dichromate gave 0.75 g. of 2-methyl-1,4-naphthoquinone (m. p. 103–105°) which did not depress the melting point of an authentic sample.

A solution of 1 g. of the aminonaphthol hydrochloride in 25 cc. of water at 75°, treated with 0.6 cc. of acetic anhydride and 0.5 g. of sodium acetate in 3 cc. of water, gave 0.98 g. of 1-acetamino-2-methyl-4-naphthol, m. p. 206–208° after crystallization from diluted ethanol. *Anal.* Calcd. for $C_{13}H_{13}O_2N$: C, 72.6; H, 6.1; N, 6.5. Found: C, 72.9; H, 6.5; N, 6.5.

The acetaminonaphthol (0.67 g.), methylated in the usual way with dimethyl sulfate and anhydrous potassium carbonate in acetone solution, gave 0.53 g. of the ether which, crystallized from diluted ethanol, melted at 198–200° and did not depress the melting point of the 1-acetamino-2-methyl-4-methoxynaphthalene prepared as previously described. *Anal.* Calcd. for $C_{14}H_{15}O_2N$: C, 73.3; H, 6.6; N, 6.1. Found: C, 73.0; H, 6.9; N, 6.1.

Preparation of the Esters of the Polycarboxylic Acids.—The naphthols were treated at room temperature with succinic anhydride (glutaric anhydride, expt. II) in pyridine solution, ether was added, and the solution was extracted successively with dilute hydrochloric acid and sodium bicarbonate (expt. I); or the reaction product was added to cold hydrochloric acid, the mixture was extracted with ether, and the acidic product, isolated by extraction with sodium bicarbonate, was crystallized from a carbon tetrachloride–chloroform (expts. I and II), or benzene–heptane solution (expt. III). The ether solution of the original reaction mixture (expt. II), after extraction with sodium bicarbonate and concentration, was diluted with carbon tetrachloride and yielded 0.8 g. of *bis*-(1-acetoxy-2-methyl-4-naphthyl) glutarate, m. p. 164–166° after crystallization from an alcohol–benzene solution. *Anal.* Calcd. for $C_{31}H_{28}O_8$: C, 70.3; H, 5.3. Found: C, 70.4; H, 5.4.

In experiment IV the hydroquinone was boiled with glutaric anhydride and 5 cc. of dimethylaniline in 25 cc. of chloroform, the solution was washed successively with dilute hydrochloric acid and sodium bicarbonate, the alkaline extract was acidified and the precipitated product crystallized from ethanol. The results are summarized in Table I.

Preparation of the Esters of the Amino Acids. (A) 1-Acetoxy-2-methyl-4-naphthyl N-Trimethylglycinate Chloride.—The acetoxynaphthol (5 g.) was treated with 4 cc. of chloroacetyl chloride and 10 cc. of dimethylaniline in 50 cc. of chloroform and, after one hour at 25°, the mixture was boiled thirty minutes. After extraction with dilute hydrochloric acid and sodium bicarbonate solution, solvent was distilled *in vacuo* and the residue, crystallized from ethanol, gave 6 g. of the chloroacetate; m. p. 103.5–104° after recrystallization from a chloroform–heptane solution. *Anal.* Calcd. for $C_{15}H_{13}O_4Cl$: C, 61.5; H, 4.5. Found: C, 61.6; H, 4.9.

A solution of 3 g. of the chloroacetate in 30 cc. of acetone containing 1.2 g. of trimethylamine gave, after twenty-four hours at room temperature, 3.3 g. of the glycinate chloride, m. p. 217° after crystallization from an alcohol–acetone solution. *Anal.* Calcd. for $C_{15}H_{22}O_4NCl$: C, 61.4; H, 6.3; N, 4.0. Found: C, 61.2; H, 6.9; N, 3.9.

(B) 2-Methyl-1,4-naphthohydroquinone *bis*-N-Trimethylglycinate Chloride.—The hydroquinone (5 g.), acylated as previously described with 7.5 cc. of chloroacetyl chloride and 13 cc. of dimethylaniline in 50 cc. of chloroform, gave 7 g. of the *bis*-chloroacetate (m. p. 109–110° after recrystallization from heptane. *Anal.* Calcd. for $C_{19}H_{12}O_4Cl_2$: C, 55.1; H, 3.7. Found: C, 54.7; H, 3.9) a portion (3.3 g.) of which, dissolved in 20 cc. of dioxane, was treated with a solution of 1.5 g. of trimethylamine in 15 cc. of acetone and, after twenty-four hours at room temperature, the deposited gummy product, triturated with acetone and crystallized from a methanol–acetone solution, yielded 1.3 g. of the *bis*-glycinate chloride, m. p. 204° after recrystallization. *Anal.* Calcd. for $C_{21}H_{30}O_4N_2Cl\cdot 2H_2O$: C, 52.4; H, 7.1; N, 5.8; Cl, 14.8. Found: C, (1) 52.4, (2) 52.6; H, (1) 6.9, (2) 7.3; N, 6.0; Cl, 14.9.

