by this combination, and normal feathering developed within five days.

Vitamin B_{12} Plus Thymine.—Chicks fed 6 μg. of vitamin B_{12} plus 25 mg. of thymine per 100 Gm. of diet, produced more red blood cells and hemoglobin than did vitamin B_{12} plus either duodenal substance or powdered stomach. However, the regenerations were not sufficient to bring the red blood cell counts and hemoglobin levels back to normal. This combination did not cure the paralysis of the chicks, but feathering returned to normal in a week's time.

Controls.—The chicks used as controls remained anemic, had poor feathering, enlarged crops, and were all paralyzed.

SUMMARY

Folic acid seemed to be the most active vitamin B_{12} potentiator tested. Its combination with vitamin B_{12} cured the induced anemia. Red blood cell counts and hemoglobin levels of the chicks fed this combination returned to normal after fourteen days of treatment. Iron did not have a significant effect when used with the combination of folic acid and vitamin B_{12}. The combination of vitamin B_{12} and thymine produced more hemopoiesis than vitamin B_{12} plus either duodenal substance or desiccated stomach, but was not sufficient to bring the red blood cell counts and hemoglobin levels back to normal. Neither duodenal substance nor desiccated stomach had an appreciable potentiating effect on vitamin B_{12}.

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Glycine Esters of Therapeutically Useful Phenols*

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In a search for water-soluble derivatives that might be free from various undesirable side effects, the preparation of glycine esters of diethylstilbestrol, methyl salicylate, and other therapeutically useful phenols, was undertaken. The esters were obtained as hydrochlorides by hydrogenolysis of the corresponding N-(benzyloxycarbonyl)-glycyl esters. The analgetic activity of methyl glycylsalicylate hydrochloride was tested by a variable radiant heat procedure and found to be equal to or slightly greater than that of acetylsalicylic acid under the conditions of the experiment.

THE PREPARATION of glycine esters of diethylstilbestrol, salicylic acid, methyl salicylate, and other therapeutically useful phenols, was undertaken in a study dealing with the general problem of water-insolubility and various side effects frequently encountered in the therapeutic use of phenolic substances. Glycine derivatives in which either the amino group or the carboxyl group is free are capable of forming water-soluble salts, and such derivatives are formed in the physiological detoxication of aromatic carboxylic acids, arylacrylic acids, arylacetic acids, and certain others (1). It is understandable, therefore, that glycine derivatives of physiologically active alcohols, phenols, and amines with low water-solubility, have been prepared because of the solubilizing effect of the glycine moiety. The glycine derivatives of phenetidin (aminophenacetin or phenocoll) (2), aniline (3), phenol (4), and dihydromorphine (4), have been found to possess the pharmacologic effect characteristic of the parent substance.

The hydrochlorides of phenolic glycinates can be prepared by hydrogenolysis of the corresponding phenolic N-(benzyloxycarbonyl)glycin-

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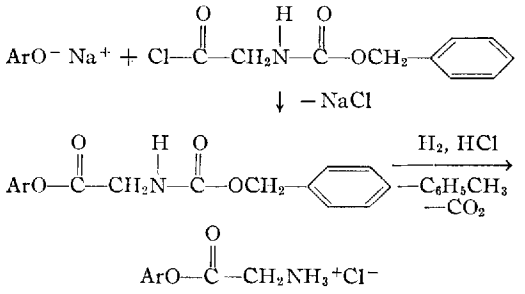
ates which result in the reaction of the phenol or its sodium salt with N-(benzyloxycarbonyl)-glycyl chloride, a reagent that is used for introduction of glycine into peptides (5).



This series of reactions was applied successfully in the preparation of diethylstilbestryl diglycinate dihydrochloride and methyl glycylsalicylate hydrochloride. Satisfactory derivatives could not be obtained from 5,7-dichloro-8-hydroxyquinoline or salicylic acid, although the glycyl ester of the latter (glycylsalicylic acid) was prepared previously by ammonolysis of chloroacetylsalicylic acid (6).

EXPERIMENTAL

All melting points are uncorrected and were observed by use of a Fisher-Johns melting point apparatus.