(C) 1-Acetoxy-2-methyl-4-naphthyl β-Alanate Hydrochloride.—A mixture of carbobenzoxy-β-alanyl chloride (prepared from 10 g. of carbobenzoxy-β-alanine), 7 g. of the acetoxynaphthol and 10 cc. of dimethylaniline in 50 cc. of chloroform was boiled for thirty minutes, the product, purified as previously described, gave 5.5 g. of the carbobenzoxy-β-alanate (m. p. 106.5–108°. *Anal.* calcd. for $C_{24}H_{23}O_6N$: C, 68.5; H, 5.5; N, 3.3. Found: C, 68.2; H, 5.6; N, 3.5), a portion (2 g.) of which, hydrogenated in the usual manner in glacial acetic acid, yielded, after treatment with hydrogen chloride, removal of the

solvent *in vacuo*, trituration with chloroform and crystallization from a methanol–ether solution, 0.65 g. of the β-alanate hydrochloride, m. p. 164–167° after crystallization from a chloroform–acetone solution. *Anal.* Calcd. for $C_{16}H_{18}O_4NCl \cdot H_2O$: C, 56.2; H, 5.9; N, 4.1. Found: C, 55.8; H, 6.1; N, 4.1.

Preparation of the Sodium Salts of the Inorganic Esters of 1-Acetoxy-2-methyl-4-naphthol.—Pyridine solutions of the acid halide and the naphthol (except that the naphthol was boiled ten minutes with a carbon tetrachloride solution of pyridine and chlorosulfonic acid in the preparation of the sulfate) were mixed (at 15–20° and 40°, respectively, in the preparation of the sodium phosphate and the thiophosphate), the product was poured onto ice (except that in the preparation of the sulfate, solvent was decanted and the residue dissolved in 30 cc. of water), the mixture was neutralized with sodium carbonate and the pure sodium salt was isolated as described below.

(A) 1-Acetoxy-2-methyl-4-naphthyl Sodium Sulfate.—The sodium carbonate solution of the product obtained from 3 g. of the naphthol and 1.5 cc. of chlorosulfonic acid was extracted with chloroform and added to an equal volume of saturated sodium chloride solution. The precipitated sulfate (3.6 g.) was recrystallized twice from water. *Anal.* Calcd. for $C_{13}H_{11}SO_6Na$: C, 49.1; H, 3.5; Na, 7.2. Found: C, 48.5; H, 3.6; Na, 7.3.

(B) 1-Acetoxy-2-methyl-4-naphthyl Sodium Phosphate.—The sodium carbonate solution of the phosphate prepared from 20 g. of the naphthol and 13 cc. of phosphoryl chloride was evaporated to dryness *in vacuo*, the residue was extracted with hot butanol, the extract was concentrated to a sirup *in vacuo*, hot isopropyl alcohol was added and the deposited salt (31.5 g.) recrystallized from a butanol–isopropyl alcohol solution, gave 20 g. of the pure phosphate. *Anal.* Calcd. for $C_{13}H_{11}O_6PNa_2 \cdot H_2O$: C, 43.6; H, 3.1. Found: C, 43.1; H, 3.5.

(C) 1-Acetoxy-2-methyl-4-naphthyl Sodium Thiophosphate.—The thiophosphate, prepared from 5 g. of the naphthol and 3.5 g. of thiophosphoryl chloride, was purified as the corresponding phosphate and gave 3 g. of pure compound. *Anal.* Calcd. for $C_{13}H_{11}SPO_3Na_2 \cdot H_2O$: C, 43.3; H, 4.1; S, 8.9. Found: C, 44.2; H, 4.8; S, 8.3.

Preparation of the Glycosides. (A) Glucosides.—Solutions of α-glucose pentaacetate in acetic acid (60 cc. and 15 cc., respectively, in expts. I and III) were saturated with hydrogen bromide. After fifteen hours at room temperature in experiment I, but after two hours in experiment II, chloroform (200 cc. and 50 cc. in the respective experiments) was added, the solutions were washed with ice-water, dried with calcium chloride and stirred twenty-four hours and seventeen hours, respectively, with the naphthol, anhydrous potassium carbonate and reagent acetone. Solvent was distilled *in vacuo* from the filtered solutions, the residue was crystallized from methanol and was recrystallized from an acetone–methanol solution in experiment I.[12] In experiment III the filtered solution of the acetylated glycoside was washed successively with 1% sodium chloride solution, 10% alkali containing a little sodium dithionite and with dilute acetic acid. Solvent was distilled *in vacuo* and the residual acetate was crystallized from ethanol.

(B) Maltosides.—In experiment II the solution of the maltose octaacetate in 260 cc. of acetic acid was added to a solution of 40 g. of hydrogen bromide in 120 cc. of acetic acid at 0° and, after thirty minutes, 200 cc. of chloroform was added, the mixture was extracted twice with ice-water and the dried solution was stirred for twenty-four hours with the naphthol, reagent acetone and anhydrous potassium carbonate. The filtered solution was washed successively with water, 2% alkali, acetic acid and solvent was evaporated *in vacuo*. The residue (m. p. 175–177° with sintering at 140° after crystallization from methanol) was purified by crystallization from a benzene–heptane solution. The acetylated bromomaltose used in experiment IV was prepared as described above from a solution of maltose octaacetate in 60 cc. of acetic acid and 26 cc. of acetic acid saturated with hydrogen bromide at 0° and the acetylated maltoside was purified by crystallization from ethanol.