N-(Benzyloxycarbonyl)glycine was prepared by the reaction of benzyloxycarbonyl chloride (benzyl chloroformate or carbobenzoxy chloride) with glycine in yields of 95% according to the procedure described by Carter, *et al.* (7), when four to five times the quantities of reagents recommended were used and the reaction mixture was left standing overnight at room temperature before isolating the product.

The N-(benzyloxycarbonyl)glycine was converted to the corresponding acid chloride by treatment with phosphorous pentachloride at low temperatures (5), and was employed at once in the reaction with phenols as described below.

Diethylstilbestryl Di[N-(benzyloxycarbonyl)glycinate].—In a solution of 2.3 Gm. (0.1 equivalent) of sodium in 200 cc. of absolute ethyl alcohol, was dissolved 13.4 Gm. (0.05 mole) of diethylstilbestrol by gently refluxing for two hours. After complete removal of the ethyl alcohol by distillation under reduced pressure, the disodium salt of diethylstilbestrol was obtained as a solid and was washed with dry ether and dried.

A mixture of 15.6 Gm. (0.05 mole) of the disodium salt of diethylstilbestrol and 100 cc. of water was cooled to 0° and treated dropwise at that temperature with a solution of 22.7 Gm. (0.1 mole) of N-(benzyloxycarbonyl)glycyl chloride in 66 cc. of dry ether. During this addition the mixture was vigorously shaken, and the agitation was continued for one-half hour at 0° after addition was complete. After the mixture had stood overnight at room temperature, the slightly yellow precipitate which had formed was filtered and washed with one portion of water and two of cold alcohol to remove any diethylstilbestrol or its disodium salt. The diethylstilbestryl di[N-(benzyloxycarbonyl)glycinate] obtained in this way was recrystallized three times from ethyl alcohol, and a 16.5-Gm. (50% of theoretical) yield of a white powder, m.p. 196.5°, was obtained.

Anal.—Calcd. for $C_{38}H_{38}N_2O_8$: C, 70.14; H, 5.89; N, 4.31. Found: C, 70.40, 70.40; H, 6.08, 6.19; N, 4.44, 4.30.

Diethylstilbestryl Diglycinate Dihydrochloride.—A solution of 5 Gm. (0.0077 mole) of diethylstilbestryl di[N-(benzyloxycarbonyl)glycinate] in 1.5 L. of absolute ethyl alcohol was prepared with gentle heating, and placed in a 3-L. three-neck flask. A palladium catalyst (palladium black) prepared from 4 Gm. of palladium chloride by reduction with formaldehyde in strongly alkaline solution (8) was added, along with 16.2 cc. (5% excess) of 1 *N* alcoholic HCl. A rapid stream of hydrogen was passed into the vigorously stirred mixture at atmospheric pressure. Hydrogen was introduced in this way for one and one-half hours while the temperature of the mixture was maintained at 70°, and for an additional six hours after heating was discontinued. After filtration of the catalyst, the ethyl alcohol was removed by distillation under reduced pressure. The brown residue was dissolved in hot absolute ethyl alcohol and left standing overnight in the refrigerator. The crystals which separated from the alcohol solution were combined with an additional crop which was precipitated by careful addition of dry ethyl acetate. By this means, repeating the recrystallization from ethyl alcohol if necessary, a 1.2-Gm. (33% of theoretical) yield of a compound believed to be diethylstilbestryl diglycinate dihydrochloride, m. p. 214°, dec., was obtained. The product was very hygroscopic and decomposed on standing to form a sticky brown mass. Low carbon and nitrogen values and high hydrogen values in the following analytical data are probably a result of the high hygroscopicity of the product.

Anal.—Calcd. for $C_{22}H_{28}Cl_2N_2O_4 \cdot H_2O$: C, 55.81; H, 5.96; N, 5.92. Found: C, 55.48, 55.40; H, 6.50, 6.55; N, 5.53, 5.54.