(C) Deacetylations.—The acetylated glycosides were treated with hot sodium methylate solutions, the solutions were acidified with acetic acid, solvent was distilled *in vacuo* and the residual glycosides were purified in (Ia) by trituration with ethyl acetate followed by crystallization from 75 cc. of water and separation from a methanol solution by dilution with ethyl acetate; (IIa) by trituration with ethyl acetate and crystallization from water; (IIIa) by trituration with chloroform (the insoluble product, 0.63 g., melted at 223–225°) and crystallization from 50% acetic acid; and (IVa) by crystallization from a butanol–ethyl acetate solution. The results are summarized in Table II.

3-Methyl-1-tetralone.—A mixture of diethyl α-phenyl-β-methylglutarate[13] (70 g.), 400 cc. of concentrated sulfuric acid and 80 cc. of water was heated on the steam-bath for three hours, poured into 500 cc. of ice-water, the solidified precipitate was crystallized from a benzene–petroleum ether solution and gave 37.5 g. of 2-methyl-4-keto-1,2,3,4-tetrahydro-1-naphthoic acid, m. p. 107–110° after recrystallization from benzene. *Anal.* Calcd. for $C_{12}H_{12}O_3$: C, 70.7; H, 5.9. Found: C, 70.8; H, 6.1.

A mixture of 46.5 g. of the naphthoic acid, 0.5 g. of cupric oxide and 47 g. of quinoline was heated seventy minutes at 200–215°, the cooled mixture was added to cold, dilute hydrochloric acid, extracted with petroleum ether, the extract was washed successively with dilute hydrochloric acid, alkali, acetic acid and, after removal of solvent, distilled to give 28.5 g. of 3-methyl-1-tetralone, b. p. 142–143° at 16 mm.; oxime, m. p. 121–122.5°.[3]

3-Methyl-1-naphthol. (a) From 2-Methyl-4-keto-1,2,3,4-tetrahydro-1-naphthoic Acid.—The acid (3 g.) and 0.5 g. of sulfur were heated to 255–265° for thirty minutes and, after addition of a small amount of copper oxide, the product was distilled at 1 mm. A benzene solution of the distillate was washed successively with sodium bicarbonate and alkali containing sodium dithionite. Upon acidification, the alkaline extract gave the naphthol which, after two crystallizations from a heptane–petroleum ether solution, melted at 88–90°, resolidified and

(12) The method of preparation of glycosides developed by Montgomery, Richtmyer and Hudson [THIS JOURNAL, **64**, 690 (1942)] also gave the acetate.

(13) Connor and McClellan, *J. Org. Chem.*, **3**, 573 (1939).

TABLE II

Expt.	Aceto-sugar, g.	Naphthol	G.	K_2CO_3, g.	Acetone, cc.	Acetate, g.	Methanol, cc.	Na, mg.	Glycoside, g.	M. p., °C.	Analyses, % Calcd. for	C	H	Found C	H
I	46.5	1-Acetoxy-2-methyl-	21	60	180	17.0				180–181	$C_{37}H_{30}O_{18}$	59.4	5.5	59.6	5.8
Ia		4-naphthol				19.0	190	120	9.2	206–208	$C_{17}H_{20}O_7$	60.7	6.0	60.3	5.5
														60.6	6.9
II	76.0	1-Acetoxy-2-methyl-	25	60	200	29.5				183–184	$C_{39}H_{46}O_{20}$	56.2	5.6	56.3	6.1
IIa		4-naphthol				10	100	75	2.7	145–150	$C_{23}H_{30}O_{12}$. H_2O	53.6	6.2	53.0 53.2	6.3 6.1
III	12.0	3-Methyl-1-naphthol	4.2	15	50	1.9				135–137	$C_{25}H_{28}O_{10}$	61.3	5.8	61.0	5.6
IIIa						1.0	10	10	0.3	223–225	$C_{17}H_{20}O_8$	63.7	6.3	63.4	6.1
IV	16.0	3-Methyl-1-naphthol	4.5	13	50	2.9				152.5–154	$C_{47}H_{44}O_{18}$	57.3	5.7	57.2	5.7
IVa						14.3	200	200	2.8	175–178	$C_{23}H_{30}O_{11}$	57.3	6.3	57.2	6.8

In experiment IIa the maltoside at first appeared to be hydrated with no definite melting point and even after twenty-four hours *in vacuo* over potassium hydroxide the compound melted over a range of 5°. Deacetylation of 60.5 g. of 1-acetoxy-2-methyl-4-naphthyl-β-maltoside heptaacetate in an atmosphere of nitrogen, using 600 cc. of methanol and 0.5 g. of sodium, gave 35 g. of crude maltoside, a portion (31.5 g.) of which was recrystallized from water and yielded 29 g. of pure product.

melted at 92.5–93°.[3] *Anal.* Calcd. for $C_{11}H_{10}O$: C, 83.5; H, 6.4. Found: C, 83.2; H, 6.6.

(b) **From 3-Methyl-1-tetralone.**—The tetralone (15 g.) was brominated in the usual manner, boiled with dimethylaniline and gave 10.2 g. of the naphthol, m. p. 87–89°.

2-Methyl-1-naphthylamine.—A suspension of 40 g. of 1-nitro-2-methylnaphthalene[14] in 160 cc. of methanol was reduced with Raney nickel at 1–3 atmospheres pressure, the filtered solutions of three similar preparations were combined, treated with 70 cc. of concentrated hydrochloric acid and yielded, after filtration and concentration, 122 g. of the hydrochloride, m. p. 228–231° with decomposition after crystallization from methanol.[15] *Anal.* Calcd. for $C_{11}H_{12}NCl$: C, 68.2; H, 6.3; N, 7.2; Cl, 18.3. Found: C, (1) 68.0, (2) 67.7; H, (1) 6.6, (2) 6.4; N, (1) 7.7, (2) 8.1; Cl, (1) 20.0, (2) 19.7.

The amine hydrochloride (5 g.), treated with 7 cc. of acetic anhydride and 25 cc. of pyridine gave 3.8 g. of the acetamino derivative (m. p. 191–192° after crystallization from benzene.[15] *Anal.* Calcd. for $C_{13}H_{13}ON$: C, 78.3; H, 6.6; N, 7.0. Found: C, (1) 78.1, (2) 78.2; H, (1) 6.6, (2) 6.1; N, (1) 7.2, (2) 7.2) and the same product was obtained by acetylating the free amine in chloroform solution.

N-(2-Methyl-1-naphthyl)-gluconamide.—A mixture of 3 g. of 1-amino-2-methylnaphthalene, 3.5 g. of δ-gluconolactone, 2 cc. of water and 4 cc. of acetic acid was heated at 100° in an atmosphere of nitrogen for eighteen hours. After dilution with chloroform and water, the precipitated product (3.4 g.) was filtered off and crystallized from 50% acetic acid, m. p. 212–214°. *Anal.* Calcd. for $C_{17}H_{21}O_6N$: C, 60.8; H, 6.3. Found: C, 60.3; H, 6.6.

1-Amino-2-naphthylacetic Acid.—A mixture of 5.5 g. of 1-nitro-2-naphthylacetic acid,[16] 75 cc. of methanol and 10 cc. of 10% sodium hydroxide was hydrogenated in the usual manner with Raney nickel at room temperature. The filtered solution, diluted with 100 cc. of water and acidified with acetic acid, yielded 3.8 g. of the amine, m. p. 238–240° (with decomposition) after crystallization from methanol. *Anal.* Calcd. for $C_{12}H_{11}O_2N$: C, 71.7; H, 5.6; N, 7.0. Found: C, (1) 71.8, (2) 72.0; H, (1) 5.7, (2) 5.6; N, (1) 6.9, (2) 7.0.

3-Methyl-1-naphthylamine Hydrochloride.—An agitated mixture of 5 g. of 3-methyl-1-naphthol, 7.5 g. of ammonium sulfite, 7.5 cc. of 28% ammonia water and 15 cc. of water was heated at 165° for sixteen hours. A benzene solution of the oily product was washed with 10% alkali and water, treated with 50 cc. of 5% hydrochloric acid and yielded 4.7 g. of the amine hydrochloride, m. p. 265–267° after crystallization from 60% hot ethanol by addition of concentrated hydrochloric acid. *Anal.* Calcd. for $C_{11}H_{12}NCl$: C, 68.2; H, 6.3; N, 7.2. Found: C, 68.3; H, 6.7; N, 8.0.

1,4-Diamino-2-methylnaphthalene. (a) **From 2-Methyl-1,4-naphthohydroquinone.**—The hydroquinone (7.8 g.), heated as above with 20 g. of ammonium sulfite, 20 cc. of 28% ammonia water and 40 cc. of water, gave an oily product which, treated as described above, yielded 5.5 g. of the diamine dihydrochloride (m. p. 287–290°; 299–301° after recrystallization from hot water by addition of concentrated hydrochloric acid. *Anal.* Calcd. for $C_{11}H_{14}N_2Cl_2$: C, 54.3; H, 5.8; N, 11.5. Found: C, 54.8; H, 6.3; N, 11.2).

The diacetamino compound (0.8 g., prepared by heating a solution of 1 g. of the dihydrochloride in 10 cc. of water and 25 cc. of acetic acid with 3 cc. of acetic anhydride and 1 g. of sodium acetate) melted at 306–308° and was identical with that prepared from the diamine obtained on reduction of the coupling product of diazotized sulfanilic acid and 1-amino-2-methylnaphthalene as described below.