Methyl N-(Benzyloxycarbonyl)glycylsalicylate.—A mixture of 23.3 Gm. (0.153 mole) of methyl salicylate and 153 cc. of 1 *N* sodium hydroxide was placed in a three-neck flask fitted with a dropping funnel, mechanical stirrer, and thermometer, and chilled to −10° by means of an ice-salt bath. A solution of N-(benzyloxycarbonyl)glycyl chloride, prepared from 32 Gm. (0.153 mole) of N-(benzyloxycarbonyl)glycine, in 100 cc. of cold dry ether, was added dropwise with vigorous stirring to the solution of the sodium salt of methyl salicylate. The addition was complete after one hour, and the mixture was stirred for one-half hour longer at 0°. The crystalline precipitate which separated after the reaction mixture had stood at room temperature for a short time was filtered, washed with water, and recrystallized from dilute alcohol. By this means a 16-Gm. (30.4% of theoretical) yield of methyl N-(benzyloxycarbonyl)glycylsalicylate, m. p. 82°, was obtained.

Anal.—Calcd. for $C_{18}H_{17}NO_6$: C, 62.95; H, 5.16; N, 4.27. Found: C, 62.76; H, 4.99; N, 4.10.

Methyl Glycylsalicylate Hydrochloride.—A solution of 8.44 Gm. (0.0246 mole) of methyl N-(benzyloxycarbonyl)glycylsalicylate in 100 cc. of absolute ethyl alcohol was treated with 25.8 cc. of 1 *N* alcoholic HCl or its equivalent, and 2 Gm. of a 10% palladium on carbon catalyst (9) was added. The mixture was shaken in hydrogen under 60-lb. pressure for two hours at room temperature. After filtration of the catalyst and removal of the alcohol by distillation under reduced pressure, the white crystalline residue was recrystallized repeatedly from mixtures of alcohol and ether. By this means, a 4.5-Gm. (40% of theoretical) yield of methyl glycylsalicylate hydrochloride, m. p. 175–177°, was obtained.

Anal.—Calcd. for $C_{10}H_{12}ClNO_4$: C, 48.81; H, 4.96. Found: C, 48.41; H, 5.08.

Methyl glycylsalicylate hydrochloride was found to be quite soluble in water and alcohol. The alcohol solution appeared to be stable, but the compound underwent hydrolysis rapidly in aqueous solution.

PHARMACOLOGY

The analgetic effect of methyl glycylsalicylate hydrochloride was tested by the variable radiant heat procedure of Hardy, Wolff, and Goodell as modified by Thorp (10, 11).

Pain threshold measurements were made on white rats which had been fasted for twenty-four hours before the test. An aqueous solution containing the desired dose was administered by stomach tube, and readings were made at fifteen, thirty, and sixty minutes after administration. Data were obtained which made possible a comparison of the maximum pain threshold readings in treated rats and average normal readings in the same rats before treatment. The increase in the pain threshold following administration of the analgesic was then expressed as a percentage of the normal pain threshold.

In a series of 17 rats receiving 200 mg. of methyl glycylsalicylate hydrochloride per Kg. of body weight a mean value of 20.9% (σ = 12.0, ϵ = 2.91) increase in pain threshold was observed. In a control series of 17 rats receiving 200 mg. of acetylsalicylic acid per Kg. of body weight, a mean value of 12.2% (σ = 5.3, ϵ = 1.3) increase in pain threshold was observed. The "*t*" value (12) calculated from these data was found to be 2.77. Therefore, it appears that methyl glycylsalicylate hydrochloride exerts an analgetic effect at least equal to that of acetylsalicylic acid and, perhaps, slightly higher under the conditions of this experiment.

SUMMARY

1. In a search for water-soluble derivatives of therapeutically useful phenols which might be free from various undesirable side effects, the hydrochlorides of the glycine esters of diethyl stilbestrol and methyl salicylate have been prepared. Unsuccessful attempts were made in this investigation to prepare similar derivatives of acetylsalicylic acid and 5,7-dichloro-8-hydroxyquinoline.

2. Hygroscopicity and instability, especially to hydrolysis, made the preparation of pure derivatives of this type rather difficult, and hindered pharmacologic studies which had been planned as part of this investigation.

3. Methyl glycylsalicylate hydrochloride appeared to exert an analgetic effect at least equal to and possibly slightly higher than that of acetylsalicylic acid in pain threshold tests of the variable radiant heat type.

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