(b) **From 4-(*p*-Sulfo-phenylazo)-1-amino-2-methylnaphthalene.**—A solution of 2.5 g. of the azo-derivative[15] in 40 cc. of 2.5% sodium hydroxide was reduced at 70° with 3.5 g. of sodium dithionite, 25 cc. of ethylene dichloride was added and concentration of the extract, followed by dilution with petroleum ether, yielded 0.7 g. of the diamine, m. p. 113–114° after crystallization from a benzene–petroleum ether solution.[17] The dihydrochloride was obtained in a similar reduction by treating the ethylene dichloride extract with 5 cc. of concentrated hydrochloric acid, evaporating to dryness and crystallizing the residue from hot water by addition of concentrated hydrochloric

(14) Prepared by the method of Fierz-David and Mannhardt, *Helv. chim. acta*, **20**, 1027 (1937).

(15) Lesser [*Ann.*, **402**, 1 (1913)] gives a value of about 230°, with decomposition, for the hydrochloride and 188° for the acetylated amine.

(16) Mayer and Oppenheimer, *Ber.*, **49**, 2110 (1916).

(17) Vesely and Kapp [*Rec. trav. chim.*, **44**, 360 (1925)] prepared this compound by reduction of 1-amino-2-methyl-4-nitronaphthalene and gave the m. p. as 111–113°.

acid, m. p. 300–301°. *Anal.* Calcd. for $C_{11}H_{14}N_2Cl_2$: C, 54.3; H, 5.8; N, 11.5; Cl, 28.4. Found: C, (1) 54.4, (2) 54.7; H, (1) 5.9, (2) 6.1; N, (1) 11.1, (2) 11.2; Cl, (1) 28.7, (2) 28.9. Similarly, a suspension of the azo-compound (3.5 g.) in 35 cc. of water was reduced with 5 g. of stannous chloride in 15 cc. of hot concentrated hydrochloric acid and gave 1.5 g. of the dihydrochloride, m. p. 297–300° after crystallization from dilute hydrochloric acid.

The diamine (5 g.), treated with 15 cc. of acetic anhydride in 35 cc. of dioxane, deposited 7.2 g. of pure 1,4-diacetamino-2-methylnaphthalene (m. p. 308–309° after crystallization from an acetic acid–benzene solution. *Anal.* Calcd. for $C_{15}H_{16}N_2O_2$: C, 70.2; H, 6.3; N, 11.0. Found: C, (1) 70.2, (2) 70.0; H, (1) 6.4, (2) 6.4; N, (1) 11.0, (2) 10.8).

(c) **From 4-(*p*-Carboxyphenylazo)-1-amino-2-methylnaphthalene.**—A solution of diazotized *p*-aminobenzoic acid (prepared from 1.4 g. of the acid) was added at 2° to a solution of 1.6 g. of 1-amino-2-methylnaphthalene in 120 cc. of water and 1.5 cc. of concentrated sulfuric acid, the precipitated azo-compound was reduced in the usual manner with palladinized carbon and the filtered solution, after appropriate manipulations, deposited 0.8 g. of the diamine (m. p. 109–111°: 0.3 g. of impure material separated from the filtrate).

1-Acetamino-2-methyl-4-aminonaphthalene.—A mixture of 14.5 g. of 1,4-diacetamino-2-methylnaphthalene, 70 cc. of ethanol and 70 cc. of concentrated hydrochloric acid was boiled for three hours and, after several hours at 5°, the deposited hydrochloride (12.9 g.) was filtered off. A solution of 14.1 g. of the crude product in 350 cc. of hot water, basified with ammonium hydroxide, deposited the free amine which, crystallized from ethylene dichloride, gave 8 g. of 1-acetamino-2-methyl-4-aminonaphthalene (m. p. 190–191°. *Anal.* Calcd. for $C_{13}H_{14}N_2O_4$: C, 72.8; H, 6.6; N, 13.1. Found: C, 73.1; H, 6.7; N, 13.1), identical with the product (0.99 g. *Anal.* Found: C, 72.9; H, 6.8; N, 13.3) obtained by reduction of 1.25 g. of 1-acetamino-2-methyl-4-nitronaphthalene[17] in 25 cc. of ethanol with Raney nickel at room temperature.

N-(1-Acetamino-2-methyl-4-naphthyl)-succinamic Acid. (A) **From N-(1-Amino-2-methyl-4-naphthyl)-succinamic Acid.**—A hot chloroform solution of 1 g. of 2-methyl-1,4-diaminonaphthalene was treated with 0.7 g. of succinic anhydride, the crude product (1.57 g.) was crystallized from ethanol and gave 1 g. of the pure succinamic acid, m. p. 192° with decomposition. *Anal.* Calcd. for $C_{15}H_{16}O_3N_2$: C, 66.2; H, 5.9; N, 10.3. Found: C, 66.4; H, 6.8; N, 10.6. The acetyl derivative, prepared from 20 mg. of the acid, 1 cc. of acetic acid and 0.1 cc. of acetic anhydride, melted at 250° (with decomposition when fused in a block preheated to 240°), resolidified and then melted at 268–270°. *Anal.* Calcd. for $C_{17}H_{18}O_4N_2 \cdot HOAc$: C, 60.9; H, 5.9; N, 7.5. Found: C, 60.8; H, 5.6; N, 7.8.

(b) **From 1-Acetamino-2-methyl-4-naphthylamine.**—A hot dioxane solution of 3 g. of the amine and 1.6 g. of succinic anhydride gave 2.7 g. of the succinamic acid, m. p. 245°. After recrystallization from acetic acid the compound melted at 250°, resolidified and melted at 269–271° (the melting point was not depressed by addition of the acetate prepared as above described). *Anal.* Calcd. for $C_{17}H_{18}O_4N_2 \cdot HOAc$: C, 60.9; H, 5.9; N, 7.5. Found: C, 61.2; H, 5.8; N, (1) 7.8, (2) 7.8. When dried at 100° and 1 mm. over potassium hydroxide, the compound lost the acetic acid of crystallization. *Anal.* Calcd. for $C_{17}H_{18}O_4N_2$: C, 65.0; H, 5.8; N, 8.9. Found: C, 64.8; H, 6.2; N, 8.9.

2-Chloromethyl-1,4-dimethoxynaphthalene.—Dimethoxynaphthalene[18] (73 g.) was dissolved in a warm solution of 80 cc. of monochloromethyl ether in 200 cc. of acetic acid and, after fifteen hours at 25°, water was added and the mixture extracted with benzene. The extract was washed with sodium bicarbonate, solvent was distilled from the dried, filtered solution, the residue was extracted with petroleum ether, the solution was treated with active carbon, filtered and solvent was distilled *in vacuo*. The residue was distilled (1 mm.; bath temperature 180–190°) and the crystalline distillate (56.5 g.), crystallized from petroleum ether, gave 50.5 g. of the pure product, m. p. 62–63°. The product from a similar preparation was analyzed. *Anal.* Calcd. for $C_{13}H_{13}O_2Cl$: C, 65.9; H, 5.4. Found: C, 66.2; H, 5.8.

2-Methyl-1,4-naphthoquinone-ω-potassium Sulfonate.—An agitated mixture of 5 g. of 2-chloromethyl-1,4-dimethoxynaphthalene, 15 cc. of methanol and a solution of 3.8 g. of sulfur dioxide in 7 cc. of 28% ammonia water and 15 cc. of water was heated at 135° for sixteen hours. After extraction with chloroform, the aqueous solution was diluted with 10 g. of potassium chloride in 30 cc. of water and the deposited salt, reprecipitated from 20 cc. of warm water by addition of 5 g. of potassium chloride in 15 cc. of water, yielded 1.1 g. of impure potassium sulfonate. *Anal.* Calcd. for $C_{13}H_{13}SO_5K$: K, 12.2. Found: K, 15.4.

A solution of 1 g. of the impure potassium salt in 5 cc. of water, treated with a solution of 2.4 g. of potassium dichromate and 2.4 cc. of concentrated sulfuric acid in 15 cc. of water, gave, after fifteen minutes at 90–100°, a solution which, diluted with 4 g. of potassium chloride in 15 cc. of water and cooled to 5° for four hours, yielded 0.46 g. of the quinone sulfonate. The salt was further purified by recrystallization from acidulated water. *Anal* Calcd. for $C_{11}H_7SO_5K$: K, 13.5. Found: K, 13.0.

The S-benzylthiuronium salt, crystallized from 50% ethanol, melted at 182–183° with decomposition. *Anal.* Calcd. for $C_{19}H_{18}N_2S_2O_5$: C, 54.5; H, 4.3; N, 6.7. Found: C, 54.4; H, 4.2; N, 7.1.

ω-*bis*-(1,4-Dimethoxy-2-methylnaphthalene)-disulfide.—A solution of 10 g. of potassium hydroxide in 150 cc. of absolute ethanol was saturated with hydrogen sulfide and boiled for ninety minutes with a suspension of 18.8 g. of 1,4-dimethoxy-2-chloromethylnaphthalene in 100 cc. of absolute ethanol, the diluted mixture was acidified, the separated oil dissolved in ether and the solution was extracted with 10% alkali. The diluted, cold extract was shaken with chloroform containing 25 g.

(18) Prepared in 91% yield by adding (in a nitrogen atmosphere) 40 cc. of dimethyl sulfate to the mixture formed by hydrogenating 16 g. of 1,4-naphthoquinone in 50 cc. of methanol, then adding a solution of 48 g. of potassium hydroxide in 100 cc. of water during thirty minutes and, after forty-five minutes at the temperature of the steam-bath, precipitating the product by addition of water. After recrystallization from ethanol, the ether (17.3 g.) melted at 86–87.5°. Sah [*Rec. trav. chim.*, **59**, 1029 (1941)] reported a yield of 58% and a m. p. of 85°.

of iodine, the chloroform solution was then washed with sodium bisulfite, evaporated to dryness *in vacuo* and the residue, crystallized from heptane–ether solution, yielded 11.2 g. of pure disulfide, m. p. 116–117°. *Anal.* Calcd. for $C_{26}H_{26}O_4S_2$: C, 66.9; H, 5.6. Found: C, 66.5; H, 5.8.

Concentration of the filtrate yielded 0.5 g. more of the disulfide and 0.4 g. was obtained from the original ether solution of the sulfhydryl compound.

In another preparation, a mixture of 48 g. of 2-chloromethyl-1,4-dimethoxynaphthalene, 15.5 g. of thiourea and 150 cc. of ethanol was boiled for two hours, a solution of 12 g. of sodium hydroxide in 120 cc. of water was added and the mixture boiled two hours longer. On acidification, the diluted product yielded the oily sulfhydryl derivative which, dissolved in chloroform, was treated with 20 g. of sodium hydroxide in 200 cc. of water, ice and 40 g. of iodine, unchanged iodine was removed with sodium bisulfite, the chloroform solution was evaporated *in vacuo* and the residue, crystallized from a heptane–ether solution, yielded 35.5 g. of the disulfide, m. p. 115–116°.

TABLE III

Expt.	Compound	Active at micrograms
1	2-Methyl-1,4-naphthoquinone	1
2	2-Methyl-1,4-naphthoquinone-ω-potassium sulfonate	>50
3	2-Methyl-3-hydroxy-1,4-naphthoquinone-ω-potassium sulfonate	>50
4	2-Methyl-1,4-naphthohydroquinone	1
5	2-Methyl-1,4-naphthohydroquinone-*bis*-hydrogen glutarate	10
6	2-Methyl-1,4-naphthohydroquinone-N-trimethylglycinate chloride	12
7	1-Hydroxy-2-methyl-4-naphthyl-β-glucoside	3
8	1-Hydroxy-2-methyl-4-naphthyl-β-maltoside	5
9	1-Acetoxy-2-methyl-4-naphthol	2
10	1-Acetoxy-2-methyl-4-naphthyl-hydrogen succinate	3
11	1-Acetoxy-2-methyl-4-naphthyl-hydrogen glutarate	4
12	1-Acetoxy-2-methyl-4-naphthyl-N-trimethylglycinate chloride	4
13	1-Acetoxy-2-methyl-4-naphthyl-β-alanate hydrochloride	4
14	1-Acetoxy-2-methyl-4-naphthyl-sodium sulfonate	6
15	1-Acetoxy-2-methyl-4-naphthyl-sodium phosphate	4
16	1-Acetoxy-2-methyl-4-naphthyl-sodium thiophosphate	10
17	2-Methyl-1-naphthol	5
18	2-Piperidinomethyl-1-naphthol	>50
19	3-Methyl-1-naphthol	5
20	3-Methyl-1-naphthyl-β-glucoside	10
21	3-Methyl-1-naphthyl-β-maltoside	20
22	3-Methyl-1-naphthyl hydrogensuccinate	10
23	2-Methyl-1-naphthylamine hydrochloride	50
24	1-Acetamino-2-methylnaphthalene	>50
25	N-(2-Methyl-1-naphthyl)-gluconamide	>50
26	1-Amino-2-naphthylacetic acid	>200
27	1-Amino-3-methylnaphthalene hydrochloride	>25
28	2-Methyl-1,4-diaminonaphthalene dihydrochloride	3
29	1-Acetamino-4-aminonaphthalene	>50
30	N-(1-Amino-2-methyl-4-naphthyl)-succinamic acid	>50

2-Methyl-3-hydroxy-1,4-naphthoquinone-ω-potassium Sulfonate.—A suspension of 5 g. of *bis*-ω-(1,4-dimethoxy-2-methylnaphthalene)-disulfide in 25 cc. of 30% hydrogen peroxide and 125 cc. of acetic acid was stirred ten hours at room temperature, the resulting solution was evaporated to dryness *in vacuo*, the residue dissolved in water, the solution washed with chloroform and the product crystallized by adding a solution of 10 g. of potassium chloride in 25 cc. of water. After two similar recrystallizations from acidulated potassium chloride solution, the crude product (2.05 g.) gave the pure salt. *Anal.* Calcd. for $C_{11}H_9SO_6K$: K, 12.8. Found: K, 12.5.

Titration[19] of the potassium salt showed the presence of an acidic group with a pK of 4.65; phthiocol, the corresponding 3-hydroxyquinone, has a value of 4.5.

A solution of 0.48 g. of the potassium salt in 15 cc. of cold water containing a drop of concentrated hydrochloric acid was treated with 0.4 g. of S-benzylthiuronium chloride in 5 cc. of water and the precipitated product, recrystallized from ethanol, gave the pure S-benzylthiuronium salt, m. p. 200–201° with decomposition. *Anal.* Calcd. for $C_{19}H_{18}O_6N_2S_2$: C, 52.5; H, 4.2; N, 6.5. Found: C, 52.4; H, 4.4; N, 6.7.

Bioassays.—Day-old chicks were used for these tests made by Drs. L. W. McElroy and J. J. Oleson as described previously.[1] In experiments 5, 6, 16 and 22 only 30, 36, 73 and 69%, respectively, of the chicks showed a clotting time of less than fifteen minutes. The results are summarized in Table III.

Summary

1. The compounds prepared by partial esterification of polycarboxylic acids with 2-methyl-1,4-naphthohydroquinone and its derivatives, esters of 1-acetoxy-2-methyl-4-naphthol formed with succinic and glutaric acids, the *bis*-hydrogen glutarate of 2-methyl-1,4-naphthohydroquinone, the hydrogen succinate of 3-methyl-1-naphthol, 1-acetoxy-2-methyl-4-naphthyl-β-alanate hydrochloride, the N-trimethylglycinate chloride and the corresponding diammonium salt were all hydrolyzed in warm aqueous solution.

2. Esters of 1-acetoxy-2-methyl-4-naphthol formed with sulfuric, phosphoric and thiophosphoric acids (showing activities of 35, 50 and 20%, respectively, of that of 2-methyl-1,4-naphthoquinone) were stable in hot solution as were the monoglucoside and the maltoside of 2-methyl-1,4-naph-

(19) The determination was made by Dr. T. H. Davies of this Laboratory.

thohydroquinone (activities about 66% of that of 2-methyl-1,4-naphthoquinone) in the absence of oxygen. The corresponding glycosides of 3-methyl-1-naphthol gave complete response in minimum concentrations of 10 and 20 micrograms. The active 1-amino- and 1,4-diamino-derivatives of 2-methylnaphthalene were almost inactivated by conversion to the monoacetyl derivatives and these compounds as well as N-(2-methyl-1-naphthyl)-gluconamide and N-(1-amino-2-methyl-4-naphthyl)-succinamic acid were inapplicable.

3. An improved method for the preparation of 3-methyl-1-naphthol and 3-methyl-1-tetralone is described.

4. The structures of the esters of acetoxy-2-methylnaphthol and of the succinamic acid of 1,4-diamino-2-methylnaphthalene were established. From these orientation determinations structures of the new compounds examined for antihemorrhagic activity were ascertained. Substituents in the methyl of 2-methylnaphthalene lower the activity of the derivatives as compared with the parent quinone; 3-methyl-1,4-dihydroxyisoquinoline was inactive.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Heats of Organic Reactions. XIV. The Digestion of β-Lactoglobulin by Pepsin

BY GOTFRED HAUGAARD AND RICHARD M. ROBERTS

It is the aim of the experiments described here to apply calorimetry to the study of proteolytic processes. During the hydrolysis of a protein peptide bonds adjacent to many different types of side-chains are broken, and the peptides and amino acids formed have widely different ionization properties. The thermochemistry of the process is thus of such complexity that it is scarcely to be hoped that measurement of the net heat evolution would yield results of thermodynamic significance. We proposed, therefore, to use the calorimeter as an indicator, and to supplement the thermal measurements by simultaneous chemical determinations.

Acid or alkali have two drawbacks as hydrolytic catalysts for our purposes. The high temperature required for a reasonable rate of reaction is impractical for calorimetric work. Furthermore, acid and alkali are relatively unspecific in their attack of peptide bonds. Enzymatic catalysis eliminates both of these difficulties. The reaction can be carried out near room temperature. Proteolytic enzymes are specific in their action; each enzyme attacks only a few types of peptide bonds, so that a smaller number of processes is involved in the reaction.

Crystalline β-lactoglobulin was chosen as the substrate for the present work. This protein is easily prepared in a salt-free condition, contains no lipid or carbohydrate, and is homogeneous in the ultracentrifuge and electrophoresis cell.

Experimental Procedure

The enzyme selected was crystalline pepsin, which has a *p*H optimum around 1.5. In this case the buffering power of hydrochloric acid is sufficient to maintain constant *p*H. Complication of the heat evolution in an unknown manner by ionization effects, as would be the case for other enzymes requiring solutions buffered near neutrality, is thus avoided.

Enzyme and substrate solutions were made up in sufficient quantity for two simultaneous digestions to be made, one in the calorimeter, to measure heat evolution, and the other separately in a thermostat at the same temperature, using the same proportions of enzyme and substrate solutions in both digestions. Samples of the latter digest were removed at frequent intervals for determination of the following quantities: amino nitrogen, nitrogen not precipitable by trichloroacetic acid, and nitrogen dialyzable through cellophane.

Lactoglobulin.—Crystalline β-lactoglobulin was prepared from raw, skim milk by a modification of the original procedure suggested to one of the authors by Dr. A. H. Palmer.[1] After removal of the casein by coagulation at *p*H 4.5, the *p*H of the whey was readjusted to 6.0–7.0. The less soluble whey proteins are first precipitated by addition of solid ammonia sulfate to half saturation.[2] Solid ammonium sulfate was added to the filtrate from this first precipitate to 80–85% saturation. This second precipitate was redissolved in a minimum amount of water, adjusted to *p*H 6.0–6.5, and the subsequent dialysis and crystallization were carried out in the manner described in Palmer's article cited above.

The crystalline β-lactoglobulin so obtained was recrystallized as follows. The crystals were suspended in water and dissolved by addition of the minimum amount

(1) Palmer, *J. Biol. Chem.*, **104**, 351 (1934).

(2) See, in this connection, M. and S. P. L. Sörensen, *Compt. rend. trav. lab. Carlsberg. Sér. chim.*, **23**, 61 (1939).